Morphometric comparison of glomerular basement membrane thickness and density of the deposits in idiopathic mesangiocapillary glomerulonephritis type I and II

M. Danilewicz and M. Wagrowska-Danilewicz
Department of Pathology (Morphometry Division), Medical University of Lodz, Poland.

SUMMARY

Morphometric comparison of glomerular basement membrane thickness and density of the depostis in idiopathic mesangiocapillary glomerulonephritis type I and III.

Fifteen renal biopsy specimens from patients with idiopathic mesangiocapillary glomerulonephritis type I (MCGN-I) and fifteen from patients with type III (MCGN-III) for whom both light and electron microscopy as well as immunofluorescence microscopy and full clinical data were available were examined quantitatively and compared with six cases of normal controls. Morphometric investigations of the electron micrographs were performed by means of a computer image analysis sys - tem to compare glomerular basement membrane (GBM) thickness and the elec - tron – microscopic density of the depostis in MCGN-I and MCGN-III as well as to study whether these parameters could correlate with the clinical data. The study revealed that the mean value of the deposit area per GBM area was in MCGN-III patients significantly increased in comparison with MCGN-I group. The mean values of the GBM thickness, however, were similar in both MCGN-I and MCGN-III groups. There were significant positive correlations between deposit area per GBM area and proteinuria in MCGN-I and MCGN-III patients. Significant positi - ve correlation was also noted between GBM thickness and proteinuria in MCGN-I, but not in MCGN-III group. We observed in MCGN-I group significant positi - ve correlations between deposit area per GBM area and hematuria as well as bet - ween GBM thickness and hematuria. Correlations between the other parameters were weak and have not reached statistical significance.

The present morphometric analysis of glomerular ultrastructure has not eluci - dated the controversy concerning whether patients with mesangiocapillary glo - merulonephritis should be further subdivided to include a «type III». Although the analysis of the electron – microscopic density of the depostis suggests morpholo - gic separateness fo these glomerulopathies, the clinicopathologic correlations do not support this differentiation.

Key words: ***Mesangiocapillary glomerulonephritis type I and III. Morphometry. Density of the deposits. Glomerular basement membrane thickness.***

Recibido: 27-XII-96.
En versión definitiva: 21-III-97.
Aceptado: 21-III-97.

Correspondencia: M. Danilewicz, M.D., Ph.D.
ul. Zamenhofa 5 m.
90-431 Lodz, Poland

INTRODUCTION

Mesangiocapillary glomerulonephritis type I (MCGN-I) is a well defined histopathologic entity that, althoufh it may be found in a variety of clinical settings[1, 2], is usually idiopathic[3]. This disease is characterized morphologically by the presence of granular, subendotelial electron-dense material presumed to be immune deposits[4].

In 1973 Burkholder et al. described mesangiocapillary glomerulonephritys type III (MCGN-III) which is characterized by the presence of subendothelial and predominant subepithelial deposits resembling those seen in membranous nephropathy[5]. This suggestion was supported by Anders, Thoenes, and their colleagues[6, 7] who argued that these patients should be regarded as comprising a distinct subgroup of patients with mesangiocapillary glomerulonephritis (MCGN). Furthermore Strife et al. reported another variant of MCGN-III with disruption of the glomerular basement membrane[8]. However, the classification of patients with biopsy features characteristic of MCGN-III has been a source of controversy. Some observers do not suggest any differences between these patients and those who have MCGN-I and prefer to classify patients with MCGN-III in the same group with those with MCGN-I[3, 9]. Moreover, common genetic basis for types I and III of MCGN has been pointed out[10].

The aim of this study is to evaluate if the GBM thickness and the electron-microscopy density of the deposits are different in MCGN type I and III. Moreover, to study the correlations between these subgroups and clinical and analytical data.

PATIENTS AND METHODS

Patients

Fifteen patients with MCGN-I and fifteen with MCGN-III of Burkholder type[5] were examined by percutaneous renal biopsy. In each case morphological diagnosis of MCGN-I and MCGN-III was established independently by two experienced nephropatologists and based on light microscopy, immunofluorescence and electron microscopy. Morphological and immunopathological findings in cases with MCGN-I and MCGN-III are summarized in table I. As a control 6 biopsy specimens of the kidneys removed because of trauma were used.

Table I. Morphological and immunopathological findings in cases with MCGN-I and MCGN-III.

