Principles of Separation: Indications and Therapeutic Targets for Plasma Exchange

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Summary
Extracorporeal “blood purification,” mainly in the form of hemodialysis has been a major portion of the clinical activity of many nephrologists for the past 5 decades. A possibly older procedure, therapeutic plasma exchange, separates and then removes plasma as a method of removing pathogenic material from the patient. In contrast to hemodialysis, therapeutic plasma exchange preferentially removes biologic substances of high molecular weight such as autoantibodies or alloantibodies, antigen-antibody complexes, and Ig paraproteins. These molecular targets may be cleared through two alternative procedures: centrifugal separation and membrane separation. This review presents operational features of each procedure, with relevance to the nephrologist. Kinetics of removal of these plasma constituents are based on the principles of separation by the apheresis technique and by features specific to each molecular target, including their production and compartmentalization in the body. Molecular targets for common renal conditions requiring therapeutic plasma exchange are also discussed in detail.


Introduction
Therapeutic plasma exchange (TPE) belongs on a list of extracorporeal therapies that nephrologists are frequently considered experts at managing. TPE is expected to be a logical therapeutic option to consider in a disease condition in which the pathogenesis is linked to a specific toxic biologic substance that has a relatively high molecular mass (>15,000 D), a slow rate of formation, and distribution in the intravascular space (Table 1) (1). In contrast to hemodialysis or hemofiltration in which many substances with lower or middle molecular weights are targeted for removal, the target with TPE is typically a single constituent of plasma (Figure 1) (2).

There are two fundamentally different technological approaches to achieve plasma exchange: separation with centrifugal forces, and separation with a filter membrane-based apparatus. They operate on different physical principles, but each is capable of efficiently fractionating plasma contents from whole blood (3) and allowing for replacement with plasma or albumin. Most clinical trial evidence on safety and efficacy of TPE has subsequently come from studies using centrifugation separation technology. Nonetheless, membrane separation is assumed to have similar efficacy in most conditions (4). Operational contrasts between centrifugation and membrane filtration, the two major technologies used to achieve TPE, are outlined in Table 2. Centrifugal apheresis separates the plasma from cellular components based on density, whereas membrane apheresis is based on molecular size. For the nephrologist to apply the TPE procedure, it is necessary to understand the methods of substance removal by both procedures and the kinetics involved.

Current Indications for TPE
The most recent American Society for Apheresis (ASFA) guidelines, published in 2010 and updated in 2013, include a growing list of category I indications for TPE, in which therapeutic apheresis is considered a first-line therapy, either alone or in conjunction with another therapy (5,6). ASFA category I indications for kidney disease from the most recent guidelines are shown in Table 3.

Principles of Centrifugal Separation
Centrifugal flow devices most commonly deliver continuous flow from the patient to the centrifuge (Figure 2A). An anticoagulant, usually citrate, is added before centrifugation, which is then followed by return of the rest of the blood components with the appropriate replacement fluid (typically albumin or plasma) so that a continuous flow extracorporeal circuit is formed (7). The functional unit is the centrifuge itself, which spins at typical speeds of 2000–2500 rpm to separate the contents of the anticoagulated blood based on the density or specific gravity of various components of blood. Nonselective plasma removal is achieved through layering of plasma near the axis of rotation, adjacent to which is a buffy coat consisting of platelets, lymphocytes, monocytes, and granulocytes in that order extending from the axis of rotation with the red blood cells forming the outermost layer (8). The efficiency of separation of the various blood components depends on the dimensions of the centrifuge and the variable speed (revolutions per minute) of the centrifuge, which creates gravitational forces, as well as the dwell time, which is the amount of time that the blood spends in the centrifuge.
of cryoglobulins (900,000). Ef

(67,000) to B-lipoprotein (2,400,000), and potentially up to that

proteins with a molecular mass ranging from that of albumin

sides of the membrane for large molecules) are at unity for

the ratio of solute concentrations between

pore size and distribution. Sieving coef

all constituents of the plasma, both pathologic and bene

change, as commonly performed, is nonselective, removing

the desired plasma clearance (9). Membrane plasma ex-

calculated blood volume will need to be processed to achieve

the layering of rejected protein solutes on the membrane inner

in vivo

Figure 1.

