Point of view

Blood filtration: new opportunities and the implications of systems biology

Medical blood filtration therapies include haemodialysis, haemofiltration and therapeutic plasmapheresis. These membrane technologies have been available for decades and the industries supporting them are mature. The therapies focus on the removal of specific components that are known (or thought) to be involved in the particular disease state.

Recent studies of the human genome and proteome have produced massive data sets that can only be interpreted using complex system science. Systems biology has revealed that many diseases are system level effects of complex biological networks involving large numbers of signaling agents and effectors. It is becoming apparent that control of such diseases will only be achieved by controlling the systems or networks that cause them. These diseases are often daunting to drug development.[1]

Theoretical, animal and clinical data suggest that blood filtration may be uniquely suited as therapy for these complex diseases. In this article the use of blood filtration in complex diseases (e.g., sepsis) will be reviewed.

A neglected therapeutic domain

Dialysis and haemofiltration target the removal of low molecular weight (MW) metabolic wastes (MW <~12 kiloDalton (kD)) associated with kidney failure (e.g., urea, MW = 60 Dalton (D); uric acid, MW = 168 D; beta 2 microglobulin, MW = 11.8 kD). Targets of plasma filtration include very high MW proteins associated with dysproteinaemic disorders (MW~900 to 2,500 kD), and lower MW proteins (IgG, MW = 150 kD) associated with autoimmune diseases that include rheumatoid arthritis and systemic lupus erythematosus.

Between these extreme target ranges are thousands of plasma molecules between 12 and 150 kD and potentially up to 900 kD that participate in many complex biologic networks. Some networks are operative to maintain health, others provide homeostatic adaptation to disease and others are pathologic. Some consider that plasma filtration, by removing all elements of plasma, should deal with these diseases. However, any benefit of plasma filtration from removal of pathologic networks may be offset by harm from removal of adaptive or homeostatic networks. In addition, the need to maintain levels of important coagulation and osmotic agents in blood requires replacement of filtered plasma with fresh plasma or albumin, adding significant risk and expense.

The domain between ~12 and 900 kD contains a large number of species that are part of adaptive molecular networks and pathologic molecular networks. The development of membrane separation media specifically designed to sieve selected pathologic networks in this range, while leaving intact other adaptive networks, could provide effective new therapies for diseases originating in this domain. Such therapies should be relatively rapid and inexpensive to develop and deploy for the following reasons.

First, membrane manufacturing processes can be readily adapted to produce membranes with the desired removal characteristics for a given target network. Blood and filtrate pumping-monitoring equipment is well developed and widely available in the health care delivery industry. New filters could be deployed on existing pump-monitoring equipment and used by methods well known to physicians and nurses.

Second, systems biology is being increasingly used by biomedical researchers to evaluate diseases resulting from systems level effects of complex molecular and cellular networks. Such diseases are often daunting to development of effective drugs,[1] but should be particularly amenable to control by properly designed filtration devices. Systems biology should allow identification of target networks of molecules for which filters may be designed.

Third, health care is under ever-increasing pressure to contain costs. Using the insights of systems biology to identify target networks, and available assets in the manufacturing and health care sectors to produce appropriate membrane filters, could lead to the development and deployment of many effective therapies at relatively low cost.

Finally, the innovative efforts of various researchers to produce novel blood depurative technologies for liver failure, amyloidosis and other applications are acknowledged. However, these complicated devices (bioreactors, albumin dialysis, etc) will require considerable money and time to develop and will be expensive to deploy in the clinical sector. These devices and methods will not be considered here.

Review of filtration process and terms

Differences between membranes

Among methods of blood filtration, pressure-driven (convective) filtration is the simplest and the most

readily adapted to the MW domain between 12 and 900 kD; it will be the focus of this review.

Membrane sieving capability is typically quantified by the molecular weight cut-off (MWCO). Definitions of MWCO vary; typically the MWCO is the MW of molecules so large that only 1 to 10% of them are transmitted through the membrane under a specific set of operating conditions. Membrane fouling, or polarisation, due to the deposition of proteins on and within the membrane can significantly reduce the rate of protein transmission. Sieving of a particular molecule by a membrane is quantified by the molecule's sieving coefficient and may be expressed by the equation:

$$SC = [X]_{ultrafiltrate} / [X]_{blood} \quad \text{Equation 1}$$

Where

SC = sieving coefficient
$[X]_{ultrafiltrate}$ = ultrafiltrate concentration of X
$[X]_{blood}$ = blood concentration of X

Typically, a molecule with an effective SC < 0.1 in a polarised membrane is considered to be ineffectively cleared by that membrane.

