Virchows Arch. A Path. Anat. and Histol. 384, 85–92 (1979)

Virchows Archiv A

Direct Immunofluorescent Studies of Urinary Casts in Human Nephropathies

Claudine Orfila*, Joel Rakotoarivony**, and Jean-Michel Suc
Groupe INSERM U 133, CHU Rangueil, F-31054 Toulouse Cedex, France

Summary. Urinary sediments from 192 patients with renal biopsy-proven nephropathy were studied in order to look for correlations between immunofluorescent urinary casts, and glomerular deposits, tubular cast deposits and selectivity of proteinuria.

Our findings for immunofluorescent urinary cast and glomerular deposits showed that the best correlation exist in lupus nephritis. In other nephropathies the correlation between urinary cast and glomerular deposits was inconstant and did not enable us to determine the type of nephropathy by immunofluorescent observation of urinary sediment. In "Minimal change" nephropathy, deposits are infrequent but in this condition good correlations are present, except for IgA. IgA is more frequently found in urinary casts than in glomeruli in some nephropathies: secretory IgA may be involved in this phenomenon.

Urinary cast deposits were more frequent than tubular cast deposits in some nephropathies studied, with the exception of IgM, which was very rare in urinary sediment ($< 20\%$).

Follow-up comparison showed that urinary cast and glomerular deposit findings were well correlated for IgM and Fibrin/Fibrinogen while no correlation was evident between urinary cast and tubular cast deposits.

The frequency of immunofluorescent urinary cast deposits seems to decrease with the increasing molecular weight of studied proteins, and increases as the selectivity of proteinuria decreases.

Key words: Urinary casts – Immunofluorescence – Nephropathies.

Introduction

Few authors have been interested by immunofluorescent (IF) studies of urinary casts (UC). Some have investigated the composition of the matrix of casts

* Attachée de recherche INSERM
** Chargé de recherche INSERM
Send offprint requests to: C. Orfila

0340-1227/79/0384/0085/$01.60

(McQueen, 1962; McQueen, 1966; McKenzie and McQueen, 1969; Rutecki et al., 1971; Imhof et al., 1972) and the nature of granules (McQueen, 1962; Rutecki et al., 1971; Orita et al., 1977) but rarely has comment on the correlation between the presence of UC and the type of nephropathy been made. Our study was performed in a large group of human nephropathies. The presence of IF/UC deposits has been investigated to answer three questions: to see if urinary sediments (US) could be an index of IF glomerular deposits or of IF tubular cast deposits, and whether the IF/US deposits could be related to the selectivity of proteinuria in nephropathies.

Material and Methods

One hundred and ninety two US from 185 patients with renal biopsy performed for various nephropathies (Table 1) and 23 US of control subjects were investigated. Seven patients have had follow-up studies of US and biopsy (varying from 2 months to 2 years).

Table 1. Renal biopsy material. (2 repeated biopsies (2 r) have been performed in 7 patients)

Minimal changes	33
IgA nephropathy	27 (2 r)
Membranous	20
Proliferative endocapillary GN	18 (2 r)
extracapillary GN	5
endo + extracapillary GN	3
Mesangiocapillary GN	11
Lupus	21 (6 r)
Chronic interstitial nephritis	15 (2 r)
Miscellaneous	39 (2 r)

Biopsy specimens were divided into two portions for light and IF microscopic studies. For histological preparations, a portion of the specimen was fixed in Dubosq-Brazil solution and 3 μ sections were prepared from paraffin – embedded material. The sections were routinely stained with hematoxylin and eosin, periodic-acid-Schiff reagent and Masson trichrome.

The second part of the specimen was embedded fresh in OCT compound (Ames Tissues Teck), rapidly frozen in liquid nitrogen. Sections 3 μ thick were cut in a Cryostat and subsequently stained with fluorescein – conjugated antisera specific for human IgM, IgA, C3, Fibrin (Hyland Laboratories), IgG, Fibrinogen (Behring Laboratories).

