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Three-dimensional analysis of glomerular morphology in patients with subtotal nephrectomy

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Three-dimensional analysis of glomerular morphology in patients with subtotal nephrectomy. Previous studies documented that single section examination of kidney tissue underestimates glomerulosclerosis and that three-dimensional examination of glomerular morphology improves recognition of the incidence and distribution of sclerotic changes within the glomerular capillary tuft. We have adopted this technique to evaluate the true frequency and the spatial extent of glomerulosclerosis in patients who were subjected to extensive renal mass reduction. We re-evaluated four kidney biopsies of patients with a solitary kidney who had undergone partial nephrectomy for renal-cell carcinoma. Histopathological examination aimed at detection of glomerular sclerotic lesions was performed on serial sections (from 75 to 93 serial sections for each biopsy, 3 μm thick) together with three-dimensional morphometric analysis of glomerular tuft and sclerotic areas using a computer-based image processing system. Results were compared with observations based on more conventional single section evaluation of the same biopsies. Among 65 glomeruli examined by three-dimensional morphometric analysis, only 8% were normal, 42% revealed segmental sclerosis and 51% global sclerosis. These results confirmed that single section evaluation grossly overestimates the number of normal glomeruli (37% vs. 8%, respectively), since the majority of glomeruli classified as normal are indeed affected by sclerotic changes in areas typically out of the section plane. The three-dimensional distribution of sclerosis is characterized by the appearance of multi-focal areas affecting a small capillary tuft volume (<10%/) which ultimately propagate to the entire capillary tuft. Despite the maintenance of renal function, at the time of biopsy in patients with extensive ablation of renal mass, the incidence of glomerulosclerosis affects almost the entire glomerular population. These data suggest sclerotic lesions initially arise as multifocal lesions within the capillary tuft, and eventually propagate to global sclerosis.

Extensive surgical ablation of renal mass in the rat is associated with hemodynamic adaptation of remnant nephrons. Specifically, a reduction in afferent arteriole resistance, which serves to increase glomerular perfusion and pressure, maintains GFR despite the reduced nephron number [1, 2]. A few weeks after renal mass ablation, however, animals develop glomerulosclerosis which is a likely consequence of renal structural damage induced by adaptive hemodynamic responses to the removal of renal parenchyma [3]. The clinical relevance of the above findings, and particularly the issue of whether humans with surgical reduction of part of the renal parenchyma are also at risk of developing glomerulosclerosis, has been questioned for the last 15 years.

In 1991 Novick and coworkers [4] studied a group of patients who had surgical reduction of more than half of their renal mass. Most were patients with solitary kidneys who subsequently underwent nephron-sparing surgery for localized renal cancer. The population studied consisted of 14 patients followed for at least five years after renal mass reduction in the solitary kidney. Of these, nine patients were proteinuric but renal function deteriorated only in two (as inferred by serum creatinine). Four patients with moderate to severe proteinuria underwent renal biopsy. The biopsy specimens showed that in one patient almost all glomeruli (98%) were affected by global sclerosis. In the other three patients a large number of glomeruli were normal (68, 87 and 75%, respectively), and the remaining glomeruli were affected by global (27, 3 and 11%) or segmental sclerosis (5, 10 and 11%, respectively). These evaluations were done on standard sections using conventional morphological technique.

We have previously reported the results of a study on the distribution of glomerular lesions in a rat model of nephrosis using three-dimensional morphometric analysis [5]. The data showed that single section examination of renal biopsy specimens remarkably overestimated the number of normal glomeruli and underestimated the actual extent of glomerulosclerosis to the point that, while only 20 to 30% of glomeruli, on average, revealed sclerotic changes by conventional single section evaluation, more than 90% had detectable glomerulosclerosis when studied by three-dimensional morphometry. Conventional evaluation of the incidence of glomerular sclerosis in human renal biopsies is not actually based on the processing of a single section, but several consecutive sections (more than 20 to 30 sections) are effectively obtained. However, despite this number of sections quantitative observations are performed considering individual sections without serial analysis of the same capillary tuft in the section series. In addition, with the precise aim to avoid the counting of the same capillary tuft in consecutive sections quantification of the number of glomeruli affected by sclerosis is restricted to sections that are considerably apart. With the present study we then took advantage of the serial section examination technique to re-analyze renal biopsies from the same four patients previously studied by Novick et al [4], who had more than 50% reduction of renal mass. All the glomeruli entirely contained in the four biopsies were

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Table 1. Demographic and renal functional data in the four individual patients

Biopsy	Identification number in [4]	Sex	Age *years*	% Of kidney remaining	Follow-up *months*	GFR at follow-up *ml/min*	Proteinuria at follow-up *g/day*
A	26	M	57	33%	86	52	6.74
B	3	M	53	67%	200	63	3.80
C	22	F	62	33%	132	dialysis	2.81
D	14	M	58	25%	110	dialysis	0.93

analyzed by three-dimensional reconstruction of the capillary tuft in order to more precisely define the extent and intraglomerular localization of sclerotic lesions. Serial section examination also permitted evaluation of the incidence of atubular glomeruli in these four bipsies.