Figure I plots the SC against MW for polydisperse (MW) dextrans, a typical test molecule for membrane characterisation studies. Membranes with MWCO of 30 kD, 50 kD and 100 kD are shown.[2] Typical nominal MWCO for medical haemofiltration membranes is 30 kD with some up to 50 kD.

Excretion of target molecules

The goal of blood filtration is to remove target molecules from blood by transmission through the membrane. The rate of removal (in mg/min) of any given target molecule, X, will depend on its concentration in ultrafiltrate (UF; $[X]_{UF}$), and the ultrafiltrate flow rate (Quf; mL/min). $[X]_{UF}$ depends on the SC_X (determined by MW of X and the MWCO of the membrane). The rate of excretion (mg/minute) of X is:

$$\text{Excretion of X}_{(mg/min)} = [X]_{uf} \times Quf \quad \text{Equation 2}$$

Combining Equation 1 and 2:

$$\text{Excretion of X}_{(mg/min)} = Quf \times [X]_b \times SC_x \quad \text{Equation 3}$$

Thus, excretion of X may be increased by increasing either Quf or SC_x.

Operating conditions, polarisation and effective sieving coefficient

Filtration performance is dependent on both the membrane materials and the device operating conditions. Materials effects are determined by the manufacturer, operating conditions (filter blood flow (Qb); Quf) are determined by the operator. For any given Qb, an increasing Quf increases the accumulation of retained material at the membrane surface (polarisation), fouling the membrane, and reducing protein transmission and

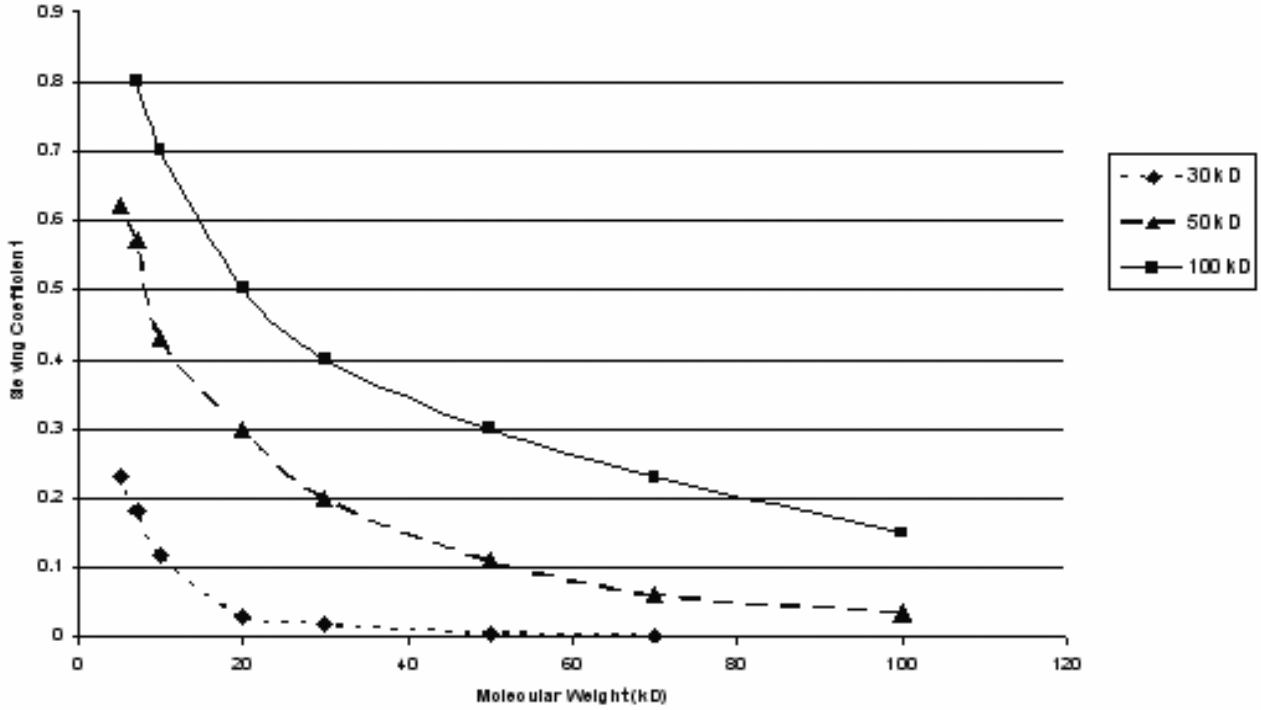

Figure 1. Asymmetric, polysulfone membranes (Filtron Technology Corp.) having MWCO of 30, 50 and 100 kD are compared using polydisperse (MW) dextran.[2]

