New Approaches to the Treatment of Dense Deposit Disease

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ABSTRACT

The development of clinical treatment protocols usually relies on evidence-based guidelines that focus on randomized, controlled trials. For rare renal diseases, such stringent requirements can represent a significant challenge. Dense deposit disease (DDD; also known as membranoproliferative glomerulonephritis type II) is a prototypical rare disease. It affects only two to three people per million and leads to renal failure within 10 yr in 50% of affected children. On the basis of pathophysiology, this article presents a diagnostic and treatment algorithm for patients with DDD. Diagnostic tests should assess the alternative pathway of complement for abnormalities. Treatment options include aggressive BP control and reduction of proteinuria, and on the basis of pathophysiology, animal data, and human studies, plasma infusion or exchange, rituximab, sulodexide, and eculizumab are additional options. Criteria for treatment success should be prevention of progression as determined by maintenance or improvement in renal function. A secondary criterion should be normalization of activity levels of the alternative complement pathway as measured by C3/C3d ratios and C3NeF levels. Outcomes should be reported to a central repository that is now accessible to all clinicians. As the understanding of DDD increases, novel therapies should be integrated into existing protocols for DDD and evaluated using an open-label Bayesian study design.

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In the past two decades, the development of new clinical treatment protocols has revolved around evidence-based guidelines. Randomized, controlled trials have become the favored metric for assessing the effectiveness of novel interventions, with anything falling below this level of certainty running the danger of being discounted.[1] For rare diseases, this requirement represents a significant challenge.

A rare disease makes the randomized, controlled study design impractical for numerous reasons: Sample size is small and geographically dispersed; the use of historical controls is often impossible; and randomization can be seen as unethical, especially in the face of significant disease morbidity.[2] Because rarity, by definition, suggests an insubstantial public health care concern, one approach to this conundrum is to avoid rare diseases in favor of more common and substantial problems. However, this option is impractical because rare diseases, in aggregate, still represent a substantial health care problem in the developed world.

There are 5000 to 6000 rare diseases, most of which are genetic in origin, and with the continued separation of broad disease categories into smaller, well-defined entities, approximately 250 new rare diseases are described each year.[3] For a disease to be considered rare in the United States, it must affect fewer than 200,000 citizens, reflecting a prevalence of approximately six per 10,000, whereas in Europe, the definition is slightly stricter: Up to five per 10,000.[4] Thus, an estimated 25 million North Americans and 30 million Europeans are afflicted with rare diseases. How, then, are therapeutic advances to be developed for these populations? This article focuses on dense deposit disease (DDD; also known as membranoproliferative glomerulonephritis type II), which is rare even among

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rare diseases, and uses DDD as a model for how new treatment guidelines can be proposed on the basis of evidence derived from animal studies and genetic and molecular data and how outcomes can be followed using Bayesian analysis.

DDD: CLINICAL PHENOTYPE

DDD affects an estimated two to three people per million. It accounts for <20% of all cases of membranoproliferative glomerulonephritis in children and only a fractional percentage of cases in adults.[5,6] The name itself is descriptive of the electron-dense transformation of the glomerular basement membranes (GBM) that occurs in a segmental, discontinuous, or diffuse pattern within the lamina densa (Figure 1). The precise composition of these altered areas remains unknown. The key complement protein, C3, is almost always seen by immunofluorescence microscopy, usually in the absence of Ig deposition. Its presence along the margins of the dense deposits produces a "railroad track" appearance, and where it outlines the mesangium, rings are seen.[7]

The classic light microscopic appearance of a membranoproliferative glomerulonephritis is seen in approximately 25% of patients.[8,9] Mild mesangial cell hypercellularity is the most common pattern (approximately 45%), but a crescentic pattern (approximately 18%) or an acute proliferative and exudative pattern (12%) also occurs.[9] In addition to glomerular dense deposits, patients develop deposits along the choriocapillaris-Bruch's membrane-retinal pigment epithelial interface, a region morphologically similar to the capillary tuft-GBM-podocyte interface (Figure 1).