Methods

Patient population

We re-evaluated four kidney biopsies of patients with a solitary kidney who had undergone partial nephrectomy for renal-cell or transitional-cell carcinoma. The mean portion of the kidney tissue resected was 60% (range 33 to 75%). All patients had kidney tissue that was considered to be both functionally and histologically normal at the time of partial nephrectomy. Patients were followed for a mean period of 132 months (range 86 to 200). Patient status deteriorated during the follow-up period in that all developed moderate-to-severe proteinuria (0.93 to 6.74 g per day) and two had dialysis-dependent renal failure. Patient demographic and renal functional data, as well correspondence of patient identification numbers in this and in the previous study are reported in Table 1.

Pathological examination

Fragments of kidney tissue were processed for light microscopy by fixation in Dubosq-Brazil fluid and embedding in paraffin. From 75 to 93 serial sections (3 μm thick) were cut on a microtome and stained with periodic acid-Schiff's reagent (PAS). Histopathological examination aimed at detection of glomerular sclerotic lesions was performed using light microscopy. For each of the four biopsies the number of normal, segmentally and globally sclerotic glomeruli was determined. Glomerular sclerosis was defined as an increase in mesangial matrix substance associated with capillary wall wrinkling and collapse. The sclerotic lesions were often but not always associated with adhesion to Bowman's capsule.

Morphometrical analysis

A section located approximately in the middle of the tissue, hereafter referred to as a "starting section," was identified for each kidney tissue fragment. Examination through all the sections preceding and following the starting section allowed us to select the glomeruli entirely contained in the tissue fragment, and these were then subjected to three-dimensional morphometrical analysis. Every section of each selected glomerulus was digitized using a CCD videocamera connected to the microscope and transferred to a computer for digital processing with general purpose image-processing software (Image 1.55, NIH, Bethesda, MD, USA). For each section the profiles of capillary tuft and sclerotic areas were manually outlined using a polygonal line and the area occupied by the polygon was automatically measured in screen pixels. Exact enlargement was used for calculation of actual values of measured surface area using digitized images of a calibration grid, as previously described [5, 6]. Volume of capillary tuft and sclerotic areas for each glomerulus were then calculated by multiplying the measured surface areas by section thickness. In addition, for each glomerulus classified to be affected by a sclerotic process, the number of sections with sclerotic areas was determined and these sections processed for evaluation of total percentage of capillary tuft volume affected by sclerosis. The number of atubular glomeruli was also estimated by inspection of all individual sections of each glomerulus.

Three-dimensional reconstruction

Images of all the sections of each glomerulus representing the glomerular tuft profiles and the profile of sclerotic areas, when present, were used for three-dimensional projection as schematically represented in Figure 1. On the images acquired from the microscope, the capillary tuft and areas of sclerosis were outlined in each section. These outlines were then used to create a side view, using a projection function, to show the localization of sclerosis areas. X-y-z coordinates of the pixels in to the profiles of glomerular tuft and sclerotic areas were also stored in the memory and used to construct a three-dimensional solid model of sclerotic lesions within the glomerular tuft.

Results

All the four examined renal tissue biopsies showed major structural abnormalities. Typical sclerosis changes of different degree are represented in Figure 2. Light microscopic findings of histological examination are summarized in Table 2. The spectrum of changes was rather broad and differed among the biopsies, with biopsy A and B showing moderate, biopsy C pronounced and biopsy D extensive glomerulosclerosis. The results of morphometrical analysis are shown in Figure 3. The distribution of capillary tuft volume tended to be bimodal. Two subsets of glomeruli were distinguished, one composed mostly of globally sclerotic glomeruli and a second in which glomeruli with segmental sclerosis predominated. Few normal glomeruli were found in either subset.

