MONASH University

THE BIOLOGICAL AND PHYSIOLOGICAL IMPACT OF THE REMOVAL OF MIDDLE MOLECULES USING A NEW TYPE OF POLYETHERSULFONE HIGH CUT-OFF MEMBRANE DURING BLOOD PURIFICATION

By

RAFIDAH BINTI ATAN

MBBS (Malaya), M. ANAES (Malaya), FANZCA, EDIC

A thesis submitted for the degree of Doctor of Philosophy at

Monash University in 2017

Jeffrey Cheah School of Medicine and Health Sciences,

Monash University Malaysia

Supervisors:

Joint Principal Supervisor:

Professor Anuar Zaini Md Zain

Jeffrey Cheah School of Medicine and Health Sciences,
Monash University Malaysia
Jalan Lagoon Selatan, 47500 Bandar Sunway,
Selangor Darul Ehsan, Malaysia

Joint Supervisor:

Professor Rinaldo Bellomo

Australia and New Zealand Intensive Care Research Centre,
Department of Epidemiology and Preventive Medicine,
Monash University,
The Alfred Centre,
[redacted]
Victoria 3004, Australia

Copyright notice

Notice 1

Notice 2

Abstract

Background

Shock states with multiorgan failure remains associated with high mortality in critically ill patients. The underlying etiology may vary but the clinical presentation remains largely similar. The underlying mechanisms are still being explored, with hypercytokinemia playing a major role in the pathogenesis. Cytokines are largely middle-molecules, and non-specific cytokine removal as adjunctive therapy is a novel approach that is still being explored. High cut-off hemofiltration is one such technique with great potential, based on simple but sound reasoning. This thesis explores prior evidence and conducts further experiments to test the hypothesis on whether high cut-off point hemofiltration will result in important physiological and biological impact, when applied as adjunctive therapy in critically ill patients in acute kidney injury and on vasopressor support.

Methods

We conducted an extensive literature search for prior evidence to support such an intervention in the form of ex-vivo, animal and human studies. We embarked on the first ever double-blind randomised controlled trial comparing high cut-off point with standard hemofiltration in 76 critically ill patients on vasopressor support who were in acute kidney injury and compared effects on vasopressor requirement. We also compared the two filters thoroughly on various aspects of clinical relevance such as effects on albumin levels, vasopressor levels and duration, hemofiltration duration and filter life. We also conducted concurrent studies on measures of biological impact such as cytokine clearance, apoptosis indices, nucleosome level and toll-like receptor expression.

Results

Our extensive literature search revealed that there were abundant evidence to support higher cytokine removal by high cut-off techniques albeit accompanied by some concerns about higher rates of albumin removal. We also uncovered some

research that supported physiological benefits such as reduction in vasopressor requirement. None of these were however randomised and blinded studies.

Our randomised controlled trial on high cut-off hemofiltration however did not find positive physiological impact of high cut-off hemofiltration when compared with standard hemofiltration as defined by hours of vasopressor-free time. Our thorough comparison on various other aspects including maximum rates and time to permanent cessation of norepinephrine, time to cessation of hemofiltration and filter life also did not find any benefit of high cut-off hemofiltration. Interestingly high cut-off hemofiltration also did not result in significant lowering of plasma albumin levels.

Our comparison on various biological effects found that although sieving by the high cut-off filter was higher for certain cytokines, this did not result in significant lowering of plasma levels of those cytokines. Some other cytokines had similar clearance and sieving as standard membranes. The effects on apoptosis indices, nucleosome levels and toll-like receptor expression were also not significantly different.

Conclusion

The concept of higher cytokine removal offered by high cut-off filters and its simplicity of application was a highly attractive idea, however our study of these filters did not find them efficacious as adjunctive therapies in critically ill patients with acute kidney injury on vasopressor therapy. As any intervention is not devoid of potential harm, studies on adjunctive approaches should investigate alternative methods that may offer better efficacy.

Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

Signature: 

Print Name: RAFIDAH ATAN

Date: 30th OCTOBER 2017

Thesis including published works declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes 6 original papers published in peer reviewed journals, 1 publication as conference proceedings and 1 currently submitted for publication. The core theme of the thesis is a study on the biological and physiological impact of high cut-off hemofiltration in critically ill patients with acute kidney injury. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the student, working within the Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, under the supervision of Professor Rinaldo Bellomo and Professor Anuar Zaini Md Zain.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

My contributions to the publications involved the following:

Publication list	Publication Title	Status	Nature and % of student contribution	Co-author name(s) Nature and % of Co-author's contribution*	Co-author(s), Monash student Y/N*
1	Techniques of extracorporeal cytokine removal: a systematic review of the literature.	Published	Literature search, screening of abstracts, selection of articles, data extraction, analysis and drafting of manuscript 50%	David Crosbie. Literature search, screening of abstracts, selection of articles 30%. Rinaldo Bellomo. Important intellectual input in terms of search strategy, final arbiter in article selection, drafting of manuscript 20%	No – for all co-authors

2	Techniques of extracorporeal cytokine removal: a systematic review of the literature on animal experimental studies.	Published	Literature search, screening of abstracts, selection of articles, data extraction, analysis and drafting of manuscript 50%	David Crosbie. Literature search, screening of abstracts, selection of articles 30%. Rinaldo Bellomo. Important intellectual input in terms search strategy, final arbiter in article selection, drafting of manuscript 20%	No – for all co-authors
3	Techniques of extracorporeal cytokine removal: a systematic review of human studies	Published	Literature search, screening of abstracts, selection of articles, data extraction, analysis and drafting of manuscript 50%	David Crosbie. Literature search, screening of abstracts, selection of articles 30%. Rinaldo Bellomo. Important intellectual input in terms search strategy, final arbiter in article selection, drafting of manuscript 20%	No – for all co-authors
4	A double-blind randomized controlled trial of high cut-off vs. standard hemofiltration in patients with vasodilatory shock and acute kidney injury	Submitted for publication	Study concept and conduct, sample and data collection, data analysis and interpretation, statistical analysis, drafting of the manuscript. 50%	Leah Peck: Study conduct, data collection 10%, John Prowle: Study concept and design 5%, Elisa Licari: Study concept and design 5%, , Glenn M. Eastwood2: study conduct 5%, Marcus Stor: study concept and design 2.5%, Hermann Goehl: study concept and design 2.5%, Rinaldo Bellomo study concept and design, data analysis and interpretation, statistical analysis, drafting of the manuscript 20%.	No – for all co-authors
5	High cut-off hemofiltration versus standard hemofiltration: effect on plasma cytokines.	Published	Study concept and conduct, sample and data collection, data analysis and interpretation, statistical analysis, drafting of the manuscript. 50%	Leah Peck. Study conduct, data collection 5%, Kumar Visvanathan. Study concept and design 7.5%, Narelle Skinner. Laboratory analysis 15%, Glenn Eastwood. Study conduct 2.5% Rinaldo Bellomo. Study concept and design, drafting of manuscript 15% Markus Storr. Study concept and design, drafting of manuscript 5% , Hermann Goehl. Study concept and design, drafting of manuscript 5%	No – for all co-authors

6	High cut-off hemofiltration versus standard hemofiltration: a pilot assessment of effects on indices of apoptosis.	Published	Study concept and conduct, sample and data collection, data analysis and interpretation, statistical analysis, drafting of the manuscript. 50%	Grazia Maria Virzi. Laboratory analysis, drafting of manuscript 12.5%. Leah Peck. Conduct of study, data collection 5%. Amutha Ramadas. Statistical analysis 2.5%. Alessandra Brocca. Laboratory analysis 7.5%. Glenn Eastwood. Conduct of study 2.5% Suneet Sood Drafting of the manuscript 2.5%, Claudio Ronco drafting of manuscript 2.5%. Rinaldo Bellomo. Study concept and design, analysis and interpretation, drafting of manuscript 10%, Hermann Goehl. Study concept, draft of manuscript 2.5%, Markus Storr. Study concept, draft of manuscript 2.5%.	No – for all co-authors
7	Nucleosome levels and toll-like receptor expression during high cut-off hemofiltration: a pilot assessment.	Published	Study concept and conduct, sample and data collection, data analysis and interpretation, statistical analysis, drafting of the manuscript. 50%	Clive May. Study concept and design 5% Simon Bailey. Laboratory analysis 10% Marcel Tanudji. Laboratory analysis 5% Kumar Visvanathan. Study concept and design, drafting of manuscript 5% Narelle Skinner. Laboratory analysis 7.5% Rinaldo Bellomo Study concept and design, drafting of manuscript 12.5% Hermann Goehl. Study concept and design. Drafting of manuscript. 5% Markus Storr. Study concept and design, drafting of manuscript 5%	No – for all co-authors

I have renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Student signature: 

Date: 30th October, 2017

The undersigned hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor signature  **Date:** 30th Oct 2017

Other PhD-related publications during the PhD period

1. World Federation of Societies of Intensive and Critical Care Medicine Congress (WFSICCM) Abstract: Rafidah Atan, John Prowle, Leah Peck, Glenn Eastwood, Rinaldo Bellomo Randomized controlled study of high cut-off point hemofiltration vs. standard hemofiltration in acute renal failure. Journal of Critical Care - December 2013 (Vol. 28, Issue 6, Page e50, DOI: 10.1016/j.jcrc.2013.09.027)

PhD-related presentations during the PhD period

1. Oral presentation: "Pilot Randomized Controlled Study of High Cut-Off Point Hemofiltration vs Standard Hemofiltration in patients with Acute Renal Failure – An Interim Report". Annual Austin Research Prize Surgery and Anaesthesia 2009, Department of Surgery, Austin Hospital 25th November, 2009
2. Poster presentation: “Randomized controlled study of high cut-off point hemofiltration vs. standard hemofiltration in acute renal failure”. 11th World Federation of Societies of Intensive and Critical Care Medicine Congress, Durban, South Africa. August 2013
3. Lecture: “Blood purification in sepsis: Theory vs. Evidence”. Annual Scientific Meeting in Intensive Care (ASMIC) 2014, Kuala Lumpur 16th August 2014
4. Lecture: “Does one filter fit all?” Annual Scientific Meeting in Intensive Care, Kuala Lumpur. 16th August, 2015
5. Lecture: “Extracorporeal blood purification for sepsis” Baxter Healthcare (Malaysia) Sdn Bhd, Kuala Lumpur. 11th January, 2016

PhD-related journal reviewing during the PhD period

1. Blood purification, 2010.
2. Inflammation Research, 2014.

Acknowledgements

I would like to acknowledge the following individuals for their help and support during my PhD journey:

My husband, Nor'azim Mohd Yunos, for his love, support, patience and guidance. You're still the best decision I've ever made in my life. Thank you for being an excellent role model for me and the boys.

My children, Anas, Muaz, Muhammad Umar and Yahya. You guys are my biggest achievements in life and I'm so proud of each and every one of you. I can pursue my career goals and have a family at the same time because you boys are trouble-free, independent and sensible.

My supervisor, Rinaldo Bellomo, for his patience and guidance. You've guided me through all stages of this journey and endured a very long journey indeed. This is despite how busy you are and how outstandingly you stand in the eyes of this world. I can't thank you enough, this thesis could not materialise without you and I apologise for all my shortcomings.

My supervisor, Anuar Zaini Mohd Zain, for his patience and guidance. Your great accomplishments and humility are an inspiration to us all.

My co-authors, colleagues, domestic helpers and extended family members who've all contributed directly or indirectly towards my ability to persevere in this journey.

Chapter 1

Introduction and literature review: High cut-off hemofiltration and extracorporeal blood purification

1.1 Hypercytokinemia in critical illness

Cytokines are small proteins that display autocrine, paracrine and endocrine functions. Their molecular weights fall within the range of middle molecules (0.5 to 60kDa). They are produced by many types of cells; of special note in the context of critical illness is the production of cytokines by immune and endothelial cells. Over the years, new cytokines continue to be discovered, while known ones display newly discovered functions. What is evident is that a particular cytokine can act on multiple sites and perform multiple functions, while at the same time different cytokines have overlapping roles. In scientific terms, they have been described as being both pleiotropic and redundant.

The study of cytokines have spanned over decades of research, resulting in thousands of scientific papers. The extensive amount of literature that supports the study of cytokines and its network is summarised in the following discussion; supported by excellent reviews including that by Bone 1996, Pinsky 2000, Cohen 2002, Dinarello 2007 and Schulte 2013. A few original research papers are also highlighted.

Cytokines play a role in almost every facet of life and are involved in roles as diverse as cell development in embryology, homeostasis in health and disease, and cancer development and prevention. Perhaps one area of extensive study relate to their effects on both innate and adaptive immunity. In the context of critical illness, much of its significance lies in the belief that production is enhanced in response to sepsis and tissue injury.

The pathways of cytokine production under these circumstances are well summarised. Essentially, infectious and non-infectious stimuli present themselves as PAMPs (pathogen-associated molecular patterns) or DAMPs (damage-associated molecular patterns or alarmins) to pattern recognition receptors, such as toll-like receptors (TLRs). The binding of PAMPs and DAMPs to TLRs initiate a complex cellular response, involving important molecules such as the transcription factor NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) which ultimately results in the synthesis and release of mediators including cytokines. The study of

cytokines in critical care however extends beyond that of its production and functions to include possible therapeutic strategies based on this knowledge.

Many cytokines play a proinflammatory role; prominent examples include TNF-alpha (tumor necrosis factor alpha) and interleukin(IL)-1; which in a homeostatic sense may serve to limit infection and minimise the effects of tissue injury. These very same effects, however, also induce undesirable effects such as an increase in vascular permeability, a drop in systemic vascular resistance and a procoagulant effect, and are blamed for the systemic inflammatory response, shock states and multiorgan dysfunction seen in critically ill patients. Selectively blocking the effects of IL-1 and TNF-alpha in sepsis and SIRS therefore seemed like an attractive concept. Results from such studies in critical illness however, have been disappointing and the characteristics of these cytokines may offer an explanation why. IL-1 and TNF-alpha are labelled as ‘early’ cytokines. They play a major role in the initiation of the cytokine cascade at the onset sepsis or SIRS. In later stages they are often undetectable in blood. As such, the failure may be because these cytokines are useful as targets in a prophylactic manner, which is hardly feasible in a clinical context.

‘Late’ cytokines such as HMGB-1 (high mobility group box-1; molecular weight 25 kDa) and MIF (macrophage migration inhibitory factor; MW 12.5kDa), both of which are also proinflammatory but released at later stages of disease and persist longer in the circulation, have also been studied as potential therapeutic targets in conditions such as sepsis; with some animal trials showing benefits in models of critical illness (Wang 2014, Kang 2014, Bloom 2016, Kerschbaumer 2012, Calandra 2000).

Other cytokines act in an anti-inflammatory manner, including IL-10, IL-4 and TGF-beta (transforming growth factor beta). These cytokines may serve to limit and counter the harmful effects of proinflammatory cytokines, and administration of these cytokines have been studied with some animal studies showing benefits. Anti-inflammatory cytokines however, are also blamed for their role in inducing anergy, immunosuppression and susceptibility to secondary infections. One of the most widely studied mechanisms in this regard is the inducement of widespread apoptosis

of mononuclear cells. Selective blocking of anti-inflammatory cytokines has therefore been studied as a therapeutic strategy in patients suffering immunosuppression.

The administration of proinflammatory cytokines such IL-7, GM-CSF (granulocyte-macrophage colony-stimulating factor) and IFN-gamma (interferon gamma) has also been studied to counter this immunosuppression. Some preliminary studies have shown support for this theory. However, concerns arise with their use in patients who are not in the phase of immunosuppression as they may in turn extend the survival of neutrophils and lead to greater harm.

Some cytokines are recognised to have both proinflammatory and anti-inflammatory roles; a prominent example includes IL-6 (Scheller 2011). Preliminary studies on selective antagonism of IL-6, as well as others on enhancement of its effects have also been reported. Some of these studies were in the context of critical illness.

In summary, it is well substantiated that both proinflammatory and anti-inflammatory effects of cytokines are essential for recovery in the setting of infection and tissue injury. At the same time, both effects may play a role in multiorgan dysfunction, secondary infections and death. At the time that our journey was undertaken, it was believed that cytokine excess i.e. hypercytokinemia was highly responsible for poor outcomes in sepsis and SIRS. Essentially this occurs by inflating either proinflammatory or anti-inflammatory effects and moving the host out of the ‘zone’ of homeostasis into a state of harmful excess.

Elevated levels of cytokines such as IL-6, IL-10 and MIF were also found to be associated with poor outcomes, which further supports the theory that hypercytokinemia is harmful (Pinsky 1993, Kellum 2007, Grieb 2010). It was also believed that excess in proinflammatory cytokines, without compensatory increase in anti-inflammatory cytokines, or vice versa, will create an imbalance and that this imbalance is also harmful (Pinsky 2000). There appeared to be a need to block or remove excess cytokines in an attempt to achieve a state of homeostasis and balance again. These theories led to the study of various therapeutic approaches to counter the effects of hypercytokinemia including cytokine removal by extracorporeal

techniques and the use of cytokine antagonists as outlined above. The former offer non-selective removal, while the latter target specific cytokines.

Treatment strategies targeting specific cytokine networks in critically ill patients have been inconclusive at best, and demonstrated harm at worst. It is evident from the above discussion that targeting specific cytokines is a complex approach as it depends much on the timing i.e. which cytokines are playing a prominent role at a particular point in time. The issue of correct targeting in fact goes beyond the issue of timing, but is also affected by the cytokine 'climate' in individual patients; which may vary due to reasons such as severity of insult, state of health and genetic predisposition.

The complexity of targeting specific cytokines and the failure of trials of this approach made the theory of non-specific removal all the more attractive. There is great logic in supporting this latter approach; the degree of non-selective cytokine removal is likely to be concentration dependent and will self-regulate any variability in cytokine responses due to disease and patient factors. By removing all excess cytokines, there is a greater likelihood that return to homeostatic balance will be achieved. In contrast, our knowledge of cytokine networks, coupled by the failure of clinical trials, suggests that targeting specific cytokines may interfere in the balance in ways that cannot be predicted.

Challenges to this logic also exist. After years of study by various groups, the question of whether removing cytokines wil confer benefit or if it will cause harm (or at best prove futile) continues to be debated with proponents on both sides (Schulte 2013, Brown 2016, Frencken 2017). There are other potential flaws in the cytokine reduction theory. Cytokines are powerful molecules and can exert effects at very low concentrations. Furthermore, what matters more may be cytokine effects at tissue level, irrespective of its plasma concentration. There also exists the perennial issue of making deductions based on cytokine levels or measurements - it is highly likely that there are mediators, which are not measured, that are playing an important part.

Our research was undertaken on the premise that hypercytokinemia is harmful due to its excess, that nonspecific removal may benefit the host by returning concentrations

to more homeostatic levels and that this approach may be successful in ways that targeting specific cytokines were not. Our proposed technique was via an extracorporeal approach. Our first step was to find evidence that cytokine removal using these approaches were in fact possible.

1.2 High cut-off hemofiltration as extracorporeal blood purification (EBP) technique

The interest in removing cytokines through the process of renal replacement therapy started more than two decades ago when cytokines were found in the hemofiltrate of patients undergoing renal replacement therapy (Bellomo et al 1993, De Vriese et al 1999). Nonspecific removal of cytokines using extracorporeal blood purification (EBP) techniques subsequently emerged as a favoured approach in dealing with hypercytokinemia, and techniques such as standard hemofiltration, high volume hemofiltration, plasmafiltration and adsorption were studied over the years.

Cytokine molecules are within the middle molecular range of 0.5 to 60kDa. Many would be too large to pass through standard hemofilters that have in vivo cut-off point of 20 to 30kDa. A modification to the technique of hemofiltration was subsequently developed in the form of filters with larger pores. High cut-off (HCO) hemofilters with in vivo cut-off points of 60 to 100kDa are likely to facilitate the passage of most cytokines, even with the phenomenon of membrane fouling i.e. adsorption of proteinaceous materials onto the filter surface that reduces its effective pore size on exposure to the circulation. It is postulated therefore that high cut-off hemofilters would offer better cytokine clearance compared to standard hemofilters.

The notion of using high cut-off hemofilters to remove cytokine is an attractive idea given the simplicity of its concept. Hemofiltration is a procedure commonly performed in the intensive care unit. The technical expertise required is not unusual and widely considered a basic skill. The dose of hemofiltration proposed when using these filters are standard doses. Cytokine removal using high cut-off hemofilters would only require the use of a new type of filter whilst other consummables and equipment remain the same. Additionally, critically ill patients suffering from sepsis and systemic inflammatory response syndrome (SIRS) often develop acute kidney injury requiring hemofiltration. Moreover, this therapy is potentially safe for continuous application, as

opposed to plasmafiltration that could remove 100 percent of cytokines but also cause significant loss of plasma proteins and therefore allowing only intermittent therapy alongside a need for plasma replacement with its associated risks.

One source of concern with the use of high cut-off hemofilters, however, is that the pore size is close to that of albumin (MW 66kDa). The occurrence of membrane fouling may help to limit albumin's passage across the filter but this aspect would require monitoring and remains a safety issue.

1.3 Aim of thesis and research questions

The aim of this thesis is therefore to study the physiological and biological effects of high cut-off hemofiltration as a technique of extracorporeal blood purification (EBP) and to attempt to answer the following research questions:

- Is there enough evidence in the literature to support the use of high cut-off hemofiltration as a technique of extracorporeal blood purification?
- Does high cut-off hemofiltration result in improved haemodynamic stability, as reflected by vasopressor free time, compared to standard hemofiltration, which would suggest positive physiological effects?
- Does high cut-off hemofiltration result in better removal of cytokines as compared to standard hemofiltration, which would suggest positive biological effects?
- Does high cut-off hemofiltration positively impact other biological effects such as apoptosis indices, nucleosome levels and toll-like receptor expression, which may be affected in this subgroup of patients, when compared to standard hemofiltration?
- Are there any concerns especially in terms of protein loss associated with the use of high cut-off hemofiltration?

1.4 Research hypothesis

We hypothesize that hemofiltration with such high cut-off membranes would achieve more rapid improvement in blood pressure and decreased norepinephrine requirements and also decreases serum cytokine concentration compared to hemofiltration with a standard membrane.

High cut off hemofiltration may also beneficially affect cytokine levels, apoptosis indices, nucleosome levels and toll-like receptor (TLR) expression when compared to standard hemofiltration.

We also hypothesize that albumin loss caused by use of high cut off hemofiltration will not cause significant drop in plasma albumin levels when compared to standard hemofiltration.

The first three publications of this thesis therefore seek to find evidence that extracorporeal blood purification with high cut-off hemofiltration will result in high cytokine removal rates. The evidence must also compare its performance to standard hemofilters as well as other EBP techniques such as plasmafiltration, adsorption, high volume hemofiltration and hybrid techniques.

An extensive systematic review using important keywords was performed and more than 2000 abstracts were screened. Only studies which measured cytokine clearance were included as this is taken to be conclusive evidence of cytokine removal. Studies identified were classified into laboratory/ex vivo studies, animal studies and human studies.

The first publication was on ex vivo studies, which are preliminary studies performed under laboratory conditions:

Atan R, Crosbie D, Bellomo R. Techniques of extracorporeal cytokine removal: a systematic review of the literature. Blood Purif. 2012;33(1-3):88-100

1.5 Publication

Original Paper

Blood Purification

Blood Purif 2012;33:88–100
DOI: 10.1159/000333845

Received: May 30, 2011
Accepted: September 28, 2011
Published online: January 13, 2012

Techniques of Extracorporeal Cytokine Removal: A Systematic Review of the Literature

Rafidah Atan[b] David Crosbie[a] Rinaldo Bellomo[a]

[a]Department of Intensive Care, Austin Hospital, Heidelberg, Vic., Australia; [b]Johor Bahru Clinical School, Monash University Sunway Campus, Johor Bahru, Malaysia

Key Words
Cytokines · Hemofiltration · Plasmapheresis · Plasma exchange · High cut-off filters · Couples plasma filtration adsorption · Continuous renal replacement therapy

Abstract
Background and Aims: Attempts at achieving cytokine homeostasis include blood purification to deliver cytokine removal. Assessment of ex vivo studies for optimal operating conditions is a vital step. ***Methods:*** We conducted a systematic search for ex vivo studies on cytokine removal using known modalities of extracorporeal circulation. We selected 29 articles and analyzed data according to clearance, sieving coefficient, ultrafiltrate concentration and percentage removal. ***Results:*** We identified four main techniques for cytokine removal: standard techniques, high cut-off (HCO) techniques, adsorption techniques and combined plasma filtration adsorption. HCO hemofiltration (HCO/HF) showed greatest consistency in cytokine removal among all approaches. Mean albumin clearance with HCO filters was 3.74 ml/min. ***Conclusion:*** Ex vivo data support the view that HCO/HF is the most consistently effective approach in terms of sieving and clearance. Further investigation of HCO/HF in randomized controlled trials in animal models and humans seems desirable.

Introduction

Cytokines are believed to play a key role in the pathophysiology of sepsis, multiorgan dysfunction and other inflammatory states seen in critically ill patients. The stimuli for cytokine release are many and may come in the form of pathogen-associated molecular patterns in the case of microbial sepsis, or damage-associated molecular patterns in the case of systemic inflammatory response syndrome inducing tissue injury [1, 2]. Overexpression leading to hypercytokinemia therefore can occur as a result of microbial sepsis or tissue injury, leading to the observation that although the initial presentation is diverse, patients often spiral into a similar pattern of shock, multiorgan failure and death.

Cytokine release following these triggers occurs through complex pathways which may include activation of the innate immune system through binding of pathogen- or damage- associated molecular patterns to pattern recognition receptors such as toll-like receptors. The mechanisms involved are still being defined but appear to share common pathways despite diverse primary underlying disease. Cytokines exist in both proinflammatory as well as anti-inflammatory forms, and a delicate balance between these two opposing forces is necessary for cytokine release to achieve its protective pur-

KARGER
Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0253–5068/12/0333–0088$38.00/0

Accessible online at:
www.karger.com/bpu

Prof. Rinaldo Bellomo
Department of Intensive Care, Austin Hospital

pose. An excess of proinflammatory cytokines contributes to the development and maintenance of the systemic inflammatory response syndrome, which will then lead to capillary leakage and multiorgan dysfunction, while excess of anti-inflammatory cytokines contributes to the development and maintenance of an immunosuppressive counter-inflammatory response syndrome [3]. These imbalances in cytokines are considered undesirable and invite attempts at restoration of homeostasis [4].

Over the last two decades, attempts were made to restore the cytokine imbalance by blocking proinflammatory effect by means of anticytokine monoclonal antibodies [5]. Unfortunately, such attempts have failed to achieve clinically detectable benefits [6]. Possible mechanisms for such failure include the inability to modulate several cytokines at the same time and the fact that the therapy removes a particular cytokine from the circulation rather than decreasing its levels to a more physiological concentration. There is also the difficulty in delivering an immunomodulating therapy that lasts 24 h a day, which can increase its effect as levels increase and decrease its effects as levels decrease.

Extracorporeal blood purification techniques carry the possibility of overcoming such limitations. Several characteristics of most cytokines make them a logical target for extracorporeal removal. They are of middle molecular weight; they are water soluble, and they are often found free of protein binding. Because of these characteristics and the belief that restoration of cytokine homeostasis is desirable, several investigators have sought to develop techniques of blood purification which can achieve some cytokine removal.

No studies have systematically assessed the efficacy of different extracorporeal techniques in achieving such goals when described under optimal operating conditions ex vivo. Such assessment is vital to develop a logical approach to the selection of the optimal technique of cytokine removal in man. Accordingly, we conducted a systematic review of all ex vivo studies of extracorporeal cytokine removal techniques.

Methods

We conducted a systematic search from April 2010 to January 2011, using the PubMed database for relevant articles on ex vivo studies on cytokine removal using known modalities of extracorporeal circulation. We then systematically assessed the efficacy of all extracorporeal techniques previously reported in the literature using these data.

Our approach at identifying relevant articles for analysis is outlined in figure 1. The following search terms were used: 'cytokine' AND 'continuous renal replacement therapy'; 'cytokine' AND 'hemofiltration'; 'cytokine' AND 'hemodiafiltration'; 'cytokine' AND 'high volume hemofiltration'; 'cytokine' AND 'adsorption'; 'cytokine' AND 'plasmapheresis'; 'cytokine' AND 'bioartificial kidney' and 'cytokine' AND 'coupled plasma filtration adsorption'. All the terms used were MeSH terms except for 'bioartificial kidney' which is a keyword search. Abstracts of articles retrieved were then screened for two inclusion criteria: ex vivo studies and the reporting of a numerical value of at least one of these measures of cytokine removal: clearance, sieving, percentage removal or concentration in the filtrate. Two independent researchers performed the search and then manually screened retrieved articles for those which meet both inclusion criteria. Abstracts which did not include enough details as well as publications with no abstracts provided were traced using library resources, and each paper was screened for inclusion criteria. We excluded review articles and articles published in languages other than English.

We selected 29 articles for detailed analysis and extracted data on these four main ways of expressing cytokine removal: clearance, sieving coefficient (SC), ultrafiltrate (UF) concentration and percentage removal. Of these four, UF concentrations were measured in only 3 papers including one which used healthy donor plasma spiked with recombinant interleukin-6 (IL-6) [7], another which simulated therapy during extracorporeal membrane oxygenation [8] and another involving blood withdrawn from adults undergoing hemodialysis (HD) [9]. As a result of this lack of uniformity, the data for UF concentration were not analyzed further and are not displayed here. This left data on clearance, SC and percentage removal for analysis. Data which were reported only in the form of graphs or figures had their numerical values estimated from the details given in the graphs. When more than one measurement was available, an average value was calculated. The information was first analyzed to seek out techniques that offer the highest rate of cytokine removal based on in vitro data. Where sufficient data were available, these techniques were then analyzed for operating characteristics which appear to offer the best rate of cytokine removal.

In terms of definition, standard techniques refer to the use of standard high-flux hemofilters at standard doses while high cut-off (HCO) techniques refer to the use of super high-flux hemofilters with a nominal cut-off point of more than 60 kDa [10]. Both techniques were further subdivided according to the underlying process involved, either convection (hemofiltration, HF), diffusion (HD) or a combination of both (hemodiafiltration; HDF). The term 'coupled plasma filtration adsorption' (CPFA) was used to refer to techniques where there is initial separation of the cellular component from the plasma component through the use of a plasma filter [11] or a highly porous super high-flux hemofilter [12] prior to exposing the filtered fluid to adsorbents for cytokine removal. Adsorption techniques included all techniques where either whole blood or plasma was exposed to a sorbent. As this review only includes ex vivo studies, some of the adsorption techniques utilizing plasma may perhaps be clinically applied as CPFA, but in this review are not counted as such.

The HCO techniques which had the highest number of ex vivo data reported in the literature were then analyzed to determine whether different approaches, i.e. HF, HD or HDF, using the HCO filter resulted in any difference in the rate of cytokine removal.

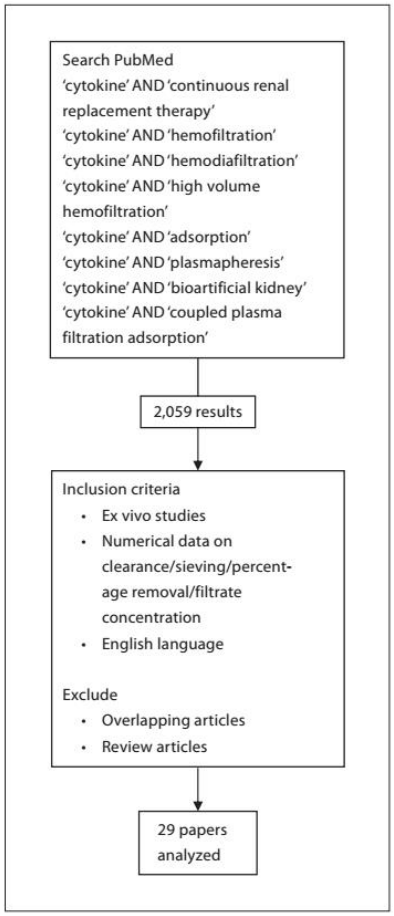


Fig. 1. Flow diagram summarizing the article review process.

Data on standard techniques are almost exclusively available for standard HF (Std HF). We then compared the efficacy of standard techniques with HCO techniques as there are enough data reported for Std HF and because the comparison between the two techniques was considered of potential clinical value. In one of the comparisons, data for HCO/HF for UF flow at 6 liters/h were excluded to achieve similar operating characteristics between HCO/HF and Std HF in an attempt to eliminate the contribution of dosage to the differences observed.

Data on percentage removal were not analyzed further because of insufficient features that were shared across experiments, making it inappropriate for us to pool the data. Furthermore, measurements done following incubation or single pass have little implications on the performance of the device in vivo or in clinical settings. We have kept to descriptive terms when commenting on the results.

We calculated means, standard deviations, median and interquartile ranges for HCO techniques and Std HF. We further highlight that, due to the limited amount of data, we have not made any statistical comparisons and have kept to descriptive terms.

Results

The data extraction process is summarized in figure 1.

We identified four main techniques: standard techniques, CCO techniques, adsorption techniques and CPFA.

Only one paper studied the use of plasmafiltration [13], while another single paper studied the BioLogic-DTPF (detoxification plasma filtration) system which is a combination of hemodiabsorption and CPFA [14]. High-volume HF is not represented in this analysis as there were no in vitro data reporting cytokine removal using this approach.

The main cytokines measured were interleukin-1β (IL-1β), IL-6, interleukin-8 (IL-8), interleukin-10 (IL-10), interleukin-1 receptor antagonist (IL-1ra) and tumor necrosis factor-α (TNF-α). Many of the HCO filter studies also included data on albumin loss [15–20].

Tables 1 and 2 and figure 2 show that data on clearance and SC were available mainly for HCO/HF, HCO/HD, Std HF and CPFA. Other techniques, such as HCO/HDF, Std HD, hemoadsorption, plasmafiltration and BioLogic-DTPF, had limited data. Data for CPFA were mainly for TNF-α. One paper on CPFA was subsequently excluded from analysis because the results displayed appeared to be erroneous as confirmed through communication with one of the authors [10].

Table 3 shows a comparison of clearance with HCO/HD versus HCO/HF. There were insufficient data to include HCO/HDF in the comparison. HCO/HD with a dialysate flow (Q_d) of more than 6 liters/h was excluded to minimize dosage differences as the comparison was aimed at studying the difference in clearance achieved with different approaches, i.e. HD versus HF using the HCO filter. Table 3 shows that, for the same operating characteristics and device code, HCO/HD appeared to achieve lower clearance compared to HCO/HF. However, as is shown in table 1, HCO/HD can achieve high rates of clearance if coupled with very high Q_d at 9–30 liters/h. Although there were insufficient data for HCO/HDF for comparison, the rate of clearance from available data appeared similar in range compared to HCO/HF and HCO/HD when combined flows [ultrafiltrate flow (Q_f) + Q_d] were 3.5–6 liters/h.

Table 1. Clearance of various cytokines and albumin in milliliters per minute according to extracorporeal technique

Technique	Device code	Q_b ml/min	Q_f ml/h	Q_d ml/h	RF	IL-1β	IL-2	IL-6	IL-8	IL-10	IL-1ra	TNF-α	Albumin
HCO/HF [19]	5	300	1,000	NA	pre	13.67		12.33	5.67	10.67		5.67	1.8
HCO/HF [13]	3	200	1,000	NA	post	0.423	4.95			10.2		11.7	
HCO/HF [17]	4	150	1,000	NA	post			16.62			17.59	13.05	8.55
HCO/HF [20]	6	300	1,000	NA	pre	11.67		9.67	4.33	15.67		6.67	1
HCO/HF [15]	1	150	1,800	NA	post	24.85		13.03	11.75		22.56	22.96	2.46
HCO/HF [15]	2	150	1,800	NA	post	23.6		14.73	11.5		21.15	24.3	5.17
HCO/HF [17]	4	150	3,000	NA	post			32.25			41.28	23.87	8.9
HCO/HF [19]	5	300	6,000	NA	pre	80.67		33.67	20.33	65.5		11	1.8
HCO/HF [20]	6	300	6,000	NA	pre	47		24.33	15.5	60.67		12	1
HCO/HD [17]	4	150	NA	1,000	NA			11.69			15.32	8.43	3.27
HCO/HD [18]	5	250	NA	1,000	NA	19.33		14.67	18.33			5	3
HCO/HD 4% alb. [17]	4	150	NA	1,000	NA			12.5			15.47	9.11	
HCO/HD [15]	1	150	NA	3,000	NA	19.6		10.6	12		19.8	12.6	2.08
HCO/HD [17]	4	150	NA	3,000	NA			23.7			29.51	16.18	7.06
HCO/HD 4% alb. [17]	4	150	NA	3,000	NA			13.44			15.98	7.26	
HCO/HD [15]	1	150	NA	5,000	NA	22		11.3	11.7		28.2	16.2	2.68
HCO/HD [18]	5	250	NA	9,000	NA	28.67		17.67	48.33			8	0.6
HCO/HD [16]	8	300	NA	12,000	NA	100		37	8			33	5
HCO/HD [16]	8	300	NA	18,000	NA	112		49	32			35	6
HCO/HD [16]	8	300	NA	30,000	NA	110		75	62			40	5
HCO/HDF [15]	1	150	500	3,000	post	26.4		19.4	17.6		30.6	18.2	2.47
HCO/HDF [15]	1	150	500	5,000	post	30		20.6	17.8		30	23.2	3.29
Std/HF [22]	16	100	900	NA	NA	11.7		2.5				0.1	
Std/HF [22]	17	100	900	NA	NA	8.9		0.7				1.3	
Std/HF [22]	18	100	900	NA	NA	7.4		1.7				0.07	
Std/HF [22]	19	100	900	NA	NA	3.3		1.1				0.2	
Std/HF [23]	12–15	150	1,800	NA	NA			2–3	2–3			0	
Std/HF [34]	27	150	1,800	NA	NA	28.8						2.9	
Std/HF [34]	28	150	1,800	NA	NA	16.5						0.24	
Std/HF [34]	29	150	1,800	NA	NA	20.1						0.96	
Std/HF [21]	9	200	2,000	NA	pre	4.3		–2	23.3	15		3.8	
Std/HD [21]	9	200	NA	2,000	NA	10.7		2.4	19	0.3		2.7	
CPFA [28]	40	200	1,000	NA	NA							0.16	
CPFA [28]	38	200	1,000	NA	NA							13.2	
CPFA [28]	39	200	1,000	NA	NA							15.6	
CPFA [12]	54	250	4,800	NA	NA	45		98.1	24.7	91.9		4.3	
Hemoadsorption [22]	9	100	NA	NA	NA	22.92		23.28				3.42	
BioLogic-DTPF [14]	35			NA	NA	18.7		19				14.3	

Device code: refer to table 6 for details on device used according to code.

Q_b = Blood flow (ml/min); Q_f = ultrafiltrate flow (ml/h); Q_d = dialysate flow (ml/h); RF = replacement fluid either before or after dilution; NA = not available or not applicable; HCO/HD 4% alb. = HCO/HD using 4% albumin as dialysate.

Other abbreviations are as indicated in the main text.

Table 2. SC of various cytokines and albumin according to extracorporeal technique

Technique	Device code	Q_b ml/min	Q_p ml/min	Q_f, ml/h	Q_d ml/h	RF	IL-1β	IL-2	IL-6	IL-8	IL-10	IL-1ra	TNF-α	Albumin
HCO/HF [17]	4	150	NA	1,000	NA	post			0.998			1.025	0.785	0.513
HCO/HF [13]	3	200	NA	1,000	NA	post	0.73	0.34			0.66		0.66	0.06
HCO/HF [19]	5	300	NA	1,000	NA	pre	0.77		0.7	0.3	0.62		0.29	0.08
HCO/HF [20]	6	300	NA	1,000	NA	pre	0.71		0.57	0.25	0.89		0.32	0.06
HCO/HF [15]	1	150	NA	1,800	NA	post	0.82		0.43	0.4		0.75	0.76	0.05
HCO/HF [15]	2	150	NA	1,800	NA	post	0.78		0.49	0.42		0.7	0.81	0.13
HCO/HF [17]	4	150	NA	3,000	NA	post			0.648			0.825	0.495	0.173
HCO/HF [19]	5	300	NA	6,000	NA	pre	0.73		0.33	0.19	0.65		0.1	0.003
HCO/HF [20]	6	300	NA	6,000	NA	pre	0.46		0.25	0.15	0.61		0.11	0.01
HCO/HD [18]	5	250	NA	NA	1,000	NA	0.77		0.57	0.78			0.18	0.11
HCO/HD [17]	4	150	NA	NA	1,000	NA			0.7			0.92	0.51	
HCO/HD 4% alb. [17]	4	150	NA	NA	1,000	NA			0.75			0.93	0.55	
HCO/HD [15]	1	150	NA	NA	3,000	NA	0.4		0.22	0.24		0.4	0.25	
HCO/HD [17]	4	150	NA	NA	3,000	NA			0.47			0.59	0.32	
HCO/HD [15]	1	150	NA	NA	5,000	NA	0.27		0.13	0.14		0.35	0.19	
HCO/HD [18]	5	250	NA	NA	9,000	NA	0.18		0.11	0.31			0.05	0.007
HCO/HD [16]	8	300	NA	NA	12,000	NA	0.49		0.2	0.02			0.15	0.01
HCO/HD [16]	8	300	NA	NA	18,000	NA	0.35		0.15	0.1			0.09	0.02
HCO/HD [16]	8	300	NA	NA	30,000	NA	0.2		0.13	0.12			0.07	0.01
HCO/HDF [15]	1	150	NA	500	2,500	post	0.53		0.39	0.34		0.6	0.36	
HCO/HDF [15]	1	150	NA	500	4,500	post	0.35		0.24	0.21		0.35	0.28	
Std/HF [22]	16	NA	100	900	NA	NA	0.78		0.17				0.18	
Std/HF [22]	17	NA	100	900	NA	NA	0.59		0.05				0.09	
Std/HF [22]	18	NA	100	900	NA	NA	0.49		0.12				0.005	
Std/HF [22]	19	NA	100	900	NA	NA	0.22		0.07				0.01	
Std/HF [13]	10	200	NA	1,000	NA	post	0.23	0.03			0.04		0.047	0.01
Std/HF [23]	12	150	NA	1,800 (pre)	NA	pre			0.01	0.1			0	
Std/HF [23]	12	150	NA	1,800 (post)	NA	post			0.02	0.09			0	
Std/HF [23]	13	150	NA	1,800 (pre)	NA	pre			0.06	0			0	
Std/HF [23]	13	150	NA	1,800 (post)	NA	post			0.04	0			0	
Std/HF [23]	14	150	NA	1,800 (pre)	NA	pre			0.12	0.11			0	
Std/HF [23]	14	150	NA	1,800 (post)	NA	post			0.07	0.1			0	
Std/HF [23]	15	150	NA	1,800 (pre)	NA	NA			0.11	0.12			0	
Std/HF [23]	15	150	NA	1,800	NA	NA			0.1	0.13			0	
Std/HF [29]	21	NA	100	1,800	NA	NA	0.14						0.13	
Std/HF [29]	22	NA	100	1,800	NA	NA	0.02						0.02	
Std/HF [29]	23	NA	100	1,800	NA	NA	0.15						0.1	
Std/HF [29]	24	NA	100	1,800	NA	NA	0.08						<0.01	
Std/HF [29]	25	NA	100	1,800	NA	NA	0.35						0.01	
Std/HF [29]	26	NA	100	1,800	NA	NA	<0.01						0.02	
Std/HF [34]	27	150	NA	1,800	NA	NA	0.96						0.098	
Std/HF [34]	28	150	NA	1,800	NA	NA	0.62						0.01	
Std/HF [34]	29	150	NA	1,800	NA	NA	0.67						0.03	
Std/HD [21]	9	200	NA	NA	2,000	NA	0.32		0.07	0.57	0.01		0.08	
Plasmafiltration [13]	11	200	NA	1,000	NA	NA	0.94	0.03			0.94		0.96	

Device code: refer to table 6 for details of device used according to code.

Q_b = Blood flow (ml/min); Q_f = ultrafiltrate flow (ml/h); Q_d = dialysate flow (ml/h); Q_p = plasma flow (ml/min); RF = replacement fluid either before or after dilution; NA = not available or not applicable; HCO/HD 4% alb. = HCO/HD using 4% albumin as dialysate.

Other abbreviations are as indicated in the main text.

Albumin loss was comparable between HCO/HF, HCO/HD and HCO/HDF except for the use of filter 4 which showed very high albumin clearance (average 8.7 ml/min) with HCO/HF (table 1). Table 1 shows that the clearance of albumin varied from 1 to above 8 ml/min with HCO filters. No data regarding albumin clearance were available for HCO filter 3 or either of the standard filters. Pooling of data on albumin loss by all HCO filters found a mean albumin clearance of 3.74 ml/min.

CPFA appeared to offer a similar level of clearance for TNF-α compared to HCO techniques, with Cole et al. [12] reporting very high levels of clearance achieved with this technique. Similarly, hemoadsorption and BioLogic-DTPF offered a level of clearances similar to HCO techniques for IL-1β and IL-6. Data available for these techniques (CPFA, hemoadsorption and BioLogic-DTPF) were, however, too limited to allow observation of clearance achieved across studies.

A comparison of clearance between Std HF and HCO/HF was then performed as there were sufficient data for both techniques and because these two techniques essentially differ only in the type of filter used (table 4). HCO/HF at UF 6 liters/h was excluded from the table to achieve more comparable blood flow (Q_b) and Q_f between the two techniques. HCO/HF appeared to offer significantly higher cytokine clearance for IL-6 and TNF-α compared to Std HF, but clearance appeared similar for IL-1β.

Data on sieving are shown in table 2. SC was greater with HCO/HF compared to Std HF for IL-1β, IL-6, IL-8 and TNF-α, while SC between the two techniques was similar for IL-10. Comparing SC achieved with different HCO techniques showed a trend towards higher levels of SC achieved with HCO/HF for IL-1β, IL-6 and TNF-α compared with other approaches. SC for IL-8 appeared similar between HCO/HF and HCO/HD. HCO/HF also showed high clearance for IL-10 and IL-1ra. Table 2 also shows that high SC is offered by plasmafiltration. In terms of operating characteristics, increases in both UF and Q_b appeared to decrease SC with HCO/HF.

Table 5 presents data as the percentage reduction in blood levels of cytokines following the intervention and is used as the main measure of cytokine removal for adsorption techniques. There was wide variation in terms of primary technique, device studied, timing of measurement of blood levels and matters relating to conduct of the experiment. Data were available mainly for IL-6 and TNF-α, with some data also available for IL-8. The removal of IL-6 appeared to be highest after 60 min of adsorption using cytosorb (divinylbenzene copolymer beads) and a mesoporous carbon module. The removal of TNF-α was highest through HF using filter 27 followed by adsorption using cytosorb. For IL-8, the highest percentage of removal at 60 min was achieved through adsorption with a mesoporous carbon module followed by Std HF with filter 13 (PAN). Mesoporous carbon modules also removed 100% of IL-1β after 60 min of exposure. IL-1ra appeared to be significantly removed as well via hemoadsorption using cytosorb. Table 6 shows a summary of the various devices utilized for extracorporeal cytokine removal.

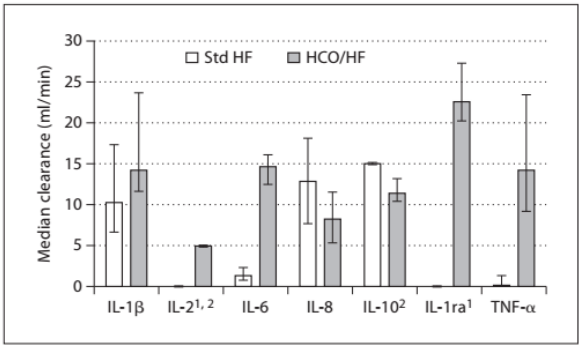


Fig. 2. Median cytokine clearance by Std HF versus HCO/HF, with whiskers indicating interquartile range. [1] No values recorded for IL-2 and IL-1ra for Std HF. [2] No whiskers shown when only one value recorded.

Discussion

Key Findings

We performed a systematic analysis of ex vivo experimentation involving different techniques of blood purification to determine their efficacy in the removal of cytokines under optimal operating conditions. Available ex vivo data on clearance and sieving suggest that a high clearance is consistently achieved with HCO techniques. In particular, HCO/HF shows the greatest consistency compared to other approaches (HCO/HD and HCO/HDF) using this filter type. HCO/HD and HCO/HDF result in less clearance than HCO/HF except when very high Q_d are used. Increasing UF flow increases clearance. In general, Std HF techniques offer insignificant removal of cytokines except for IL-1β (17 kDa) and, perhaps, IL-8 (8 kDa). Albumin clearance with the use of HCO filters ranges from 1 to 8.9 ml/min with a mean value of 3.74 ml/min.