As a histologically defined disease, DDD lacks unequivocal diagnostic serologic markers of disease activity, although most patients are positive for C3 nephritic factor (C3NeF).[6,10] This is an autoantibody that recognizes neoantigenic epitopes on C3bBb, the C3 convertase of the alternative pathway of complement. C3 convertases cleave C3 into C3b and C3a and thereby instigate and amplify the complement cascade. By stabilizing this normally labile convertase, C3NeF impedes the physiologic regulation of C3bBb by the regulators of the complement activation family and factor I. Nearly 80% of patients with DDD have evidence of alternative pathway dysregulation as reflected by low C3 levels and detectable C3 degradation products, such as C3d, in their serum.[10]

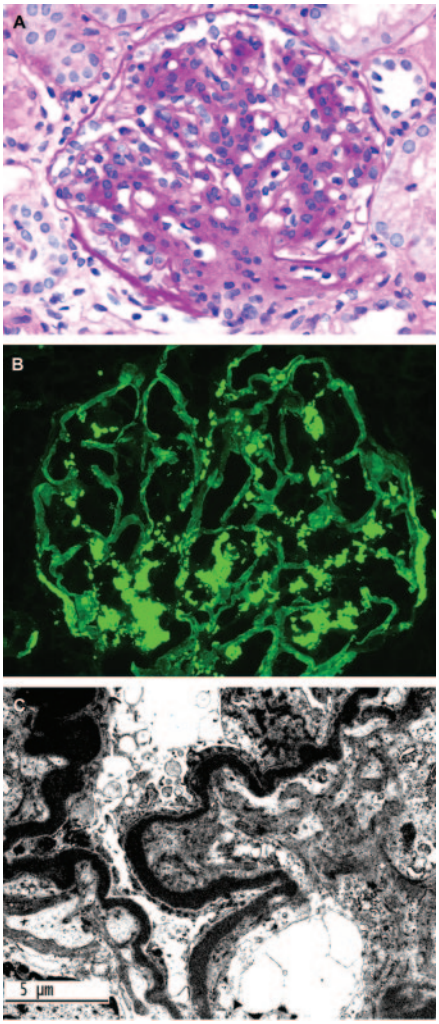

Figure 1. Histopathology of dense deposit disease (DDD). (A) The classic light microscopic appearance showing a membranoproliferative pattern (seen in approximately 25% of patients; periodic acid-Schiff stain). (B) C3 in loops and mesangial areas. The prominent granular deposits in the mesangium result in rings of immunofluorescence that are characteristic of DDD (fluorescein-conjugated anti-C3 antibody stain). (C) Electron photomicrograph showing highly electron-dense transformation of the glomerular basement membranes diagnostic of DDD (unstained). Magnifications: ×400 in A and B; ×5000 in C.

DDD affects female individuals slightly more frequently than male individuals. The DDD Database, a patient-parent–driven epidemiologic study, reports a 3:2 female:male bias among the 56 patients with DDD that it has accrued.[11] This database also reports that progression to ESRD occurs in approximately half of patients who have carried the diagnosis for at least 10 yr, in agreement with data reported by other investigators.[12,13] The North American Pediatric Renal Trials and Collaborative Studies (NAPRTCS) database outcomes are similar. Of the 119 registered children with DDD, 81 have progressed to ESRD (personal communication, William Harmon, MD, Children's Hospital, Boston, MA; March 2, 2007). From the DDD Database, it seems that progression to ESRD develops rapidly, usually within 4 yr of diagnosis, and is the more likely outcome in younger (≤10 yr) than older patients ($P = 0.006$; Figure 2). Girls may have a more aggressive disease course than boys ($P = 0.16$).

There have been fewer than 200 transplants in patients with DDD.[14] Five-year allograft survival approximates 50%, which is significantly worse than the NAPRTCS database as a whole ($P = 0.001$). Living-related donor grafts fair better than deceased-donor grafts ($P < 0.005$). Histologic evidence of recurrent DDD develops in nearly all grafts and is the predominant cause of graft failure in 15 to 50% of transplant recipients.[6,15] Graft loss typically occurs within 2.5 years of transplantation. There are few data to suggest that any therapeutic interventions have an impact on reversing this course, although isolated reports have described the use of plasmapheresis, which seems to be of equivocal benefit.[16,17] The impact of genetics on graft survival has not yet been explored.

ANIMAL STUDIES AND DDD

The first animal model in which DDD was described was the Norwegian York-

shire breed of piglets.[18,19] Affected piglets seemed healthy at birth but after a few weeks failed to thrive as a result of a rapidly progressive glomerulonephritis that inevitably led to death (median 37 d; $n = 25$). Hegasy *et al.*[20] showed that the molecular basis for kidney failure was a point mutation leading to an isoleucine-to-arginine change at amino acid position 1166 (I1166R), which resulted in a nonfunctional factor H gene product. The factor H gene, *CFH*, encodes a soluble member of the regulators-of-complement-activation family that acts at the level of the C3 and C5 convertases. The I1166R mutation effectively impedes extracellular release of factor H, resulting in a decrease in serum factor H levels and unchecked and deregulated activation of the alternative pathway of complement.

