Proteinuria as Diagnostic Marker after Human Kidney Transplantation

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Summary
Molecular weight analyses of urinary proteins in 34 patients following cadaveric kidney transplantation were performed by SDS–PAA-electrophoresis in order to diagnose transplant complications. A micromolecular ‘tubular’ proteinuria (mw 70–10,000) was found in all post-operative urines. Later on during clinically normal periods the patients exhibited an unphysiological proteinuria of mw 70–40,000. Recurrence of tubular proteinuria was associated with rejection episodes and acute kidney failure. Twelve patients developed a macromolecular glomerular proteinuria (mw > 60,000), caused by recurrent glomerulonephritis, glomerular rejection disease or renal vein thrombosis. Steroid treatment reduced the glomerular permeability for macromolecules above mw 65,000.

Introduction
Renal protein handling seems to depend largely on the protein’s molecular weight (Waldmann & Strober, 1974). The glomerular filter clears the serum of proteins according to their molecular radius, while most proteins are reabsorbed by the tubular cells (Hardwicke et al, 1970). Damage to the glomerular filter system causes macromolecular (mw 60–1,000,000) protein excretion, and diseases of the tubules and interstitial tissues lead to proteinurias composed from micromolecules of mw 70–10,000 (Boesken et al, 1973). Polyacrylamid (PAA)-electrophoresis with detergents (SDS) has proved to be an easy method for separating proteins by their molecular weight. It allows the clear distinction between ‘glomerular’ and ‘tubular’ types (Pesce et al, 1972; Boesken et al, 1973), as well as differentiating various nephropathies based on their histological diagnosis. This study was done to differentiate the uncomplicated post-transplantation course from rejection crises and recurrent renal diseases.
PATIENTS AND METHODS

All 34 patients received cadaveric kidneys because of end-stage kidney failure caused by chronic glomerulonephritis (16), pyelonephritis (4) and various other nephropathies. Prior to the transplant 18 patients were nephrectomised. Thirteen cases were followed up immediately after the operation. About 300 urine specimens were analysed at different intervals for quantitative and molecular weight analysis of the urinary proteins, the last ones being performed 2–46 months after transplantation. Clinical diagnoses were independent of these urine analyses. Kidney biopsies were examined by Prof. Bohle, Tübingen, and Doz Dr Mittermayer, Freiburg.

All methods regarding urine concentration, SDS–PAA-disc electrophoresis and gel evaluation have been described elsewhere (Boesken et al, 1973).

RESULTS

As seen already in acute and chronic nephropathies the transplanted kidney excretes proteins in various patterns (Figure 1) –

1. Glomerular proteinuria in a
   (a) selective (mw 60–120,000) or (Fig. 1 c, d)
   (b) unselective form (mw 60–1,000,000) (b)
2. Tubular proteinuria mw 10–70,000 (e)
3. Proteinuria mw 40–70,000 (f)
4. Mixed type of 1 + 2 (g)
5. Mixed type of 1 + 3 (h)

The protein mobility in these electrophoreses was related linearly to the logarithm of their molecular weight. Differentiation of mixed-type proteinurias was achieved by calculating the ratio of micro- to macromolecular proteins GTPR (Boesken et al, 1973). The glomerular selectivity was roughly characterised by the ratio of 7s–IgG to transfer in the urine. For easier identification protein bands were enumerated as G1–G6 in glomerular and T1–T6 in micromolecular tubular proteinurias (Figure 1).

Post-operative phase

The uncomplicated post-operative course of six transplanted nephrectomised patients was characterised by a tubular proteinuria A–T5 of 3,200(800–6,900)mg/day (Figure 2A). Gradually during the following three weeks the proteins of mw 10–40,000 T3–5 disappeared from the urine in parallel to a decreasing total proteinuria. The pattern A–T2/3 (mw 70–40,000) appeared to be a typical finding of the late phase in well-functioning transplants (19 patients,
Figure 1. The different types of urinary protein excretion. The relative mobility (RM), molecular weight and numbers of the protein bands are indicated. (a) physiological pattern; (b) unselective; (c), (d), more selective glomerular proteinurias; (e) tubular micromolecular proteinuria; (f) proteinuria A – T₂/₃ ; (g) mixed proteinuria (b + e); (h) mixed proteinuria (b + f).

