**Clinical implications of biocompatibility in blood purification membranes**

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Raymond M. Hakim. MD. PhD
Vanderbilt University Medical Center

**Introduction**

There are several types of hemodialysis membranes such as the cellulose base membranes, cellulose acetate, polyacrylonitrile (PAN), polymethylmethacrylate (PMMA), and polysulphone (PS) and others currently in clinical use. Structurally, cellulosic membranes consist of repeating polysaccharide units, reminiscent of bacterial and yeast cell wall lipopolysaccharides. It is not, therefore, surprising that a humoral pathway that participates in the defense against bacterial infection, namely the complement pathway, is activated during dialysis with these membranes. The other membranes differ from cellulosic membranes and from each other with respect to chemical structure and surface charge. However, in general, synthetic membranes have a markedly reduced ability to activate complement or other cellular elements and are considered biocompatible membranes.

Although the complement pathway has been studied most closely, it is important to emphasize that other humoral pathways are also activated during dialysis. For example, the activation of the coagulation pathway is well known and is only blunted by relatively large doses of heparin. More recently, the activation of the kallikrein–kininogen (contact) pathways by dialysis membranes have also been demonstrated and is presumed to be the pathway implicated in the recent reports of anaphylactic reactions in dialysis patients converting enzyme inhibitors and PAN membrane. However, differences in the ability of these membranes to activate the coagulation and the contact pathways have not been adequately studied.

In addition to these humoral pathways, there is now evidence that cellular components of blood also participate in these interactions, including activation of platelets with subsequent generation of thromboxane, the release of leukocyte proteinase enzymes, generation of interleukin-1 from monocytes and most importantly, the generation of reactive oxygen species from activated neutrophils. The humoral pathways and cellular elements interact with each other and the activation of one pathway often leads to the activation of another. However, it is important to note that cellular pathways have been shown to be activated by direct contact with cellulosic type membranes, via specific carbohydrate receptors.

**Basic mechanisms of blood–membrane interactions**

**Activated pathways**

Before considering the clinical consequences of blood–membrane interaction it is useful to describe briefly the different systems involved in the blood–membrane interactions during dialysis.

1. **Complement.** The detection of products of complement activation from the common pathway (C3a and C5a, the so called anaphylatoxins) without detectable levels of C4a generated from the classical pathway suggests that complement activation proceeds via the alternate pathway.
Among the different types of dialysis membranes, new cellulosic membranes activate complement to the greatest degree. Maximum complement activation occurs at 15 minutes after initiation of dialysis and lasts up to 90 minutes. The peak at 15 minutes reflects the balance between the activation within the extracorporeal circuit and clearance of these products of complement via a large pool of cellular receptors in the blood. As dialysis proceeds, the rate of complement activation decreases. The mechanisms of passivation of these surfaces after initiation of dialysis has not been well defined but probably includes specific deposition of complement fragments such as C3b, as well as non-specific deposition of fibrin on the activating sites of the dialyzer membrane surface.

There is also increasing evidence that in addition to the C3a and C5a fragments, the membrane attack complex, C5b-9 can actually participate in the process of cellular activation. These polymeric aggregates of C5b, C6, C7, C8, and C9 combine together often on the cell surface and can cause lethal cell lysis or sublethal injury to the surface of cells, and are appropriately called the membrane attack complex. In addition, they also seem to participate in other cellular activation processes.

2. Coagulation and kallikrein pathways. Although the complement pathway of activation has been the aspect of biocompatibility that has been most thoroughly studied, it is clear that other closely related pathways are activated during blood-membrane interaction. The usual requirements for heparin anticoagulation during hemodialysis have made direct study of components of this cascade difficult, although some studies suggest that certain membranes are more thrombogenic than others. A comparison of different membranes with respect to their ability to activate Hageman factor (factor XII) in vitro shows that PAN membranes activate Hageman factor to a greater degree than do other membranes. Furthermore, the differential ability of dialysis membranes to activate Hageman-factor-dependent pathways, as assessed by the formation of kallikrein and the subsequent generation of bradykinin, has also been shown.

Other humoral substances may be important. Using a sheep model, investigators implicated prostanoid activation products such as thromboxane in mediating pulmonary hypertension. When sheep were pretreated with cyclo-oxygenase inhibitors, the pulmonary effects of cellulosic-activated blood were abrogated.