<table>
<thead>
<tr>
<th>Table 1. Ideal target molecule characteristics for therapeutic plasma exchange</th>
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<tr>
<td>Identified etiologic agent or toxic substance</td>
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<tr>
<td>High molecular mass ($\geq$15,000 D)</td>
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<td>Slow rate of formation</td>
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<td>Low turnover</td>
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Molecular Targets

Intensive plasma exchange is used in the treatment of diseases in which the pathogenesis is associated with abnormal circulating pathogenic autoantibodies (10), para-

proteins, non-Ig proteins, antigen-antibody complexes, al-

loantibodies, endogenous toxins, exogenous poisons, or uniden-
tified toxic plasma constituents in some cases (11). The ability to achieve and maintain reduced concentrations of target molecules with frequent plasma exchange treat-
ments has been demonstrated for a limited number of dis-
ease states. Clinical responses have more frequently been evalu-
ated in the absence of measurement of changes in concen-
tration of pathogenic target molecules.

The primary factors governing removal of target sub-
stances in TPE include the volume of distribution, the $t_{1/2}$, and the potential for rebound of the target to elevated levels in the vascular space after the procedure. Increases in plasma volume exchanged during one TPE procedure yield diminishing return, due to removal of a fixed pro-
portion (65%–70%) of the existing substance and dilution of the plasma level by the replacement fluid used for TPE. Therefore, the per cent decrease in plasma concentration expected during a plasma exchange diminishes as higher total plasma volume is removed: exchange of the first plasma volume (approximately 3 L in a 70-kg individual with a normal hematocrit level) would remove 63%, the next single plasma volume 23%, and the third plasma vol-

ume only 9% (Figure 3) (1,2,9,12,13). In addition, if the siev-
ing coefficient is below unity, removal becomes less efficient. Net reduction will be affected by the redistribution from extravascular to intravascular compartments, by rates of synthesis, and by volumes of distribution approaching and then exceeding the calculated plasma volume (2). The plasma $t_{1/2}$ of the molecular target affects the speed with which the plasma concentration rebounds after the TPE pro-
cedure. The quick rebound in levels for a molecule with a short $t_{1/2}$ would necessitate more frequent TPE. Furth-
more, transfer of target molecules is ongoing between in-
tercellular, interstitial, and intravascular spaces. These interactions between compartments, analogous to the two-

pool model for small solutes, are diagrammed in Figure 4.

These clearances and compartmental features, which will determine the number and frequency of TPEs required, relate ultimately to characteristics of the molecular target associated with the disease. If the antibody has a long $t_{1/2}$ levels in the absence of removal will persist despite cessation of endoge-

ous production. A $t_{1/2}$ of 5 days means that the fractional

Figure 1. | Effectiveness of extracorporeal therapies in relationship to the size of target substances. Molecular masses (in kilodaltons) are as indicated. Sieving coefficients (calculated as the ratio of solute concentrations between filtrate and blood sides of the membrane for large molecules) are essentially at unity for proteins with a molecular mass ranging from that of albumin to B-lipoprotein (2,400,000 D), and potentially up to that of cryoglobulins (900,000 D). At the high end, exclusion of platelets (1–2 μm) and rejection of very high molecular mass proteins (3,000,000 D) are characteristic (2). VitB12, vitamin B12.
turnover of IgM will be 19% per day, so that when production is stopped, disappearance is fairly prompt. For IgG, the t\(_{1/2}\) of 22 days results in only 7% turnover per day, and a longer disappearance curve (13). With TPE, pathogenic IgM molecules can be effectively depleted: because IgM is large (approximately 970,000 D) and resides 90% intravascularly, clearance will be efficiently achieved by one or two plasma exchanges (11), with minimal rebound in serum level. Most autoantibody-mediated diseases, however, are related to production of IgG, a molecule roughly one-eighth the size and residing 30%–45% in the extravascular space. With such a large plasma volume, under otherwise identical circumstances, several plasma exchanges will be required to remove the Ig from the circulation and the interstitial space and to achieve meaningful clearance (9). For IgG-mediated processes, for example, six TPEs would be expected to decrease circulating IgG levels to one-fifth or one-sixth of the baseline levels.

