Original paper

Difficulties in differentiating thin basement membrane disease from Alport syndrome

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We examined a group of 83 patients (57 children and 26 adults) with thin basement membrane disease and 17 patients with Alport syndrome. We compared the clinical data and, above all, the morphological patterns of both disease entities, with particular focus on not very advanced changes which might lead to a misdiagnosis due to the non-detection of the early stages of Alport syndrome.

Key words: thin basement membrane disease, Alport syndrome.

Introduction

Erythrocyturia is a frequent symptom of three glomerulopathies – thin basement membrane disease, IgA nephropathy and Alport syndrome. IgA nephropathy generally does not pose diagnostic difficulties. Diagnosis is based on histopathological examination and immunofluorescence. There may, however, occur difficulties in differentiating thin basement membrane disease from the early stages of Alport syndrome.

The typical pattern of thin basement membrane disease is a thinning of the lamina densa of the glomeruli to under 200 nm, affecting most of the nephron loop [1, 2, 3].

Characteristic for the Alport syndrome are an uneven thickness as well as structural changes of the basement membrane, more precisely the lamina densa, such as loosening and, above all, splitting [4, 5], with small dense inclusions referred to as "breadcrumbs" between the layers [6].

This syndrome can also be accompanied by light microscopic changes, such as the presence of immature glomeruli and of foam cells in the kidney interstitium, but these are not typical features of the syndrome.

In some cases of Alport syndrome only minor ultrastructural abnormalities are observed, for example a mere thinning of the lamina densa or a loosening of its structure in some segments only. These symptoms are more frequent in women. In children, initially minor changes progress with time. The aforementioned observations were confirmed by animal experiments [7, 8]. There have also been individual cases of Alport syndrome in which the symptoms did not include any changes in the basement membranes [9]. In some cases of thin basement membrane disease, on the other hand, there may occur a loosening of the structure and even a duplication of the lamina densa.

The above examples show that the differentiation of these two diseases can be difficult. This difficulty was one of the reasons to start looking for differences in genetic research [9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19]. The first were Habib *et al.* [9]. They were followed by other researchers: Aarons *et al*. [10], Lang *et al*. [11], Lamprecht *et al.* [12], Lemmink *et al*.

Table I. Clinical signs of thin basement membrane disease

Clinical signs	Children (n = 57) ♀29, ♂28	Adults (n = 26) ♀14, ♂12
Isolated erythrocyturia	33	6
Hematuria => isolated erythrocyturia	1	–
Acute renal insufficiency => isolated erythrocyturia	1	–
Erythrocyturia and proteinura	4	8
Nephrotic syndrome and erythrocyturia	2	–
Nephrotic syndrome =>erythrocyturia and proteinuria	–	2
Hematuria and proteinuria	–	1
Proteinuria	2	2
Nephrotic syndrome	14	7

[13], Pigneras *et al*. [14], and others [15, 16, 17, 18, 19, 20, 21, 22, 23, 24]. It was established that both diseases are accompanied by mutations in the COL4A3/COL4A4 genes. However, based on the available results of the research on the mutations in the COL4A3/COL4A4 genes, no clear diagnostic criteria could be defined in order to distinguish thin basement membrane disease, autosomal dominant Alport syndrome and autosomal recessive Alport syndrome. Of diagnostic importance is the lack of reaction with anti chains α3, α4 and α5 collagen type IV in the basement membranes of renal glomeruli and tubules (and the lack of reaction from the anti-α5 chain in the skin).

Consequently, the diagnosis of thin basement membrane disease is more difficult than might be assumed. In clinical assessments and in morphological diagnoses the disease is often not taken into consideration.

In routine diagnosis, the most readily available and surest examination procedure is electron microscopy. However, even a complete set of such tests does not always allow one to make a definite diagnosis.

Aim of the study

Based on tissue material obtained by kidney biopsies, we decided to compare the morphological patterns, especially the electron microscopic images of thin basement membrane disease and Alport syndrome, with particular focus on characteristics which might point to an early stage of Alport syndrome.