Urinary sediment material was used for IF studies with a techniqe described previously (Orfila et al., 1977). For IF studies of renal biopsies and US the same antisera were used; these appeared to be monospecific as judged by immunoelectrophoresis against normal human serum and/or normal human plasma. Slides were examined with a Leitz Ortholux microscope employing an HBO 200 light source.

Proteinuria selectivity pattern was investigated by semi-quantitative immunoelectrophoresis (Scheidegger 1955). We distinguished: "physiological" (Ph) proteinuria corresponding to normal urine and "tubular" (Tub) proteinuria with molecules of molecular weight (MW) less than 50,000. The term of "selective" (S) proteinuria was used when albumin was higher than 80% and all proteins less than 90,000 MW. "Moderately selective" (MS) proteinuria when there are average quantities of globulin and "poorly selective" (PS) when MW of proteins are more than 90,000.

Results

A) Basic Study

Analytical and detailed study will be limited to some types of nephropathy in which a sufficient number of cases was investigated.

"Minimal Change" (Mc) Nephropathy. 33 observations.

We observed traces of proteins in UC and glomeruli but IgA was more frequently found in UC than in glomeruli (160%).

The ratio of UC to Tubular casts (TC) deposits is 6/1 for Fibrin/Fibrinogen, 16/6 for IgG, but TC deposits of IgM (1/3) and IgA (8/15) are more frequent than UC deposits.

Endocapillary Proliferative (ECP) Glomerulonephritis. 18 observations.

The ratio of UC to glomerular deposits is relatively high for IgG (10/15), Fibrin/Fibrinogen (8/14) and low for IgM (2/8) and C3 (3/13) but IgA was more often observed in UC than in glomeruli: 8/7 (114%).

The ratio of UC to TC deposits is 10/2 for IgG, 8/2 for Fibrin/Fibrinogen, 8/6 for IgA but low for IgM 2/3 and no C3 deposits are observed in TC.

Mesangiocapillary (MC) glomerulonephritis with sub-endothelial deposits: 11 observations.

The ratio of UC to glomerular deposits is 6/10 for IgG, 8/11 for C3 and 9/11 Fibrin/Fibrinogen, 2/7 for IgM, IgA was more frequent in UC than in glomeruli: 9/8 (112,5%).

The ratio of UC to TC deposits is 9/2 for Fibrin/Fibrinogen, 6/3 for IgG, 9/5 for IgA, and 2/2 for IgM and no C3 deposits are observed in TC.

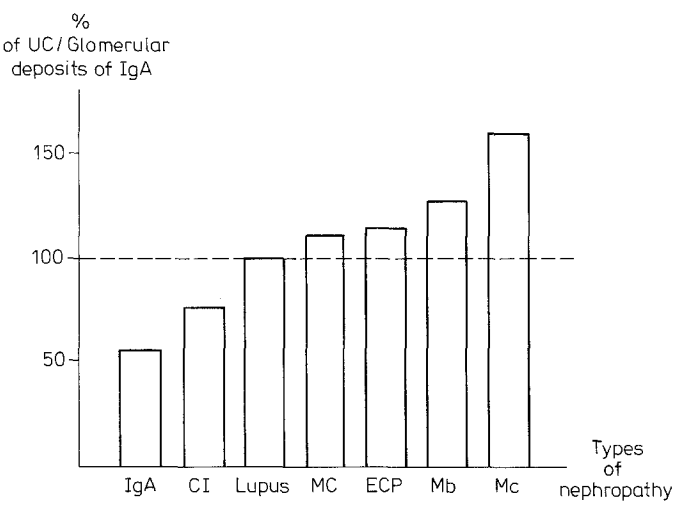

Fig. 1. Ratio of urinary cast to glomerular deposits of IgA in some nephropathies