Table 3. Comparison of clearance between HCO/HD and HCO/HF (HCO/HDF was excluded from comparison due to lack of data; comparisons were only performed for HCO techniques and Std HF due to lack of data for other techniques)

Technique	Device code	Q_b ml/min	Q_f, ml/h	Q_d, ml/h[1]	IL-1βb	IL-2	IL-6	IL-8	IL-10	IL-1ra	TNF-α	Albumin
HCO/HD [17]	4	150	NA	1,000			11.69			15.32	8.43	3.27
HCO/HD [18]	5	250	NA	1,000	19.33		14.67	18.33			5	3
HCO/HD 4% alb. [17]	4	150	NA	1,000			12.5			15.47	9.11	
HCO/HD [15]	1	150	NA	3,000	19.6		10.6	12		19.8	12.6	2.08
HCO/HD [17]	4	150	NA	3,000			23.7			29.51	16.18	7.06
HCO/HD 4% alb. [17]	4	150	NA	3,000			13.44			15.98	7.26	
HCO/HD [15]	1	150	NA	5,000	22		11.3	11.7		28.2	16.2	2.68
Mean ± SD		164 ± 38		2,429 ± 1,512	20.3 ± 1.5		14 ± 4.5	14 ± 3.7		20.7 ± 6.5	10.7 ± 4.4	3.6 ± 2
Median		150		3,000	19.6		12.5	12		17.9	9.1	3
IQR (Q_d ≤6 liters/h)		(150, 150)		(1,000, 3,000)	(19.5, 20.8)		(11.5, 14.1)	(11.9, 15.2)		(15.6, 26.1)	(7.8, 14.4)	(2.7, 3.3)
HCO/HF [19]	5	300	1,000	NA	13.67		12.33	5.67	10.67		5.67	1.8
HCO/HF [13]	3	200	1,000	NA	0.42	4.95			10.2		11.7	
HCO/HF [17]	4	150	1,000	NA			16.62			17.59	13.05	8.55
HCO/HF [20]	6	300	1,000	NA	11.67		9.67	4.33	15.67		6.67	1
HCO/HF [15]	1	150	1,800	NA	24.85		13.03	11.75		22.56	22.96	2.46
HCO/HF [15]	2	150	1,800	NA	23.6		14.73	11.5		21.15	24.3	5.17
HCO/HF [17]	4	150	3,000	NA			32.25			41.28	23.87	8.9
HCO/HF [19]	5	300	6,000	NA	80.67		33.67	20.33	65.5		11	1.8
HCO/HF [20]	6	300	6,000	NA	47		24.33	15.5	60.67		12	1
Mean ± SD		222 ± 75	2,511 ± 2,083		28.8 ± 27.1		19.6 ± 9.3	11.5 ± 6	32.5 ± 28	25.7 ± 10.6	14.6 ± 7.3	3.8 ± 3.3
Median		200	1,800		23.6		15.7	11.6	15.7	21.86	12	2.13
IQR		(150, 300)	(1,000, 3,000)		(12.7, 35.9)		(12.9, 26.3)	(7.1, 14.6)	(10.7, 60.7)	(20.3, 27.2)	(11, 23)	(1.6, 6)

HCO/HDF was excluded from comparison due to lack of data. Comparisons were only performed for HCO techniques and Std HF due to lack of data for other techniques. Device code: refer to table 6 for details of device used according to code. Q_b = Blood flow (ml/min); Q_f = ultrafiltrate flow (ml/h); Q_d = dialysate flow (ml/h); NA = not available or not applicable; HCO/HD 4% alb. = HCO/HD using 4% albumin as dialysate; IQR = interquartile range. Other abbreviations are as indicated in the main text.

[1] HCO/HD with Q_d >6,000 ml/h were excluded to achieve similar operating characteristics between HCO/HF and HCO/HD.

Table 4. Comparison of clearance between Std HF and HCO/HF[1]

Technique	Device code	Q_b, ml/min	Q_f, ml/h	Q_d ml/h	IL-1β	IL-2	IL-6	IL-8	IL-10	IL-1ra	TNF-α
Std HF [22]	16	100	900	NA	11.7		2.5				0.1
Std HF [22]	17	100	900	NA	8.9		0.7				1.3
Std HF [22]	18	100	900	NA	7.4		1.7				0.07
Std HF [22]	19	100	900	NA	3.3		1.1				0.2
Std HF [23]	12, 13, 14, 15	150	1,800	NA			2–3 (2.5)	2–3 (2.5)			0
Std HF [34]	27	150	1,800	NA	28.8						2.9
Std HF [34]	28	150	1,800	NA	16.5						0.24
Std HF [34]	29	150	1,800	NA	20.1						0.96
Std HF [21]	9	200	2,000	NA	4.3		–2	23.3	15		3.8
Mean ± SD		133 ± 35	1,422 ± 499		12.6 ± 8.7		1.1 ± 1.7	12.9 ± 14.7			1.1 ± 1.4
Median		150	1,800		10.3		1.4	12.9			0.24
IQR		(100, 150)	(900, 1,800)		(6.6,17.4)		(0.8, 2.3)	(7.7, 18.1)			(0.1, 1.3)
HCO/HF [19]	5	300	1,000*	NA	13.67		12.33	5.67	10.67		5.67
HCO/HF [13]	3	200	1,000*	NA	0.423	4.95			10.2		11.7
HCO/HF [17]	4	150	1,000*	NA			16.62			17.59	13.05
HCO/HF [20]	6	300	1,000*	NA	11.67		9.67	4.33	15.67		6.67
HCO/HF [15]	1	150	1,800*	NA	24.85		13.03	11.75		22.56	22.96
HCO/HF [15]	2	150	1,800*	NA	23.6		14.73	11.5		21.15	24.3
HCO/HF [17]	4	150	3,000*	NA			32.25			41.28	23.87
Mean		200 ± 71	1,514 ± 756		14.8 ± 10		16.4 ± 8.1	8.3 ± 3.9	12.2 ± 3	25.6 ± 10.6	15.5 ± 8.1
Median		175	1,257		14.3		14.7	8.3	11.4	22.6	14.3
IQR		(150, 250)	(1,000, 1,800)		(11.7, 23.6)		(12.5, 16.1)	(5.3, 11.6)	(10.4, 13.2)	(20.3, 27.2)	(9.2, 23.4)

Comparisons were only performed for HCO techniques and Std HF due to lack of data for other techniques. Device code: refer to table 6 for details of device used according to code. Q_b = Blood flow (ml/min); Q_f = ultrafiltrate flow (ml/h); Q_d = dialysate flow (ml/h); NA = not available or not applicable; IQR = interquartile range. Other abbreviations are as indicated in the main text.

[1] HCO/HF with Q_f >3,000 ml/h were excluded to achieve similar operating characteristics between Std HF and HCO/HF.

Other techniques which offer high clearance of cytokines include CPFA, BioLogic-DTPF, hemoadsorption and plasmafiltration. For most of these techniques, however, there are insufficient ex vivo data to demonstrate consistent efficacy across studies [24–27].

Relation to Previous Literature

There are no other reviews in the literature which have studied ex vivo cytokine clearance, sieving or UF concentration or percentage removal data for the comparison of different techniques of blood purification.

Significance of Study Findings

Multiorgan failure secondary to shock states is a common cause of death in critically ill patients. Advancement in the field of intensive care has not resulted in improved outcomes for this cohort [38]. The accompanying hypercytokinemia is believed to be harmful, and nonspecific removal of cytokines seems desirable [39].

The use of blood purification as adjunctive therapy in various shock states for the purpose of cytokine removal has been studied for more than a decade [40]. Despite biological evidence to suggest that this may be a worthwhile approach, details of implementation, such as the best technique and operative characteristics to titrate the rate of removal, remain unclear [4]. Through this review, we now provide some basis for the design of randomized controlled clinical trials. In particular by demonstrating that high cytokine removal is consistently achieved with HCO/HF, we show that, at this time, one of the logical approaches to the treatment of cytokinemia by extracorporeal techniques would be by means of HCO/HF. High-

Table 5. Percentage removal of various cytokines according to extracorporeal techniques

Technique	Device	Q_b ml/min	Q_p ml/min	Q_f ml/h	IL-1β	IL-6	IL-8	IL-10	IL-1α	TNF-α	
Std HF [23]	13	150		1,800		45	65			20	at 60 min
Std HF [23]	13	150		1,800		40	90			–5	at 60 min
Std HF [23]	15	150		1,800		35	15			0	at 60 min
Std HF [23]	15	150		1,800		35	20			5	at 60 min
Std HF [23]	14	150		1,800		20	30			15	at 60 min
Std HF [23]	14	150		1,800		20	40			–5	at 60 min
Std HF [23]	12	150		1,800		5	30			0	at 60 min
Std HF [23]	12	150		1,800		–2	25			20	at 60 min
Std HF [34]	27	150		1,800	25	52				80	at 60 min
Std HF [35]	30		200	600						30	at 60 min
Std HF [35]	31		200	600						7	at 60 min
Std HF [35]	27		200	600						15	at 60 min
Std HF [34]	27	150		1,800						60	at 150 min
Std HF [34]	28	150		1,800						10	at 150 min
Std HF [34]	29	150		1,800						15	at 150 min
Std HDF (0.9% saline dialysate) [37]	32	25				56				58	single pass
Std HDF (2% albumin dialysate) [37]	32	25				77				81	single pass
Std HF [29]	21	100		1,800	3.3					0	single pass
Std HF [29]	22	100		1,800	0					30	single pass
Std HF [29]	23	100		1,800	0					0	single pass
Std HF [29]	24	100		1,800	3.15					0	single pass
Std HF [29]	25	100		1,800	2					32	single pass
Std HF [29]	26	100		1,800	11					0	single pass
Adsorption/mesoporous carbon module [30]	41		0.5		100	92	96			28	at 60 min
Adsorption/non-mesoporous carbon module [30]	42		0.5		8	5	30			5	at 60 min
Hemoadsorption/Cytosorb [32]	50	0.2				90		80		60	at 60 min
Hemoadsorption/Cytosorb [32]	50	0.4				90		80		60	at 60 min
Hemoadsorption/Cytosorb [32]	50	0.8				90		80		60	at 60 min
Hemoadsorption/Cytosorb [32]	50	1.2				90		80		60	at 60 min
Hemoadsorption/Cytosorb [38]	50	0.8				82		72		50	at 60 min
Adsorption/Amberlite XAD-7 [35]	52		30			40				16	at 60 min
Adsorption/activated charcoal [35]	36		30			30				16	at 60 min
Adsorption/CTR [36]	53	1				30				17	at 60 min
Hemoadsorption/Cytosorb [32]	50	0.2				100		100		89	single pass
Hemoadsorption/Cytosorb [32]	50	0.4				100		100		89	single pass
Hemoadsorption/Cytosorb [32]	50	0.8				100		100		89	single pass
Hemoadsorption/Cytosorb [32]	50	1.2			1.25	100		100		0	single pass
Hemoperfusion/ihuman serum albumin [33]	51	0.13				8				33	single pass
Immunoadsorption/CF-X [31]	43					98	69	88		100	incubated 60 min
Immunoadsorption/Immunosorba TR [31]	44					26	80	52		93	incubated 60 min
Immunoadsorption/Immunosorba PH [31]	45					10	72	0.5		68	incubated 60 min
Immunoadsorption/Selesorb [31]	46					37	85	83		1	incubated 60 min
Immunoadsorption/Lixelle [31]	47					72	90	57.5		87	incubated 60 min
Immunoadsorption/MPCF-XM20 [31]	48					97	42	96		100	incubated 60 min
Immunoadsorption/MPCF-XN20 [31]	49					74	30	66		98	incubated 60 min
Hemoadsorption/Cytosorb [32]	50					100		100		10	incubated 2 h
Hemoadsorption/Cytosorb [32]	50					100		100		100	incubated 2 h
Hemoadsorption/Cytosorb [32]	50					61		71		9	incubated 60 min
Adsorption/Amberlite XAD-7 [27]	52					40			16	8	incubated 60 min
Adsorption/DHP1 charcoal [27]	36					26			40	10	incubated 60 min
CPFA [12]	54	250		80 ml/min	90	72	100	70		7	single pass

Values displayed are percentages from either single-pass experiments, following incubation for a specified period of time or measurements taken during flow techniques. For flow techniques, values displayed were measurements done at either 60 or 150 min. Device code: refer to table 6 for details of device used according to code. Q_p = Plasma flow (ml/min). Other abbreviations are as indicated in the main text or table 6.

Table 6. Details of devices used for extracorporeal cytokine removal according to codes

Filter/device type	Device code	Filter/device name	Filter/device type	Filter size, m^2	Cut-off point[a], kDa
HCO	1	Cytoflux	polysulfone sieving coefficient of 0.05	1.4	
HCO	2	Cytoflux	polysulfone sieving coefficient of 0.13	1.4	
HCO	3	Sureflux FH150	Cellulose triacetate	1.5	
HCO	4	Polyflux P2SX	polyamide	1.27	100
HCO	5	Polyflux P2SH	polyamide		100
HCO	6	FB-110FH	cellulose triacetate	1.1	60
HCO	8	Polyflux P5SH	polyamide	2.2	
Standard	9	AN69	PAN	0.8	
Standard	10	UT700	Cellulose triacetate	0.7	
Standard	12	Polyflux 11	polyamide	1.1	30
Standard	13	AN69	polyacrylonitrile	1.3	30
Standard	14	CT90	cellulose triacetate	0.9	30
Standard	15	F60	polysulfone	1.2	30
Standard	16	APF06S	polyacrylonitrile		
Standard	17	CH-0.6L	PMMA	*	
Standard	18	UT500S	Cellulose triacetate	*	
Standard	19	PSC07	polysulfone	*	
Standard	20	PSF700	polysulfone	*	
Standard	21	Amicon D-20	polysulfone	0.25	
Standard	22	FH88H	polyamide	2.0	
Standard	23	Fresenius F-40	polysulfone	0.65	
Standard	24	Baxter CA-210	cellulose acetate	2. 1	
Standard	25	AN69S	polyacrylonitrile	0.5	
Standard	26	Renal System HF250	polysulfone	0.25	
Standard	27	AN69HF	polyacrylonitrile	0.60	
Standard	28	FH66D	polyamide	0.60	
Standard	29	BL624	polysulfone	1.0	
Standard	30	AV600	polysulfone	1.35	30
Standard	31	Diafilter20	polysulfone	0.40	
Standard	32	PAS	polyalkyl sulfone	0.3	
Sorbent	36	DHP1	activated charcoal		
Sorbent	41	Mesoporous activated carbon module	activated carbon from phenolic resin based beads in monolithic form		
Sorbent	42	Non-mesoporous carbon module	activated carbon from phenolic resin based beads in monolithic form		
Sorbent	43	CF-X	cellulose beads crosslinked with hexamethylene-di-isocyanate		
Sorbent	44	Immunosorba TR	polyvinyl alcohol gel crosslinked with tryptophan		
Sorbent	45	Immunosorba PH	polyvinyl alcohol gel crosslinked with phenylalanine		
Sorbent	46	Selesorb	cellulose beads crosslinked with dextran sulfate		
Sorbent	47	Lixelle	cellulose beads crosslinked with hexadecyl alkyl chain		
Sorbent	48	MPCF-XM20	CF-X coated with MPC (2-methacryloyloxyethyl phosphorylcholine) copolymer		
Sorbent	49	MPCF-XN20	CF-X coated with MPC (2-methacryloyloxyethyl phosphorylcholine) copolymer		
Sorbent	50	Cytosorb	divinylbenzene copolymer		
Sorbent	51	iHSA	purified human serum albumin linked to macroporous acrylic beads (EUPERGIT)		
Sorbent	52	XAD7	Amberlite		
Sorbent	53	CTR	cellulose beads covalently bound to hexadecyl alkyl chain		
Plasmafilter/sorbent	38	Albuflow AF01/MDS	polysulfone/cellulose microparticles	1.0	
Plasmafilter/sorbent	39	plasmaFlux PSu 1S/MDS	polysulfone/cellulose microparticles	0.3	
Plasmafilter/sorbent	40	FX60/MDS	polysulfone/cellulose microparticles	1.4	
Plasmafilter/sorbent	54	PS9002/Charcoal	polysulfone (cut-off 150 kDa) with albumin SC of 1/detoxyl 3 (135 g of carbon)		
Plasmafilter/sorbent	35	BioLogic-DTPF	plasmafilter/70 g powdered charcoal	0.35	
Plasmafiltration	11	MPS05	polyethersulfone plasma filter	0.5	

Devices used for CPFA are displayed as plasmafiltration device/adsorption device. [a] Cut-off points only presented when specifically stated by authors.
* Information not available.

er clearance is also observed with HCO/HF compared to diffusive technique using HCO (HCO/HD) despite the same class of filter being used. One explanation is that this may be similar to the observation that convective treatment is more effective at removing molecules that are in the middle to higher range of molecular weight when using standard hemofilters compared to diffusive treatments using the same class of filters. For example, the antibiotic vancomycin, which has a molecular weight of approximately 1.1 kDa, appears to be removed effectively by convection with membranes that have a cut-off value of 20–30 kDa, but is removed to a very limited degree if diffusive techniques are applied using the same membranes. These same principles may apply to HCO membranes, but to a higher level of molecular weight values, and explain the difference observed.

Finally, although other techniques, such as CPFA, BioLogic-DTPF, hemoadsorption and plasmafiltration, also demonstrate significant ability to clear cytokines, these techniques come at the price of added complexity, increased cost, requirement of specialized equipment and specialized expertise. Plasmafiltration, for example, has the added complexity of requiring replacement with donor plasma or albumin-containing solution. It is therefore suitable only for short-duration therapy and intermittent application. HCO/HF, on the other hand, is essentially similar to Std HF, which is currently widely applied but using a different type of filter. Available data for other techniques also remain insufficient and are much less extensive than the data available for HCO/HF and could be further studied in animal experiments and man.

Accordingly, HCO/HF seems a logical first-line extracorporeal therapy for the treatment of cytokinemia in man at this point in time based on efficacy, ease and feasibility of implementation. The albumin losses demonstrated ex vivo also suggest clinical feasibility.

Strengths and Limitations

Ex vivo experimentation is the starting point for future developments, and we have performed the first literature review on ex vivo data on the subject of extracorporeal cytokine removal. This review was preceded by an extensive literature search with two researchers conducting eight sets of literature search independently and uncovering more than 2,000 articles for screening. Clearance, SC and percentage removal as measures of cytokine removal are directly related to the efficacy of the device being studied, as opposed to measurements of plasma concentration which are affected by other factors, such as endogenous production and clearance/decay. Although the studies lack homogeneity, we were able to find some common grounds for comparison and, where appropriate, have made adjustments while performing the comparison to achieve some form of logical comparability. We hope that this analysis will provide clues for the best approach to in vivo work.

Despite the above strengths, this analysis has many limitations, which include limited data for analysis and lack of homogeneity in the conduct of the studies. This makes pooling the data questionable despite our attempts at achieving similarity in terms of operative characteristics. We acknowledge that even after removing extreme flow rates to achieve equivalence, there may not be enough uniformity to allow accurate generic class statements. We also acknowledge that despite our conclusion that there is a lack of difference in albumin losses between different approaches using the HCO filter, some in vitro and in vivo works have concluded that differences do exist [15, 17, 41]. Our opinion is that such differences between convective and diffusive clearance would be logical for large molecules. Finally, expressing data as percentage reductions, given the concentration-dependent removal, is imperfect and may misleadingly suggest that a technique performs better than another when in fact, in a given experiment, the concentration of solute was higher and concentration-dependent removal similarly higher as a consequence. Such percentage reductions must, therefore, be interpreted with caution.

We have excluded studies in languages other than English for practical reasons and thus might have excluded some pertinent publications. Data on adsorption, a technique of extracorporeal blood purification that is gaining much prominence, are underrepresented as most studies involving adsorption do not report clearance, and measurement of SC is irrelevant for this technique. Ex vivo studies do not operate under the same complex systems as animal or human studies, and results may not be reproducible in experiments involving the complete protoplasm.

Future Studies

Our analysis is limited to ex vivo work. It seems desirable to explore the data supporting such work by application of the techniques to animal models or their use in humans.

Randomized controlled trials in such models and in humans are crucial to the advancement of this type of blood purification.

Conclusions

In conclusion, ex vivo data support the view that HCO/HF offers significant removal of cytokines by extracorporeal therapy. HCO/HF is also consistently effective in terms of sieving and clearance and is clearly more efficacious than Std HF despite similarities in ease of implementation. HCO/HF as well as other techniques offering similar efficacy of cytokine removal in ex vivo studies offer potential for meaningful cytokine removal in man and should be further studied in animal experiments and in human studies.

References

1 Adib-Conquy M, Cavaillon JM: Stress molecules in sepsis and systemic inflammatory response syndrome. FEBS Lett 2007;581:3723–3733.
2 Rubartelli A, Lotze MT: Inside, outside, upside down: damage-associated molecular-pattern molecules (DAMPs) and redox. Trends Immunol 2007;28:429–436.
3 Bone RC: Sir Isaac Newton, sepsis, SIRS, and CARS. Crit Care Med 1996;24:1125–1128
4 Kellum JA, Bellomo R, Mehta R, Ronco C: Blood purification in non-renal critical illness. Blood Purif 2003;21:6–13.
5 Fisher CJ Jr, Dhainaut JF, Opal SM, Pribble JP, Balk RA, Slotman GJ, et al: Recombinant human interleukin 1 receptor antagonist in the treatment of patients with sepsis syndrome. Results from a randomized, double-blind, placebo-controlled trial. Phase III rhIL-1ra Sepsis Syndrome Study Group. JAMA 1994;271:1836–1843.
6 Opal SM, Fisher CJ Jr, Dhainaut JF, Vincent JL, Brase R, Lowry SF, et al: Confirmatory interleukin-1 receptor antagonist trial in severe sepsis: a phase III, randomized, double-blind, placebo-controlled, multicenter trial. The Interleukin-1 Receptor Antagonist Sepsis Investigator Group. Crit Care Med 1997;25:1115–1124.
7 Hansen TG, Tønnesen E, Toft P, Bendtzen K: Interleukin-6 (IL-6) is not removed from plasma during experimental haemofiltration. Acta Anaesthesiol Scand 1998;42:1129.
8 Skogby M, Adrian K, Friberg LG, Mellgren G, Mellgren K: Influence of hemofiltration on plasma cytokine levels and platelet activation during extra corporeal membrane oxygenation. Scand Cardiovasc J 2000;34:315–320.
9 Schindler R: Elimination of cytokines from plasma by ultrafiltration using conventional polysulfone or DIAPES membranes. Contrib Nephrol. Karger, Basel, 2003, vol138, pp 37–42.
10 Haase M, Bellomo R, Morger S, Baldwin I, Boyce N: High cut-off point membranes in septic acute renal failure: a systematic review. Int J Artif Organs 2007;30:1031–1041.
11 Tetta C, Cavaillon JM, Schulze M, Ronco C, Ghezzi PM, Camussi G, Serra AM, Curti F, Lonnemann G: Removal of cytokines and activated complement components in an experimental model of continuous plasma filtration coupled with sorbent adsorption. Nephrol Dial Transplant 1998;13:1458–1464.
12 Cole L, Bellomo R, Davenport P, Tipping P, Uchino S, Tetta C, Ronco C: The effect of coupled haemofiltration and adsorption on inflammatory cytokines in an ex vivo model. Nephrol Dial Transplant 2002;17:1950–1956.
13 Bordoni V, Bolgan I, Brendolan A, Crepaldi C, Gastaldon F, D'intini V, et al: Caspase-3 and -8 activation and cytokine removal with a novel cellulose triacetate super-permeable membrane in an in vitro sepsis model. Int J Artif Organs 2003;26:897–905.
14 Steczko J, Ash SR, Blake DE, Carr DJ, Bosley RH: Cytokines and endotoxin removal by sorbents and its application in push-pull sorbent-based pheresis: the BioLogic-DTPF system. Artif Organs 1999;23:310–318.
15 Mariano F, Fonsato V, Lanfranco G, Pohlmeier R, Ronco C, Triolo G, et al: Tailoring high-cut-off membranes and feasible application in sepsis-associated acute renal failure: in vitro studies. Nephrol Dial Transplant 2005;20:1116–1126.
16 Lee WC, Uchino S, Fealy N, Baldwin I, Panagiotopoulos S, Goehl H, et al: Super high flux hemodialysis at high dialysate flows: an ex vivo assessment. Int J Artif Organs 2004;27:24–28.
17 Morgera S, Klonower D, Rocktäschel J, Haase M, Priem F, Ziemer S, et al: TNF-alpha elimination with high cut-off haemofilters: a feasible clinical modality for septic patients? Nephrol Dial Transplant 2003;18:1361–1369.
18 Uchino S, Bellomo R, Morimatsu H, Goldsmith D, Davenport P, Cole L, et al: Cytokine dialysis: an ex vivo study. ASAIO J 2002;48:650–653.
19 Uchino S, Bellomo R, Goldsmith D, Davenport P, Cole L, Baldwin I, et al: Super high flux hemofiltration: a new technique for cytokine removal. Intensive Care Med 2002;28:651–655.
20 Uchino S, Bellomo R, Goldsmith D, Davenport P, Cole L, Baldwin I, et al: Cytokine removal with a large pore cellulose triacetate filter: an ex vivo study. Int J Artif Organs 2002;25:27–32.
21 Cole L, Bellomo R, Davenport P, Tipping P, Ronco C: Cytokine removal during continuous renal replacement therapy: an ex vivo comparison of convection and diffusion. Int J Artif Organs 2004;27:388–397.
22 Teraoka S, Mineshima M, Hoshino T, Ishimori I, Kaneko I, Sato Y, et al: Can cytokines be removed by hemofiltration or hemoadsorption? ASAIO J 2000;46:448–451.
23 Bouman CS, van Olden RW, Stoutenbeek CP: Cytokine filtration and adsorption during pre- and postdilution hemofiltration in four different membranes. Blood Purif 1998;16:261–268.
24 Glogowski KR, Stammers AH, Niimi KS, Tremain KD, Muhle ML, Trowbridge CC: The effect of priming techniques of ultrafiltrators on blood rheology: an in vitro evaluation. Perfusion 2001;16:221–228.
25 Skogby M, Adrian K, Friberg LG, Mellgren G, Mellgren K: Influence of hemofiltration on plasma cytokine levels and platelet activation during extra corporeal membrane oxygenation. Scand Cardiovasc J 2000;34:315–320.
26 Nagaki M, Hughes RD, Lau JY, Williams R: Removal of endotoxin and cytokines by adsorbents and the effect of plasma protein binding. Int J Artif Organs 1991;14:43–50.
27 Weber V, Hartmann J, Linsberger I, Falkenhagen D: Efficient adsorption of tumor necrosis factor with an in vitro set-up of the microspheres-based detoxification system. Blood Purif 2007;25:169–174.
28 Goldfarb S, Golper TA: Proinflammatory cytokines and hemofiltration membranes. J Am Soc Nephrol 1994;5:228–232.
29 Sandeman SR, Howell CA, Mikhalovsky SV, Phillips GJ, Lloyd AW, Davies JG, et al: Inflammatory cytokine removal by an activated carbon device in a flowing system. Biomaterials 2008;29:1638–1644.

30 Oda S, Hirasawa H, Shiga H, Nakanishi K, Matsuda K, Nakamura M, et al: Cytokine adsorptive property of various adsorbents in immunoadsorption columns and a newly developed adsorbent: an in vitro study. Blood Purif 2004;22:530–536.
31 Song M, Winchester J, Albright RL, Capponi VJ, Choquette MD, Kellum JA: Cytokine removal with a novel adsorbent polymer. Blood Purif 2004;22:428–434.
32 Zimmermann M, Busch K, Kuhn S, Zeppezauer M: Endotoxin adsorbent based on immobilized human serum albumin. Clin Chem Lab Med 1999;37:373–379 .
33 van Bommel EF, Hesse CJ, Jutte NH, Zietse R, Bruining HA, Weimar W: Cytokine kinetics (TNF-α, IL-1β, IL-6) during continuous hemofiltration: a laboratory and clinical study. Contrib Nephrol. Karger, Basel, 1995, vol 116, pp 62–75.
34 Nagaki M, Hughes RD, Keane HM, Lau JY, Williams R: In vitro plasma perfusion through adsorbents and plasma ultrafiltration to remove endotoxin and cytokines. Circ Shock 1992;38:182–188.
35 Taniguchi T, Hirai F, Takemoto Y, Tsuda K, Yamamoto K, Inaba H, Sakurai H, Furuyoshi S, Tani N: A novel adsorbent of circulating bacterial toxins and cytokines: the effect of direct hemoperfusion with CTR column for the treatment of experimental endotoxemia. Crit Care Med 2006;34:800–806.
36 Awad SS, Sawada S, Soldes OS, Rich PB, Klein R, Alarcon WH, et al: Can the clearance of tumor necrosis factor alpha and interleukin 6 be enhanced using an albumin dialysate hemodiafiltration system? ASAIO J 1999;45:47–49.
37 Kellum JA, Song M, Venkataraman R: Hemoadsorption removes tumor necrosis factor, interleukin-6, and interleukin-10, reduces nuclear factor-κB DNA binding, and improves short-term survival in lethal endotoxemia. Crit Care Med 2004;32:801–805.
38 Schefold JC, Hasper D, Jörres A: Organ crosstalk in critically ill patients: hemofiltration and immunomodulation in sepsis. Blood Purif 2009;28:116–123.
39 Lever A, Mackenzie I: Sepsis: definition, epidemiology, and diagnosis. BMJ 2007;335:879–832.
40 Schetz M, Ferdinande P, Van den Berghe G, Verwaest C, Lauwers P: Removal of pro-inflammatory cytokines with renal replacement therapy: sense or nonsense? Intensive Care Med 1995;21:169–176.
41 Morgera S, Slowinski T, Melzer C, Sobottke V, Vargas-Hein O, Volk T, et al: Renal replacement therapy with high-cutoff hemofilters: impact of convection and diffusion on cytokine clearances and protein status. Am J Kidney Dis 2004;43:444–453.

1.6 Summary of literature review on ex-vivo studies

The literature review on ex-vivo studies found good evidence that HCO hemofiltration is one of the better techniques for cytokine removal. Its rate of clearance as well as sieving coefficients (SC) of various cytokines were greater than that of standard techniques and similar to more complicated techniques such as hemoadsorption, plasmafiltration and hybrid techniques. Hemofiltration as a modality appear to offer higher clearance compared to hemodialysis. Albumin loss appeared comparable between different modalities of HCO techniques i.e. continuous hemofiltration versus continuous hemodialysis.

No date restrictions were applied and the earliest relevant article traced back to 1991. As the studies were conducted under artificial systems, many of the measurements were performed after a single pass through the extracorporeal system. Those involving artificial circulation were applied over a short duration only. Some of the adsorption techniques involved incubation for a limited period. These laboratory settings, although necessary to pave the way for further studies, may not predict what will occur in live circulation. Phenomenon such as membrane fouling and saturation of adsorption devices may not be reflected under these laboratory conditions.

We proceeded to perform a second systematic review of the literature on animal studies:

Atan R, Crosbie D, Bellomo R. Techniques of extracorporeal cytokine removal: a systematic review of the literature on animal experimental studies. Int J Artif Organs. 2013 Mar;36(3):149-58.

Additional searches were performed due to the time lag between the first publication and the second, as well as honouring requests from reviewers. This second publication therefore involved a screening of more than 3000 abstracts.

1.7 Publication

Int J Artif Organs 2013; 00 (00): 000-000 DOI: 10.5301/ijao.5000128

REVIEW ARTICLE

Techniques of extracorporeal cytokine removal: A systematic review of the literature on animal experimental studies

Rafidah Atan[1], David Crosbie[2], Rinaldo Bellomo[2]

[1] Johor Bahru Clinical School, Monash University Sunway Campus, Bukit Azah, Johor Bahru, Johor - Malaysia
[2] Department of Intensive Care, Austin Hospital, Heidelberg, Melbourne - Australia

ABSTRACT

Background and Aims: *Extracorporeal cytokine removal may be desirable. We sought to assess extracorporeal blood purification (EBP) techniques for cytokine removal in experimental animal studies.*
Methods: *We conducted a targeted, systematic search and identified 17 articles. We analyzed cytokine clearance, sieving coefficient (SC), ultrafiltrate (UF) concentration, and percentage removal. As this review concerns technical appraisal of EBP techniques, we made no attempts to appraise the methodology of the studies included. Results are in descriptive terms only.*
Results: *Applying predicted clearance for 80 kg human, high volume hemofiltration (HVHF) techniques and plasmafiltration (PF) showed the highest rates of cytokine removal. High cutoff (HCO)/HF and PF techniques showed modest ability to clear cytokines using low to medium flows. Standard hemofiltration had little efficacy. At higher flows, HCO/HF achieved clearances between 30 and 70 ml/min for IL-6 and IL-10. There was essentially no removal of tumor necrosis factor (TNF)-alpha outside of PF.*
Conclusions: *Experimental animal studies indicate that HVHF (especially with HCO filters) and plasmafiltration have the potential to achieve appreciable IL-6 and IL-10 clearances. However, only PF can remove TNF-alpha reliably.*

KEY WORDS: *Cytokines, Hemofiltration, Plasmafiltration, Sepsis, Clearance, Interleukins*

Accepted: July 6, 2012

INTRODUCTION

The role of cytokines in the pathophysiology of various acute inflammatory states is continuously being defined (1). The stimuli for cytokine release are many and may come in the form of pathogen-associated molecular patterns (PAMPs) in the case of microbial sepsis, or damage-associated molecular patterns (DAMPs) in the case of systemic inflammatory response syndrome (SIRS) induced tissue injury (2, 3). Cytokines are not normally present in large amounts in the circulation, so the presence of excessive amounts may herald the development of multiorgan dysfunction and high mortality (4). Additionally, imbalances between pro-inflammatory and anti-inflammatory cytokines occur at different stages of critical illness and are believed to contribute to a poor outcome (5).

Over the last few decades, attempts have been made to develop therapies that will help to regulate the level of cytokines in the circulation and restore cytokine balance. Such therapies should ideally be able to modulate several cytokines at the same time and decrease cytokinemia to levels associated with optimal outcomes rather than aim for complete elimination (6). As the timing and degree of cytokine release may vary under different circumstances even in the same patient, ideally these therapies should be continuous and self-adjustable to cytokine levels.

In theory, extracorporeal blood purification (EBP) techniques approach this ideal (7). Several characteristics of most cytokines make them a logical target for extracorporeal removal. They are of middle molecular weight; they are water soluble; they are often found free of protein binding (8). Because of these characteristics and the belief that restoration of cytokine homeostasis is desirable, several investigators have sought to develop techniques of EBP which can achieve cytokine removal (9). We have recently assessed the efficacy of different EBP techniques at cytokine removal in *ex vivo* conditions (10). The relevance of such data to removal *in vivo*, however, remains poorly understood. In particular, no studies have systematically assessed the efficacy of different extracorporeal techniques in achieving such goals in the *in vivo* setting of experimental animal studies. The additional assessment of EBP techniques in preclinical studies is vital to the development of a logical approach to the selection of the optimal technique of cytokine removal in humans. Accordingly, we conducted a systematic review of all experimental animal studies of EBP techniques for cytokine removal.

METHODS

We conducted a systematic search using the Pubmed and Embase databases for relevant articles on *in vivo* animal experimental studies on cytokine removal using known modalities of EBP. We then systematically assessed the efficacy of all EBP techniques previously reported in the literature using these data.

Our approach at identifying relevant articles for analysis is outlined in Fig. 1.

The following search terms were used: 'cytokine' AND 'continuous renal replacement therapy'; 'cytokine' AND 'hemofiltration'; 'cytokine' AND 'hemodiafiltration'; 'cytokine' AND 'high volume hemofiltration'; 'cytokine' AND 'adsorption'; 'cytokine' AND 'plasmapheresis'; 'cytokine' AND 'bioartificial kidney' and 'cytokine' AND 'coupled plasma filtration adsorption'. To ensure no relevant articles were missed, further searches were conducted using the term 'interleukin', 'interleukin-1', 'interleukin-2', 'interleukin-6', 'interleukin-8', 'interleukin-10', 'interleukin-18' and 'tumor necrosis factor alpha'. All these terms were combined using the term 'AND' with the terms 'hemofiltration', 'hemodiafiltration', 'adsorption', 'plasmapheresis', 'renal replacement therapy',

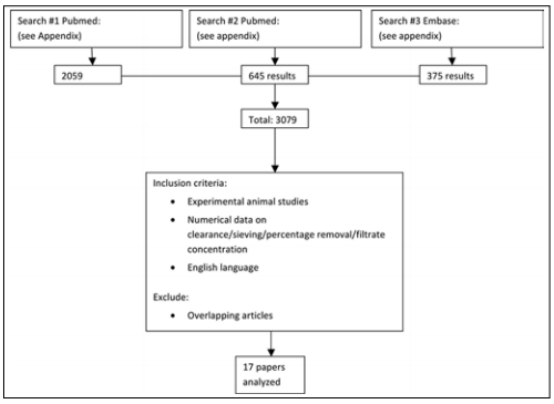


Fig. 1 - *Flow diagram summarizing manuscript review process.*

'bioartificial kidney', 'high volume hemofiltration', 'CPFA' and 'coupled plasma filtration adsorption'. All the terms used were MESH and Emtree terms except for 'bioartificial kidney', 'high volume hemofiltration', 'CPFA' and 'coupled plasma filtration adsorption' which are keyword searches as neither MESH nor Emtree terms for these exists. 'Renal replacement therapy' is a MESH term but not an Emtree term. All MESH and Emtree terms were also searched as keyword searches. Non-MESH or non-Emtree terms were searched as keyword searches in the respective databases using both British and American English spelling.

Abstracts of articles retrieved were then screened for two inclusion criteria: experimental animal studies and the reporting of a numerical value of at least one of these measures of cytokine removal: clearance, sieving, percentage removal, or concentration in the filtrate. Two independent researchers performed the search and then manually screened retrieved articles for those which meet both inclusion criteria. Abstracts that did not include enough details as well as publications with no abstracts were traced using library resources and each paper was screened for inclusion criteria. We excluded review articles and articles published in languages other than English.

As this review is concerned with technical appraisal of each EBP technique in the terms described above and not clinical outcomes such as survival, we have not made attempts at appraising the methodology of each study identified. We sought to identify all articles that have been published on the subject so that a fair conclusion can be attempted

without overlooking any particular technique. Studies are only excluded if the results appear to be a duplication. There were also real difficulties in appraising the quality of the individual studies as the animals, techniques, and operating characteristics that they used were rather varied. Data which were reported only in the form of graphs or figures had their numerical values estimated from the details given in the graphs. When more than one measurement was available, an average value was calculated. In an attempt to assimilate data from different animal experiments into a degree of homogeneity, a predicted CL in an 80 kg human was then calculated based on the CL and SC data. The CL values achieved using various devices and operating characteristics were then compared using this predicted data. The information was then analyzed to seek out techniques that offer the highest rate of cytokine removal based on experimental animal data. Where sufficient data were available, these techniques were then analyzed for operating characteristics that appeared to offer the best rates of cytokine removal.

In an attempt to derive meaning and a degree of uniformity that could be applied clinically to experiments that were conducted using a range of small, medium, and large animals, we calculated a "predicted clearance" in an 80 kg human. The calculation was done in the following manner: from the data given in the papers, the UF rate in ml/kg per hr was calculated for the animal subjects and then the equivalent UF rate for an 80 kg human was derived in ml/min. The product of the sieving coefficient (SC) from the animal studies and ultrafiltration (UF) rate in ml/min gave the value of CL in ml/min for an 80 kg human. Where data on UF concentration, pre- and postfilter plasma concentration were available, SC was calculated using the formula: 2 x UF concentration / (prefilter concentration + postfilter concentration) and CL derived from the calculated SC. For plasmafiltration or selective plasma filtration where data were only available in the form of CL, SC was first derived through CL divided by plasmafiltration rate in ml/min. The product of this derived SC and the derived rate of plasmafiltration flow (ml/min) in an 80 kg human equals CL. Data on percentage removal was not analyzed further because of insufficient data and insufficient shared features across experiments.

Due to the limited amount of data, we did not calculated means, standard deviations, medians, or interquartile ranges. Rather than make any statistical comparisons, we kept to descriptive terms.

RESULTS

The data extraction process is summarized in Figure 1. In terms of definitions, the term "standard technique" was used to refer to the use of standard high flux hemofilters at standard doses of filtrate flow (<25 ml/kg per hr), while "high cutoff techniques" refer to the use of super high-flux hemofilters with a nominal cutoff point of more than 60 kDa (11). The term, "high volume hemofiltration" (HVHF) was used to refer to techniques of hemofiltration at doses higher than 50 ml/kg per hr. HVHF using HCO filters are labeled as HCO/HVHF and classified under HCO hemofiltration. The term "plasmafiltration" was used to refer to techniques involving the passing of blood through a large pore filter that resulted in filtration of plasma, where this filtered plasma was discarded and replaced by another source of colloid/plasma (plasmaexchange). The term "selective plasma filtration" was used to refer to techniques that involve initial plasmafiltration, followed by passage of the filtered plasma through a second selective plasma filter which then separates plasma components according to their size prior to elimination and subsequent replacement. "Adsorption techniques" included all techniques where either whole blood or plasma was exposed to a sorbent. "Modified ultrafiltration" refers to a technique of ultrafiltration initiated upon the termination of cardiopulmonary bypass.

We identified only 17 articles that fulfilled our selection criteria and proceeded to detailed analysis and extraction of data using four main ways of expressing cytokine removal: clearance (CL), sieving coefficient (SC), ultrafiltrate (UF) concentration, and percentage removal.

Most of the studies were conducted on animals in the weight range of 20 kg to 40 kg with only three studies conducted in small animals (rats) and one on a large animal species (ponies). The majority of studies (nine) involved between 15 to 36 animals; one study each involved a sizable number of 48 and 84 animals and only three studies involved 10 or fewer animals. The models studied were mostly endotoxic shock (10 experiments); others were pancreatitis (3), fulminant hepatic failure (2), and cardiopulmonary bypass (1). Ten studies looked at hemofiltration using standard hemofiltration filters and only two were conducted using high cutoff filters. Out of these studies on hemofiltration, four studied hemofiltration at doses defined as high volume hemofiltration (1 of the high cutoff group and 3 of the standard hemofiltration groups). Only two experiments studied

plasmafiltration techniques and only one experiment each studied adsorption (direct hemoperfusion) and modified ultrafiltration in relation to cardiopulmonary bypass.

In summary, we identified four main techniques: 1. standard techniques; 2. high cutoff (HCO) techniques; 3. adsorption techniques; and 4. plasma filtration techniques. Standard techniques and HCO techniques include both hemofiltration at standard doses as well as hemofiltration at high volume doses according to current definitions.

The cytokines measured were interleukin-1b (IL-1b), interleukin-6 (IL-6), interleukin-8, interleukin-10 (IL-10), and tumor necrosis factor-alpha (TNF-alpha). None of the studies included data on albumin loss. These studies were done on a mixture of small to large animals and the experimental conditions under which studies were conducted were acute pancreatitis, acute fulminant hepatic failure, gram negative sepsis, and cardiopulmonary bypass with sternotomy, all of which are known precipitants of hypercytokinemia. Tables I and II show data on clearance (CL) and sieving coefficient (SC) extracted from the animal experiments. One paper on HCO hemofiltration (HCO/HF) was subsequently excluded from analysis because the results displayed appeared to be a case of duplication (12).

Comparison of predicted clearance in an 80 kg human (Tab. III) showed that HF using standard filters at flows of 100 ml/kg per hr (labeled as Std/HVHF) achieved good clearance of IL-10 but no TNF-alpha clearance. Standard HF at standard flows (Std/HF) only achieved little CL of all studied cytokines; frequent filter changes or prophylactic versus late commencement of HF did not affect clearance. HCO techniques were able to achieve modest CL of IL-6 and IL-10 at moderately high flows of 45 ml/kg per hr. However, another paper on HCO filters reported low CL for IL-1b and zero clearance for IL-6 and TNF-alpha despite very high flows at 120 ml/kg per hr. Plasmafiltration (PF) techniques were the only techniques able to achieve reliable modest-to-good clearance of TNF-alpha. For both

TABLE I - CLEARANCE (CL) DATA FROM ANIMAL STUDIES

Technique	Filter code	Qb	Qf	Qp	Pre/ post	IL-1b	IL-6	IL-10	TNF-alpha	Animal	n	Weight
HCO/CVVH[13]	1	UF(ml/min) × 3.5	45 ml/kg/hr		post		18.5	12		Pigs	6	20-30 kg
HCO/HVHF[18]	8	2 ml/kg/min	2 ml/kg/min		pre	0*	0		0	Ponies	5	110-220 kg
Std/HVHF[20]	6	No details	6000 ml/hr						0	Dogs	5	28.7 + /-7.2 kg
Std/HVHF[19]	9	200 ml/min	1750 ml/hr		pre				0	Dogs	8	19-22 kg
Std/CAVH[15]	5		14.1 ml/hr			0			0	Rats	6	400-500 g
Std/CAVH + TSI (5 mg/kg)[15]	5		40.8 ml/hr			0			0	Rats	12	400-500 g
Std/CAVH[16]	5		10-22 ml/hr						0	Rats	6	400-470 g
Std/CVVH[17]	6	No details	3000 ml/hr						0	Dogs	5	30.2 + /-5.5 kg
Std/CVVH[17]	7	No details	3000 ml/hr						0	Dogs	5	30.2 + /-5.5 kg
Std/CVVH[20]	6		3000 ml/hr						0	Dogs	5	28.7 + /-7.2 kg
Std/CVVH[21]	10	100	1000 ml/hr						0	Pigs	7	30.7-45.7 kg
Selective plasma filtration[14]	2	120 ml/min	10 ml/min	20					8	Pigs	7	17-28 kg
Selective plasma filtration[14]	3	120 ml/min	10 ml/min	20					10	Pigs	7	17-28 kg
Plasmafiltration[14]	4	120 ml/min	20 ml/min						10	Pigs	6	17-28 kg

HCO = high cutoff; CVVH = continuous veno venous hemofiltration; Std = standard; CAVH = continuous arteriovenous hemofiltration; VHF = high volume hemofiltration; TSI = thromboxane synthase inhibitor; Qb = blood flow; Qf = ultrafiltrate flow; Qp = plasma flow in ml/min; Pre/post = refers to predilution or postdilution respectively; Blood pressure+ = blood pressure dependent; No details = details not given in the paper; Filter code = refer to Tab. IV; * = reported only as IL-1; n = number of animals in the subgroup subjected to each operating characteristics.

TABLE II - SIEVING COEFFICIENT (SC) DATA FROM ANIMAL STUDIES

Technique	Filter code	Qb	Qf	Pre/ post	IL-1b	IL-6	IL-8	IL-10	TNF-alpha	Animal	n	Weight (kg)	Other
HCO/CVVH[13]	1	UF(ml/ min) × 3.5	45 ml/kg per hr	post		0.835		0.54		Pigs	6	20-30	
HCO/HVHF[18]	8	2 ml/kg/ min	2 ml/kg per min	pre	0.06*	0			0.23	Ponies	5	110-220	
Std/HVHF[22]	11		100 ml/kg per hr	pre				0.573		Minipigs	12	31.2 + /-3.4	Late, change
Std/HVHF[22]	11		100 ml/kg per hr	pre				0.563		Minipigs	12	31.2 + /-3.4	Early, change
Std/HVHF[23]	11		100 ml/kg per hr	pre				0.48-0.55		Minipigs	12	21-30	#Late, no change
Std/CVVH[22]	11		20 ml/kg per hr	pre				0.57		Minipigs	12	31.2 + /-3.4	Late, no change
Std/CVVH[22]	11		20 ml/kg per hr	pre				0.585		Minipigs	12	31.2 + /-3.4	Early, no change
Std/CVVH[22]	11		20 ml/kg per hr	pre				0.565		Minipigs	12	31.2 + /-3.4	Late, change
Std/CVVH[22]	11		20 ml/kg per hr	pre				0.55		Minipigs	12	31.2 + /-3.4	Early, change
Std/CVVH[23]	11		20 ml/kg per hr	pre				0.48-0.55		Minipigs	12	21-30	#Late, no change
Std/CVVH[23]	11		20 ml/kg per hr	pre				0.48-0.55		Minipigs	12	21-30	#Late, no change
Std/CVVH[27]	15	70-130	20 ml/kg per hr						0.65	Pigs	12	37 + /-7	Therapeutic
Std/CVVH[27]	15	70-130	20 ml/kg per hr						0.55	Pigs	12	37 + /-7	Prophylactic
Std/CVVH[28]	16	150	1000 ml/hr	post		0.301			0.024	Pigs	6	27-32	
MUF after CPB[26]	14	10-15 ml/ kg/min	No details			0.69	0.47		0.14	Piglets	12	8.4 + /-0.4	

HCO = high cutoff; CVVH = continuous veno venous hemofiltration; Std = standard; MUF = modified ultrafiltration; CPB = cardiopulmonary bypass; Qb = blood flow; Qf = ultrafiltrate flow; Pre/post = refers to predilution or postdilution respectively; Filter code = refer to Tab. IV; Late = commencement of hemofiltration after decline of total peripheral resistance of 30%; Early = prophylactic i.e. immediately after the induction of pancreatitis using sodium taurocholate; Change/no change = refers to whether filters are changed every 12 hr. Therapeutic = after mean arterial pressure decreased 20% below baseline; Prophylactic = CVVH simultaneous with induction of insult; * = reported only as IL-1; # = according to criteria set in reference 22 regarding 'late' vs. 'early' commencement of CVVH; some data are reported as both CL and SC; n = number of animals in the subgroup subjected to each operating characteristics.

standard techniques and PF techniques, rate of clearance increased with increasing the filtration rate. Selective PF did not appear to confer additional improvement in cytokine CL when compared to standard PF.

There was only one animal study that assessed adsorption and measured the rate of cytokine removal. This study, which used a β2 microglobulin adsorption column (Lixelle) through direct hemoperfusion (DHP), displayed adsorption rate or percentage of cytokine removed over the course of 180 minutes. The values displayed were averaged from six readings and showed an approximate adsorption rate of 30% for IL-1b, IL-6 and TNF-alpha (data not shown).