Table II. Clinical signs of Alport syndrome

Clinical signs	Infants (n = 9)	Adults (n = 8)
Erythrocyturia	3	1
Erythrocyturia and proteinuria	–	4
Proteinuria	1	2
Nephritic syndrome	4	1
Renal insufficiency	1	–

Material and methods

The research material comprised biopsy specimens of kidneys of 83 patients (57 children and 26 adults) with a diagnosis of thin basement membrane disease and of 17 patients (9 children and 8 adults) with a diagnosis of Alport syndrome. The control group (for the evaluation of the thickness of the lamina densa of the basement membranes) comprised 11 biopsy samples of 11 patients with minimal change disease.

In the group of children with thin basement membrane disease were 29 girls aged 2 to 16 years and 28 boys aged 2 to 17 years. The group of adults with the same diagnosis was composed of 14 women aged 23 to 70 years and 12 men aged 19 to 70 years. Among the 9 children with Alport syndrome were 7 boys from 7 to 17 years of age and 2 girls of 2 and 10 years. The group of adults with Alport syndrome consisted of 5 men aged 21 to 46 years and 3 women aged 24 to 49 years (Table I).

Thirteen children with thin basement membrane disease and 3 patients with Alport syndrome (including 2 children) had a family history of kidney diseases. In most cases, the family members were diagnosed with erythrocyturia. Clinical symptoms are presented in Table II.

Histological examinations

Part of the biopsy samples fixated in 10% neutral formalin solution were routinely processed. The material was immersed in paraffin, cut by microtome into sections of 3-5 μm and mounted on microscope slides. The preparations were stained with haematoxylin and eosin, impregnated with silver halides in

accordance with Jones' method and the PAS reaction was carried out.

Electron microscopy examinations

The material collected for ultrastructural studies was routinely fixed in a 3.6% buffered glutaraldehyde solution with pH of 7.4. Next, the material was embedded in Epon 812 epoxide resin. Photographic documentation was carried out in the column of an Opton 900 transmission electron microscope.

Immunofluorescence assays

Immunofluorescence slices of 5-6 μm were fixed in a cold 1:1 mixture of alcohol and acetone for 10 minutes, dried and rinsed three times in PBS. Next, the specimens were incubated with sera containing antibodies against IgA, IgG, IgM, complement proteins C3, C4, C1q, and fibrinogen, labelled with fluorescein 5-isothiocyanate (FITC). Following incubation, the slices were rinsed three times in PBS and covered in glycerol under cover slips. The presence, composition and locations of the deposits of immunoglobulins, complement proteins and fibrinogen were assessed by fluorescence microscopy.

Measurement of the thickness of the lamina densa

In order to assess the thickness of the lamina densa of the basement membrane, three measurements were performed in the same places by means of the computing environment MATLAB (www.mathworks.com) semiautomatic method. Measurements were carried out in different sections of the nephron loop of the glomerulus capillaries and in sections immediately neighbouring mesangial regions.

All the glomeruli in a sample (1 to 4 glomeruli) were subjected to a morphometric analysis and in each an average of 5 sections in all the vascular loops were evaluated. Microphotographs were made with the following enlargements: 3500×, 4400×, 5600×, 7000×, 8750×, 10 500×, 11 500×.

Statistical analysis

In view of the lack of a normal distribution of some variables (Shapiro-Wilk test, $p < 0.05$) and the small quantity of data, only non-parametric statistical methods based on median tests were applied for the statistical analyses. The differences in the thickness measurement results between the groups were analysed by means of the Kruskal-Wallis test, which is the non-parametric equivalent of the one-way analysis of variance (ANOVA). For post-hoc analysis the conservative Dunn's multiple comparison test was applied. The Wilcoxon signed-rank test was used in order to compare the lamina densa thickness measurements obtained by the semiautomatic method in the different groups. The predetermined significance level for the analyses was $p < 0.05$. In the data description the levels $p < 0.05$ and $p < 0.001$ were indicated, if they occurred.

Results

Thin basement membrane disease

The light microscopic examinations did not reveal any significant changes, either in the group of children or in the group of adults with thin basement membrane disease. They were described as mesangial hypercellularity (not confirmed by the electron microscopic examination) or as non-specific abnormalities. In two children – a 2-year-old girl and a 7-year-old boy – symptoms of immature renal glomeruli were observed, and in one girl – who did not show any abnormalities of the glomeruli – the presence of foam cells and renal interstitial fibrosis. In 4 children, mesangial glomerulonephritis was suspected, due to the presence of deposits of IgA and IgM in 1 case, IgM and C1q in 1 case, IgA and M as well as C3 in 1 case, and IgA and C3 in 1 case. The deposits were vestigial, and their presence was not confirmed by the electron microscopic examination. There was also no confirmation of an increase of the number of mesangial cells. Such trace deposits were also observed in 4 adults – of IgA, IgG, IgM, C3 and C1q in 1 case, of IgA, IgM, C3 and C4 in 1 case, and of IgM and C3 in 2 cases. In these cases, the possibility of mesangial glomerulonephritis could also be excluded by electron microscopy examination due to lack of mesangial hypercellularity.