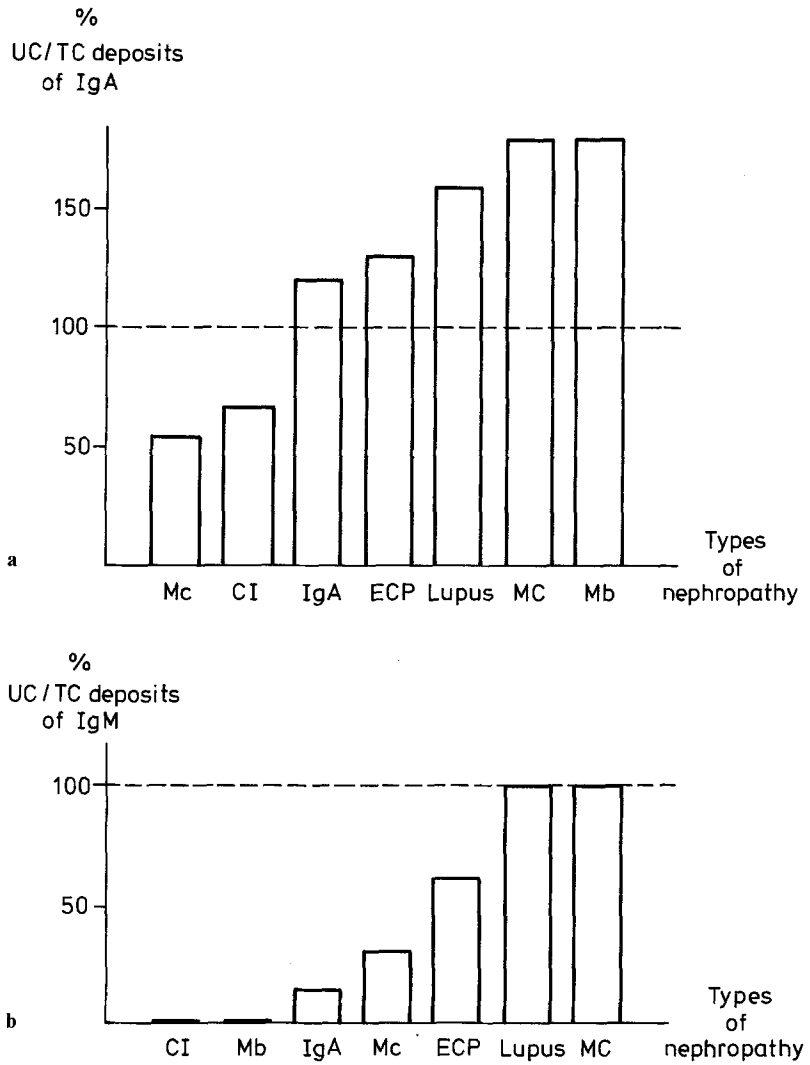

Fig. 2. **a** Ratio of urinary cast to tubular cast deposits of IgA in some nephropathies. **b** Ratio of urinary cast to tubular cast deposits of IgM in some nephropathies

Membranous (Mb) Glomerulonephritis. 20 observations.

The ratio of UC to glomerular deposits is relatively low for IgG (9/20), Fibrin/Fibrinogen and C3 (4/20), low for IgM (0/1) but UC deposits of IgA are more frequent than glomerular deposits: 9/7 (128,5%).

The ratio of UC to TC deposits is high for IgG (9/12) and C3 (4/1) Fibrin/Fibrinogen (4/2), IgA (9/5) and no IgM deposits are observed in UC.

IgA Nephropathy. 27 observations.

The ratio of UC to glomerular deposits is relatively high for IgG (18/27),

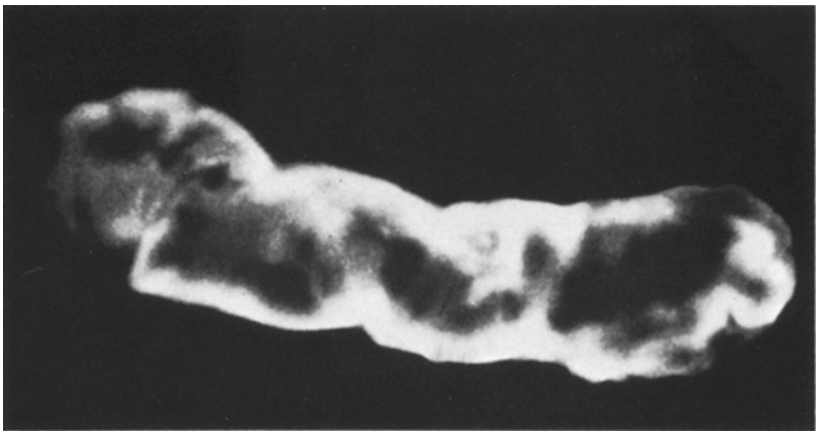

Fig. 3. Urinary cast stained by FITC-Labelled anti-IgG serum (× 1,000)