effective SC. Thus, the ability to enhance clearance by increasing Quf is often limited by polarisation.

Membranes are designed to provide optimal performance within a particular range of Qb and Quf. Stable patients tolerate Qb of ~10% of their cardiac output; patients in shock often cannot tolerate this, and require lower Qb. This lower Qb may limit achievable Quf.

Existing haemofiltration methods were designed with membranes, Qb and Quf effective to remove the target molecules of kidney failure. Use of conventional haemofiltration beyond these design limits (e.g., at very high Quf) invites degraded performance; in particular this may result in a marked decline in the ability to clear large molecules due to the high degree of fouling.

Clinical investigators seeking to develop new treatments using blood filtration typically have only Quf as a parameter to manipulate. The MW range of recognised inflammatory mediators in sepsis is from 8 (IL-8) to 90 kD (endotoxin). Polarisation of existing haemofilters (e.g. MWCO = 30 to 50kD) typically reduces their effective sieving coefficients by 30 to 50%; increasing Quf may further degrade performance. This is limiting in at least two ways. First, if increased excretion of X is sought by increasing Quf, SC_X decreases as polarisation layer accumulates. Second, if a network of molecules is targeted (X_1, X_2, X_3…X_i), they will exist across some spectrum of MW, and the larger X_i may not be sieved at all.

If the neglected domain of filtration is to be developed, then membranes with appropriately designed MWCO will need to be produced.

Complex system science and sepsis

Complex networks or systems are extremely common in nature. Complex physiologic systems include metabolism, neuronal networks, and the immune response. The study and use of complex systems science has been the preserve of physicists, mathematicians, engineers and theoretical biologists. Until recently, applications of complex systems science to biomedical studies have been limited. However, the decoding of the human genome and those of other organisms is bringing the study of complex systems to the forefront in biology and medicine.[3]

Massive amounts of data have resulted from genomic and proteomic studies. There are millions of potential interactions between the genome and the proteins it produces, which in turn regulate the genome and other vital systems. Isolated effects of a single gene or protein often have no relevance to gene/protein function in a whole system context. It is as a whole system that plants, animals, and humans interact with both external and internal stresses. In medicine, an increasing number of human diseases are recognised to have their pathologic mechanisms and/or principal manifestations linked to the activity of complex biological systems. Designing effective therapy for such diseases will require an understanding of complex system structure (topology) and dynamics.

Complex system topology

A complex network or system is a collection of items with connections between the items. The connections are variously called edges (mathematics), links (computer science, e.g., a URL), ties (sociology, e.g., kinship, friendship, business) or bonds (physics, e.g., electrostatic or gravitational forces). In this paper, the terms vertex and edge are used.

Complex systems are inherently difficult to understand because of many complications on many levels. First, at the level of whole systems, networks may overlap. Second, edges exhibit a variety of characteristics. They can have different weights, directions, and functions; and they can evolve over time. Third, vertices and edges are diverse and are often structurally and dynamically complex. Macrophages, T cells, endothelial cells, neutrophils and platelets are physical vertices in the innate immune response that support the function of inflammation. In addition to their morphologic and functional diversity, they exhibit functional complexity. Each cell is itself a complex dynamic system, containing many vertices including receptors (cell surface receptors; intracellular domains) and signaling molecules (JAK, STAT; NFkB, IkB etc.), each with various functional edges. Vertices evolve both by aging and by responding and adapting to environmental (e.g., pathogen associated molecular patterns) and network (cytokines) influences. Fourth, meta-complications occur when various complications influence each other. In a critically injured or infected patient, the catabolism resulting from the effects of the network of counter regulatory hormones may thin the intestinal barrier, allowing translocation of bacteria at a time when innate immunity has cycled into an immune suppression. This sets the stage for secondary infection and often death.