	MCGN-I n = 15	MCGN-III n = 15
Light microscopy		
Diffuse mesangial hypercellularity*	15	15
Thickening of the capillay wall	15	15
Focal and segmental sclerosis	7	2
Lobular accentuation	9	3
Focal tubular atrophy and/or focal intersticial fibrosis	12	6
Electron microscopy		
Increase in mesangial cells	15	15
Increase in mesangial matrix	15	15
Mesangial interposition	15	8
Subendothelial electron-dense deposits	15	15
Numerous subepithelial electron-dense deposits	–	15
Small subepithelial electron-dense desposists	2	–
Mesangial electron-dense deposits	6	4
Immunofluorescence		
Granular IgG deposits of the peripheral capillary wall	11	6
Granular IgG deposits of the peripheral capillary wall and mesangium	4	1
Granular IgM deposits of the peripheral capillary wall	2	–
Granular C_3 deposits of the peripheral capillary wall	12	10
Granular C_3 deposits of the peripheral capillary wall and mesangium	3	5

*more then three cells per mesangial region in a thin 2 to μ section at a distance from vascular pole.

Light microscopy

The tissue specimens were embedded in paraffin, sections cut precisely at 4μ, and stained by hematoxylin and eosin, periodic acid-Shiff (PAS)-alcian blue, trichrome light green (Masson), and by silver impregnation (Jones).

Immunofluorescence microscopy

The tissue was snap frozen, sectioned at 5 μ and fixed in 95% alcohol for 10 min. Sections incubated with FITC-antisera (Hoechst) to human IgG, IgA, IgM and complement (C3) were viewed on Carl Zeiss (Jena) NU-2 microscope, using and HBO 200 lamp and proper filters.

Electron microscopy

Tissue was fixed in glutaraldehyde, post-fixed in 1% osmium tetroxide, embedded in epon and sectioned on a LKB ultratome. Sections were stained by lead citrate and uranyl acetate, and viewed in a JEM 100B electron microscope.

MORPHOMETRIC

Electron micrographs of all patients with MCGN-I, MCGN-III and controls were studied morphometrically. One glomerulus from each specimen was photographed at × 10,000.

Histological morphometry was performed by means of image analysis system consisting of a Pentium 75 MHz IBM-compatible computer equipped with an optical mouse, AVer 2000 card (frame grabber, true-color, real-time), produced by ADDA Technologies (USA), and primax flatbed scanner. This system was programmed (program MultiScan, produced by CSS-Poland) to calculate in semiautomatic mode:

– The surface are of a structure using stereological net (with regulated number of points).

– The distance between two points.

Four negatives from each case were enlarged to a uniform size 12.7 × 17.8 cm and than scanned in Primax flatbed scanner at resolution 600 × 1.200 dpi. A calibration gird was similarly scanned to calibrate morphometric measurements.

The images of the electron micrographs were saved serially in the memory of a computer, and then quantitative examinations had been carried out. The quantitative examination included the following glomerular parameters:

1. The summed area of osmophilic immune deposits per cross-sectional area of the capillary basement membrane (the mesangial deposits were neglected). This parameter was measured using point counting method which is an adaptation of the principles of Weibel[11]. The point spacing being 0.33 μ. Total number of the points of a net was 144, and total area was 13.7 sq. μ. The percentage of electron-dense deposits area was an expression of the number of points overlying these deposits as a percentage of the total points counted.

2. The basement membrane thickness. This parameter was measured in each micrograph using a simple method introduced by McLay et al.[12] at four representative points from the overlying epithelial cell plasma membrane to the opposing endothelial cell plasma membrane, including elements involved with electron-dense deposits.

STATISTICAL METHODS

Differences between groups were tested using Mann-Whitney's U test. The clinico-morphological correlations were based on detailed case sheet data analysis with particular reference to serum creatinine at biopsy and to quantitation of hematuria and proteinuria. Correlation coefficients were calculated using Spearman's method. Results were deemed statistically significant if $p < 0,05$.

RESULTS

Clinical features of the patients with MCGN-I and MCGN-III at the time of biopsy are given in table II. Most of our patients were young adults and the mean age was 34.8 in MCGN-I group and 33.2 in MCGN-III group. Male predominance was noticeable in

Table II. Clinical findings at the time of biopsy in cases with MCGN-I and MCGN-III.

