

## Renal Biopsy: History and Perspectives

### Abstract

Renal biopsy (RB) is a valuable diagnostic tool. This review discusses RB used in Russia in research and practice since the 1980s. Among others the role of RB in the following conditions is analyzed: glomerulo- and pyelonephritis, arterial hypertension, alcoholism, diabetes mellitus and urinary tract malformations in children. The material used in certain studies was unique: wedge or core biopsies in hydronephrosis, acute and chronic pyelonephritis. The collection of RBs was associated with risk, in particular, intra-operative biopsies in acute pyelonephritis and hydronephrosis. Morphological overdiagnosis of mesangioproliferative glomerulonephritis resulted in the overtreatment with steroids and cytostatica in some cases. The main conclusion is that, considering possible adverse effects, RB for research should not exist as such; it must always be performed according to clinical indications. If a patient gives informed consent to research on renal tissue obtained for diagnostic purposes, it can be done, provided that enough tissue remains for the diagnostics. High level of integrity, quality of specimens and of their examination must be a precondition for the use of RB in research and practice.

### Keywords

Renal biopsy • Pyelonephritis • Glomerulonephritis • Alcoholism • Russia

### Introduction

Renal biopsy (RB) is a valuable diagnostic tool; it was broadly used in the former Soviet Union (SU). RBs were taken for research from patients with glomerulonephritis (Gn), acute and chronic pyelonephritis, amyloidosis, renovascular hypertension (from both kidneys in some studies) [1-6] other hypertension (essential or of unknown etiology) [6-9] alcohol-related disorders [10-19] diabetes mellitus [20] rheumatoid arthritis [21] from children with

### Review Article

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urinary tract anomalies including those combined with hydronephrosis or pyelonephritis [22-26]

The I.M. Sechenov Medical Academy (recently renamed University) in Moscow has been the leading institution in the field of nephrology and nephropathology. Numerous textbooks, manuals and journal articles emerged from the Academy; many of them are cited in this review [1-3,5,10-17,20,22,23,25-64] RBs were taken in an operating room under conditions of sterility. As for morphological methods, the electron microscopy was not always used for diagnostics. Nevertheless, about one third of the biopsy cylinder was embedded in epoxy resin. The semi-thin resin sections were made for research but were not used for diagnostics, the latter being performed mainly on the basis of paraffin sections and immunofluorescence for immunoglobulins, complement fractions and fibrinogen. For diagnostic purposes, a part of the biopsy cylinder was fixed in formalin and embedded in paraffin. The histological stains mostly used for the diagnostics were hematoxylin and eosin (H&E), Periodic acid-Schiff (PAS) and Congo red (for the identification of amyloid). On the contrary to the international practice, silver stains were not used for the routine diagnostics in the Sechenov Medical

Academ [34-42] Later on, the silver, Masson's trichrome, other stains and immunohistochemical methods started to be used in diagnostic centers. The histological specimens and paraffin blocks were preserved in archives that were used for research; some archives were in disarray and certain specimens could not be found. [65]

### **Pyelonephritis**

In the studies by Kirillov, [27,28] excisional (wedge) RBs were sampled in the course of kidney-preserving operations such as lithotomy from patients with chronic or acute (including purulent) pyelonephritis. In the international literature, pyelonephritis is not listed among conditions where RB is indicated, while acute inflammation, infection and hydronephrosis are generally considered to be contraindications. In particular, wedge biopsy from the kidney in acute pyelonephritis is associated with a risk of abscess formation. In another study of acute and chronic pyelonephritis, a core biopsy from renal medulla and a wedge from the cortex were taken concomitantly. [63] In the studies from the same Institution, [1,29] RBs were collected from patients with chronic pyelonephritis and hydronephrosis, while conclusions were based on linear correlations between ultrastructural morphometric and clinical indices. However, statistical significance of the correlation coefficients in this and some similar studies was overstated. A comparison with the reference table [66] demonstrated that many claimed P-values were overstated being too high for the given correlate on coefficients and the number of correlation pairs in the dissertation [29] and journal articles [1,30-32] details, images and documentary evidence are in the book. [67] In a later study, "cytomembranes of the interstitial tissue of renal medullary layer" were studied using core RBs collected during lithotomy operations from patients with urolithiasis and secondary pyelonephritis. [68] The presence of the "medullary layer" in the specimens indicates that RBs were quite deep with a risk of calyx perforation. Core RBs were taken from patients with pyelonephritis also by other researchers. [69] Fine-needle RB in acute pyelonephritis was performed and recommended. [70]

