Diseases and histological normality of the renal glomerulus: a clinicopathological study

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SYNOPSIS Thirty-seven percutaneous renal biopsies showing no significant abnormalities on light microscopy were studied electron optically and by immunofluorescence when available. Assessment of the pathological material was followed by analysis of the patients’ clinical notes, and a clinicopathological correlation was carried out.

Twenty-three patients fulfilled the clinical criteria of minimal change disease; 10 did not behave clinically as minimal change and showed immune complex deposition; two had benign recurrent haematuria; and two had myelomatosis.

Our study shows that if diagnosis is based solely on the light microscope appearances of renal biopsy, diseases other than minimal change are likely to be overlooked. Accuracy of diagnosis in structural terms requires additional immunofluorescence and electron microscopic study; final clinical diagnosis also requires careful follow-up, and repeat biopsy may be necessary.

For over 60 years it has been known that there are patients, both children and adults, with the nephrotic syndrome in whom no significant glomerular changes can be seen by light microscopy of biopsy specimens. These patients have constituted the problem of minimal change glomerulonephritis (lipoid nephrosis, foot process disease, ‘nil’ disease), in which it was found difficult to equate clinical severity with apparently normal histological glomerular appearances (Robson, 1972).

The introduction of electron microscopy showed that in minimal change glomerulonephritis there is partial or complete fusion of the epithelial cell foot processes (Farquhar et al, 1957a and b; Folli et al, 1958; Movat and McGregor, 1959; Bencosme and Bergman, 1962), and that this is the one consistent morphological abnormality (Pollak et al, 1968; Spargo and Seymour, 1972; Jao et al, 1973).

However, it has recently been realized that other glomerular lesions not readily detectable by light microscopy may cause proteinuria or the nephrotic syndrome (Hopper et al, 1970; Muehrcke and Pirani, 1972; Jao et al, 1973; Hyman and Burkholder, 1974).

We have therefore reviewed renal biopsy specimens submitted over a seven-year period and selected those in which the glomeruli showed no significant histological abnormality for detailed study by electron microscopy, and immunofluorescence when available. Our findings were subsequently correlated with the clinical findings.

Material and Methods

A total of 308 percutaneous biopsies were reviewed. Of these, 37 (12.0%) were considered as showing histologically normal glomeruli or, at most, marginally increases in mesangial cells and/or matrix. All biopsy specimens contained a minimum of five untraumatized glomeruli, the majority more than 10.

From 28 of these cases, processed material was studied electron optically; in 12 cases, electron microscopy was augmented by immunofluorescence studies. Seven patients underwent repeat biopsies. Assessment of the pathological material was followed by analysis of the patients’ clinical notes, and a clinicopathological correlation was carried out.

TECHNIQUES

Immediately on removal of the biopsy cores, small fragments were cut from both ends and fixed for electron microscopy. The remainder was bisected...
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half being used for light microscopy and half for immunofluorescence studies.

1 **Light Microscopy**

Tissue was fixed in mercuric formalin, dehydrated, and embedded in Paraplast. Sections of 2 mm maximum thickness were routinely stained with haematoxylin and eosin (H and E), periodic acid-Schiff (PAS), Martius-scarlet-blue (MSB), and methenamine silver (MeS).

2a **Transmission Electron Microscopy**

Primary fixation was in buffered 2.5% glutaraldehyde, followed by repeated washing in cacodylate buffer. After post-fixation in buffered osmium tetroxide, the tissue was dehydrated and embedded in Araldite. 0.5 mm thick sections were stained with alkaline toluidine blue for tissue recognition, and thin sections containing glomeruli were mounted on grids, double stained with uranyl acetate/lead citrate, and examined electron optically.

2b **Scanning Electron Microscopy**

Small fragments of tissue were fixed for 4 hours in 2.5% glutaraldehyde followed by washing in three changes of cacodylate buffer over 24 hours and storage in buffer wash at 4°C. After dehydration in graded acetones, ranging from 30% to 100% in 10% increments, the tissue was critical point dried from liquid CO₂. Dried tissue blocks were fastened to stubs with colloidal silver and coated with gold in a Polaron Sputter coater. Coated blocks were examined in a Cambridge S4-10 SEM at a gun potential of 20 kV.