N. of cases	Microhematuria	Gross hematuria	Proteinuria			Nephrotic syndrome	Renal function impairment[1]	Hypertension (>90/160)
			<1 g/24	1-2 g/24 h	2-3,5 g/24 h			
MCGN-I	2	13	2	1	6	6	4	13
MCGN-III	2	10	–	2	5	8	2	11

[1]Serum creatinine > 1.5 mg%

MCGN-III, but not in MCGN-I group. At the time of renal biopsy, a high percentage of patients with MCGN-I and MCGN-III showed nephrotic syndrome or heavy proteinuria. Clinical renal impairment (serum creatinine greater than 1.5 mg/100 ml) was noted in 4 MCGN-I patients and in 2 MCGN-III. Elevated blood pressure was observed in 13 MCGN-I and in 11 MCGN-III cases. Hematuria accompanied proteinuria in 15 MCGN-I and 12 MCGN-III patients.

The morphometric data of the deposit area per GBM area and GBM thickness appear from table III. The mean value of the deposit area per GBM area was in MCGN-III patients significantly increased in comparison with MCGN-I group ($p < 0.03$). The mean values of the GBM thickness were similar in both MCGN-I and MCGN-III groups (p = NS). In accord with qualitative descriptions there was in both groups a dintinct thickening of the GBM in comparison with normal controls (respectively: $p < 0.05$ and $p < 0.02$). The correlations between deposit area per GBM area and serum creatinine, proteinuria and hematuria as well as between GBM thickness and these parameters are shown in table IV. There were significant positive correlations between deposit area per GBM area and proteinuria (n = 15, r = 0.66, $p < 0.01$ in MCGN-I and n = 15, r = 0.57, $p < 0.03$ in MCGN-III). Significant positive correlation was also noticed between GBM thickness and proteinuria in MCGN-I, but not in MCGN-III group (respectively: n = 15, r = 0.52, $p < 0.05$ and n = 15, r = –0.99, p = NS). We observed in MCGN-I group significant positive correlations between deposit area per GBM area and hematuria as well as between GBM thickness and hematuria (respectively: n = 15, r = 0,58, $p < 0.03$ and n = 15, r = 0.53, $p < –.05$). In MCGN–III group these correlations were weak and not significant. Correlations between deposit area per GBM area and serum creatinine and between GBM thickness and serum creatinine were also weak and not significant.

Table III. Deposit area per GBM area and GBM thickness in patients with MCGN-I and MCGN-III.

	Sex		Age		Deposit area/GBM area		GBM thickness (nm)	
No	MCGN-I	MCGN-III	MCGN-I	MCGN-III	MCGN-I	MCGN-III	MCGN-I	MCGN-III
1	F	M	49	30	0.1	0.14	275.9	521.2
2	M	M	30	28	0.12	0.15	496.2	482.3
3	F	F	34	33	0.2	0.2	707.4	712.6
4	F	M	42	32	0.12	0.32	468.5	602.1
5	F	M	42	44	0.11	0.19	347.8	320.7
6	M	F	27	38	0.1	0.45	297.1	365.3
7	F	M	35	26	0.12	0.14	400.2	322.4
8	M	M	46	19	0.22	0.27	906.4	531.8
9	M	M	40	34	0.12	0.12	387.9	634.2
10	F	M	27	41	0.19	0.3	727.6	890.5
11	M	F	22	47	0.11	0.15	313.5	567.3
12	F	F	39	36	0.16	0.18	812.6	1,004.7
13	M	F	36	24	0.31	0.11	1,112.3	423.5
14	M	M	28	45	0.16	0.35	724.7	360.9
15	M	F	26	21	0.11	0.17	446.5	747.8
$\bar{X}$ ± SD			34.8 ± 8.1	33.2 ± 8.7	0.15 ± 0.05	0.2 ± 0.1	561.6 ± 253.8	565.8 ± 205.4
					p < 0.03*		p > 0.05 (NS)*	
Control (n = 6)			37.8 ± 8.9				338.8 ± 57.3	
							p < 0.05**	p < 0.02**

*Between MCGN-I and MCGN-III group, **Between MCGN-I or MCGN-III and controls. $\bar{X}$ ± SD mean ± standard deviation.

