Misdiagnosing renal amyloidosis as minimal change disease

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ABSTRACT

Background. Minimal change disease (MCD) accounts for 10–15% of all adult nephrotic syndrome cases and requires normal renal histology by light microscopy and negative immunohistology. Foot process effacement on electron microscopy (EM) is typical. Renal amyloid deposits demonstrate pathognomonic green birefringence when viewed under cross-polarized light after staining tissue with Congo red (CR) and may reveal fibrils on EM. Late diagnosis and delayed treatment of renal amyloidosis negatively impact on renal and patient survival.

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Methods. A retrospective analysis was performed on 2116 patients referred to the National Amyloidosis Centre between 2001 and 2013, in whom renal amyloidosis was confirmed histologically. Twenty-seven of these patients had renal histology initially interpreted to be MCD.

Results. Among 26 patients in whom biopsy specimens and/or reports were retrieved, the median age at MCD diagnosis was 62 years and presenting proteinuria averaged 7.8 g/24 h. The median time period between the two diagnoses was 241 days (range: 20–2632 days). MCD was diagnosed without CR in 17/26 (65%) biopsies, but all specimens contained amyloid on retrospectively stained biopsies. MCD was diagnosed without EM in 17/26 (65%) cases and all of 10 such biopsies subsequently demonstrated fibrils. Sixteen patients were subjected to two or more renal biopsies when their proteinuria proved steroid refractory.

Conclusion. This study highlights the need to stain renal biopsies from proteinuric adults with CR, examine them under cross-polarized light and perform EM wherever possible. If the suspicion of renal amyloidosis remains high, despite apparent negative histology, specimens should be reviewed at specialist centres before undertaking a second kidney biopsy.

Keywords: Congo red, electron microscopy, minimal change disease, renal amyloidosis

INTRODUCTION

Renal amyloidosis and minimal change disease (MCD) typically present with heavy proteinuria and the nephrotic syndrome (NS). Despite their similar clinical presentations, therapeutic interventions aimed at inducing clinical remission differ substantially between these conditions. Similarly, outcomes differ markedly between patients with these two renal lesions; amyloidosis is usually a multi-system, progressive disease with a poor renal and overall prognosis, whereas MCD is typically a non-scarring, renal-isolated disease that remits following commencement of immunosuppressive therapy and has a good renal and overall prognosis.

MCD accounts for ∼90% of cases of NS in children who are <10 years old, 50% of cases among children between 10 and 18 years and 10–15% of adults presenting with NS [1]. Histologically, it is characterized by a lack of any detectable abnormality on light microscopy (LM), other than possible slight mesangial prominence. On electron microscopy (EM), there is epithelial cell foot process effacement. Immunohistochemical staining in MCD may sometimes demonstrate low-intensity staining for C3 and IgM [2] but is often negative. Because MCD accounts for 90% of cases of NS in young children and is typically steroid responsive, the usual practice in those presenting with NS from this age group is to initiate treatment with corticosteroids without recourse to a diagnostic kidney biopsy [3]. In contrast, adults who present with NS usually undergo a diagnostic kidney biopsy before commencement of disease-modifying therapy. Only ∼75% of adults with MCD are steroid responsive and there may be a delay of 3–4 months before the response is achieved; total steroid exposure, with tapering, is usually around 6 months [2, 3]. The Kidney Disease: Improving Global Outcomes (KDIGO) recommend calcineurin inhibitors (CNIs) and cyclophosphamide in those intolerant of high-dose corticosteroids or those who are steroid unresponsive [3].

Renal amyloidosis is rare in children but accounts for 2–5% of cases of NS in young adults, and 11–13% in middle and old age [4]. Amyloidosis is a histological diagnosis. The presence of extracellular, amorphous eosinophilic deposits on bright-field LM after staining with haematoxylin and eosin may alert the pathologist to the possibility of amyloid as may the presence of 8–15 nm diameter, randomly orientated, non-branching fibrils on EM [5]. However, demonstration of deposits showing green birefringence when viewed under cross-polarized light after staining of tissue with Congo red (CR) dye is the definitive histological test [6]. Identification of amyloid deposits within the kidneys must be followed in all cases by tests to determine the amyloid fibril protein. In clinical practice, this has traditionally been achieved with immunohistochemistry and/or immunofluorescence, although, more recently, proteomic analysis of microdissected amyloid deposits has been used to determine the amyloid fibril protein with great accuracy [5]. Many types of amyloid affect the kidneys, including AL in which the amyloid fibrils are composed of monoclonal immunoglobulin light chains or fragments thereof, and AA in which the fibrils are composed of the acute-phase reactant serum amyloid A (SAA) protein. A diagnosis of AL amyloidosis should be suspected when there is light chain restriction in the glomeruli, interstitium and/or vessels following staining of renal tissue with antibodies against kappa and lambda immunoglobulin light chains. There are a number of other proteins that can deposit as amyloid in the kidneys such as apolipoprotein A-I, apolipoprotein A-II, fibrinogen Aα chain, gelsolin, lysozyme and leukocyte chemotactic factor 2.