3 **Immunofluorescence**

4 mm thick cryostat sections were incubated for 30 minutes with commercially prepared FITC conjugated antisera to IgA, IgG, IgM, IgE, fibrinogen, and the C₃ fraction of complement, and then viewed under ultraviolet excitation. Control sections were pretreated with unconjugated antihuman antiserum, the specificity of which was checked at intervals by immunoelectrophoresis.

**Results**

1 **Light Microscopy**

Glomerular appearances ranged from normal to mild prominence of mesangial components only (figs 1 and 2).

2a **Transmission Electron Microscopy**

Of the 28 cases studied, 17 showed significant fusion of the epithelial cell foot processes (fig 3), often with

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Fig 1  **Histologically normal glomeruli in first biopsy specimen from patient 4 (cf figs 11 and 12).**

PAS x 350
a slightly increased vesiculation of visceral epithelial cells. None disclosed granular or fibrillar deposits in, or other abnormality of, their capillary basement membranes or mesangial areas. These cases were categorized as 'foot process disease' (FPD).

In eight cases, granular electron dense deposits, indicating immune complexes, were present in focally thickened basement membranes. These complexes, though not numerous, were predominantly epimembranous in distribution (fig 4) but occasionally involved the mesangia or adjacent basement membranes (fig 5). On this basis, these were classified as immune complex disease (ICD).

In two cases (28 and 34), fibrillar material similar to amyloid with a focal epimembranous and mesangial distribution was present (fig 6).

In one case (11), glomeruli appeared normal.

2b SCANNING ELECTRON MICROSCOPY (SEM)
Limited tissue availability from the renal cores restricted SEM to a few specimens only. The main advantage of this technique lay in providing high resolution topographical views of glomerular structure in which fusion of epithelial foot processes and collapse of epithelial cells on to underlying basement membranes were seen to advantage (figs 7 and 8).

3 IMMUNOFLOURESCENCE (IF)
This was available in 12 cases. Of these, six were negative to all antisera used; the remaining six cases disclosed weak positivity to a range of labelled antisera (figs 9 and 10).

PATHOLOGICAL CLASSIFICATION (table 1)
Based on EM and IF evidence, 17 of the 37 cases were classified as FPD. Extensive EM study of this group failed to reveal immune complex deposits within glomeruli, and IF studies for localizing immunoglobulins, when performed, were negative.

Nine cases were classified as ICD. Eight of these showed electron dense granular deposits, and IF, when done, was positive; in one case (25), IF was weakly positive, but no material was available for EM.

One case (11), from which IF was not available, was classified as normal on EM evidence only.

Two cases (28 and 34), although negative to all fluorescent antisera, were classified as 'amyloid disease', as already described.

In eight cases, classification was not possible because of insufficient tissue or unavailability of EM or IF evidence.

CLINICAL DATA
The clinical notes and follow-up records of the
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Fig 3  Foot process disease showing fusion of foot processes (arrows) of epithelial cells (ep) along outer borders of normal basement membranes. cl = capillary lumen. TEM × 6250
Fig 4  Limited epimembranous immune complex deposition (indicated by arrows) in glomerular basement membranes. Overlying epithelial cells (ep) show extensive foot process fusion. cl = capillary lumen.
JEM \times 12500
Fig 5  Immune complexes (arrows) within basement membranes in mesangial area (mes). Epithelial cells (ep) show fusion of foot processes. TEM × 12 500
Fig 6  Focal infiltrate of fibrillar amyloid (arrows) which has elevated epithelial cell (ep) from glomerular basement membrane (bm), below which lie the capillary lumen (cl) and lining endothelium (en). TEM × 37 500
Fig 7  Normal glomerular capillary showing primary and secondary processes (open arrows) of epithelial cells external to glomerular basement membrane (closed arrows), with portion of lining endothelium (en). SEM x 10 000
Fig 8  Glomerular capillaries in foot process disease showing loss and fusion of foot processes (open arrows) external to glomerular basement membranes (closed arrows) and portions of endothelial linings (en). SEM x 9000
Fig 9  Foci of fluorescent IgM in immune complex deposits in glomerular mesangia. IF × 350

Fig 10  Focal fluorescent deposits of complement (C3) in immune complexes in basement membrane and mesangia. IF × 400
Table I  Pathological classification

FPD = foot process disease; ICD = immune complex disease; Unclass = unclassifiable; 'Amyloid' = fibrillar protein present, with a fine structure similar to amyloid.