Network topology is the relationship of the edges and vertices of the network, typically drawn as maps; examples include electrical circuit diagrams and metabolic charts. Mapping becomes unwieldy or impossible as the number of vertices and edges increases beyond a few hundred. Representation and manipulation of complex system topology is achieved using mathematical graph theory. The starting point in the mathematical representation of a network is the determination of the distribution of the number of edges between vertices. The number of edges (k) for a vertex is its degree of connectedness. For example, a well-

known model network is the regular NK model network. One implementation of the NK model is an array of 100,000 light bulbs (each bulb is a vertex; the number of vertices, n = 100,000) each with two (k = 2) edges (wires).[4] Most natural networks are irregular with vertices of various degrees. Most biological networks appear to exhibit scale free topology with relatively few vertices of high degree (e.g., k = 8 to 40) and many more vertices of lower degree (e.g., k = 1 to 4).[5] The probability (p) that any vertex has k edges is given as a power law function:

$$p \sim k^{-\alpha} \qquad \text{Equation 4}$$

Other topologies exhibit other probabilities, e.g., random, exponential, Poisson etc.[6]

Application of systems biology and engineering principles to evaluating therapeutic strategy

System biology perspective of anti-sepsis drug development

Over the past thirty years, numerous drugs have been developed to neutralise some specific inflammatory mediator involved in sepsis. Over 10,000 patients have been enrolled in dozens of studies and trials to evaluate these drugs for survival benefit; none have been successful.[7] Meta-analysis of 18 trials of six different agents concluded that beneficial effects of these drugs "…if present, are small (an ~10% reduction in mortality rate)."[8]

Some sepsis agents have actually increased mortality. A randomised controlled trial (RCT) was done of human monoclonal immunoglobulin M antibody (MAB-T88) against enterobacterial common antigen (ECA) in patients with septic shock and presumed Gram-negative infection. MAB-T88 was associated with a non-significant increase in mortality rate (37% MAB-T88; 34% placebo; p = .36) in the whole study group which approached significance (mortality 37.2% MAT-T88; 26.5% placebo, p = .08) in the subgroup with ECA infection.[9] Tumor necrosis factor (TNF) receptor (TNFR) fused to the Fc portion of IgG1 (TNFR:Fc) neutralises TNF alpha. A RCT revealed a mortality of 30% in the placebo group, 48% in the middle dose group and 53% in the high dose group (p=0.02).[10] Lethal results have also been observed with other agents in animal studies.[11-13]

The only successful anti-sepsis drug is recombinant human activated protein C (rhAPC; Xigris™). In a RCT mortality in the placebo group was 30.8% and in the rhAPC group, 24.7% (P=0.005).[14] Xigris™ is the first and only drug approved for marketing by the US Food and Drug Administration as a specific sepsis therapy. Limited acceptance of Xigris™ by the medical community is manifest in sales that have fallen well short of projections, apparently due to the drug's price (~$6,800/treatment), modest survival benefit, and concerns about cost effectiveness.[15-17]

Systems biology, sepsis drugs and therapeutic strategy. The innate immune response is essential to host survival; disable it and the host dies of infection or injury. The systemic inflammatory response syndrome (SIRS, the clinical expression of pro-inflammatory activity) has its counter balancing response, compensatory anti-inflammatory response syndrome (CARS).[18-20] CARS can also be extreme, resulting in immune suppression and secondary infection, which can be lethal in two ways. First, the pathogen effects cause death. Second, the infection triggers a second pro-inflammatory response, resulting in death from sepsis. A mixed antagonist response syndrome (MARS), with elements of SIRS and CARS is also recognized,[18,19] so SIRS, CARS, or MARS, when excessively amplified, are also lethal. What strategy could control septic inflammation, but maintain the adaptive and protective function of innate immunity?

Target elimination is the appropriate strategy in some diseases (e.g., cancer). In sepsis, however, the system from which disease results (innate immune system) is also essential for survival, ablation is not a reasonable goal. Anti-sepsis drugs sought to neutralise the function of a perceived "key mediator." The general lack of benefit, and occasional lethal effects, can be understood by considering drug effect on inflammatory network topology.