DISCUSSION

Key findings

We performed a systematic analysis of animal experimentation involving different techniques of EBP to determine

TABLE III - PREDICTED CLEARANCE (ML/MIN) IN 80 KG HUMAN USING SC DATA

Technique	Filter code	Qb (ml/min)	Qf# (ml/hr)	Qf# (ml/kg per hr)	Pre/ post	IL1b	IL6	IL10	TNFa	Notes
HCO/CVVH[13]	1	UF (ml/min) × 3.5	3600	45	post		50.1	32.4		
HCO/HVHF[18]	8	2 ml/kg per min	9600	120	pre	9.6	0			
Std/HVHF[22]	11		8000	100	pre			76.4		late, change
Std/HVHF[22]	11		8000	100	pre			75.1		early, change
Std/HVHF[23]	11		8000	100	pre			68.7		late, change
Std/HVHF[20]	6		16725	209					0	
Std/HVHF[19]	9	200	6829	85	pre				0	
Std/CVVH[22]	11		1600	20	pre			15.2		late, no change
Std/CVVH[22]	11		1600	20	pre			15.6		early, no change
Std/CVVH[22]	11		1600	20	pre			15.1		late, change
Std/CVVH[22]	11		1600	20	pre			14.67		early, change
Std/CVVH[23]	11		1600	20	pre			13.7		late, no change
Std/CVVH[23]	11		1600	20	pre			13.7		late, change
Std/CAVH[15]	5		2480	31		0			0	
Std/CAVH + TSI (5 mg/kg)[15]	5		7280	91		0			0	
Std/CAVH[16]	5		2942	36.8					0	
Std/CVVH[17]	6		7947	100					0	
Std/CVVH[17]	7		7947	100					0	
Std/CVVH[20]	6		8362	104					0	
Std/CVVH[21]	10	100	2094	26.2					0	
Std/CVVH[27]	15	70-130	1600	20					17.3	late (drop in MAP 20%)
Std/CVVH[27]	15	70-130	1600	20					14.7	early
Std/CVVH[28]	16	150	2712	33.9			13.6		1.1	
Selective plasma filtration[14]	2	120	2136	26.7					28.48	
Selective plasma filtration[14]	3	120	2136	26.7					35.6	
Selective plasma filtration[25]	13	100	672	8.4			10.8		10.9	
Plasmafiltration[14]	4	120	4264	53.3					71.1	

Calculation was done in the following manner: from the data given in the papers, the UF rate in ml/kg per hr was calculated and then the equivalent UF rate for an 80 kg human was derived in ml/min. The product of the SC (from the animal studies) and UF rate in ml/min gave the value of CL in ml/min for an 80 kg human. SC is taken to be zero if CL is zero from CL data. For plasmafiltration/selective plasma filtration = SC derived from CL data as CL/Qf in ml/min. Product of SC and rate of Qf in ml/min in human equals CL.
HCO = high cutoff; CVVH = continuous veno venous hemofiltration; Std = standard; CAVH = continuous arteriovenous hemofiltration; HVHF = high volume hemofiltration; TSI = thromboxane synthase inhibitor; Qb = blood flow; Qf = ultrafiltrate flow; Qp = plasma flow in ml/min; # = derived value for 80 kg man; Pre/post = refers to predilution or postdilution respectively; Filter code = refer table 4; Late = commencement of hemofiltration after decline of total peripheral resistance of 30%; Early = prophylactic i.e., immediately after the induction of pancreatitis using sodium taurocholate; Change/no change = refers to whether filters are changed every 12 hr.

TABLE IV - FILTER/DEVICE CODE AND DETAILS

Ref no.	Filter no.	Filter name	Filter type	Cutoff	Filter size	Other details
13	1	Sureflux FH 70	Cellulose triacetate	80 kDa	0.7 m^2	
14	2	Cascadeflo AC-1770	Cellulose diacetate			SC for albumin 0.85
14	3	Cascadeflo AC-1730	Cellulose diacetate			SC for albumin 0.7
14	4	PF 2000N	Plasmafilter			
15, 16	5	Amicon minifilter	Polysulfone			
17, 20	6	Renaflo II PSHF 700G	Polysulfone	40 kDa	0.71 m^2	
17	7	Multiflow 100	AN69		0.85 m^2	
18	8	Toray Filtryzer BK-1.6F	PMMA	75 kDa	1.6 m^2	
19	9	Multiflow 60	AN69		0.45 m^2	
21	10	Multiflow 60P	Polyamide		0.6 m^2	
22, 23	11	Prisma M60	AN69S			
24	12	β2 microglobulin adsorption column	Lixelle	Pore size 80 μm		BMG adsorbent 1 ml/100 g BW
25	13	Ultrason 6020	Polyethersulfone	100 kDa	0.6 m^2	
26	14	COBE HC 700	Polyarylethersulfone			
27	15	Ultraflux AV 600S	Polysulfone	30 kDa		
28	16	Amicon-20	Polysulfone		0.4 m^2	

PMMA = polymethylmethacrylate; BMG = β2 microglobulin

their efficacy in the removal of cytokines during animal experiments. There were limited data available, but a comparison of these techniques using data from animal studies suggested that appreciable clearances of IL-6 and IL-10 were achieved with very high volume hemofiltration using standard filters. High cutoff hemofiltration offered appreciable clearances of IL-6 and IL-10 at filtration rates of 45 ml/kg per hr. Std/HF at low filtration rates achieve negligible cytokine removal. None of these techniques, however, removed TNF-alpha. Plasmafiltration was the only technique to achieve clearance of TNF-alpha.

Relation to previous literature

There are no other reviews in the literature that have studied experimental animal cytokine clearance data for comparison. However, we have previously published a systematic review of cytokine removal by EBP techniques when assessed *ex vivo* (10). In this regard, our analysis of animal data reveals that the *ex vivo* findings may not apply to these techniques when used in experimental studies.
In particular, while many studies of HCO/HF and some studies of Std/HF reported *in vivo* TNF-alpha removal with clearances up to a mean of 25 ml/min for HCO/HF, the animal studies could not confirm this efficacy. In fact, they showed that essentially only PF can be expected to reliably remove TNF-alpha. In contrast, the clearances achieved for IL-6 and IL-10 with HCO/HF were broadly similar in the *ex vivo* studies and in the animal studies. Additionally, while there were essentially no *ex vivo* data on IL-10 clearance with Std/HF, eight animal studies showed appreciable IL-10 clearance with both high-volume or low-volume Std/HF. In contrast, while IL-1 beta has been well studied *ex vivo* and removal has been shown to be substantial, no robust evidence for such removal exists in animal experiments.

Significance of study findings

Through this review, we now provide some basis for the design of randomized, controlled, clinical trials. In particular, by demonstrating that high cytokine removal is achieved by using higher UF rates, we show that one logical approach to the treatment of cytokinemia by EBP techniques should involve the use of higher flows.

Flows of 100 ml/kg per hr if applied to humans (8 l/hr in an 80 kg human), however, would require the use of large amounts of replacement fluids, involve, technical difficulties, expose patients to other risks (e.g., hypophosphatemia) and require catheters that can deliver very high (400 ml/min) blood flows. This will significantly increase cost, nursing care burden, and large fluid shifts, with potential for electrolyte imbalances, especially if this therapy is provided around the clock. A pulse therapy at 100 ml/kg per hr might be more feasible but would result in intermittent high removal of cytokines with periods of no cytokine removal.

Ultrafiltration flow rates of 45 ml/kg per hr using high cutoff filters appear more feasible for continuous application but may result in lesser degree of cytokine removal compared to continuous therapy at 100 ml/kg per hr of ultrafiltration. Therefore, a combination of middle-level (40-50 ml/kg per hr) flows with the use of a more porous filter may be able to achieve similar clearances at a lower cost with little added nursing care burden and may offer a safer approach. However, even such techniques cannot be expected to effectively remove TNF-alpha. The findings of this review also further support the observation that standard hemofiltration using standard flows cannot achieve meaningful cytokine removal. Another technique that achieves good cytokine clearance is plasmafiltration. It is also the only technique that can reliably remove TNF-alpha. However, plasmafiltration is suitable only for short duration therapy and intermittent application, which may be inadequate for hypercytokinemia, a condition that requires therapy around the clock. The requirement for plasma replacement using donor plasma or albumin may also prove costly and invites added risk.

Strengths and limitations

Animal experimentation is an important step towards future developments and we have performed the first literature review on experimental animal data on the subject of extracorporeal cytokine removal. This review adds a clinical perspective to a previous assessment of cytokine removal *ex vivo* and demonstrates that the *ex vivo* data, although valuable, are not reliably predictive of performance in the experimental animal setting. CL, SC, and percentage removal as measures of cytokine removal are directly related to the efficacy of the device being studied, as opposed to measurements of plasma concentration which are affected by other factors such as endogenous production and decay .

Despite the above strengths, this analysis has many limitations, including limited data for analysis and lack of homogeneity in the performance of the studies. This makes pooling the data questionable despite our attempts at achieving normalization. We have excluded studies in language other than English for practical reasons and thus might have excluded some publications. Data on adsorption, a technique of extracorporeal blood purification that is gaining much prominence, are underrepresented as most studies involving adsorption do not report CL, while measurement of SC is irrelevant for this technique. Live animal studies operate under a more complex system compared to *ex vivo* experimentation, but major differences may exist in the biological system compared to humans, such as differences in volume of distribution between species, that may jeopardize the conclusion. For these reason, the results may not be reproducible in experiments involving human beings.

Future studies

Our analysis is limited to experimental animal work. It seems desirable to explore the data supporting such work by application of the techniques in humans. Systematic assessment of the human evidence may be useful.

CONCLUSIONS

In conclusion, experimental animal data support the view that cytokine removal by HVHF offers a substantial rate of IL-6 and IL-10 removal by extracorporeal therapy. HCO hemofiltration at moderate doses may achieve comparable clearances. However, neither approach will effectively remove TNF-alpha, which can only be reliably removed by plasmafiltration. Further systematic assessment of the efficacy of these techniques in humans seems desirable.

APPENDIX

The following search strategies were displayed by Pubmed (under ‘Search details’) for the following terms:

Search 1

‘cytokine’ AND ‘continuous renal replacement therapy’;

("cytokines" (MeSH Terms) OR "cytokines" (All Fields) OR "cytokine" (All Fields)) AND continuous (All Fields) AND ("renal replacement therapy" (MeSH Terms) OR ("renal" (All Fields) AND "replacement" (All Fields) AND "therapy" (All Fields)) OR "renal replacement therapy" (All Fields))
'cytokine' AND 'hemofiltration'
("cytokines" (MeSH Terms) OR "cytokines" (All Fields) OR "cytokine" (All Fields)) AND ("haemofiltration" (All Fields) OR "hemofiltration" (MeSH Terms) OR "hemofiltration" (All Fields))
'cytokine' AND 'hemodiafiltration';
("cytokines" (MeSH Terms) OR "cytokines" (All Fields) OR "cytokine" (All Fields)) AND ("haemodiafiltration" (All Fields) OR "hemodiafiltration" (MeSH Terms) OR "hemodiafiltration" (All Fields))
'cytokine' AND 'high volume hemofiltration'
("cytokines" (MeSH Terms) OR "cytokines" (All Fields) OR "cytokine" (All Fields)) AND "high volume hemofiltration" (All Fields) – 35 retrieved
("cytokines" (MeSH Terms) OR "cytokines" (All Fields) OR "cytokine" (All Fields)) AND high (All Fields) AND volume (All Fields) AND ("haemofiltration" (All Fields) OR "hemofiltration" (MeSH Terms) OR "hemofiltration" (All Fields)) - 74 retrieved
'cytokine' AND 'adsorption'
("cytokines" (MeSH Terms) OR "cytokines" (All Fields) OR "cytokine" (All Fields)) AND ("adsorption" (MeSH Terms) OR "adsorption" (All Fields))
'cytokine' AND 'plasmapheresis'
("cytokines" (MeSH Terms) OR "cytokines" (All Fields) OR "cytokine" (All Fields)) AND ("plasmapheresis" (MeSH Terms) OR "plasmapheresis" (All Fields))
'cytokine' AND 'bioartificial kidney'
("cytokines" (MeSH Terms) OR "cytokines" (All Fields) OR "cytokine" (All Fields)) AND bioartificial (All Fields) AND ("kidney" (MeSH Terms) OR "kidney" (All Fields))
'cytokine' AND 'coupled plasma filtration adsorption'
("cytokines" (MeSH Terms) OR "cytokines" (All Fields) OR "cytokine" (All Fields)) AND coupled (All Fields) AND ("plasma" (MeSH Terms) OR "plasma" (All Fields)) AND ("filtration" (MeSH Terms) OR "filtration" (All Fields)) AND ("adsorption" (MeSH Terms) OR "adsorption" (All Fields))
("cytokines" (MeSH Terms) OR "cytokines" (All Fields) OR "cytokine" (All Fields)) AND "coupled plasma filtration adsorption" (All Fields)

Search 2

(interleukin OR interleukin-1 OR interleukin-2 OR interleukin-6 OR interleukin-8 OR interleukin-10 OR interleukin-18 OR tumor necrosis factor alpha)
AND
(renal replacement therapy OR hemofiltration OR hemodiafiltration OR adsorption OR plasmapheresis OR 'bioartificial kidney' OR 'CPFA' OR 'combined plasma filtration adsorption' OR 'high volume hemofiltration' OR 'high volume haemofiltration'
Limits: Animal studies

Search 3

(cytokine OR interleukin OR interleukin-1 OR interleukin-2 OR interleukin-6 OR interleukin-8 OR interleukin-10 OR interleukin-18 OR 'tumor necrosis factor alpha')
AND
('renal replacement therapy' OR hemofiltration OR hemodiafiltration OR adsorption OR plasmapheresis OR 'bioartificial kidney' OR 'CPFA' OR 'combined plasma filtration adsorption' OR 'high volume hemofiltration' OR 'high volume haemofiltration'
Limits: Animal studies

Address for correspondence:
Prof. Rinaldo Bellomo
Department of Intensive Care
Austin Hospital
[redacted]

REFERENCES

1. Lacy P, Stow JL. Cytokine release from innate immune cells: association with diverse membrane trafficking pathways. Blood. 2011 Jul 7;118(1):9-18.
2. Adib-Conquy M, Cavaillon JM. Stress molecules in sepsis and systemic inflammatory response syndrome. FEBS Lett. 2007 Jul 31;581(19):3723-3733.
3. Rubartelli A, Lotze MT. Inside, outside, upside down: damage-associated molecular-pattern molecules (DAMPs) and redox. Trends Immunol. 2007;28(10):429-436.

4. Pinsky MR, Vincent JL, Deviere J, Alegre M, Kahn RJ, Dupont E. Serum cytokine levels in human septic shock. Relation to multiple-system organ failure and mortality. Chest. 1993;103(2):565-575.
5. Bone RC. Sir Isaac Newton, sepsis, SIRS, and CARS. Crit Care Med 1996;24(7):1125-1128.
6. Schefold JC, Hasper D, Jörres A. Organ crosstalk in critically ill patients: hemofiltration and immunomodulation in sepsis. Blood Purif 2009;28(2):116-123.
7. Kellum JA, Bellomo R, Mehta R, Ronco C. Blood Purification in Non-Renal Critical Illness. Blood Purif 2003; 21(1):6-13.
8. Bellomo R. Blood purification in sepsis: reasonable scientific hypothesis or pipe dream? Crit Care Resusc. 2001;3(3): 202-205.
9. Nakada TA, Hirasawa H, Oda S, Shiga H, Matsuda K. Blood purification for hypercytokinemia. Transfus Apher Sci. 2006;35(3):253-264.
10. Atan R, Crosbie D, Bellomo R. Techniques of extracorporeal cytokine removal: a systematic review of the literature. Blood Purif. 2012;33(1-3):88-100.
11. Haase M, Bellomo R, Morgera S, Baldwin I, Boyce N. High cutoff point membranes in septic acute renal failure: A systematic review. Int J Artif Organs 2007;30(12): 1031-1034.
12. Lambermont B, Delanaye P, Dogné JM, Ghuysen A, Janssen N, Dubois B, et al. Large-pore membrane hemofiltration increases cytokine clearance and improves right ventricular-vascular coupling during endotoxic shock in pigs. Artif Organs. 2006;30(7):560-564.
13. Delanaye P, Lambermont B, Dogné JM, Dubois B, Ghuysen A, Janssen N, et al. Confirmation of high cytokine clearance by hemofiltration with a cellulose triacetate membrane with large pores: an *in vivo* study. Int J Artif Organs. 2006;29(10): 944-948.
14. Ho DW, Fan ST, To J, Woo YH, Zhang Z, Lau C, et al. Selective plasma filtration for treatment of fulminant hepatic failure induced by D-galactosamine in a pig model. Gut. 2002;50(6):869-876.
15. Heidemann SM, Sarnaik AP. Protective effects of a thromboxane synthetase inhibitor and continuous arteriovenous hemofiltration in rat endotoxic shock. Prostaglandins Leukot Essent Fatty Acids. 1997;56(6):473-478.
16. Heidemann SM, Ofenstein JP, Sarnaik AP. Efficacy of continuous arteriovenous hemofiltration in endotoxic shock. Circ Shock. 1994;44(4):183-187.
17. Rogiers P, Zhang H, Pauwels D, Vincent JL. Comparison of polyacrylonitrile (AN69) and polysulphone membrane during hemofiltration in canine endotoxic shock. Crit Care Med. 2003;31(4):1219-1225.
18. Veenman JN, Dujardint CL, Hoek A, Grootendorst A, Klein WR, Rutten VP. High volume continuous venovenous haemofiltration (HV-CVVH) in an equine endotoxaemic shock model. Equine Vet J. 2002;34(5):516-522.
19. Bellomo R, Kellum JA, Gandhi CR, Pinsky MR, Ondulik B. The effect of intensive plasma water exchange by hemofiltration on hemodynamics and soluble mediators in canine endotoxemia. Am J Respir Crit Care Med. 2000;161(5):1429-1436.
20. Rogiers P, Zhang H, Smail N, Pauwels D, Vincent JL. Continuous venovenous hemofiltration improves cardiac performance by mechanisms other than tumor necrosis factor-alpha attenuation during endotoxic shock. Crit Care Med. 1999;27(9): 1848-1855.
21. Ishihara S, Ward JA, Tasaki O, Brinkley WW, Seraile LG, Pruitt BA Jr, et al. Effects of long-term hemofiltration on circulating mediators and superoxide production during continuous endotoxin administration. J Trauma. 1999;46(5): 894-899.
22. Yekebas EF, Strate T, Zolmajd S, Eisenberger CF, Erbersdobler A, Saalmüller A, et al. Impact of different modalities of continuous venovenous hemofiltration on sepsis-induced alterations in experimental pancreatitis. Kidney Int. 2002;62(5): 1806-1818.
23. Yekebas EF, Eisenberger CF, Ohnesorge H, Saalmüller A, Elsner HA, Engelhardt M, et al. Attenuation of sepsis-related immunoparalysis by continuous veno-venous hemofiltration in experimental porcine pancreatitis. Crit Care Med. 2001;29(7):1423-1430.
24. Nakatani T, Tsuchida K, Fu O, Sugimura K, Takemoto Y. Effects of direct hemoperfusion with a beta2-microglobulin adsorption column on hypercytokinemia in rats. Blood Purif. 2003;21(2):145-151.
25. Rozga J, Umehara Y, Trofimenko A, Sadahiro T, Demetriou AA. A novel plasma filtration therapy for hepatic failure: preclinical studies. Ther Apher Dial. 2006;10(2):138-144.
26. Atkins BZ, Danielson DS, Fitzpatrick CM, Dixon P, Petersen RP, Carpenter AJ. Modified ultrafiltration attenuates pulmonary-derived inflammatory mediators in response to cardiopulmonary bypass. Interact Cardiovasc Thorac Surg. 2010;11(5):599-603.
27. Yekebas EF, Treede H, Knoefel WT, Bloechle C, Fink E, Izbicki JR. Influence of zero-balanced hemofiltration on the course of severe experimental pancreatitis in pigs. Ann Surg. 1999;229(4):514-522.
28. Bottoms G, Fessler J, Murphey E, Johnson M, Latshaw H, Mueller B, Clark W, Macias W. Efficacy of convective removal of plasma mediators of endotoxic shock by continuous veno-venous hemofiltration. Shock. 1996;5(2): 149-154.

1.8 Summary of literature review on animal studies

Animal studies did not provide much data on actual clearance measured and the number of cytokines studied were few. Most of the data were in the form of sieving coefficient (SC) and mainly involved two cytokines, IL-10 and TNF-alpha. Most studies involved animals within the weight range of 20 to 40 kg.

Our calculated predicted clearance for an 80kg human based on SC showed that high cut-off hemofiltration may offer higher clearance when compared to standard hemofiltration at standard doses. The highest clearance were however achieved by standard filters when delivered as high volume hemofiltration; the doses used however were not clinically feasible. TNF-alpha, which is a large molecule (54 kDa) appeared to be removed only via plasmafiltration techniques.

Animal studies are a step higher than laboratory studies, providing live circulation and in-vivo systems, however there may be major biological differences compared to humans, as well as differences in volume of distribution that may have an effect on cytokine clearance.

We proceeded with our final literature review, on human studies:

Atan R, Crosbie DC, Bellomo R. Techniques of extracorporeal cytokine removal: A systematic review of human studies. Ren Fail. 2013 Sep; 35(8):1061-70.

Similarly, an extension to the original search was done due to the time lag between this publication and the first literature search.

1.9 Publication

This is the authors accepted manuscript of an article published as the version of record in Renal Failure 19th July 2013. http://www.tandfonline.com/ http://dx.doi.org/10.3109/0886022X.2013.815089

Techniques of extracorporeal cytokine removal: A systematic review of human studies.

Rafidah Atan[1], David CA Crosbie[2], Rinaldo Bellomo[3,4]

1. Jeffrey Cheah School of Medicine and Health Sciences, Monash University, No 8 Jalan Masjid Abu Bakar, 80100 Johor Bahru, Johor, Malaysia
2. Department of Intensive Care, Northern Hospital, Epping, Melbourne, Victoria, Australia
3. Australian and New Zealand Research Centre Monash University, Melbourne, Victoria, Australia
4. Department of Intensive Care, Austin Hospital, Studley Rd, Heidelberg, Melbourne, Victoria 3084, Australia

Corresponding author

Prof. Rinaldo Bellomo

Department of Intensive Care, Austin Hospital, Studley Rd, [redacted] Australia

Tel: [redacted]5992; Fax: [redacted]

E-mail: [redacted]

Abstract

Background and Aims: Hypercytokinemia is believed to be harmful and reducing cytokine levels is considered beneficial. Extracorporeal blood purification (EBP) techniques have been studied for the purpose of cytokine reduction. We aimed to study the efficacy of various EBP techniques for cytokine removal as defined by technical measures.

Methods: We conducted a systematic search for human clinical trials which focused on technical measures of cytokine removal by EBP techniques. We identified 41 articles and analysed cytokine removal according to clearance (CL), sieving coefficient (SC), ultrafiltrate (UF) concentration and percentage removed.

Results: We identified the following techniques for cytokine removal: standard hemofiltration, high volume hemofiltration (HVHF), high cut-off (HCO) hemofiltration, plasma filtration techniques, adsorption techniques, ultrafiltration (UF) techniques relating to cardiopulmonary bypass (CPB), extracorporeal liver support systems and hybrid techniques including combined plasma filtration adsorption. Standard filtration techniques and UF techniques during CPB were generally poor at removing cytokines (median CL for interleukin 6 [IL-6]: 1.09 mL/min, TNF-alpha 0.74 mL/min). High cut-off techniques consistently offered moderate cytokine removal (median CL for IL-6: 26.5 mL/min, interleukin 1 receptor antagonist [IL-1RA]: 40.2 mL/min). Plasmafiltration and extracorporeal liver support appear promising but data are few. Only one paper studied combined plasma filtration and adsorption and found low rates of removal. The clinical significance of the cytokine removal achieved with more efficacious techniques is unknown.

Conclusion: Human clinical trials indicate that high cut-off hemofiltration techniques, and perhaps plasmafiltration and extracorporeal liver support techniques are likely more efficient in removing cytokines than standard techniques.

Introduction

Multiorgan dysfunction syndrome (MODS) results in high mortality despite advances in intensive care.[1,2] Variations in etiology, whether induced by microbials or tissue injury, often result in a similar pattern of deterioration.[3] The stimulus for cytokine activation occurs through both pathogen-associated molecular patterns (PAMPS) or damage-associated molecular patterns (DAMPS) initiating common pathways which will ultimately lead to hypercytokinemia.[4]

Although cytokines play a role in limiting damage and helping the process of wound healing, the excessive presence of cytokines in the circulation is believed to be harmful. Thus, reducing its level to a more homeostatic range is believed to improve outcome.[5,6] The use of cytokine antibodies to counteract hypercytokinemia has been found ineffective, and even harmful in critically ill patients.[7,8] Another potential approach is the use of extracorporeal techniques for the purpose of cytokine removal. [9,10,11] Cytokines are water soluble middle molecules (molecular weight 0.5 to 60kDa) which exist in free form in the circulation. These characteristics make them suitable targets for removal by extracorporeal blood purification (EBP) techniques, yet no systematic analysis has been performed to understand which technique and which filtration devices achieve the highest level of efficiency of cytokine removal in critically ill patients.

Methods

We conducted a systematic search using Pubmed database up to November 2012, for relevant articles on human studies on cytokine removal using known modalities of EBP. We then systematically assessed the efficacy of all EBP techniques previously reported in the literature using these data.

Our approach at identifying relevant articles for analysis is outlined in Figure 1.

The following search terms were used: “cytokine” AND “continuous renal replacement therapy”; “cytokine’ AND ‘hemofiltration”; “cytokine’ AND ‘hemodiafiltration”; “cytokine” AND “high volume hemofiltration”; “cytokine” AND “adsorption”; “cytokine” AND “plasmapheresis”; “cytokine” AND “bioartificial kidney” and “cytokine” AND “coupled plasma filtration adsorption”. All the terms used were MESH terms except for

“continuous”, “bioartificial kidney”, “high volume hemofiltration” and “coupled plasma filtration adsorption” which are keyword searches.

Abstracts of articles retrieved were then screened for two inclusion criteria: human experimental studies and the reporting of a numerical value of at least one of these measures of cytokine removal: clearance, sieving, percentage removal or concentration in the filtrate. Two independent researchers performed the search and then manually screened retrieved articles for those which met both inclusion criteria. Abstracts which did not include enough details as well as publications with no abstracts provided were traced using library resources and each paper screened for inclusion criteria. We excluded review articles and articles published in language other than English.

We used four main ways of expressing cytokine removal: clearance (CL), sieving coefficient (SC), ultrafiltrate (UF) concentration and percentage removed. As this review is concerned with technical aspects of cytokine removal and not patient outcome, we did not focus on survival or other clinical outcomes.

In terms of definitions, we used the term “standard technique” to refer to the use of standard high flux hemofilters (nominal cut-off point of 30 to 40 kDa) at standard doses of filtrate flow (<25 ml/kg/hr), while the term “high cut off techniques” was used to refer to the use of super high flux hemofilters with a nominal cut off point of greater than 60kDa[11]. The term “high volume hemofiltration” (HVHF) was used to refer to techniques of hemofiltration using standard hemofilters at doses higher than 50ml/kg/hr. HVHF using standard filters was labeled as Std/HVHF and classified under standard hemofiltration. The term “plasmafiltration” was used to refer to techniques involving the passing of blood through a large pore plasmafilter that resulted in filtration of plasma, where this filtered plasma was discarded and replaced by another source of colloid/plasma. The term liver extracorporeal support was used to refer to the use of devices in liver failure for the purpose of blood purification where blood was dialyzed across an albumin-impermeable membrane (MARS) or where plasma separation was followed by adsorption (Prometheus). The term “Adsorption techniques” included all techniques where either whole blood or plasma was exposed to a sorbent. The term “Combined plasma filtration adsorption” (CPFA) was used to refer to techniques where

there was initial plasma separation followed by the filtrate being exposed to an adsorption device. The term CPFA was also used to refer to a technique in which the proposed mechanism was filtration or diafiltration using a filter that offered a degree of cytokine adsorption. A few techniques relating to cytokine removal during cardiopulmonary bypass were identified; conventional ultrafiltration (CUF) which referred to ultrafiltration performed during the rewarming phase, modified ultrafiltration (MUF) which referred to ultrafiltration after separation from bypass and zero balanced ultrafiltration (ZBUF) which referred to ultrafiltration commenced after 15 minutes of CPB. Other techniques were labelled as “UF in bypass” with a description of how the technique was performed.

Data which were reported only in the form of graphs or figures had their numerical values estimated from the details given in the graphs. When more than one measurement was available, an average value was calculated. Where both UF concentration and plasma concentration are provided for the same time period, SC was taken as the fraction of UF over plasma concentration. CL was then calculated as the product of SC and ultrafiltration rate. The information on CL, SC and percentage removed was analysed to seek out techniques that offered the highest rate of cytokine removal based on human studies. Where sufficient data were available, these techniques were then analysed for operating characteristics which appeared to offer the best rate of cytokine removal.

Due to the limited amount of data, we only calculated medians and interquartile ranges for cytokines of which three of more values had been identified. We did not make any statistical comparisons due to the limited number of observations and the variation in operational characteristics.

Results

The data extraction process is summarized in Figure 1.

We identified the following main approaches: standard hemofiltration, high volume hemofiltration (HVHF), high cut-off (HCO) hemofiltration, plasma filtration techniques, adsorption techniques, ultrafiltration (UF) techniques relating to cardiopulmonary bypass

(CPB), extracorporeal liver support systems and hybrid techniques e.g. combined plasma filtration and adsorption (CPFA). The number of papers studying a particular technique as well as the total number of patients who were studied according to each technique is shown in Table 1. Many articles studied more than one technique and also measured the levels of multiple cytokines. A few papers reported on hybrid therapies such as combined plasma filtration adsorption[32], adsorption combined with standard hemodiafiltration[14] and plasmafiltration combined with standard hemodiafiltration. [47]

Standard techniques include both hemofiltration using standard filters at standard doses[13,17,18,20,22,23,35,39,40,41,43,49,51] as well as hemofiltration at high volume doses[19,49] according to current definitions; with the latter labeled as HVHF. Standard or high cut-off techniques included continuous hemofiltration,[13,15,16,17,18,20,22,23,35,39,40,41,43,49,51] continuous hemodialysis [15,18,27,45] and continuous hemodiafiltration.[21,24,25,26,36]

The main cytokines measured in the clinical studies were interleukin-1b (IL-1b), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-10 (IL-10), tumor necrosis factor-alpha (TNF-alpha) and interleukin-1 receptor antagonist (IL-1Ra) Other cytokines measured were interleukin-2 (IL-2), interleukin -2 receptor (IL-2R), interleukin-6 receptor (IL-6R) and soluble TNF-alpha receptors I and II (sTNFαRI and sTNFαRII). One paper[52] studied many other cytokines and details are included in the footnote of Table 1. Two of the high cut-off studies and a plasmafiltration study also included data on albumin loss.[15,16,42]

Tables 2 and 3 show data on clearance (CL) and sieving coefficient (SC) extracted from human studies respectively. The percentage removed data shown in Table 4. The number of patients that contributed data to each measurement is shown for each technique and treatment characteristics. Table 5 shows a list of all devices studied including other relevant details when reported.

The standard techniques achieved low clearance, for all cytokines measured even when combined with high volume hemofiltration. Std/HF techniques also had overall poor SC for various cytokines, mostly in the range of less than 0.1 to 0.2 regardless of operating characteristics. Some exceptions include IL-8, IL-1β and IL-1Ra although the ranges were very wide with some studies finding very poor SC. The percentage removed data

shown in Table 4 demonstrated that removal of cytokine was poor for standard techniques even when combined with high volume hemofiltration.

HCO techniques were more consistent in offering moderate to high degree of cytokine clearance, for all cytokines measured. For illustration, the median value of CL for IL-6 using standard HF (Std/HF) was 1.09 mL/min while the corresponding median value of CL for IL-6 for HCO technique (HCO/HF) was 26.5 mL/min (refer Table 2.1). CL using HCO techniques seemed to improve with increasing UF flows from 1 L/hr to 2.5 L/hr. HCO with continuous hemofiltration (HCO/HF) was comparable to continuous hemodialysis (HCO/HD) in terms of cytokine removal, however albumin loss was significantly different between these two modes (more than doubled with HF) when UF flows are increased from 1L/hr to 2.5 L/hr. HCO techniques consistently showed high SC of close to unity for IL-6 and IL-1Ra . Albumin SC for HCO techniques was reported in one paper and found to be 0.026.[16] Among the cytokines studied, the SC for TNF-alpha using HCO techniques appear to be consistently very low.

There were no studies involving plasma filtration that provided clearance values. Plasmafiltration showed a SC of around unity for IL-6 and G-CSF, and moderately high SC for leukemia inhibitory factor (LIF). This is however coupled with a SC of unity for albumin which is expected from the characteristics of the technique.[42] Another study found removal of 40% for IL-18 with plasmafiltration, with or without added continuous hemodiafiltration.[47]

For data on adsorption, perhaps due to the nature of the technique, only percentage removed data was reported; with direct hemoperfusion resulting in around 25% removal for IL-1β, IL-6 and IL-1Ra and about 50% removal for IL-8 and TNF-alpha.[46]

Only one paper looked at cytokine clearance with ultrafiltration during cardiopulmonary bypass and zero CL was achieved for all cytokines studied.[28] Ultrafiltration techniques during CPB has reported unusual and implausible figures of SC exceeding 1 for TNF-alpha.[28,30,48] This may indicate extracorporeal-circuit-induced formation of TNF-alpha or an error with measurements. However, the overall removal of all other cytokines as measured by SC was poor (less than 0.1) with this technique. Only one study on this

technique reported percentage removed and found 28% removal of IL-6 and 59% removal of IL-8.[31]

Only clearance values were reported for the extracorporeal liver support systems. The molecular adsorption recycling system (MARS) and Prometheus were the only techniques overall which showed high CL for TNF-alpha ranging from 25 to 29ml/min.[12] The Prometheus system also achieved high CL for IL-10 (46ml/min) and moderately high CL for sTNFαRII (12 ml/min), while MARS achieved moderate CL with both IL-8 (17ml/min) and IL-10 (16ml/min).

Similarly not all measurements were reported for the hybrid techniques. Only one paper evaluated coupled plasmafiltration adsorption (CPFA) and found excellent percentage removal for IL-10 and TNF-a (close to 100%).[32] There were a number of other hybrid techniques described.[14,47,50] Other hybrid techniques generally found low levels of cytokine removal. Standard hemodiafiltration using a filter capable of adsorption found low SC with the technique.[14] Standard HDF combined with plasmafiltration found only 38.8% removal of IL-18 and zero removal of IL-6. SHEDD-fA (sustained high efficiency daily diafiltration using a mediator adsorbing membrane) which utilizes a combination of hemodiafiltration and adsorption found low levels of removal of IL-6 (21%) with single pass measurements, and this is only when levels of IL-6 in the blood were more than 50pg/ml with zero removal with lower blood levels of IL-6.[50]

Discussion

Key findings

We performed a systematic analysis of human clinical studies involving different techniques of EBP to determine their efficacy in the removal of cytokines. We found the high cut-off techniques consistently achieved moderate to high cytokine clearance as demonstrated by CL and SC values. In contrast, standard techniques or ultrafiltration techniques appeared to be inefficient or unreliable in removing cytokines even when coupled with high volume hemofiltration. Plasmafiltration achieved high removal of cytokines, as expected, but this clearance was predictably coupled with high albumin loss. CPFA and adsorption techniques showed promising results based on percentage

removed data, although only one paper for each technique of could be identifed. Hemodiafiltration using filters capable of adsorbing mediators did not offer a high degree of removal through single pass and is largely understudied. Finally, extracorporeal liver support systems may also remove cytokines.

Relation to previous literature

To our knowledge, there are no other reviews of all human studies in the literature which have assessed objective, technical measurements of cytokine removal such as CL, SC and percentage removed for comparison. We had earlier published two systematic reviews on the same topic focusing on ex-vivo cytokine removal and cytokine removal in animal studies respectively.[53,54] The findings of the human studies reported here are broadly consistent with the findings of these two previous systematic reviews.

Significance of study findings

Despite an appreciable number of publications studying EBP techniques or devices in ex-vivo, animal experiments and human studies, details of the ideal operative characteristics to ensure the highest efficacy of cytokine removal have not been clearly outlined. Our reviews suggests that high cut-off techniques may be most consistent in offering moderate to high cytokine removal regardless of operating characteristics. Other techniques which also offer significant cytokine clearance include extracorporeal liver support, plasmafiltration and adsorption techniques but their complexity is greater and the number of studies less . Some of these complex techniques require expertise, special equipment, are expensive and cannot be employed around the clock. High cut-off techniques on the other hand use standard hemodialysis or hemofiltration equipment and standard flows of ultrafiltrate (all of which are widely available worldwide) with the only difference being the use of a filter with larger pores. The operating characteristics and the expertise required to initiate this treatment, although remains essential, are largely similar to that employed during standard continuous renal replacement therapies providing advantages for the use of high cut-off technique in terms of feasibility. More importantly, high cut-off techniques also appear to be one of the safest at a clinical level. High volume hemofiltration for example can result in hypophosphatemia, and loss

of circuit in CPFA which occurs due to clotting, especially if recurrent, can result in significant blood loss. Albumin loss caused by the high cut-off techniques on the other hand, can be replaced by infusing albumin solutions.

Strengths and limitations

The strength of this review is that it is the first to comprehesively assess all techniques of extracorporeal blood purification for their ability to remove cytokines in humans . This information is crucial for the further evolution of blood purification technology as a potential tool to modulate inflamation in sepsis. The limitations of this review include exclusion of articles in languages other than English and the inability to perform statistical comparisons due to the paucity of studies. Some techniques such as adsorption are under-represented as measures relating to clearance and sieving are not relevant to these techniques. The studies included have marked variability in other aspects of treatment and clinical circumstances as well as limited numbers of patients studied. Thus the external validity of our findings is limited. Finally, the clincial significance of cytokine removal like that of electrolyte changes[55, 56] remains unknown.

Conclusions

In conclusion, our systematic review on EBP techniques found that HCO techniques, plasmafiltration and extracorporeal liver support system are able to significantly remove cytokines. Adsorption and CPFA techniques show promise although the data on these techniques are limited. Because of the technical simplicity of HCO techniques, they may represent the most appropriate technique for randomized controlled trials of cytokine removal by EBP.

Figure 1: Flow diagram summarizing the manuscript identification and selection process

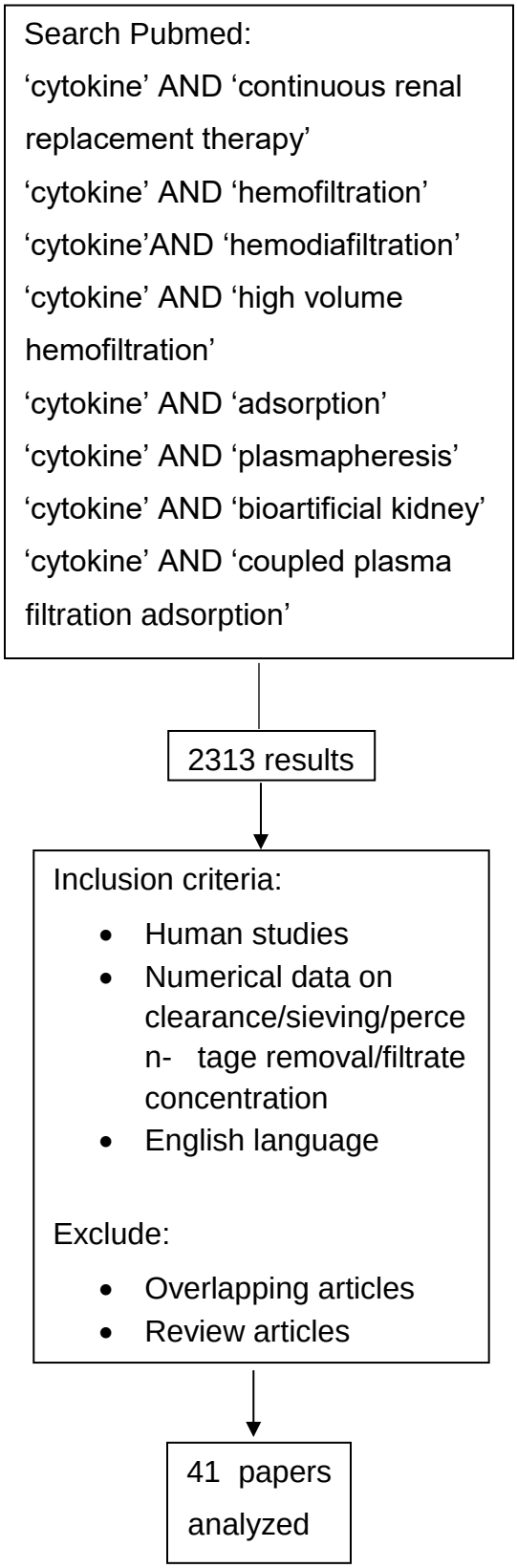


Table 1: Number of papers and total number of patients studied for each technique

Technique	No of papers studying technique	Total no of patients exposed to technique
Standard continuous hemofiltration (Std/HF)	13[13,17,18,20,22,23,35,39,40,41,43,49,51,]	201
Standard continuous hemodialysis (Std/HD)	3[18,27,45]	28
Standard continuous hemodiafiltration (Std/HDF)	5[21,24,25,26,36]	72
Standard high volume hemofiltration (Std/HVHF)	2[19,49]	26
High cut-off continuous hemofiltration (HCO/HF)	3[13,15,16]	48
High cut-off continuous (HCO/HD)	1[15]	12
Plasmafiltration	2[42,47]	22
Combined plasma filtration adsorption (CPFA)	1[32]	10
Ultrafiltration during cardiopulmonary bypass (UF in CPB)	3[28,37,44]	41
Extracorporeal liver support (MARS and Prometheus)	1[12]	8
Adsorption techniques using direct hemoperfusion (Adsorption/DHP)	2[46,52]	14
Conventional ultrafiltration (CUF)	3[29,30,31]	54
Modified ultrafiltration (MUF)	4[30,33,34,38]	80
Zero-balance ultrafiltration (ZBUF)	1[48]	15
Combined standard hemodiafiltration and adsorption (Std HDF+Adsorption)	1[14]	5
Adsorption via sustained high efficiency daily diafiltration using a mediator adsorbing membrane (Adsorption/SHEDD-fA)	1[50]	25
Combined standard hemodiafilration and plasmafiltration (Std HDF + PF)	1[47]	5

Table 2: Clearance (CL) data according to techniques, devices and treatment characteristics

Technique	Device code	n	Qb (mL/min)	Qf (mL/hr)	Qd (mL/hr)	RF	IL1-b	IL2	IL2R	IL6	IL6R	IL8	IL9	IL10	IL1RA	TNFa	sTNFaRI	sTNFaRII	Albumin
Std/HF[13]	4	10		2500		post				0.2					4.43				
Std/HF[17]	7	13	100-150	2000			2.17			1.8						1.47			
Std/HF[18]	8	11	150-200	2000		post				3.3				0		0			
Std/HF[20]	8	13	150	1000						4.92						0			
Std/HF[22]	10	33	150-200	1000		post	7.03	0	0.43	0.56	0	2.19		0	6.62	3.57		0	
Std/HF[39]	12	16		2000						0						0			
Std/HF[51]	41	38	180	2000-2400		pre				1.97		3.33							
Std/HD[18]	8	12	150-200		2000					1.9				0		0			
Std/HD[27]	35	1			2000											0			
Std/HDF[21]	9	9	~100		1000	pre									4	0.15	0.77	0.12	
Std/HDF[24]	12	10	150		1000					1.38		2.74							
Std/HDF[25]	9	20	92	525	1000	pre				2.71						0.069			
Std/HDF[26]	12	18	150		1000		25.1									21.3			
Std/HVHF[19]	34	15		7800						2.01						3.88			
HCO/HF[13]	3	20		2500		post				38.3					39				
HCO/HF[15]	2	6		1000		post				8.43					16.23	0			0.13
HCO/HF[15]	2	6		2500		post				24.6					41.43	0			0.87
HCO/HF[16]	6	16	150	1000		post				14.67						0			
HCO/HD[15]	2	6			1000	post				9					15.43	0			0.16
HCO/HD[15]	2	6			2500	post				21.8					28.33	0			0.35
Adsorption/DHP[52¤]	42	9	80-100				24.3			22.4		0.26	14.6	14	6.93				
UF in bypass[28^]	14	20		1000			0			0		0							
MARS[12*]	1	8	200		18000[#]					3		17		16		29	2		
Prometheus[12*]	2	8	200		18000[#]					4		3		46		25	12		

All clearance values are in mL/min

n = number of patients studied for each technique and treatment characteristics

^ ultrafiltration conducted throughout the whole duration of cardiopulmonary bypass

*cell-free extracorporeal liver support systems
[#] 300ml/min
¤ Other clearances (mL/min) obtained from paper: IL-12 47.3, IL-17A 25.1, FGF basic 31.4, G-CSF 16.1, IFN-ɣ 15.1, PDGF-bb 26.3, VEGF 50.1, TGF-β 5.32
Refer Table 4 for Device code
RF = replacement fluid either pre or postdilution; Qb = blood flow; Qf = ultrafiltration rate; Qd = dialysate flow
HCO=high cut-off, HF=hemofiltration,HD=hemodialysis, HDF=hemodiafiltration, Std=standard,HVHF=high volume hemofiltration,DHP=direct hemoperfusion,UF=ultrafiltration,MARS= Molecular Adsorbents Recirculation System

Table 2.1: Summary of CL values for Std/CVVH and HCO/CVVH

Technique	Device code	n	Qb (mL/min)	Qf (mL/hr)	Qd (ml/hr)	RF	IL1-b	IL2	IL2R	IL6	IL6R	IL8	IL10	IL1ra	TNFa	sTNFaRI	sTNFaRII	Albumin
HCO/HF[13]	3	20		2500		post				38.3				39				
HCO/HF[15]	2	6		1000		post				8.43				16.23	0			0.13
HCO/HF[15]	2	6		2500		post				24.6				41.43	0			0.87
HCO/HF[16]	6	16	150	1000		post				14.67					0			
Median										26.49				40.22				
IQR										(13.1,28.0)				(27.6,40.2)				

Technique	Device code	n	Qb (mL/min)	Qf (mL/hr)	Qd (ml/hr)	RF	IL1-b	IL2	IL2R	IL6	IL6R	IL8	IL10	IL1RA	TNFa	sTNFaRI	sTNFaRII	Albumin
Std/HF[13]	4	10		2500		post				0.2				4.43				
Std/HF[17]	7	13	100-150	2000			2.17			1.8					1.47			
Std/HF[18]	8	11	150-200	2000		post				3.3			0		0			
Std/HF[20]	8	13	150	1000						4.92					0			
Std/HF[22]	10	33	150-200	1000		post	7.03	0	0.43	0.56	0	2.19	0	6.62	3.57		0	
Std/HF[39]	12	16		2000						0					0			
Std/HF[51]	41	38	180	2000-2400		pre				1.97		3.33						
Median										1.09					0.735			
IQR										(0.38,2.64)					(0,1.47)			

Table 3: Sieving coefficient (SC) data for different techniques and devices with specific treatment characteristics

Technique	Device code	n	Qb (mL/min)	Qf (mL/h)	Qd (mL/h)	RF	IL1-b	IL2	IL2R	IL6	IL6R	IL8	IL10	IL1ra	TNFa	sTNFa R1	sTNFa RII	Alb	GCSF	LIF
Std/HF[13]	4	10		2500		post				0.007				0.1						
Std/HF[17]	7	13	100-150	2000			0.073			0.067					0.053					
Std/HF[22]	10	33	150-200	1000		post	0.42	0	0.05	0.04	0	0.12	0	0.41	0.22		0			
Std/HF[23]	11	16	150	2000		post	0.02			0	0.62				0					
Std/HF[40]	25	13	250-300	2000-4000		pre	0.33								0.16					
Std/HF[41]	26	7	200	2500		post						0.62								
Std/HF[43]	28	15	100-200	25.4 - 44.3 ml/min		post	0.22			0.18			0	0.28	0.16	0.006	0.003			
Std/HF[35]	20	5		2000			0.18			0		0.25			0					
Std/HF[39]	12	16		2000						0					0					
Std/HF[51]	41	38	180	2000-2400		pre				0.05		0.09								
Std/HD[45]	20	16		2000		post	0.09			0		0.68			0					
Std/HDF[36]	21	15	100-150	10.4-4.3 ml/min	10-30 ml/min	post						0.19			0.18					
Std/HDF[21]	9	9	~100		1000	pre								0.45	0.02	0.09	0.01			
Std/HDF[25]	9	20	92	525	1000	pre				0.27					0.017					
HCO/HF[13]	3	20		2500		post				0.9				0.92						
HCO/HF[15]	2	6		1000		post				0.92				1	0					
HCO/HF[15]	2	6		2500		post				> 0.92				1	0					
HCO/HF[16]	6	16	150	1000		post				0.82					0			0.026		
HCO/HD[15]	2	6			1000	post				0.92				1	0					
HCO/HD[15]	2	6			2500	post				>0.92				1	0					
PF[42]	27	14		*a*						0.93								0.99	1.29	0.66
UF in bypass[28]^	14	20		1000			0			0		0			2.3					