To better illustrate the frequency and extent of glomerulosclerosis, X-axis projections of reconstructed glomeruli classified as segmentally sclerotic are represented in Figure 4. The continuous line in the Figure denotes the position of the starting section. Only 8 glomeruli showed sclerotic lesions in the starting section, while in 19 glomeruli the starting section did not cross areas affected by the sclerotic process (see also Table 1). However, all these 19 glomeruli presented signs of sclerosis out of the starting one. In addition, serial section analysis revealed that only 5 glomeruli, out

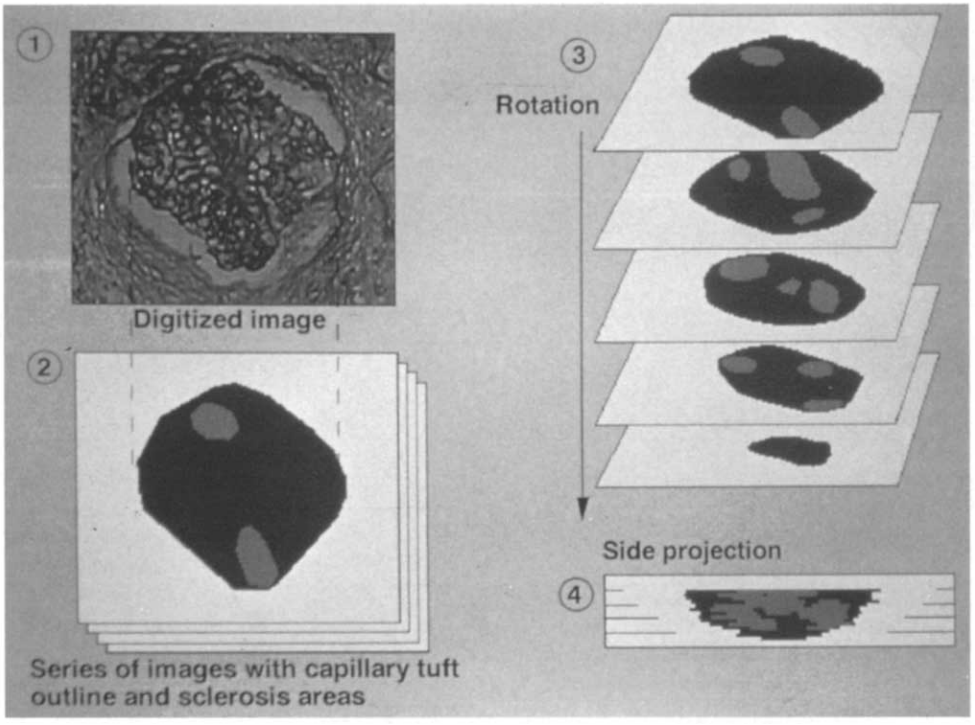

Fig. 1. *Schematic representation of the technique used for three-dimensional reconstruction of glomerular tufts.* Digitized images from the microscope (1) were used to outline the glomerular capillary tuft and areas of sclerosis if present (2). A side view (parallel to the section plane) of the capillary tuft was then created by the projection function of Image 1.55 using all the sections of each glomerulus. The projection function represents the capillary tuft volume as transparent surface with internal localization of the sclerosis areas.

of the 65 examined, were found to be completely free from sclerosis. This is in sharp contrast with the results of single-section evaluation by which as many as 24 glomeruli were classified as normal (Table 2). Figure 5 illustrates the relationship between glomerular capillary tuft volume and percentage of capillary tuft affected by sclerosis for each individual glomerulus. No statistically significant correlation was found between these two parameters. Globally sclerotic glomeruli showed small values for tuft volume, not exceeding 2.0 $\mu m^3 \times 10^{-6}$. Hypertrophic glomeruli (tuft volume larger than 3.0 $\mu m^3 \times 10^{-6}$) showed spread values of capillary tuft volume. Of interest, as shown in Figure 4 and in Table 3, a considerable number of glomeruli showed sclerotic lesions distributed in different areas of the capillary tuft. This condition is represented in details in Figure 6. Digitized images of all the sections of two representative glomeruli of biopsy A were used to generate three-dimensional drawings of the outer surface of the capillary tuft volume and the independent areas of sclerosis. The relation between the volume of sclerosis and the number of sclerotic areas in all individual reconstructed glomeruli is shown in Table 2. Several separated sclerotic areas (from 1 to 8) were detected in those glomeruli less affected by the sclerotic process (total sclerosis volume ranging from 0 to 30% of tuft volume), while glomeruli (33 in number out of 40) which had only a single area of sclerosis affecting more than 30% or less than 90% of whole tuft volume were classified as globally sclerotic. Of interest, there were no glomeruli with sclerotic areas affecting less than 30% or more than 90% of their volume. These data suggest that obsolescence of the glomerular tuft develops as a fast transition from multifocal small lesions to a single large area of sclerosis.