Here, we highlight a cohort of patients diagnosed with MCD on renal biopsy following a proteinuric clinical presentation, in whom a subsequent diagnosis of renal amyloidosis was established. We investigate the validity of the initial diagnosis of MCD and attempt to establish whether ‘standardized’ operating procedures in the histology laboratory might enable a greater detection rate of renal amyloid deposition.

MATERIALS AND METHODS

Study design and patients

A retrospective analysis of 2116 patients referred to the UK National Amyloidosis Centre (NAC) with amyloid on renal biopsy between January 2001 and July 2013 was conducted. Those patients in whom an initial diagnosis of MCD had been made were identified through a search of patient referral letters and corroborated in all such cases by original biopsy reports. This constitutes the study population.

Patient demographics at diagnosis of renal amyloidosis, disease-modifying therapeutic interventions, time between the
diagnosis of MCD and diagnosis of renal amyloid, as well as clinical outcomes were evaluated in the study population.

The study was carried out in accordance with the Declaration of Helsinki and approved by the Royal Free Hospital Ethics Committee.

**Histology**

Kidney biopsy samples ( unstained formalin-fixed, paraffin-embedded blocks and stained slides) and histology reports that had been given a diagnosis of MCD (from the study population) were obtained. Six micrometer-thick renal sections were cut and stained with alkalinized CR by the method developed by Puchtler et al. [6]. All such samples along with previously stained slides were viewed under cross-polarized light by two independent observers at the NAC to determine the presence of renal amyloid deposits that initially had been missed (Figure 1a–c). In each case where amyloid was present, identification of the fibril protein type was sought using a standard panel of antibodies.

Wherever possible, EM reports were obtained and/or EM pictures were reviewed or EM was retrospectively undertaken on the relevant biopsy samples in order to identify the presence or absence of fibrils with the kidney tissue (Figure 1d).

**RESULTS**

Twenty-seven patients were identified in whom the initial renal histology was reported as MCD prior to subsequent identification of renal amyloid. Patient characteristics at the time of review at the NAC, shortly after the diagnosis of amyloidosis, are detailed in Table 1. Thirteen patients were female and the median age at the time of diagnosis of MCD was 62 years (range: 32–73 years). The median time lapse between the diagnosis of MCD and that of renal amyloidosis was 241 days and the range was from 20 to 3632 days. The amyloid type was AL in 25 cases and AA in 2 cases.

By the time of review at the NAC, the median proteinuria was 6.9 g/24 h (range: 0.3–12.2 g) and the median eGFR was 64 mL/min (range: 6–191 mL/min). Extra-renal amyloid

**FIGURE 1**: Renal histology showing amorphous material within the glomeruli staining with CR at ×100 magnification (a), and a single glomerulus with CR deposits at ×400 magnification (b), apple green birefringence under cross-polarized light after staining with CR in the same glomerulus at ×400 magnification (c) and EM showing randomly orientated fibrils (d).
deposits were identified by SAP scintigraphy (Figure 2) and/or echocardiography in 21/27 (78%) cases at the initial NAC evaluation and nine (33%) patients had a moderate or large total body amyloid burden by SAP scintigraphic criteria [7] at this time (Table 1, and Figure 3a and b). 10 out of 25 (40%) patients with AL amyloidosis had significant cardiac involvement and the 2 patients with AA amyloidosis had liver involvement; these are known to be poor prognostic features in the respective amyloid types [8, 9].

With a median follow-up in the whole cohort of 3.3 years, 18/27 (67%) patients died, and 7/27 (26%) patients required dialysis (including two who were dialysis dependent at the time of the diagnosis of renal amyloidosis). The median estimated time to dialysis from the time of the initial MCD diagnosis was 8.6 years and from diagnosis of renal amyloidosis was 8.3 years (Figure 4a). The median patient survival by Kaplan–Meier analysis was 5.2 years (range: 0.8–8.4) from diagnosis of MCD and 4.7 years (range: 0.3–6.4) from diagnosis of renal amyloidosis (Figure 4b).