The growing list of conditions with pathogenic autoantibodies identified by modern molecular biology range from myasthenia gravis (versus the acetylcholine receptor) and the Miller-Fisher variant of acute Guillain-Barré syndrome (antibody to the GQ1b ganglioside), to autoimmune dilated cardiomyopathy (antibodies to the B-1 adrenergic receptor) and hepatitis C–related cryoglobulinemia (rheumatoid factor, IgM antibody to IgG). The vast majority of renal indications for TPE involve Ig removal. Meaningful depletion of smaller, shorter-lived constituents such as monoclonal free light chains or even smaller and shorter-lived substances such as free light chains and complement fragments has been harder to demonstrate.

### Therapeutic Targets in Disease States

Although the TPE procedure is a relatively simple and mechanical one, its effectiveness among diverse conditions...
remains variable, even as a greater number of potential pathogenic disease mediators have been identified. Examples of pathogenic target molecules for TPE in kidney disease are listed in Table 4. Antibodies specific to heparin and platelet factor 4 are a hallmark of heparin-induced thrombocytopenia. TPE can effectively reduce heparin-induced thrombocytopenia antibody titers, measured by platelet factor 4–polyvinyl sulfate ELISA or heparin-induced platelet aggregation, to low or negative levels. However, ASFA recommendations are at level 2C, a weak recommendation with low to very low quality evidence, because data are limited to small numbers of case series and reports (5). The target antigen in the neurologic syndrome neuromyelitis optica (NMO) is now understood to be the aquaporin 4 water channel (14). The serum antibody binds to the central nervous system microvessels pia, sub, and Virchow-Robin spaces. As a result, the immune system destroys myelin in the optic nerve and spinal cord. TPE reduces inflammation during attacks of neuritis. The antibody may be a distinguishing biomarker for NMO versus multiple sclerosis and other conditions. However, only 21 of 37 patients had NMO antibodies present in one series (15). In other conditions, such as idiopathic dilated cardiomyopathy, in which autoantibodies to myocardial antigens are present in most patients, heterogeneity of clinical study outcomes may relate to the autoantibody assays in use.

The same variability applies to renal indications for TPE autoantibody-induced forms of GN that do not appear to
benefit equally from TPE (4). For example, plasma exchange is not currently indicated for primary idiopathic membranous glomerulopathy recently described in association with antibodies reactive to M-type phospholipase A2 receptors on podocyte foot processes, a chronic condition with a slow and continuous disease process. Effectiveness of the procedure may also vary with disease activity, particularly with anti-glomerular basement membrane (GBM) disease or rapidly progressive GN. The rationale for TPE in IgA nephropathy is for the removal of circulating pathogenic IgA molecules or immune complexes; despite studies demonstrating that TPE can reduce levels of both, clinical benefit appears to be restricted to early acute disease and may not halt disease progression (5). In deposition diseases such as amyloidosis, removal of monoclonal Ig light chains (amyloid light chain amyloidosis) or
β₂-microglobulin (dialysis-related amyloidosis) may be inadequate given the chronic nature of the underlying process of deposition. In many cases, reduction in pathogenic molecules may only result in clinical remission if the underlying process of overproduction is also reduced.

### Anti-GBM Antibodies

Goodpasture’s syndrome is an organ-specific autoimmune disease mediated by anti-GBM antibodies (16). Anti-GBM disease remains the prototypic renal indication for TPE. The inciting factor is unknown. Almost all patients have anti-GBM antibodies detectable in their blood. Although anti-GBM titer elevations are not established as predictive factors for kidney outcomes, they correlated significantly with the severity of morphologic changes on renal biopsy in one study (17).

In Goodpasture’s syndrome, anti-GBM antibodies are directed against a region of the α3 domain of type IV collagen in the kidney and lung (18–21). Anti-GBM autoantibodies have been shown to be pathogenic through passive transfer experiments. Linear deposition of antibodies against the GBM occurs, and passive transfer in a primate model induces GN (22). Immunoreactivity of circulating Goodpasture autoantibodies to several NC 1 domains of collagen IV has been reported (20), perhaps triggered by conformational changes in the quaternary structure of the noncollagenous domain. Most patients with anti-GBM disease have circulating detectable IgG antibodies, primarily IgG1. In a recent report on the target antigens in the disease, all 57 affected patients had natural anti-GBM antibodies, although there were some differences in the target antigens (23). Anti-GBM antibodies detected by ELISA are typically in the 30–120 U/ml range (19). A recent single-center report of 221 patients treated over 10 years found that serum levels of anti-GBM antibodies were an independent predictor of patient death (23).