40 urines). Eight patients not nephrectomised before transplantation, additionally had proteinuria for 10–100 (35) days (Figure 2B), probably from their glomerulonephritic kidneys. Haematuria produced an unselective glomerular pattern.

Alterations of the post-transplant pattern A – T₂/₃ were estimated as pathological irrespective of the total protein excretion.

Recurrence of Tubular Proteinuria

Twelve of 14 acute rejection crises diagnosed clinically in 12 patients were accompanied by a temporary recurrence of a mainly tubular proteinuria A – T₄/₅; on three occasions the urine was not altered. The total protein excretion was below 100 mg/day in 3,100–500 mg/day in six and 1,000–1,700 mg/day in three cases. By chance, two urines were proved to contain tubular proteins ten days before the clinical symptoms.

Six patients developed a permanent tubular proteinuria as a sign of a chronic rejection, histologically associated in three with interstitial infiltration and fibrosis. In two patients without signs of rejection, cytomegalic infection was
associated with a tubular proteinuria of \( \text{mw} \ 70-10,000 \). In one the kidney revealed interstitial cell infiltration as in cytomegalonius. The same micromolecular proteinuria was seen in acute kidney failure in two transplants, verified histologically.

Two patients, and 17 months after transplantation, revealed the clinical signs of chronic rejection together with tubular proteinuria. The histological diagnosis was malignant nephrosclerosis.

**Recurrence of Glomerular Proteinurias**

Nine patients revealed a permanent mainly glomerular proteinuria in the late phase after transplantation, three as a transient symptom. While in the latter group glomerular rejection episodes might have been the cause for increased glomerular permeability, in five of the nine patients the pathogenesis was determined by biopsy or autopsy. Beginning at the second month after transplantation one patient exhibited a rather selective glomerular proteinuria up to 9 g/day, caused by renal vein thrombosis (Figure 2C). A recurrent proliferative glomerulonephritis was assumed in one case because of histological similarities between the diseased and transplanted kidneys. After a typical post-transplant proteinuria pattern a glomerular proteinuria up to 6 g/day developed, revealing different stages of selectivity (Figure 3A). In two more patients the biopsy also showed proliferative glomerular changes. In one however recurrent disease was excluded because of preceding pyelonephritis and a glomerular form of rejection was assumed. While an unselective glomerular proteinuria (A–G\(_5\)) of 250 mg/day together with the typical post-transplant pattern A–T\(_{2/3}\) (Figure 3C) could be detected as early as four weeks post-operatively, clinical symptoms with overt proteinuria of 6.5 g/day and renal insufficiency occurred after 12 months, leading to nephrectomy after four more months. One patient with rejection symptoms and a mainly glomerular proteinuria of 2 g/day during the third month revealed a severe necrotizing vasculitis and mesangial thickening.

Among the four patients without histological diagnosis one revealed a permanent unselective glomerular proteinuria of 2 g/day for 18 months without deterioration of kidney function. Two of these patients, now 27 and 40 months after transplantation have exhibited mild symptoms of rejection for several months. In both, an unselective glomerular protein excretion is accompanied by tubular proteinuria A–T\(_{4/5}\). One patient had a selective glomerular proteinuria of 1 g/day together with a reduction of the creatinine clearance.

**Proteinuria after Steroid Treatment**

When high doses of steroids were given for treatment of rejection crises effects on both tubular and glomerular proteinuria were observed. The reduction of micromolecular protein excretion from A–T\(_5\) to A–T\(_{2/3}\) was expected with the
Figure 2. Numbers on top indicate days after transplantation; at the bottom, proteinuria in mg/day. For protein band identification refer to Figure 1.