Results obtained on the incidence of atubular glomeruli are reported in Table 2. Forty percent of all glomeruli examined were either atubular or with completely destroyed connection between Bowman's capsule and the tubule. Among the 5 normal glomeruli 2 were without the tubule while among the 27 partially sclerotic glomeruli 4 were atubular. As expected, the incidence of atubular glomeruli was even more important in globally sclerotic glomeruli, since in 20 out of 33 glomeruli analyzed the tubule was absent or destroyed.

Discussion

The present results document that humans who underwent surgical reduction of more than 50% of their renal mass, and were followed for at least five years, developed extensive renal lesions. Actually, out of a total number of 65 glomeruli studied by three-dimensional reconstruction of the entire tuft, 60 glomeruli showed some degree of sclerosis, either segmental or global, while only 5 had no lesions. Comparative analysis performed using the conventional single-section examination versus the whole glomerular tuft reconstruction indicates that the former is a very inaccurate method to evaluate the true extent of glomerulosclerosis. This is exemplified by the fact that a significant number of glomeruli that were classified as normal by conventional morphology did have instead various degrees of sclerosis when studied by the reconstruction technique. By contrast, glomeruli classified as globally sclerotic with conventional analysis were indeed completely sclerotic by three-dimensional reconstruction. This indicates that in such glomeruli there were no portion of normal structure remaining.