Table IV. Spearman rank order correlations between selected parameters in MCGN-I and MCGN-III

Correlation between	MCGN-I	MCGN-III
GBM thickness and haematuria	n = 15, r = 0.53, p < 0.05	n = 12, r = –0.26, P – NS
GBM thickness and proteinuria	n = 15, r = 0.52, P < 0.05	n = 15, r = –0.09, P – NS
GBM thickness and serum creatinine	n = 15, r = 0.23, P = NS	n = 15, r = 0.43, P – NS
Deposit area per GBM area and haematuria	n = 15, r = 0.58, p < 0.03	n = 12, r = –0.09, P – NS
Deposit area per GBM area and proteinuria	n = 15, r = 0.66, p < 0.01	n = 15, r = 0.57, p < 0.03
Deposit area per GBM area and serum creatinine	n = 15, r = 0.41, P - NS	n = 15, r = 0.01, P – NS

DISCUSSION

In MCGN-III the demonstration of epimembranous immune complex deposits as a prominent morphologic feature by light or electron microscopy or immunofluorescence microscopic techniques usually allows morphologic differentiation from MCGN-I[13]. However, the clinical observations, in terms of presentation and prognosis do not necessarily support this distinction. An exception is that, C3 nephritis factor is rarely detectable in MCGN-III[13]. Although, as might be expected, our morphometric investigations showed distinct thickening of the GBM in both MCGN-I and MCGN-III groups in comparison with normal controls, this thickening, was similar in MCGN-I and MCGN-III patients. These findings may support pont of view of Cameron et al.[3] and Taguhi et al.[9] who preferred to classify patients with «type III» disease together with those who had type I. On the other hand, in accordance with the initial ultrastructural diagnostic criteria, the mean value of the deposit area per GBM area was in MCGN-III patients significantly increased in comparison with MCGN-I group. These results agree with observations of Burkholder et al.[5], Anders et al.[6, 7] and Strife et al.[8] that morphologic distinction between types I and III mesangiocapillary glomerulonephritis can be made with certainty.

Although we are aware that out morphometric analysis of glomerular ultrastructure has not elucidated controversy concerning whether patients with mesangiocapillary glomerulonephritis should be further subdivided to include a «type III», we can confirm evident differences between the electron -microscopic density of the deposits in types I and III of this glomerulopathy.

The analysis of the clinico-morphological correlations has provided some interesting insights into the nature of the GBM dysfunction in cases with MCGN. Especially, we found strong positive correlations between deposit area per GBM area and proteinuria in both MCGN-I and MCGN-III groups. In available literature we found no data documenting this correlation in mesangiocapillary glomerulonephritis, but similar results were reported in membranous glomerulopathy[14-16]. In MCGN-III this correlation may be partially clarified applying cytochemical techniques. Using these technics, morphologists have identified a local alteration in the composition and ultrastructure of the glomerular basement membrane adjacent to subepithelial immune complexes in experimental (Heymann) membranous glomerulopathy. The ultimate access of albumin to Bowman's space occurred specially in areas where the overlying epitelial foot processes had become detached from the lamina rara externa of the GBM[17, 18]. Although our correlative study suggest that in MCGN-I similar alteration of the GBM may be caused by subendothelial deposits, the local mechanisms leading to this alteration remain to be shown.

Significant positive correlation was also noticed between GBM thickness and proteinuria in MCGN-I, but not in MCGN-III group. These fundings suggest, that in MCGN-I, in which thickening of the GBM mainly dependes on subendothelial deposits, not density but rather location of the deposits may play a role in this process. Our study pointed out that in MCGN-I both deposit area per GBM are and GBM thickness positively correlated with hematuria. It is worth pointing our that the subendothelial deposits are often accompanied by hematuria[3]. This supposition is also supported by observation of Swainson et al.[19] who noticed that focal damaging of the GBM usually occurred in relation to subendothelial deposits. Surprisingly, in membranous glomerulopathy these correlations suggested that thickening of the GBM may be the efficient barrier for the erytrocytes[14]. Although in MCGN-III these correlations also tended to be negative, they unfortunately have not reached statistical significance. On the other hand, our study revealed in both MCGN-I and MCGN-III groups positive, but not significant correlations between GBM thickness and serum creatinine. In contrast, Shemesh et al.[20] found that in membranous glomerulopathy capillary wall tickness tended to be related directly to the glomerular filtration rate and not inversely is might be expected. We wish to emphasise, however, that this relationship was also not significant.

In conclusion we can confirm that degree of proteinuria was positively correlated with the density of the deposits in both MCGN-I and MCGN-III groups. It is also worth pointing out that in MCGN-I positive correlations existed between density of the subendothelial deposits as weel as GBM thickness and hematuria. The present morphometric analysis of glomerular ultrastructure has not elucidated the controversy concerning whether patients with mesangiocapillary glomerulonephritis should be further subdivided to include a «type III». Although the analysis of the electron -microscopic density of the deposits suggests morphologic separateness of these glomerulopathies, the clinico-pathologic correlations do not support this differentiation. Probably, both subtypes of MCGN are the same entity, only with ultrastructural differences. Finally, it seems that the results of this investigation indicates that MCGN should be divided in the subgroups I and III, depending on the initial classification criteria.