### **Alcoholism**

Among patients with supposed alcohol-related disorders, biopsies were collected from kidneys, pancreas, liver, lung, salivary glands, stomach and skin, repeatedly in

some cases. [10,12,15] Intraoperative lung biopsies were taken at surgeries for suppurative lung diseases. [64] Some RBs used for the morphological studies of alcoholism were collected according to clinical indications but in many cases specimens from different organs were taken for research without sufficient indications. The attitude to patients with alcohol use disorders in the Russian healthcare has sometimes been less responsible with lower procedural quality assurance; last reviewed in. [71] There is an opinion, shared by the author, that RB for research should not exist as such; it must always be performed according to clinical indications.

It was concluded on the basis of a series of RB studies that a generalized cytoskeleton abnormality with accumulation of filaments of intermediate type in macrophages, epithelial and other cells is typical for the damage by ethanol or the "alcoholic disease." [10,14,15] It is known that Mallory bodies, seen in alcoholic hepatitis and some other liver conditions, contain filaments of intermediate type; however, such generalizations have never been confirmed by other researchers. In any case, the cytoskeleton can be studied in experiments or post mortem. Another example: RBs were collected from patients with chronic alcoholism and nephritic symptoms, whereas "intracapillary proliferative glomerulonephritis" was diagnosed in all cases. [18] In a later study by the same researchers, the histopathological findings in 40 from 43 patients with alcoholism and nephritic symptoms were morphologically classified as membranoproliferative (mesangiocapillary) Gn; while in 29 from 31 patients with nephritic symptoms without alcoholism "fibroplastic" Gn was diagnosed [19] The striking difference between the two groups is indicative of the data trimming. Other invasive procedures (celiacography, endoscopic cholangiopancreatography etc.) were applied in alcoholics without clear indications. [12] In the author's opinion, repeated biopsies from different organs, doubtful morphological descriptions and interpretations, call in question the indications for RB at least in a part of the studied patients.

### **Glomerulonephritis (Gn)**

In the Russian-language literature RB has been generally regarded to be indicated in suspected Gn [33,72,73] or "always when it can influence therapy or estimate prognosis." [74] In the internationally used handbooks,

RB in isolated proteinuria and/or microhematuria without abnormal urine sediment or signs of progressive renal disease is generally regarded to be not indicated. Indications for RB are sometimes formulated more liberally; but an obvious prerequisite must be a high quality of morphological examination. The utility of RB must be considered in the context of the patient's needs in terms of diagnosis, prognosis and therapy. [75] In Russia, RBs were sometimes collected from patients with "inactive nephritic" or latent clinical forms of supposed Gn, i.e. in cases with isolated proteinuria and/or hematuria. [34-36,43-46,76] At the same time, the classifications of Gn has been different from those used internationally, which interfered with the implementation of guidelines from the foreign literature. For example, IgA nephropathy was not considered to be a separate entity; it was not mentioned even in the article from the Sechenov Academy dedicated to the "hematuric form" of Gn. [37] IgA nephropathy was usually diagnosed on RB as mesangioproliferative Gn (MG) and treated with corticosteroids and/or cytotoxic drugs.[33,38-45] In later editions controversies can be found; for example, in the textbook, [77] IgA nephropathy and Berger's disease are discussed separately and different treatments are recommended. IgA nephropathy as a separate entity was criticized as a "manifestation of a classificational crisis. [77-78] Original classifications of Gn were proposed. [46] It should be mentioned apropos that in the latter study morphometric methods proposed earlier [47-48] have been used without references. In the National Manual, probably the most authoritative Russian-language edition in nephrology, IgA nephropathy and MG are discussed in one chapter titled "Mesangioproliferative (IgA) glomerulonephritis" as follows (from Russian): "The term IgA nephropathy is used to designate an entity, the morphological equivalent of which is MG." [49] This is partly at variance with the known fact that glomeruli in IgA nephropathy may be normal at light microscopy or show segmental mesangial proliferation confined to some glomeruli (focal proliferative Gn), diffuse mesangial proliferation (such as in MG) or, rarely, crescentic Gn. Healing of focal lesions can result in focal glomerulosclerosis.

