*Point of view*

# Blood purification in sepsis: reasonable scientific hypothesis or pipe dream?

Septic shock, severe sepsis and the multiorgan dysfunction syndrome (MODS), collectively, remain the commonest cause of death in the intensive care unit (ICU). It is not surprising that clinicians have been looking for new therapies beyond surgical drainage of the septic focus and early and appropriate antibiotic therapy.[1] Lack of success with several trials of immunomodulation in the 1990s has led to frustration, which has led to scepticism about our ability to turn our insights into the immunology and molecular biology of septic shock into clinically meaningful improvements in outcome. Sceptics argue that the immune system is too complex to be successfully manipulated, that animal models are irrelevant to human sepsis and that clinical intervention in humans is typically too late. They also argue that single mediator manipulation is pointless, that clinical outcome is too dependent on other variables, and that ICU studies that use mortality as the primary outcome measure are doomed to failure.[2]

This therapeutic nihilism can be easily applied to the application of extracorporeal blood purification therapy (EBPT) in the treatment of sepsis. In this article I will argue that such scepticism, although scientifically healthy, is premature, as I believe that the next decade will deliver the first trials demonstrating real clinical benefits of EBPT in ICU patients.

**The immune response and blood purification**

Our understanding of the immune response to sepsis is increasing and has been recently summarized in three excellent reviews.[3-5] A teleological interpretation of the findings so far would indicate that our body is the equivalent of an immunological minefield where bacteria, fungi or viruses can trigger an explosion. This interaction between microbe and host has two potential outcomes: a) the response kills the microbe, or b) the response kills the microbe and the host.

The system of innate immunity has evolved over millions of years and is older than the history of the mammalian species. It is effective because it typically results in the death of the intruder, an effect which is useful to the species and decreases the risk of disease transmission. In such a system, the infected individual either dies or the microbe is killed, thus decreasing the risk of contagion. However, the response is not always beneficial to the individual who may die as a consequence of the immune mechanism.

Only very recently in the development of the mammalian species, has it become possible (and desirable) for individuals to survive massive septic injury. The arrival of intensive care units, safe anaesthesia, surgical asepsis, surgical drainage, and antibiotics are indeed very new events in the history of the immune system. If the life of mammals were the equivalent of a 24-hour clock, such advances would represent the equivalent of a millisecond! All of a sudden we are trying to change the human immune system, to do now what we, rather than it, think is beneficial. It is not surprising that we are having trouble in reversing millions of years of evolution.

The immune system is complex[6,7] and one might despair that manipulation would ever be possible. Yet in other conditions we can manipulate it effectively, and to the patient's benefit. Successful organ transplantation is proof of this newly acquired ability. We simply need to learn how to manipulate its response to major infection. Once we do, adjuvant therapies for sepsis will become established and variable in nature (just like immunosuppression) as we target and effectively modify various steps in this immune response. Inevitably, therapy for septic shock will then resemble the armamentarium now available for the treatment of organ rejection (e.g. corticosteroids, cyclosporin, tacrolimus, sirolimus, OKT3, mycophenolate, azathioprine etc.). Will EBPT be part of this armamentarium?

**What are the targets for extracorporeal blood purification therapy?**

If EBPT is to advance, we need to generate hypotheses about its biological targets and evolve technologies that can significantly affect such biological targets. We must test their effectiveness in relevant animal models, apply them to human sepsis in phase I/II trials and then, finally, conduct one or more phase III trials to demonstrate their efficacy. This process is difficult, time-consuming and extraordinarily expensive. The major problem, at this stage, remains the lack of specific molecular targets. A similar lack for specific targets has held back the evolution of extracorporeal liver support techniques.

Several soluble mediator systems have been proposed as targets for manipulation by EBPT in sepsis. They include eicosanoids, leukotrienes, complement, cytokines, platelet activating factor, oxidants, chemokines, other potentially important small peptides and vasogenic substances, and perhaps even uraemic toxins. What is uncertain is their biological hierarchy. Which system is most important? Which system is the primary

trigger of the cascade? Which system is activated and at what time? When is a system important and when is it of little clinical impact? More importantly, which system or systems sustain the septic or inflammatory state at the time when clinical intervention is logistically feasible? We still have very limited answers to these questions and yet they are fundamental. Our increasing understanding in this area will prove vital to further logical developments in EBPT. Such increased understanding will depend heavily on developments in clinical and molecular immunology.