3. Neutrophils. The effect of blood-membrane interactions can be seen with cellular elements of blood as well. Neutropenia occurs maximally at 15 minutes from the initiation of dialysis with new cellulosic membranes, and complement activation has been shown to be directly related to this phenomenon. Neutrophils have receptors for C3b, C5a, and CD11b/CD18, the latter being a marker of a family of receptors responsible for neutrophil adhesion. Indeed, it is the increased expression of CD11b/CD18 receptors that is primarily responsible for the transient leukopenia resulting from the leukosequestration of these cells in the pulmonary capillaries and other vascular beds such as the glomerulus. When complement activation is attenuated, as during dialysis with biocompatible membranes, the transient leukopenia is also attenuated.

One of the more important products of neutrophil activation is the superoxide anions (O2−, OH− and H2O2), also known as reactive oxygen species. While reactive oxygen species are important in the host defense mechanism, they can also be potent mediators of cellular damage. In recent years, free-radical-induced injury has been implicated in myocardial tissue injury during ischaemia and reperfusion, cerebral ischaemia and pulmonary fibrosis. Several studies have suggested that these species can cause significant endothelial cell injury, particularly when granulocytes are in close proximity to endothelial cells. While these processes do not lead to acute clinical symptoms, it is important to recall that this process occurs chronically (three times a week) in patients dialyzed with non-biocompatible membranes.

4. Monocytes. Monocytes have also been implicated in blood-membrane interactions. Activation of monocytes leads to the release of interleukin-1 (IL-1), and the hypothesis that IL-1 is elaborated from monocytes during hemodialysis has been
presented\textsuperscript{10}. This molecule has numerous potential biological effects including the induction of fever, neutrophilia, increased hepatic synthesis of acute-phase reactants, and release of $\beta$-microglobulin\textsuperscript{26}. In addition, metabolism of muscle protein as well as lymphocyte activation have also been demonstrated to be direct effects of interleukin-1. Monocytes have specific receptors for complement products and complement activation has been shown to result in increased transcription of IL-1 and TNF-α\textsuperscript{27,28}. Therefore, it is likely that some membranes such as cellulosic or cellulose-based, which activate complement, may lead to enhanced activation of monocytes and increased production of interleukin-1 due to these activated complement components and one or more "second" signal. Recent studies have also indicated that monocytes have a receptor that can be activated directly by such membranes\textsuperscript{14,28}. Therefore, a direct monocyte-membrane interaction is likely to be enhanced by complement activation from blood-membrane interaction.

Finally, there is some evidence to suggest that acetate may play a role in the formation of interleukin-1\textsuperscript{29}. Whether this is a direct role of acetate or due to the presence of small-molecular-weight endotoxin material crossing from the dialysate to the blood surface of the membranes is not clear at present. However, this latter putative interaction is an example of the expanded definition one must employ when one considers the issues of biocompatibility of dialysis membranes.

5. Lymphocytes. Lymphocytes have been considered as passive bystanders in the complex blood-membrane interaction that occurs when complement is activated with some dialysis membranes. However, recent evidence suggests that they may participate in these interactions, particularly when they are exposed chronically to dialysis with cellulosic or cellulosic base membrane. For example, it was shown recently that lymphocytes harvested from patients dialyzed for as little as two weeks on cellulosic or cellulosic base membrane have an increased $\beta_{2}$-m release when placed in culture\textsuperscript{30}, more importantly, a substantial percentage of these cells no longer express $\beta_{2}$-m or HLA on their surfaces. Dialysis with a biocompatible membrane restores the lymphocytes to their baseline condition\textsuperscript{30}. Equally important, other studies have shown that lymphocytes that are chronically exposed to cellulosic or cellulosic base membrane are unable to express the maximum number of IL-2 receptors and are therefore less responsive to immune stimulation. However, the correlation of these findings with clinical outcome is still lacking. Finally, defects in NK cell function have been reported in patients on long-term cellulosic or cellulosic base membrane\textsuperscript{31}.

It has become apparent that another aspect of biocompatibility which must be taken into account concerns the ability of the membrane to adsorb material from the blood. In this regard, Cheung et al have shown the PAN membranes to adsorb C\textsubscript{3a} and C\textsubscript{5a} to a greater extent than cellulosic or cellulosic base, and a similar pattern has been found for bradykinin as well\textsuperscript{32}. Thus, it is conceivable that a membrane might be better tolerated not only because it causes less activation of substances such as complement, but also by virtue of its ability to bind and remove potentially vasoactive substances from the circulation.