Hundreds of cytokines and other inflammatory mediators function as edges in the inflammatory network, supporting the function of various vertices by conveying information that amplifies or suppresses elements of the response. Anti-sepsis drugs neutralise only one (e.g., IL-1ra-IL-1; platelet activating factor (PAF) antagonist) of these edge-vertex combinations, or a non-hub subsystem (e.g., ibuprofen-prostaglandins). As this is below the 5% threshold of vertex neutralisation needed to affect communication in the network, no effect would be predicted by systems biology,[7] and none has been seen in clinical trials.[7,8] The consistent failure of multiple sepsis drugs appears to result from the robustness and adaptability of the innate immune system.

TNF is activated very early in the inflammatory response, is highly connected to many vertices and activates many secondary edges. Thus, TNF has the characteristics of a hub. The mechanism of the dose related increase in mortality with the TNFR:Fc is uncertain. However, if TNF, a hub, malfunctioned under the influence of TNFR:Fc, then, as predicted for scale free networks,[21] fragmentation of the innate immune network would be expected, with death from underlying

infection the result.

rhAPC (Xigris™) differs from other sepsis drugs in its strategy: it does not neutralise any vertex or edge. In sepsis, the naturally occurring anticoagulant, Protein C, is consumed; mortality varies inversely with plasma levels of protein C.[22] The loss of APC allows clotting of small and medium blood vessels leading to microcirculatory arrest, organ failure, and death.[23] Administration of rhAPC restores homeostasis. The coagulation network interacts with the innate immune system; rhAPC administration was associated with reduced plasma levels of IL-6. The effects of rhAPC are probably best understood at the tissue level: partial restoration of anticoagulant homeostasis prevents complicating ischaemia. Its modest reduction of inflammation reflects modest immune system modulating effects.[24] Unfortunately, the impact of this strategy, though positive, is very limited.

Systems biology is sufficient to explain the general failure of existing sepsis drugs. Whether it actually does awaits further developments in the field.

Engineering control methods in complex systems

The configuration of a robust and adaptive immune response varies with the inciting perturbation and as the response evolves. Response variability between septic patients is marked due to variability in initial insults and comorbid conditions in combination with the range of genetic polymorphisms.[25] Many cytokines interact synergistically with one another.[26,27] This synergy powerfully amplifies cytokine effects and is consistent with non-linear dynamics typical of biological signalling systems.[28]

In designing a control strategy for sepsis, there are three general goals. First, the adaptability and robustness of the innate immune response must be preserved. Second, excess system activity must be reduced. Third, the control strategy must be effective regardless of which inflammatory network components are active in a patient.

Engineering methods have been used for decades to deal with immunologic problems. First, engineers have modelled immune responses in order to optimise vaccination and other disease control methods.[29,30] Second, engineers use the immune system as a model of a complex, distributed, autonomous system. Immune system topology and surveillance 'methods' have been used to develop computer network security systems and related applications.[31]

Control of aggregate system activity is a common engineering problem. One method for overall system control requires that the flow of information on a critical number of edges in the system be reduced, but not interrupted. This preserves system topology and adaptability, but reduces overall activity.[32] By analogy to the NK model, the goal is to leave all light bulbs and wires intact, but turn down the current to the system. System topology is preserved (as is robustness and adaptability), but the intensity of the light and heat is reduced.

With inflammation, a method is required that can reduce the flow of information (inflammatory mediators) along all edges, regardless of which edges are active at any given time. This should reduce aggregate system activity, while topology and adaptability remain intact.[32] Of available methods, blood filtration with a membrane designed to sieve all recognised cytokines and other inflammatory mediators seems like the best strategy to meet these criteria.

Haemofilter membrane design and therapeutic capability in sepsis

Haemofiltration in sepsis

Haemofiltration was designed to treat acute renal failure (ARF) in critically ill patients. In septic patients, haemofiltration resulted in unanticipated improvements in cardiopulmonary function,[33,34] which prompted study of haemofiltration as therapy for sepsis and septic shock. Studies vary considerably in Quf, duration of treatment, pre-haemofiltration resuscitation and haemofiltration techniques and equipment, among other things.[35] An ADQI working group found no conclusive evidence supporting efficacy in sepsis, and recommended haemofiltration only be used in sepsis as an investigational procedure.[36]

Evidence for a survival benefit of haemofiltration in sepsis is limited, but of interest.