BIBLIOGRAFIA

1. Habib R, Levy M: Membranoproliferative glomerulonephritis. In: Hamburger J, Crosnier J, Grunfeld J (eds): *Nephrology*. John Wiley & Sons, 507-534, New York, 1979.
2. Kim Y, Michael AF: Idiopathic membranoproliferative glomerulonephritis. *Annu Rev Med* 31: 273-288, 1980.
3. Cameron JS, Turner DR, Heaton J, Williams GD, Ogg CS, Chantler C, Haycock GB, Hicks J: Idiopathic mesangiocapillary glomerulonephritis. Comparison of types I and II in children and adults and long-term prognosis. *Am JMed* 74: 175-192, 1983.
4. Ford DM, Briscoe DM, Shanley PF, Lum GM: Childhood membranoproliferative glomerulonephritis type I: Limited sterois therapy. *Kidney Int* 41: 1606-1612, 1992.
5. Burkholder PM, Marchand A, Krueger RP: Mixed membranous and proliferative glomerulonephritis. A correlative light, immunofluorescence and electron microscopic study. *Lab In - vest* 23: 459-479, 1979.
6. Anders D, Thoenes W: Basement membrane changes in membranoproliferative glomerulonephritis. A light and electron microscopic study. *Virchows Arch* 369: 87-109, 1975.
7. Anders D, Agricola B, Sippel M, Thoenes W: Basement membrane changes in membranoproliferative glomerulonephritis II. Characterisation of a third type by silver impregnation of ultra thin sections. *Virchows Arch* 376: 1-19, 1977.
8. Strife CF, McEnery PT, McAdams AJ, West CD: Membranoproliferative glomerulonephritis with disruption of the glomerular basement membrane. *Clin Nephrol* 7: 65-72, 1977.
9. Taguchi T, Bohle A: Evaluation of change with time of glomerular morphology in membranoproliferative glomerulonephritis: a serial biopsy study of 33 cases. *Clin Nephrol* 31: 297-306, 1989.
10. Bakkaloglou A, Soylemezoglu O, Tinaztepe K, Saatci U, Soylemezogly F: Familial membranoproliferative glomerulonephritis. *Nephrol Dial Transplant* 10: 21-24, 1995.
11. Weibel ER: *Stereological methods. Vol 1. Practical methods for biological morphometry.* Academic Press 101-161, London, New York, Toronto, Sydney, San Francisko, 1979.
12. McLay ALC, Jackson R, Meyboom F, Jones JMB: Glomerular basement membrane thinning in adults: Clinicopathological correlations of a new diagnostic approach. *Nephrol Dial Transplant* 7: 191-199, 1992.
13. Holley KE, Donadio JV: Membranoproliferative glomerulonephritis. In: Tisher CC, Brenner BM (eds.): *Renal Pathology* vol. 1. J. B. Lippincott Company, 228-264. Philadelphia, 1989.
14. Danilewicz M, Wagrowska-Danilewicz M: Idiopathic membranous glomerulopathy. Quantitative study of the subepithelial deposits and glomerular basement membrane including clinico-morphological correlations. *J Nephrol* 3: 139-143, 1996.
15. Honkanen E, Tornroth T, Gronhagen-Riska C, Sankila R: Natural history, clinical course and morphological evolution of membranous nephropathy. *Nephrol Dial Trasplant* suppl. 1: 35-41, 1992.
16. Tornroth T, Honkanen E, Pettersson E: The evolution of membranous glomerulonephritis reconsidered: new insights from a study on relapsing disease. *Clin Nephrol* 28: 107-117, 1987.
17. Schneeberger EE, Grupe WE: The ultrastructure of the glomerular slit diaphragm in autologous immune complex nephritis. *Lab Invest* 34: 398-308, 1976.
18. Schneeberger EE, O'Brien MA, Grupe WE: Altered glomerular permeability in Munich-Wistar rats with autologous immune complex nephritis. *Lab Invest* 40: 227-236, 1979.
19. Swaison CP, Robson JS, Thomson D, MacDonald MK: Mesangiocapillary glomerulonephritis: A long-term study of 40 cases. *J Pathol* 141: 449-68, 1983.
20. Shemesh O, Ross JC, Deen WM, Grant GW, Myers BD: Nature of the glomerular capillary injury in humans membranous glomerulopathy. *J Clin Invest* 77: 868-877, 1986.