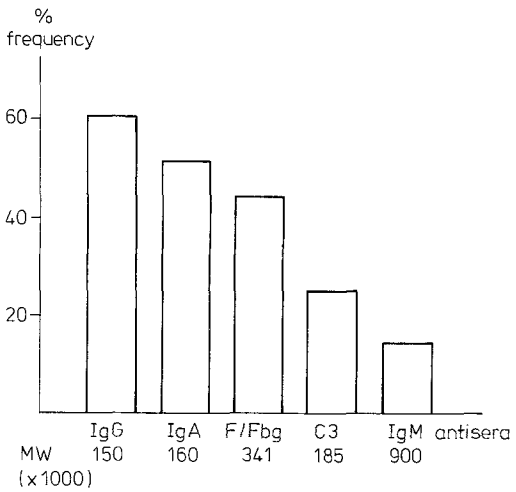

Fig. 4. Overall frequency of urinary sediment deposits (immunofluorescence) 192 cases

Fibrin/Fibrinogen (12/22), low for C3 (4/26) and IgM (1/11). The relatively low frequency (15/27) of IgA deposits in UC is noticeable.

The ratio of UC and TC deposits is 15/3 for Fibrin/Fibrinogen, 18/13 for IgG, 15/12 for IgA but low for IgM, there are more IgM deposits in TC than in UC (1/7).

Lupus Nephritis. 21 observations.

The ratio of UC to glomerular deposits is high for IgA: 16/16, IgG: 16/19, Fibrin/Fibrinogen: 13/16 and lower for C3: 14/20 and IgM: 7/17.

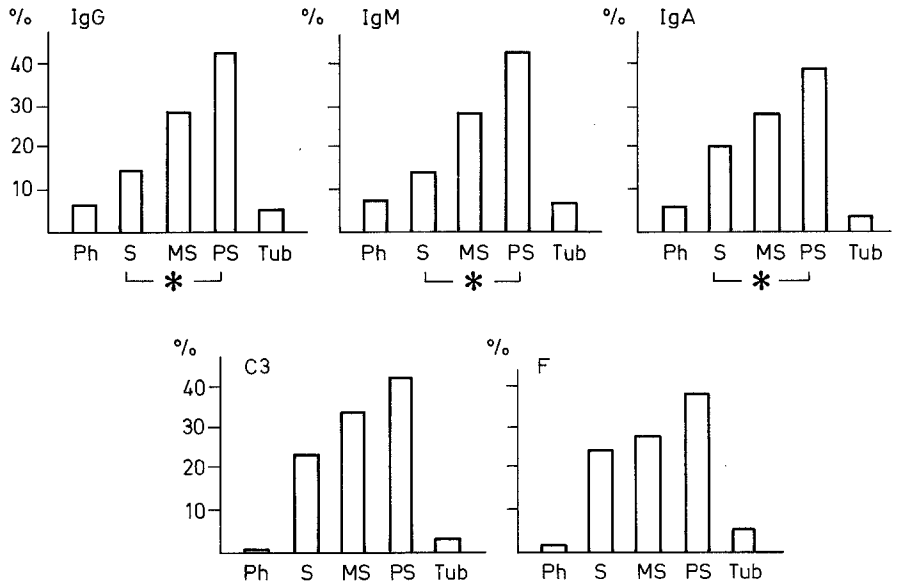

Fig. 5. Overall frequency of urinary sediment deposits correlated with immunoelectrophoretic pattern selectivity of proteinuria $P < 0.01$

The ratio of UC to TC deposits is high for C3 (14/3) IgG (16/9), IgA (16/10) IgM (7/7) and no Fibrin/Fibrinogen deposits are observed in TC.