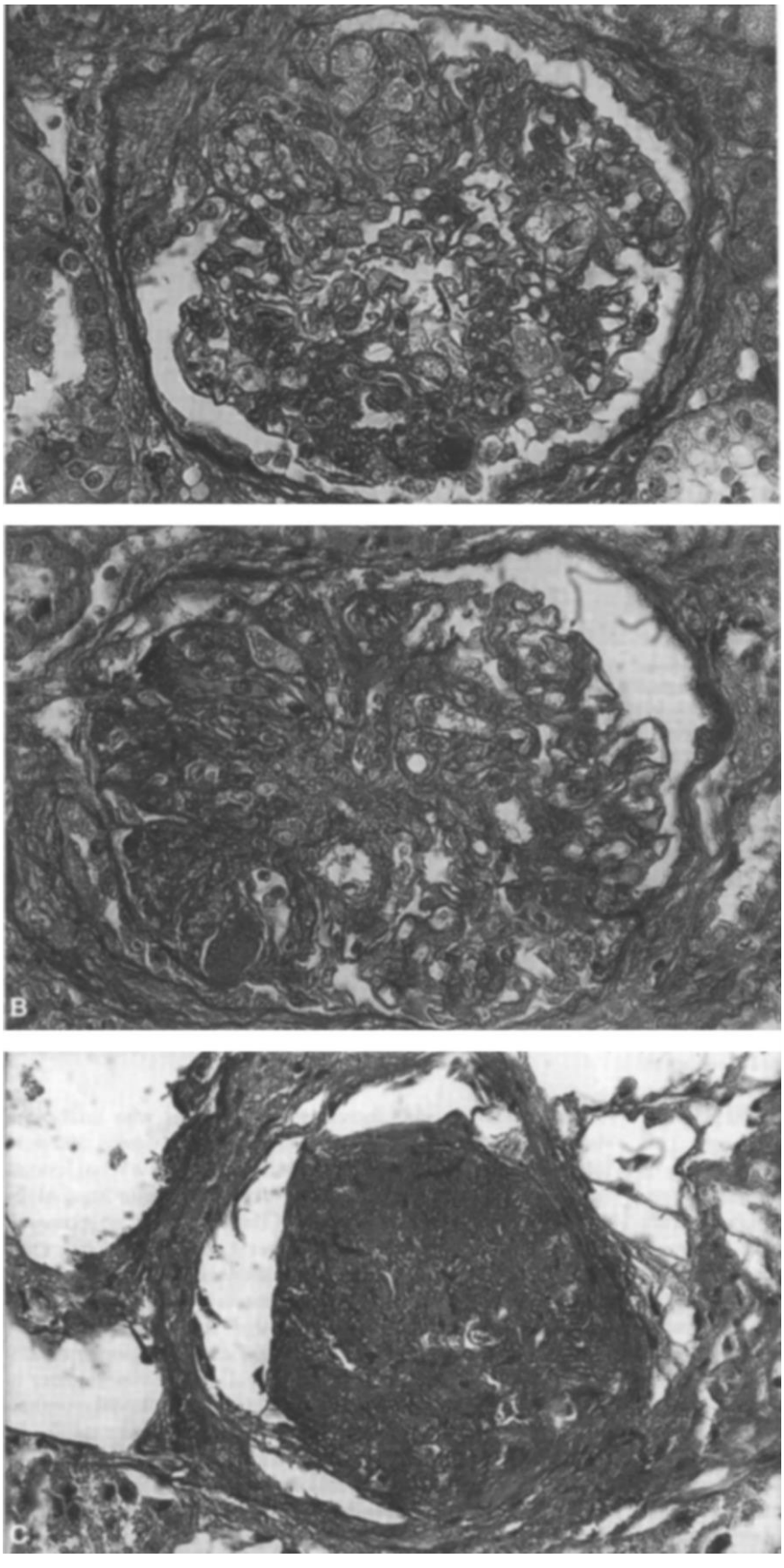

Fig. 2. *Representative photomicrographs at light microscopy of glomerular capillary tuft affected by different degree of sclerosis.* Rather mild sclerotic changes (**A**), diffuse and extensive sclerotic changes (**B**) and global sclerosis (**C**).

Table 2. Incidence of sclerosis according to single-section and serial-section morphologic analysis of glomerular structure, and incidence of atubular glomeruli (numbers in parenthesis), in four biopsies from patients with solitary kidney and partial nephrectomy

Biopsy	Number of glomeruli (atubular)	Single-section analysis			Serial-section analysis		
		Normal	Segmental sclerosis	Global sclerosis	Normal	Segmental sclerosis	Global sclerosis
A	26 (6)	17	4	5	3 (1)	18 (1)	5 (4)
B	3 (0)	2	1	—	—	3 (0)	—
C	22 (10)	4	2	16	2 (1)	4 (1)	16 (8)
D	14 (10)	1	1	12	—	2 (2)	12 (8)
Total no.	65 (26)	24	8	33	5 (2)	27 (4)	33 (20)
Percentage 100%	(40%)	37%	12%	51%	8%	42%	51%

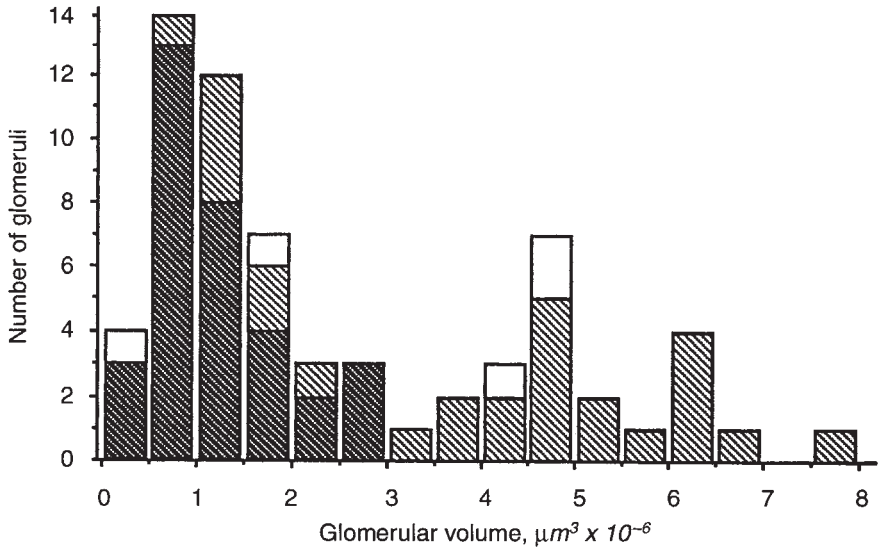

Fig. 3. *Distribution of glomerular capillary tuft volume and incidence of segmental and global sclerosis in glomeruli analyzed by serial section reconstruction in the four biopsies studied.* Symbols are: (□) normal glomeruli; (▨ up r down l stripe) glomeruli with segmental sclerosis; (▩ dark up r down l stripe) glomeruli with global sclerosis.

Specific comparisons about the incidence of sclerosis in individual patients obtained using the two methods are difficult to perform, since the number of glomeruli that could be examined in the two studies was highly variable among the four biopsies. In detail, in biopsies B and D only 3 and 14 glomeruli were present with their entire volume, respectively. However, some estimation can be made of the frequency of sclerosis in the total glomerular population that was studied. In the previous study among 234 glomeruli examined, 47% were classified as normal, 47% as affected by global sclerosis and only 5% as affected by segmental sclerosis. In the present investigation involving 65 glomeruli from the same four biopsies, the percentage of normal glomeruli was only 8% and that of segmentally sclerosed was 42%. This indicates that single section evaluation overestimates the number of normal glomeruli and greatly underestimates the number of glomeruli affected by segmental sclerosis. Of interest, the percentage of globally sclerotic glomeruli was remarkably similar in the two studies, 47% and 51% in the previous and in the present investigation, respectively.