Although the DDD Norwegian Yorkshire pigs are no longer available (sperm has been stored), a mouse with a targeted deletion of Factor H ($Cfh^{-/-}$) has been made.[21] Deletion of factor H, like its intracellular retention, results in uncontrolled activation of the alternative pathway of complement, evidenced in these mice by significantly reduced concentrations of C3 and the presence of C3 breakdown products in the homozygous mutants. $Cfh^{-/-}$ mice also develop renal disease characterized by the deposition of C3 on glomerular capillary walls, mesangial hypercellularity with marked matrix expansion, peripheral capillary loop thickening with the deposition of periodic acid-Schiff–positive material, and double-contouring of the GBM, entirely consistent with the diagnosis of DDD and in concordance with the histology that develops in the porcine kidney. However, unlike the Norwegian Yorkshire pig, the factor H–deficient mouse has only 25% 8-mo mortality.

Mouse mutants that are null for both factors H and B ($Cfh^{-/-}.Cfb^{-/-}$) have a normal renal phenotype, as would be predicted from the alternative pathway complement cascade, because factor B is necessary for the formation of C3bBb, the alternative pathway convertase (Figure 3). The absence of factor B in the $Cfh^{-/-}.Cfb^{-/-}$ mutant precludes formation of this convertase, making the absence of factor H inconsequential. This finding also suggests that uncontrolled activation of C3 is an absolute requirement for the development of DDD and is consistent with the observation that C3 deposition in the GBM is evident before the appearance of the dense deposits.[21]

Cleavage of C5 by C5 convertase is the last enzymatic step in the complement cascade (Figure 3). Of the two forms of C5 convertase, one (the alternative pathway convertase) is formed by addition of

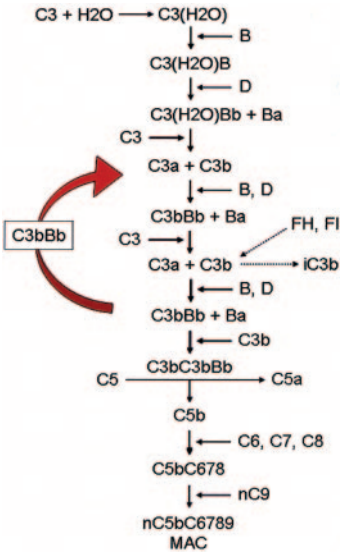

Figure 3. The alternative pathway is constitutively active at low levels through the hydrolysis of the thioester in C3 to C3(H2O). Hydrolyzed C3 combines with factor B, and in the presence of factor D, C3(H2O)Bb is formed. This intermediate convertase leads to the production of C3a and C3b from C3, and C3b enters the C3bBb amplification loop. Amplification on soluble C3bBb occurs with low efficiency because free C3b is rapidly inactivated by factors H and I. However, if C3b binds covalently to surfaces or as a covalent dimer to fluid-phase IgG, then it is partially protected from inactivation. In its dimeric form (C3bC3bIgG), it is seven to 10 times more efficient in generating a C3 convertase than surface-bound monomeric C3b.[71] The very same enzyme on surfaces or on IgG in the fluid phase becomes a C5 convertase by acquiring an additional C3b in its vicinity, which increases the affinity of the enzyme for C5. Here we show in red just one of the possible amplification routes, which seems to be the most relevant in DDD (see text). In the absence of factor H to control levels of C3b in the fluid phase, the $Cfh^{-/-}$ mouse mutant develops DDD. Because factor B is critical to the formation of C3bBb, its absence in the $Cfh^{-/-}.Cfb^{-/-}$ mutant rescues the disease phenotype and DDD does not develop. In the $Cfh^{-/-}.C5^{-/-}$ mutant and the $Cfh^{-/-}$ mutant treated with anti-C5 antibodies, the degree of kidney disease is decreased compared with the degree of kidney disease seen in the $Cfh^{-/-}$ mutant.