<table>
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Table II  Clinical data and final clinical diagnoses

NS = nephrotic syndrome; PrU = proteinuria; Haemat = haematuria; H/T = hypertension; AlbU = albuminuria; BJPrU = Bence-Jones proteinuria; SRMC = steroid responsive minimal change; CMC = clinical minimal change; Not MC = not minimal change clinically; BRH = benign recurrent haematuria.
patients were reviewed; relevant data, including the final clinical diagnoses, are summarized in table II.

They comprised 21 men and 16 women, their ages ranging from 11 to 66 years, with the following presenting symptoms:

Nephrotic syndrome 27
Proteinuria 6
Haematuria 2
Proteinuria and hypertension 1
Albuminuria and Bence Jones proteinuria 1

ASO titres were recorded on admission in 23 cases; in only one case (11) was it significantly raised (>300 Todd units).

Renal function in terms of serum creatinine levels was assessed at presentation in all cases. Values ranged from 27 to 248 μmol/l (0.3 to 2.8 mg/100 ml); only four cases (4, 20, 21 and 28) were above the accepted upper limit of normal (133 μmol/l; 1.5 mg/100 ml).

Thirteen patients were given what is now considered adequate steroid therapy, ie, at least 100 mg of prednisolone on alternate days. Of these, eight who responded were classified as steroid responsive minimal change (SRMC). Fifteen patients received inadequate or no steroid therapy but went into spontaneous remission with or without subsequent relapses (clinical minimal change—CMC); 10 failed to respond to steroids or failed to behave clinically as minimal change (not MC); two had benign recurrent haematuria (BRH); and two were cases of myeloma.

REPEAT BIOPSIES
Repeat biopsies were performed on seven of the 37 cases and related to clinical progress or response to therapy (table III).

Of the two cases (4 and 27) presenting with immune complex disease, one progressed to a severe membranous glomerulopathy and renal failure in the 18 months between biopsies (figs 11 and 12). The other showed no significant structural or immunological change during the inter-biopsy period of 15 months despite clinical deterioration.

Four patients (10, 14, 27, and 37) presented with foot process disease. Two proceeded to moderate deposition of IgM containing complexes within mesangial areas (fig 13) after intervals of 24 and 56 months respectively. Two others registered no structural or immunological change in intervals of 19 and 36 months between biopsies; one of these experienced clinical remission following adjustment of steroid dosage after repeat biopsy.

In one case (15), lack of immunofluorescence data and absence of glomeruli from first biopsy EM fragments prevented classification; repeat biopsy 22 months later disclosed significant IgM and C3 containing complexes within glomerular basement membranes.

DEATHS (table IV)
Five of the 37 patients studied have died.

Three succumbed to myocardial ischaemia; of these, two with foot process disease showed no histological abnormality of glomeruli in necropsy specimens which were not, however, subjected to IF or EM studies. The third myocardial ischaemic death involved a patient with established immune complex disease, but no necropsy was performed.

A fourth death resulted from massive pulmonary embolus one month after biopsy which had disclosed foot process disease; histology of renal tissue taken at necropsy showed normal glomerular appearances. The fifth death, affecting a patient with glomerular 'amyloid' deposits in his biopsy specimen, resulted from a terminal leukaemic manifestation of myelomatosis.

CLINICOPATHOLOGICAL CORRELATION (table V)
Clinically, the 37 patients fell into four main groups. Twenty-three fulfilled the clinical criteria of minimal change disease; two had benign recurrent haematuria; two had myelomatosis; and 10 had primary glomerular diseases which failed to respond to adequate steroids and which did not behave clinically as minimal change.