Comparisons of percentages of glomerular diseases, diagnosed by RB in Moscow and Rostock in Germany (Table 1), [17,79] are suggestive of the regular

overdiagnosis of Gn in the former. Old equipment, such as Reichert microtomes from the 1930s, was used in many institutions. The author of this book participated in the research using epoxy resin sections cut by a modern LKB pyramitome with glass knives [2,3,5] after that he found it difficult to examine diagnostic paraffin sections, less clearly visualizing basement membranes and mesangial matrix. The paraffin slides were relatively thick, the thickness being uneven. Occasionally overstained thick sections can mimic a glomerular capillary wall thickening. This is apparently the reason why membranous Gn was diagnosed in Moscow more than twice as frequently as in Rostock (Table 1).

The diagnosis of MG was used broadly, encompassing 49-60.8% of all Gn cases diagnosed by RB. [50,51] As mentioned above, epoxy resin sections and silver impregnation were not used for the diagnostics, while electron microscopy was applied only occasionally. Using these methods, the collecting box of MG could have been partly sorted out, excluding from it some cases morphologically bordering on the norm i.e., isolated proteinuria and/or hematuria without renal or systemic disease, not requiring immunosuppressive therapy. In such cases, histologically are often detected only minor glomerular abnormalities: mild mesangial widening and hypercellularity, scarce deposits of immunoglobulins and complement. In conditions of insufficient quality of histological specimens, without silver impregnation and electron microscopy, such changes were sometimes overestimated and Gn overdiagnosed. As mentioned above, RBs were collected from patients with the "inactive nephritic" or latent clinical types of Gn i.e., minimal proteinuria and/or hematuria. [33-36,74] As a result of the histological overdiagnosis of Gn, some patients were treated by corticosteroids and cytotoxic drugs such as

Condition	Moscow	Rostock
Diffuse Gn	81.7	59.3
MG	55.5	40.2
Membranous Gn	9.2	4.1
Minor glomerular abnormalities	7.1	20.8 (1978-83) 30 (1990-99)

**Table 1.** Percentages of glomerular diseases diagnosed by RB in Moscow and Rostock. [17,79]

azathioprin, cyclophosphamide or chlorambucil [38-44] without sufficient indications.

### **Congenital conditions**

The dubious concept of hypoplastic renal dysplasia was developed on the basis of pediatric RBs, described as follows: "Racemosely arranged glomeruli with single capillary loops, abundant rounded cells freely lying in the cavity of a capsule; single mesangial cells; irregular enlargement, loosening, and thinning of the basement membrane", narrow extracapillary space, glomeruli having irregular form and singular capillary loops or total absence of capillaries, [46,52] which has no analogues in the international literature. The terms "renal hypoplasia" and "dysplasia" are used in the literature with different meanings. In the author's opinion, the descriptions were at least in part based on tangential sections of glomeruli, which is evident looking at the illustrations in the articles partly reproduced and commented. [67,80] It was recommended to the authors to verify their concept counting glomeruli "with singular capillary loops" in autopsy or nephrectomy specimens, but it was not done.

The common feature of these and some other works is the presentation of ultrastructural findings without comparison with light-microscopic images, whereas variants of the norm and artefacts have been interpreted as specific pathological phenomena. For example, hypoplastic dysplasia was diagnosed by electron microscopy in 8 from 34 randomly selected patients aged 9-54 years with nephrotic syndrome and histologically minimal glomerular changes. [53] At the same time, there was not a single case of Alport syndrome or thin basement membrane nephropathy, having some morphological features in common with the "hypoplastic dysplasia" within the meaning of the papers, [46,52] in 4440 RBs overviewed in the study. [50] These two conditions constituted  $\geq 1\%$  of all renal diseases diagnosed by RB in Rostock. [79] The concept of hypoplastic dysplasia was discussed with clinicians collecting biopsies, which could have caused confusion and interfered with the diagnosis of Alport syndrome. The diagnosis of this condition is of importance for genetic consultation of patients.