Animal trials. A conventional haemofilter (MWCO = 50 kD) was studied in a swine model of lethal *Staphylococcus* sepsis. Animals were identically prepared and studied in pairs, one actively filtered, the other sham filtered (blood circulated through filter and circuit, but UF line clamped). The endpoint was survival time. Survival time in actively filtered pigs was 59 to 312% longer than that in sham filtered pigs depending on system operating characteristics.[37]

These same investigators evaluated the effect of filtration using a membrane with a MWCO = 100 kD; this MWCO is expected to remove all known inflammatory mediators. Again animals were studied in pairs, both actively filtered, both with the same Qb and Quf, one with a conventional filter (MWCO=50 kD) the other with the 100 kD MWCO membrane. Animals filtered with the 100 kD MWCO membrane had a survival time 84% longer than animals filtered with a conventional membrane, and ~506% longer than sham-filtered animals in the previous study.[38] Thus, in this animal model, a substantial survival benefit was shown with a conventional haemofilter (MWCO = 50 kD); this

benefit was nearly doubled using the 100 kD MWCO membrane.

Clinical studies. Most clinical studies are too variable to allow detailed analysis. However, one study (short term, high volume haemofiltration (STHVH)) used strict entry criteria, a strict pre-haemofiltration resuscitation protocol, and a consistent haemofiltration protocol.[39]

Honore *et al*,[39] developed STHVH as a maximally aggressive method of haemofiltration given available haemofilters, fluid pumping and monitoring equipment, and methods. As with most other studies, the only parameter available for manipulation was Quf. A prospective, interventional (no control group) study was undertaken in 20 patients with refractory septic shock. Septic patients entering the ICU were evaluated for enrollment. Resuscitation (fluids; cardiotonic drugs; monitoring) was guided by protocol. Patients failing to achieve and maintain haemodynamic endpoints were diagnosed with refractory septic shock and were candidates for STHVH. STHVH was performed using a polysulfone haemofilter (Fresenius; MWCO = 35 kD) and a peristaltic blood pump to perform an isovolaemic exchange of 35 liters in 4 hours. This exchange volume was selected because it is the maximum ultrafiltrate flow rate (Quf) achievable on available equipment.

Endpoints for STHVH were haemodynamic response and survival. A patient was a haemodynamic responder if haemodynamic parameters improved within the 4 hours of STHVH as follows: by 2 hours, cardiac index increase >= 50%, mixed venous saturation increase >= 25%; by 4 hours pH >7.3, epinephrine dose reduction => 50%. Patients failing to achieve all these values were non-responders. Observed mortality rates were compared between responders and non-responders, and between the whole group and mortality predicted by illness severity scores (APACHE II, SAPS). In the study cohort (n = 20), the predicted mortality rate was 79%, the observed mortality rate, 55% ($p < 0.05$), for a relative reduction in mortality (RRM) of 30.4%. Among the eleven haemodynamic responders, nine survived (~18% mortality). Among non-responders, all were dead within 24 hours (100% mortality). Responders and non-responders were evaluated retrospectively with respect to body weight (on ICU admission) and delay time (from ICU admission to beginning STHVH).

The median delay time was significantly greater in non-responders (13.8 hours (9.6 - 17.5)) than responders (6.8 hours (3.25 - 12)). This is consistent with previous studies demonstrating that delays in effective treatment in sepsis increase mortality.[40,41] Delay time resulted from patient variability, some patients deteriorating rapidly, others more gradually.

The mean body weight was significantly less ($p < .05$) in responders (66.2 ± 8.4 kg) than in non-responders (82.6 ± 13.4 kg). Retrospective calculation of the Quf dose (liters of UF/kg body weight/4 hour treatment) in the two groups revealed an average Quf dose in the response group about 24% higher than in the non-response group.

This dose effect was not expected. At the time of this study, dose adjustment of Quf to body weight was not done. Ronco *et al*,[42] in a randomised, prospective trial, done concurrently with the STHVH study, evaluated three Quf doses in ICU patients with ARF. Dose (ml/kg/hr) Groups were: Gp I = 20; Gp II = 35; Gp III = 45 mL/kg/hr. Duration of haemofiltration varied with the duration of ARF. Survival was significantly higher in Gp II and III compared to Gp I. In a small subgroup of septic patients, Gp III patients tended to have better survival than Gp I and II patients.

In sepsis, body size is not a risk factor for morbidity or mortality, except in the morbidly obese patient. None of the STHVH patients were obese. Thus, Honore *et al*,[39] and Ronco *et al*,[42] independently demonstrated the importance of dose adjusting Quf to patient body weight.