The inspection of total glomerular volume using serial section examination also permitted quantification of the incidence of atubular glomeruli. It has been reported that a small percentage of glomeruli are not connected to proximal tubule in normal humans (3 to 4%) while in several renal diseases, characterized by glomerular and tubulointerstitial injury, this percentage importantly increases [7]. In our present study we confirmed that glomerular damage is associated with a rather high frequency of atubular glomeruli; in fact, 40% of all examined glomeruli were without connection with the tubule. As previously described [7], we also observed that when sclerotic changes in a glomerulus are more important, more frequently the glomerulus is without the tubule.

The results of this and previous [5] studies indicate that serial section morphometrical evaluation permits investigation of the three-dimensional extension of sclerosis lesions. This approach is useful for precise quantification of normal glomeruli versus glomeruli affected by segmental sclerosis. Extension of this technique to routine morphological evaluation, however, is not yet recommended since the technique is very time consuming. Despite the fact that as yet it must remain a research tool only, we can anticipate that rapid developments in the area of computer-based imaging will advance this field in the near future.

After the first description of glomerulosclerosis in rats undergoing extreme surgical ablation of renal mass, the question of whether these findings also occurred in humans was widely debated. Evidence that renal ablation in dogs [8] did not produce renal dysfunction was taken as an argument in favor of the species-specificity of the lesions described in rats. The present study extends the previous observations of Novick and coworkers that patients with a reduction in renal mass, despite maintaining

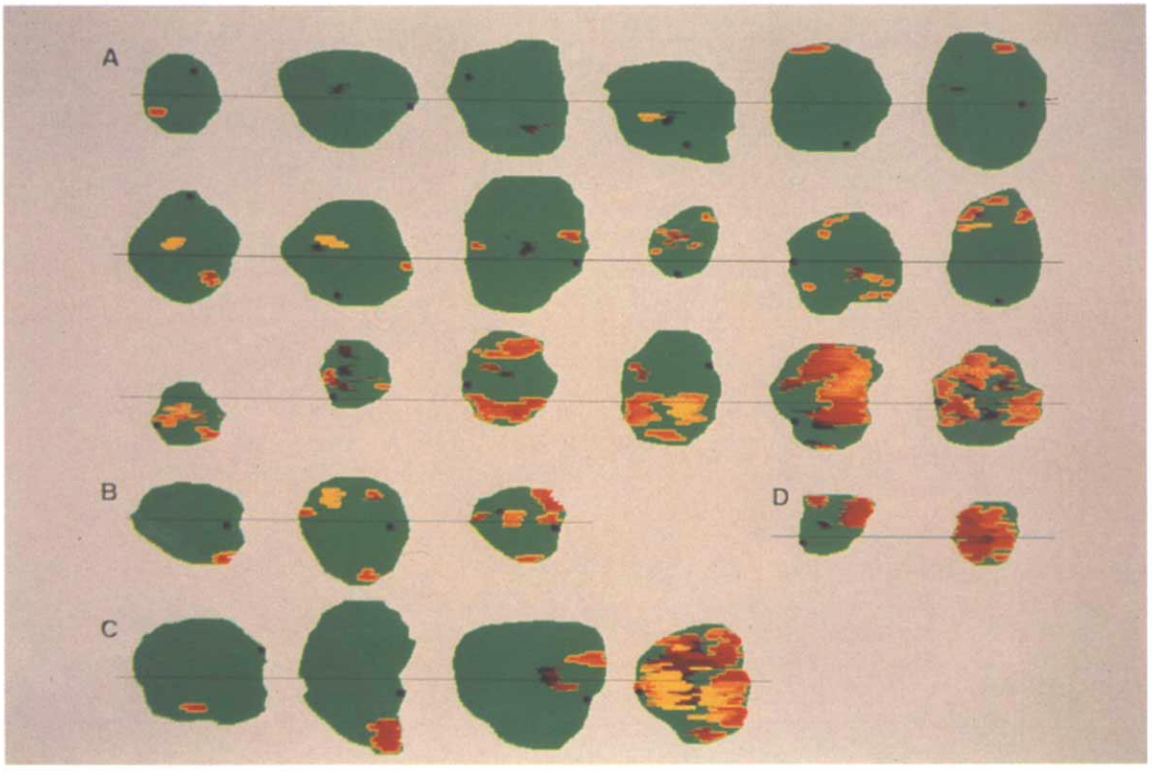

Fig. 4. *Spatial distribution of focal areas of sclerosis in segmentally sclerotic glomeruli from the four biopsies analyzed (***A, B, C** *and* **D***).* Glomerular capillary tuft (in green) and sclerotic areas (in light and dark red) are projected parallel to the section plane. Black line represents the projection of the starting section. Black dots represent the position of the vascular pole. Reproduction of this figure in color is made possible by a grant from Ciba Medical Department, Varese, Italy.