Later on, the same researchers (and their followers) applied the term hypoplastic dysplasia to the glomerular changes in congenital hydronephrosis and other renal

abnormalities in children, interpreting them as an inborn nephropathy affecting a major part of glomeruli [22,25,26,53,54] A regular combination of two prima facie unrelated conditions: an inborn glomerulopathy affecting a major part of glomeruli, and hydronephrosis related to an abnormality of the ureteropelvic junction, seems to be improbable. Glomerular changes in hydronephrosis caused by the urine retention (collapse of the glomerular tuft with the widening of the urinary space) are different from those described within the concept of "hypoplasia" and "dysnephrogenesis" as per the article.[54] For this research, 167 intra-operative RBs from children with urogenital malformations, plus RBs for the control group from adult urological patients, were collected within the framework the research<sup>[55]</sup> with questionable indications and enhanced risk at least in a part of the cases.

### **Renal and pancreatic biopsies in diabetes mellitus**

The same group of researchers collected pancreatic excision biopsies 5×5 mm in the course of the surgical operations of "pancreatic blood shunting into the systemic blood flow in insulin-dependent diabetics" discussed in the preceding paper. [81] From the same patients, core RBs were taken [56] Apart from several reports from the former SU, no analogues of this surgical treatment of diabetes mellitus were found in the literature. In the studies of RBs from diabetics, Gn and mesangiolytic were designated as consecutive stages of diabetic glomerulosclerosis. [57] Ultrastructural descriptions included frequent mesangial interposition with displacement of mesangial cells to the periphery of glomerular capillary loops and formation of double-contour basement membranes, [57,58] which is at variance with usual descriptions. In particular, the morphological picture of Gn, if detected in a diabetic patient, is usually interpreted as a superimposed condition possibly needing a special therapy. [82,83] It should be commented that in diabetes mellitus, RB is generally indicated for patients under the suspicion of a renal disease other than diabetic nephropathy, in particular, when they present with severe proteinuria. [75,84] It is important to diagnose a non-diabetic renal condition, in particular, membranoproliferative Gn (characterized by the mesangial interposition), where the immunosuppressive therapy should be considered. The interpretation of morphological picture of Gn as a characteristic phenomenon or a stage of diabetic nephropathy is potentially misleading.

## Renovascular hypertension summarizing discussion

RB in renovascular (named also vasorenal in Russia) hypertension was discussed previously with documentary evidence of manipulated statistics. [67] Some risk for patients was caused by bilateral renal biopsies taken for the research. [29,59-62] Some details of this research should be commented: "Mathematical model of renovascular hypertension, [61,62,85] "Renal endocrine system" and its "stereotype cyclic changes" in various renal diseases. [32] Corresponding English language summaries, available also in PubMed, deserve to be quoted:

"Comparison of the findings of clinical, instrumental, and laboratory examination of patients with vasorenal (renovascular) hypertension with the results of morphological analysis of renal bioptic material showed that multivariate regression analysis of the parameters of examination of the patients provides for authentic calculation of the quantitative index of nephroarteriosclerosis - the vascular index of the afferent arterioles of the renal glomeruli. The calculated values of the vascular index for both kidneys are criteria for choosing the method of operative intervention in vasorenal hypertension. [85]

Comment: At that time, I worked at the same department and examined, among others, biopsy specimens from patients with renovascular hypertension; they were usually small, most of them contained no more than 1-3 glomeruli and arterioles, while some specimens contained none of these structures at all. Most of the specimens were unsuitable for a reliable morphometric assessment, let alone "choosing the method of operative intervention in vasorenal hypertension." [85] I informed thereof the chief researcher and other participants of the study.