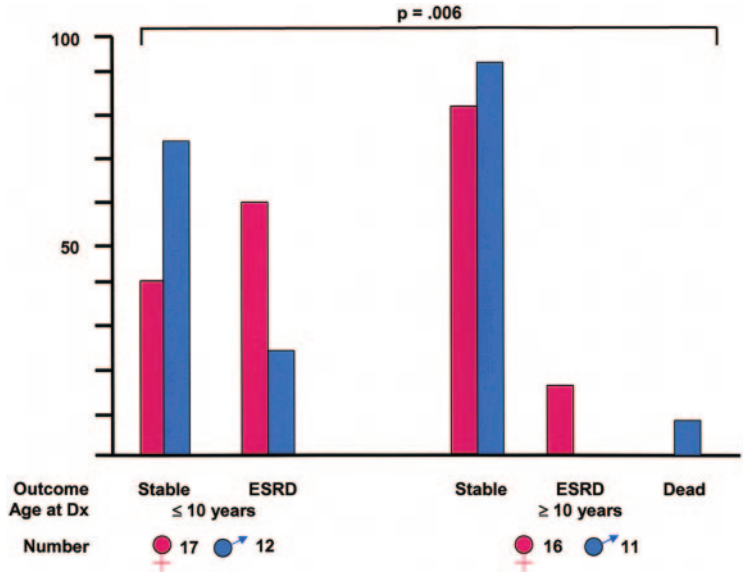

Figure 2. Age at diagnosis *versus* outcome (stable or ESRD). Patients who are ≤10 yr of age are more likely to progress to ESRD than are older patients ($P = 0.006$). Progression to ESRD typically occurs within 4 yr of diagnosis.

C3b to the C3 convertase, C3bBb. This trimolecular C5 convertase (*i.e.*, C3bC3bBb) converts C5 into C5a and C5b. C5b, in turn, complexes with C6 and C7, which recruit C8 and trigger binding and polymerization of C9 to form C5b-9, the membrane attack complex (MAC). MAC creates pores in membranes that are not protected by complement regulators and promote destruction of pathogenic organisms or immune complex–coated cells.

Although serum convertases that are formed with monomeric C3b are inefficient in converting C5 into C5a and C5b, in DDD, the continued cleavage of C3 and the formation of C3b-C3b dimers on the GBM is a particularly effective mechanism for promoting the formation of C5 convertase at this site.[22] To determine the effect of C5 and downstream proteins of the complement cascade in DDD, Pickering *et al.* studied the renal phenotype in $Cfh^{-/-}.C5^{-/-}$ mice and in 12-mo-old animals observed less severe renal disease with reduced mortality and reduced glomerular cellularity as compared with age-matched $Cfh^{-/-}$ mice. However, the proteinuria at 12 mo did not differ between the $Cfh^{-/-}$ and $Cfh^{-/-}.C5^{-/-}$ mice, suggesting that chronic deposition of C3 along the GBM alone is sufficient to disrupt the glomerular permeability barrier.[23]

Suspecting that renal inflammation during DDD flare-ups may critically depend on C5 activation, they next explored the effect of C5 inhibition using a monoclonal anti-C5 antibody and found that it protected $Cfh^{-/-}$ mice that were exposed to a nephrotoxic insult triggered by nephrotoxic serum.[23] Administration of anti-C5 antibody completely prevented the development of proteinuria and glomerular neutrophil influx. Similar experiments performed on $Cfh^{-/-}.C6^{-/-}$ mice were accompanied by marked neutrophil infiltration and proteinuria not significantly different from that seen in $Cfh^{-/-}$ animals, indicating that it is cleavage of C5 to the anaphylatoxin C5a, as opposed to the generation of MAC, that accounts for the glomerular neutrophil influx and albuminuria in $Cfh^{-/-}$ mice during heterologous nephritis.[23]

In aggregate, animal data firmly place fluid-phase dysregulation of the alternative pathway of complement as the triggering pathophysiologic event in DDD. During disease progression, solid-phase activation of downstream complement proteins, in particular cleavage of C5 to C5a and C5b, contributes to the injury.

GENETICS OF DDD

DDD is a complex genetic disease. Only a few families in which more than one member has DDD have been identified, although there are several families in which multiple members have a variety of other autoimmune diseases such as Celiac disease, thyroiditis, and type 1 diabetes.[6] Included in the latter group of families is one in which there are identical twins, one with DDD and the other with type 1 diabetes, suggesting that in the presence of a permissive genotype, environmental factors may be important determinants of disease phenotype.

Of the genes associated with DDD, the most robust data are available for factor H. Consistent with animal data implicating deletion of this gene in dysregulation of the alternative pathway and the development of a DDD renal phenotype, one family of consanguineous parentage has been reported in which two siblings' DDD was diagnosed by renal biopsy.[24] Both children were positive for C3NeF and had low C3 and alternative pathway–mediated hemolysis (APH) 50 levels with increased levels of the C3 degradation product C3d. (APH 50 measures total hemolytic activity of the alternative pathway.) Mutation screening of the factor H gene, *CFH*, showed that the affected children were homozygous for the deletion of a lysine residue at position 224 (ΔK224).