All subsequent analyses were carried out on 26 of the 27 patients because neither the initial biopsy nor the report for one patient could be retrieved. Out of the 26 (65%) patients, 17 were diagnosed with MCD without CR staining of kidney biopsies having been performed (Figure 5a). All 17 biopsies did, however, contain amyloid deposits that were evident when sections were stained with CR by the method of Puchtler et al. [6] and viewed under cross-polarized light at the NAC. Interestingly, 1 of these 17 biopsies had been stained with Sirius red at the local hospital and was reported as equivocal for amyloid despite a ‘final diagnostic’ report indicating MCD (Figure 5a).

The remaining nine biopsies had been stained with CR locally but thought not to show amyloid. We were able to retrieve five of these biopsy samples; four were found to show amyloid deposits that were evident when sections were stained with CR by the method of Puchtler et al. [6] and viewed under cross-polarized light at the NAC. Interestingly, 1 of these 17 biopsies had been stained with Sirius red at the local hospital and was reported as equivocal for amyloid despite a ‘final diagnostic’ report indicating MCD (Figure 5a).

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The remaining nine biopsies had been stained with CR locally but thought not to show amyloid. We were able to retrieve five of these biopsy samples; four were found to show amyloid on CR staining at the NAC, but one did not show green birefringence after CR staining although there was evidence of renal amyloid on the basis of EM, corroborated by SAP scintigraphy. The diagnosis of MCD was made without EM in 17 of 26 patients in this series. In the remainder, EM was performed but was reported not to show fibrils thus supporting a diagnosis of MCD. Interestingly, 9/26 (35%) patients had neither CR staining nor EM performed prior to being diagnosed with MCD (Figure 5b). EM was retrospectively undertaken on 10 of

Table 1. Patient characteristics at the time of initial review at the NAC

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gender, M/F</th>
<th>Age, years</th>
<th>Amyloid subtype</th>
<th>Presenting eGFR, mL/min/1.73 m²</th>
<th>Presenting proteinuria, g/24 h</th>
<th>Presenting amyloid load on SAP scan</th>
<th>Extra-renal organ involvement at the time of diagnosis of amyloid</th>
<th>Renal replacement therapy required?</th>
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<td>1</td>
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<td>38</td>
<td>AL</td>
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<td>51</td>
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<td>40</td>
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<td>43</td>
<td>12.23</td>
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17 biopsy specimens that did not have EM performed initially. This revealed the presence of fibrils, typical of amyloid, in every case; all 10 of these biopsy specimens were also found to contain amyloid by CR staining at NAC review. Similarly, the diagnosis of MCD was made without immunofluorescence staining with antibodies to kappa and lambda immunoglobulin light chains in 13 of 26 patients in this series; among those that were stained in this manner however, none reportedly showed kappa or lambda light chain restriction.

Sixteen patients underwent a second, and one patient underwent a third renal biopsy before the diagnosis of amyloidosis was made. Of these 16 (88%) patients, 14 did in fact have demonstrable amyloid on their first renal biopsy when this was re-stained and/or reviewed at the NAC.

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**DISCUSSION**

We report here 27 patients with renal disease in whom an initial diagnosis of MCD was revised to renal amyloidosis following reassessment of their kidney biopsies. This cohort highlights several distinct and important reasons for delay in establishing a diagnosis of renal amyloidosis. Most commonly (17 of 26 available renal biopsies), the diagnosis of MCD was made in the absence of staining with CR dye or EM. Amyloid was identified in every one of these cases when they were correctly stained and viewed under cross-polarized light, corroborated by all available EM images. In a smaller number of cases, there was an initial failure to identify amyloid despite CR staining and/or EM. We do not know whether the CR-stained specimens were viewed under cross-polarized light or simply under brightfield light, but the latter seems likely. These data highlight the need for renal biopsy specimens obtained from proteinuric adults to be stained with CR and viewed under cross-polarized light. A diagnosis of MCD cannot be made without exclusion of amyloid using this ‘gold standard’ diagnostic test. EM should also be undertaken on renal biopsy specimens from proteinuric adults wherever possible, particularly when MCD is suspected, with the presence of fibrils alerting the pathologist to the possibility of amyloid or alternative fibrillar pathology. It is noteworthy
that in one of these 17 cases, Sirius red staining was used. Although Sirius red has been reported to result in ‘stronger’ staining that might be ‘more noticeable’ than CR, it stains experimentally induced amyloid deposits only weakly and is known to stain connective tissue non-specifically [10]; it is thus inadequate for exclusion of amyloid. The finding of kappa or lambda light chain restriction on immunofluorescence microscopy can undoubtedly alert the pathologist to the possibility of AL amyloidosis [11] and it is perhaps surprising that this was not detected in any of the 13 biopsies in this series thus stained. Similarly, the finding of a plasma cell dyscrasia on routine pre-biopsy serum or urine immunoelectrophoresis and/or serum free light chain assay should alert the pathologist to the possibility of AL amyloidosis.

