C4d Staining in the Diagnosis of C3 Glomerulopathy

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In 2010 we suggested the term C3 glomerulopathy for glomerular disease in which there was isolated deposition of C3 in glomeruli in the absence of immunoglobulins and of components of the classic pathway of complement activation.1 The presence of such isolated C3 implies that complement has been activated via the alternative pathway and alerts the clinician to the need to investigate the complement system for genetic or acquired complement dysregulation. C3 glomerulopathy may have a range of appearances by light microscopy and electron microscopy. Many cases show a membranoproliferative pattern but some are mesangial proliferative and some show prominent endocapillary hypercellularity emphasizing the importance of making a diagnosis based on pathogenesis rather than pure morphology. On electron microscopy, cases can be divided into those which show very osmiophilic dense transformation of the GBM (dense deposit disease) and those without that appearance which have been designated C3 glomerulonephritis.2 Although the original concept was based on the presence of isolated C3 it is clear that, in practice, this is too stringent a definition and that, if cases where the underlying pathology is alternative pathway activation are not to be missed, a wider net is needed. This was clearly shown by a study from Hou et al.3 They looked at cases of dense deposit disease in which it is generally accepted that the glomerular changes result from abnormal control of the alternative pathway of complement. They found that if they used a stringent criterion of only C3 with no immunoglobulins then 50% of cases diagnosed as dense deposit disease on electron microscopy would not be classified as C3 glomerulonephritis. In order to identify the great majority of cases in which the patient requires investigation of the complement pathway they suggested that the best cut-off would be dominant C3 with C3 staining at least two orders of magnitude more intense than any other immune reactant. This was accepted by a 2013 consensus report2 and it was suggested that this could be given the designation ‘glomerulonephritis with dominant C3’.

In this issue of JASN, Sethi and colleagues4 propose that staining for C4d may be useful in distinguishing C3 glomerulopathy from other types of glomerular injury. Most renal pathologists will be familiar with staining for C4d in transplant biopsies. When complement is activated by antibodies through the classic pathway, there is cleavage of C4 and C4d becomes covalently bound at the site of activation. In transplant biopsies it is a long-lasting marker that identifies sites at which antibodies have bound to the graft endothelium and activated complement.5,6 C4 cleavage also occurs in the lectin pathway of complement activation but not in the alternative pathway. Hence Sethi and coworkers hypothesized that C4d should be absent in cases of C3 glomerulopathy and would be a useful diagnostic tool. They therefore studied C4d staining in a range of biopsies with antibody-mediated glomerulonephritis or C3 glomerulopathy. Cases of antibody-mediated glomerulonephritis generally showed bright C4d staining. A notable exception was IgA nephropathy, where it is already recognized that, in many cases, C3 deposition appears to reflect alternative pathway activation; in IgA nephropathy the presence of C4d, almost certainly as a result of the lectin pathway, is associated with a worse outcome.7,8 In the cases that they had already classified as C3 glomerulopathy, C4d was negative in 24 of 30 biopsies and there was only 1+ C4d in the remaining 6. So C4d behaved as they had predicted in these relatively clear-cut cases. Perhaps the most interesting results are in the cases where classification was more problematic. Thus they describe two cases that showed a C3 dominant pattern of staining but the patients had what they call ‘ill-defined autoimmune disease’. In these cases the C4d staining was 1–2+. It is possible that cases like these may represent abnormal alternative pathway control where the clinical presentation has been triggered by immune complex deposition. Also interesting was a case that showed weak staining for IgG κ and bright staining for C3. In this case there was bright C4d staining, indicating that the monoclonal immunoglobulin was indeed the likely cause of the C3 deposition. It is notable in this case that immunofluorescence after pronase digestion of paraffin-embedded material showed stronger staining for monoclonal IgG than on immunofluorescence. This phenomenon of ‘masked’ immunoglobulin deposits that are detected by immunofluorescence on protease-digested paraffin sections but not on frozen sections is being increasingly recognized.9,10 In Messias’s series10 14 of the 20 cases with masked immunoglobulin deposits were C3 dominant by routine immunofluorescence and could have been misdiagnosed as C3 glomerulopathy. It would be interesting to know whether they would have been identified by C4d staining.
An area of particular interest is the relationship of post-infectious glomerulonephritis (PIGN) and C3 glomerulopathy. There is overlap in both clinical presentation and morphologic appearances. It is not unusual for presentation of C3 glomerulopathy, both dense deposit disease and C3 GN, to occur after an infection\(^1\) or to show exacerbations during infections.\(^2\) PIGN has traditionally been thought of as a disease triggered by glomerular immune complex deposition but in many cases there is glomerular C3 deposition without immunoglobulin.\(^3\) In addition PIGN and C3 glomerulopathy may both show prominent sub-epithelial humps on electron microscopy. This overlap means that it may be very difficult to decide on morphology alone whether a biopsy is a typical PIGN that will resolve, or whether it represents a C3 glomerulopathy due to an underlying complement abnormality that will lead to persistent glomerulonephritis. In the C3 glomerulopathy consensus\(^4\) it was noted that the distinction may require the patient to be followed for several months to see if there is the typical resolution expected with PIGN. Therefore it is of great interest to ask how C4d staining behaves in cases of PIGN. Sethi and colleagues examined 13 biopsies of post-infectious GN and found that six were negative for C4d. In most, but not all, cases the C4d staining appeared to mirror the IgG staining. Unfortunately they do not present follow-up data for these cases so it remains to be established whether the presence of C4d and/or IgG helps to distinguish cases of apparent PIGN with different clinical courses.

Renal pathology is an art rather than an exact science and those of us who practice it have to weigh up evidence from morphology, immunohistochemistry and clinical features in order to make a diagnosis that will allow the nephrologist to judge the best way to investigate and manage the patient. By recognizing cases of C3 glomerulopathy we alert the clinician to the need for investigation of the complement system and it is to be hoped that in the not too distant future these cases may be amenable to targeted inhibition of the complement pathway at the level of C3 activation. The level of suspicion on a renal biopsy for the disease process of C3 glomerulopathy will depend on the interpretation of light microscopy, immunohistochemistry, electron microscopy, and clinical history. In some cases there are features, as with the EM appearances in dense deposit disease, that are almost pathognomonic, but in other cases careful consideration of all the features is necessary. The paper by Sethi \textit{et al.} shows that C4d staining is a useful addition to our toolbox that will let us refine further our ability to recognize cases of C3 glomerulopathy.

REFERENCES