UF in bypass[37µ]	22	11								<0.001		0.004								
UF in bypass[44¤]	39	16								1.246										
CUF [30]	16	11		62 ml/min						0.035			poor		1.01					
CUF [30]	17	10		42 ml/min						0.037			poor		2.72					
CUF [29]	15	10	100-120				0.67			0.04		0.22			0.9				0.04	
MUF[30]	16	11		82 ml/min						0.005			0.1		2.22					
MUF[30]	17	9		93 ml/min						0.003			poor		2.01					
MUF[34]	12	20								0.03		0.12								
MUF[38]	23	20	10-15 ml/kg/min												0.23					
MUF[33]	38	20	300-400 (during MUF)	*						0.25		0.06	0							
ZBUF[48]	32	15		#			0.39			0.019					194					
Std/HDF +adsorption[14]	5	10	90-130	2000	NA	pre	0.05			0.03		0.07			0					

n = number of patients studied for each technique and treatment characteristics

a = 100ml/kg for first 4-6hrs and then 150ml/kg over 28 to 30hrs

^ = ultrafiltration conducted throughout the whole duration of cardiopulmonary bypass

µ = from aortic cross clamp until end of CPB

¤ = from aortic cross clamp until within 5 minutes of clamp removal

*1200 to 1800 ml total during MUF

Fluid removal of 1L every 10min until 3 L/m2 BSA removed

Refer Table 4 for Device code

RF = replacement fluid either pre or postdilution; Qb = blood flow; Qf = ultrafiltration rate; Qd = dialysate flow

HCO=high cut-off, HF=hemofiltration,HD=hemodialysis, HDF=hemodiafiltration, Std=standard, PF= plasmafiltration, UF = ultrafiltration, CUF= conventional ultrafiltration, MUF= modified ultrafiltration, ZBUF= zero balance ultrafiltration

Table 4: Percentage removal data for different cytokines

Technique	Device code	n	Qb(ml/min)	Qf(ml/H)	Qd (ml/min)	RF	IL1-b	IL2	IL6	IL8	IL10	IL1ra	IL18	TNFa
CUF[31]	18	23	100	100-300ml/kg					28	59				
Std/HF[49]	25	11	200	1000		pre		0		0	3.15			0
Std/HVHF[49]	33	11	300	6000		1/3pre 2/3post		0		0	3.45			0
Adsorption/DHP[46]	29	5	100				24.7		24.65	54		28.5		52.25
PF[47]	31	8	80	***b***					0				42.9	
CPFA[32]	19 + 36 + 37	10	155	***a***							99.8			99.6
Std HDF +PF[47]	30(+31)	5	100 (CHDF) + 80 (PE)	***b***					0				38.8	
Adsorption/SHEDD-fA[50]	40	25	150	1500		post			21					

n = number of patients studied for each technique and treatment characteristics

a = 30-40 ml/min(Qp); (32-38ml/min UF+dialysate outflow)

b =3.6-4.0L plasma exchanged per session

Refer Table 4 for Device code

RF = replacement fluid either pre or postdilution; Qb = blood flow; Qf = ultrafiltration rate; Qd = dialysate flow

cut-off, HF=hemofiltration,HD=hemodialysis, HDF=hemodiafiltration, HVHF=high volume hemofiltration, Std=standard, PF= plasmafiltration, CUF= conventional ultrafiltration, CPFA= combined plasmafiltration adsorption, DHP= direct hemoperfusion, PD = peritoneal dialysisMUF= modified ultrafiltration, ZBUF= zero balance ultrafiltration, SHEDD-fA= sustained high efficiency daily diafiltration using a mediator adsorbing membrane

Table 5: Device codes

Reference	Device name	Device code	Filter type	Filter size (m^2)	Molecular cut-off point	Features
12	MARS	1	albumin impermeable membrane+charcoal+anion exchange resin+dialyzer		60kDa	*
12	Prometheus	2	albumin filter(polysulfone) + neutral resin adsorber + anion exchange adsorber + dialyzer(polysulfone)		250kDa	#
13,15	P2SH	3	polyamide	1.1	60kDa in vivo	
13,16	Polyflux 11s	4	polyamide	1.1	30kDa	
14	Multiflow 60 + polymyxin B	5	AN69 (polyacrylonitrile) with Polymyxin B immobilized fibre	0.6		
16	PSH1	6	Polyamide	0.6	60kDa	
17	Prisma	7	AN69	0.9	35-40kDa	
18,20	Multiflow 60	8	AN69	0.6	40kDa	
21,25	AN69HF	9	AN69			
22	AV600	10	polysulfone	1.35	30kDa	
23	FH66	11	polyamide			
24,26,34	AN69S	12	polyacrylonitrile			
28	650 SF 1.3	14	polyacrylonitrile		30kDa	
29	Diafilter 20	15	polysulfone			
30	Jostra BC20	16	polyamide	0.2		
30	Jostra BC60	17	polysulfone	0.65		
31	DHF02	18	polyethersulfane	0.25		
32	MPS 07	19	polyethersulfone	0.7		
35	FH66D	20	polyamide			
36	AN69	21	polyacrylonitrile	1.6		
37	PF40	22	polysulfone		40kDa	
38	Bently Hemoconcentrator	23	polysulfone	0.3		
40	AN69	25	AN69	1.2		
41	BL627	26	polysulfone			
42	PF1000	27	polypropylene	0.14		
43	Multiflow 100	28	AN69	0.9	35-40kDa	

46	Lixelle	29	β2-microglobulin adsorption column (cellulose beads + hexadecyl groups)		30kDa	
47	Panflow APF06S	30	polyacrylonitrile			
47	Plasmaflow OP08W	31	plasma filter			
48	HPH1400	32	polysulfone	1.3	65kDa	
49	Filtral 16	33	AN69	1.6		
19	Flat plate filter (15 parallel membranes)	34	polyacrylonitrile	0.43	30-40kDa	
27	Hemoflow F60	35	polysulfone			
32	BLS 627	36	polysulfone	1.2		
32	Amberchrom	37	reverse phase styrenic polymer resin	surface 600 to 800m^2/g		
33	Diafilter D30-NR	38	polysulfone			
44	Hemocor HPH1000	39	polysulfone			
50	Filtrizer BG-PO	40	polymethylmethacrylate			
51	NI-PRO UF-205	41	cellulose triacetate	1.9		
52	Toraymyxin 20R	42	polymyxin B			

*20%human serum albumin as dialysate which then passes thru columns of charcoal and anion exchange resins. Water soluble substances cleared by low flux dialyzer in a secondary circuit

following separation plasma passes through two columns containing different adsorbents. Water soluble substances cleared by high-flux dialyzer directly inserted into the blood circuit

References:

1. Beale R, Reinhart K, Brunkhorst F, Dobb G, Levy M, Martin G. Promoting Global Research Excellence in Severe Sepsis (PROGRESS): Lessons from an International Sepsis Registry. Infection 2009; 37:222-232.
2. Cabre L, Mancebo J, Solsona JF, et al. Multicenter study of the multiple organ dysfunction syndrome in intensive care units: The usefulness of Sequential Organ Failure Assessment scores in decision making. Intensive Care Med 2005;31:927–933
3. Bone RC. Sir Isaac Newton, sepsis, SIRS, and CARS. Crit Care Med 1996;24:1125-8.
4. Cinel I, Opal SM. Molecular biology of inflammation and sepsis: a primer. Crit Care Med 2009;37:291-304
5. Schefold JC, Hasper D, Jörres A. Organ crosstalk in critically ill patients: hemofiltration and immunomodulation in sepsis. Blood Purif 2009;28:116-23
6. Ronco C, Tetta C, Mariano F, Wratten ML, Bonello M, Bordoni V et al. Interpreting the Mechanisms of Continuous Renal Replacement Therapy in Sepsis: The Peak Concentration Hypothesis. Artificial Organs 2003; 27:792–801.
7. Fisher CJ Jr, Dhainaut JF, Opal SM, Pribble JP, Balk RA, Slotman GJ et al. Recombinant human interleukin 1 receptor antagonist in the treatment of patients with sepsis syndrome. Results from a randomized, double-blind, placebo-controlled trial. Phase III rhIL-1ra Sepsis Syndrome Study Group. JAMA 1994;271:1836-43.
8. Opal SM, Fisher CJ Jr, Dhainaut JF, Vincent JL, Brase R, Lowry SF, et al: Confirmatory interleukin-1 receptor antagonist trial in severe sepsis: a phase III, randomized, doubleblind, placebo-controlled, multicenter trial. The Interleukin-1 Receptor Antagonist Sepsis Investigator Group. Crit Care Med 1997;25:1115–1124.
9. Kellum JA, Bellomo R, Mehta R, Ronco C. Blood purification in non-renal critical illness. Blood Purif. 2003;21:6-13
10. Venkataraman R, Subramanian S, Kellum JA. Clinical review: extracorporeal blood purification in severe sepsis. Crit Care 2003;7:139-45.
11. Ronco C, Inguaggiato P, D'Intini V, Cole L, Bellomo R, Poulin S et al. The role of extracorporeal therapies in sepsis. J Nephrol. 2003;16 Suppl 7:S34-41
12. Stadlbauer V, Krisper P, Aigner R, Haditsch B, Jung A, Lackner C et al. Effect of extracorporeal liver support by MARS and Prometheus on serum cytokines in acute-on-chronic liver failure. Crit Care 2006;10:R169

13. Morgera S, Haase M, Kuss T, Vargas-Hein O, Zuckermann-Becker H, Melzer C et al. Pilot study on the effects of high cutoff hemofiltration on the need for norepinephrine in septic patients with acute renal failure. Crit Care Med 2006;34:2099-104
14. Peng Y, Yuan Z, Li H. Removal of inflammatory cytokines and endotoxin by veno-venous continuous renal replacement therapy for burned patients with sepsis. Burns 2005;31:623-8.
15. Morgera S, Slowinski T, Melzer C, Sobottke V, Vargas-Hein O, Volk T et al. Renal replacement therapy with high-cutoff hemofilters: Impact of convection and diffusion on cytokine clearances and protein status. Am J Kidney Dis 2004;43:444-53.
16. Morgera S, Rocktäschel J, Haase M, Lehmann C, von Heymann C, Ziemer S et al. Intermittent high permeability hemofiltration in septic patients with acute renal failure. Intensive Care Med 2003;29:1989-95.
17. Dahaba AA, Elawady GA, Rehak PH, List WF. Procalcitonin and proinflammatory cytokine clearance during continuous venovenous hemofiltration in septic patients. Anaesth Intensive Care 2002;30:269-74.
18. Kellum JA, Johnson JP, Kramer D, Palevsky P, Brady JJ, Pinsky MR. Diffusive vs. convective therapy: effects on mediators of inflammation in patient with severe systemic inflammatory response syndrome. Crit Care Med 1998;26:1995-2000.
19. Sanchez-Izquierdo JA, Perez Vela JL, Lozano Quintana MJ, Alted Lopez E, Ortuño de Solo B, Ambros Checa A. Cytokines clearance during venovenous hemofiltration in the trauma patient. Am J Kidney Dis. 1997; 30: 483-8.
20. Sander A, Armbruster W, Sander B, Daul AE, Lange R, Peters J. Hemofiltration increases IL-6 clearance in early systemic inflammatory response syndrome but does not alter IL-6 and TNF alpha plasma concentrations. Intensive Care Med. 1997;23:878-84.
21. van Bommel EF, Hesse CJ, Jutte NH, Zietse R, Bruining HA, Weimar W. Impact of continuous hemofiltration on cytokines and cytokine inhibitors in oliguric patients suffering from systemic inflammatory response syndrome. Ren Fail 1997;19:443-54.
22. Heering P, Morgera S, Schmitz FJ, Schmitz G, Willers R, Schultheiss HP et al. Cytokine removal and cardiovascular hemodynamics in septic patients with continuous venovenous hemofiltration. Intensive Care Med 1997;23:288-96.
23. Hoffmann JN, Hartl WH, Deppisch R, Faist E, Jochum M, Inthorn D. Effect of

hemofiltration on hemodynamics and systemic concentrations of anaphylatoxins and cytokines in human sepsis. Intensive Care Med 1996;22:1360-7.

24. Bellomo R, Tipping P, Boyce N. Interleukin-6 and interleukin-8 extraction during continuous venovenous hemodiafiltration in septic acute renal failure. Ren Fail. 1995;17:457-66.
25. van Bommel EF, Hesse CJ, Jutte NH, Zietse R, Bruining HA, Weimar W. Cytokine kinetics (TNF-alpha, IL-1 beta, IL-6) during continuous hemofiltration: a laboratory and clinical study. Contrib Nephrol 1995;116:62-75.
26. Bellomo R, Tipping P, Boyce N. Continuous veno-venous hemofiltration with dialysis removes cytokines from the circulation of septic patients. Crit Care Med 1993;21:522-6.
27. Byrick RJ, Goldstein MB, Wong PY. Increased plasma tumor necrosis factor concentration in severe rhabdomyolysis is not reduced by continuous arteriovenous hemodialysis. Crit Care Med 1992;20:1483-6.
28. Antunes N, Dragosavc D, Petrucci Junior O, Oliveira PP, Kosour C, Blotta MH et al. The use of ultrafiltration for inflammatory mediators removal during cardiopulmonary bypass in coronary artery bypass graf surgery. Rev
Bras Cir Cardiovasc 2008;23:175-82.
29. Brancaccio G, Villa E, Girolami E, Michielon G, Feltri C, Mazzera E et al. Inflammatory cytokines in pediatric cardiac surgery and variable effect of the hemofiltration process. Perfusion 2005;20:263-8.
30. Berdat PA, Eichenberger E, Ebell J, Pfammatter JP, Pavlovic M, Zobrist C et al. Elimination of proinflammatory cytokines in pediatric cardiac surgery: analysis of ultrafiltration method and filter type. J Thorac Cardiovasc Surg 2004;127:1688-96
31. Dittrich S, Aktuerk D, Seitz S, Mehwald P, Schulte-Mönting J, Schlensak C et al. Effects of ultrafiltration and peritoneal dialysis on proinflammatory cytokines during cardiopulmonary bypass surgery in newborns and infants. Eur J Cardiothorac Surg 2004;25:935-40
32. Ronco C, Brendolan A, Lonnemann G, Bellomo R, Piccinni P, Digito A et al. A pilot study of coupled plasma filtration with adsorption in septic shock. Crit Care Med 2002;30:1250-5

33. Kiziltepe U, Uysalel A, Corapcioglu T, Dalva K, Akan H, Akalin H. Effects of combined conventional and modified ultrafiltration in adult patients. Ann Thorac Surg. 2001;71:684-93
34. Bogă M, Islamoğlu, Badak I, Cikirikçioğlu M, Bakalim T, Yağdi T et al. The effects of modified hemofiltration on inflammatory mediators and cardiac performance in coronary artery bypass grafting. Perfusion 2000;15:143-50
35. Hoffmann JN, Werdan K, Hartl WH, Jochum M, Faist E, Inthorn D. Hemofiltrate from patients with severe sepsis and depressed left ventricular contractility contains cardiotoxic compounds. Shock 1999;12:174-80
36. Toft P, Kehler D, Brandslund I I, Tønnsen E. The immunological effects of continuous veno-venous haemodiafiltration in critically ill patients. Crit Care 1999;3:159-165
37. Watanabe T, Sakai Y, Mayumi T, Shimomura T, Song MH, Tajima K et al. Effect of ultrafiltration during cardiopulmonary bypass for pediatric cardiac surgery. Artif Organs 1998;22:1052-5
38. Wang W, Huang HM, Zhu DM, Chen H, Su ZK, Ding WX. Modified ultrafiltration in paediatric cardiopulmonary bypass. Perfusion 1998;13:304-10
39. Hoffmann JN, Faist E, Deppisch R, Hartl WH, Inthorn D. Hemofiltration in human sepsis: evidence for elimination of immunomodulatory substances. Contrib Nephrol 1995;116:76-9
40. Xie H, Ji D, Gong D, Liu Y, Xu B, Zhou H et al. Continuous veno venous hemofiltration in treatment of acute necrotizing pancreatitis. Chin Med J (Engl) 2003;116:549-53
41. Mariano F, Tetta C, Guida G, Triolo G, Camussi G. Hemofiltration reduces the serum priming activity on neutrophil chemiluminescence in septic patients. Kidney Int. 2001;60:1598-605
42. Reeves JH, Butt WW, Shann F, Layton JE, Stewart A, Waring PM et al. Continuous plasmafiltration in sepsis syndrome. Plasmafiltration in Sepsis Study Group. Crit Care Med. 1999;27:2096-104
43. De Vriese AS, Colardyn FA, Philippé JJ, Vanholder RC, De Sutter JH, Lameire NH. Cytokine removal during continuous hemofiltration in septic patients. J Am Soc Nephrol 1999;10:846-53
44. Clar A, Bowers MC, Larson DF. Derivation of sieving coefficients to determine the efficacy of the hemoconcentrator in removal of four inflammatory mediators

produced during cardiopulmonary bypass. ASAIO J. 1997;43:163-70

45. Hoffmann JN, Hartl WH, Deppisch R, Faist E, Jochum M, Inthorn D. Hemofiltration in human sepsis: evidence for elimination of immunomodulatory substances. Kidney Int. 1995;48:1563-70
46. Tsuchida K, Takemoto Y, Sugimura K, Yoshimura R, Nakatani T. Direct hemoperfusion by using Lixelle column for the treatment of systemic inflammatory response syndrome. Int J Mol Med. 2002;10:485-8
47. Yonekawa C, Nakae H, Tajimi K, Asanuma Y. Effectiveness of combining plasma exchange and continuous hemodiafiltration in patients with postoperative liver failure. Artif Organs. 2005;29:324-8
48. Tallman RD, Dumond M, Brown D. Inflammatory mediator removal by zero-balance ultrafiltration during cardiopulmonary bypass. Perfusion. 2002;17:111-5
49. Cole L, Bellomo R, Journois D, Davenport P, Baldwin I, Tipping P. High-volume hemofiltration in human septic shock. Intensive Care Med. 2001;27:978-86
50. Nishida O, Nakamura T, Kuriyama N, Hara Y, Yumoto M, Shimomura Y et al. Sustained high-efficiency daily diafiltration using a mediator-adsorbing membrane (SHEDD-fA) in the treatment of patients with severe sepsis. Contrib Nephrol. 2011;173:172-81
51. Schilder L, Nurmohamed SA, ter Wee PM, Girbes AR, Beishuizen A, Paauw NJ et al. Effect of anticoagulation regimens on handling of interleukin-6 and -8 during continuous venovenous hemofiltration in critically ill patients with acute kidney injury. Cytokine. 2012;60:601-7
52. Oishi K, Mimura-Kimura Y, Miyasho T, Aoe K, Ogata Y, Katayama H et al. Association between cytokine removal by polymyxin B hemoperfusion and improved pulmonary oxygenation in patients with acute exacerbation of idiopathic pulmonary fibrosis. Cytokine. 2013;61:84-9
53. Atan R, Crosbie D, Bellomo R. Techniques of extracorporeal cytokine removal: a systematic review of the literature. Blood Purif. 2012;33:88-100
54. Atan R, Crosbie D, Bellomo R. Techniques of extracorporeal cytokine removal: A systematic review of the literature on animal experimental studies. Int J Artif Organs 2013; 36: 149-158

55. Brain M, Anderson M, Parkes S, Fowler P. Magnesium flux during continuous venous hemodiafiltration with heparin and citrate anticoagulation. Crit Care Resus. 2012; 14:274-282.
56. Brain M, Parkes S, Fowler P, Robertson I, Brown A. Calcium flux in continuous venovneous hemodiafiltration with heparin and citrate anticoagulation. Crit Care Resusc. 2011;13:72–81.

1.10 Summary of literature review on human studies

Our literature review on extracorporeal cytokine removal and human studies reported findings which were mostly similar compared to previous reviews on laboratory and animal studies. HCO hemofiltration appeared to result in higher clearance of cytokines compared to standard hemofiltration. Higher clearance is further achieved when HCO hemofiltration is applied with ultrafiltration rates of 2500ml/hour; similar to the proposed dose for renal replacement therapy. Higher albumin loss was reported with HCO *hemofiltration* as opposed to HCO *hemodialysis* by one of the studies. HCO hemofiltration however also achieved higher clearance of cytokines compared to HCO hemodialysis. None of these studies on HCO filters however were conducted as blinded randomised control trials. High volume hemofiltration using standard filters, as opposed to findings from animal studies, did not increase clearance. Clearance of TNF-alpha was generally poor except when using liver extracorporeal support devices.

From the findings of our extensive series of systematic reviews, we concluded that high cut off hemofiltration using standard doses of ultrafiltration (25 ml/kg/H) gives the best combination of an effective yet simplistic and widely available technique that does not require unusual levels of expertise. This technique has the potential to significantly remove cytokines close to the degree of plasmafilters but unlike plasmafiltration is suitable for continuous application, offering yet another desirable feature of an EBP technique. The improved clearance by virtue of larger pore sized filters eliminates the need for high volume hemofiltration and issues that arise from the need for large amounts of replacement fluids. The higher loss of albumin at the price of higher clearance appear to occur at a rate that can be resolved by albumin administration. HCO hemofiltration will also double as renal replacement therapy for patients in acute kidney injury (AKI).

Additional theoretical advantages of nonspecific removal of cytokines, as that offered by HCO hemofiltration, include the ability to self-adjust to changing levels of cytokines and the ability to modulate several cytokines at the same time. Finally, regardless of its ability to remove cytokines at a clinically significant rate, hemofiltration may still offer advantages through other as yet unexplained mechanism.

There was good evidence therefore to support the pursuit of our research question further by conducting a double-blind randomised clinical trial comparing HCO hemofiltration to standard hemofiltration at standard operating conditions for renal replacement therapy. As the risk benefit rationalisation of instituting hemofiltration for the purpose of cytokine clearance was not yet established, the indication to initiate this therapy remained standard conditions for initiating hemofiltration in the setting of AKI.

Chapter 2

The physiological impact of high cut-off hemofiltration

2.1. Introduction

Our extensive literature review on in vitro, animal and clinical studies confirms that the high cut-off membrane is able to remove significant amounts of cytokine as measured by its clearance. Compared to other extracorporeal techniques that also offer significant cytokine removal, HCO hemofiltration appear to offer the best balance in terms of high cytokine clearance, ease of administration, safety and wide availability in terms of equipment and expertise. All this leads to a strong indication to embark on a trial to study the effects of HCO hemofiltration on an appropriate target population i.e. critically ill patients suffering from conditions predisposing to hypercytokinemia, in a real clinical setting.

In an attempt to answer our research questions on the physiological and biological impact of HCO hemofiltration in hypercytokinemic patients, we conducted a phase II equivalent double-blind, randomised controlled trial comparing high cut-off point hemofiltration with standard hemofiltration involving 72 critically ill patients who were haemodynamically unstable requiring vasopressor infusion. Patients recruited also fulfilled standard indications for renal replacement therapy as the accepted indication for hemofiltration in critically ill patients is still severe acute kidney injury. Commencing hemofiltration on the basis of suspected hypercytokinemia alone is controversial with unclear safety concerns.

Clinical studies on blood purification at the time our study was undertaken were still in its early stages, mostly measuring surrogate outcome measures like haemodynamic improvement and reduction in norepinephrine requirement. The findings of these early trials were encouraging toward high cut-off hemofiltration but none of these clinical trials were based on a blinded comparison. The degree of albumin loss as shown by some studies may occur up to a moderate degree, but generally not accompanied by a precipitous drop in plasma albumin levels. These earlier studies therefore appeared to support the safety of the intervention.

We attempted to assess the physiological impact of HCO hemofiltration in our study by studying its effects on hemodynamic stability through vasopressor infusion free-time as the primary outcome measure.

A total of four interim analyses were performed in a blinded fashion throughout the conduct of our trial. The first was conducted at 20 patients and the results presented for the Austin Research Prize. The purpose of the interim analyses was to demonstrate safety in the conduct of the trial and a comparison of baseline characteristics, time off norepinephrine, ICU (intensive care unit) outcome and plasma levels of albumin were performed. There were no difference between the two groups in any of the comparisons but the ICU mortality difference between the two groups were quite marked, with 3 survivors in one group versus 7 survivors in the other (p value = 0.17). This lead to a planned second safety interim analysis at 40 patients.

The interim analysis performed at 40 patients worryingly showed a significant difference in ICU mortality between the two groups (p=0.011). The mortality rate for the group that was faring worse (60 to 65 percent) however was not higher than expected for this cohort of haemodynamically unstable patients in acute renal failure. The severity scoring of the affected group was also higher although this did not reach the level of significance. The difference in mortality therefore was attributed to chance. This was especially after taking into consideration the fact that the mortality rate for the group that was faring better was implausibly low at around 20 to 25 percent. Recruitment was continued with another planned interim analysis at 50 patients.

The interim analysis at 50 patients again showed a significant difference between the two groups (p=0.02) but again the mortality rate for the group with the higher mortality (56 to 60%) was not higher than expected for the cohort of sick patients therefore allowing recruitment of patients to continue. The mortality rate for the group faring better remained implausibly low at 20 to 25 percent. The difference was again attributed to chance and another blinded interim analysis was planned at 60 patients.

At 60 patients, the difference in mortality between the two groups was found to be insignificant confirming our suspicion that any earlier difference in mortality was due to chance. A decision was made to increase the number of patients recruited to 76 to account for protocol violations or recruitment errors. A total of 76 patients were successfully enrolled.

Our paper has been submitted for publication and is presented below:

2.2 Publication

A double-blind randomized controlled trial of high cut-off vs. standard hemofiltration in critically ill patients with acute kidney injury

Rafidah Atan[1], Leah Peck[2], John Prowle[3,4], Elisa Licari[5], Glenn M. Eastwood[2], Markus Storr[6], Hermann Goehl[6], Rinaldo Bellomo[2,7]

1. Clinical School Johor Bahru, Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, Malaysia
2. Department of Intensive Care, Austin Hospital, Heidelberg, Victoria, Australia
3. Adult Critical Care Unit and Department of Renal & Transplant Medicine, The Royal London Hospital, Barts Health NHS Trust, London, UK.
4. William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, UK.
5. Department of Intensive Care, Alfred Hospital, Prahran, Victoria, Australia
6. Gambro Dialysatoren GmbH, Research & Development, Hechingen, Germany
7. School of Medicine, The University of Melbourne, Parkville, Melbourne, Victoria, Australia

Correspondence to:

Rinaldo Bellomo

Department of Intensive Care, Austin Hospital

[redacted]

Tel: [redacted]

Fax: [redacted]

Email: [redacted]

Abstract

Background: In critically ill patients with acute kidney injury (AKI) receiving vasopressors, high cytokine levels may sustain the shock state. High cut-off (HCO) hemofiltration achieves greater cytokine removal in ex-vivo and in animal models and may reduce the duration of shock but may also increase albumin losses.

Methods: We conducted a phase II double-blind randomized controlled trial comparing continuous veno-venous high cut-off hemofiltration (CVVH-HCO) to standard hemofiltration (CVVH-Std) in 76 critically ill vasopressor-dependent patients with AKI. The primary outcome measure was norepinephrine-free time within the first seven days of treatment.

Results: The median hours of norepinephrine-free time at day seven were 32 (0, 110.8) for CVVH-HCO and 56 (0, 109.3) hours (p=0.520) for CVVH-Std. In-hospital mortality was 55.6% with CVVH-HCO vs. 34.2% with CVVH-Std [adjusted OR 2.49 (95% CI 0.81 to 7.66; p=0.191)]. Moreover, there was no significant difference in time to cessation of norepinephrine (p=0.358), time to cessation of hemofiltration (p=0.563) and filter life (p=0.21). Serum albumin levels (p=0.112) were similar and the median dose of intravenous albumin given was 90 (20, 212) grams for CVVH-HCO and 80 (15, 132) grams for CVVH-Std (p=0.252).

Discussion: In critically ill patients with AKI, CVVH-HCO did not reduce the duration of vasopressor support or mortality or change albumin levels compared to CVVH-Std.

Keywords: high cut-off filter, super high flux filter, acute kidney injury, sepsis, SIRS, critical illness, hemofiltration, blood purification

Introduction

Shock states with accompanying acute kidney injury (AKI) are a leading cause of death in critically ill patients(Hotchkiss et al., 2016), with a mortality rate of approximately 60%(Uchino et al., 2005). In this setting, high cytokine levels are believed to contribute to sustained vasodilatation, continued multi-organ dysfunction syndrome (MODS) and mortality (Bosmann & Ward, 2013; Schulte, Bernhagen, & Bucala, 2013). They appear to do so through complex effects on inflammation, immunity, and coagulation pathways (Morgera et al., 2006). Accordingly, they have been the target of therapeutic interventions for more than two decades.

For example, antibodies against key cytokines such as TNF-alpha and IL-1 as well as analogues to cytokine antibodies such as IL-1ra have been studied as adjunctive treatment of septic shock (Dinarello, 2001). However, such treatments have been unsuccessful. This lack of success with the targeting of specific individual molecules has suggested the need to test broader approaches including nonspecific extracorporeal cytokine removal (Ronco et al., 2003; Schefold, Hasper, & Jorres, 2009). In this regard, the application of blood purification therapies has been proposed as a way of returning cytokines to more homeostatic levels (R. Atan, D. Crosbie, & R. Bellomo, 2013). Various blood purification techniques aimed at cytokine clearance have been explored for such purposes over the last 20 years including standard hemofiltration, adsorption techniques, plasmapheresis and hybrid techniques (R. Atan, D. C. Crosbie, & R. Bellomo, 2013). Unfortunately, many of these techniques have also been unsuccessful. Such lack of success, however, may, at least in part, be related to the use of membranes that can only achieve low levels of cytokine removal due to their limited porosity.

Most cytokines have molecular sizes that range between 8kDa and 60kDa, while standard hemofiltration membranes have nominal cut-off points of somewhere between 10 to 30 kDa. These observations logically suggest the need of more targeted membrane characteristics to achieve greater levels of cytokine removal. In this regard, high cut-off (HCO) filters, also known as super high flux filters have been developed and tested. Such membranes have larger nominal pore sizes ranging between 60 to 150kDa and offer better removal of cytokines ex-vivo (Boschetti-de-Fierro, Voigt, Storr, & Krause, 2013). In this regard, some early studies have shown promising results with

these HCO filters in the treatment of sepsis and AKI and demonstrated a degree of safety (Morgera et al., 2006; Morgera et al., 2003; Morgera et al., 2004).

In light of the above considerations, we designed and performed a phase II double-blind randomized study comparing standard veno-venous hemofiltration (CVVH-Std) with HCO hemofiltration (CVVH-HCO) in critically ill patients with AKI requiring vasopressor support (ClinicalTrial.gov/NCT00912184). We hypothesized that there would be a difference in norepinephrine requirements expressed as hours of norepinephrine-free time within the first week of treatment.

Methodology

The study was approved by the Austin Hospital Human Research Ethics Committee and performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

We randomized patients in a 1 to 1 ratio to receive either standard continuous veno-venous hemofiltration (CVVH-Std) or high cut off continuous veno-venous hemofiltration (CVVH-HCO) within 12 hours of a decision to commence hemofiltration. Randomization was achieved through random allocation generated by a computer program using permuted block sizes undisclosed to recruiting personnel. Concealment of allocation was achieved through opaque sealed envelopes labeled with sequential numbers.

We included patients on norepinephrine for hemodynamic support who required hemofiltration for AKI. The criteria for initiating hemofiltration included either oliguria (<100ml/6h) unresponsive to fluid resuscitation, hyperkalemia of more than 6.5 mmol/L, severe acidemia of less than pH of 7.2, serum urea of more than 25 mmol/L, serum creatinine of more than 300 µmol/L or clinically significant organ edema in the setting of acute renal failure (e.g. pulmonary edema). Patients were recruited if the clinician anticipated that the patient would require hemofiltration for at least 72 hours.

We excluded patients who were less than 18 years of age and those in whom the treating clinician believed that death was likely within 24 hours. We also excluded patients who had been treated with hemofiltration or other dialysis during the same hospital admission, were on maintenance dialysis prior to admission or were pregnant or breastfeeding. The study was conducted in a general intensive care unit within a

major tertiary hospital. Informed consent was obtained from the legally responsible person (next of kin or person with medical power of attorney).

Following enrolment, patients were randomly allocated to either continuous venovenous hemofiltration (CVVH) with custom manufactured polyethersulfone standard hemofilters (CVVH-Std) with nominal cut-off point of 30kDa or CVVH with polyethersulfone high cut-off filters (CVVH-HCO) with nominal cut-off point of 100kDa (P2SH filters, 1.12m^2, Gambro, Hechingen, Germany). Patients, health-care personnel and researchers were blinded to treatment allocation. The two types of filters were indistinguishable in appearance.

For each treatment, the technical settings were the following: blood flow at 200 ml/min, ultrafiltration rate at 25 ml/kg/h rounded to the nearest 100 ml with bicarbonate-buffered replacement fluids. The choice of anticoagulation was left to the discretion of the treating clinician. However, the mainstay of anticoagulation mode was low dose pre-filter heparinization. Each treatment was applied until a maximum of 14 days or until cessation of continuous renal replacement therapy or death or discharge from ICU, whichever occurs earlier. Other aspects of treatment continued according to clinical needs and standard care. Filters were changed only upon clotting or termination of renal replacement therapy.

The primary outcome measure for this study was cumulative hours of alive and norepinephrine-free time within the first week after randomization. This was done to compensate for the competing risk of mortality (if a patient died while on norepinephrine, the following days after death contributed zero norepinephrine-free hours to the outcome).

Assuming a standard deviation for the primary outcome equal to 50% of the mean, we estimated that a sample of 36 patients in each group (total 72) would carry an 80% power of detecting a 25% difference in norepinephrine-free time in the first week at an alpha of 0.05. An additional four patients were recruited to account for possible loss to follow-up or protocol violations or technical failures.

A biological outcome study was also conducted as part of this assessment (change in the levels of each of three key cytokines; IL-1, IL-6 and IL-10) and has been previously reported (Atan et al., 2016).

Due to the potential loss of albumin with HCO hemofiltration, additional outcomes studied included the percentage change in serum albumin levels and the total amount of albumin in grams administered intravenously for each patient over the first seven days. If fresh frozen plasma was administered, its albumin concentration was estimated as 3.3g per liter (Ewalenko, Deloof, & Peeters, 1986).

We also measured filter life, maximum rate of vasopressor infusion per day in micrograms/min and duration of hemofiltration. For maximum rate of vasopressor analysis, the highest rate recorded on the last day alive was carried forward to Day 7. Three blinded interim analyses were conducted after 20, 40 and 50 patients respectively with the aim of stopping the trial if either group had a mortality rate of more than 60%.

Statistical analysis

Analysis was performed according to a modified intention to treat principle which required both randomization and treatment initiation. Non-normal data were log-transformed to enable the use of parametric statistics and non-parametric tests were applied if the data remained non-parametric. Baseline characteristics were compared using the Mann Whitney U test for continuous data or Fisher's exact test for categorical data. Norepinephrine free time in the first week, intravenous albumin administration and filter life comparisons were performed using the Mann-Whitney U test. Changes in median serum albumin levels over time and maximum rate of vasopressor infusion per day comparisons were analyzed using repeated measures analysis of variance (RMANOVA). Time to cessation of norepinephrine and time to cessation of hemofiltration were analyzed using the log-rank test. As a degree of imbalance in baseline characteristics is common in pilot studies such as this, post hoc outcome adjustments using logistic regression analysis were performed and included key baseline variables (APACHE 3) and those variables with greatest baseline imbalance.

Results

We randomized 76 patients with 38 patients assigned to each group. Two patients were subsequently excluded; one patient had received prior hemofiltration during the same hospital admission and therefore fulfilled exclusion criteria, and another died shortly after recruitment and randomization but before treatment was commenced, leaving 74 patients for a modified intention to treat analysis.

Baseline features and process of care

The baseline characteristics of the study patients are shown in Table 1. The median norepinephrine rate at commencement in was 13 µg/min (6, 29) for CVVH-HCO and 13 µg/min (5, 23.5) for CVVH-Std (p=0.668). Several variables differed at baseline including oliguria, international normalized ratio (INR) and blood lactate levels. However, APACHE II, APACHE III and SOFA scores were similar between the two groups. A total of 226 filters were used for CVVH-HCO group with a median filter life of 9 (4, 17) hours vs 269 filters for CVVH-Std group with a median filter life of 10 (5.5, 19.8) hours (p= 0.21). No anticoagulation was used for 119 (52.7%) CVVH-HCO filters and 118 (43.9%) CVVH-Std filters, mostly due to contraindications. Other anticoagulation types were unfractionated heparin, regional heparinisation, citrate, and low molecular weight heparin and prostaglandin infusion.

Outcomes

Median cumulative norepinephrine-free time over seven days was 32 hours (0, 110.8) for CVVH-HCO and 56 hours (0, 109.3) for CVVH-Std after randomization (p = 0.520) . Figure 1 shows norepinephrine free time (hours) per group per day for the first seven days. The maximum norepinephrine rates of infusion per day (micrograms per minute) were similar for both groups (Figure 2; p=0.750). Tables showing median values and interquartile ranges for both Figure 1 and Figure 2 are provided in the Appendix (Table 1a and Table 2a). Changes in serum albumin levels within the first seven days were not significantly different between the two groups (p=0.192) (Figure 3). The median dose of intravenous albumin given over the first seven days were 90 (20, 212) grams for CVVH-HCO and 80 (15, 132) grams for CVVH-Std (p=0.252).

There was no difference in time to permanent cessation of norepinephrine in survivors (Figure 4; p=0.358)) and time to permanent cessation of hemofiltration in survivors (Figure 5; p=0.563) within the full 14 days of treatment period. Median time to permanent cessation of norepinephrine in survivors could not be calculated as less than 50% of subjects achieved this event in both groups.

The unadjusted odds ratio for ICU mortality with HCO hemofiltration was 2.17 (95% CI 0.84 to 5.58; p=0.109) for ICU mortality and 2.40 (95% CI 0.94 to 6.15; p= 0.067) for in hospital mortality. The adjusted odds ratio (lactate, INR, serum albumin and APACHE 3)

was 2.13 (95% CI 0.69 to 6.65; p=0.191) for ICU mortality and 2.49 (95% CI 0.81 to 7.66; p=0.112) for in-hospital mortality (Table 2).

Discussion

Key findings

We conducted a pilot, phase II, double blind, randomized, controlled trial of high cut-off (HCO) hemofiltration (CVVH-HCO) compared with standard hemofiltration (CVVH-Std) in critically ill patients with AKI, using the primary outcome of alive norepinephrine-free time (hours) within the first week of treatment. We found no difference in median alive norepinephrine-free time between the two groups. There were also no differences between the two groups in terms of mortality, serum albumin levels, intravenous albumin administration, duration of hemofiltration, duration of norepinephrine infusion and filter life. Finally, the adjusted odds ratio for ICU and in-hospital mortality was not lowered by CVVH-HCO.

Relationship to previous studies

Previous clinical studies involving HCO filters suggested benefits from their use in terms of increased cytokine clearance and attenuation of the inflammatory response (Haase et al., 2007; Kade, Lubas, Rzeszotarska, Korsak, & Niemczyk, 2016; Morgera et al., 2003; Morgera et al., 2004). Morgera et al studied hemodynamic effects of HCO hemofiltration by comparing rates of norepinephrine infusion (Morgera et al., 2006) and found greater reduction in norepinephrine requirements in the HCO group following adjusted analysis. This study, however, was limited to a 48-hour period and was an open-label randomized trial. These investigators reported some changes in two selected cytokines. We previously found higher clearance of IL-6 and IL-8 with CVVH-HCO, but not for other cytokines. There was overall higher cytokine sieving and clearance with CVVH-HCO, but this did not translate into a significantly greater reduction in plasma cytokine levels with CVVH-HCO compared with CVVH-Std (14). No investigators have yet conducted double blind randomized controlled trials of HCO filters in this setting.

Implications of study findings

Our study implies that there is no beneficial effect of CVVH-HCO on vasopressor therapy in critically ill patients with AKI. Moreover, it implies that HCO does not lead to significant differences in serum albumin levels or albumin requirement compared to

standard CVVH. In addition, it implies that there are no advantages in other important outcomes such as the level of vasopressor support, time to cessation of vasopressor therapy, time to cessation of hemofiltration and filter life. Finally, our study implies that there are no mortality advantages with CVVH-HCO. In their aggregate, these findings do not support a role for HCO-hemofiltration in critically ill patients in the presence of AKI.

Strengths and limitations

Our study has several strengths. To our knowledge, it is the first trial of blood purification for the treatment of critically ill patients on vasopressor support and in AKI conducted in a double-blind fashion. This design attenuated the risk of performance and ascertainment bias. The risk of selection bias was further attenuated by concealed allocation. The inclusion and exclusion criteria were reflective of the population of interest for possible larger studies making our findings relevant to similar patients elsewhere and thus providing a degree of external validity. Patients were enrolled within 12 hours of a decision to start hemofiltration, a target that is clinically realistic and feasible and enabled an early effect if one was present. We included patients requiring vasopressor therapy due to various presumed etiologies, which reflects actual clinical practice as the treatment of shock states in critically ill patients remains similar irrespective of etiology, and there may be difficulty in differentiating etiology and mechanisms at the start of the critical illness (Khanna et al., 2017). As such, although our patients had a degree of heterogeneity, this may be an advantage, as our findings have broad clinical relevance to critically ill patients requiring vasopressor therapy.

Our study carries some limitations. Sample size was small, although adequately powered for our primary outcome of interest and the largest sample size so far for the assessment of HCO filters so far. Thus, we cannot definitively comment on any possible mortality effects. However, within the limitations of a phase II study, the signal available is in favour of standard CVVH and does not support any beneficial effect of HCO-therapy on mortality and even suggests a potential for harm. We included a heterogeneous population of patients with vasoplegia and not all patients had septic shock. However, a recent extensive review indicates that the biological high cytokine response to sepsis is likely similar to that associated with non-infective insults because damage associated molecular patterns (DAMPs) trigger essentially identical cellular

responses as pathogen associated molecular patterns (PAMPs) (1). The baseline characteristics were not fully balanced, as is common with pilot studies, and this may have affected the results. Statistical adjustments, however, showed no differences in favour of HCO filtration. We did not use high volume exchanges. However, the recent IVOIRE study (Joannes-Boyau et al., 2013) showed that such increased levels of dose intensity did not affect the outcomes of sepsis-associated vasodilatory shock. Finally, we did not compare the effects of the two interventions on antibiotic levels. However, antibiotics area all relatively small molecules that will move freely across both filter types and would not be affected differently by HCO vs. Std CVVH.

Conclusions

In conclusion, we found that treatment of critically ill patients with severe acute kidney injury receiving vasopressor support with HCO hemofiltration did not result in higher alive norepinephrine-free time at one week compared to patients treated with standard hemofiltration. Other secondary outcomes also showed no beneficial effects of HCO hemofiltration including the findings in our previous publication on the effects of HCO hemofiltration on cytokines level. Within the limitations of a pilot phase II trial, our study does not support further investigation of CVVH-HCO in critically patients in acute kidney injury who are on vasopressor therapy.

Conflict of interest statement

Rinaldo Bellomo has received travel support and consultancy fees from Gambro, BBraun and Baxter Health care.