ASFA categorizes anti-GBM disease as a category 1 indication for TPE. A low to very low quality evidence, for diffuse alveolar hemorrhage, and category 1A, strong recommendation based on high quality evidence, for dialysis independence (5). Successful treatment of anti-GBM GN couples removal of pathogenic autoantibodies from the circulation with suppression of further autoantibody production. Early implementation of plasma exchange is essential because glomerular injury becomes irreversible once the patient is dialysis dependent. Antibody titers promptly decrease with plasma exchange and immunosuppression. In a frequently cited small randomized clinical trial, anti-GBM antibodies disappeared roughly twice as fast as in the control group (24). The trial suggested a trend toward a superior outcome in the plasma exchange group; however, its small size (n=20) and somewhat higher level of kidney function at entry in the plasma exchange group make interpretation difficult. Limited data suggest a correlation between the level of anti-GBM antibody and severity of morphologic changes in the kidney (17,24). Undetectable antibody levels are the goal of TPE therapy in anti-GBM disease. TPE achieves a rapid decline in anti-GBM antibodies (4). In the largest single-center report, the combination of plasma exchange with corticosteroids and cyclophosphamide had a beneficial effect on renal and patient outcomes compared with treatment without plasma exchange (24). Anti-GBM antibody titers should be monitored regularly and apheresis should be stopped when none are detectable, typically after 10–14 treatments.

### Thrombotic Thrombocytopenic Purpura

The “idiopathic” acquired form of thrombotic thrombocytopenic purpura (TTP) is considered to be an autoimmune condition caused by formation of inhibitory autoantibodies (usually IgG) against the ADAMTS13 (a disintegrin-like and metalloprotease with thrombospondin type 1 motifs-13) protease (25–30). ADAMTS13 is the vWf-cleaving metalloprotease that is deficient in activity either by a congenital genetic mutation or an acquired autoimmune condition in TTP (25,28,29). The low activity of this protease leads to
large vWF multimers, which allow platelet aggregation in response to intravascular shear stress and form the critical nidus of microthrombi formation in the arterioles (31). A relatively large (102 patients) randomized controlled clinical trial has shown the superiority of TPE over plasma infusion alone to treat TTP (30). The inhibitory IgG autoantibodies against the ADAMTS13 protease are the target in this case. An additional benefit of TPE performed with fresh frozen plasma, not albumin, replacement is the ability to administer large volumes of plasma containing the deficient ADAMTS-13 enzyme (30). In rare cases of TTP due to a genetic lack of ADAMTS-13 and no inhibitor, plasma alone may be sufficient for treatment.

Rapidly Progressive GN
TPE is currently established as an effective therapy for ANCA-positive rapidly progressive GN in patients with advanced kidney impairment (serum creatinine > 5.7 mg/dl) or dialysis dependence, and in those with diffuse alveolar hemorrhage, with no benefit over immunosuppression alone for those with mild disease (5). However, all clinical trials have included both pauci-immune (ANCA-associated) and immune complex GN. There are no clinical trials reporting on only patients with idiopathic immune complex GN. A frequently cited large randomized clinical trial for immune complex nephritis due to SLE showed no benefit of addition of TPE to a standard steroid/cyclophosphamide regimen (32). ANCA has a high molecular weight, low volume of distribution, low turnover rate, and a long $t_{1/2}$ and may be pathogenic in pauci-immune GN. In the MEPEX trial by the European Vasculitis Study Group, randomization to the plasma exchange treatment arm reduced risk of dialysis dependence at 1 year (33).

Myeloma Cast Nephropathy
Multiple myeloma and Waldenström’s macroglobulinemia are malignant plasma cell disorders characterized by the production of monoclonal Igs by clones of B lymphocytes. The overproduction of monoclonal Ig types follows in parallel the overproduction of monoclonal Igs by clones of B lymphocytes. The most frequently found in Waldenström’s macroglobulinemia, characterized by production of monoclonal IgM molecules. A single plasmapheresis treatment effectively reduced IgM levels by 46% and serum viscosity by 45% in one recent report, with a reversal of hyperviscosity-related retinopathy and retinal hemodynamic parameters (39).