A. Uncomplicated early posttransplant course in a nephrectomised patient. Note the gradual disappearance of the micromolecular proteins T-4,5.

B. Early course in a patient with the diseased kidneys still in function. Beside the typical tubular proteinuria (9, 16, 24) the patient excretes glomerular proteins until day 34.

C. In this patient the typical post-operative proteinuria (1, 9) was replaced by a tubular proteinuria A-T5 as sign of an acute rejection (26). He was treated with steroids leading to a decreased glomerular permeability for macromolecular proteins. Note on day 32 the missing albumin excretion. While macromolecules reappeared until day 46, the tubular microproteins persisted at least to day 87. The selective glomerular proteinuria (176) was caused by a renal vein thrombosis.
Figure 3. Recurrent glomerular proteinuria. The numbers on top indicate days after transplantation; at the bottom, proteinuria in mg/day.

A. Recurrent proliferative glomerulonephritis. After a post-operative tubular (1) and late-phase protein excretion (55) a glomerular proteinuria appeared. The micromolecular bands (108-143) indicate additional interstitial rejection reaction.

B. Transient selective glomerular proteinuria together with a late-phase proteinuria A-T2/3. Note the missing band of IgG (band G-4, 5).

C. Glomerular rejection disease: an unselective glomerular proteinuria was detected (23) after an uncomplicated early course (3-15), clinical rejection symptoms were noted around day 300. Histologically severe vascular and glomerular alterations were seen.
improvement of the interstitial rejection reaction. Additionally however in seven cases a temporary selective disappearance of macroproteins from the urine including the physiologically excreted albumin was observed, in two cases as early as four days after therapy. Remarkably, proteins $T_{1-3}$ (mw 60–40,000) did not change their excretion pattern. Macroproteins gradually reappeared after several weeks. These results were controlled by immunochemical quantitation of single urinary proteins (Figure 2C).

DISCUSSION

As seen already in chronic nephropathies (Boesken et al, 1973) and shown now in the transplanted kidney the finding of glomerular- or tubular-type proteinurias reflects the localisation of damage to the nephron. Perioperative kidney ischaemia as well as interstitial processes lead to micromolecular tubular proteinuria, caused probably by reabsorption failure. Damage to the glomerular filter irrespective of a glomerular or vascular pathogenesis leads to macromolecular protein excretion.

The amount of total urinary protein or of albumin (Weise and Bockhorn, 1972) after transplantation does not appear to be a good indicator of kidney damage, as shown by very low quantities of tubular proteins in rejection crises. A change of protein pattern however was often an early sign, preceding acute rejections by a few days or the clinical symptoms of chronic glomerular rejection disease for nearly one year.

In patients nephrectomised before transplantation the immediate post-operative proteinuria was classified clearly as a tubular one, in accordance with the findings of Debray-Sachs (1970) and Revillard et al (1970). In later stages no patient in our group resumed a physiological protein excretion, but always the pattern $A-T_{2-3}$ (mw 70–40,000) (Figure 1, a + f), possibly in accordance with an increased Ig–L-chain-proteinuria (Epstein et al, 1968). Permanent immuno-suppressive therapy or ischaemic damage might cause this finding. Different tubular patterns in various stages of tubular damage (Figure 2A) might reflect a selective type of tubular reabsorption.