Figure legends

Figure 1: Norepinephrine-free time (hours) per day per group: Day 1 to Day 7

Figure 2: Median highest norepinephrine infusion rates per day

Figure 3: Median serum albumin (g/L): Day 1 to Day 7

Figure 4: Time to cessation of norepinephrine infusion within the first 7 days

Figure 5: Time to CRRT cessation within 14 days

Table 1: Baseline characteristics

	CVVH-HCO (n=36)	**CVVH-Std (n=38)**	**p value**
Age in years	65.4 (52.4, 74.2)	70.4 (62.1,77.2)	0.084
Male sex, n (%)	17 (47.2)	26 (68.4)	0.065
Weight in kg	78 (67.8, 85.8)	80 (70, 86.3)	0.606
Source of admission, n (%)			
Emergency department	11 (30.6)	12 (31.6)	0.925
Operation room	8 (22.2)	6 (15.8)	
Ward	13 (36.1)	15 (39.5)	
Other hospitals	4 (11.1)	5 (13.2)	
APACHE 2	25 (18.3, 29)	23.5 (21, 28.5)	0.940
APACHE 3	88.5 (69, 116.8)	86 (70, 106.5)	0.758
SOFA Cardiovascular	4 (4, 4)	4 (4, 4)	0.789
SOFA Respiratory	2 (2, 3)	2.5 (2, 3)	0.577
SOFA Kidney	3 (2, 4)	3 (2, 4)	0.084
SOFA Coagulation	0 (0, 2)	0 (0, 2)	0.782
SOFA Liver	2 (1, 3)	1 (0, 2)	0.055
Baseline creatinine in micromoles/L	87.5 (70.3, 104.8)	91 (70.5, 113)	0.411
MAP at enrolment in mmHg	70 (70, 78.8)	75 (70, 80)	0.934
RIFLE score at commencement, n (%)			
R	4 (11.1)	1 (2.6)	0.378
I	8 (22.2)	7 (18.4)	
F	24 (66.7)	29 (76.3)	
Did not fulfil criteria	0	1 (2.6)	
Inclusion criteria met at randomisation, n (%):			
Oliguria (less than 100mls urine output over six hours)	28 (77.8)	21 (55.3)	0.025
Hyperkalemia > 6.5 mmol/L	2 (5.6)	4 (10.5)	0.675
pH < 7.2	11 (30.6)	14 (36.8)	0.568
Urea > 25 mmol/L	11 (30.6)	16 (42.1)	0.302

Creatinine > 300 micromoles/L	13 (36.1)	13 (34.2)	0.864
Oedema (clinically significant oedema)	7 (19.4)	9 (23.7)	0.662
Reason for commencing vasopressor infusion, n (%)			
Septic shock	20 (55.6)	21 (55.3)	1.0
Cardiogenic shock	9 (25)	10 (26.3)	
Hypovolemic shock	1 (2.8)	1 (2.6)	
Others	6 (16.7)	6 (15.8)	
INR	1.6 (1.4, 2.2)	1.3 (1.2, 1.9)	0.006
APTT in secs	36.5 (29, 47)	34 (27, 44.5)	0.389
WCC x 10^9/L	17.2 (12.4, 22.7)	13.1 (7.8, 21.4)	0.161
Platelet count x 10^9/L	153 (66.3, 229.8)	161.5 (94.3, 317.3)	0.131
Serum urea in mmol/L	16.4 (10.6, 25.9)	23.1 (10.2, 32.4)	0.220
Serum creatinine in micromoles/L	252 (184.8, 368.8)	257.5 (200.5, 391)	0.795
Serum albumin in g/L	30 (24, 34.8)	26 (22, 30.5)	0.092
Blood lactate in mmol/L	4 (2.4, 6.2)	2.1 (1.3, 4)	0.002
Blood pH	7.34 (7.24, 7.41)	7.33 (7.25, 7.42)	0.934
Blood bicarbonate levels in mmol/L	20.4 (15.9, 24)	21.5 (16.1, 27.5)	0.323
Base excess in mmol/L	-4.25 (-10.98, 0)	-4 (-9.85, 0.75)	0.608
Norepinephrine rate at commencement in micrograms/min	13 (6, 29)	13 (5, 23.5)	0.668
Mechanical ventilation, n (%)	26 (72.2)	27 (71.1)	0.911

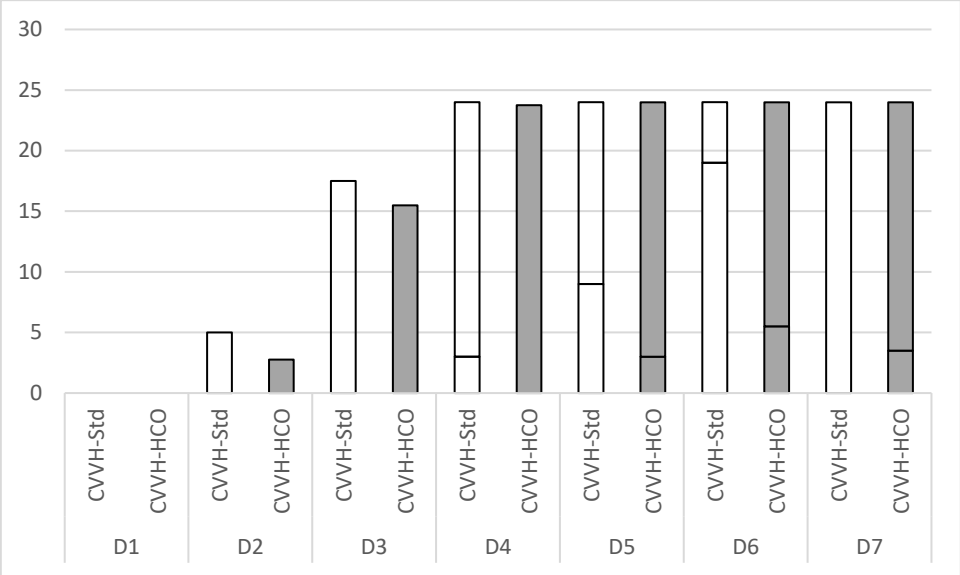
CVVH-HCO (high cut-off group); CVVH-Std (control/standard group)
Values = median (Q1, Q3); except when indicated as n (%)

Table 2: ICU and hospital mortality in the study groups

	CVVH-HCO n (%)	CVVH-Std n (%)	Unadjusted OR (95% CI)	Adjusted OR# (95% CI)
ICU mortality	18 (50)	12 (31.6)	2.17 (0.84 to 5.58) p = 0.109	2.13 (0.69 to 6.65) p = 0.191
Hospital mortality	20 (55.6)	13 (34.2)	2.40 (0.94 to 6.15) p = 0.067	2.49 (0.81 to 7.66) p = 0.112

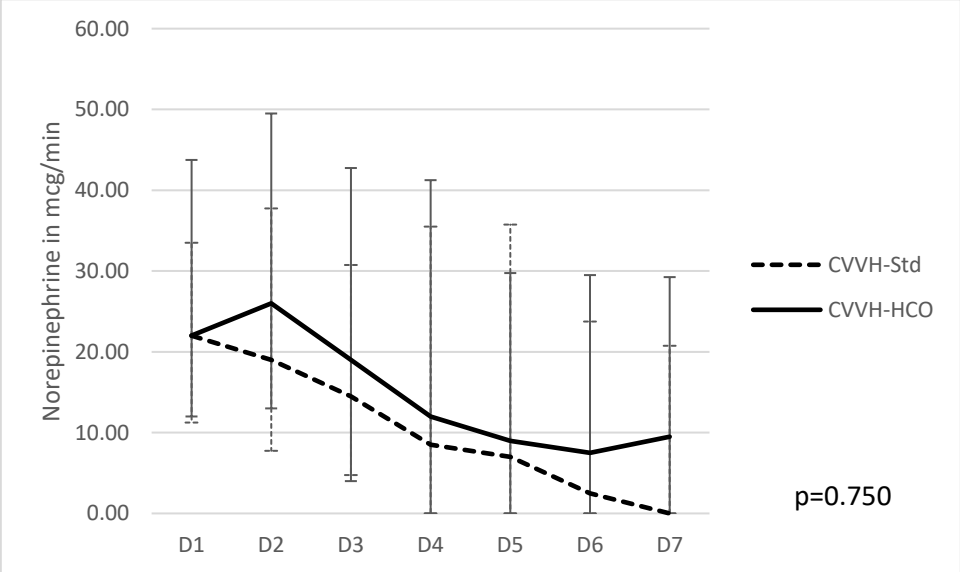
Adjusted to INR, Alb, Lactate and APACHE 3

Figure 1: Norepinephrine free time (hours) per day per group: Day 1 to Day 7



Values are median (middle line), Q1 (lower margin) and Q3 (upper margin)

Figure 2: Median highest norepinephrine infusion rates per day

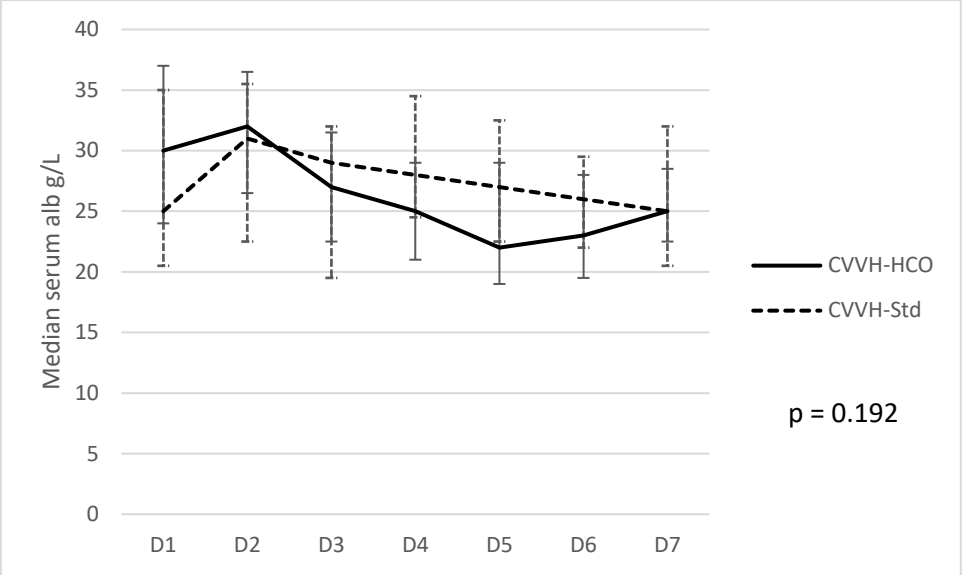


CVVH-HCO: high cut-off group; CVVH-Std: control/standard group

Error bars indicate interquartile ranges

n = 36 (CVVH-HCO); 38 (CVVH-Std)

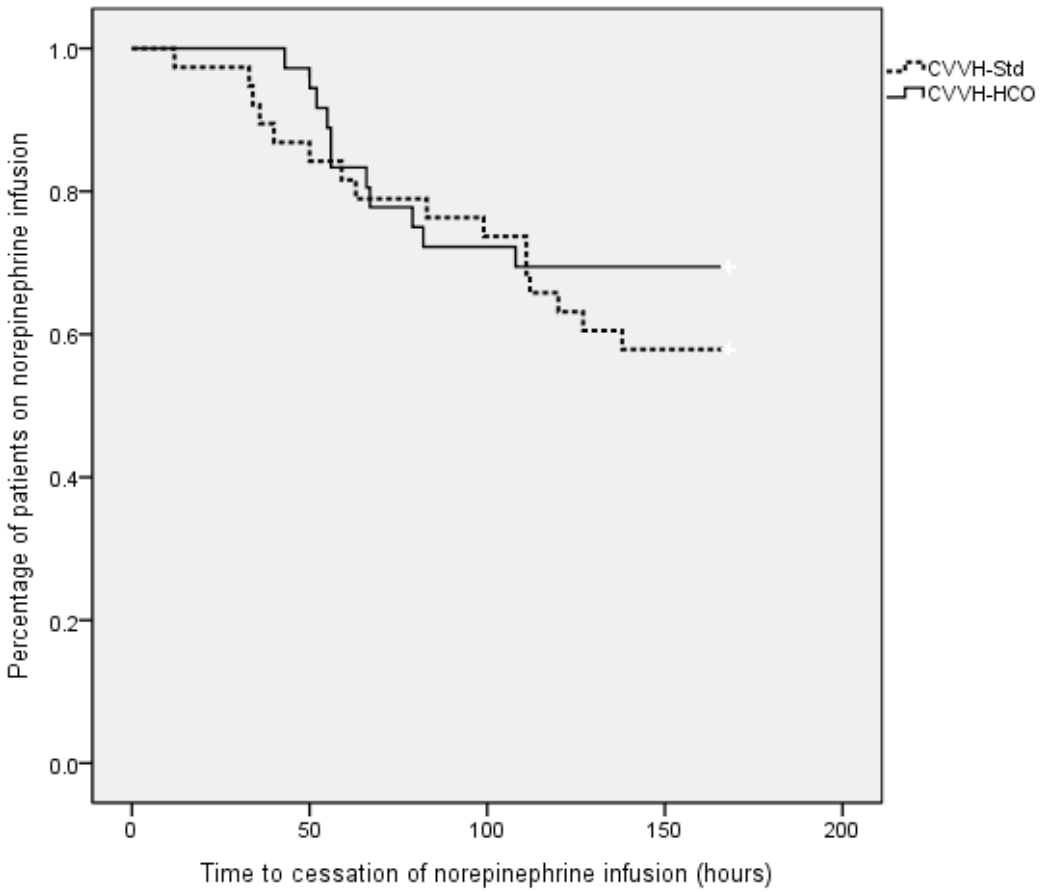
Figure 3: Median serum albumin (g/L): Day 1 to Day 7



CVVH-HCO: high cut-off group; CVVH-Std: control/standard group

Error bars indicate interquartile ranges

Figure 4: Time to cessation of norepinephrine infusion within 7 days



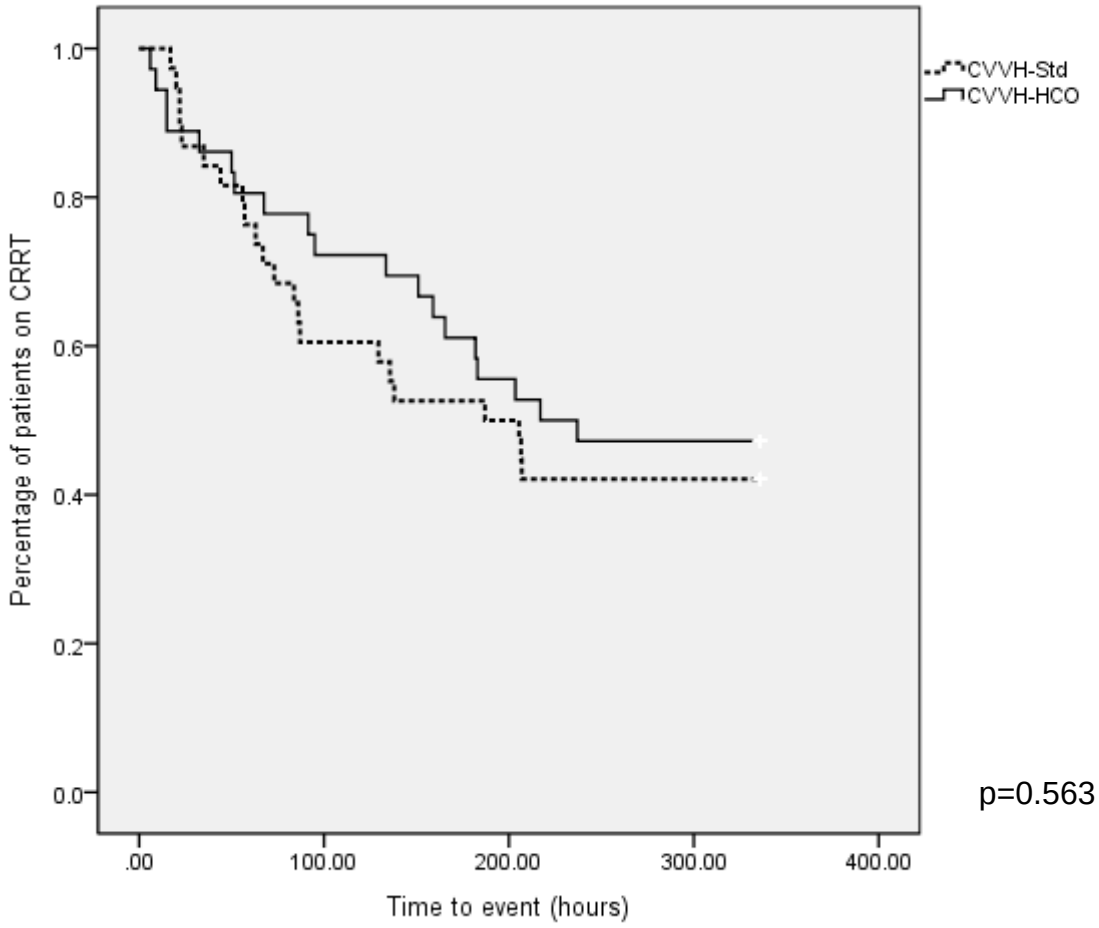
p=0.358

The median time cannot be computed as the curve did not drop to 0.5 and below.

CVVH-HCO: high cut-off group; CVVH-Std: control/standard group

Event = permanent cessation of norepinephrine infusion in survivors

Figure 5: Time to CRRT cessation within 14 days



p=0.563

CVVH-HCO: high cut-off group; CVVH-Std: control/standard group

Event = permanent cessation of CRRT in survivors

Appendix

Table 1a: Norepinephrine free time (hours) per day for the first seven days.

	CVVH-Std	CVVH-HCO
D1	0 (0, 0)	0 (0, 0)
D2	0 (0, 5)	0 (0, 2.75)
D3	0 (0, 17.5)	0 (0, 15.5)
D4	3 (0, 24)	0 (0, 23.75)
D5	9 (0, 24)	3 (0, 24)
D6	19 (0, 24)	5.5 (0, 24)
D7	24 (0, 24)	3.5 (0, 24)

Values are medians (Q1, Q3)

Table 2a: Highest norepinephrine rates of infusion (micrograms per minute) per day for the first seven days.

	CVVH-Std	CVVH-HCO
D1	22 (11.25, 33.5)	22 (12, 43.75)
D2	19 (7.75, 37.75)	26 (13, 49.5)
D3	14.5 (4.75, 30.75)	19 (4, 42.75)
D4	8.5 (0, 35.5)	12 (0, 41.25)
D5	7 (0, 35.75)	9 (0, 29.75)
D6	2.5 (0, 23.75)	7.5 (0, 29.5)
D7	0 (0, 20.75)	9.5 (0, 29.25)

Values are medians (Q1, Q3)

References

1. Hotchkiss RS, Moldawer LL, Opal SM, Reinhart K, Turnbull IR, Vincent JL. Sepsis and septic shock. Nature reviews Disease primers. 2016;2:16045.

2. Uchino S, Kellum JA, Bellomo R, Doig GS, Morimatsu H, Morgera S, et al. Acute renal failure in critically ill patients: a multinational, multicenter study. Jama. 2005;294(7):813-8.

3. Bosmann M, Ward PA. The inflammatory response in sepsis. Trends in immunology. 2013;34(3):129-36.

4. Schulte W, Bernhagen J, Bucala R. Cytokines in sepsis: potent immunoregulators and potential therapeutic targets--an updated view. Mediators of inflammation. 2013;2013:165974.

5. Morgera S, Haase M, Kuss T, Vargas-Hein O, Zuckermann-Becker H, Melzer C, et al. Pilot study on the effects of high cutoff hemofiltration on the need for norepinephrine in septic patients with acute renal failure. Critical care medicine. 2006;34(8):2099-104.

6. Dinarello CA. Anti-cytokine therapies in response to systemic infection. The journal of investigative dermatology Symposium proceedings. 2001;6(3):244-50.

7. Schefold JC, Hasper D, Jorres A. Organ crosstalk in critically ill patients: hemofiltration and immunomodulation in sepsis. Blood purification. 2009;28(2):116-23.

8. Ronco C, Tetta C, Mariano F, Wratten ML, Bonello M, Bordoni V, et al. Interpreting the mechanisms of continuous renal replacement therapy in sepsis: the peak concentration hypothesis. Artificial organs. 2003;27(9):792-801.

9. Atan R, Crosbie D, Bellomo R. Techniques of extracorporeal cytokine removal: a systematic review of the literature on animal experimental studies. The International journal of artificial organs. 2013;36(3):149-58.

10. Atan R, Crosbie DC, Bellomo R. Techniques of extracorporeal cytokine removal: a systematic review of human studies. Renal failure. 2013;35(8):1061-70.

11. Boschetti-de-Fierro A, Voigt M, Storr M, Krause B. Extended characterization of a new class of membranes for blood purification: the high cut-off membranes. The International journal of artificial organs. 2013;36(7):455-63.

12. Morgera S, Rocktaschel J, Haase M, Lehmann C, von Heymann C, Ziemer S, et al. Intermittent high permeability hemofiltration in septic patients with acute renal failure. Intensive care medicine. 2003;29(11):1989-95.

13. Morgera S, Slowinski T, Melzer C, Sobottke V, Vargas-Hein O, Volk T, et al. Renal replacement therapy with high-cutoff hemofilters: Impact of convection and diffusion on cytokine clearances and protein status. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2004;43(3):444-53.

14. Atan R, Peck L, Visvanathan K, Skinner N, Eastwood G, Bellomo R, et al. High cut-off hemofiltration versus standard hemofiltration: effect on plasma cytokines. The International journal of artificial organs. 2016;39(9):479-86.

15. Ewalenko P, Deloof T, Peeters J. Composition of fresh frozen plasma. Critical care medicine. 1986;14(2):145-6.

16. Haase M, Bellomo R, Baldwin I, Haase-Fielitz A, Fealy N, Davenport P, et al. Hemodialysis membrane with a high-molecular-weight cutoff and cytokine levels in sepsis complicated by acute renal failure: a phase 1 randomized trial. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2007;50(2):296-304.

17. Kade G, Lubas A, Rzeszotarska A, Korsak J, Niemczyk S. Effectiveness of High Cut-Off Hemofilters in the Removal of Selected Cytokines in Patients During Septic Shock Accompanied by Acute Kidney Injury-Preliminary Study. Medical science monitor : international medical journal of experimental and clinical research. 2016;22:4338-44.

18. Khanna A, English SW, Wang XS, Ham K, Tumlin J, Szerlip H, et al. Angiotensin II for the Treatment of Vasodilatory Shock. The New England journal of medicine. 2017.

19. Joannes-Boyau O, Honore PM, Perez P, Bagshaw SM, Grand H, Canivet JL, et al. High-volume versus standard-volume hemofiltration for septic shock patients with acute kidney injury (IVOIRE study): a multicentre randomized controlled trial. Intensive care medicine. 2013;39(9):1535-46.

2.3 Published abstract

Preliminary results of the study were also presented as a poster during the 11th World Federation of Societies of Intensive and Critical Care Medicine Congress, in Durban, South Africa, between 28th Aug to 1st Sept 2013. This presentation resulted in the following abstract.

tracheal extubation. According to the findings of the current study, since nebulized budesonide has no systemic complications of IV corticosteroid, it can be used as the first choice in reducing the complications attributed to extubation.
Conclusion: No significant difference was found between the two groups. Considering the very low systemic absorption of nebulized budesonide, however, we recommend it for prevention of post-extubation complications instead of IV dexamethasone.

http://dx.doi.org/10.1016/j.jcrc.2013.09.026

Randomized controlled study of high cut-off point hemofiltration vs. standard hemofiltration in acute renal failure

Rafidah Atan[a], *John Prowle*[b], *Leah Peck*[b], *Glenn Eastwood*[b], *Rinaldo Bellomo*[b]
[a]*Monash University Sunway Campus, Johor Bahru, Malaysia*
[b]*Austin Hospital, Melbourne, Australia*

Introduction: Cytokines participate in the pathogenesis of shock states. High cut-off (HCO) hemofilters with larger pores allow cytokine removal, potentially modifying outcomes. Albumin, however, may be lost in the filtrate, and a fall in plasma albumin is a possible side effect.
Objective: To compare the effect of HCO versus standard hemofilters (HF) on vasopressor requirement and serum albumin levels.

Methods: We conducted a randomized, double-blind, controlled study and recruited 76 adults with acute kidney injury and hemodynamic instability requiring vasopressor support to receive 25 mL/kg per hour hemofiltration with either HCO or standard filters, for a maximum of 14 days. Albumin levels were monitored daily. The primary outcome measure was noradrenaline free time in the first week. Median values of continuous variables were compared with the Mann-Whitney *U* test and categorical variables with the Fisher exact or c2 tests, as appropriate [1].
Results: Median albumin levels during days of treatment was 24.5 g/L (HCO) versus 26 g/L (standard group) ($P = .41$). The median number of noradrenaline-free hours in the first week was 337 hours in HCO versus 328 hours in the standard group ($P = .71$). Hospital mortality rates were 55.3% in the high cut-off group vs. 34.2% in controls ($P = .07$).
Conclusion: There were no statistically significant differences in albumin levels or noradrenaline-free time in the first week or in hospital mortality. No benefits of HCO could be identified.

Reference

[1] Haase M, Bellomo R, Morgera S, Baldwin I, Boyce N. High cut-off point membranes in septic acute renal failure: a systematic review. Int J Artif Organs 2007 Dec;30(12):1031–41.

http://dx.doi.org/10.1016/j.jcrc.2013.09.027

The results presented appear to differ from the main publication as the calculation presented in the abstract were cumulative norepinephrine-free hours per group per day. In the full paper, calculations were based on number of norepinephrine-free hours per patient over the first seven days. Despite the two different approaches to analysis, the overall findings remain the same.

2.4. Summary

The findings of our main study for this thesis could not find any physiological advantage of HCO hemofiltration compared with standard hemofiltration. This coupled with higher cost of filters and the potential loss of albumin pushes us to the conclusion that HCO hemofiltration is not superior to standard hemofiltration in critically ill patients on vasopressors and in AKI. Further studies should look at other potential adjuncts to treatment.

Our analysis of the physiological effects of the HCO filter was rather thorough. Apart from vasopressor free-time, we also looked at other outcomes that may act as an advantage of this newer intervention. These included effects on the rate of norepinephrine infusion per day, filter life, ICU mortality and in-hospital mortality. We also compared time to permanent cessation of vasopressor therapy and time to permanent cessation of hemofiltration in survivors over an extended period of treatment time i.e. 14 days. Although the study was not powered for the purpose of these additional outcomes, we could not find any signal to indicate physiological superiority of HCO hemofiltration over standard hemofiltration. In fact for many of these outcomes there may be a signal towards harm.

As this was a phase II equivalent study, we also embarked on the concurrent study of possible mechanisms, should we have observed an advantageous effect. As such, the main secondary outcome was a study on the effects of HCO on cytokine clearance and plasma cytokine levels. In subsequent papers, we present our findings on the effects of the intervention on apoptosis indices, nucleosomes and toll-like receptor expression.

Chapter 3

The biological impact of high cut-off hemofiltration

3.1. Introduction

The study of any EBP technique is not complete without an assessment of its biological impact on important mediators. Studies on EBP techniques should report both its effects on reduction of target molecules, as well as effects of the therapy on cellular immunity. Our study, which is a phase II equivalent analysis of HCO hemofiltration therefore also seeks to obtain data on plasma cytokine levels, apoptosis indices, nucleosome levels and toll-like receptor (TLR) expression as a measure of biological impacts of the intervention.

The number of patients involved in these sub-studies is small. Due to processing requirements of the blood samples, we could only include patients that were admitted during office hours. Many of the patients who contributed data also did not survive through all three timelines as the patients recruited were severely ill and had a predicted baseline mortality of around 60%. Despite these challenges faced during the conduct of the study, we analysed the data obtained to observe any trends of the responses studied.

Our first publication in this series studied the effects of HCO hemofiltration versus standard hemofiltration on plasma levels of key cytokines.

There is however a caveat in linking physiological effects to cytokine sieving and cytokine plasma levels. It comes in the fact that we're measuring effects of the intervention on cytokines in the plasma, when the most important effect may be what occurs at the tissue level. Furthermore, the effects of the intervention on clinical outcomes may be influence by mechanisms other than cytokine removal. Despite this, it makes great sense to measure the effects of HCO hemofiltration on plasma cytokines, as this was the mechanism underlying the proposed benefit of this intervention and substantiated by our previous systematic reviews.

We therefore present our findings on the effects of HCO hemofiltration on cytokine clearance and plasma cytokine levels in the next publication; while appreciating at the same time that regardless of the effects of HCO hemofiltration on plasma cytokines and its removal, the most important effects remain clinical outcome measures.

Atan R, Peck L, Visvanathan K, Skinner N, Eastwood G, Bellomo R, Storr M, Goehl H. High cut-off hemofiltration versus standard hemofiltration: effect on plasma cytokines. Int J Artif Organs. 2016 Nov 11;39(9):479-486.

3.2 Publication: Effects on plasma cytokines

IJAO
ISSN 0391-3988

Int J Artif Organs 2016; 39(9): 479-486
DOI: 10.5301/ijao.5000527

ORIGINAL RESEARCH ARTICLE

High cut-off hemofiltration versus standard hemofiltration: effect on plasma cytokines

Rafidah Atan[1], Leah Peck[2], Kumar Visvanathan[3], Narelle Skinner[3], Glenn Eastwood[2], Rinaldo Bellomo[2,4], Markus Storr[5], Hermann Goehl[5]

[1] Clinical School Johor Bahru, Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, Bandar Sunway - Malaysia
[2] Department of Intensive Care, Austin Hospital, Heidelberg, Victoria - Australia
[3] St. Vincent's Hospital, University of Melbourne, Fitzroy, Melbourne - Australia
[4] School of Medicine, University of Melbourne, Parkville, Victoria - Australia
[5] Gambro Dialysatoren GmbH, Research & Development, Hechingen - Germany

ABSTRACT

Purpose: To study the effects of continuous veno-venous hemofiltration (CVVH) with high cut-off filters (CVVH-HCO) on plasma cytokine levels, sieving coefficient and clearance compared to CVVH using standard filters (CVVH-Std) in a nested cohort within a double-blind randomized controlled trial in severe acute kidney injury (AKI) patients.

Methods: We measured plasma and post-filter levels of IL-6, TNF-alpha, IL-8, IL-1 beta, RANTES, IL-10, IFN-gamma and IFN-alpha in both study groups. We also measured cytokine levels in the ultrafiltrate and calculated sieving coefficients and clearances.

Results: By 72 hours of treatment, IL-6 had decreased during both treatments (p = 0.009 and 0.005 respectively). In contrast, IL-10 had decreased with CVVH-Std (p = 0.03) but not CVVH-HCO (p = 0.135). None of the other cytokines showed changes over time. There were also no significant between group differences in plasma levels for each cytokine over the 72-hour treatment period. For all cytokines combined, however, the median sieving coefficient was higher for CVVH-HCO (0.31 vs. 0.16; p = 0.042) as was the mass removal rate by ultrafiltration (p = 0.027). While overall combined cytokine levels had fallen to 62.2% of baseline at 72 hours for CVVH-HCO (p<0.0001) and to 75.9% of baseline with CVVH-Std (p = 0.008) there were no between group differences.

Conclusions: CVVH-HCO achieved greater combined sieving coefficient and mass removal rate by ultrafiltration for a group of key cytokines than CVVH-Std. However, this effect did not differentially lower their plasma level over the first 72 hours. Our study does not support the use of CVVH-HCO to lower cytokines in critically ill patients with AKI.

Keywords: Acute kidney injury, Apoptosis, Hemofiltration, High cut-off filters, Sepsis

Introduction

Cytokines are polypeptides that play an important role during sepsis and the systemic inflammatory response syndrome (SIRS) (1). Their production is induced by noxious stimuli from both infective and noninfective etiology (2) and is necessary to combat infection and contain tissue injury (3). During such responses, numerous cytokines are produced with beneficial as well as adverse effects (3, 4). Some cytokines play specific roles, but most exhibit a degree of redundancy and have overlapping functions with other cytokines(4).

In critically ill patients with multiorgan dysfunction syndrome (MODS), overproduction of cytokines, or a hypercytokinemic state, is believed to be a type of maladaptive response to infection or injury, which contributes to adverse outcomes in critically ill patients (5). Accordingly, in the past 2 decades, there has been great interest in attenuating this response by reducing hypercytokinemia either through specific antagonists or via nonspecific removal (6, 7).

Specific antagonist antibodies towards cytokines have not been effective in sepsis or multiorgan failure (8, 9). However, nonspecific removal of cytokines may return the balance of factors toward homeostasis, so that the beneficial effects of cytokines can be achieved without the adverse impact of hypercytokinemia (10, 11). In this regard, a particular hemofiltration technique using high cut-off (HCO) filters (12) appears to

Accepted: October 8, 2016
Published online: November 10, 2016

Corresponding author:
Prof. Rinaldo Bellomo
Department of Intensive Care
Austin Hospital
[redacted]

achieve superior cytokine removal when compared to hemofiltration using conventional high-flux membranes in ex vivo, animal, and some human experiments (13-15). HCO hemofiltration also has added advantages compared to more complex blood purification techniques in terms of wide availability, simplicity and potential cost-effectiveness and established use in sepsis and MODS-associated acute kidney injury (16).

Accordingly, we conducted an exploratory, biological, nested, cohort study within a larger double-blind, randomized, controlled trial, involving acute kidney injury (AKI) in patients with MODS. We aimed to compare the effects of hemofiltration using high cut-off (HCO) versus standard hemofilters on the blood levels, sieving coefficient and clearances of several cytokines of different molecular weight.

Methods

We performed an exploratory investigation in a nested cohort of patients within a larger double-blind, randomized, parallel-group, controlled trial (ClinicalTrials.gov/ NCT00912184) comparing continuous veno-venous hemofiltration with high cut-off filters (CVVH-HCO) to continuous veno-venous hemofiltration with standard high-flux membranes (CVVH-Std).

The study was approved by the Austin Hospital Human Research Ethics Committee (H2008/03400). Written informed consent was obtained from patients or the person responsible.

All study patients had acute kidney injury (AKI) with underlying shock requiring vasopressor infusion and were recruited within the first 12 hours of commencement of hemofiltration. Patients were randomized to either CVVH-HCO using polyethersulfone filters with a nominal cut-off point of 100 kDa (P2SH filters, 1.12 m^2; Gambro) or to CVVH-Std using custom-manufactured, control polyethersulfone filters with a nominal cutoff point of 30kDa (10, 11). The study filters were identical in appearance and surface area.

The settings for CVVH were a blood flow (Qb) of 250 mL/min, an ultrafiltration (UF) dose of 25 mL/kg/h with pre-dilution and the exclusive use of bicarbonate buffered replacement fluids. We excluded patients who were on maintenance dialysis and those who had received hemofiltration during the same hospital admission. The filters were only changed when clotting occurred. The mode of anticoagulation was left to the discretion of the treating physicians.

Due to rapid processing requirements for some of the samples, we could only sample bloods during daytime. A large number of recruited patients were admitted after hours and samples were obtained only from 26 patients, with only 14 patients providing complete prefilter and prereplacement fluid blood sampled at baseline (T0), at 24 hours after initiation of CVVH (T24) and between 72 to 96 hours after initiation of CVVH (T72). In addition, blood was also sampled from the post filter port at T24 at the same time as the arterial blood and ultrafiltrate collection.

Blood samples were immediately centrifuged at 3,500 rpm for 5 minutes and the resulting plasma was stored at -70°C degrees until the time of measurement. As the study was double-blind in nature, at the time of sampling we were unable to identify whether the samples were from the intervention or the control group.

Cytokine analysis

Plasma was thawed and centrifuged at 1000 xg for 10 minutes prior to detection of cytokines using multiparameter Luminex Bead Assays (Invitrogen). Luminex Singleplex bead kits were combined to create a multiplex assay for human RANTES (regulated on activation, normal T expressed and secreted protein), interferon-alpha (IFN-alpha), interleukin-10 (IL-10) and interferon-gamma (IFN-gamma) and plasma assayed using the Human Extracellular Protein Buffer Reagent Kit (Thermo-Fisher) according to the manufacturer's specifications. Luminex Ultrasensitive Singleplex bead kits were combined to create a multiplex assay for human tumor necrosis factor alpha (TNF-alpha), interleukin-6 (IL-6), interleukin-8 (IL-8) and interleukin-1 beta (IL-1 beta) and plasma assayed using the Human Ultrasensitive Cytokine Buffer Reagent Kit (Thermo Fisher) according to the manufacturer's specifications. For measurement, a Bio-Plex 200 Suspension Array System (Bio-Rad Laboratories) was used with Bio-Plex Manager Software. Cytokine concentrations were determined from standard curve fitting software using a 5-parameter algorithm.

Statistical analysis

Descriptive statistics were performed. Baseline characteristics were compared using the Mann-Whitney U-test for continuous data and chi-square analysis or Fisher's exact test for categorical data. Data on plasma cytokine at T0, T24 and T72 were normalized according to percentage change at the respective timelines compared to T0 (baseline = 100%). Normalized cytokine levels were log-transformed and analyzed using repeated measures analysis of variance (RMANOVA). Within group changes in cytokine levels over time were analyzed using nonparametric ANOVA (Friedman's test). The sieving coefficient (SC) was calculated at T24 as ultrafiltrate concentration over arterial concentration. Clearance (CL) in mL/min was calculated as the product of SC and ultrafiltration. Median values of SC and CL were calculated for all cytokines in both groups. Prefilter and postfilter samples were analyzed as percentage change in post-filter plasma levels compared to arterial levels at T24. Percentage changes across the filter between the 2 groups were compared using MannWhitney U-test. Statistical significance was defined as a $p<0.05$.

Results

A total of 26 patients were sampled at time 0 but a complete set of samples were only obtained from 15 patients, who survived to all 3 timelines; 1 patient fulfilled an exclusion criterion and was subsequently excluded, leaving 6 in the CVVH-HCO group and 8 in the CVVH-Std group. Their baseline characteristics are shown in Table I. Many patients did not receive anticoagulation due to contraindications. When coagulation was used, the main mode was low-dose, systemic heparin. None of the patients received anticoagulation in the form of citrate, which may affect the porosity of the filter.

Cytokine levels were severely elevated at baseline for most patients, except for IL-1 beta, IFN-gamma and IFN-alpha, which showed generally low levels in all patients. Plasma levels for

TABLE I - Baseline characteristics and outcome of study patients

	HCO (n = 6)	Sth HF (n = 8)	p value
Age (y)	57.8 (46.4, 64.3)	66.8 (63.6, 76.4)	0.228
Sex (M/F)	5/1	5/3	0.580
Weight (kg)	85.5 (72.5, 94.8)	80.1 (77.5, 91.3)	0.755
APACHE 2	19.5 (12, 27)	23 (17.3, 23.3)	0.755
APACHE 3	63.5 (54.3, 87)	77.5 (65.3, 95)	0.573
SOFA cardiovascular	4 (4, 4)	4 (3.75, 4)	0.491
SOFA respiration	3 (2.25, 3)	3 (2, 3.25)	0.852
SOFA renal	2.5 (2, 3)	2.5 (2, 4)	1.000
SOFA coagulation	0.5 (0, 2.5)	0 (0, 0.25)	0.345
SOFA liver	2.5 (1.25, 3)	1 (0, 1)	0.073
Baseline creatinine within one year of date of admission (µmol/L)	86.5 (55.8, 106)	80 (69.8, 96.8)	0.662
RIFLE score at commencement of CRRT (R/I/F)	0/2/4	1/2/5	0.656
Shock etiology (Sepsis/Cardiogenic/Other)	3/2/1	3/3/2	0.881
Baseline mean arterial pressure at enrolment (mmHg)	70 (66.3, 70)	75 (70, 80)	0.142
Ventilated at enrolment (Y/N)	5/1	6/2	1.0
Serum urea at enrolment (mmol/L)	13.8 (10.9, 18.3)	20 (9.7, 26)	0.852
Serum creatinine at enrolment (µmol/L)	247.5 (220.5, 266.3)	200 (171.5, 255.5)	0.414
Blood lactate at enrolment (mmol/L)	2.7 (2.4, 4.9)	1.5 (1.1, 1.7)	0.001
Blood pH at enrolment	7.37 (7.31, 7.39)	7.38 (7.32, 7.42)	0.491
Serum albumin at enrolment (g/dL)	34 (33.3, 37.8)	24.5 (20.3, 27.3)	0.008
Noradrenaline infusion at enrollment (mcg/min)	17.5 (13.3, 23.3)	6.5 (3, 11.5)	0.108
ICU mortality %	33.3%	37.5%	1.0
Hospital mortality %	50%	37.5%	1.0
*IL-6 (pg/mL)	192.63	491.85	1.0
*TNF-alpha (pg/mL)	16.15	24.83	0.085
*IL-8 (pg/mL)	417.99	251.48	0.609
*IL-1 beta (pg/mL)	0.97	0.88	0.727
*RANTES (pg/mL)	696.42	1339.97	0.018
*IL-10 (pg/mL)	60.87	49.23	0.851
*IFN-gamma (pg/mL)	1.12	1.12	0.687
*IFN-alpha (pg/mL)	0	0	0.767

Data displayed are medians (interquartile range) for patients involved in mixed anova analysis.
* For cytokine data, all patients were included; CVVH-HCO (n = 11), CVVH-Std (n = 14).
* Typical range from literature for baseline cytokine levels in multiorgan failure/severe sepsis (in pg/ml).
IL-6 = 656 to 2375 (24,25); TNF-alpha = 16 to 33 (24, 25); IL-8 = 261 to 1439 (24, 26); IL-1 beta = 3.8 to 66.02 (24, 26); IL-10 = 227 to 638 (25,26); IFN-gamma = 225 to 275 pg/ml (27).

IFN-gamma and IFN-alpha were negligible or undetected in most patients and were not analyzed further (Tab. I).

Sieving coefficients (SC) and clearances are presented in Table II. CVVH-HCO achieved high SC for IL6 (SC 1.20) and IL8 (SC 0.68); with a CL of 44.28 mL/min and 26.38 mL/min, respectively. CVVH-HCO achieved only low SC for other cytokines. SC values and clearances were generally low for all cytokines with CVVH-Std. For the combined SC and CL of all cytokines, we found significant differences between CVVH-HCO and CVVH-Std both SC ($p = 0.042$), but not for CL ($p = 0.06$) (Tab. II) and a significant overall decrease in plasma levels at T24 and T72 for both CVVH-HCO ($p<0.0001$) and CVVH-Std ($p = 0.008$) (Tab. III). Across filters, levels decreased nonsignificantly only for IL-1 beta and IL-10 (Tab. IV).

TABLE II - Sieving coefficient (SC) and filtrate clearances (CL) for all cytokines

Cytokine (approximate reported MW)	Median SC (IQR)	Median CL mL/min (IQR)
IL-6 (22 to 28kDa)	HCO = 1.20 (0.63,1.41) Std = 0.27 (0.09, 1.18) p = 0.142	HCO = 35.97 (21.89, 41.33) Std = 8.97 (2.70, 39.30) P = 0.210
TNF-alpha (monomeric 17.5 kDa; trimeric 52 kDa)	HCO = 0.28 (0.06, 0.39) Std = 0.30 (0.12, 0.55) p = 0.681	HCO = 8.41 (2.14, 11.45) Std = 7.97 (4.15, 21.17) p = 0.758
IL-8 (8 kDa)	HCO = 0.68 (0.51, 1.18) Std = 0.45 (0.38, 0.79) p = 0.299	HCO = 26.04 (14.70, 39.07) Std = 15.00 (12.11, 25.12) p = 0.351
IL-1 beta (17.5 kDa)	HCO = 0.04 (0.03, 0.89) Std = 0.18 (0.16, 0.21) p = 1.0	HCO = 1.31 (0.89, 29.98) Std = 6.52 (5.18, 7.22) p = 1.0
RANTES (8 kDa)	HCO = 0.015 (0.004, 0.05) Std = 0 (0, 0.01) p = 0.114	HCO = 0.58 (0.13, 1.45) Std = 0 (0, 0.44) p = 0.114
IL-10 (17 to 21 kDa)	HCO = 0.31 (0.11, 0.32) Std = 0 (0) p = 0.114	HCO = 9.66 (3.42, 11.91) Std = 0 (0) p = 0.114
All cytokines combined (CVVH-HCO n = 42; CVVH-Std n = 54)	HCO = 0.31 (0.04, 0.68) Std = 0.16 (0.001, 0.45) p = 0.042	HCO = 10.68 (1.08, 26.04) Std = 5.40 (0.02, 15) p = 0.055

IFN-gamma = 20 to 25 kDa; IFN-alpha = 20 to 22 kDa.
For individual cytokines, number of observations: CVVH-HCO n = 7; CVVH-Std n = 9.
For all cytokines combined, number of observations: CVVH-HCO = 42, CVVH-Std = 54. P values obtained through Mann-Whitney *U*-test.

TABLE III - Plasma levels at T24 and T72 for all cytokines

Cytokine	Group	T24 (IQR) (% compared to T0)	T72 (IQR) (% compared to T0)	p value
IL-6	CVVH-HCO	86.1 (57.4,107.6)	42.7 (18.1,51.4)	p = 0.009
	CVVH-Std	52.2 (17.2, 103)	50.4 (36.4, 72.8)	p = 0.072
TNF-alpha	CVVH-HCO	116.6 (91.2,145.8)	81.7 (65.2,132.7)	p = 0.311
	CVVH-Std	103.4 (82.5,175.8)	106.5 (84.4,149.4)	p = 0.687
IL-8	CVVH-HCO	60.8 (26.2, 104.8)	52.9 (22.3, 82.7)	p = 0.069
	CVVH-Std	93.4 (54.9, 139.7)	101 (48.7, 131.2)	p = 0.882
IL-1 beta	CVVH-HCO	85.7 (69.5, 129.4)	84.9 (41.5, 139)	p = 0.607
	CVVH-Std	130.6 (65.2, 162.9)	75.9 (49.7, 123.4)	p = 0.607
RANTES	CVVH-HCO	99.1 (67, 112.1)	112.6 (59.f3, 165.1)	p = 1.0
	CVVH-Std	70.4 (54.7, 99.3)	78 (62.2, 319.5)	p = 0.325
IL-10	CVVH-HCO	97.5 (68, 122.5)	51.9 (24, 70.3)	p = 0.135
	CVVH-Std	78.7 (70.5, 137.2)	54.4 (33.1, 89.1)	p = 0.03
All cytokines combined	CVVH-HCO	94.1 (63.1, 131.3)	62.2 (39, 99.2)	p<0.0001
	CVVH-Std	84.1 (58.3, 155.7)	75.9 (41.3, 117.1)	p = 0.008

Values are expressed as percentage compared to baseline values i.e. T0 = 100%.
For individual cytokines, number of observations: CVVH-HCO n = 6; CVVH-Std n = 8.
For all cytokines combined, number of observations: CVVH-HCO = 36, CVVH-Std = 4 8. P values obtained through Friedman test.

TABLE IV - Median % change across the filter for all cytokines*

Cytokine	Median % change across the filter (post – pre)/pre (IQR)	p value (CVVH-HCO vs. CVVH-Sth)
IL6	CVVH-HCO = 29.25 (-6.19, 45.19)	0.181
	CVVH-Std = 0.97 (-10.96, 7.58)	
TNFalpha	CVVH-HCO = 7.02 (-34.43, 67.73)	0.864
	CVVH-Std = 8.99 (-26.81, 45.14)	
IL-8	CVVH-HCO = 19.92 (3.05, 95.16)	0.181
	CVVH-Std = 8.4 (-8.71, 25.72)	
IL-1beta	CVVH-HCO = neg 10.16 (-21.48, 12.98)	0.689
	CVVH-Std = -neg 16.22 (-36.50, 5.91)	
RANTES	CVVH-HCO = 16.71 (-11.49, 159.26)	0.955
	CVVH-Std = 35.58 (-9.83, 59.83)	
IL-10	CVVH-HCO = neg 26.66 (-46.29, -5.32)	0.388
	CVVH-Std = neg 3.56 (-44.37, 14.74)	
All cytokines combined	CVVH-HCO = 2.92 (neg 16.89, 34.19)	0.489
	CVVH-Std = 0.205 (neg 15.8, 24.04)	

*A positive value indicates an increase, a negative value indicates a decrease. For individual cytokines, number of observations: CVVH-HCO n = 6; CVVH-Std n = 9.
For all cytokines combined, number of observations: CVVH-HCO = 36, CVVH-Std = 54. P values obtained through Mann-Whitney *U*-test.

Mass balances were also calculated for all cytokines (Tab. V). We assumed a hematocrit of 25% for all patients. There are significant differences in mass removal rate by ultrafiltration (MUF) for IL-6 and when all cytokines are combined. There are no differences for other measures of mass transfer, namely mass removal by adsorption and total mass transfer. Negative values for mass removal by adsorption may indicate release of cytokines into the circulation or sampling errors.

Changes in normalized plasma levels of various cytokine levels over T0, T24 and T72 are shown in Figures 1, 2 and 3 for IL-6, IL-10 and all cytokines combined and in the supplementary appendix for the other cytokines (see Supplementary Figures A, B, C, D available online as supplementary material at www.artificial-organs.com). There were no statistically significant differences in plasma levels over 3 timelines between the 2 groups for other cytokines analyzed (Tab. III).

Discussion

Key findings

In an exploratory study, we measured the sieving coefficient values, clearances, transfilter changes and plasma levels, of 8 cytokines from patients with AKI and multiorgan dysfunction syndrome (MODS) from a nested cohort within a double-blind, randomized, controlled trial comparing CVVH with high cut-off filters (CVVH-HCO) to CVVH with standard high-flux filters (CVVH-Std). We found that the sieving coefficient values and clearances were high for cytokines like IL-6 and IL-8 using CVVH-HCO but not CVVH-std and that for all cytokine combined, SC and mass removal by ultrafiltration were higher with CVVH-HCO. However, we also found that cytokine levels did not decrease significantly across either type of filters and that there was no difference in the percentage change in combined plasma cytokine levels over time between CVVH-HCO and CVVH-Std.

Relationship to previous studies

Previous systematic reviews found evidence of higher rates of cytokine removal with CVVH-HCO (13-15). However, no double-blind, randomized, controlled trial had previously compared the SC, clearances and plasma levels of multiple cytokine with CVVH-HCO versus CVVH-St over 72 hours of observation. However, Morgera et al had compared HCO hemofiltration with standard hemofiltration in septic patients in AKI over a 48-hour period, in a randomized but unblended study, and found statistically significant higher clearance of IL-6 and IL-1ra by HCO hemofiltration (17), with a value of 51 mL/min in an earlier study (18). Our results for IL-6 clearance are similar. We also found statistically significant reduction in IL-6 levels within both CVVH-HCO and CVVH-Std but this effect was not found for other cytokines like IL-8 with similar clearances. This suggests that any declines in plasma levels observed were not significantly influenced by the type of filter and clearances. Morgera et al also reported SC for IL-6 with HCO hemofiltration to approximate unity throughout a 12-hour treatment period (19). Our earlier systematic review on human studies found SC of around 0.9 for IL-6. In this study, we found a similarly high SC for IL-6 around unity. These observations support the likely accuracy of our measurements. Our study, however, studied a much greater number of other cytokines as well. In this regard, however, we found that apart from IL-8, other cytokines did not achieve high SC and clearance values with CVVH-HCO.

In our study, plasma levels for IFN-gamma and IFN-alpha were negligible or undetected in most patients. A review of the literature found both evidence for enhanced stimulation of interferon production as well as suppression of levels in septic patients (20-22). One study found an inverse relationship between plasma levels of IFN-gamma and severity of sepsis (23). Large variations in plasma levels were also reported in the literature for other types of cytokines in studies involving groups of patients similar to ours. This heterogeneity may be

TABLE V - Mass balances for all cytokines: Total mass removal rate (MTR), Mass removal rate by ultrafiltration (MUF) and Mass removal rate by adsorption (MAD)

Cytokine (approximate reported MW)	Total mass removal rate (MTR) (pg/min)	Mass removal rate by ultrafiltration (MUF) (pg/min)	Mass removal or addition rate by adsorption or generation (MAD) (pg/min)
IL-6 (22 to 28kDa)	HCO = 1438.35 (-4984.43, 3882.6)	HCO = 7184.69 (3225.9, 19905)	HCO = -8616 (-22054.17, 4.80)
	Std = 1217.5 (475.5, 5312.1)	Std = 530 (364, 1614)	Std = +251.77 (-874.5, 5141.7)
	p = 0.388	p = 0.008	p = 0.05
TNF-alpha (monomeric 17.5 kDa; trimeric 52 kDa)	HCO = 609.3 (-351.3, 3351.3)	HCO = 114.87 (104.27, 129)	HCO = +501.24 (-492.3, 3233.4)
	Std = 490.68 (-114, 1461.17)	Std = 142.3 (72.6, 171.67)	Std = +377.22 (-299.67, 1318.83)
	p = 1.0	p = 0.456	p = 1.0
IL-8 (8 kDa)	HCO = 159.22 (-2672.1, 3539.4)	HCO = 4058.25 (1849.88, 11500.77)	HCO = -3039.9 (-3647.4, 138.67)
	Std = 921.67 (294.5, 3351.03)	Std = 983.33 (872.27, 2519.65)	Std = -60.48 (-1369, 873.03)
	p = 0.607	p = 0.181	p = 0.113
IL-1 beta (17.5 kDa)	HCO = 88.98 (3, 175.4)	HCO = 3.15 (2.55, 3.88)	HCO = +79.24 (-0.3, 171.52)
	Std = 37.17 (18.82, 54.23)	Std = 2.67 (2.4, 3.07)	Std = +34.5 (16.6, 51.17)
	p = 0.776	p = 0.456	p = 0.955
RANTES (8 kDa)	HCO = 5971.72 (-44703.9, 20784.15)	HCO = 307.43 (46.8, 1018.02)	HCO = +5833.13 (-47437.2, 19766.13)
	Std = 4816.7 (-30828.5, 34960)	Std = 0 (0,319.3)	Std = +4816.7 (-30828.5, 34960)
	p = 0.689	p = 0.328	p = 0.607
IL-10 (17 to 21 kDa)	HCO = 3318.92 (1052.43, 5688.40)	HCO = 409.5 (0, 635.95)	HCO = +3000.94 (1052.43, 4441.85)
	Std = 1231.17 (293.83, 2297.4)	Std = 0 (0,0)	Std = 1231.17 (293.83, 2297.4)
	p = 0.181	p = 0.066	p = 0.272
All cytokines combined (CVVH-HCO n = 36; CVVH-Std n = 54)	HCO = 680.05 (-541.5, 3768.15)	HCO = 368.34 (31.64, 2355.3)	HCO = -79.24 (-2691.45, 1700.31)
	Std = 559.93 (18.82, 3392.68)	Std = 127.9 (2.22, 530)	Std = +174.15 (-299.67, 3180.33)
	p = 0.542	p = 0.027	p = 0.229

For individual cytokines, number of observations: CVVH-HCO n = 6; CVVH-Std n = 9.
For all cytokines combined, number of observations: CVVH-HCO = 36, CVVH-Std = 54. P values obtained through Mann-Whitney *U*-test.

due to variations in disease severity and cytokine response of individuals and highlights the complexity of performing cytokine studies in critically ill patients.

Implications of study findings

Our study shows that the plasma of patients with severe AKI in the setting of SIRS and MODS has high levels of some cytokines and low levels of others in a complex and variable pattern. For cytokines like IL-6, however, which are markedly elevated, our study implies that even techniques such as CVVH-HCO with clearances (50 mL/min) that would typically lower creatinine and urea levels in AKI patients have a limited impact on blood levels or first-pass removal. Our observations also logically imply that the production of some cytokines must, therefore, be proportionately higher than that of creatinine and urea. Finally, they further suggest that much higher clearances are likely necessary to lower elevated cytokine levels in patients with MODS and AKI.