On review of the medical literature, there is a previous case report of AL amyloidosis diagnosed as MCD [12]. The diagnosis of renal amyloidosis in this nephrotic patient was reached only after 2 years and three kidney biopsies. By this time the patient had become dialysis dependent and had been exposed to steroids, cyclosporine A, cyclophosphamide, azathioprine and tacrolimus without improvement. A subsequent review of all three renal biopsies with CR staining, EM and immunostaining showed the presence of amyloid by CR staining in the last two, associated in each case with lambda light chain restriction; the first biopsy was again indicative of MCD alone. The authors proposed three possible explanations: first, the patient had both diseases; second, this was a morphological variant of a light chain deposition disease indistinguishable from MCD on LM alone; and third (favoured), there was amyloid all along but too scanty to detect. Although amyloid fibrillogenesis by proteins must, by definition, involve a phase of pre-fibrillar aggregate formation, there is little evidence to support the idea of clinical disease before amyloid has supervened. It is thus more likely that a sampling error is responsible for the failure to identify renal amyloid on the very rare occasions that it may be missed despite correct staining of tissue specimens viewed under the correct conditions. Jones et al. [13], in 1986, published a report of two nephrotic patients initially misdiagnosed with MCD who were then found to have scanty amyloid deposits on biopsy review. Re-examination, even by LM, proved useful in these cases and interestingly, a few scattered, silver-positive, epimembranous spicules of variable morphology were identified in certain glomeruli, which were subsequently found to contain amyloid when the CR-stained tissue and EM were reviewed. The authors reiterate the importance of remembering that MCD is a diagnosis of exclusion, and in their cohort of nephrotic patients, from 1972 to 1982, renal amyloidosis was 1.5 times more frequent than MCD.

There was a median time lapse of 241 days between the diagnoses of MCD and renal amyloidosis among these patients. The renal and overall outcomes in this group were poor with an eventual need for renal replacement therapy in 26% and the median survival from diagnosis of amyloid of 4.7 years. AL amyloidosis is a progressive disease, which, in the absence of therapy, is almost universally fatal, but chemotherapy directed towards the underlying clonal plasma cell disease is increasingly successful. Prior to being diagnosed with renal amyloid, patients received steroids, often in combination with cyclophosphamide, CNIs or azathioprine in order to bring about resolution of their nephrotic-range proteinuria. In each case, the reason for reviewing the initial kidney biopsy or undertaking a repeat kidney biopsy was a failure to respond to therapy. Taken together, these data support the need for review of biopsy specimens in a specialist centre whenever the suspicion of renal amyloidosis remains high. Examples might include a patient with a plasma cell dyscrasia diagnosed with MCD who fails to respond to steroid therapy, or a patient with a plasma cell dyscrasia diagnosed with MCD who has significant cardiac or hepatic disease. Another situation in which review of histology by a specialist centre is warranted is when EM pictures show fibrils but the precise diagnosis remains unclear.

In conclusion, the treatment of amyloidosis with appropriate chemotherapeutic or biological agents in a timely fashion can halt ongoing amyloid deposition and prevent progression to ESRD and the requirement for renal replacement therapy [14, 15]. Furthermore, early diagnosis may mean a reduction in the likelihood of infectious and thrombotic complications associated with a prolonged nephrotic state [14]. Finally, correctly diagnosing renal amyloidosis will prevent inappropriate, ineffectual and potentially harmful use of drugs such as steroids, cyclophosphamide and/or CNIs. This study highlights the importance of staining renal biopsy tissue from adult nephrotic patients with CR dye and viewing the specimens under cross-polarized light. Whenever the clinical suspicion of renal amyloidosis remains high, tissue blocks should be forwarded to specialist amyloidosis centres. In addition, renal

![Image](https://academic.oup.com/ndt/article/29/11/2120/1808503/fig5)

**Figure 5**: Histological staining patterns. (a) Proportion of patients whose initial renal biopsy was stained with CR and those in whom the result was equivocal. (b) Proportion of patients whose biopsies were reviewed under EM.
tissue should be obtained for EM wherever possible, at the very least so that the pathologist can revert to analysis of EM pictures whenever the clinical picture demands it.

**CONFLICT OF INTEREST STATEMENT**

All of the authors confirm that the results presented in this paper have not been published previously in whole or part, except in abstract format. R.H.S is a clinical research fellow supported by The Royal Free London NHS Foundation Trust. None of the other authors declare any conflict of interest.

**REFERENCES**


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