The second fundamental issue is whether the putative target substances can be removed by the currently available EBPT's. It appears, at this time, that the majority of these molecular targets are water-soluble. This is fortunate, because current technology has evolved from renal replacement therapy, which is relatively efficient in removing water-soluble molecules and relatively inefficient in removing lipid soluble molecules. Unfortunately, even for the inflammatory water-soluble molecules, we have a very limited understanding of our ability to remove the appropriate biological targets. This lack of knowledge must be further addressed for rational modifications in technology to take place.

Nevertheless, this lack of knowledge has not impeded kidney replacement therapy. For close to 50 years, we have been able to provide clinically effective chronic renal replacement therapy despite our lack of understanding of what the "uraemic toxins" (i.e. therapeutic targets) are.[8,9] We simply know that short-term uraemic toxins have a low molecular weight and are water-soluble. Yet we have been able to develop a therapy (e.g. dialysis) that can preserve life for years. We have also identified an easily measured marker (e.g. urea) that appears to reflect the state or concentration of such small molecules sufficiently in the uraemic patient. The challenge is now to develop a similar approach in humans with severe sepsis, with EBPT removing all possible septic mediators and measuring EBPT performance by means of one or two marker molecules that are indicative of the efficacy (and safety) of such immune manipulation.

**What can current extracorporeal blood purification therapy remove?**

In the previous section, potential targets for EBPT were discussed and highlighted the fact that, at the present time, all known inflammatory systems are legitimate targets for EBPT. Given this view, two questions become obvious: a) can we remove mediators from all systems, and if not, b) which mediators can we remove and by how much?

The logical answer at this time would appear that we can remove some, and maybe many, non-protein bound small water-soluble mediators of immune dysfunction (e.g. small peptides, vasogenic amines, uraemic toxins, and eicosanoids)[10-14] and that we can do so with a reasonable level of efficiency. Such removal, however, is probably insufficient in intensity to be clinically meaningful. In a recent randomized controlled trial,[15] Ronco and colleagues tested the hypothesis that increasing EBPT (in this case continuous venovenous haemofiltration) in ICU patients with multi-organ failure inclusive of acute renal failure (ARF) would affect mortality. They found that increasing the rate of plasma water exchange rate from approximately 1.5 L/hr to approximately 2.5 L/hr or to 3 L/hr reduced mortality by >30% ($p = 0.0013$). This study demonstrated that modulation of a blood purification therapy decreases mortality in MODS with acute renal failure.

The findings of this trial have tremendous clinical and biological implications. Although the results are confined to ARF, they demonstrate that type and dose of EBPT can affect mortality in a subset of critically ill patients. Secondly, they demonstrate that we need to explore the correct dose for all types of EBPT before we dismiss its clinical value. Current EBPT may be biologically "correct" even in sepsis but "underdosed" as the current intensity of CRRT and the properties of available CRRT membranes may be inadequate for severe sepsis in the absence of renal failure. Thus, we still need to explore what the "right" dose of currently available EBPT is in sepsis and MODS with ARF, just as much as we need to explore new technologies and the appropriate immune targets.

The above study by Ronco and colleagues also proves an important biological point: that the removal of small to medium-size water-soluble molecules is desirable in MODS with associated ARF. We do not know yet whether this is true in the absence of ARF or in isolated sepsis. We also do not know whether the effect is dependent on the removal of uraemic toxins, other "immune" toxins or both. If the effect on mortality were dependent on the removal of uraemic toxins only, such findings would then suggest that acute uremia independently increases mortality in MODS. These findings would add uraemic toxins to the list of possible modulators of the immune response. In fact, several laboratory and human investigations support the view that uraemic toxins have an important effect on immune function.[16-18]

From several animal and human investigations we also know that using current technology our ability to remove middle molecules is limited.[19] Convective extraction is probably better than diffusive removal, but the amounts are still small and may not have any biological or clinical impact.[20] We do know, however, that membrane adsorption of these molecules is

substantial, particularly early (i.e. first 4 hours) in the life of the membrane.[21] Such absorption can temporarily reduce the concentration of some cytokines and complement anaphylatoxins (e.g. C3a and C5a) and could have biological and clinical significance.