Strengths and limitations

Our study has important strengths. To our knowledge, it is the first double-blind trial of high cut-off hemofilters, under

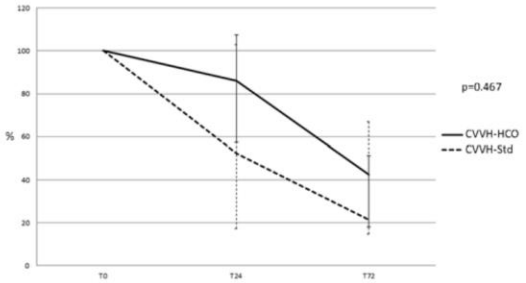


Fig. 1 - Comparison of changes in IL-6 plasma levels with CVVH-HCO and CVVH-std. P value: comparison of change over time between the two groups. T0 = pre-filter plasma at 0 to 12 hours after randomization; T24 = pre-filter plasma at 24 hours after randomization; T72 = pre-filter plasma at 72 to 96 hours after randomization. CVVH-HCO = high cut-off group; CVVH-Std = control/standard group.

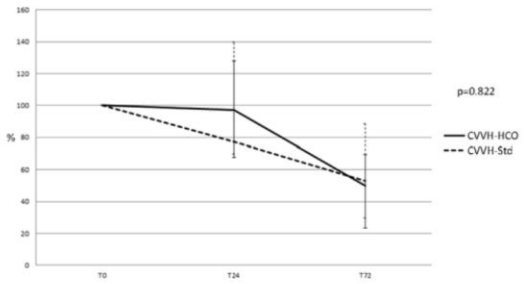


Fig. 2 - Comparison of changes in IL-10 plasma levels with CVVH-HCO and CVVH-std. P value: comparison of change over time between the two groups. T0 = pre-filter plasma at 0 to 12 hours after randomization; T24 = pre-filter plasma at 24 hours after randomization; T72 = pre-filter plasma at 72 to 96 hours after randomization. CVVH-HCO = high cut-off group; CVVH-Std = control/standard group.

real clinical conditions, in critically ill AKI patients requiring vasopressor support. We included patients with hypercytokinemic states from infective and noninfective etiology for 2 main reasons: this is a study centered on cytokine removal; and it is frequently difficult to confirm the exact underlying etiology at early stages of critical illness. This approach would better reflect usage of these filters under clinical conditions. We analyzed several cytokines that are representative of pro-inflammatory and anti-inflammatory biological responses. We measured these cytokines at baseline and monitored changes over a 72-hour period, providing an extended assessment of changes. We analyzed cytokine levels in the ultrafiltrate to measure SC and CL by HCO filters. Finally, we studied changes in plasma cytokine levels across the filter.

Our study also carries some limitations. Our sample size was small, thus limiting our power to detect differences. However, the use of multiple comparisons, assessment of SCs, clearances, changes across the filter, plasma levels and ANOVA analysis all

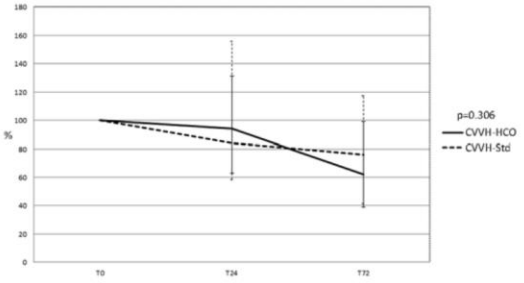


Fig. 3 - Comparison of changes in overall cytokine plasma levels with CVVH-HCO and CVVH-std, P value: comparison of change over time between the two groups. T0 = pre-filter plasma at 0 to 12 hours after randomization; T24 = pre-filter plasma at 24 hours after randomization; T72 = pre-filter plasma at 72 to 96 hours after randomization. CVVH-HCO = high cut-off group; CVVH-Std = control/standard group.

together increased our ability to detect any differences between the groups, if present. The baseline characteristics indicated some imbalances, which may explain some of our findings. We did not measure some important mediators such as high mobility group box-1 (HMGB-1) and macrophage migration inhibitory factor (MIF), but given our findings, it is unlikely that these large cytokines would have been affected differently. Measurements may have occurred at different times during the filter life, with variable membrane fouling, which may have affected cytokine clearance. However, HCO filters were expected to largely overcome such limitations. They did not appear to do so.

Conclusions

In conclusion, in patients with AKI and MODS, we confirmed high clearance of IL-6 and IL-8 with CVVH-HCO, but not for other cytokines. Despite clearances above those necessary to lower urea and creatinine levels in AKI and higher overall cytokine clearances than CVVH-Std, we could not find an overall significantly greater reduction in plasma cytokine levels with CVVH-HCO compared with CVVH-Std. Our findings imply that cytokine removal technologies need to look beyond CVVH-HCO to lower systemic cytokine levels in patients with MODS and AKI (24-27).

Disclosures

Financial support: This study was supported by the Austin Hospital Intensive Care Trust Fund.
Conflict of interest: Rinaldo Bellomo has received travel support and consultancy fees from Gambro, BBraun and Baxter Healthcare.

References

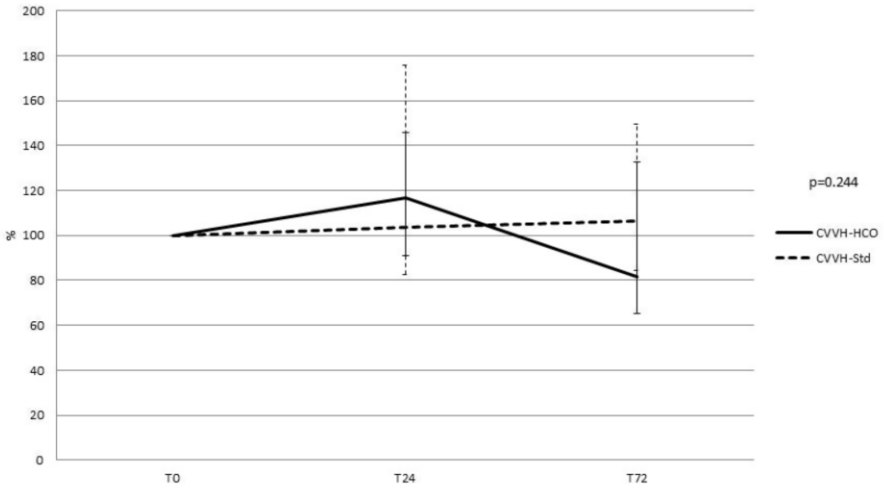
1. Schulte W, Bernhagen J, Bucala R. Cytokines in sepsis: potent immunoregulators and potential therapeutic targets-an updated view. Mediators Inflamm. 2013;2013:165974.
2. Hirsiger S, Simmen HP, Werner CM, Wanner GA, Rittirsch D. Danger signals activating the immune response after trauma. Mediators Inflamm. 2012;2012:315941.

3. Annane D, Bellissant E, Cavaillon JM. Septic shock. Lancet. 2005;365(9453):63-78.
4. Bone RC. Sir Isaac Newton, sepsis, SIRS, and CARS. Crit Care Med. 1996;24(7):1125-1128.
5. Pinsky MR, Vincent JL, Deviere J, Alegre M, Kahn RJ, Dupont E. Serum cytokine levels in human septic shock. Relation to multiple-system organ failure and mortality. Chest. 1993;103(2):565-575.
6. Bosmann M, Ward PA. The inflammatory response in sepsis. Trends Immunol. 2013;34(3):129-136.
7. Honore PM, Jacobs R, Joannes-Boyau O et al. Newly designed CRRT membranes for sepsis and SIRS--a pragmatic approach for bedside intensivists summarizing the more recent advances: a systematic structured review. ASAIO J. 2013;59(2):99-106.
8. Fisher CJ Jr, Slotman GJ, Opal SM, et al; IL-1RA Sepsis Syndrome Study Group. Initial evaluation of human recombinant interleukin-1 receptor antagonist in the treatment of sepsis syndrome: a randomized, open-label, placebo-controlled multicenter trial. Crit Care Med. 1994;22(1):12-21.
9. Morris PE, Zeno B, Bernard AC, et al. A placebo-controlled, double-blind, dose-escalation study to assess the safety, tolerability and pharmacokinetics/pharmacodynamics of single and multiple intravenous infusions of AZD9773 in patients with severe sepsis and septic shock. Crit Care. 2012;16(1):R31.
10. Venkataraman R, Subramanian S, Kellum JA. Clinical review: extracorporeal blood purification in severe sepsis. Crit Care. 2003;7(2):139-145.
11. Schefold JC, Hasper D, Jörres A. Organ crosstalk in critically ill patients: hemofiltration and immunomodulation in sepsis. Blood Purif. 2009;28(2):116-123.
12. Boschetti-de-Fierro A, Voigt M, Storr M, Krause B. Extended characterization of a new class of membranes for blood purification: the high cut-off membranes. Int J Artif Organs. 2013;36(7):455-463.
13. Atan R, Crosbie D, Bellomo R. Techniques of extracorporeal cytokine removal: a systematic review of the literature. Blood Purif. 2012;33(1-3):88-100.
14. Atan R, Crosbie D, Bellomo R. Techniques of extracorporeal cytokine removal: a systematic review of the literature on animal experimental studies. Int J Artif Organs. 2013;36(3):149-158.
15. Atan R, Crosbie DC, Bellomo R. Techniques of extracorporeal cytokine removal: a systematic review of human studies. Ren Fail. 2013;35(8):1061-1070.
16. Uchino S, Kellum JA, Bellomo R, et al; Beginning and Ending Supportive Therapy for the Kidney (BEST Kidney) Investigators. Acute renal failure in critically ill patients: a multinational, multicenter study. JAMA. 2005;294(7):813-818.
17. Morgera S, Haase M, Kuss T, et al. Pilot study on the effects of high cutoff hemofiltration on the need for norepinephrine in septic patients with acute renal failure. Crit Care Med. 2006;34(8):2099-2104.
18. Morgera S, Slowinski T, Melzer C, et al. Renal replacement therapy with high-cutoff hemofilters: Impact of convection and diffusion on cytokine clearances and protein status. Am J Kidney Dis. 2004;43(3):444-453.
19. Morgera S, Rocktäschel J, Haase M, et al. Intermittent high permeability hemofiltration in septic patients with acute renal failure. Intensive Care Med. 2003;29(11):1989-1995.
20. Lauw FN, Simpson AJ, Prins JM, et al. Elevated plasma concentrations of interferon (IFN)-gamma and the IFN-gamma-inducing cytokines interleukin (IL)-18, IL-12, and IL-15 in severe melioidosis. J Infect Dis. 1999;180(6):1878-1885.
21. Rigato O, Salomao R. Impaired production of interferon-gamma and tumor necrosis factor-alpha but not of interleukin 10 in whole blood of patients with sepsis. Shock. 2003;19(2):113-116.
22. Cabioglu N, Bilgic S, Deniz G et al. Decreased cytokine expression in peripheral blood leukocytes of patients with severe sepsis. Arch Surg. 2002;137(9):1037-1043; discussion 1043.
23. Jekarl DW, Kim JY, Lee S, et al. Diagnosis and evaluation of severity of sepsis via the use of biomarkers and profiles of 13 cytokines: a multiplex analysis. Clin Chem Lab Med. 2015;53(4):575-581.
24. Hoffmann JN, Hartl WH, Deppisch R, Faist E, Jochum M, Inthorn D. Hemofiltration in human sepsis: evidence for elimination of immunomodulatory substances. Kidney Int. 1995;48(5):1563-1570.
25. Kellum JA, Johnson JP, Kramer D, Palevsky P, Brady JJ, Pinsky MR. Diffusive vs. convective therapy: effects on mediators of inflammation in patient with severe systemic inflammatory response syndrome. Crit Care Med. 1998;26(12):1995-2000.
26. Heering P, Morgera S, Schmitz FJ, et al. Cytokine removal and cardiovascular hemodynamics in septic patients with continuous venovenous hemofiltration. Intensive Care Med. 1997;23(3):288-296.
27. Peng Z, Pai P, Hong-Bao L, Rong L, Han-Min W, Chen H. The impacts of continuous veno-venous hemofiltration on plasma cytokines and monocyte human leukocyte antigen-DR expression in septic patients. Cytokine. 2010;50(2):186-191.

Supplementary figures:

Appendix Figure A

TNF-alpha plasma levels: CVVH-HCO vs. CVVH-Std



Comparison of change in TNF-alpha plasma levels over three timelines between the two groups.

Values are medians; normalised to percentage change compared to baseline. Baseline (T0) is 100%

T0 = pre-filter plasma at 0 to 12 hours after randomization

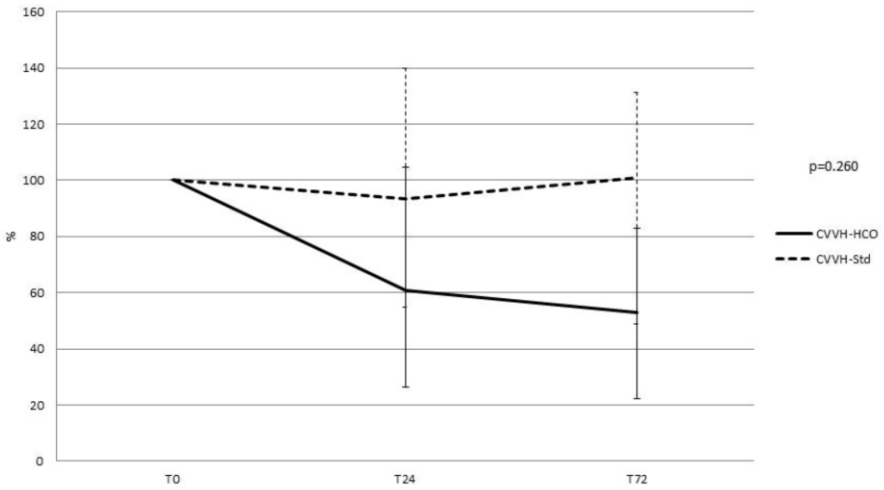
T24= pre-filter plasma at 24 hours after randomization

T72= pre-filter plasma at 72 to 96 hours after randomization

CVVH-HCO: High cut-off group; CVVH-Std: control/standard group

Appendix Figure B

IL-8 plasma levels: CVVH-HCO vs. CVVH-Std



Comparison of change in IL-8 plasma levels over three timelines between the two groups. Values are medians; normalised to percentage change compared to baseline. Baseline (T0) is 100%

T0 = pre-filter plasma at 0 to 12 hours after randomization

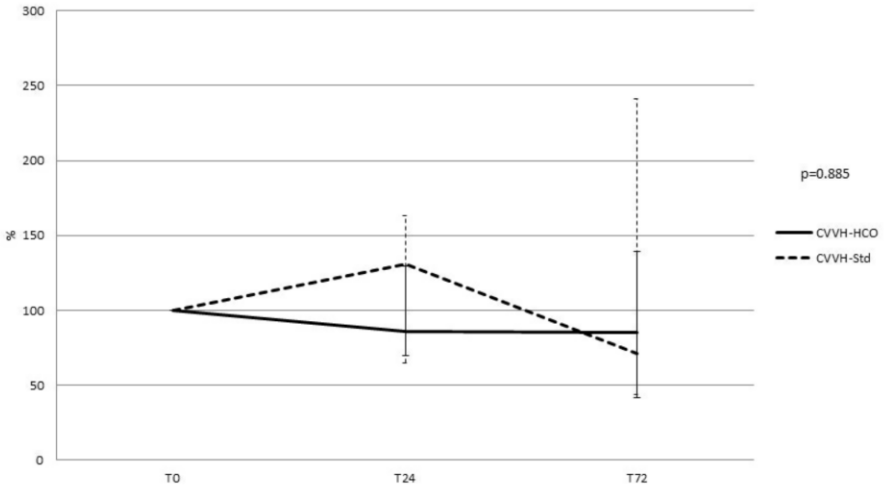
T24= pre-filter plasma at 24 hours after randomization

T72= pre-filter plasma at 72 to 96 hours after randomization

CVVH-HCO: High cut-off group; CVVH-Std: control/standard group

Appendix Figure C

IL-1 beta plasma levels: CVVH-HCO vs. CVVH-Std



Comparison of change in IL-1 beta plasma over three timelines between the two groups. Values are medians; normalised to percentage change compared to baseline. Baseline (T0) is 100%

T0 = pre-filter plasma at 0 to 12 hours after randomization

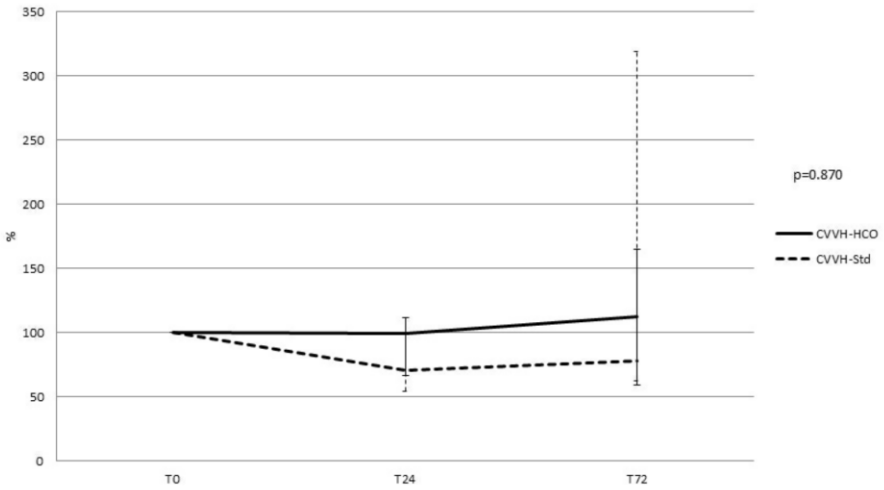
T24= pre-filter plasma at 24 hours after randomization

T72= pre-filter plasma at 72 to 96 hours after randomization

CVVH-HCO: High cut-off group; CVVH-Std: control/standard group

Appendix Figure D

RANTES plasma levels: CVVH-HCO vs. CVVH-Std



Comparison of change in RANTES plasma levels over time between the two groups. Values are medians; normalised to percentage change compared to baseline. Baseline (T0) is 100%

T0 = pre-filter plasma at 0 to 12 hours after randomization

T24= pre-filter plasma at 24 hours after randomization

T72= pre-filter plasma at 72 to 96 hours after randomization

CVVH-HCO: High cut-off group; CVVH-Std: control/standard group

3.3 Summary of effects on plasma cytokines

Our double-blind study did not support that HCO hemofiltration resulted in a higher reduction in plasma cytokine levels compared to standard techniques when conducted over a 72 hour period of observation.

HCO hemofiltration may offer higher SC and mass removal via ultrafiltration for cytokines such as IL-6 and IL-8 but these two measures are reflective of single pass phenomenon. When plasma levels are plotted over three timelines, the trends observed do not suggest a sustained or significant effect over a 72 hour period superior to that of standard hemofiltration. This lack of an effect may indicate that the rate of removal, although high is disproportionate to even higher rates of cytokine production. Removal rates over a 72 hour period may be further affected by the phenomenon of membrane fouling. The reduction in plasma levels with standard hemofiltration for IL-10 and IL-6 despite low or zero sieving can be explained by spontaneous rates of decay.

Our observations confirmed that molecules with higher molecular weight (MW) tends to be filtered better by HCO filters compared to standard hemofilters however it is not true for all cytokines. RANTES for example have low MW but sieving is poor for both filters. Factors other than MW therefore may have affected sieving into play including the molecular structure and molecular charges. Plasma levels of cytokines also appear to be affected by factors additional to SC as it there is no strong correlation between sieving and rate of reduction in plasma levels over the 72 hour observation period. Possible factors affecting plasma levels may include rates of endogenous production, rates of production upon exposure to the filter, rates of clearance by the filter and spontaneous decay in the circulation.

3.4 Publication: Effects on apoptosis indices

The plasma of hypercytokinemic patients has been shown to contain mediators, which induce apoptosis of monocytes and also that of other cells. In this substudy, we aimed to analyse whether the levels of these mediators were differentially affected by HCO hemofiltration as compared to standard hemofiltration. Plasma of patients in our study was incubated with U937 monocytes and the effects of the plasma on apoptotic indices were assessed.

Original Paper

Blood Purification

Blood Purif 2014;37:296–303
DOI: 10.1159/000363220

Received: February 25, 2014
Accepted: April 25, 2014
Published online: August 1, 2014

High Cut-Off Hemofiltration versus Standard Hemofiltration: A Pilot Assessment of Effects on Indices of Apoptosis

Rafidah Atan[a] Grazia M. Virzi[b, c] Leah Peck[d] Amutha Ramadas[a] Alessandra Brocca[b, c] Glenn Eastwood[d] Suneet Sood[a] Claudio Ronco[b, c] Rinaldo Bellomo[d, e] Hermann Goehl[f] Markus Storr[f]

[a]Clinical School Johor Bahru, Jeffrey Cheah School of Medicine and Health Sciences, Monash University Malaysia, Johor Bahru, Malaysia; [b]Department of Nephrology, Dialysis and Transplant, San Bortolo Hospital, San Bortolo, and [c]International Renal Research Institute Vicenza, IRRIV, Vicenza, Italy; [d]Department of Intensive Care, Austin Hospital, Heidelberg, Vic., and [e]Australian and New Zealand Intensive Care Research Centre, Melbourne, Vic., Australia; [f]Gambro Dialysatoren GmbH, Research and Development, Hechingen, Germany

Key Words
Sepsis · Acute kidney injury · Hemofiltration · High cut-off filters · Apoptosis

Abstract
Objectives: To measure plasma pro-apoptotic and pro-necrotic activity in severe acute kidney injury (AKI) patients within a randomized controlled trial of continuous veno-venous hemofiltration with high cut-off filters (CVVH-HCO) versus standard filters (CVVH-Std). ***Methods:*** We measured pro-apoptotic and pro-necrotic plasma activity by trypan blue exclusion cell viability assay, detection of DNA fragmentation, and by determination of caspase-3 activity and annexin V-based apoptosis and necrosis detection assay. ***Results:*** Compared to no apoptosis or necrosis after incubation with healthy plasma, 14–18% of cells showed apoptosis and 4–8% showed necrosis after incubation with plasma from AKI patients. When comparing different measures of pro-apoptotic or pro-necrotic activity, CVVH-HCO and CVVH-Std showed no differential effects on such activity, which remained high over the first 3 days of treatment. However, using annexin V-FITC, there was a significant drop in pro-apoptotic activity across the filter for the CVVH-HCO group ($p = 0.043$) but not for the CVVH-Std group ($p = 0.327$) and a significant difference between the two groups (CVVH-HCO vs. CVVH-Std $p = 0.006$). ***Conclusions:*** Patients with severe AKI have increased pro-apoptotic and pro-necrotic activity. Although on single-pass effect assessment, CVVH-HCO was superior to CVVH-Std in decreasing annexin V-FITC-assessed pro-apoptotic activity, there was no overall attenuation of such activity during the first 3 days of treatment.

Introduction

Apoptosis is believed to play an important role in the pathogenesis of acute kidney injury (AKI) [1, 2]. Although acute tubular necrosis has been conventionally accepted to be the main process of cell injury in AKI and its most common histopathological manifestation, its presence or absence or the degree of necrosis correlate poorly with illness severity and outcome. This observation suggests that other forms of cell injury or death such as apoptosis may be a potentially important mechanism

KARGER

E-Mail karger@karger.com
www.karger.com/bpu

0253–5068/14/0374–0296$39.50/0

Prof. Rinaldo Bellomo
Department of Intensive Care
Austin Hospital
[redacted]
E-Mail [redacted]

of tubular cell injury [3]. In fact, apoptosis and necrosis may occur simultaneously, represent different aspects or moments in a continuum of injury, overlap, and share common pathways [4, 5].

An increased production of factors which induce pro-apoptotic and pro-necrotic activity in patients with the systemic inflammatory response syndrome (SIRS) and/or with severe sepsis may contribute to a high incidence of AKI in such patients. Moreover, although apoptosis serves many important homeostatic functions, it may become dysregulated in SIRS/sepsis and may contribute to the multiorgan dysfunction syndrome (MODS) [6]. Attempts at attenuating the excessive pro-apoptotic and/or pro-necrotic activity of plasma from septic patients may be logically expected to have beneficial effects on the severity of AKI [7] and MODS. However, no reliable ways of decreasing pro-apoptotic and pro-necrotic activity in plasma have been developed so far. On the other hand, experimental findings show that pro-inflammatory molecules may be removed by techniques of extracorporeal blood purification, making an approach based on blood purification of research interest [8, 9].

Among techniques of extracorporeal blood purification, we have recently shown that those based on high cut-off [10, 11] (also named high-permeability or super high-flux) filters appear to achieve superior blood purification performance when compared to conventional commercial high-flux membranes. These differences have been confirmed in systematic reviews of in vitro [12], animal [13] and human [14] studies. However, the effect of treatment with such HCO membranes on the pro-apoptotic or pro-necrotic activity of plasma obtained from patients with severe AKI and MODS remains unknown.

Accordingly, we aimed to compare the effects of hemofiltration using high cut-off versus standard hemofilters on plasma levels of pro-apoptotic and pro-necrotic activity in a nested cohort of patients from a double-blind randomized controlled trial comparing these two techniques.

Methods

We performed a pilot investigation in a cohort of patients nested within a larger randomized double-blind controlled trial (ClinicalTrials.gov/NCT00912184) comparing continuous veno-venous hemofiltration with high cut-off filters (CVVH-HCO) with continuous veno-venous hemofiltration using standard high-flux membranes (CVVH-Std). The study was performed in the Department of Intensive Care, Austin Hospital, Heidelberg, Vic., Australia, and was approved by the Austin Hospital Human Research Ethics Committee (H2008/03400). Written informed consent was obtained from patients or the person responsible.

All study patients had AKI with underlying shock requiring vasopressor infusion and were recruited within the first 12 h of commencement of hemofiltration. Patients were then randomized to either CVVH-HCO using polyethersulfone filters with a nominal cut-off point of 100 kDa (P2SH filters, 1.12 m^2; Gambro, Hechingen, Germany) or to CVVH-Std using custom manufactured control polyethersulfone filters with a nominal cut-off point of 30 kDa [10, 11]. The study filters were identical in appearance and surface area.

The settings for CVVH were a blood flow of 250 ml/min, an ultrafiltration dose of 25 ml/kg/h with pre-dilution and the exclusive use of bicarbonate-buffered replacement fluids. We excluded patients who were on maintenance dialysis and those who had received hemofiltration during the same hospital admission.

Due to rapid processing requirements, we could only sample bloods for this nested study from patients during the daytime. As a large number of patients were admitted after hours, we could only obtain samples from 26 patients, with only 16 patients providing complete pre-filter and pre-replacement fluid blood sampled at baseline (T0), at 24 h after initiation of CVVH (T24) and between 72 and 96 h after initiation of CVVH (T72). In addition, blood was also sampled from the post-filter port at T24 at the same time as the arterial blood collections. Blood samples were immediately centrifuged at 3,500 rpm for 5 min and the resulting plasma was stored at −70°C until the time of measurement. As the study was double blind in nature, we were unable to identify at the time of sampling whether the samples were from the intervention or the control group. All specimens were shipped at −20°C to the International Renal Research Institute (IRRV), Vicenza, Italy, for analysis.

U937 Cell Culture

We used the U937 cell culture for assessment of apoptotic and necrotic activity. The human cell line U937 is a monocytic precursor cell line derived from histiocytic lymphoma [15]. The U937 cells were grown in complete liquid phase medium (RPMI 1640; International PBI) supplemented with 10% heat-inactivated (30 min at 56°C) fetal calf serum, 2 mM L-glutamine, 100 IU/ml penicillin and 100 μg/ml streptomycin (Sigma Chemical Co.). The U937 cells were maintained in a controlled atmosphere (5% CO_2) incubator at 37°C and passaged every second or third day. Cell viability was 99%, as assessed by trypan blue exclusion (Sigma Chemical Co.).

Induction of Apoptosis

U937 cells were plated at 3×10^6 cells/well in 6-well plates, and incubated with 80% RPMI 1640 medium (with 2 mM L-glutamine, 100 IU/ml penicillin and 100 mg/ml streptomycin) and 20% of plasma from Austin Hospital patients and healthy controls in standard condition (at 37°C in 5% CO_2 for 24 h). Before use in experiments, U937 cells were washed twice in Dulbecco's PBS (without calcium and magnesium; pH 7.4).

Trypan Blue Exclusion Cell Viability Assay

U937 cells were treated with patient plasma and the ability of the compound to induce apoptosis was evaluated at 24 h. Untreated cells were maintained in an identical manner and used as a control. 50 μl of cell culture was added to 50 μl of trypan blue (Sigma Chemical Co.) exclusion dye and examined under microscope. The level of cell viability was expressed as percentage of the total cell population at 20× magnification.

Table 1. Baseline characteristics and outcome of study patients

	HCO (n = 7)	Std HF (n = 8)	p value
Age, years	61.1 (49.1, 75.3)	66.8 (63.6, 76.4)	0.536
Male/female, n	6/1	5/3	0.57
Weight, kg	80 (71.5, 93.5)	80.1 (77.5, 91.3)	0.96
APACHE-2	24 (13, 28)	23 (17.3, 23.3)	0.96
APACHE-3	72 (54.5, 104.5)	77.5 (65.3, 95)	0.87
SOFA cardiovascular	4 (4, 4)	4 (3.75, 4)	0.46
SOFA respiration	3 (2, 3)	3 (2, 3.25)	0.536
SOFA renal	3 (2, 3.5)	2.5 (2, 4)	0.867
SOFA coagulation	0 (0, 2)	0 (0, 0)	0.463
SOFA liver	2 (1, 3)	1 (0, 1)	0.097
Baseline creatinine within 1 year of date of admission, μmol/l	88 (61.5, 104)	80 (69.8, 96.8)	0.78
RIFLE score at commencement of CRRT (R/I/F), n	0/2/5	1/2/5	0.63
Shock etiology (sepsis/cardiogenic/other), n	3/3/1	3/3/2	0.875
Baseline mean arterial pressure at enrolment, mm Hg	70 (65, 70)	75 (70, 80)	0.094
Ventilated at enrolment (yes/no), n	5/2	6/2	1.0
Serum urea at enrolment, mmol/l	16 (11.2, 23.4)	20 (9.7, 26)	0.87
Serum creatinine at enrolment, μmol/l	252 (228, 327)	200 (172, 256)	0.23
Blood lactate at enrolment, mmol/l	2.9 (2.45, 4.95)	1.48 (1.1, 1.69)	0.001
Blood pH at enrolment	7.35 (7.3, 7.39)	7.38 (7.32, 7.42)	0.397
Serum albumin at enrolment, g/dl	34 (31.5, 36.5)	24.5 (20.3, 27.3)	0.006
Noradrenaline infusion at enrollment, μg/min	17 (8, 21.5)	6.5 (3, 11.5)	0.189
ICU mortality, %	42.9	37.5	1.0
Hospital mortality, %	57.1	37.5	0.62

Data displayed are medians (interquartile range) unless specified otherwise. APACHE = Acute Physiology and Chronic Health Evaluation; SOFA = Sequential Organ Failure Assessment; R/I/F = risk/injury/failure; CRRT = continuous renal replacement therapy; ICU = intensive care unit.

Evaluation of Apoptosis

Since cell growth inhibition and presence of apoptosis bodies were indicative of apoptosis induction, we examined the characteristic features of apoptosis by different methods.

Detection of DNA Fragmentation

Apoptosis is characterized by DNA fragmentation, showing a ladder-like pattern, and nuclear fragmentation in several smaller fragments. Untreated and plasma treated U937 cells (1×10^6 cells) were harvested and washed with Dulbecco's PBS. The DNA fragmentation assay was performed using an Apoptotic DNA Ladder Extraction KIT (BioVision) according to the manufacturer's protocol. DNA ladder fragmentation was detected by electrophoresis on 1.2% agarose gel staining with SYBR® Safe (Invitrogen); the bands were visualized under ultraviolet light and photographed.

Determination of Caspase-3 Activity

U937 cells were assayed for activation of caspase-3, an effector caspase able to cleave various cytoplasmic or nuclear substrates which leads to many morphological features of apoptotic cell death. Caspase-3 concentration was measured by Human Caspase-3 Instant ELISA Kit (eBioscience) with a fluorometric assay.

U937 cells (1×10^6 cells) incubated with plasma for 24 h were processed according to the manufacturer's instructions and finally caspase-3 levels were measured in cell lysates at 450 nm in the VICTOR4 Multilabel Plate Reader (PerkinElmer Life Sciences). The amount of caspase-3 (ng/ml) was calculated from the standard curve according to the manufacturer's protocol. Each experiment was performed in triplicate. Standard samples ranged from 0.16 to 10.0 ng/ml. Human Caspase-3 Instant ELISA Kit sensitivity is 0.12 ng/ml.

Annexin Apoptosis Detection Assay

The Annexin V-FITC Kit is an apoptosis detection kit based on the binding properties of annexin V to phosphatidylserine (PS) and on the DNA-intercalating capabilities of propidium iodide (PI). One of the morphological changes of the apoptotic cells is the appearance on the surface of the cell of PS, a negatively charged phospholipid usually located in the inner leaflet of the plasma membrane. In the early phase of apoptosis, the integrity of the cell membrane is maintained but the cells lose the asymmetry of their membrane phospholipids [16–19]. PS becomes exposed at the cell surface and forms one of the specific signals for recognition and removal of apoptotic cells by macrophages [17, 20]. Cells were washed twice with cold PBS and then resuspended in 0.5 ml of PBS at a concentration of 1×10^6 cells/ml. Transfer of 100 μl of solution to a 5-ml culture tube was performed and 5 μl of annexin V-FITC and 2.5 μl PI (Beckman Coulter, Brea, Calif., USA) were then added. The cells were gently vortexed and incubated for 10 min at room temperature (25°C) in the dark. 400 μl of 1× binding buffer

was added to each tube. Analysis was performed by Navios Flow Cytometer (Beckman Coulter) to identify the subpopulations of the apoptosis cells within 1 h. We also used as negative control the untreated cells and as positive control the cells treated with healthy donor plasma. Apoptotic cells were gated and enumerated by identifying those cells that exhibited FITC and PI staining.

Annexin V-FITC labeled was used to quantitatively determine the percentage of cells within population that were undergoing apoptosis. PI was used to distinguish necrosis from non-necrosis cells. The biparametric analysis shows three distinct populations: the viable cells which have low FITC and low PI signal, the apoptotic cells which have high FITC and low PI signal, and the necrotic cells which have high FITC and a high PI signal. Negative controls to set up compensation and quadrants encompassed unstained cells, cells stained with annexin V-FITC alone (for FL-1 fluorescence) and cells stained with PI alone (detected in FL-4). A minimum 20,000 events were collected on each sample.

Statistical Analysis

Baseline characteristics were analyzed using the Mann-Whitney U test for continuous data and χ^2 analysis for categorical data. Data on pro-apoptotic mediators were log transformed and analyzed using repeated-measures analysis of variance (ANOVA). Pre- and post-filter samples at T24 were compared using the Mann-Whitney U test for between-group comparison and Wilcoxon signed-rank test for within-group comparison. Statistical significance was defined as $p < 0.05$.

Results

We obtained a complete set of samples from 16 patients; however, 1 patient was subsequently excluded for meeting an exclusion criteria. A total of 15 patients were finally analyzed; 7 in the CVVH-HCO group and 8 in the CVVH-Std group. In general, except for baseline differences in blood lactate ($p = 0.001$) and serum albumin levels ($p = 0.006$), the two groups were broadly similar (table 1).

In monocytes treated for 24 h with patients' plasma, the results showed DNA ladder formation with different molecular weight fractions, suggesting the presence of apoptotic activity in both groups (fig. 1).

Pro-apoptotic activity was markedly elevated compared to controls and seen in between 14 and 18% of cells. The changes in plasma levels of pro-apoptotic activity over T0, T24 and T72 using annexin V-PI FITC analysis are shown in figure 2. Pro-apoptotic activity showed a non-significant trend toward lower levels over the three timelines ($p = 0.227$). For the control group a similar non-significant decline in pro-apoptotic activity over the first 24 h was followed by an increase toward baseline level at T72. For pro-necrotic activity (fig. 3) there was also a marked difference compared to control with between 4 and 8% of cells being affected. Both groups showed a

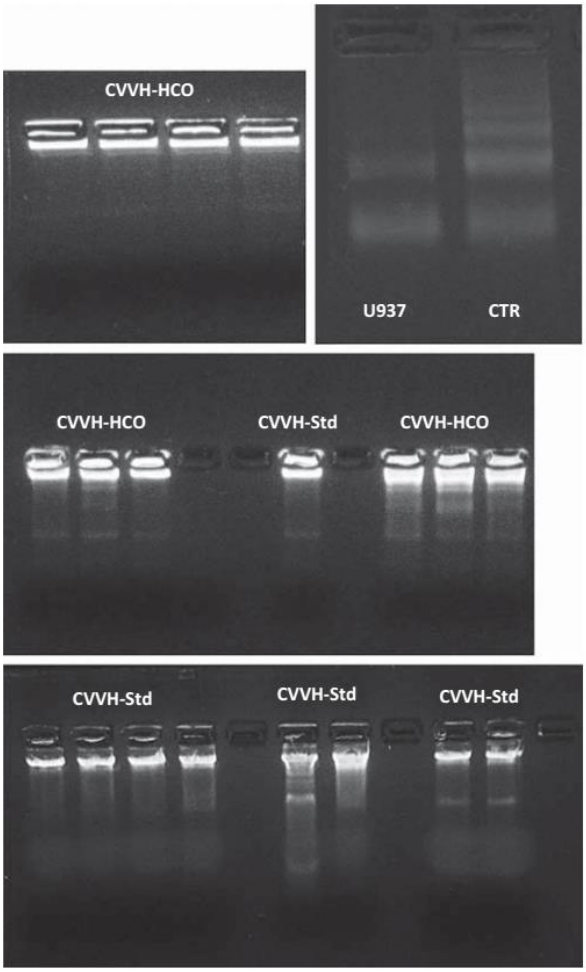


Fig. 1. DNA ladder fragmentation, as detected by electrophoresis on 1.2% agarose gel staining, from monocytes (U937) after 24 h of incubation with plasma from patients of both groups (CVVH-HCO and CVVH-Std). The biochemical hallmark of apoptosis is the fragmentation of the genomic DNA, an irreversible event that commits the cell to die. In many systems, this DNA fragmentation has been shown to result from the activation of an endogenous nuclear endonuclease. This enzyme selectively cleaves DNA at sites located between nucleosomal units generating mono- and oligonucleosomal DNA fragments. These DNA fragments reveal, upon agarose gel electrophoresis, a distinctive ladder pattern consisting of multiple DNA subunits as shown in the figure. The bands were visualized under ultraviolet light and photographed.

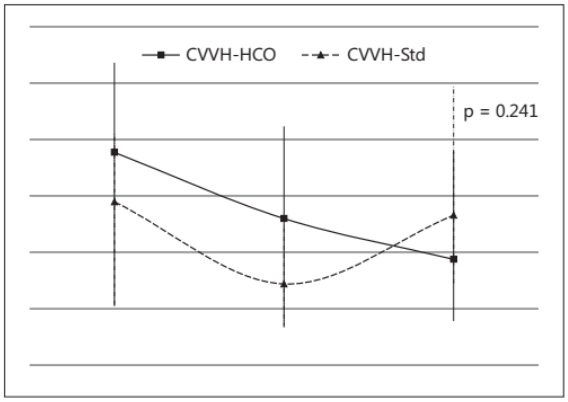


Fig. 2. Median percentage (%) of U937 cells showing apoptosis according to annexin V-FITC analysis following incubation with plasma from patients at T0, T24 and T72 in CVVH-HCO compared to CVVH-Std.

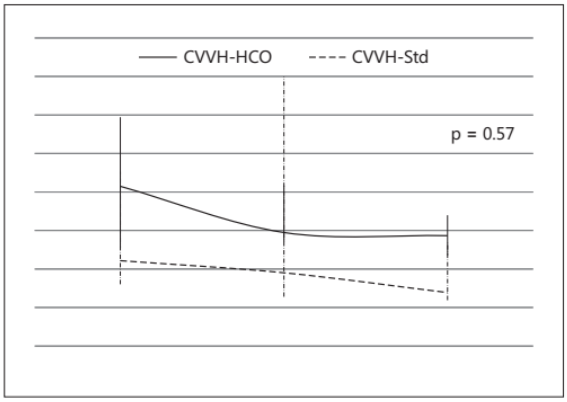


Fig. 3. Median percentage (%) of U937 cells showing necrosis according to annexin V-FITC analysis following incubation with plasma from patients at T0, T24 and T72 in CVVH-HCO compared to CVVH-Std.

small non-significant decline over the first 3 days (CVVH-HCO, p = 0.301; CVVH-Std, p = 0.304).

For apoptosis analysis using caspase-3 activation (fig. 4), both groups showed a similar trend of initial decrease in the first 24 h followed by an increase to near baseline levels at day 3.

There was no statistically significant difference in time-group interaction using repeated-measures ANOVA for all three tests of pro-apoptotic or pro-necrotic activity (annexin V-FITC apoptosis, p = 0.24; necrosis, p = 0.57; caspase-3 activation, p = 0.28). For all three types of measurements, there were no significant differences between the two groups at all three time points.

A total of 5 patients from the CVVH-HCO group and all 8 patients from the CVVH-HF group also had post-filter samples taken at T24. Analysis of post-filter samples obtained at T24 showed that there was no significant difference in levels between the two groups for all types of analyses (annexin V-FITC apoptosis, p = 0.435; necrosis, p = 0.354; caspase-3 activation, p = 0.354).

When pre- versus post-filter levels of pro-apoptotic factors were compared using annexin V-FITC, there was a significant drop across the filter for the CVVH-HCO group (fig. 5; p = 0.043) which was not seen with the CVVH-Std group (fig. 5; p = 0.327) and a significant difference between the two groups (CVVH-HCO vs. CVVH-Std, p = 0.006). The above effects were not seen for necrosis analysis using annexin V-FITC (CVVH-HCO, p = 0.893; CVVH-Std, p = 0.161) or apoptosis analysis through caspase-3 activation (CVVH-HCO, p = 0.893; CVVH-Std, p = 0.327).

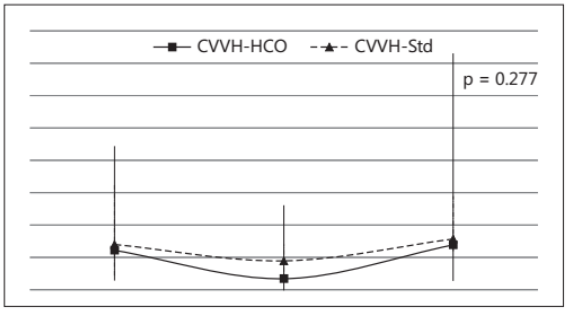


Fig. 4. Median percentage (%) of U937 cells showing apoptosis according to caspase-3 activation following incubation with plasma from patients at T0, T24 and T72 in CVVH-HCO compared to CVVH-Std.

Discussion

Key Findings

Using multiple techniques, we measured indices of pro-apoptotic and pro-necrotic activity in plasma from 15 patients with AKI and MODS obtained as a nested cohort within a double-blind randomized controlled trial comparing CVVH with high cut-off filters (CVVH-HCO) to CVVH with standard high-flux filters (CVVH-Std). We found that such plasma exhibited high levels of both pro-apoptotic and pro-necrotic activity at baseline and that such activity showed only a negligible trend to a

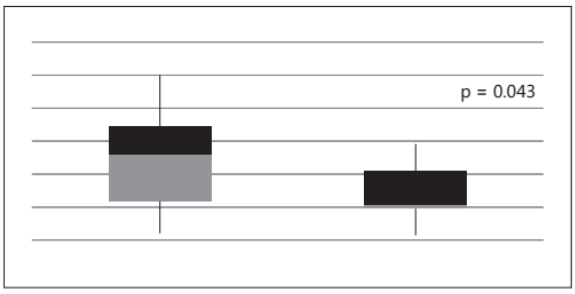


Fig. 5. Percentage (%) of U937 cells showing apoptosis according to annexin V-FITC analysis following incubation with plasma sampled at T24 from the CVVH-HCO group: pre-filter vs. post-filter. Dark shaded area indicates the separation of above and below the median value.

decrease over the 3 days of assessment, while caspase activation remained unchanged. There was, however, a significant drop in pro-apoptotic activity across the filter at T24 for the high cut-off group using one of the methods for analysis which was significantly different from the lack of any effect with CVVH-Std.

Relationship to Previous Studies

There is some evidence that high permeability hemofiltration might remove pro-apoptotic factors [8], however whether its rate of removal when compared to CVVH-Std translates into a reduction in pro-apoptotic activity in plasma remains untested. Bordoni et al. [8] compared standard hemofiltration with high permeability hemofiltration and found that high permeability hemofiltration resulted in higher levels of pro-apoptotic factors in the ultrafiltrate. Although this may indicate greater removal of these mediators by the high permeability group, levels of pro-apoptotic mediators in the plasma were not different between the two groups. There were key differences between our study and that by Bordoni et al [8]. The latter was an ex vivo experiment, on a sepsis model, using human whole blood spiked with lipopolysaccharide to induce apoptosis. In addition, the super high-flux (high cut-off) type made from cellulose triacetate and two other standard filters made from cellulose triacetate and polyethersulfone, respectively. Plasma and ultrafiltrate obtained from the experiment were then analyzed for pro-apoptotic activity using U937 cells.

In our study, patients were randomized in a double-blind method to the two differential treatments in real time, as clinical interventions, over a period of at least 72 h. Furthermore, in our study, the two study filters were made of similar material and indistinguishable to the naked eye providing excellent conditions for a double-blind study. We are not aware of other similar double-blind trials.

The level of pro-apoptotic and pro-necrotic activity in the plasma of our study patients was high. Using similar cell-based annexin V-FITC assays, other investigators have reported varying percentages in the degree of monocyte apoptosis seen in similar groups of patients. Bilbault et al. [21] reported 15.7% monocyte apoptosis in severe sepsis patients, Giamarellos-Bourboulis et al. [22] reported 27.6–55.4% monocyte apoptosis in septic shock patients, and Weiss et al. [23] reported a range of 10–20% in a mix of severe sepsis and septic shock patients. Our finding of apoptosis at a median range of 14–18% is in keeping with some of these observations.

Implications of Study Findings

Our study shows that the plasma of patients with severe AKI in the setting of SIRS and MODS is toxic. When such plasma is incubated with monocytes, it can induce apoptosis in close to 1 cell in every 6 and necrosis in close to 1 cell in every 25. Such toxicity is not significantly attenuated by the passage of time over the first 3 days despite the application of CVVH-Std. When CVVH-HCO is applied, no gains are achieved despite a single-pass effect to diminish such activity, a beneficial effect which is greater in magnitude with CVVH-HCO than with CVVH-Std. Our findings demonstrate that during AKI and MODS, factors circulate in the plasma, which are injurious to cells; that these factors can be removed to a degree during single pass through the HCO filters but also that such removal is insufficient to achieve an effect on plasma toxicity. They imply that different blood purification technologies need to be developed if attenuation of pro-apoptotic and pro-necrotic activity is an important therapeutic target. They also imply that studies of blood purification techniques, which confine themselves to the measurement of commonly studied soluble mediators and do not assess the effect of such techniques on the pro-apoptotic and pro-necrotic activity of plasma, may well provide a misleading impression of biological efficacy.

There are other blood purification techniques under investigation, such as hemoadsorption/hemoperfusion and combined plasma filtration adsorption (CPFA). Some are currently being investigated in clinical trials. The COMPACT trial [24] of CPFA in sepsis failed to improve outcome and COMPACT2 is currently recruiting patients, using a higher dose of treatment. The EUPHRATES trial [25] of polymyxin B hemoperfusion is also currently recruiting patients, following the initial supportive

findings from the first EUPHAS trial [26]. The effects of the above techniques on the clearance of pro-apoptotic and pro-necrotic mediators will likely be an important area of mechanistic investigation.

Strengths and Limitations

Our study has important strengths. To our knowledge, it is the first trial to study the effect of blood purification on pro-apoptotic and pro-necrotic factors in man. We measured such activity at baseline and over 3 days, thus providing an extended view of such activity in critically ill patients with AKI and MODS treated with CVVH. We used several techniques and types of assays to estimate pro-apoptotic activity and their findings are all broadly concordant. Moreover, we performed our study within the context of a double-blind randomized controlled trial comparing CVVH-Std with a more advanced and promising blood purification technique [12–14] based on higher permeability membranes (CVVH-HCO), thus removing the effect of selection bias. Our findings may have implications for the development of future techniques of blood purification in MODS.

Sepsis is a highly complex phenomenon and novel underlying mechanisms are often identified. This high cut-off hemofiltration study was performed based on the basis of several premises: that the membrane has improved capability to remove mediators; that non-specific clearance of water-soluble middle molecules would be better than targeting individual mediators, and that smaller studies of such therapy had indicated some benefit either biochemically or clinically. These preliminary observations justified a double-blind randomized controlled trial. Most blood purification therapies are still at the experimental stage and it is equally important to know which therapies do not work, as all novel interventions may pose potential harm.