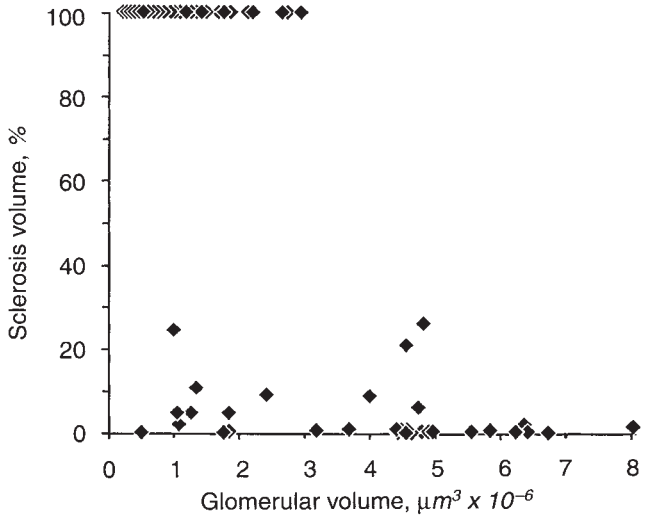

Fig. 5. *Relation between glomerular capillary tuft volume and fractional volume of sclerosis in reconstructed glomeruli from the four biopsies analyzed.*

Table 3. Distribution of glomeruli as a function of the degree of sclerosis and of the number of sclerotic areas in each glomerulus

Number of sclerotic areas per glomerulus	Percentage of capillary tuft affected by sclerosis				
	0–10%	10–20%	20–30%	30–90%	90–100%
1	7	—	—	—	33
2	5		—	—	—
3	1	1	—	—	—
4	2	—	—	—	—
5	5	—	1	—	—
6	2	—	1	—	—
7	1	—	—	—	—
8	—	—	1	—	—

stable renal function for some years, are at increased risk of glomerulopathy, and the nature and the extent of the lesions that involve most of the remnant glomeruli are also defined. The clinical relevance of these findings rests on the observation that the extent of glomerulosclerosis in rat and human is qualitatively and quantitatively comparable if the same proportion of renal parenchyma is removed. These findings indicate, at variance to initial skepticism [8], that the rat is a valid model for study human ablation-mediated renal injury. Therefore, reduction of renal mass in the rat appears to be an appropriate model for clarifying the natural history of progressive renal disease in humans and to more extensively explore the effectiveness of novel treatments that may prevent or retard renal disease progression.

The present analysis failed to show a relationship between glomerular volume and percentage of capillary tuft affected by sclerosis at the individual glomerular level. Previous studies in the rat [9, 10] suggested that ablation of renal mass initially induced glomerular enlargement, which is then followed by shrinkage and eventual complete sclerosis of the glomerular tuft. At variance to these experimental observations, our present analysis of the kidney tissue at a single time point does not allow definite