In 15 children and 8 adults exponents of focal segmental glomerulosclerosis were observed, in the case of only 4 children and 4 adults in the light microscopic examination and in all the other cases exclusively in the electron microscopic. These changes were less severe.

For all patients, the electron microscopic examination revealed typical features of thin basement membrane disease, namely a thinning of the lamina densa to under 200 nm in over 80% of the capillary loops (Fig. 1). In addition, in 16 children and 4 adults isolated minor local rarefactions of the structure of the lamina densa were discovered (Fig. 2).

Alport syndrome

In the group with diagnosis of Alport syndrome, the morphological pattern – both in the light and the electron microscopic image – was clearly more diverse than in the previous group.

In 10 patients, the light microscopic examination revealed not very pronounced changes described as mesangial hypercellularity or non-specific lesions. In 4 cases focal segmental glomerulosclerosis (FSGS)

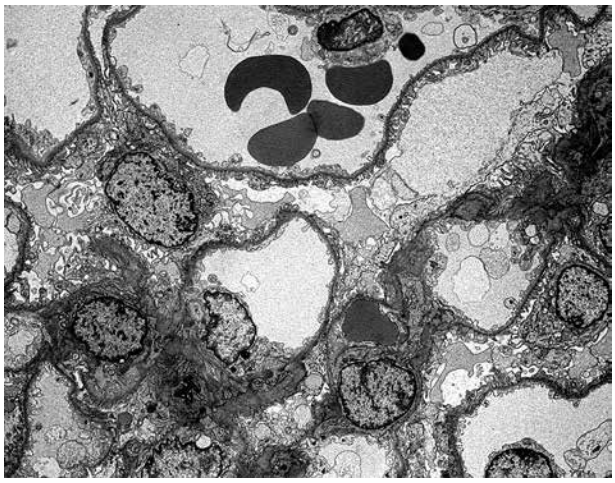

Fig. 1. A fragment of renal glomerulus from a patient with thin basement membrane disease. Lamina densa thinning within all capillary loops. Electron microscopy, magnification 4000×

was diagnosed. In all 4 cases this diagnosis was confirmed by the electron microscopic examination. The histological examinations showed in all cases numerous foam cells in the interstitium (Fig. 3).

In 1 case the immunofluorescence test revealed the presence of IgA, IgM and C3 deposits, due to which the possibility of IgA nephropathy was taken into consideration. The electron microscopic examination confirmed the presence of deposits as well as mesangial hypercellularity and resulted in a diagnosis of IgA nephropathy imposed on the changes typical for Alport syndrome.

In this group, there was a 24-year-old female patient who had been diagnosed 13 years previously with thin basement membrane disease. From the age of 2 she had erythrocyturia and proteinuria. The first biopsy confirmed only exponents of thin basement membrane disease (extensive thinning of the lamina densa). However, the light microscopic examination revealed the presence of foam cells and focal interstitial fibrosis. This patient has a family history of

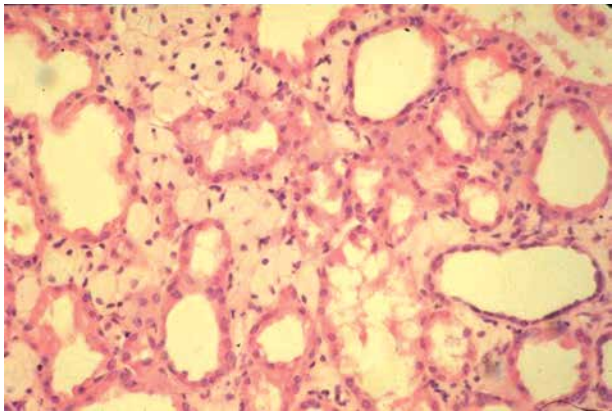

Fig. 3. Alport syndrome. Aggregates of foam cells within the renal interstitium. HE staining, magnification 400×