Acute and chronic rejections with interstitial damage exhibited mainly tubular proteinurias accompanied by macromolecular proteins. This is in contrast to glomerular proteinurias in rejections (Laterre et al, 1970; Revillard et al, 1970) which in part may be due to a different interpretation of mixed proteinurias. In our patients with rejections and mixed proteinurias (six of 14), the amount of tubular protein was far greater than glomerular proteinuria. Glomerular proteinuria in excess of tubular proteinuria was confined to patients with histological changes in the glomeruli, who usually did not exhibit the symptoms of an acute rejection. The frequent finding (12 of 34) of mainly glomerular proteinurias might be explained by a mild glomerular rejection reaction. This pathogenetic process may cause chronic rejection of kidneys which show glomerular prolifera-
tion or severe vascular alteration (3 patients). According to Porter (1974) recurrent glomerulonephritis and glomerular rejection disease may not be differentiated histologically. Our few cases do not allow a clear statement regarding the value of protein patterns to distinguish these two disease entities. The difference however between selective and unselective glomerular proteinurias should reflect pathogenetic differences, for example the glomerular reaction to immune complexes produces the unselective type. One of our patients with the selective proteinuria suffered from renal vein thrombosis.

Treatment with high doses of steroids reduces glomerular excretion of serum proteins including albumin, but not excretion of the tubular proteins. Decreased glomerular permeability by altered membrane structure (Huang & Kalant, 1968) or increased reabsorption by the glomerular cells (Germuth et al, 1968) might be responsible.

In conclusion the molecular weight analysis of urinary proteins by SDS–PAA-electrophoresis permits the following statements —

(a) During the first three weeks the transplanted kidney excretes micromolecular proteins due to tubular dysfunction. This pattern may be disturbed by haematuria or by filtration by the native diseased kidneys.
(b) Rejection episodes are accompanied by tubular or glomerular proteinurias. In the typical acute rejection, and in chronic rejections verified by interstitial infiltration, a mainly tubular form was detected.
(c) While transient glomerular proteinurias might reflect glomerular rejection reactions, neither the histological examination nor the molecular weight pattern of the permanent glomerular proteinurias could clearly distinguish between recurrent glomerulopathies and glomerular rejection reactions.
(d) This analysis might give diagnostic hints in chronic complications regarding localisation in the nephron in advance of the clinical symptoms. SDS–PAA-deselectrophoretic analysis therefore seems to be a suitable tool for surveillance of patients with kidney transplants.

Acknowledgement

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References

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Open Discussion

RAVNSKOV (Sweden) Your main conclusion from this paper, that electrophoresis of urinary proteins is of value in prediction of outcome of rejection crisis, is in conflict with most other papers in this field. I therefore want to make a comment and ask a question. I will concentrate mainly on low-molecular-weight proteins. The excretion of LMW proteins depends on many factors, the most important of which are their plasma concentrations since, especially after transplantation, these may change several thousand per cent in the course of a few days, and the glomerular filtration rate, simply because a kidney with many nephrons will excrete more protein than a kidney with a few nephrons in spite of the same single nephron damage. These two factors cannot be evaluated by studying electrophoretic patterns. If you want to get an expression of the events at the nephron level, you have to determine the level of a specific protein in the urine and in the plasma, and thereafter to calculate the protein/creatinine clearance rate. Such studies showed that the protein/creatinine clearance ratio was inversely related to the glomerular filtration rate, which may explain the tubular patterns which you have observed during rejection crisis, but didn't show that protein analysis was of any value in predicting a rejection crisis. My question is: have you any immunochemical analyses which can confirm your findings?

BOESKEN Regarding the glomerular type of proteinuria we have controlled all our results by the usual protein clearances with IgG, transferrin and albumin and we got a good correlation between the glomerular selection type and the patterns shown here in the electrophoresis. We did not look at tubular proteinuria and we cannot correlate this with microglobulin clearances. However the pattern might be more valuable despite varying amounts of proteins excreted. You saw the very high tubular-type proteinuria directly after transplantation, up to 7 g and usually you don't see any tubular proteinuria in chronic disease.
above 2 g/day. This might reflect the different GFR, which however cannot explain micromolecular proteinuria, because their tubular concentration decreases. Any tubular-type proteinuria therefore must be caused by tubular dysfunction independent of GFR.