Pathologically, 14 of the 23 cases clinically diagnosed as minimal change showed foot process fusion only on electron microscopy and were therefore classified as 'foot process disease'. Six were unclassifiable because no material was available for electron microscopy. Of the remaining three, one had positive immunofluorescence and complexes on electron microscopy (case 33); the second gave positive immunofluorescence but provided no material for electron microscopy (case 25); the third had electron dense complexes but no glomeruli were available for immunofluorescence (case 6). These three cases were thus classified pathologically as immune complex disease. It is interesting to note
Fig 11  First biopsy from case 4 showing limited immune complex deposition (arrows) along epimembranous aspect of glomerular basement membrane. Epithelial cell (ep) shows foot process fusion; endothelium (en) lines capillary lumen (cl) (cf fig 1). TEM × 14 250
Fig 12. Repeat biopsy from case 4 showing continuous immune complex deposits (arrows) in thickened glomerular basement membranes. Epithelial cells (ep) show extensive foot process fusion; basement membranes are separated from capillary lumina (cl) by fenestrated endothelium (cf figs 1 and 11). TEM × 18 750.
Fig 13  Repeat biopsy from case 10 showing IgM containing complexes (arrows) in the mesangial areas (mes). Part of a mesangial cell nucleus occupies the upper left. En = capillary endothelium. TEM × 25 000
that case 6 had a nephrotic syndrome associated with seropositive rheumatoid disease, and that her nephrosis cleared with a change in antirheumatoid therapy which had not included gold treatment. Case 25 had had a transient nephrotic episode at the age of 2 years, and a recurrence in 1971 when aged 13 years. On both occasions remissions occurred spontaneously and rapidly. The biopsy (taken towards the end of the second episode) showed a mild increase in mesangial cells and matrix, possibly representing the late stage of a resolving acute glomerulonephritis. Case 33, who also had a short nephrotic episode with spontaneous and permanent remission, probably also exemplified transient immune complex deposition rather than true minimal change glomerulonephritis.

The two patients with benign recurrent haematuria gave classical clinical histories. In one (case 19), tissue was not available for either immunofluorescence or electron microscopic study. The other (case 11) was biopsied in clinical remission and proved normal electron optically.

Biopsy specimens from both patients with myelomatosis (cases 28 and 34) proved negative to immunofluorescence, but electron optically both showed mesangial and subendothelial fibrillar deposits similar to 'amyloid'.

Of the 10 other cases diagnosed clinically as having primary glomerular diseases other than minimal change, six were classified pathologically as immune complex disease, and one (case 15) was unclassifiable because no material was available for electron microscopic or immunofluorescent studies. Repeat biopsy in this instance, however, confirmed the clinical impression of immune complex disease. The remaining three were classified pathologically as foot process disease (cases 10, 14, and 17), but, because of their clinical courses, two (10 and 14) were rebiopsied (vide supra) to disclose immune complex disease. The third case (17), a young man who presented in 1970 with asymptomatic proteinuria and mild hypertension, has remained clinically well despite proteinuria and hypertension which have remained at constant levels.

Discussion

Minimal change glomerulonephritis (lipoid nephrosis, foot process disease, idiopathic nephrotic syndrome) is a well documented clinical and pathological entity. Clinically, the heavy proteinuria or nephrotic syndrome respond favourably to adequate steroid therapy in 95-100% of cases (Cameron, 1970; Lancet, 1972; Jao et al, 1973; Cameron et al, 1974; Mallick, 1975). It often runs a relapsing course, 40-60% of patients showing recurrence of proteinuria following steroid withdrawal (Cameron et al, 1974), and spontaneous remissions are common.