This issue was explored in a recent investigation by our group.[22] Using a randomized cross-over design in patients with established septic shock and acute renal failure, we tested the hypotheses that increasing the ultrafiltration rate in continuous venovenous haemofiltration (CVVH) from 1 to 6 L/hr would a) increase the removal of several cytokines and of C3a and C5a, b) lower their serum concentration, and c) decrease vasopressor requirements. We found that increasing the ultra-filtration rate led to a significant decrease in complement anaphylatoxin levels (Figure 1) as well as a reduction in IL-10 concentration. However, we also found that such reduction was not mediated by convective removal, but rather by membrane adsorption, which increased with an increase in ultrafiltration. This study demonstrated the mechanisms at work during EBPT using current technology as well as the limitations of such technology. Thus, we need to explore more efficient ways of removing these "middle" molecules, as they may play an important role in the maintenance of organ dysfunction and systemic inflammation.

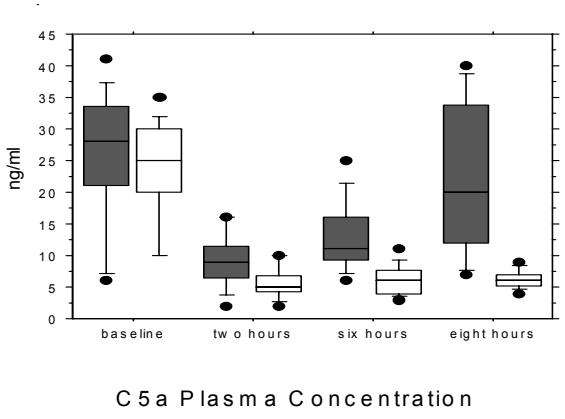


**Figure 1.** Box plot graph presenting the effect of high volume haemofiltration (white) on serum C5a when compared with standard CVVH (black) in patients with septic shock.

Several approaches are worthy of further investigation. One of them is plasmapheresis.[23] However, current plasmafilters only allow plasmafiltration rates of 30 - 50 mL/min (it is unclear whether this rate of blood purification is sufficient) and require the administration of fresh frozen plasma (FFP). This approach is costly and the supply of FFP too limited to support widespread application in sepsis. A logical approach would be to use sorbents to purify the plasma removed during filtration before returning it to the patient. This approach, aptly named coupled plasma filtration adsorption (CPFA) is currently being actively explored in animals and humans.[24]

A further approach to EBPT would be to increase the porosity of membranes in order to improve "middle" molecular clearance. Such large pore haemofiltration (super high-flux haemofiltration) has now been tested in animals with promising results.[25] Phase I studies are under way. It could also be that using even current technology, increasing ultrafiltration rates would add clinically important benefits as suggested by Ronco *et al*.[15] This degree of haemofiltration could be increased further (i.e. high-volume haemofiltration or HVHF) to optimize blood purification in septic patients. This approach has been tested in animals and pilot studies are now testing its efficacy in human beings.[26] Indeed, the study referred to previously[22] as a demonstration of the mechanisms of mediator removal, also demonstrated that HVHF led close to a 70% decrease in vasopressor requirements with only 8 hours of treatment.

**Can new therapies alter the outcome of critical illness and severe sepsis?**

While there has been much scepticism that major outcomes (especially mortality) of MODS or sepsis or critical illness can ever be modified, there have been some recent inroads. For example, low-volume ventilation increases survival in ARDS (anti-inflammatory effect or lung-specific effect given the lowering of IL-6 levels in patients receiving low-volume ventilation?).[27] Also activated protein C[28] has been demonstrated to increase survival in a large randomized, placebo controlled multicentre study of patients with severe sepsis and organ dysfunction as has the yet unpublished MONARCS trial.[29] The paradigm that so called "adjuvant" or "supportive" therapies do not change outcome has been proven wrong.

If EBPT has a clinically relevant effect, once the technology and doses are right and the appropriate large trials have been completed, such effect may well be seen.

**Summary**

EBPT represents a promising new approach to the adjuvant treatment of severe sepsis, septic shock and MODS. The technology is rapidly evolving and pilot animal and human studies are now taking place to prepare the ground for phase II and phase III trials. The rationale for EBPT is scientific and based on our current understanding of the immune response to severe infection. The initial data are encouraging. The correct technology and molecular targeting, however, are still being explored. Once the best technology and dose have been determined, we will be able to really test whether

EBPT is just a pipe dream or a reasonable scientific hypothesis.

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