STHVH from the design perspective

Designers of a device for some specific purpose contemplate an envelope of high efficacy, outside of which efficacy is reduced or lost. Thus, the boundaries of a device's performance envelope are typically of more interest than its average performance.[43] For STHVH, envelope boundaries are assessed with a scattergram using clinically relevant parameters (delay time; body weight) to define boundaries.

In Figure 2, each of the 20 patients is plotted against delay time (DT; ordinate) and body weight (BW; abscissa). The responder survivors (closed boxes), responder deaths (open circles) and non-responder deaths (open boxes). Survivors are constrained by BW and DT. All patients less than 81 Kg, treated within 8.25 hours survived. However, one patient (BW = 73 Kg) treated at 13.5 hours survived and two patients (67 & 63 Kg) treated at 12 and 16.5 hours responded, but died.

Figure 2 reveals an envelope of efficacy bounded by BW = ~81 kg and delay time = ~8 hours. In this envelope, patient survival was 100%; outside the envelope survival was 1 of 12. This suggests that STHVH in catecholamine resistant, refractory septic shock is extremely effective, but this effect is constrained by BW and DT. Appropriate redesign of STHVH methods or devices to expand its envelope should provide a survival benefit for patients of all body weights and lengthen the delay time in which therapy can begin.

The small number of patients and the retrospective nature of some observations limit the conclusions of this analysis. The envelope of DT-BW interaction is

probably more complicated than it appears here. However, if this is only a rough approximation of the potential of haemofiltration in severe sepsis, then two conclusions are supported.

First, haemofiltration as a potentially effective sepsis therapy cannot be ignored.

Second, current filter or pump designs are not adequate to treat all patients. Redesign is needed to expand the performance envelop.

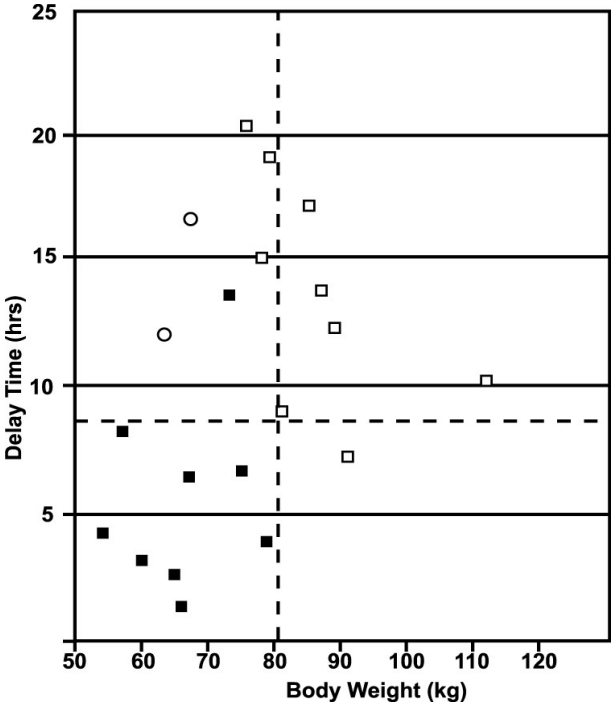

Figure 2. The delay time (DT) and body weight (BW) plotted for each of the 20 patients (Honore *et al*,[39]).

Haemofiltration redesigned for sepsis

STHVH may be redesigned at two points: the pumps, to allow higher Quf dose; or the filter, to make it more effective.

Pump redesign. Increasing Quf by pump redesign is technically simple. If 0.53 liters/kg/4 hours is the effective dose of ultrafiltrate in sepsis when using a conventional filter, then the average non-responder (BW = 82.6 Kg) requires an exchange of 43.8 liters (10.9 liters/hour) and the largest patient (112 kg) would require an exchange of ~59 liters (14.75 liters/hour). The following problems exist with these high flow rates.

First, fluid balance errors are a major source of risk for patient injury or death in haemofiltration; the higher the exchange volume, the higher the risk. Designing fluid balancing and monitoring systems to control this risk is expensive. Health care systems worldwide are under marked constraints to limit expenditures. If acceptably safe equipment were developed, its acquisition would be costly.

Second, if high Quf is necessary, then the therapy may be limited only to those centers (e.g. tertiary hospitals) who could manage the cost and risk. This introduces severe delays in starting therapy in any patient not fortunate enough to present to such a center.