Pathologically, the minimal change lesion is
associated with no significant light microscope abnormalities (Robson, 1972; Grishman and Churg, 1973; Jao et al., 1973; Cameron et al., 1974) and with partial or complete fusion of the epithelial cell foot processes at the electron microscope level (Pollak et al., 1968; Spargo and Seymour, 1972; Jao et al., 1973). Many authors now believe that the minimal change lesion is not mediated by immune complex deposition; thus complexes are not seen electron optically, and immunofluorescence is typically negative (Michael et al., 1964; Drummond et al., 1966; Pollak et al., 1968; Hopper et al., 1970; Habib and Kleinknecht, 1971; Berger et al., 1971; Morel-Maroger et al., 1972; Hoyer et al., 1972; Muehrcke and Pirani, 1972; Jao et al., 1973; Lewis et al., 1973; Morel-Maroger et al., 1973; Hyman and Burkholder, 1974). In this series, three patients (6, 25, and 33), in whom a clinical diagnosis of minimal change had been made, showed morphological evidence of immune complex deposition. Probably these cases represent spontaneously resolving immune complex glomerulonephritis rather than true minimal change disease, a situation which is liable to occur in any clinical series of cases of 'minimal change' glomerulopathies.

We have excluded from this series all renal biopsies showing focal and segmental glomerular sclerosis (segmental hyalinosis), an entity which is currently regarded as separate and distinct (Abramowicz et al., 1970; Churg et al., 1970; White et al., 1970; Nagi et al., 1971; Habib and Kleinknecht, 1971; Cameron, 1972; Lancet, 1972; Jao et al., 1973; Berlyne, 1974; Cameron et al., 1974; Jenis et al., 1974; Hyman and Burkholder, 1974). It is appreciated, however, that focal glomerulosclerosis in the early stages affects primarily juxtaglomerular glomeruli which may escape a small or superficial biopsy (Churg et al., 1970; White et al., 1970; Lancet, 1972; Jao et al., 1973; Jenis et al., 1974; Hyman and Burkholder, 1974) but the clinical course differs from that of minimal change disease, and rebiopsy may provide the correct diagnosis.

In respect of benign recurrent haematuria, the underlying lesion is a focal proliferative glomerulonephritis (Ross, 1960; Bodian et al., 1965; Ayoub and Vernier, 1965; Ferris et al., 1967; Singer et al., 1968; Cameron, 1972; Heptinstall, 1973; Mallick, 1975).

Ten of our 37 cases (27%) showing no significant abnormalities on light microscopy subsequently developed membranous glomerulonephritis. Clinically, these cases (2, 4, 10, 14, 15, 17, 26, 27, 31, and 35) have shown a gradual progression of their renal disease and have failed to respond to steroids. Six (2, 4, 26, 27, 31, and 35) were diagnosed as immune complex disease on electron microscopy of first biopsies, and this was confirmed in two (4 and 27) by repeat biopsies. In case 15, neither IF nor EM was performed on the first biopsy, but repeat biopsy confirmed immune complex disease. In three patients (10, 14, and 17) diagnosed pathologically as having foot process disease, clinical follow-up indicated a disease other than minimal change. Repeat biopsies from two of them (10 and 14) showed conclusive evidence of immune complex disease; repeat biopsy in the third (17) is under consideration.

In two cases, therefore, repeat biopsies were necessary to establish the correct pathological diagnosis. This again raises the question whether minimal change glomerulonephritis may transform into membranous glomerulonephritis (Hopper et al., 1970). Neither patient, however, behaved clinically as a subject of minimal change disease, and we agree with recent authors who state that this transformation does not take place (Hopper et al., 1970; Cameron, 1972; Robson, 1972). Possibly these cases represent false negative results related to biopsy sampling in a disease (membranous glomerulonephritis), which may not affect all glomeruli equally, particularly in the early stages. Unfortunately, immunofluorescence was not available for assessment of the biopsies in question, a point which emphasizes the value of this technique, particularly when applied to several glomeruli, in distinguishing early membranous from minimal change disease.

Conclusions

We agree with Muehrcke and Pirani (1972), Mac-Donald (1973), and Jao et al. (1973) that if diagnosis of glomerular disease by percutaneous biopsy based solely on light microscope appearances diseases other than minimal change are likely to be overlooked. Accuracy of diagnosis, in structural terms, requires additional immunofluorescence and electron microscopic study. Final clinical diagnosis also requires careful follow-up, and repeat biopsy may be necessary.

We acknowledge with thanks the help of members of the technical staff of the University Department of Pathology with histological preparations.

References


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