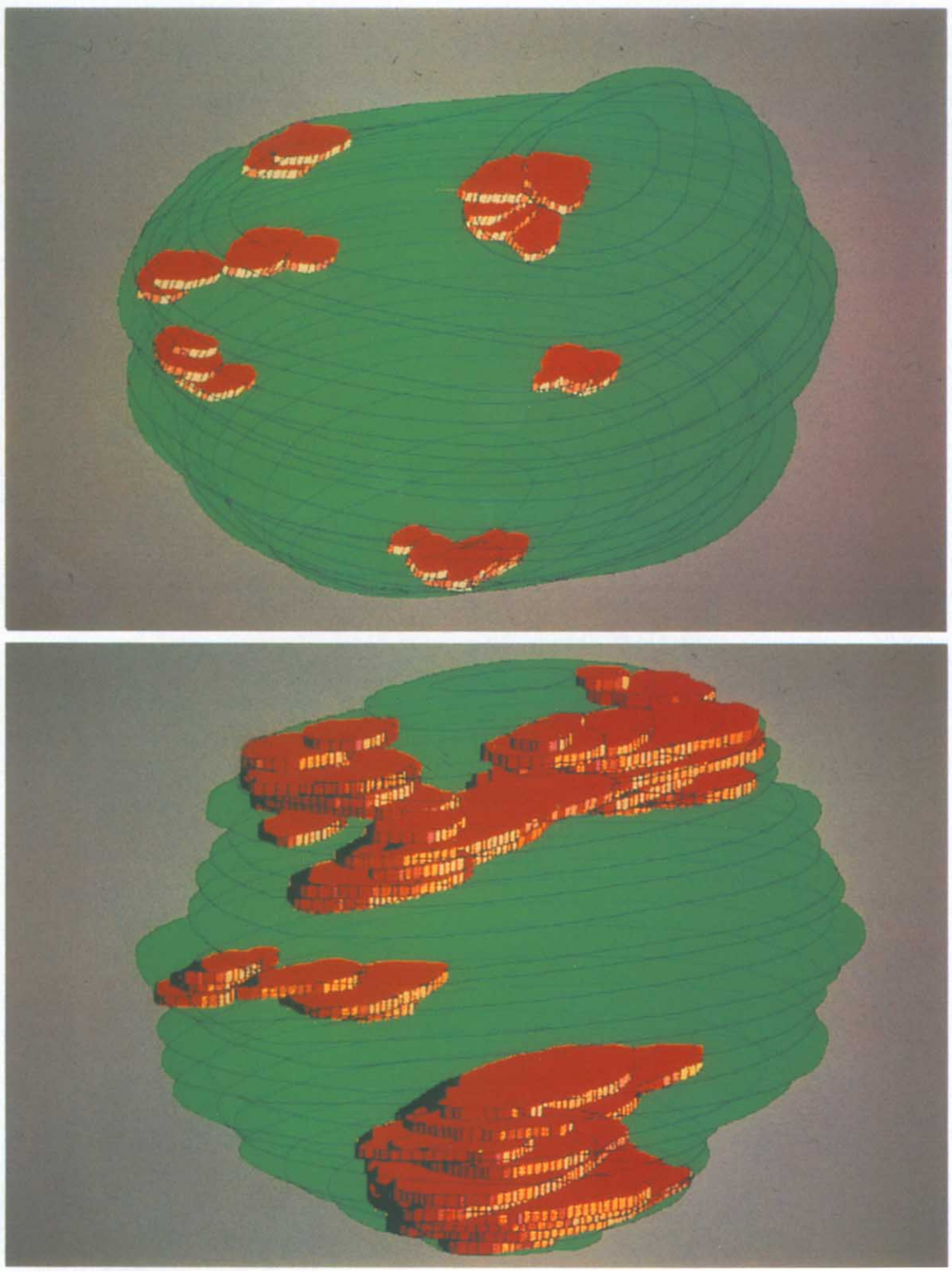

Fig. 6. *Three-dimensional representation of the location of sclerotic areas (in red) within the capillary tuft volume (the green transparent surface around the capillary tuft) of two representative glomeruli from biopsy A.* These two glomeruli are also represented in Figure 4, respectively as the fifth glomerulus in the second line and the third glomerulus in the third line. Reproduction of this figure in color is made possible by a grant from Ciba Medical Department, Varese, Italy.

conclusions to be drawn about the dynamic process of glomerular network remodeling after extensive renal ablation. However, two observations can be made considering our volume measurements. First that early sclerosis lesions were comparably present in large as well as in small glomeruli (Fig. 5), suggesting that glomerular hypertrophy did not consistently preceed development of glomerular sclerosis. Secondly, the glomerular tuft does shrink when the glomerulus become completely sclerosed, as shown by data reported in Figure 3.

Within the limit of the assumption that the pattern of the distribution of sclerotic areas in less affected versus highly affected glomeruli clarifies how glomerulosclerosis developed initially and then further progresses, the present study offers some completely unexpected indications. Three-dimensional reconstruction of glomeruli with less than 30% of the tuft volume affected by sclerosis (presumably at the early phase of the pathological process) shows several independent sclerotic areas randomly distributed within the capillary tuft volume. Independent growth of such multiple areas to confluence is the likely anatomical equivalent of the progression of glomerular damage observed in 33 out of 65 glomeruli examined. In addition, analysis of the percentage of tuft volume affected by sclerosis indicates two extreme conditions: several glomeruli had small areas of sclerosis that together involved less than 30% of the whole capillary tuft, and several other glomeruli were almost completely obsolescent with a sclerosis volume of 100%. However, no glomeruli were found with intermediate values of glomerulosclerosis. On the basis of this observation, we speculate that global sclerosis develops by a process of rapid transition from an initial phase of many little sclerotic areas to a phase of massive propagation of the initial lesions that become confluent enough to involve the entire capillary tuft volume.

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