**Clinical studies on the effects of blood-membrane interactions**

1. Chronic hemodialysis patients

It is evident that the number of pathways which are activated have the potential for producing many side-effects in patients during hemodialysis. Hemodialysis-associated acute events related to these interactions have been described. For example, acute anaphylactic type reactions have been described in patients on converting enzyme inhibitors and some synthetic membranes, because of the simultaneous activation of the contact pathway, which leads to the formation of bradykinin and the inhibition of kininase enzyme by the converting enzyme inhibitors\textsuperscript{33}.

Similarly, the "first use syndrome" which has been described in patients dialyzed with new cellulosic membranes, has been ascribed to the acute and vigorous complement activation that is present in a subset of chronic hemodialysis patients\textsuperscript{33}. 
Although a role for allergic reactions to ethylene oxide has also been described\(^{34,35}\), recent data from the Center for Disease Control, which reports on such reactions described a 2-fold higher odds ratio (p<0.001) for patients on cellulose-based membranes than for synthetic membranes which are also sterilized with ethylene oxide (Table 1).

Other long-term adverse symptoms may also correlate with the extent of complement activation\(^{36-38}\). One-third fewer adverse reactions occur during dialysis over large numbers of treatments in patients dialyzed with biocompatible membranes, and these patients had less than half the length of hospitalization from cardiovascular and infectious causes compared to patients dialyzed with cellulosic or cellulosic base membranes. Finally, reuse of cellulosic dialysis membranes with formaldehyde which alternates the extent of complement activation was among the independent variables associated with reduced death rates, in a 5-year analysis of more than 4000 dialysis patients\(^{39}\).

The leukosequestration associated with cellulosic membranes is not merely a passive phenomenon. During the leukosequestration, many of the lysosomal granules containing enzymes such as lactoferrin, proteolytic elastase, and alpha-1 proteinase inhibitor are released\(^9\). Indeed, emerging evidence suggests that these events, such as release of intracellular enzymes and reactive oxygen species, also lead to chronic side-effects. In a recent study, the response of neutrophils to phagocytic challenges was considerably weaker when they were isolated in patients dialyzed with biocompatible membranes. This defect was also associated with a greater incidence of infections in patients on cellulosic membranes\(^{40}\).

### Table 1: Effect of dialyzer membrane and dialyzer reuse on reporting of new dialyzer syndrome, United States, 1993

<table>
<thead>
<tr>
<th>Factor</th>
<th>Total centers</th>
<th>New dialyzer Syndrome</th>
<th>% Reporting</th>
<th>log logistic regression model*</th>
<th>Odds ratio</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membrane types</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cuprophann</td>
<td>1346</td>
<td>31</td>
<td>1.9</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
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<tr>
<td>Cellulose acetate</td>
<td>1293</td>
<td>28</td>
<td>1.3</td>
<td>0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regenerated cellulose</td>
<td>404</td>
<td>35</td>
<td>2.0</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polysulfone†</td>
<td>1188</td>
<td>28</td>
<td>1.0</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cellulose triacetate</td>
<td>154</td>
<td>26</td>
<td>1.0</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAN†</td>
<td>72</td>
<td>24</td>
<td>1.0</td>
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<tr>
<td>Dialyzer reuse</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>No</td>
<td>614</td>
<td>21</td>
<td>1.0</td>
<td>—</td>
<td></td>
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</tr>
<tr>
<td>Yes</td>
<td>1686</td>
<td>29</td>
<td>1.5</td>
<td>0.002</td>
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<td></td>
</tr>
</tbody>
</table>

PAN denotes polyacrylonitrile; PMMA denotes polymethylmethacrylate.