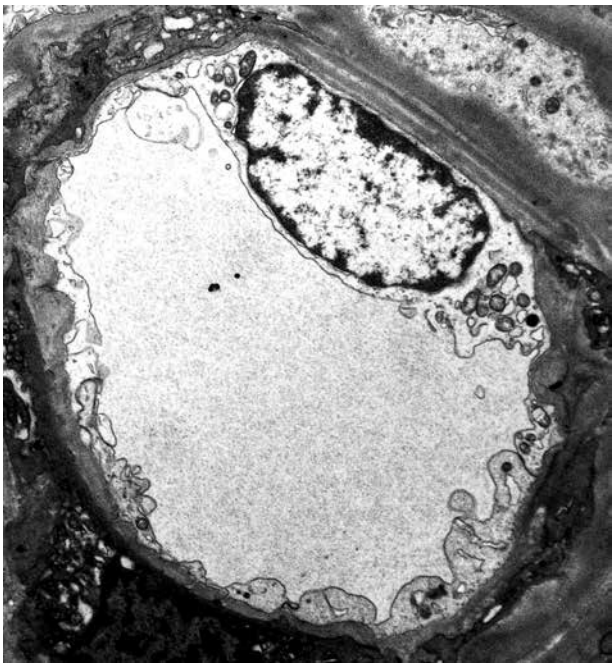

Fig. 2. Capillary loop of renal glomerulus affected by thin basement membrane disease. In some segments, lamina densa of normal thickness with duplication. Thinning present in the remaining parts of the loops. Electron microscopy, magnification 5600×

erythrocyturia. Her sister underwent biopsies at the age of 12 and again at the age of 17. The first biopsy revealed exponents of thin basement membrane disease and minor segmental glomerulosclerosis. The second biopsy showed that the glomerulosclerosis had not intensified and no other abnormalities were discovered apart from the thinning of the membranes.

For the entire group with a diagnosis of Alport syndrome the electron microscopic image clearly pointed to this syndrome, although the different changes were not equally developed. In all cases uneven thickness of the lamina densa was observed – with sections of less than 200 nm and significantly thickened sections (1337 nm). In most cases the capillary loops showed changes in the structure of the lamina densa – numerous loosenings over large sections, splitting and, above all, local formation of several layers, separated from each other by small dense particles (Fig. 4). The surface of the lamina densa, especially the subepithelial layer, was uneven over large sections and intermeshed in some places. Moreover, mesangial matrix expansion was found in 7 cases.

No differences of intensity of the aforementioned changes depending on the clinical symptoms or the age of the patients were noted.

Minimal change disease – control group

In all cases there were discovered ultra-structural changes that are typical for minimal change disease:

disappearance of podocyte foot processes over more than 75% of the surface of the capillaries and microvilli-related surface changes in many podocytes, directed toward Bowman's capsule.

The lamina densa of the basement membrane of most of the capillary loops was of normal thickness. Only in a few isolated sections, a thickness of maximum 200 nm was observed. None of the cases showed a loosening of the structure or a duplication of the lamina densa.

Measurement of the thickness of the lamina densa of the basement membrane

The results of the measurements of the thickness of the lamina densa revealed significant differences between thin basement membrane disease and Alport syndrome, as illustrated by Table III, which also contains the measurement results from the control group (MCD).

Discussion

In the specialist literature, thin basement membrane disease and Alport syndrome are often discussed together, especially due to the frequent occurrence of thin membranes in the Alport syndrome, above all in the early stages of the disease. In this phase, the morphological differentiation of the two diseases can be very difficult [25, 26]. Both cause similar clinical symptoms: erythrocyturia, erythrocyturia and proteinuria, proteinuria, nephrotic syndrome. In our material, patients with thin basement membrane disease were relatively frequently diagnosed with proteinuria. The high percentage of patients with thin basement membrane disease diagnosed with proteinuria may be explained by the fact that, in the case of proteinuria or proteinuria and erythrocyturia, material for electron microscopic tests is usually secured, whereas in the case of isolated erythrocyturia often no material is secured for such tests. If such tests were carried out in all the relevant cases, the number of diagnosed cases of thin basement membrane disease would be significantly higher and the percentage of patients with proteinuria consequently correspondingly lower. The extra-renal symptoms typical for Alport syndrome – hearing loss and eye abnormalities