K244 is located within the complement regulatory region in the fourth of the 20 short consensus repeats (SCR) of factor H. Functional studies of factor H ΔK224 have shown that binding to heparin, C3d, and human umbilical vein endothelial cells is not altered, consistent with its intact C-terminal recognition and cell-binding properties. However, binding to C3b is weak; consequently, both co-factor activity of factor H ΔK224 in the presence of factor I and decay-accelerating activity are markedly reduced.[24]

Most patients with DDD do not have disease-causing mutations in *CFH*; however, several alleles of both *CFH* and the complement factor H–related 5 gene (*CFHR5*) are preferentially associated with DDD.[25–27] Of these associations, one of the potentially most interesting is the tyrosine-402-histidine (Y402H) polymorphism. The frequency of the factor H H402 variant is increased in both DDD and age-related macular degeneration, which may be germane because patients with DDD develop early-onset macular drusen.[28–30]

The Y402H polymorphism lies in SCR7. This SCR contributes to one of at least three glycosaminoglycan (GAG)-recognition sites in factor H and participates in binding to C-reactive protein and a number of pathogens that sequester factor H for protection against complement. Structural studies have shown that the substitution occurs toward the center of SCR7, well away from boundaries with SCR8 and 9, and that the three-dimensional structures of both allotypic variants are otherwise identical.[31] Nevertheless, binding studies indicate that the Y402H change alters the specific types of GAG that are recognized by this particular site, which is interesting in view of the fact that mutations disrupting SCR20 affect binding to C3d/C3b and are linked to another rare kidney disorder, atypical hemolytic uremic syndrome.[32–34] *In vitro* functional studies have shown that binding to both human umbilical vein endothelial cells and C-reactive protein is reduced for the H402 variant of factor H as compared with the Y402 variant.[33,34] Heparin-binding assays of the H402 and Y402 variants produce equivocal results.

Significant associations with DDD have also been found with the two common allotypes of C3, glycine 102 (G102) and arginine 102 (R102), designated C3F (fast, G102) and C3S (slow, R102) on the basis of differences in electrophoretic motility.[35,36] C3F is the less common variant and is found in only 20% of

white, 5% of black, and 1% of Asian individuals.[37–39] It is in linkage disequilibrium with a second polymorphism of C3, leucine314proline (L314P): R102 preferentially segregates with P314 and G102 preferentially segregates with L314.[40] An increased prevalence of C3F has been linked to a number of immune-mediated diseases, including IgA nephropathy,[41] systemic vasculitis,[42] and unspecified glomerulonephritis.[43] We have found that the uncommon C3 haplotype—C3 G102/P314—is associated with DDD, consistent with other reports.[39,40]

To identify additional associations between DDD and other complement-related genes, we completed a single-nucleotide polymorphism–based first-pass analysis of approximately 80 genes in 20 patients with DDD and more than 100 control subjects. For 17 genes, one or more exonic and/or intronic SNP generated $P < 0.05$ with >10% association.

In aggregate, these data suggest that most patients with DDD segregate particular variants of several complement or related genes. The functional impact of these variants may be to alter the kinetics of complement regulation or to expose novel amino acid epitopes that facilitate formation of autoantibodies such as C3NeF, with the common outcome being dysregulation of the alternative pathway of complement. The consequence is unchecked damage to unprotected extracellular matrices such as the GBM and Bruch's membrane.

DIAGNOSIS OF DDD

A renal biopsy is essential to diagnose DDD, with the pathognomonic feature being electron-dense deposits along the GBM that are resolved by electron microscopy.[6] Immunofluorescence staining for C3 is almost always positive in capillary loops and in mesangial areas; staining for Ig is usually negative.

Once a diagnosis of DDD is made, the status of the complement system should be documented by ordering CH50, APH 50, C3, C3d, C4, and FH; C3NeF should be measured; and *CFH* should be screened for mutations using bidirectional sequencing (Figure 4). Complement protein measures in DDD are distinctive, with most patients having only low C3 levels, whereas properdin, C5, and other terminal proteins are within the normal range. Factor H levels can be low, as has been reported with missense mutations in the coding sequence that block protein secretion from the endoplasmic reticulum.[25] (For a list of laboratories providing these tests, please contact the correspondence author.)

TREATMENT GUIDELINES FOR DDD

Most treatment guidelines for DDD are primarily based on case series before 1995.[44–48] Recent animal and genetic data, however, suggest that novel interventions should be coupled with nonspecific treatments to retard progression of glomerular disease. Treatment options should reflect and be driven by diagnostic test results.

Nonspecific Treatments

Nonspecific measures that are effective in slowing progression of numerous chronic glomerular diseases include aggressive BP control and reduction of proteinuria.[6] Angiotensin-converting enzyme (ACE) inhibitors and angiotensin II type 1 receptor blockers are first-line agents to decrease proteinuria, improve renal hemodynamics, and possibly limit leukocyte infiltration in the kidney.[49,50] Although not widely used in children, in the presence of hyperlipidemia, lipid-lowering agents such as hydroxymethylglutaryl CoA reductase inhibitors may also delay progression of renal disease, correct endothelial cell dysfunction, and alter long-term atherosclerotic risks.[51,52]

The use of steroid therapy is probably not effective in DDD,[6] although it is extremely effective in a form of glomerulonephritis called juvenile acute nonproliferative glomerulonephritis, which can be confused with DDD.[53] The two diseases can be distinguished clinically, because DDD is typically associated with C3NeF-induced hypocomplementemia, often with nephrotic syndrome and hypertension, whereas in juvenile acute nonproliferative glomerulonephritis, C3 levels remain at the lower limit of normal.