Chronic Interstitial (CI) Nephritis without evident obstruction of urinary tract: 15 observations.

The ratio of UC to glomerular deposits is 7/9 for IgA, 7/14 for IgG, 5/13 for Fibrin/Fibrinogen, 4/11 for C3 and 0/5 for IgM.

The ratio of UC to TC deposits is 4/1 for C3, 5/2 for Fibrin/Fibrinogen, 7/3 for IgG. But TC deposits of IgA (7/11) and IgM are more frequent than UC deposits (0/4).

If IgA nephropathy is excluded it appears that in the most frequent GN we have studied (Fig. 1) IgA UC deposits are more frequent than IgA glomerular deposits in "minimal change", membranous, endocapillary proliferative and mesangiocapillary GN in decreasing order of frequency.

We observed that TC deposits of IgA are more frequent than UC deposits only in CI and Mc nephropathy (Fig. 2a) and that TC deposits of IgM are always more frequent than UC deposits (Fig. 2b).

B) Follow-Up Study

In follow-up studies of UC deposits, seven patients had undergone two renal biopsies (Table 1) and the evolution of deposits in US and renal deposits have been compared using the five antisera.

The correlation between UC and glomerular deposits is good for IgM and Fibrin/Fibrinogen (6/7), and poor for IgA and C3 (2/7) and IgG (3/7).

There was no correlation between UC and TC deposits.

C) Immunofluorescent Urinary Cast Deposits and Selectivity of Proteinuria

In the studies of all UC deposits (whatever the type of nephropathy) IgG (Fig. 3) is more frequently found (61%) then IgA (52%) and other proteins studied (Fig. 4). The MW of these proteins is shown in Fig. 4. Except for Fibrin/Fibrinogen (341,000), it is evident that the frequency of protein deposits decreases when MW increases.

For each protein tested, we have observed the highest frequency in "poorly selective" proteinuria (Fig. 5). The difference observed between "poorly selective" and "selective" proteinuria is significant ($P < 0{,}01$) for IgG, IgM, IgA but not for Fibrin/Fibrinogen and C3.

Discussion

Immunofluorescent studies of US have been made by some authors in order to examine the chemical nature of the matrix of the cast, composed of Tamm-Horsfall mucoprotein (McQueen, 1962; McKenzie and McQueen, 1969; Rutecki et al., 1971). The presence of immunoglobulins in granules of UC has been demonstrated by some investigators (McQueen, 1962; Rutecki et al., 1971; Orita et al., 1977). The investigation of the relationship between IF urinary cast deposits versus glomerular deposits in various nephropathies has rarely been performed (Orfila et al., 1977).

In lupus nephritis, it seems that frequent UC deposits of IgG, IgA and Fibrin/Fibrinogen are noticeable and that the relationships between IF urinary casts and glomerular deposits of immunoglobulin are good in this type of nephritis. The relationships between UC and glomerular deposits are less constant in other nephropathies studied.

Another important fact is that UC deposits of IgA are more frequent than glomerular deposits in some nephropathies. In our study, we have not investigated US with anti-secretory IgA anti-serum, but some authors (Tourville et al., 1969; McCoy et al., 1974; Dobrin et al., 1975) have demonstrated secretory IgA in renal tubular cells and casts from patients with morphological evidence of significant renal damage. The frequent deposits of IgA observed in UC might thus be secretory IgA. This has been shown by Popli et al. 1978 who observed a high incidence of IgA in TC and lack of IgA deposits elsewhere, concluding that there is tubular secretion of IgA.

The small number of follow-up studies have shown relatively good correlation between UC and renal deposits, which must be correlated by further studies. It has been shown (Kaufman et al., 1970) that urinary infection increases the levels of urinary IgA, but in our cases no episode of urinary infection was found at the time of renal biopsy.