* Adjusted for center size (1-40, 41–80, ≥81 patients)
† High flux membranes

### Table 2: Effect of type of membrane used for hemodialysis on outcome of patients with acute renal failure.

<table>
<thead>
<tr>
<th></th>
<th>BCM*</th>
<th>BICM*</th>
<th>P-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients combined</td>
<td>37</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Recovery of renal function</td>
<td>23  (62%)</td>
<td>13  (37%)</td>
<td>0.04</td>
</tr>
<tr>
<td>Survival</td>
<td>21  (57%)</td>
<td>13  (37%)</td>
<td>0.11</td>
</tr>
<tr>
<td>Patients non-oliguric at initiation of HD</td>
<td>20</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Became oliguric during hemodialysis</td>
<td>8  (40%)</td>
<td>15  (75%)</td>
<td>0.047</td>
</tr>
<tr>
<td>Recovery of renal function</td>
<td>17  (85%)</td>
<td>8   (40%)</td>
<td>0.003</td>
</tr>
<tr>
<td>Survival</td>
<td>16  (80%)</td>
<td>8   (40%)</td>
<td>0.01</td>
</tr>
<tr>
<td>Patients oliguric before hemodialysis</td>
<td>17</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Recovery of renal function</td>
<td>6   (33%)</td>
<td>5   (33%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Survival</td>
<td>5   (29%)</td>
<td>5   (33%)</td>
<td>0.83</td>
</tr>
</tbody>
</table>

* BCM, biocompatible membrane; BICM, bioincompatible membrane
† P-value from exact logistic regression after adjustment for APACHE II score.
In addition to differences in symptoms, biocompatibility may have specific adverse clinical consequences. Recent studies have clearly shown that the incidence of amyloid bone disease is much higher in patients who have been dialyzed with a cellulose type membrane than with a biocompatible membrane41). The effects of the cellulose membrane may be due, not only to its inability to clear or adsorb β2 microglobulin, but also to its ability to increase β2m release by mononuclear cells and endothelial cells via the action of complement activation products. In addition, there is evidence that polymerization of β2m is favored by the presence of ROS and proteases, products of neutrophils activated by complement products43).

2. Acute renal failure

A more recent finding is the effect of biocompatibility on the resolution of acute renal failure. Although sequestration of activated neutrophils in the lung parenchyma during dialysis with cellulose membrane is a well-known phenomena, it is important to recall that activated neutrophils adhere to other vascular beds, wherever there is adequate blood flow. With this in mind, recent animal studies of experimentally induced ischemic acute renal failure have shown that such animals exposed (not dialyzed) to a cellulose type membrane have a slower resolution of their acute renal failure than animals exposed to a biocompatible membrane44).

Such a hypothesis has been recently tested in the clinical setting in a prospective randomized study of the effect of the dialysis membrane on the recovery of patients with acute renal failure45). The two membranes used were both low flux, with one membrane associated with vigorous complement activation and the other with modest complement activation. Survival and recovery of renal function were targeted as outcome parameters. In an initial study of 72 patients with acute renal failure well matched for severity of illness, patients dialyzed with the biocompatible membranes had a 62% renal recovery and a 57% survival, compared to 37% renal recovery and survival in patients dialyzed with the membrane associated with complement activation. These differences in recovery and survival were even more marked in patients who were non-oliguric (>400 ml/day) at the start of their dialysis treatment. In this subset of non-oliguric patients, the percent surviving their acute illness in the group dialyzing with the biocompatible membrane was twice the percent of patients dialyzing with the biocompatible membrane, whereas in the subset of patients with oliguric renal failure, there was no membrane effect on recovery or survival.

The results of this single center study has recently been confirmed in a multicenter study, with larger number of patients and published in abstract form46).

Preliminary work is also being pursued on the possibility that biocompatibility of the dialysis membrane may impact on the decline of residual renal function. Recent studies have shown that patients who start peritoneal dialysis retain their residual renal function for much longer periods of time than patients starting hemodialysis. The possibility that this may be due to the bioincompatibility of the dialysis membrane is suggested by animal studies of rats with 5/6 nephrectomy and exposed chronically to dialysis membranes with different biocompatibilities47). Again, such a hypothesis appears to be supported with clinical studies48).

Finally, the biocompatibility of the dialysis membranes is likely to be an important factor in the differences in mortality between chronic hemodialysis patients dialyzed with cellulose membranes and those dialyzed with synthetic membranes. Based on a large sample of prevalent patients in which adjustments were made for multiple co-morbid factors as well as the dose of dialysis, the mortality rate of patients dialyzed with cellulose membranes was found to be 20% higher than similar patients dialyzed with synthetic biocompatible membranes49). The contribution of flux is however a confounding variable.

Biocompatibility is thus an important concern that involves the entire hemodialysis treatment, plays a major role in the well-being of patients and may have short-term and long-term clinical implications50). With an expanded knowledge of blood-membrane interactions, improvement in the safety of the treatment itself and in the patient’s quality of
life will be possible.

References
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