Strategies to reduce C3NeF in DDD using mycophenolate mofetil to inhibit differentiation, maturation, and allostimulatory function of B and T lymphocytes or rituximab, a chimeric IgG1 mAb that specifically targets the CD20 surface antigen expressed on B lymphocytes, have not been studied.[6] The use of rituximab may be justified in patients who are positive for C3NeF, do not have a mutation in *CFH*, and show evidence of C3 consumption (Figure 4). Standard rituximab protocols for the treatment of renal disease should be used, following C3NeF levels and complement assays to document any response.[54]

Disease-Specific Treatments

In patients with defined pathologic mutations of *CFH* (and perhaps those carrying *CFH* risk alleles), specific treatment guidelines should include infusion of fresh frozen plasma or plasmapheresis and exchange with plasma, rather than albumin, to provide functionally intact factor H (recombinant factor H is not currently available). The siblings reported by Licht *et al.*[24] were treated with infusions of 10 to 15 ml of fresh frozen plasma per kg body weight at 14-d intervals, a dosing schedule based on the measured half-life of factor H of 6 d.[55] Except for one episode of mild hypotension and a few episodes of nonspecific abdominal pain that was responsive to antihistamines, the treatment has been well tolerated and kidney function has been shown to be preserved. Of historical note, transfusion of normal porcine plasma to affected Norwegian Yorkshire piglets also inhibited complement activation and increased survival.[56]

An additional DDD-specific treatment that is supported by animal data is the use of an anti-C5 antibody such as eculizumab (Soliris; Alexion Pharmaceuticals, Cheshire, CT) to decrease C5a-mediated glomerular damage. Its development was based on a murine prototype (N19-8), which almost completely inhibits terminal complement complex formation and C5a release *in vitro*.[57] Safety and efficacy of eculizumab

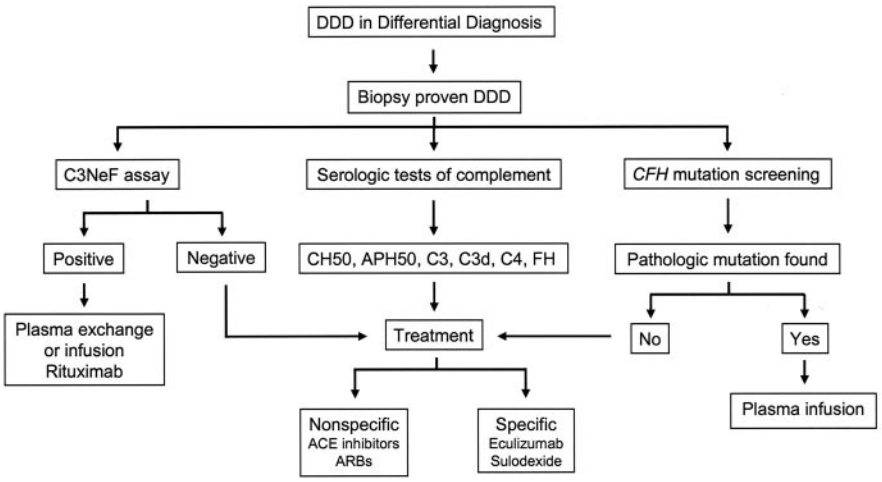

Figure 4. Flow diagram illustrating the diagnostic evaluation and treatment of a patient with DDD. The diagnosis is made by renal biopsy. Serologic tests of complement should be obtained, C3NeF should be assayed, and *CFH* should be screened for mutations. In the presence of C3NeF, removal or dilution of the autoantibody should be considered *via* plasma exchange or infusion, and anti–B cell agents such as rituximab might be valuable. In the presence of pathologic mutations in *CFH* that lead to absent or dysfunctional factor H protein, plasma infusion should be considered (with the use of recombinant factor H in the future). In addition, nonspecific treatment should be aimed at controlling BP and proteinuria. Other treatments that should be considered include eculizumab (an anti-C5 antibody [see Figure 3]) and sulodexide (a heparanase inhibitor [see Figure 5]). The criterion for treatment success should be prevention of disease progression as determined by maintenance of or prevention of decrease in renal function. The secondary criterion should be normalization of activity levels of the alternative complement pathway as measured by C3/C3d ratios and C3NeF levels. After having reached a clinical steady state, reasonable follow-up steps could be monthly for the first 3 to 6 mo, every 2 mo for the rest of the first year, and subsequently every 6 mo, adjusting clinical monitoring if a flare in disease activity occurs.