Cryoglobulinemia
Cryoglobulins consist of abnormal γ globulins and complement components, with a molecular mass of approximately 200 kD, with the unusual property of precipitating in serum or plasma when chilled and redissolving when warm. When analyzed immunologically, the most common form involves a polyclonal Ig, usually IgM or IgA, having rheumatoid factor activity, and is commonly due to chronic viral infections such as hepatitis C or HIV. Complications may include hyperviscosity syndrome. Pegylated IFN-α and ribavirin are now considered the standard of care for hepatitis C management, the major cause of mixed cryoglobulinemia (40). The addition of TPE allows efficient removal of cryoglobulins, and may also improve immune complex solubility by altering the antigen–antibody ratio (41). It is most appropriate for patients with active moderate to severe cryoglobulinemia, including membranoproliferative GN, neuropathy, arthralgias, or ulcerating purpuric lesions (42). Case series and reports, but no randomized trials, support the use of TPE in cryoglobulinemia, performed with warmed lines and replacement fluids to prevent precipitation (43, 44).

Recurrent FSGS
Researchers recently identified soluble urokinase receptor in the plasma of patients with recurrent FSGS, capable
of inducing profound albuminuria when incubated with isolated rat glomeruli (38,39). It may be causative in the proteinuria through a mechanism that includes lipid-dependent activation of the α5β3 integrin (44,45). One risk factor for recurrence is elevated permeability activity detected through an in vitro assay (44,46). The factor appears to be a small protein with one or more glycation sites, with an apparent molecular mass of approximately 50 kD (44). Galactose affinity chromatography has identified cardiotoxin-like cyto- kinase-1 present in the circulation at up to 100 times normal in patients with recurrent FSGS, in the active fraction. Pretransplant TPE may be effective in preventing or delaying recurrence of FSGS in high-risk patients (44,47). Nonrandomized clinical trials and case series indicate that removal of the factor by TPE is associated with partial or complete remission of the nephrotic syndrome in recurrent disease (44,46,48).

**Hemolytic Uremic Syndrome**

There is no compelling evidence that TPE generally benefits patients with diarrhea-associated hemolytic uremic syndrome (HUS), although some patients in the recent European outbreak responded (49). In contrast, TPE has been first-line treatment for atypical HUS (5), although without prospective trials. A growing list of genetic mutations and polymorphisms are now known to predispose to atypical HUS, most commonly involving complement regulators or activators, including complement factor H, complement factor I, and membrane cofactor proteins (50). Recent guidelines recommend TPE for removing circulating complement regulatory components or autoantibodies, while replacing deficient factors with fresh frozen plasma, the strongest recommendation being for those with factor H autoantibodies (51).

**Kidney Transplantation**

The use of plasmapheresis in renal transplantation is generally in one of two scenarios; (1) the use as part of a pretransplantation protocol to decrease immunologic risk of renal allograft rejection (anti-HLA antibody “desensitization” or ABO blood group incompatible transplantation), consequently expanding access to renal transplantation; and (2) use as part treatment for antibody-mediated rejection after transplantation (52). ESRD patients who have high panel-reactive antibodies or donor-specific antibodies undergo desensitization, a complex medical regimen in which donor-specific antibodies are eliminated and production of new antibodies is inhibited to decrease immunologic risk and allow safer transplantation (52–58). Acute humoral rejection of renal allografts is caused by alloantibodies that cause kidney injury in the peritubular capillaries, resulting in endothelial damage, loss of capillary patency, ischemia, and proliferation of myofibroblasts. TPE is used as part of the treatment of acute humoral rejection (52).

Because of the conditions treated and the operational features inherent to extracorporeal TPE, an understanding of target molecules and the kinetics of their removal by the procedure is valuable to the nephrologist. Centrifugal and membrane separation each achieve the goals of plasma exchange, through contrasting technology. Although a growing number of target molecules of pathophysiologic relevance to human diseases are being identified, quantitative methodology has yet to be applied in most cases. In contrast to the uremic syndrome, in which multiple molecules of diverse size accumulate and require removal by dialysis, conditions typically treated by TPE involve a single dominant disease mediator. The application of concepts such as “adequacy,” familiar to the nephrologist, needs to be evaluated if clinical outcomes of TPE are to be better understood.

**Disclosures**

M.E.W. is a consultant for Sanofi Aventis and Nephrogenex. R.A.B. serves on the advisory boards for Octapharma and Alexion.

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