Our study carries some limitations. The sample size was small, thus limiting our power to detect significance for anything but large differences. The baseline characteristics indicated some imbalances between the two groups, namely serum albumin and blood lactate at enrolment. This high intra- and inter-individual variability may limit our ability to detect differences. However, there was no consistent trend to indicate any differential effect of CVVH-HCO compared with CVVH-Std. Moreover, we used multiple techniques to detect and effect and all were concordant in showing lack of effect. Finally, such techniques are laborious and larges studies can only be justified if pilot investigations such as ours show clear trends suggestive of a possible effect. No such consistent trends were observed. We did not measure other mediators of inflammation. Knowledge of their levels may have helped understand the relationship between such mediators and pro-apoptotic activity. We intend to focus future studies on such relationships. We did not measure pro-apoptotic activity in the ultrafiltrate. We reasoned that the demonstration of such activity in the absence of changes in the cell toxicity of plasma would be of little clinical relevance. One of the challenges faced in the study of apoptosis is the choice of diagnostic methods used. Often combinations of techniques are necessary as no single technique is able to differentiate apoptosis exclusively from other causes of cell death such as necrosis [27]. These techniques would include DNA agarose gel electrophoresis which identifies apoptosis through the classical appearance of ladder pattern DNA fragmentation, caspase-3 activation which occurs during the final pathway of the apoptosis process, and the use of annexin V which binds to PS expressed on cell surfaces during the apoptosis process. The addition of PI to annexin V assay helps to improve differentiation between necrosis and apoptosis.

Other techniques exist to assess plasma for the presence of pro-apoptotic and pro-necrotic activity. However, we reasoned that the aggregate to the above techniques, if consistent, would provide a sufficient understanding of such activity in our patients. The clear activation of such pathways and their concordance support our approach.

Finally, this experiment is focused on mechanisms that promote cell injury by studying levels of such activity in plasma. It remains unclear at this stage whether a relationship exists between plasma levels of pro-apoptotic and pro-necrotic factors and the degree of cell in injury in vital organs.

Conclusions

In conclusion, we found that patients with AKI and MODS have strong pro-necrotic activity and even stronger pro-apoptotic activity in plasma. When such patients are exposed to CVVH-Std and such activity measured over the first 3 days of treatment, no clear decrease in activity can be seen. When such patients are randomized to receive CVVH-HCO, despite a single-pass effect showing diminished activity, no overall benefit emerges in terms of reduction of either activity in circulating plasma. If blood purification technology is to attenuate such toxic plasma activity, novel approaches beyond CVVH-HCO need to be developed.

Acknowledgement

This study was supported by the Austin Hospital Intensive Care Trust Fund.

Disclosure Statement

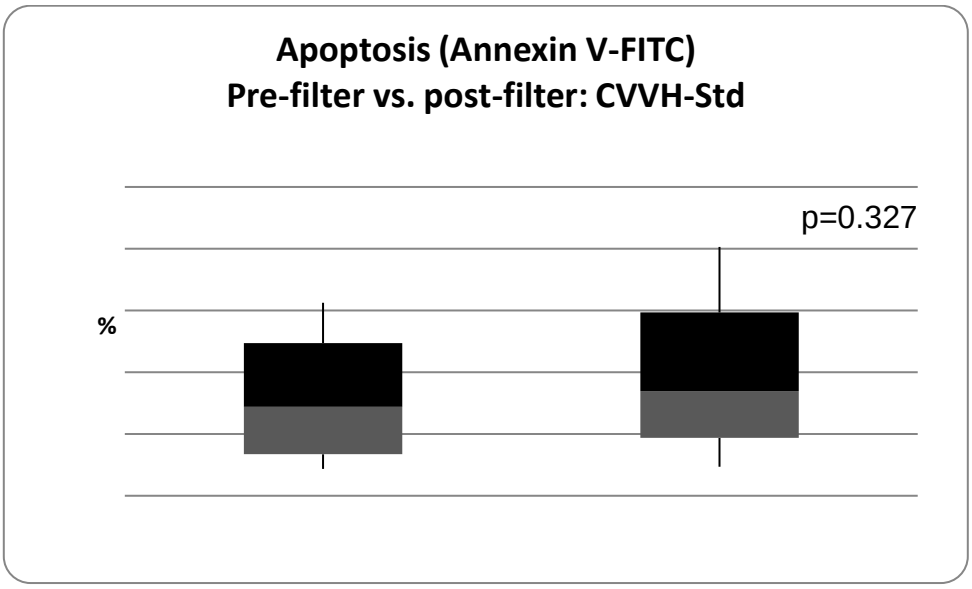
R. Bellomo has received travel support and consultancy fees from Gambro.

References

1 Lydon A, Martyn JA: Apoptosis in critical illness. Int Anesthesiol Clin 2003;41:65–77.
2 Wan L, Bagshaw SM, Langenberg C, Saotome T, May C, Bellomo R: Pathophysiology of septic acute kidney injury: what do we really know? Crit Care Med 2008;36(suppl 4):S198–S203.
3 Rosen S, Heyman SN: Difficulties in understanding human 'acute tubular necrosis': limited data and flawed animal models. Kidney Int 2001;60:1220–1224.
4 Kaushal GP, Basnakian AG, Shah SV: Apoptotic pathways in ischemic acute renal failure. Kidney Int 2004;66:500–506.
5 Linkermann A, De Zen F, Weinberg J, Kunzendorf U, Krautwald S: Programmed necrosis in acute kidney injury. Nephrol Dial Transplant 2012;27:3412–3419.
6 Hotchkiss RS, Swanson PE, Freeman BD, Tinsley KW, Cobb JP, Matuschak GM, Buchman TG, Karl IE: Apoptotic cell death in patients with sepsis, shock, and multiple organ dysfunction. Crit Care Med 1999;27:1230–1251.
7 Jacobs R, Honore PM, Joannes-Boyau O, Boer W, De Regt J, De Waele E, et al: Septic acute kidney injury: the culprit is inflammatory apoptosis rather than ischemic necrosis. Blood Purif 2011;32:262–265.
8 Bordoni V, Bolgan I, Brendolan A, Crepaldi C, Gastaldon F, D'intini V, et al: Caspase-3 and -8 activation and cytokine removal with a novel cellulose triacetate super-permeable membrane in an in vitro sepsis model. Int J Artif Organs 2003;26:897–905.
9 Cantaluppi V, Assenzio B, Pasero D, Romanazzi GM, Pacitti A, Lanfranco G, et al: Polymyxin-B hemoperfusion inactivates circulating proapoptotic factors. Intensive Care Med 2008;34:1638–1645.
10 Boschetti-de-Fierro A, Voigt M, Storr M, Krause B: Extended characterization of a new class of membranes for blood purification: the high cut-off membranes. Int J Artif Organs 2013;36:455–463.
11 Morgera S, Rocktäschel J, Haase M, Lehmann C, von Heymann C, Ziemer S, Priem F, Hocher B, Göhl H, Kox WJ, Buder HW, Neumayer HH: Intermittent high permeability hemofiltration in septic patients with acute renal failure. Intensive Care Med 2003;29:1989–1995.
12 Atan R, Crosbie D, Bellomo R: Techniques of extracorporeal cytokine removal: a systematic review of the literature. Blood Purif 2012;33:88–100.
13 Atan R, Crosbie D, Bellomo R: Techniques of extracorporeal cytokine removal: a systematic review of the literature on animal experimental studies. Int J Artif Organs 2013;36:149–158.
14 Atan R, Crosbie DC, Bellomo R: Techniques of extracorporeal cytokine removal: a systematic review of human studies. Ren Fail 2013;35:1061–1070.
15 Sundström C, Nilsson K: Establishment and characterization of a human histiocytic lymphoma cell line (U937). Int J Cancer 1976;17:565–577.
16 Martin SJ, Reutelingsperger CP, McGahon AJ, Rader JA, van Schie RC, LaFace DM, Green DR: Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: inhibition by overexpression of Bcl-2 and Abl. J Exp Med 1995;182:1545–1556.
17 Fadok VA, Voelker DR, Campbell PA, Cohen JJ, Bratton DL, Henson PM: Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages. J Immunol 1992;148:2207–2216.
18 Diaz C, Schroit AJ: Role of translocases in the generation of phosphatidylserine asymmetry. J Membr Biol 1996;151:1–9.
19 Homburg CH, de Haas M, von dem Borne AE, Verhoeven AJ, Reutelingsperger CP, Roos D: Human neutrophils lose their surface Fc gamma RIII and acquire Annexin V binding sites during apoptosis in vitro. Blood 1995;85:532–540.
20 Verhoven B, Schlegel RA, Williamson P: Mechanisms of phosphatidylserine exposure, a phagocyte recognition signal, on apoptotic T lymphocytes. J Exp Med 1995;182:1597–1601.
21 Bilbault P, Lavaux T, Lahlou A, Uring-Lambert B, Gaub MP, Ratomponirina C, et al: Transient Bcl-2 gene down-expression in circulating mononuclear cells of severe sepsis patients who died despite appropriate intensive care. Intensive Care Med 2004;30:408–415.
22 Giamarellos-Bourboulis EJ, Routsi C, Plachouras D, Markaki V, Raftogiannis M, Zervakis D, et al: Early apoptosis of blood monocytes in the septic host: is it a mechanism of protection in the event of septic shock? Crit Care 2006;10:R76.
23 Weiss M, Elsharkawi M, Welt K, Schneider EM: Transient leukocytosis, granulocyte colony-stimulating factor plasma concentrations, and apoptosis determined by binding of Annexin V by peripheral leukocytes in patients with severe sepsis. Ann NY Acad Sci 2003;1010:742–747.
24 Livigni S, Bertolini G, Rossi C, Ferrari F, Giardino M, Pozzato M, Remuzzi G: Efficacy of coupled plasma filtration adsorption in patients with septic shock: a multicenter randomised controlled clinical trial. BMJ Open 2014;4:e003536.
25 Rachoin JS, Foster D, Dellinger RP: Endotoxin removal: how far from the evidence? From EUPHAS to EUPHRATES. Contrib Nephrol. Basel, Karger, 2010, vol 167, pp 111–118.
26 Cruz DN, Antonelli M, Fumagalli R, Foltran F, Brienza N, Donati A, Malcangi V, Petrini F, Volta G, Bobbio Pallavicini FM, Rottoli F, Giunta F, Ronco C: Early use of polymyxin B hemoperfusion in abdominal septic shock: the EUPHAS randomized controlled trial. JAMA 2009;301:2445–2452.
27 Havasi A, Borkan SC: Apoptosis and acute kidney injury. Kidney Int 2011;80:29–40.

Supplementary Figure

Figure 5a: Percentage (%) of U937 cells showing apoptosis according to Annexin V-FITC analysis following incubation with plasma sampled at T24 from CVVH-Std group of patients: Pre-filter vs. Post-filter



Pre-filter T24 = pre-filter plasma at 24 hours after randomization

Post-filter T24= post-filter plasma at 24 hours after randomization

CVVH-HCO: High cut-off group; CVVH-Std: control/standard group

3.5 Effects on apoptosis indices: Summary

In this study on the effects on pro-apoptotic and pro-necrotic factors, a significant reduction with passage across the filter is observed for pro-apoptotic factors with CVVH-HCO using one of the measurement techniques (annexin V FITC). Similar reductions were not observed with another measurement (caspase-3) or for pro-necrotic activity. Any reduction that was observed did not translate into a significant reduction when observed over time. Prefilter vs. postfilter changes are more reflective of a single pass phenomenon. When levels are charted over a 72 hour period, there was no signal to support higher removal of pro-apoptotic and pro-necrotic factors using CVVH-HCO compared to CVVH-Std.

3.6 Effects on nucleosome levels and toll-like receptor (TLR) Expression: Publication

Nucleosomes result from DNA fragmentation and are an indirect marker of apoptosis, as direct measurement of the phenomenon is difficult. Our last publication compared the effects of the two interventions on nucleosome levels. It also has a small subsection on toll-like receptor (TLR2 and TLR4) expression.

The number of samples was small and as such this publication was in the form of a short paper.

Nucleosome levels and toll-like receptor expression during high cut-off haemofiltration: a pilot assessment

Rafidah Atan, Clive May, Simon R Bailey, Marcel Tanudji, Kumar Visvanathan, Narelle Skinner, Rinaldo Bellomo, Hermann Goehl and Markus Storr

ABSTRACT

Objectives: To measure plasma nucleosome levels and expression of toll-like receptors (TLRs) in a pilot cohort of patients with severe acute kidney injury (AKI) within a randomised controlled trial of continuous venovenous haemofiltration with high cut-off filters (CVVH-HCO) v standard filters (CVVH-std).

Methods: We measured plasma nucleosome levels using the Cell Death Detection ELISA PLUS (10X) assay kit. We analysed plasma levels for correlation with disease severity and compared the effects of CVVH-HCO and CVVH-std on plasma nucleosome levels over the first 72 hours. We studied cell surface TLR expression on CD14-positive monocytes in a subcohort of CVVH-HCO patients.

Results: We did not detect nucleosomes in normal human plasma, but found elevated nucleosome levels in patients with severe AKI. Nucleosome levels at randomisation correlated weakly with Acute Physiology and Chronic Health Evaluation III scores (Pearson $\rho = 0.475$, $P = 0.016$). Treatment with CVVH-HCO or CVVH-std had no effect on nucleosome levels over 72 hours. The mean fluorescence intensity (MFI) ratios of TLR2 and TLR4 expression were elevated throughout the 72-hour period (range for TLR2, 0.97–3.98; range for TLR4, 0.91–10.18) and did not appear to decrease as a result of treatment with CVVH-HCO.

Conclusions: Nucleosome concentration was elevated in the plasma of patients with severe AKI and mildly correlated with disease severity, but was not affected by treatment with CVVH-HCO or CVVH-std. Similarly, levels of TLR2 and TLR4 expression did not decrease over time during CVVH-HCO treatment.

Crit Care Resusc 2015; 17: 239–243

Apoptosis[1,2] likely contributes to organ injury in the setting of sepsis and systemic inflammatory response syndrome (SIRS).[3] Direct assessment of apoptosis is difficult[4-6] and one important marker is DNA fragmentation into nucleosomal units,[7,8] which may predict outcomes.[9] Recent research also established the crucial role of toll-like receptors (TLRs) in sepsis and SIRS.[10-17]

High cut-off (HCO) haemofilters[18] have improved performance in removing middle molecules (0.5 to 60kDa).[19-21] We designed a trial involving HCO filters (continuous venovenous haemofiltration [CVVH]-HCO) in critically ill patients with acute kidney injury (AKI) requiring vasopressor support. In a subset of patients, we performed a pilot assessment on plasma nucleosome levels and changes in TLR4 and TLR2 expression.

Methods

Our pilot investigation was nested within a randomised, double-blind, controlled trial (ClinicalTrials.gov/NCT00912184) approved by the Austin Hospital Human Research Ethics Committee (H2008/03400). Written informed consent was obtained from the patient or person responsible.

Patients with AKI and underlying shock requiring vasopressor infusion were recruited within 12 hours of commencing haemofiltration. Patients were randomised to either CVVH-HCO, using polyethersulfone filters with a nominal cut-off point of 100 kDa (Polyflux P2SH filters, 1.12 m^2, Gambro), or to standard CVVH (CVVH-std), using custom-manufactured control polyethersulfone filters with a nominal cut-off point of 30 kDa. The two study filters were identical in appearance and surface area.

The settings for CVVH included a blood flow of 200 mL/min, an ultrafiltration dose of 25 mL/kg/h and use of predilution bicarbonate-buffered replacement fluids. We excluded patients on maintenance dialysis and those who received CVVH during the same hospitalisation. Arterial blood samples were taken at baseline (T0), at 24 hours after initiation of CVVH (T24), and 72–96 hours after initiation of CVVH (T72). Blood was also sampled from the postfilter port at T24.

Measurement of plasma nucleosomes

We used the Cell Death Detection ELISA PLUS (10X) kit (Roche Diagnostics). A 20 μL volume of the sample was added to each well of streptavidin-coated 96-well plates, and 80 μL of the immunoreagent added, containing antihistone-biotin and antiDNA-POD monoclonal antibodies. The plates were incubated for 2 hours at 20°C on a shaker at 300 rpm, washed, and the reaction was developed with 2,2′-azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt solution (ABTS) (a water-soluble horseradish peroxidase substrate). Stop solution was added after 10–20

Table 1. Baseline characteristics and outcomes of study patients

Characteristic	High cut-off haemofiltration ($n = 6$)	Standard haemofiltration ($n = 7$)	*P*
Median age, years (IQR)	57.8 (46.4–64.3)	65 (62.3–72.2)	0.295
Sex (male/female), *n*	5/1	5/2	1.0
Median weight, kg (IQR)	85.5 (72.5–94.75)	80 (75–87.6)	0.731
Median APACHE II score (IQR)	19.5 (12–27)	23 (16.5–23.5)	0.731
Median APACHE 3 score (IQR)	63.5 (54.25–87)	72 (60.5–90.5)	0.731
Median SOFA score (IQR)			
Cardiovascular	4 (4–4)	4 (3.5–4)	0.445
Respiratory	3 (2.25–3)	3 (2–3.5)	1.0
Renal	2.5 (2–3.75)	2 (2–3.5)	0.731
Coagulation	0.5 (0–2.5)	0 (0–0.5)	0.445
Liver	2.5 (1.25–3)	0.5 (0–1)	0.093
Total of 5 SOFA scores	13 (10.5–14.75)	9 (8.5–11.5)	0.051
Median baseline creatinine within 1 year of date of admission, µmol/L (IQR)	86.6 (55.75–106)	80 (66.5–101.5)	0.628
Median RIFLE scores at start of CRRT (R/I/F)	0/2/4	1/2/4	0.629
Shock etiology (sepsis/cardiogenic/other), *n*	3/2/1	2/3/2	0.719
Median baseline mean arterial pressure at enrolment, mmHg (IQR)	70 (66.25–70)	75 (70–77.5)	0.234
Ventilated at enrolment (yes/no), *n*	5/1	5/2	1.0
Median serum urea at enrolment, mmol/L (IQR)	13.8 (10.93–18.33)	22.6 (13.65–26.9)	0.534
Median serum creatinine at enrolment, µmol/L (IQR)	247.5 (220.5–266.3)	217 (179–256)	0.628
Median blood lactate at enrolment, mmol/L (IQR)	2.7 (2.43–4.93)	1.4 (1.1–1.76)	0.002*
Median blood pH at enrolment (IQR)	7.37 (7.31–7.39)	7.34 (7.30–7.42)	0.731
Median international normalised ratio at enrolment (IQR)	1.85 (1.45–2.25)	1.3 (1.2–1.35)	0.022*
Median activated partial thromboplastin time at enrolment, seconds (IQR)	44 (31.25–47)	30 (24–37)	0.295
Median serum albumin at enrolment, g/dL (IQR)	34 (33.25–37.75)	27 (21.5–27.5)	0.014*
Median noradrenaline infusion at enrolment, µg/min (IQR)	17.5 (13.25–23.25)	5 (3–8)	0.051
Intensive care unit mortality, %	33.33%	28.57%	1.00
Hospital mortality, %	50%	28.57%	0.592

IQR = interquartile range. APACHE = Acute Physiology and Chronic Health Evaluation. SOFA = sequential organ failure assessment. RIFLE = risk, injury, failure, loss of kidney function, end-stage kidney disease. CRRT = continuous renal replacement therapy. * Statistically significant.

minutes (once adequate colour development was present) and plates were then read on a colorimetric plate reader (Synergy H1, BioTek) at 405 nm, with a reference wavelength of 490 nm. Nucleosome levels from one healthy human volunteer were also measured.

Validation

We conducted assay validation with purified nucleosomes provided in the kit as positive control. Values were normalised to the absorbance of the positive control sample and expressed as relative units.

To establish the intra-assay coefficient of variation, the positive control sample was analysed five times on three separate plates. To determine the interassay coefficient of variation, five different dilutions of the control nucleosomes were assayed in triplicate on three separate plates. The coefficients were defined by the standard deviation as a percentage of the mean. Each sample was assayed in triplicate on each plate (taking the mean value), and the mean and standard error from three separate assay runs were calculated.

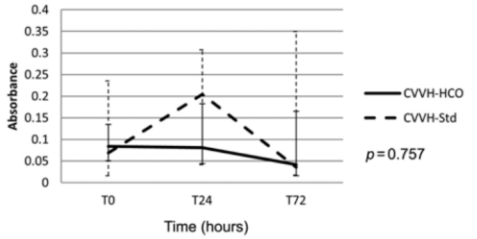
Measurement of nucleosomes from patients with sepsis

We added a 20 µL volume of the sample to each well in triplicate, and added 80 µL of immunoreagent. The assay then proceeded as described above. None of the samples gave values outside the working range of the assay.

Toll-like receptor analysis

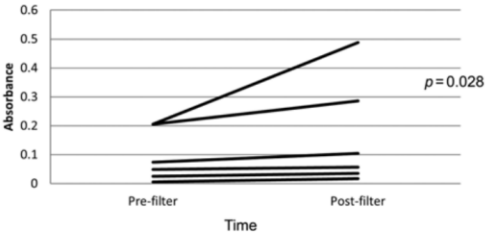
To measure peripheral blood mononuclear cell (PBMC) TLR expression, we performed cell surface staining on frozen PBMCs using human CD14-allophycocyanin/cyanine7

Figure 1. Median nucleosome levels in plasma at T0, T24 and T72, patients on CVVH-HCO v patients on CVVH-std*



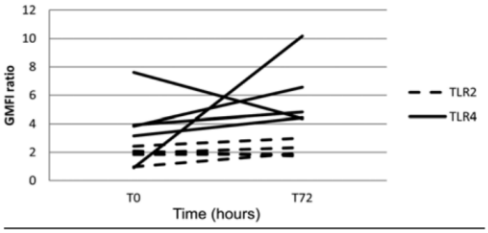
T0 = prefilter plasma at 0–12 hours after randomisation. T24 = prefilter plasma at 24 hours after randomisation. T72 = prefilter plasma at 72–96 hours after randomisation. CVVH-HCO = high cut-off continuous venovenous haemofiltration. CVVH-std = control/standard continuous venovenous haemofiltration. Absorbance = $[A_{405\,nm} - A_{490\,nm}]$.
* Nucleosome levels not detected in normal human plasma.

Figure 2. Median nucleosome levels in plasma sampled at 24 hours from the CVVH-HCO patients, prefilter v postfilter



CVVH-HCO = high cut-off continuous venovenous haemofiltration. Absorbance = $[A_{405\,nm} - A_{490\,nm}]$. Pre-filter = prefilter plasma at 24 hours after randomisation. Post-filter = postfilter plasma at 24 hours after randomisation.

Figure 3. TLR2 and TLR4 expression, T0 v T72 (CVVH-HCO)



CVVH-HCO = high cut-off continuous venovenous haemofiltration. GMFI = geometric mean channel fluorescence intensity (in normal control = 2.03). T0 = prefilter plasma at 0–12 hours after randomisation. T72 = prefilter plasma at 72–96 hours after randomisation.

(CD14-APCcy7), TLR2-fluorescein isothiocyanate (TLR2-FITC), and TLR4-R-phycoerythrin (TLR4-PE) conjugated monoclonal antibodies, as previously described.[22] Expression was assessed on the CD14+ monocyte. Relative fluorescence intensity was expressed as a ratio of the geometric mean fluorescence intensity (MFI) of the test sample to that of an isotype control stained sample.

Statistical analysis

Baseline characteristics were analysed using the Mann–Whitney *U* test for continuous data and χ^2 analysis for categorical data. Data on nucleosome levels were analysed using repeated-measures analysis of variance for between-group analysis. The Friedman test was used for within-group analysis. Prefilter v postfilter samples at T24 were compared using the paired *t* test. Correlations were analysed using the Pearson correlation test and statistical significance was defined as $P < 0.05$.

Results

We obtained samples from 13 patients for nucleosome analysis: six in the CVVH-HCO group and seven in the CVVH-std group. There were significant baseline differences between the groups in blood lactate levels ($P = 0.002$), serum albumin levels ($P = 0.014$) and international normalised ratio for prothrombin time ($P = 0.022$) (Table 1).

Validation experiments

Serially diluting the nucleosomes in assay buffer produced a linear decrease in absorbance down to a 1:50 dilution, and the nucleosomes in the positive control sample could be detected at a 1:100 dilution. Dilution in 10% or 25% human plasma did not affect the linearity of the assay. The assay showed excellent reproducibility, with an intra-assay coefficient of variation of 2.07 ± 0.22 %, and an interassay coefficient of variation of 6.96 ± 0.53 %.

Measurement of plasma nucleosomes

Nucleosomes were not detected in the plasma of the normal volunteer. There were no significant changes in median plasma levels over 72 hours within either group (CVVH-HCO, $P = 0.607$; CVVH-std, $P = 1.00$). There was also no significant difference in plasma levels over 72 hours between the two groups ($P = 0.757$) (Figure 1). There was a statistically significant but weak correlation between nucleosome levels at baseline and Acute Physiology and Chronic Health Evaluation (APACHE) III scores ($\rho = 0.475$; $P = 0.016$).

At T24, we analysed 15 samples for prefilter v postfilter nucleosome levels (six patients from the CVVH-HCO group [Figure 2] and nine patients from the CVVH-std group) and found that nucleosome levels increased significantly across

the filter for the CVVH-HCO group ($P = 0.016$), but did not in the CVVH-std group ($P = 0.294$)

TLR2 and TLR4 expression in the CVVH-HCO group

We analysed five patients in the CVVH-HCO group for TLR2 and TLR4 expression at T0 and T72 (Figure 3). We also analysed five patients for changes in TLR2 and TLR4 expression across the filter at T24 (prefilter v postfilter). TLR2 expression was increased in 40%–60% of samples (MFI ratio range, 0.97–3.98) compared with a reference MFI ratio of 2.0 (SD, 0.66). TLR4 expression showed a greater increase, involving 95% of analysed samples. The MFI ratio for TLR4 ranged from 0.91 to 10.18, compared with a normal reference MFI ratio of 1.55 (SD, 1.81).

There was no reduction in TLR2 and TLR4 expression over 72 hours of treatment. There was no reduction in median TLR2 and TLR4 expression due to passage of blood through the filter. There was no significant correlation between plasma nucleosome levels, TLR2 ($P = 0.304$) or TLR4 ($P = 0.235$) expression, or between TLR2 and TLR4 levels at baseline ($P = 0.068$).

Discussion

Key findings

Nucleosome levels were elevated and correlated weakly with APACHE III scores at randomisation. Treatment over 72 hours did not reduce plasma nucleosome levels, which increased following passage across HCO filters. TLR2 and TLR4 expression was also increased and did not decrease over 72 hours of treatment with CVVH-HCO.

Implications

Previous studies found correlation between nucleosome levels and patient outcome as well as disease severity.[9,23] We also found that nucleosome levels mildly correlated with illness severity. Our study, which was conducted under conditions of double-blind randomisation, found no effects of CVVH-HCO on circulating nucleosomes, which was similar to other interventions.[23-25] We previously found that HCO haemofiltration is superior in removing middle molecules.[19-21] Our findings suggest a lack of effect on nucleosome levels when using HCO filters, despite their increased pore size. We also found no effect on both TLR2 and TLR4 levels.

In summary, we could not find any benefit of HCO haemofiltration in terms of reducing a key aspect of the underlying apoptotic process.

Strengths and limitations

Our measurements over 72 hours of treatment provided an extended view of the effects of the interventions, but our sample size was small and subject to a high risk of errors.

Conclusions

Our pilot assessment of the effect of CVVH-HCO on plasma nucleosome levels and TLR expression found increased levels of proapoptotic mediators in patients with severe AKI and multiorgan failure, and weak correlation with illness severity. We found that CVVH-HCO had no effect on overall plasma nucleosome levels. Our findings also suggest that previous assessments of blood purification techniques focused on cytokines failed to capture changes in important components of the innate immune response to injury.

Competing interests

None declared.

Author details

Rafidah Atan, Intensivist, PhD Candidate[1]
Clive May, Principal Research Fellow[2]
Simon R Bailey, Senior Lecturer, Pre-Clinical Veterinary Sciences[3]
Marcel Tanudji, Research and Development Project Manager[4]
Kumar Visvanathan, Associate Professor,[5] Infectious Diseases Physician and Associate Professor,[6] and Adjunct Appointment[7]
Narelle Skinner, Research Associate[6]
Rinaldo Bellomo, Co-Director[8]
Hermann Goehl, Scientific Director, Special Membranes Project[9]
Markus Storr, Department Head, Research and Development[10]

1 Johor Bahru Clinical School, Monash University Malaysia, Johor, Malaysia.
2 Howard Florey Institute, Univerisity of Melbourne, Melbourne, VIC, Australia.
3 Faculty of Veterinary Science, University of Melbourne, Melbourne, VIC, Australia.
4 Sirtex Medical Ltd, Sydney, NSW, Australia.
5 Southern Clinical School, Faculty of Medicine, Nursing and Health Sciences, Monash University, Melbourne, VIC, Australia.
6 St. Vincent's Hospital, University of Melbourne, Melbourne, VIC, Australia.
7 Laboratory of Clinical Immunology/Microbiology, Rockefeller University, New York, NY, United States.
8 Australian and New Zealand Intensive Care Research Centre, Melbourne, VIC, Australia.
9 Department of Intensive Care, Austin Hospital, Melbourne, VIC, Australia.
10 Gambro Dialysatoren GmbH, Research and Development, Hechingen, Germany.

Correspondence: rinaldo.bellomo@austin.org.au

References

1 Kellum JA, Bellomo R, Mehta R, Ronco C. Blood purification in non-renal critical illness. *Blood Purif* 2003; 21: 6-13.

2 Rimmelé T, Kellum JA. Clinical review: blood purification for sepsis. *Crit Care* 2011; 15: 205.

3 Hotchkiss RS, Swanson PE, Freeman BD, et al. Apoptotic cell death in patients with sepsis, shock, and multiple organ dysfunction. *Crit Care Med* 1999; 27: 1230-51.

4 Lerolle N, Nochy D, Guérot E, et al. Histopathology of septic shock induced acute kidney injury: apoptosis and leukocytic infiltration. *Intensive Care Med* 2010; 36: 471-8.

5 Niu G, Chen X. Apoptosis imaging: beyond annexin V. *J Nucl Med* 2010; 51: 1659-62.

6 Atan R, Virzi GM, Peck L, et al. High cut-off hemofiltration versus standard hemofiltration: a pilot assessment of effects on indices of apoptosis. *Blood Purif* 2014; 37: 296-303.

7 Bortner CD, Oldenburg NB, Cidlowski JA. The role of DNA fragmentation in apoptosis. *Trends Cell Biol* 1995; 5: 21-6.

8 Zeerleder S, Zwart B, Wuillemin WA, et al. Elevated nucleosome levels in systemic inflammation and sepsis. *Crit Care Med* 2003; 31: 1947-51.

9 Chen Q, Ye L, Jin Y, et al. Circulating nucleosomes as a predictor of sepsis and organ dysfunction in critically ill patients. *Int J Infect Dis* 2012; 16: e558-64.

10 Medzhitov R. Toll-like receptors and innate immunity. *Nat Rev Immunol* 2001; 1: 135-45.

11 Lorne E, Dupont H, Abraham E. Toll-like receptors 2 and 4: initiators of non-septic inflammation in critical care medicine? *Intensive Care Med* 2010; 36: 1826-35.

12 Medzhitov R, Preston-Hurlburt P, Janeway CA Jr. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. *Nature* 1997; 388: 394-7.

13 O'Neill LA, Golenbock D, Bowie AG. The history of Toll-like receptors — redefining innate immunity. *Nat Rev Immunol* 2013; 13: 453-60.

14 Kawai T, Akira S. The roles of TLRs, RLRs and NLRs in pathogen recognition. *Int Immunol* 2009; 21: 317-37.

15 Fenhammar J, Rundgren M, Hultenby K, et al. Renal effects of treatment with a TLR4-inhibitor in conscious septic sheep. *Crit Care* 2014; 18: 488.

16 Fenhammar J, Rundgren M, Forestier J, et al. Toll-like receptor 4 inhibitor TAK-242 attenuates acute kidney injury in endotoxemic sheep. *Anesthesiology* 2011; 114: 1130-7.

17 Lin Q, Li M, Fang D, et al. The essential roles of Toll-like receptor signaling pathways in sterile inflammatory diseases. *Int Immunopharmacol* 2011; 11: 1422-32.

18 Boschetti-de-Fierro A, Voigt M, Storr M, Krause B. Extended characterization of a new class of membranes for blood purification: the HCO membranes. *Int J Artif Organs* 2013; 36: 455-63.

19 Atan R, Crosbie D, Bellomo R. Techniques of extracorporeal cytokine removal: a systematic review of the literature. *Blood Purif* 2012; 33: 88-100.

20 Atan R, Crosbie D, Bellomo R. Techniques of extracorporeal cytokine removal: a systematic review of the literature on animal experimental studies. *Int J Artif Organs* 2013; 36: 149-58.

21 Atan R, Crosbie DC, Bellomo R. Techniques of extracorporeal cytokine removal: a systematic review of human studies. *Ren Fail* 2013; 35: 1061-70.

22 Visvanathan K, Skinner NA, Thompson AJ, et al. Regulation of Toll-like receptor-2 expression in chronic hepatitis B by the precore protein. *Hepatology* 2007; 45: 102-10.

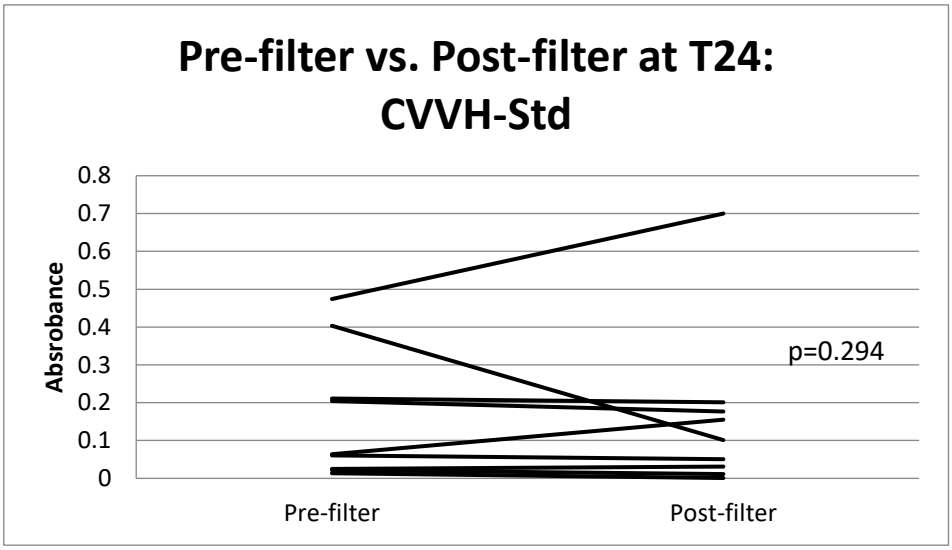
23 Zeerleder S, Stephan F, Emonts M, et al. Circulating nucleosomes and severity of illness in children suffering from meningococcal sepsis treated with protein C. *Crit Care Med* 2012; 40: 3224-9.

24 D'Auria F, Rovere-Querini P, Giazzon M, et al. Accumulation of plasma nucleosomes upon treatment with anti-tumour necrosis factor-alpha antibodies. *J Intern Med* 2004; 255: 409-18.

25 Stoetzer OJ, Fersching DM, Salat C, et al. Prediction of response to neoadjuvant chemotherapy in breast cancer patients by circulating apoptotic biomarkers nucleosomes, DNAse, cytokeratin-18 fragments and survivin. *Cancer Lett* 2013; 336: 140-8. ❑

Supplementary Material

Figure 3: Median nucleosome levels in plasma sampled at T24 from CVVH-Std group of patients: Pre-filter vs. Post-filter*



* Nucleosome levels not detected in normal human plasma

Absorbance = absorbance [$A_{405nm} – A_{490nm}$]

Pre-filter = pre-filter plasma at 24 hours after randomization

Post-filter = post-filter plasma at 24 hours after randomization

CVVH-Std: control/standard group

3.7 Effects on nucleosome levels and TLR expression: Summary

In this small substudy, nucleosome levels from DNA fragmentation were confirmed to be high in our study patients with shock states and acute injury. There is a weak correlation between illness severity as defined by APACHE III scores and nucleosome levels at baseline.

Observations in the CVVH-HCO group indicate that overall there is increase in nucleosome levels when blood crosses this filter. The numbers are too small for us to come to conclusions, but seem to suggest that CVVH-HCO results in higher apoptosis when blood crosses this filter in contrast to the findings of our earlier paper on apoptosis indices. There were no significant within and between group differences in nucleosome levels over 72 hours.

TLR2 and TLR 4 levels were also increased in majority of patients studied. We could not see any beneficial trends on TLR2 and TLR4 levels from treatment with CVVH-HCO.

3.8 Summary of studies on physiological and biological effects

At this juncture, it seems appropriate to summarise key aspects of the studies presented in Chapters 2 and 3 as they were all supported by the same RCT. We will review the strengths and weaknesses of the RCT including its substudies and present a short overall conclusion.

The strength of the RCT supporting these studies is in their low bias, high validity and strong methodology.

To our knowledge, this was the first study comparing high cut-off hemofiltration with standard hemofiltration in a randomised, double-blind manner. The two filters were indistinguishable to the naked eye and eliminated performance bias. During the whole conduct of the study, there was no identifiable way that the two groups could have been differentiated. Allocation sequence was only revealed at the time of data analysis after study completion. The removal of performance bias helped to ensure that any differences in outcome would have occurred as a result of the intervention.

This was also the first study to compare the two treatments under real-life conditions. From earlier studies, it was clear that the potential target group for high cut-off

hemofiltration were patients in hypercytokinemic conditions. The target population in our trial were patients in shock states, which would fulfil this prerequisite. Our systematic reviews also found ample evidence to suggest greater cytokine removal using HCO filters in these conditions.

The only established and accepted indication for hemofiltration is acute kidney injury in the presence of clear indications such as oliguria, fluid overload, severe acidosis or severe azotemia. Starting hemofiltration on the basis of hypercytokinemia alone was not an accepted indication despite its attractive concept. For this reason, our patients were not only in shock states, but also in acute kidney injury with at least one clear indication to commence hemofiltration. This offers the best balance between exploring extended indications and safety concerns.

Our study also provided real-life conditions in terms of duration of treatment. All previous trials, which were not blinded, studied the treatment over a shorter period of time, or following first pass circulation. To fully study the efficacy of this technique, we needed to study its use over a longer, more clinically relevant period of time i.e. at least 72 hours. Additionally, our patients were treated with the study filters for a maximum of two weeks or until death or recovery occurred. This reflects usage under real conditions. The decision to limit the duration of therapy to a maximum of two weeks was to reduce the cost of the trial. We were confident however, that if any benefit of high cut-off hemofiltration existed, it would be evident following a much shorter duration of intervention than the two weeks maximum period applied in our study.

Biomarkers were also studied over a 72-hour period. This offered a more sustained period of observation that provided a good balance between clinical relevance, feasibility and safety. The amount of blood removed from these patients for the purpose of measurement of biomarkers over the 72 hour period was reasonable and would not have endangered the patients.

The rationale for the conduct of this study was highly important. For many years, the subject of cytokine removal was studied as a specific intervention in hypercytokinemic states. High cut-off hemofiltration, as highlighted in earlier discussions in this thesis, offer distinct advantages over other more expensive and technically challenging methods. The method of application was highly intuitive and did not require specific training beyond standard training of those working with critically ill patients. Our double

blind randomised controlled trial was an opportunity to study this intervention compared to a standard intervention, under real conditions.

Our study had some limitations. The physiological and biological effects that were studied were all surrogate in nature. However, studies on surrogate outcomes are often necessary initial steps prior to large scale trials. A phase II assessment to monitor safety and efficacy is appropriate for any intervention that has potential for side effects and especially one that would involve critically ill and unstable patients. If our study successfully confirmed the benefits of high cut-off hemofiltration on important surrogate outcomes, we could then justify progression to a larger scale randomised controlled study.

Although this was a randomised trial, the baseline characteristics were not perfectly balanced. This was an unfortunate limitation of a phase II trial, due to the smaller number of subjects enrolled, and was especially enhanced in the case of the substudies as they involved an even smaller number of observations. For the mortality outcomes, we attempted to adjust for the differences but imbalances may still have had a measurable impact.

The baseline mortality rate for patients included in our study was very high and in the range of 60 to 70%. This posed real difficulties in completing the 72 hour observation period during the measurements of biomarkers. Many of our patients did not survive this timeline leading to a reduced number of observations.

Some of the biomarkers measured in this study, namely toll-like receptor measurements required fresh bloods. This reduced the opportunity of sampling bloods to that which only occurred during office hours. Many of our patients were admitted after hours and during weekends. This greatly reduced the number of observations.

The location of the laboratory measuring the samples was 25 km away from the intensive care unit that was treating the study patients. Initially, couriers were employed to transfer the blood samples, however when it was discovered that this caused an unacceptable delay, the bloods were subsequently transferred by the investigators themselves. The number of observations for TLR measurements was so low because many of the samples that were obtained were spoilt due to duration required for transfer of the samples, which were solved only by self-transportation. The number of

observations was further reduced by the fact that many of the patients did not survive the 72-hour period of observation. The small number of observations in the substudies on biological impact allowed us to comment only in terms of the signal observed.

At the end of conducting the above studies however, we were not able to demonstrate any benefit of high cut-off hemofiltration in terms of its biological and physiological impact.

Further discussions, including that on future directions will be explored in the next concluding chapter.

Chapter 4

Conclusions

4.1: Conclusions of the thesis

To conclude I would like to attempt to answer the research questions asked at the start of the thesis:

- Is there enough evidence in the literature to support the use of high cut-off hemofiltration as a technique of extracorporeal blood purification?
  Our extensive systematic reviews on this subject conducted under various conditions concluded that there was evidence from ex-vivo, animal and non-randomised human studies to support this theory.
- Does high cut-off hemofiltration result in improved haemodynamic stability, as reflected by vasopressor free time, compared to standard hemofiltration, which would suggest positive physiological effects?
  The findings of our blinded randomised controlled trial however did not find improved vasopressor-free time or other positive physiological effects of clinical relevance offered by high cut-off hemofiltration.
- Does high cut-off hemofiltration result in better removal of cytokines as compared to standard hemofiltration, which would suggest positive biological effects?
  Yes and no. Yes, there is evidence to support better sieving coefficient for cytokines such as IL-6 and IL-8. No, there is no evidence to support better sieving of other cytokines. Finally, regardless of the degree of removal offered for respective cytokines, we did not find evidence that this resulted in a reduction in plasma levels as a result of high cut-off hemofiltration.
- Does high cut-off hemofiltration positively impact other biological effects such as apoptosis indices, nucleosome levels and toll-like receptor expression, which may be affected in this subgroup of patients, when compared to standard hemofiltration?
  Within the limits of a small number of observations, we were not able to find any signal to indicate a positive impact of high cut-off hemofiltration in any of these indices.
- Are there any concerns especially in terms of protein loss associated with the use of high cut-off hemofiltration?
  Despite its initial and logical concern, any increase in protein loss did not translate to a significant reduction in plasma albumin levels compared to standard hemofilters. This is in the background of similar intravenous albumin administered to both groups.

Our detailed and rather extensive study on high cut-off hemofiltration as treatment for critically ill patients in shock states and acute kidney injury requiring hemofiltration did not find any benefits of the intervention despite its initial promise and highly logical approach.

4.2 Strengths and weaknesses of the thesis

This thesis has multiple strengths. The flow of the argument is straightforward and started with a literature review that was systematic and extensive, to establish a strong case for the pursuit of this subject. Thousands of abstracts were screened and the results were divided into three different levels of study; ex-vivo, animal and human studies, depicting important steps in the development of a new technology. The methodological requirements of systematic reviews also meant that another reviewer was involved in the search process. It was highly unlikely that important articles were missed at the point the searches were conducted.

Additionally, due to the time lag, the search was extended for the second review article to include new timelines. We were also required by the reviewers to repeat the search involving another database i.e. Embase. This extensive revision however produced a very small number of additional articles, which did not alter the direction of our conclusions. This gave us confidence that the original search was thorough enough.

The three reviews were all published, and has collectively received a respectable number of citations despite the fact that this remains a rather niche area of study. New citations of the reviews continue to emerge and we hope that this indicates that our reviews were of some value to other researchers.

The systematic reviews also had some limitations. One limitation was that it was a study on numerical values of various measures of clearance. Studies that only presented changes in plasma levels were excluded. We opted against studying plasma cytokine levels as this was subject to even greater variability. As discussed in our papers, changes in plasma levels may be due to factors which are unrelated to clearance by the device involved.

Adsorption devices were also under-represented in the reviews. Although this was not our specific area of study, any information on this particular intervention would have been valuable to current researchers. The low representation of adsorption devices

could be due to a number of reasons. Many were studies on endotoxin clearance which was not our measure of interest, while others looked only at plasma cytokine levels. Additionally, the lower number of hits for adsorption may be due the fact that hemofiltration was a more heavily studied approach at the time the searches were performed. Adsorption devices however, are current popular areas of study on the subject of blood purification.

As the first publication was in 2011, many of the studies included in the first systematic review were dated. The search was extended to November 2012 during our second and third paper, but that was still over five years ago. The subject of this thesis however involved mainly hemofiltration. The findings of the systematic reviews related to hemofiltration were unlikely to be affected as the number of studies on cytokine removal via hemofiltration techniques have significantly declined over recent years. We also mainly interrogated one database, namely Medline. However as highlighted in a preceding argument, we repeated the search using a second database. Yet, the yield from additional searches was very low. When we balanced the value of studying different databases versus the feasibility of doing so in view of the large number of abstracts to be screened, we are of the opinion that this would not have changed our conclusions.

The argument presented by the thesis then proceeded to the next section – an investigation on the effects of high cut-off hemofiltration under real conditions, in an appropriate target population.

The strengths and weaknesses of the RCT component of the thesis and its resulting substudies have been extensively discussed at the end of chapter 3. In summary, the main strengths of the RCT were its high validity and low bias design. At the time of writing, it was the only double blind RCT ever conducted using the high cut-off filter.

The outcomes studied, on both biological and physiological effects were fairly extensive and covered important aspects of potential interests to fellow researchers in this area of study.

The study was on an important application of the high cut-off filter i.e. CVVH, which remains one of the commonest modalities of renal replacement therapy used in critically

ill patients with acute kidney injury. The subjects recruited were reflective of the intended population i.e. patients with manifestations of hypercytokinemia.

The weaknesses of this section of thesis is summarised in the following points. The main RCT and its substudies looked at outcomes that were surrogate in nature. This step however is necessary in the course of establishing the effectiveness of any intervention. As it was a phase II equivalent, the numbers involved were small; furthermore many patients died due to the severity of their disease and that further reduced the number of observations especially for the smaller substudies. All of these issues may have interfered with the strength of the conclusions.

We included the level of important cytokines in our study but did not include important cytokines such as HMGB-1(high mobility group box 1), which is observed to rise later and persist longer (Wang 1999, Fink 2007). HMGB-1 is both passively released and actively secreted in sepsis and non-sepsis conditions (Yang 2015). It has a molecular weight of 25 kDa; potentially removable by the HCO filter. HMGB-1 levels were also reported to be altered as the result of EBP in an animal study (Peng 2012). The impact of this omission, including that of other possibly important mediators is minor due to the negative study results, but would have offered an additional contribution to the body of knowledge about this important molecule.

This thesis by publication is supported by an adequate number of papers; six published papers, one published abstract and one already submitted for publication.

4.3 Significance

The first part of the thesis on literature review was done extensively and established that there was a strong case to pursue HCO hemofiltration as adjunct therapy in sepsis and SIRS. The second part of the thesis, in the form of substudies supported by the RCT helped to answer this important question: Can HCO hemofiltration work under real conditions?

4.4 Future directions

A metaanalysis on extracorporeal blood purification concluded that blood purification in sepsis decreased mortality compared to no blood purification (Zhou 2013). The paper however combined all modalities into one entity, raising questions on the logic of pooling of data, as well as clinical applicability of the conclusion. On the other hand,

another review suggested that at our current stage of knowledge, it is unlikely that targeting cytokines will lead to patient benefit (Brown 2016).

We were not able to demonstrate any benefit of high cut-off hemofiltration from studying its biological and physiological impact. We do not recommend that further clinical studies on cytokine removal involve this particular device. We cannot justify recommending that this study progresses to a larger study.

In terms of future directions in the use of HCO filters, one promising area is its use in myeloma cast nephropathy. A recently published RCT on this subject however did not conclusively establish its efficacy in this regard, as no benefit was found in its primary outcome of hemodialysis independence at three months (Bridoux 2017). The authors however found benefit with extended analyses at six months and 12 months, and called for further studies to be conducted.

Rhabdomyolysis is another clinical condition currently studied for potential application of the HCO filter. The MW of myoglobin is 16.7 kDa, which would allow passage through standard filters. A Cochrane review in 2014, involving only three studies, found no advantage of standard CRRT over no CRRT in this condition (Zeng 2014); although essentially what the review highlighted was the lack of trials in this area. A number of case studies claimed greater clearance of myoglobin with HCO filters (Naka 2005, Albert 2012, Heyne 2012); perhaps the larger pore size of the high cut-off filter may help to protect efficacy compared to standard filters when membrane fouling occurs. The rationale for using the HCO filter therefore lies in its greater ability to remove myoglobin molecules and possibly reducing the risk of kidney injury in this condition. One RCT on the use of HCO filter in rhabdomyolysis was found registered with ClinicalTrials.gov, with myoglobin plasma levels after 48 hours as its primary outcome. The study has finished recruiting patients but has yet to be published (ClinicalTrials.gov Identifier: NCT01467180).