have been tested by Hillmen *et al.*[58] in patients with paroxysmal nocturnal hemoglobinuria (PNH). In a double-blind, randomized, placebo-controlled, multicenter phase III trial involving 87 patients, these investigators observed stabilization of hemoglobin levels in nearly 50% of patients who were on eculizumab (21 of 43) *versus* none in the placebo group (0 of 44). Patients in the treatment group received infusions of 600 mg of eculizumab every week for 4 wk, followed thereafter by a maintenance dose of 900 mg of eculizumab every 2 wk for the duration of the study. Serious adverse events were reported in four patients in the eculizumab group and nine patients in the placebo group but were not considered to be treatment related. The most common adverse events reported in the eculizumab group were headache, nasopharyngitis, back pain, and nausea, with headache and back pain occurring more frequently in the eculizumab group than in the placebo group (Figure 5). (Note: Eculizumab has now been approved by the Food and Drug Administration for PNH.)

The use of sulodexide is another treatment that may slow disease progression in DDD. Sulodexide is a combination of two GAGs—an electrophoretically fast-moving low molecular weight heparin (80% by weight) and dermatan sulfate (20%)—and can be given orally, subcutaneously, or by intravenous injection. It has profibrinolytic and antithrombotic properties and is an effective inhibitor of heparanase, a β-D-endoglycosidase.[59] Glomerular heparanase expression is increased in DDD and contributes to disease pathogenesis by selectively degrading the negatively charged GAG side chains of heparan sulfate proteoglycans within the GBM or at the surface of podocytes and the glomerular endothelium (Figure 5). This leads to altered permselective properties, loss of glomerular epithelial and endothelial cell anchor points, or liberation of heparan sulfate–bound factors, such as growth factors, chemokines, and cytokines.[60,61] Desulfation of critical GAG also weakens interactions with factor H, which may prove pathogenic in individuals with factor H mutations that attenuate GAG binding.

Upregulation of glomerular heparanase expression has been observed in several other experimental and human glomerular diseases,[62–64] and its inhibition seems to be beneficial at least in animal models.[60,61] Glomerular heparanase expression is augmented by reactive oxygen species, angiotensin II, and proinflammatory cytokines.[65] In *in vitro* models of activated glomerular endothelial cells, heparanase expression not only is increased but also is associated with structural changes to cell surface heparan sulfates.[66] Heparan sulfates on glomerular endothelium also play a prominent role during inflammation and in local complement activation and regulation.[66,67]

Sulodexide may therefore have multiple effects that could make it effective in DDD, including inhibition of glomerular heparanase activity and interference with binding of leukocytes and/or activated complement components to glomerular endothelium. It is approved in Europe to treat vascular thrombotic conditions, and there are recent data to support its use in diabetes. It has been used in several small phase II studies to treat early diabetic nephropathy and can induce an additional 40 to 70% reduction in albuminuria in individuals with tight glycemic and BP control. There are two ongoing clinical trials to evaluate its effect in diabetes (Phase III: http://www.clinicaltrials.gov/ct/gui/show/NCT00130208; Phase IV: http://www.clinicaltrials.gov/ct/show/NCT00130312). At dosages of 200 mg/d, sulodexide has no anticoagulant properties and has an excellent safety profile.[68] An international study to test its efficacy in DDD is planned, and as newer structurally well-defined GAG-

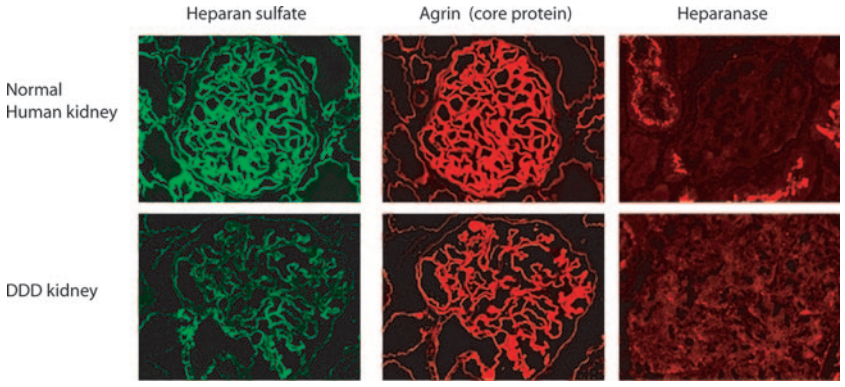

Figure 5. In DDD, glomerular basement membrane staining of heparan sulfate is decreased and heparanase expression is enhanced. Staining for the agrin core protein remains unchanged. Tubular expression of heparanase is high in both DDD and controls.

based therapeutics are made available, it may be possible to opt for agents with specific anti-heparanase or GAG-replacing functions. Participation, although open to all, will require patient consent and institutional review board approval.