The correlations between immunofluorescent UC and TC deposits were poor. UC deposits were often more frequent than TC deposits except for IgM (UC deposits of IgM were observed in 20% of all cases). Firstly we may point out that immunoglobulin deposits have been seen in TC in some nephropathies not mediated by immunological mechanisms (Popli et al., 1978) and secondly renal biopsy specimens are frequently cortical and TC are usually observed in collecting tubules (more frequent in the medullary area). Renal biopsy sections

were thin (3 μ), and thus the probability of observing TC in this site is less than in aliquots of centrifuged urine.

With regard to the correlation between UC deposits and the immuno-electrophoretic pattern of proteinuria, the IF deposits in UC are more frequent in "poorly selective" proteinuria, whatever the type of protein tested or the type of nephropathy studied. The difference between the types of "poorly selective" and "selective" proteinuria is statistically significant for those immunoglobulins studied. IgG is the protein which is the most frequently observed in UC deposits, although the MW of this protein is the smallest. The small MW could explain the frequent incidence of the finding of this molecule and the low incidence of an large MW protein, such as IgM.

In conclusion, this study shows that the nature of the deposits found in US mainly reflects glomerular permeability, as expressed by the selectivity of proteinuria. In Mc and lupus nephritis glomerular and US deposits are also well correlated.

Acknowledgments. We thank Mr. J.F. de Boissezon and Mr. M. Abbal (INSERM U 100) for immunoelectrophoretic studies of proteinuria, Miss C. Sabardu and Mr. C. Mora for the preparation of the manuscript.

A part of this study was presented at the VIIe International Congress of Nephrology, Montreal, 22 June 1978.

References

Dobrin, R.S., Knudson, F.E., Michael, A.F.: The secretory immune system and renal disease. Clin. Exp. Immunol. **21**, 318–328 (1975)

Imhof, P.R., Hushak, J., Schumann, G., Dukor, P., Wagner, J., Keller, H.M.: Excretion of urinary casts after the administration of diuretics. Br. Med. J. **2**, 199–202 (1972)

Kaufman, D.B., Katz, R., McIntosh, R.M.: Secretory IgA in urinary tract infection. Br. Med. J. **4**, 463–465 (1970)

McCoy, R.C., Abramowsky, C.R., Tischer, C.C.: IgA nephropathy. Am. J. Path. **76**, 123–140 (1974)

McKenzie, J.K., McQueen, E.G.: Immunofluorescent localization of Tamm-Horsfall mucoprotein in human kidney. J. Clin. Path. **22**, 334–339 (1969)

McQueen, E.G.: The nature of urinary cast. J. Clin. Pathol. **15**, 367–373 (1962)

McQueen, E.G.: Composition of urinary casts. Lancet **i**, 397–398 (1966)

Orfila, C., Mignon-Conté, M., De Boissezon, J.F., Suc, J.M.: Study of the correlation between glomerular and urinary sediment deposits using immunofluorescent technique. Biomedicine **27**, 89–92 (1977)

Orita, Y., Imai, N., Ueda, N., Aoki, K., Sugimoto, K., Ando, A., Fujiwara, Y., Hirano, S., Abe, H.: Immunofluorescent studies of urinary casts. Nephron **19**, 19–25 (1977)

Popli, S., Lange, C.F., Buckingham, J.M., Viol, G.W., Geis, W.P., Hano, J.E.: Heavy immunoglobulin-A deposits in casts in the tubular lumina of end-stage kidneys. Kidney Int. **14**, 659, 1978

Rutecki, G.J., Goldsmith, C., Schreiner, G.E.: Characterization of proteins in urinary casts. N. Engl. J. Med. **284**, 1049–1052 (1971)

Scheidegger, J.J.: Une microméthode de l'immunoélectrophorèse. Int. Arch. Allergy **7**, 103–110 (1955)

Tourville, D.R., Adler, R.H., Bienenstock, J., Tomasi, T.B.: The human secretory immunoglobulin system: immunohistological localization of IgA secretory "piece", and lactoferrin in normal human tissues. J. Exp. Med. **129**, 411 (1969)

Received April 9, 1979