In terms of future directions in blood purification and cytokine removal, important current modalities include adsorption techniques. This focus is supported by the findings of the systematic review by Zhou et al. which highlighted that the benefit of blood purification in sepsis was mainly driven by studies on adsorption and plasma exchange (Zhou 2013). While the use of plasma exchange in this regard continue to be hampered by concerns regarding costs and safety, adsorption devices seem to be current hot topics

in our search for an effective blood purification device. Prominent examples include Polymyxin-B (PMX) and CytoSorb® hemoperfusion devices.

Polymyxin-B hemoperfusion (PMX) is possibly the most studied adsorption technique in clinical trials, resulting in a number of well designed studies including multicentre randomised trials. In 2009, an RCT involving patients in septic shock found significant benefits in the application of PMX, in terms of hemodynamic improvements and mortality (Cruz 2009). The study however was not conclusive as it was terminated early due to meeting a stopping rule after recruiting only about 50% of the intended number of patients. Subsequent RCTs however found conflicting results. The ABDO-MIX trial, a multicentre RCT studying PMX in patients with abdominal sepsis, found no difference in mortality at day 28; but also, with some concern, a non significant increase in mortality in the PMX group at day 90 (Payen 2015). The EUPHRATES trial, an even larger multicentre RCT, also found no difference in mortality at day 28 (Klein 2014, Iba 2017). Mortality benefit however, was found when a post-hoc subgroup analysis was attempted, initiating calls for further studies. The full results of this study are yet to be published.

CytoSorb, a biocompatible polymer, has also received significant attention. Important recent publications on this topic include an RCT, involving patients in septic shock and ARDS (acute respiratory distress syndrome), studying the effects of the intervention on IL-6 plasma levels. The researchers however found no difference in IL-6 plasma levels in the CytoSorb group when compared to no hemoperfusion. The crude 60-day mortality analysis were also higher in the treatment group, with no difference found following adjusted analysis (Schadler 2017). Another recent paper on CytoSorb published findings from a multicentre registry on CytoSorb use, involving 198 patients from 130 centres from 22 countries. Preliminary results reported improved observed mortality compared to predicted mortality with markedly reduced IL-6 levels following treatment (Friesecke 2017). It is clear that evidence supporting clinical application of CytoSorb is still lacking and mostly in preliminary stages.

Cytokine removal as adjunctive therapy in critical illness remains an attractive concept to some researchers. The excitement is somewhat dampened due to a series of negative trials; even those reporting positive effects failed to do so in a definitive manner. Our state of knowledge on cytokine networks and characteristics of important

mediators however, continue to expand. Failures of clinical trials, although disappointing, contribute important knowledge and progress, and may provide crucial insights in deciding our future directions. We hope that our work has also contributed to this journey.

References

Adib-Conquy M, Cavaillon JM. Stress molecules in sepsis and systemic inflammatory response syndrome. FEBS Lett. 2007 Jul 31;581(19):3723-33.

Albert C, Haase M, Bellomo R, Mertens PR. High cut-off and high-flux membrane haemodialysis in a patient with rhabdomyolysis-associated acute kidney injury. Critical care and resuscitation : journal of the Australasian Academy of Critical Care Medicine. 2012;14(2):159-62.

Allam R, Scherbaum CR, Darisipudi MN et al. Histones from dying renal cells aggravate kidney injury via TLR2 and TLR4. J Am Soc Nephrol. 2012 Aug;23(8):1375-88.

Annane D, Bellissant E, Cavaillon JM. Septic shock. Lancet (London, England). 2005;365(9453):63-78.

Antunes N, Dragosavc D, Petrucci Junior O, Oliveira PP, Kosour C, Blotta MH et al. The use of ultrafiltration for inflammatory mediators removal during cardiopulmonary bypass in coronary artery bypass graf surgery. Rev Bras Cir Cardiovasc 2008;23:175-82.

Atkins BZ, Danielson DS, Fitzpatrick CM, Dixon P, Petersen RP, Carpenter AJ. Modified ultrafiltration attenuates pulmonary-derived inflammatory mediators in response to cardiopulmonary bypass. Interact Cardiovasc Thorac Surg. 2010 Nov;11(5):599-603.

Awad SS, Sawada S, Soldes OS, Rich PB, Klein R, Alarcon WH et al. Can the clearance of tumor necrosis factor alpha and interleukin 6 be enhanced using an albumin dialysate hemodiafiltration system? ASAIO J. 1999 Jan-Feb;45(1):47-9.

Beale R, Reinhart K, Brunkhorst F, Dobb G, Levy M, Martin G. Promoting Global Research Excellence in Severe Sepsis (PROGRESS): Lessons from an International Sepsis Registry. Infection 2009; 37:222-232.

Bellomo R. Blood purification in sepsis: reasonable scientific hypothesis or pipe dream? Crit Care Resusc. 2001 Sep;3(3):202-5.

Bellomo R, Kellum JA, Gandhi CR, Pinsky MR, Ondulik B. The effect of intensive plasma water exchange by hemofiltration on hemodynamics and soluble mediators in canine endotoxemia. Am J Respir Crit Care Med. 2000 May;161(5):1429-36.

Bellomo R, Tipping P, Boyce N. Continuous veno-venous hemofiltration with dialysis removes cytokines from the circulation of septic patients. Crit Care Med 1993;21:522-6.

Bellomo R, Tipping P, Boyce N. Interleukin-6 and interleukin-8 extraction during continuous venovenous hemodiafiltration in septic acute renal failure. Ren Fail. 1995;17:457-66.

Berdat PA, Eichenberger E, Ebell J, Pfammatter JP, Pavlovic M, Zobrist C et al. Elimination of proinflammatory cytokines in pediatric cardiac surgery: analysis of ultrafiltration method and filter type. J Thorac Cardiovasc Surg 2004;127:1688-96.

Bernard GR, Francois B, Mira JP, Vincent JL, Dellinger RP, Russell JA, Larosa SP, Laterre PF, Levy MM, Dankner W, Schmitt N, Lindemann J, Wittebole X. Evaluating the efficacy and safety of two doses of the polyclonal anti-tumor necrosis factor-α fragment antibody AZD9773 in adult patients with severe sepsis and/or septic shock: randomized, double-blind, placebo-controlled phase IIb study*. Crit Care Med. 2014 Mar;42(3):504-11.

Bilbault P, Lavaux T, Lahlou A, Uring-Lambert B, Gaub MP, Ratomponirina C et al. Transient Bcl-2 gene down-expression in circulating mononuclear cells of severe sepsis patients who died despite appropriate intensive care. Intensive Care Med. 2004 Mar;30(3):408-15.

Bloom J, Sun S, Al-Abed Y. MIF, a controversial cytokine: a review of structural features, challenges, and opportunities for drug development. Expert opinion on therapeutic targets. 2016;20(12):1463-75.

Bogă M, Islamoğlu, Badak I, Cikirikçioğlu M, Bakalim T, Yağdi T et al. The effects of modified hemofiltration on inflammatory mediators and cardiac performance in coronary artery bypass grafting. Perfusion 2000;15:143-50.

Bone RC. Immunologic dissonance: a continuing evolution in our understanding of the systemic inflammatory response syndrome (SIRS) and the multiple organ dysfunction syndrome (MODS). Ann Intern Med. 1996 Oct 15;125(8):680-7.

Bone RC. Sir Isaac Newton, sepsis, SIRS, and CARS. Crit Care Med. 1996 Jul;24(7):1125-8.

Bordoni V, Bolgan I, Brendolan A, Crepaldi C, Gastaldon F, D'intini V et al. Caspase-3 and -8 activation and cytokine removal with a novel cellulose triacetate super-permeable membrane in an in vitro sepsis model. Int J Artif Organs. 2003 Oct;26(10):897-905.

Bortner CD, Oldenburg NB, Cidlowski JA. The role of DNA fragmentation in apoptosis. Trends Cell Biol. 1995 Jan;5(1):21-6.

Boschetti-de-Fierro A, Voigt M, Storr M, Krause B. Extended characterization of a new class of membranes for blood purification: the high cut-off membranes. Int J Artif Organs. 2013 Jul;36(7):455-63.

Bosmann M, Ward PA. The inflammatory response in sepsis. Trends in immunology. 2013;34(3):129-36.

Bottoms G, Fessler J, Murphey E, Johnson M, Latshaw H, Mueller B, Clark W, Macias W. Efficacy of convective removal of plasma mediators of endotoxic shock by continuous veno-venous hemofiltration. Shock. 1996 Feb;5(2):149-54.

Bouman CS, van Olden RW, Stoutenbeek CP. Cytokine filtration and adsorption during pre- and postdilution hemofiltration in four different membranes. Blood Purif. 1998;16(5):261-8.

Brain M, Anderson M, Parkes S, Fowler P. Magnesium flux during continuous venovenous haemodiafiltration with heparin and citrate anticoagulation. Crit Care Resusc. 2012 Dec;14(4):274-82.

Brain M, Parkes S, Fowler P, Robertson I, Brown A. Calcium flux in continuous venovenous haemodiafiltration with heparin and citrate anticoagulation. Crit Care Resusc. 2011 Jun;13(2):72-81.

Brancaccio G, Villa E, Girolami E, Michielon G, Feltri C, Mazzera E et al. Inflammatory cytokines in pediatric cardiac surgery and variable effect of the hemofiltration process. Perfusion 2005;20:263-8.

Bridoux F, Carron PL, Pegourie B, Alamartine E, Augeul-Meunier K, Karras A, et al. Effect of High-Cutoff Hemodialysis vs Conventional Hemodialysis on Hemodialysis Independence Among Patients With Myeloma Cast Nephropathy: A Randomized Clinical Trial. Jama. 2017;318(21):2099-110.

Brown KA, Brown GA, Lewis SM, Beale R, Treacher DF. Targeting cytokines as a treatment for patients with sepsis: A lost cause or a strategy still worthy of pursuit? International immunopharmacology. 2016;36:291-9.

Byrick RJ, Goldstein MB, Wong PY. Increased plasma tumor necrosis factor concentration in severe rhabdomyolysis is not reduced by continuous arteriovenous hemodialysis. Crit Care Med 1992;20:1483-6.

Cabioglu N, Bilgic S, Deniz G et al. Decreased cytokine expression in peripheral blood leukocytes of patients with severe sepsis. Archives of surgery (Chicago, Ill : 1960). 2002;137(9):1037-43; discussion 43.

Cabre L, Mancebo J, Solsona JF, et al. Multicenter study of the multiple organ dysfunction syndrome in intensive care units: The usefulness of Sequential Organ Failure Assessment scores in decision making. Intensive Care Med 2005;31:927–933.

Cantaluppi V, Assenzio B, Pasero D, Romanazzi GM, Pacitti A, Lanfranco G et al. Polymyxin-B hemoperfusion inactivates circulating proapoptotic factors. Intensive Care Med. 2008 Sep;34(9):1638-45.

Calandra T, Echtenacher B, Roy DL, Pugin J, Metz CN, Hültner L, et al. Protection from septic shock by neutralization of macrophage migration inhibitory factor.

Chaput C, Zychlinsky A. Sepsis: the dark side of histones. Nat Med. 2009 Nov;15(11):1245-6.

Chen Q, Ye L, Jin Y et al. Circulating nucleosomes as a predictor of sepsis and organ dysfunction in critically ill patients. Int J Infect Dis. 2012 Jul;16(7):e558-64.

Cinel I, Opal SM. Molecular biology of inflammation and sepsis: a primer. Crit Care Med 2009;37:291-304.

Clar A, Bowers MC, Larson DF. Derivation of sieving coefficients to determine the efficacy of the hemoconcentrator in removal of four inflammatory mediators produced during cardiopulmonary bypass. ASAIO J. 1997;43:163-70.

Cohen J. The immunopathogenesis of sepsis. Nature. 2002;420(6917):885-91.

Cole L, Bellomo R, Davenport P, Tipping P, Ronco C. Cytokine removal during continuous renal replacement therapy: an ex vivo comparison of convection and diffusion. Int J Artif Organs. 2004 May;27(5):388-97.

Cole L, Bellomo R, Davenport P, Tipping P, Uchino S, Tetta C, Ronco C. The effect of coupled hemofiltration and adsorption on inflammatory cytokines in an ex vivo model. Nephrol Dial Transplant. 2002 Nov;17(11):1950-6.

Cole L, Bellomo R, Journois D, Davenport P, Baldwin I, Tipping P. High-volume hemofiltration in human septic shock. Intensive Care Med. 2001;27:978-86.

Cruz DN, Antonelli M, Fumagalli R, Foltran F, Brienza N, Donati A, Malcangi V, Petrini F, Volta G, Bobbio Pallavicini FM, Rottoli F, Giunta F, Ronco C. Early use of polymyxin B hemoperfusion in abdominal septic shock: the EUPHAS randomized controlled trial. JAMA. 2009 Jun 17;301(23):2445-52.

Dahaba AA, Elawady GA, Rehak PH, List WF. Procalcitonin and proinflammatory cytokine clearance during continuous venovenous hemofiltration in septic patients. Anaesth Intensive Care 2002;30:269-74.

D'Auria F, Rovere-Querini P, Giazzon M et al. Accumulation of plasma nucleosomes upon treatment with anti-tumour necrosis factor-alpha antibodies. J Intern Med. 2004 Mar;255(3):409-18.

De Vriese AS, Colardyn FA, Philippé JJ, Vanholder RC, De Sutter JH, Lameire NH. Cytokine removal during continuous hemofiltration in septic patients. J Am Soc Nephrol 1999;10:846-53.

Delanaye P, Lambermont B, Dogné JM, Dubois B, Ghuysen A, Janssen N et al. Confirmation of high cytokine clearance by hemofiltration with a cellulose triacetate membrane with large pores: an in vivo study. Int J Artif Organs. 2006 Oct;29(10):944-8.

Diaz C, Schroit AJ. Role of translocases in the generation of phosphatidylserine asymmetry. J Membr Biol. 1996 May;151(1):1-9.

Dinarello CA. Anti-cytokine therapies in response to systemic infection. The journal of investigative dermatology Symposium proceedings. 2001;6(3):244-50.

Dinarello CA. Historical insights into cytokines. Eur J Immunol. 2007 Nov;37 Suppl 1:S34-45.

Dinarello CA. Proinflammatory cytokines. Chest. 2000 Aug;118(2):503-83.

Dittrich S, Aktuerk D, Seitz S, Mehwald P, Schulte-Mönting J, Schlensak C et al. Effects of ultrafiltration and peritoneal dialysis on proinflammatory cytokines during cardiopulmonary bypass surgery in newborns and infants. Eur J Cardiothorac Surg 2004;25:935-40.

Ewalenko P, Deloof T, Peeters J. Composition of fresh frozen plasma. Critical care medicine. 1986;14(2):145-6.

Fadok VA, Voelker DR, Campbell PA, Cohen JJ, Bratton DL, Henson PM. Exposure of phosphatidylserine on the surface of apoptotic lymphocytes triggers specific recognition and removal by macrophages. J Immunol. 1992 Apr 1;148(7):2207-16.

Fenhammar J, Rundgren M, Forestier J, Kalman S, Eriksson S, Frithiof R. Toll-like receptor 4 inhibitor TAK-242 attenuates acute kidney injury in endotoxemic sheep. Anesthesiology. 2011 May;114(5):1130-7.

Fenhammar J, Rundgren M, Hultenby K et al. Renal effects of treatment with a TLR4-inhibitor in conscious septic sheep. Crit Care. 2014 Sep 3;18(5):488.

Fink MP. Bench-to-bedside review: High-mobility group box 1 and critical illness. Critical care (London, England). 2007;11(5):229.

Fisher CJ Jr, Dhainaut JF, Opal SM, Pribble JP, Balk RA, Slotman GJ et al. Recombinant human interleukin 1 receptor antagonist in the treatment of patients with sepsis syndrome. Results from a randomized, double-blind, placebo-controlled trial. Phase III rhIL-1ra Sepsis Syndrome Study Group. JAMA 1994;271(23):1836-43.

Fisher CJ, Jr., Slotman GJ, Opal SM et al. Initial evaluation of human recombinant interleukin-1 receptor antagonist in the treatment of sepsis syndrome: a randomized, open-label, placebo-controlled multicenter trial. Critical care medicine. 1994;22(1):12-21.

Frencken JF, van Vught LA, Peelen LM, Ong DSY, Klein Klouwenberg PMC, Horn J, et al. An Unbalanced Inflammatory Cytokine Response Is Not Associated With Mortality Following Sepsis: A Prospective Cohort Study. Critical care medicine. 2017;45(5):e493-e9.

Friesecke S, Trager K, Schittek GA, Molnar Z, Bach F, Kogelmann K, et al. International registry on the use of the CytoSorb(R) adsorber in ICU patients : Study protocol and preliminary results. Medizinische Klinik, Intensivmedizin und Notfallmedizin. 2017.

Giamarellos-Bourboulis EJ, Routsi C, Plachouras D, Markaki V, Raftogiannis M, Zervakis D et al. Early apoptosis of blood monocytes in the septic host: is it a mechanism of protection in the event of septic shock? Crit Care. 2006;10(3):R76.

Glogowski KR, Stammers AH, Niimi KS, Tremain KD, Muhle ML, Trowbridge CC. The effect of priming techniques of ultrafiltrators on blood rheology: an in vitro evaluation. Perfusion. 2001 May;16(3):221-8.

Goldfarb S, Golper TA. Proinflammatory cytokines and hemofiltration membranes. J Am Soc Nephrol. 1994 Aug;5(2):228-32.

Grieb G. Macrophage migration inhibitory factor (MIF): a promising biomarker. 2010;23(4):257-64.

Haase M, Bellomo R, Baldwin I, Haase-Fielitz A, Fealy N, Davenport P, et al. Hemodialysis membrane with a high-molecular-weight cutoff and cytokine levels in sepsis complicated by acute renal failure: a phase 1 randomized trial. American journal of kidney diseases : the official journal of the National Kidney Foundation. 2007;50(2):296-304.

Haase M, Bellomo R, Morger S, Baldwin I, Boyce N. High cut-off point membranes in septic acute renal failure: A systematic review. Int J Artif Organs 2007; 30(12): 1031-41

Hansen TG, Tønnesen E, Toft P, Bendtzen K. Interleukin-6 (IL-6) is not removed from plasma during experimental hemofiltration. Acta Anaesthesiol Scand. 1998 Oct;42(9):1129.

Havasi A, Borkan SC. Apoptosis and acute kidney injury. Kidney Int. 2011 Jul;80(1):29-40.

Heering P, Morgera S, Schmitz FJ, Schmitz G, Willers R, Schultheiss HP et al. Cytokine removal and cardiovascular hemodynamics in septic patients with continuous venovenous hemofiltration. Intensive Care Med 1997;23:288-96.

Heidemann SM, Ofenstein JP, Sarnaik AP. Efficacy of continuous arteriovenous hemofiltration in endotoxic shock. Circ Shock. 1994 Dec;44(4):183-7.

Heidemann SM, Sarnaik AP. Protective effects of a thromboxane synthetase inhibitor and continuous arteriovenous hemofiltration in rat endotoxic shock. Prostaglandins Leukot Essent Fatty Acids. 1997 Jun;56(6):473-8.

Heyne N, Guthoff M, Krieger J, Haap M, Haring HU. High cut-off renal replacement therapy for removal of myoglobin in severe rhabdomyolysis and acute kidney injury: a case series. Nephron Clinical practice. 2012;121(3-4):c159-64.

Hirsiger S, Simmen HP, Werner CM, Wanner GA, Rittirsch D. Danger signals activating the immune response after trauma. Mediators of inflammation. 2012;2012:315941.

Ho DW, Fan ST, To J, Woo YH, Zhang Z, Lau C et al. Selective plasma filtration for treatment of fulminant hepatic failure induced by D-galactosamine in a pig model. Gut. 2002 Jun;50(6):869-76.

Hoffmann JN, Faist E, Deppisch R, Hartl WH, Inthorn D. Hemofiltration in human sepsis: evidence for elimination of immunomodulatory substances. Contrib Nephrol 1995;116:76-9.

Hoffmann JN, Hartl WH, Deppisch R, Faist E, Jochum M, Inthorn D. Effect of hemofiltration on hemodynamics and systemic concentrations of anaphylatoxins and cytokines in human sepsis. Intensive Care Med 1996;22:1360-7.

Hoffmann JN, Hartl WH, Deppisch R, Faist E, Jochum M, Inthorn D. Hemofiltration in human sepsis: evidence for elimination of immunomodulatory substances. Kidney international. 1995;48(5):1563-70.

Hoffmann JN, Werdan K, Hartl WH, Jochum M, Faist E, Inthorn D. Hemofiltrate from patients with severe sepsis and depressed left ventricular contractility contains cardiotoxic compounds. Shock 1999;12:174-80.

Homburg CH, de Haas M, von dem Borne AE, Verhoeven AJ, Reutelingsperger CP, Roos D. Human neutrophils lose their surface Fc gamma RIII and acquire Annexin V binding sites during apoptosis in vitro. Blood. 1995 Jan 15;85(2):532-40.

Honore PM, Jacobs R, Joannes-Boyau O et al. Newly designed CRRT membranes for sepsis and SIRS--a pragmatic approach for bedside intensivists summarizing the more recent advances: a systematic structured review. ASAIO journal (American Society for Artificial Internal Organs : 1992). 2013;59(2):99-106.

Hotchkiss RS, Moldawer LL, Opal SM, Reinhart K, Turnbull IR, Vincent JL. Sepsis and septic shock. Nature reviews Disease primers. 2016;2:16045.

Hotchkiss RS, Swanson PE, Freeman BD, Tinsley KW, Cobb JP, Matuschak GM, Buchman TG, Karl IE. Apoptotic cell death in patients with sepsis, shock, and multiple organ dysfunction. Crit Care Med. 1999 Jul;27(7):1230-51.

Huang H, Evankovich J, Yan W et al. Endogenous histones function as alarmins in sterile inflammatory liver injury through Toll-like receptor 9 in mice. Hepatology. 2011 Sep 2;54(3):999-1008.

Iba T, Fowler L. Is polymyxin B-immobilized fiber column ineffective for septic shock? A discussion on the press release for EUPHRATES trial. Journal of intensive care. 2017;5:40.

Ishihara S, Ward JA, Tasaki O, Brinkley WW, Seraile LG, Pruitt BA Jr et al. Effects of long-term hemofiltration on circulating mediators and superoxide production during continuous endotoxin administration. J Trauma. 1999 May;46(5):894-9.

Jacobs R, Honore PM, Joannes-Boyau O, Boer W, De Regt J, De Waele E et al. Septic acute kidney injury: the culprit is inflammatory apoptosis rather than ischemic necrosis. Blood Purif. 2011;32(4):262-5.

Jekarl DW, Kim JY, Lee S et al. Diagnosis and evaluation of severity of sepsis via the use of biomarkers and profiles of 13 cytokines: a multiplex analysis. Clinical chemistry and laboratory medicine. 2015;53(4):575-81.

Joannes-Boyau O, Honore PM, Perez P, Bagshaw SM, Grand H, Canivet JL, et al. High-volume versus standard-volume hemofiltration for septic shock patients with acute

kidney injury (IVOIRE study): a multicentre randomized controlled trial. Intensive care medicine. 2013;39(9):1535-46.

Kade G, Lubas A, Rzeszotarska A, Korsak J, Niemczyk S. Effectiveness of High Cut-Off Hemofilters in the Removal of Selected Cytokines in Patients During Septic Shock Accompanied by Acute Kidney Injury-Preliminary Study. Medical science monitor : international medical journal of experimental and clinical research. 2016;22:4338-44.

Kang R, Chen R, Zhang Q, Hou W, Wu S, Cao L, et al. HMGB1 in health and disease. Molecular aspects of medicine. 2014;40:1-116.

Kaushal GP, Basnakian AG, Shah SV. Apoptotic pathways in ischemic acute renal failure. Kidney Int. 2004 Aug;66(2):500-6.

Kawai T, Akira S. The roles of TLRs, RLRs and NLRs in pathogen recognition. Int Immunol. 2009 Apr;21(4):317-37.

Kellum JA, Bellomo R, Mehta R, Ronco C. Blood purification in non-renal critical illness. Blood Purif. 2003;21(1):6-13.

Kellum JA, Johnson JP, Kramer D, Palevsky P, Brady JJ, Pinsky MR. Diffusive vs. convective therapy: effects on mediators of inflammation in patient with severe systemic inflammatory response syndrome. Crit Care Med. 1998;26(12):1995-2000.

Kellum JA, Kong L, Fink MP, Weissfeld LA, Yealy DM, Pinsky MR, et al. Understanding the inflammatory cytokine response in pneumonia and sepsis: results of the Genetic and Inflammatory Markers of Sepsis (GenIMS) Study. Arch Intern Med. 2007;167(15):1655-63.

Kellum JA, Song M, Venkataraman R. Hemoadsorption removes tumor necrosis factor, interleukin-6, and interleukin-10, reduces nuclear factor-kappaB DNA binding, and improves short-term survival in lethal endotoxemia. Crit Care Med. 2004 Mar;32(3):801-5.

Khanna A, English SW, Wang XS, Ham K, Tumlin J, Szerlip H, Busse LW, Altaweel L, Albertson TE, Mackey C, McCurdy MT, Boldt DW, Chock S, Young PJ, Krell K, Wunderink RG, Ostermann M, Murugan R, Gong MN, Panwar R, Hästbacka J, Favory R, Venkatesh B, Thompson BT, Bellomo R, Jensen J, Kroll S, Chawla LS, Tidmarsh

GF, Deane AM; ATHOS-3 Investigators. Angiotensin II for the Treatment of Vasodilatory Shock. N Engl J Med. 2017 Aug 3;377(5):419-430.

Kiziltepe U, Uysalel A, Corapcioglu T, Dalva K, Akan H, Akalin H. Effects of combined conventional and modified ultrafiltration in adult patients. Ann Thorac Surg. 2001;71:684-93.

Klein DJ, Foster D, Schorr CA, Kazempour K, Walker PM, Dellinger RP. The EUPHRATES trial (Evaluating the Use of Polymyxin B Hemoperfusion in a Randomized controlled trial of Adults Treated for Endotoxemia and Septic shock): study protocol for a randomized controlled trial. Trials. 2014;15:218.

Kono H, Rock KL. How dying cells alert the immune system to danger. Nat Rev Immunol. 2008 Apr;8(4):279-89.

Lacy P, Stow JL. Cytokine release from innate immune cells: association with diverse membrane trafficking pathways. Blood. 2011 Jul 7;118(1):9-18.

Lambermont B, Delanaye P, Dogné JM, Ghuysen A, Janssen N, Dubois B et al.. Large-pore membrane hemofiltration increasescytokine clearance and improves right ventricular-vascular coupling duringendotoxic shock in pigs. Artif Organs. 2006 Jul;30(7):560-4.

Lauw FN, Simpson AJ, Prins JM et al. Elevated plasma concentrations of interferon (IFN)-gamma and the IFN-gamma-inducing cytokines interleukin (IL)-18, IL-12, and IL-15 in severe melioidosis. The Journal of infectious diseases. 1999;180(6):1878-85.

Lee WC, Uchino S, Fealy N, Baldwin I, Panagiotopoulos S, Goehl H et al. Super high flux hemodialysis at high dialysate flows: an ex vivo assessment. Int J Artif Organs. 2004 Jan;27(1):24-8.

Lerolle N, Nochy D, Guérot E et al. Histopathology of septic shock induced acute kidney injury: apoptosis and leukocytic infiltration. Intensive Care Med. 2010 Mar;36(3):471-8.

Lever A, Mackenzie I. Sepsis: definition, epidemiology,and diagnosis BMJ 2007;335:879-832.

Lin Q, Li M, Fang D, Fang J, Su SB. The essential roles of Toll-like receptor signaling pathways in sterile inflammatory diseases. Int Immunopharmacol. 2011 Oct;11(10):1422-32.

Linkermann A, De Zen F, Weinberg J, Kunzendorf U, Krautwald S. Programmed necrosis in acute kidney injury. Nephrol Dial Transplant. 2012 Sep;27(9):3412-9.

Livigni S, Bertolini G, Rossi C, Ferrari F, Giardino M, Pozzato M, Remuzzi G. Efficacy of coupled plasma filtration adsorption (CPFA) in patients with septic shock: A multicenter randomised controlled clinical trial. BMJ Open. 2014 Jan 8;4(1):e003536.

Lorne E, Dupont H, Abraham E. Toll-like receptors 2 and 4: initiators of non-septic inflammation in critical care medicine? Intensive Care Med. 2010 Nov;36(11):1826-35.

Lydon A, Martyn JA. Apoptosis in critical illness. Int Anesthesiol Clin. 2003 Winter; 41(1):65-77.

Mariano F, Fonsato V, Lanfranco G, Pohlmeier R, Ronco C, Triolo G et al. Tailoring high-cut-off membranes and feasible application in sepsis-associated acute renal failure: in vitro studies. Nephrol Dial Transplant. 2005 Jun; 20(6):1116-26.

Mariano F, Tetta C, Guida G, Triolo G, Camussi G. Hemofiltration reduces the serum priming activity on neutrophil chemiluminescence in septic patients. Kidney Int. 2001;60:1598-605.

Martin SJ, Reutelingsperger CP, McGahon AJ, Rader JA, van Schie RC, LaFace DM, Green DR. Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus: inhibition by overexpression of Bcl-2 and Abl. J Exp Med. 1995 Nov 1;182(5):1545-56.

Medzhitov R, Preston-Hurlburt P, Janeway CA Jr. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. Nature. 1997 Jul 24;388(6640):394-7.

Medzhitov R. Toll-like receptors and innate immunity. Nat Rev Immunol. 2001 Nov;1(2):135-45.

Morgera S, Haase M, Kuss T et al. Pilot study on the effects of high cutoff hemofiltration on the need for norepinephrine in septic patients with acute renal failure. Critical care medicine. 2006;34(8):2099-104.

Morgera S, Haase M, Kuss T, Vargas-Hein O, Zuckermann-Becker H, Melzer C, et al. Pilot study on the effects of high cutoff hemofiltration on the need for norepinephrine in septic patients with acute renal failure. Critical care medicine. 2006;34(8):2099-104.

Morgera S, Klonower D, Rocktäschel J, Haase M, Priem F, Ziemer S et al. TNF-alpha elimination with high cut-off haemofilters: a feasible clinical modality for septic patients? Nephrol Dial Transplant. 2003 Jul;18(7):1361-9.

Morgera S, Rocktäschel J, Haase M, Lehmann C, von Heymann C, Ziemer S, Priem F, Hocher B, Göhl H, Kox WJ, Buder HW, Neumayer HH. Intermittent high permeability hemofiltration in septic patients with acute renal failure. Intensive Care Med. 2003 Nov;29(11):1989-95.

Morgera S, Slowinski T, Melzer C, Sobottke V, Vargas-Hein O, Volk T et al. Renal replacement therapy with high-cutoff hemofilters: Impact of convection and diffusion on cytokine clearances and protein status. Am J Kidney Dis. 2004 Mar;43(3):444-53.

Morris PE, Zeno B, Bernard AC et al. A placebo-controlled, double-blind, dose-escalation study to assess the safety, tolerability and pharmacokinetics/pharmacodynamics of single and multiple intravenous infusions of AZD9773 in patients with severe sepsis and septic shock. Critical care (London, England). 2012;16(1):R31.

Nagaki M, Hughes RD, Keane HM, Lau JY, Williams R. In vitro plasma perfusion through adsorbents and plasma ultrafiltration to remove endotoxin and cytokines. Circ Shock. 1992 Nov;38(3):182-8.

Nagaki M, Hughes RD, Lau JY, Williams R. Removal of endotoxin and cytokines by adsorbents and the effect of plasma protein binding. Int J Artif Organs. 1991 Jan;14(1):43-50.

Naka T, Jones D, Baldwin I, Fealy N, Bates S, Goehl H, et al. Myoglobin clearance by super high-flux hemofiltration in a case of severe rhabdomyolysis: a case report. Critical care (London, England). 2005;9(2):R90-5.

Nakada TA, Hirasawa H, Oda S, Shiga H, Matsuda K. Blood purification for hypercytokinemia. Transfus Apher Sci. 2006 Dec;35(3):253-64.

Nakatani T, Tsuchida K, Fu O, Sugimura K, Takemoto Y. Effects of direct hemoperfusion with a beta2-microglobulin adsorption column on hypercytokinemia in rats. Blood Purif. 2003;21(2):145-51.

Nishida O, Nakamura T, Kuriyama N, Hara Y, Yumoto M, Shimomura Y et al. Sustained high-efficiency daily diafiltration using a mediator-adsorbing membrane (SHEDD-fA) in the treatment of patients with severe sepsis. Contrib Nephrol 2011;173:172-81.

Niu G, Chen X. Apoptosis imaging: beyond annexin V. J Nucl Med. 2010 Nov;51(11):1659-62.

Oda S, Hirasawa H, Shiga H, Nakanishi K, Matsuda K, Nakamura M et al. Cytokine adsorptive property of various adsorbents in immunoadsorption columns and a newly developed adsorbent: an in vitro study. Blood Purif 2004;22(6):530-6.

Oishi K, Mimura-Kimura Y, Miyasho T, Aoe K, Ogata Y, Katayama H et al. Association between cytokine removal by polymyxin B hemoperfusion and improved pulmonary oxygenation in patients with acute exacerbation of idiopathic pulmonary fibrosis. Cytokine. 2013;61:84-9.

O'Neill LA, Golenbock D, Bowie AG. The history of Toll-like receptors - redefining innate immunity. Nat Rev Immunol. 2013 Jun;13(6):453-60.

Opal SM, Fisher CJ Jr, Dhainaut JF, Vincent JL, Brase R, Lowry SF et al. Confirmatory interleukin-1 receptor antagonist trial in severe sepsis: a phase III, randomized, double-blind, placebo-controlled, multicenter trial. The Interleukin-1 Receptor Antagonist Sepsis Investigator Group. Crit Care Med. 1997 Jul;25(7):1115-24.

Payen DM, Guilhot J, Launey Y, Lukaszewicz AC, Kaaki M, Veber B, et al. Early use of polymyxin B hemoperfusion in patients with septic shock due to peritonitis: a multicenter randomized control trial. Intensive care medicine. 2015;41(6):975-84.

Peng Y, Yuan Z, Li H. Removal of inflammatory cytokines and endotoxin by veno-venous continuous renal replacement therapy for burned patients with sepsis. Burns 2005;31:623-8.

Peng Z, Pai P, Hong-Bao L, Rong L, Han-Min W, Chen H. The impacts of continuous veno-venous hemofiltration on plasma cytokines and monocyte human leukocyte antigen-DR expression in septic patients. Cytokine. 2010;50(2):186-91.

Peng ZY, Wang HZ, Carter MJ, Dileo MV, Bishop JV, Zhou FH, et al. Acute removal of common sepsis mediators does not explain the effects of extracorporeal blood purification in experimental sepsis. Kidney international. 2012;81(4):363-9.

Pinsky MR, Vincent JL, Deviere J, Alegre M, Kahn RJ, Dupont E. Serum cytokine levels in human septic shock. Relation to multiple-system organ failure and mortality. Chest. 1993 Feb;103(2):565-75.

Pinsky MR. Pro- and antiinflammatory balance in sepsis. Current Opinion in Critical Care. 2000;6(6):411-5.

Rachoin JS, Foster D, Dellinger RP. Endotoxin removal: how far from the evidence? From EUPHAS to EUPHRATES. Contrib Nephrol. 2010;167:111-8.

Reeves JH, Butt WW, Shann F, Layton JE, Stewart A, Waring PM et al. Continuous plasmafiltration in sepsis syndrome. Plasmafiltration in Sepsis Study Group. Crit Care Med. 1999;27:2096-104.

Rigato O, Salomao R. Impaired production of interferon-gamma and tumor necrosis factor-alpha but not of interleukin 10 in whole blood of patients with sepsis. Shock (Augusta, Ga). 2003;19(2):113-6.

Rimmelé T, Kellum JA. Clinical review: blood purification for sepsis. Crit Care. 2011;15(1):205.

Rogiers P, Zhang H, Pauwels D, Vincent JL. Comparison of polyacrylonitrile (AN69) and polysulphone membrane during hemofiltration in canine endotoxic shock. Crit Care Med. 2003 Apr;31(4):1219-25.

Rogiers P, Zhang H, Smail N, Pauwels D, Vincent JL. Continuous venovenous hemofiltration improves cardiac performance by mechanisms other than tumor necrosis factor-alpha attenuation during endotoxic shock. Crit Care Med. 1999 Sep;27(9):1848-55.

Ronco C, Brendolan A, Lonnemann G, Bellomo R, Piccinni P, Digito A et al. A pilot study of coupled plasma filtration with adsorption in septic shock. Crit Care Med 2002;30:1250-5.

Ronco C, Inguaggiato P, D'Intini V, Cole L, Bellomo R, Poulin S et al. The role of extracorporeal therapies in sepsis. J Nephrol. 2003;16 Suppl 7:S34-41.

Ronco C, Tetta C, Mariano F, Wratten ML, Bonello M, Bordoni V, et al. Interpreting the mechanisms of continuous renal replacement therapy in sepsis: the peak concentration hypothesis. Artificial organs. 2003;27(9):792-801.

Rosen S, Heyman SN. Difficulties in understanding human "acute tubular necrosis": limited data and flawed animal models. Kidney Int. 2001 Oct;60(4):1220-4.

Rozga J, Umehara Y, Trofimenko A, Sadahiro T, Demetriou AA. A novel plasma filtration therapy for hepatic failure: preclinical studies. Ther Apher Dial. 2006 Apr;10(2):138-44.

Rubartelli A, Lotze MT. Inside, outside, upside down: damage-associatedmolecular-pattern molecules (DAMPs) and redox. Trends Immunol. 2007 Oct;28(10):429-36.

Sanchez-Izquierdo JA, Perez Vela JL, Lozano Quintana MJ, Alted Lopez E, Ortuño de Solo B, Ambros Checa A. Cytokines clearance during venovenous hemofiltration in the trauma patient. Am J Kidney Dis. 1997; 30: 483-8.

Sandeman SR, Howell CA, Mikhalovsky SV, Phillips GJ, Lloyd AW, Davies JG et al. Inflammatory cytokine removal by an activated carbon device in a flowing system. Biomaterials. 2008 Apr;29(11):1638-44.

Sander A, Armbruster W, Sander B, Daul AE, Lange R, Peters J. Hemofiltration increases IL-6 clearance in early systemic inflammatory response syndrome but does not alter IL-6 and TNF alpha plasma concentrations. Intensive Care Med. 1997;23:878-84.

Schadler D, Pausch C, Heise D, Meier-Hellmann A, Brederlau J, Weiler N, et al. The effect of a novel extracorporeal cytokine hemoadsorption device on IL-6 elimination in septic patients: A randomized controlled trial. PloS one. 2017;12(10):e0187015.

Schefold JC, Hasper D, Jorres A. Organ crosstalk in critically ill patients: hemofiltration and immunomodulation in sepsis. Blood purification. 2009;28(2):116-23.

Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. The pro- and anti-inflammatory properties of the cytokine interleukin-6. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research. 2011;1813(5):878-88.

Schetz M, Ferdinande P, Van den Berghe G, Verwaest C, Lauwers P. Removal of pro-inflammatory cytokines with renal replacement therapy: sense or nonsense? Intensive Care Med. 1995 Feb;21(2):169-76.

Schilder L, Nurmohamed SA, ter Wee PM, Girbes AR, Beishuizen A, Paauw NJ et al. Effect of anticoagulation regimens on handling of interleukin-6 and -8 during continuous venovenous hemofiltration in critically ill patients with acute kidney injury. Cytokine. 2012;60:601-7.

Schindler R. Elimination of cytokines from plasma by ultrafiltration using conventional polysulfone or DIAPES membranes. Contrib Nephrol. 2003;(138):37-42.

Schulte W, Bernhagen J, Bucala R. Cytokines in sepsis: potent immunoregulators and potential therapeutic targets--an updated view. Mediators of inflammation. 2013;2013:165974.

Skogby M, Adrian K, Friberg LG, Mellgren G, Mellgren K. Influence of hemofiltration on plasma cytokine levels and platelet activation during extra corporeal membrane oxygenation. Scand Cardiovasc J. 2000 Jun;34(3):315-20.

Song M, Winchester J, Albright RL, Capponi VJ, Choquette MD, Kellum JA. Cytokine removal with a novel adsorbent polymer. Blood Purif. 2004;22(5):428-34.

Stadlbauer V, Krisper P, Aigner R, Haditsch B, Jung A, Lackner C et al. Effect of extracorporeal liver support by MARS and Prometheus on serum cytokines in acute-on-chronic liver failure. Crit Care 2006;10:R169.

Steczko J, Ash SR, Blake DE, Carr DJ, Bosley RH. Cytokines and endotoxin removal by sorbents and its application in push-pull sorbent-based pheresis: the BioLogic-DTPF System. Artif Organs. 1999 Apr;23(4):310-8.

Stoetzer OJ, Fersching DM, Salat C et al. Prediction of response to neoadjuvant chemotherapy in breast cancer patients by circulating apoptotic biomarkers nucleosomes, DNAse, cytokeratin-18 fragments and survivin. Cancer Lett. 2013 Aug 9;336(1):140-8.

Sundström C, Nilsson K. Establishment and characterization of a human histiocytic lymphoma cell line (U-937). Int J Cancer. 1976 May 15;17(5):565-77.

Tallman RD, Dumond M, Brown D. Inflammatory mediator removal by zero-balance ultrafiltration during cardiopulmonary bypass. Perfusion. 2002;17:111-5.

Taniguchi T, Hirai F, Takemoto Y, Tsuda K, Yamamoto K, Inaba H, Sakurai H, Furuyoshi S, Tani N. A novel adsorbent of circulating bacterial toxins and cytokines: the effect of direct hemoperfusion with CTR column for the treatment of experimental endotoxemia. Crit Care Med. 2006 Mar;34(3):800-6.

Teraoka S, Mineshima M, Hoshino T, Ishimori I, Kaneko I, Sato Y et al. Can cytokines be removed by hemofiltration or hemoadsorption? ASAIO J. 2000 Jul-Aug;46(4):448-51.

Tetta C, Cavaillon JM, Schulze M, Ronco C, Ghezzi PM, Camussi G, Serra AM, Curti F, Lonnemann G. Removal of cytokines and activated complement components in an experimental model of continuous plasma filtration coupled with sorbent adsorption. Nephrol Dial Transplant. 1998 Jun;13(6):1458-64.

Toft P, Kehler D, Brandslund I I, Tønnsen E. The immunological effects of continuous veno-venous haemodiafiltration in critically ill patients. Crit Care 1999;3:159-165.

Tsuchida K, Takemoto Y, Sugimura K, Yoshimura R, Nakatani T. Direct hemoperfusion by using Lixelle column for the treatment of systemic inflammatory response syndrome. Int J Mol Med. 2002;10:485-8.

Uchino S, Bellomo R, Goldsmith D, Davenport P, Cole L, Baldwin I et al. Cytokine removal with a large pore cellulose triacetate filter: an ex vivo study. Int J Artif Organs. 2002Jan;25(1):27-32.

Uchino S, Bellomo R, Goldsmith D, Davenport P, Cole L, Baldwin I et al. Super high flux hemofiltration: a new technique for cytokine removal. Intensive Care Med. 2002 May; 28(5):651-655.

Uchino S, Bellomo R, Morimatsu H, Goldsmith D, Davenport P, Cole L et al. Cytokine dialysis: an ex vivo study. ASAIO J. 2002 Nov-Dec;48(6):650-3.

Uchino S, Kellum JA, Bellomo R et al. Beginning and Ending Supportive Therapy for the Kidney (BEST Kidney) Investigators. Acute renal failure in critically ill patients: a multinational, multicenter study. JAMA. 2005 Aug 17;294(7):813-8A.

Uchino S, Kellum JA, Bellomo R, Doig GS, Morimatsu H, Morgera S, et al. Acute renal failure in critically ill patients: a multinational, multicenter study. Jama. 2005;294(7):813-8.

van Bommel EF, Hesse CJ, Jutte NH, Zietse R, Bruining HA, Weimar W. Cytokine kinetics (TNF-alpha, IL-1 beta, IL-6) during continuous hemofiltration: a laboratory and clinical study. Contrib Nephrol 1995;116:62-75.

van Bommel EF, Hesse CJ, Jutte NH, Zietse R, Bruining HA, Weimar W. Impact of continuous hemofiltration on cytokines and cytokine inhibitors in oliguric patients suffering from systemic inflammatory response syndrome. Ren Fail 1997;19:443-54.

Veenman JN, Dujardint CL, Hoek A, Grootendorst A, Klein WR, Rutten VP. High volume continuous venovenous hemofiltration (HV-CVVH) in an equine endotoxaemic shock model. Equine Vet J. 2002 Jul;34(5):516-22.

Venkataraman R, Subramanian S, Kellum JA. Clinical review: extracorporeal blood purification in severe sepsis. Crit Care 2003;7:139-45.

Verhoven B, Schlegel RA, Williamson P. Mechanisms of phosphatidylserine exposure, a phagocyte recognition signal, on apoptotic T lymphocytes. J Exp Med. 1995 Nov 1;182(5):1597-601.

Visvanathan K, Skinner NA, Thompson AJ et al. Regulation of Toll-like receptor-2 expression in chronic hepatitis B by the precore protein. Hepatology. 2007 Jan;45(1):102-10.

Wang H, Bloom O, Zhang M, Vishnubhakat JM, Ombrellino M, Che J, et al. HMG-1 as a late mediator of endotoxin lethality in mice. Science (New York, NY). 1999;285(5425):248-51.

Wang H, Liu D. Baicalin inhibits high-mobility group box 1 release and improves survival in experimental sepsis. Shock (Augusta, Ga). 2014;41(4):324-30.

Wang W, Huang HM, Zhu DM, Chen H, Su ZK, Ding WX. Modified ultrafiltration in paediatric cardiopulmonary bypass. Perfusion 1998;13:304-10.

Wan L, Bagshaw SM, Langenberg C, Saotome T, May C, Bellomo R. Pathophysiology of septic acute kidney injury: what do we really know? Crit Care Med. 2008 Apr;36(4 Suppl):S198-203.

Watanabe T, Sakai Y, Mayumi T, Shimomura T, Song MH, Tajima K et al. Effect of ultrafiltration during cardiopulmonary bypass for pediatric cardiac surgery. Artif Organs 1998;22:1052-5.

Weber V, Hartmann J, Linsberger I, Falkenhagen D. Efficient adsorption of tumor necrosis factor with an in vitro set-up of the microspheres-based detoxification system. Blood Purif. 2007;25(2):169-74.

Weiss M, Elsharkawi M, Welt K, Schneider EM. Transient leukocytosis, granulocyte colony-stimulating factor plasma concentrations, and apoptosis determined by binding of annexin V by peripheral leukocytes in patients with severe sepsis. Ann N Y Acad Sci. 2003 Dec;1010:742-7.

Xie H, Ji D, Gong D, Liu Y, Xu B, Zhou H et al. Continuous veno venous hemofiltration in treatment of acute necrotizing pancreatitis. Chin Med J (Engl) 2003;116:549-53.

Xu J, Zhang X, Monestier M, Esmon NL, Esmon CT. Extracellular histones are mediators of death through TLR2 and TLR4 in mouse fatal liver injury. J Immunol. 2011 Sep 1;187(5):2626-31.

Xu J, Zhang X, Pelayo R et al. Extracellular histones are major mediators of death in sepsis. Nat Med. 2009 Nov;15(11):1318-21.

Yang H, Wang H, Chavan SS, Andersson U. High Mobility Group Box Protein 1 (HMGB1): The Prototypical Endogenous Danger Molecule. Molecular Medicine. 2015;21(Suppl 1):S6-s12.

Yekebas EF, Eisenberger CF, Ohnesorge H, Saalmüller A, Elsner HA, Engelhardt, M et al. Attenuation of sepsis-related immunoparalysis by continuous veno-venous hemofiltration in experimental porcine pancreatitis. Crit Care Med. 2001 Jul;29(7):1423-30.

Yekebas EF, Strate T, Zolmajd S, Eisenberger CF, Erbersdobler A, Saalmüller A et al. Impact of different modalities of continuous venovenous hemofiltration on sepsis-induced alterations in experimental pancreatitis. Kidney Int. 2002 Nov;62(5):1806-18.

Yekebas EF, Treede H, Knoefel WT, Bloechle C, Fink E, Izbicki JR. Influence of zero-balanced hemofiltration on the course of severe experimental pancreatitis in pigs. Ann Surg. 1999 Apr;229(4):514-22.

Yonekawa C, Nakae H, Tajimi K, Asanuma Y. Effectiveness of combining plasma exchange and continuous hemodiafiltration in patients with postoperative liver failure. Artif Organs. 2005;29:324-8.

Zeerleder S, Stephan F, Emonts M et al. Circulating nucleosomes and severity of illness in children suffering from meningococcal sepsis treated with protein C. Crit Care Med. 2012 Dec;40(12):3224-9.

Zeerleder S, Zwart B, Wuillemin WA et al. Elevated nucleosome levels in systemic inflammation and sepsis. Crit Care Med. 2003 Jul;31(7):1947-51.

Zeng X, Zhang L, Wu T, Fu P. Continuous renal replacement therapy (CRRT) for rhabdomyolysis. The Cochrane database of systematic reviews. 2014(6):Cd008566.

Zhou F, Peng Z, Murugan R, Kellum JA. Blood Purification and Mortality in Sepsis: A Meta-analysis of Randomized Trials. Critical care medicine. 2013;41(9):2209-20.

Zimmermann M, Busch K, Kuhn S, Zeppezauer M. Endotoxin adsorbent based on immobilized human serum albumin. Clin Chem Lab Med. 1999 Mar;37(3):373-9.