FOLLOWING TREATMENT OUTCOMES

Treatment with rituximab, plasma exchange or infusion, eculizumab, or sulodexide should be initiated in the presence of end organ damage (proteinuria/hematuria) and be continuous for 6 to 12 wk. The primary criterion for success at the end of this period should be prevention of disease progression (either maintenance of or prevention of decrease in renal function) as measured by the degree of proteinuria/hematuria. Secondary criteria for success should be normalization of activity levels of the alternative complement pathway and reduction in C3NeF levels (Figure 4). If these outcomes are achieved, then treatment should be continued with adjustment to clinical monitoring in response to flares in disease activity. Assessing efficacy, however, will be difficult, if not impossible, if a prospective, double-blinded study design is used; there are simply too few patients. Other investigators have considered this problem and concluded that a Bayesian approach is a reasonable alternative to evaluate treatment outcomes for rare and orphan diseases.[2]

There are two essential differences between the Bayesian and the double-blinded approach. The first difference is that a Bayesian study design allows the investigator to have some opinion about the probable outcome of the trial. This preconception is expressed in a terms of a *prior probability* of a successful outcome (something greater than 0.5). The traditional clinical trial, in contrast, assumes the likelihood of a successful or an unsuccessful outcome to be equal (*i.e.*, 50:50).

If one assumes, for example, that eculizumab has a measurable beneficial effect on patients with PNH 75% of the time, then one might expect the effect of eculizumab in DDD to be similar, making the prior probability of success 0.75. This assumption moves the expected distribution of outcomes to the positive side and allows the investigator to make decisions with fewer observations when comparing eculizumab-treated with non–eculizumab-treated patients with DDD. Thus, the investigator, being limited in available patients, takes advantage of the fact that treatments are offered with a reasonable expectation of a positive outcome.

Because the estimation of prior probability is subjective and can be affected by animal data and results of trials for related disorders, different investigators will assume different prior probabilities. Some investigators may assume that the effect of eculizumab on DDD is 0.80, whereas others might assume it to be 0.50 (no effect). One approach to addressing variability in prior probability is to take the average suggested from a group of investigators who are familiar with DDD or in dealing with the drug being tested.

The second difference between the Bayesian and traditional double-blinded clinical trial is that the Bayesian trial is open-ended. Decisions about continuance and efficacy are made as every data point is collected. This concept is a natural outgrowth of Wald's method of sequential analysis, which minimizes the sample size required for decision making.[69,70] The Bayesian approach codifies the intuitive decision making of an investigator when treating a rare disease. If, for example, the first three treatments are a success, then one would be inclined to continue; if they fail, then one would be inclined to stop treatment.

Because DDD is very rare, treatments and outcomes should be reported on all patients on the DDD Outcome Database (http://genome.uiowa.edu/ddd). This resource, which is available to all health care personnel, is intended to provide an up-to-date assessment of outcomes as related to treatment protocols. Ultimately, by registering patients on the DDD Outcome Database, physicians will be able to offer care on the basis of a collective experience with a large number of cases.

CONCLUSION

DDD is the prototypical rare disease, affecting only two to three people per million. It causes significant morbidity, leading to ESRD within 10 yr in 50% of people who are younger than 10 yr at diagnosis. Renal transplantation is not a reliable treatment option, because up to 50% of recipients eventually lose their graft as a result of disease recurrence.

All patients who receive a diagnosis of DDD should undergo a standard battery of tests, including review of renal biopsies, serum markers of complement activity, screening for C3NeF, and genetic testing of *CFH*. These tests focus on assessing the alternative pathway of complement for abnormalities. Treatments and outcomes should be followed by

monitoring indices of renal function and serum levels of complement activity.

Data should be reported to a central repository that is accessible in real time to all clinicians. This reporting system will allow "best available" therapies to be used in patient treatment. As our understanding of DDD increases and novel treatments develop (*e.g.*, recombinant factor H), the use of these treatments should be integrated into DDD protocols that are continually analyzed and evaluated in an open-label Bayesian study design.

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This article is dedicated to patients with DDD with the hope and expectation that the ideas contained herein will lead to the eventual development of therapies to treat this disease. We hope that this model for studying DDD can be applied to other rare diseases as well.

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DISCLOSURES

None.

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