Another summary reads as follows:

"The renin-angiotensin (juxtaglomerular apparatus - JGA) and prostaglandin (interstitial cells (IC) of renal medulla and nephrocytes of collecting tubules (NCT) systems of the kidneys were studied in 72 patients (renal biopsies, nephrectomy, morpho-functional correlations) with the nephrogenic arterial hypertension (vasorenal hypertension, chronic glomerulonephritis, pyelonephritis). Histologic and electron-microscopic methods were used;

the renin activity was determined in the peripheral blood and blood from the renal veins. The results were analyzed mathematically and statistically using an original program. It is shown that stereotype cyclic changes develop in the endocrine renal system of patients with renal hypertension and that they reflect the stages of initial hyperfunction (ultrastructural hyperplasia of JGA cells with appearance of numerous immature granules; ultrastructural moderate hyperplasia of medulla IC; increase of blood renin activity), discoordination of functions (progressing JGA hyperfunction and depletion of prostaglandin synthetic function of medulla IC; compensatory activation of NCT; further increase of the blood renin activity) and depletion (atrophy and fibroblastic transformation of the JGA of the majority of nephrons and of medulla IC). The stages of renal endocrine system alterations in the arterial hypertension are the manifestation of compensatory and adaptive response. Morphofunctional analysis with the use of morphometry and mathematical statistics are necessary for the objective evaluation of this response." [32]

In the late 1980s, I searched through the archive of ultrastructural images on photographic paper and glass plates and found approximately 20-30 images of juxtaglomerular cells with secretory granules and rhomboid protogranules showing similar structure, probably originating from a limited number of patients and experimental animals. These photographs were used as illustrations in the dissertation, [29] journal articles and books. There was not enough material for a reliable morphometric and statistical assessment of the form parameter characterizing "the elliptical shape of the granules in the JGA epithelioid cells", relative volume of secretory granules, and other ultrastructural morphometric indices issued in the dissertation and articles. [29,31,59] Human renomedullary interstitial cells, bona fide suitable for assessment of prostaglandin synthesis, were absent in the archive. There were only a few doubtful ultrastructural images, repeatedly used as illustrations in different publications. The phenomenon referred to in the above-cited summary as a "compensatory activation of nephrocytes of collecting tubules (NCT)" - a proposed morphologic equivalent of the enhanced synthesis of prostaglandins or other antihypertensive factors, has never been satisfactorily illustrated.

The data about “stereotype cyclic changes in the endocrine renal system” [32] in glomerulonephritis, pyelonephritis, and other renal conditions, and about the “calculated values of the vascular index for both kidneys” as criteria for choosing the method of operative intervention in vasorenal hypertension [85] have never been confirmed by other researchers. According to papers discussed above, renal tissues from patients with chronic pyelo- and glomerulonephritis underwent ultrastructural morphometry. Additionally, a large number of cases of renovascular hypertension (both kidneys) were reportedly analyzed. [29,32,59.] It should be commented that corresponding quantities of representative sets of ultrastructural images have never existed. I observed how this morphometry was performed: it was done using ultrastructural images on the photographic paper about 10 cm in size, by means of a ball-point pen connected to an image analyzing system. Only the granule-containing cells were analyzed morphometrically; the mean level of granularity was not determined even for a single JGA, let alone representative assessment of different JGA from the same patient. Characteristically, secretory granules were measured together with nonspecific lipofuscin-like granules, known to be a “Potential source of confusion when estimating the degree of granularity.” [86]

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## Conclusion

The RB material used in certain studies discussed above was unique e.g., wedge or core biopsies in hydronephrosis, acute and chronic pyelonephritis. The collection of RBs for the studies was associated with risk; while some research results have been unreliable and the quality level suboptimal. Apart from the articles discussed here, no other studies based on RB in hydronephrosis and acute pyelonephritis are known to us, while in chronic pyelonephritis no other studies performed abroad since the 1960s have been found. In particular, taking wedge biopsies from kidney in acute pyelonephritis may result in abscess formation. The overdiagnosis of mesangioproliferative glomerulonephritis resulted in the overtreatment of some patients with steroids and cytostatica. In conclusion, RB for research should not exist as such; it must always be performed according to clinical indications. If a patient gives informed consent to research on renal tissue obtained for diagnostic purposes, it can be done, provided that enough tissue remains for the diagnostics. In conclusion, high level of integrity, quality of specimens and of their examination must be a precondition for the use of RB in research and practice.

## Conflict of Interest

The author declares that he has no conflict of interest.

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