Third, as Quf is increased, polarisation and fouling increase, both of which may reduce protein clearance. The diminishing return created by this interaction will create its own envelope, beyond which further Quf increases will have no benefit, only increased risk.

Fourth, while redesigned pump equipment may theoretically allow patients of all sizes to be treated, the limited MWCO would not lengthen available delay time.

Filter redesign. Effective clearance of target molecules is the key function of filtration. The SC of the target molecules determines Quf needed for desired clearance (Equation 3). The MWCO determines the MW range of molecules to be cleared. Thus, the membrane, with its designed pore size distribution, is the key element in the filtration system. A membrane filter designed with a MWCO sufficiently high to usefully sieve all recognised inflammatory mediators addresses both the problem of body weight and delay time.

Body weight. In figure 1, for molecules of MW <12 kD, their SC through the 100 kD MWCO membrane are about 4 to 6 times more than their SC through the comparable 30 kD membrane. Equation 4 is useful to calculate the Quf in a 100 kD MWCO membrane, and the Quf in a 30 kD MWCO membrane, each of which provides equal clearance rates of molecules in MW range <12 kD. For the same clearance, the 100 kD MWCO membrane requires a Quf 15 to 23% that of the 30 kD membrane. Thus, use of a 100 kD MWCO membrane filter in patients with severe septic shock should allow similar results to those attained with STHVH (using a 35 kD MWCO membrane) at less than 25% of the Quf dose. This has the following results:

1. Quf dose is sharply reduced as is the risk of fluid balance errors and cost.
2. The need for pump redesign and/or acquisition of new capital equipment by hospitals is eliminated.
3. Patients of nearly all body weights can be adequately treated.
4. Lower Quf for a given blood flow will provide a higher effective MWCO.
5. Permissible delay time will probably not be lengthened by this effect alone.

Delay time. Septic inflammation is self-amplifying over time. After a certain period, irreversible, vital organ injury is reached, death results. How long this period is (i.e., acceptable delay time) depends on host factors, resuscitation, and the inciting events.

Engineering control theory suggests that the greater the level of amplification in a dynamic system, the greater is the number of edge flows that will need to be reduced to rapidly abate excess system activity. Thus, the longer the delay time, or the more severe the level of inflammatory amplification, the greater the number of edge flows (e.g., more different cytokines and other inflammatory mediators) that will need to be removed to reduce system activity. A membrane with a MWCO allowing clearance of all recognised inflammatory mediators should be maximally effective in modulating severe septic inflammation. This should materially prolong the available delay time. Thus, a membrane with an adequate MWCO e.g., 100 to 150 kD, is probably the only way to usefully lengthen permissible delay time.

Current models of septic inflammation focus on system topography, not the dynamics of the flows. Thus, the application of engineering control theory to design of sepsis therapy is theoretical. If large pore filtration is effective in sepsis, and if effects on system dynamics are a significant part of this effect, then the following should be observed:

1. A major survival benefit should be achieved at a Quf dose <<25% of the effective dose in STHVH.
2. Delay time, in which survival can still be achieved, should be substantially prolonged.

Conclusion

The basic concepts of complex system science have been reviewed and used to evaluate the general failure of sepsis drugs and the potential benefits of blood filtration as sepsis therapy. The following points are provisionally supported.

- A domain of plasma molecules exists between about 12 kD and 900 kD that are operative in various biologically complex diseases. These diseases may be uniquely amenable to control by adequately designed blood filters, however, current therapeutic blood filtration devices are not adequate.
- The rapidly evolving knowledge of systems biology should allow networks of target molecules to be identified, allowing design of appropriate blood filtration devices.
- Existing manufacturing capability and health care delivery infrastructure could be leveraged to provide relatively inexpensive production and rapid deployment of novel therapies.
- Engineers have evaluated immunology as a complex system for decades, using engineering control theory in their models. These methods may be useful in developing therapeutic strategy and devices.
- The innate immune system, in particular the inflammatory response that causes sepsis and septic shock, is reviewed as an example of a biologically complex disease, and as a target for development of therapeutic strategy and devices.

If a large pore haemofilter, when adequately tested in septic shock, is highly effective, then two benefits will result. First, an effective therapy for sepsis will be realised. Second, the results should motivate further exploration of systems biology as a guide to designing therapy, both filtration and pharmaceutical.

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