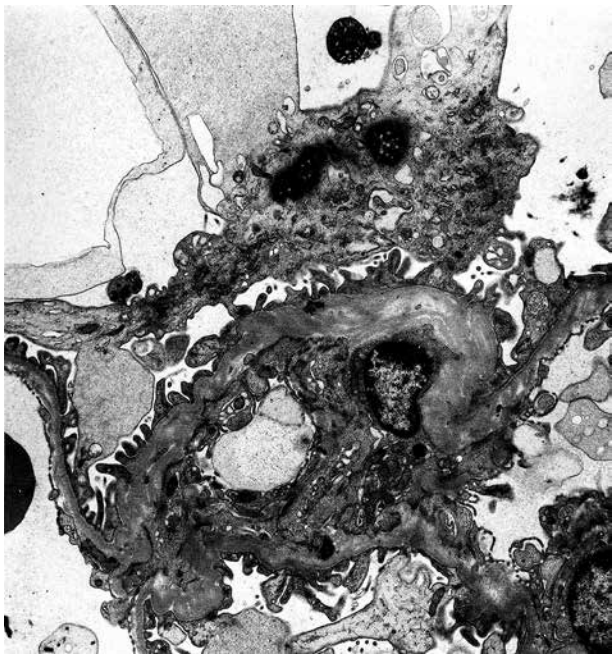

Fig. 4. Alport syndrome. Capillary lamina densa of uneven thickness. Segmental loosening of the structure. Within the thickened segment a duplication of the lamina densa. Electron microscopy, magnification 1500×

– are not always developed, and if they are developed they appear only later in life [27, 28].

The diagnostic difficulties in the early stages of Alport syndrome are well illustrated by the above described case of a female patient who was first diagnosed with thin basement membrane disease and developed symptoms of Alport syndrome 13 years later. The first biopsy revealed the presence of foam cells in the interstitium, but this was not a sufficient argument to suggest the possibility of the Alport syndrome. It is to be considered, though, if this change, given the simultaneous occurrence of thin membranes, should not be taken into account as possibly pointing to an early stage of Alport syndrome.

On the other hand, in 2 cases of thin basement membrane disease exponents of immature glomeruli were observed. These are characteristic for the Alport syndrome and should not be considered as a sign for a threatening development of Alport syndrome.

Also to be taken into consideration are patients with thin basement membrane disease who show

Table III. Descriptive statistics measurments of the thickness of lamina densa [nm] depending on the group

	N	Mean	Median	SD	Min-Max
MCD	11	249.2	190.2	180.4	56.3-555.0
TBMD children	57	179.4	142.0	103.6	51.7-553.0
TBMD adults	26	148.1	103.3	113.9	55.7-523.7
AS	17	157.5	157.5	359.0	107.1-1337.0

MCD – minimal change disease; TBMD – thin basement membrane disease; AS – Alport syndrome

subtle changes such as segmental loosening of the structure of the lamina densa or its uneven contour.

Another interesting fact in connection with the analysed material is the stronger development of other morphological changes, including light microscopic changes, in patients with Alport syndrome than in patients with thin basement membrane syndrome. It is not a significant factor, but the occurrence of such changes together with extensive thinning of the lamina densa should dictate caution concerning the prognosis.

Thin basement membrane disease does not always have a good prognosis; it is therefore unjustified to describe this disease as "mild erythrocyturia", and patients diagnosed with this disease must remain under permanent monitoring.

Conclusions

Based on an analysis of the specialist literature data and the results of our own observations derived from the comparison of the morphological characteristics of both diseases, it can be said that in cases when thin basement membrane disease is diagnosed, the following changes should be taken into consideration: presence of even minor sections of thickened lamina densa, unevenness of the surface of the lamina densa, changes in the structure of the lamina densa, presence of interstitial changes, and development of other glomerular abnormalities, especially sclerosis.

These changes can be significant for the detection of the Alport syndrome in its early stages of development.

The research results for both diseases indicate the necessity to perform kidney biopsies in each case of erythrocyturia, which is often neglected due to the opinion that erythrocyturia is not a burdensome symptom. Absolutely necessary are also electron microscopic examinations, which should be compulsory for every kidney biopsy.

The authors declare no conflict of interest.

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