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What you should know about the work-up of a renal biopsy

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To obtain relevant clinical information from a renal biopsy is an interdisciplinary task, requiring close cooperation between clinician and pathologist. The better the nephrologist's understanding, the more rewarding the information from the pathologist. Based on our experience, we will discuss some practical points which are not often known or are poorly handled by our clinical partners.

Since these points require close interaction between clinicians and pathologists, some guidelines concerning the procedure and work-up of routine kidney biopsy have been established by the Renal Pathology Society [1].

Standard procedures for renal biopsy handling and processing were established at the European Union Consensus Meeting, which took place on February 25, 2000 in Vienna, Austria; these procedures cover the following aspects [1]:

- Taking the biopsy
- Transferring the material
- Dividing the sample
- Preserving the tissue
- Cutting the biopsy sample
- Staining the biopsy sample
- Reporting the finding
- Establishing the diagnosis

If these points are taken into consideration, there is an ~40–50% chance that the results of the kidney biopsy will have a direct consequence for patient management [2–6].

What size of the biopsy is minimal and optimal for the pathologist?

The first important question on which the opinions of the nephrological and pathological communities are divided concerns the necessary size of the kidney biopsy. We recommend obtaining two biopsy cylinders with a minimal length of 1 cm and a diameter of at least 1.2 mm. The quality of a renal biopsy depends on the size, i.e. the number of glomeruli: it is generally agreed that ~10–15 glomeruli are optimal; very often 6–10 glomeruli are sufficient and in some cases even one glomerulus is enough to make a diagnosis. However, as correctly pointed out by Corwin et al. [7] ‘If the percentage of glomerular involvement in a biopsy is used to determine the severity of a focal glomerular lesion, a small biopsy sample size will lead to considerable misclassification of disease
severity. In addition, a small biopsy sample size will make the exclusion of focal disease difficult’. Therefore, the pathologist has to check the size and quality of the biopsy sample under a light microscope before he decides how to process the material. One of the cylinders should be fixed and used for light microscopy and the second one for immunohistology, electron microscopy and additional investigations.

**What clinical information is indispensable to the pathologist?**

To answer this delicate question, we wish to quote a statement from computer technology: ‘One cannot feed in garbage and get out fruit juice’. The absence of clinical information is a sore point in many partnerships between clinicians and pathologists. In an ideal world, the pathologist obtains information on the clinical history, recent laboratory values in particular urine (proteinuria, haematuria, leukocyturia, cylindruria) and serum [urea, creatinine, cholesterol, total protein, creatine clearance, C3, C4, anti-nuclear antibody (ANA), anti-neutrophil cytoplasmic antibodies (ANCA), anti-glomerular basement membrane (GBM), argininosuccinate lyase (ASL)], presence of diabetes mellitus or hypertension or other systemic diseases, other parameters of interest (if available) and current therapy (if any).

**How should a renal biopsy be handled?**

This depends very much on whether a local pathologist is available or whether biopsy samples have to be mailed. Potential media are isotonic NaCl for fast local transport of the material—the ideal procedure which permits all different types of further processing by the pathologist: cryopreservation of one part of the material for immunofluorescence, fixation with paraformaldehyde (PFA) or formaldehyde (4%, buffered, pH 7.2–7.4) and, in parallel, fixation of a small part of the material for immunohistology, electron microscopy and additional investigations.

Another small portion (if available) will be used for fixation with glutaraldehyde for examination by electron microscopy (this is optional).

ii. The biopsy is put directly into a fixative (PFA or formaldehyde) and mailed to the pathologist. The pathologist will then embed the material in paraffin. This permits routine stains to be performed as well as immunohistology by the indirect method (APAAP or ABC) using the same material. A small piece of the formalin-fixed cylinder can also be worked-up for subsequent electron microscopy.

**What you should know about how the pathologist works-up the renal biopsy**

After embedding in paraffin, it is recommended to cut several serial sections, i.e. 8–16 paraffin sections (2–3 μm thick) per biopsy, which will then be used for the following light microscopic and immunohistochemical staining procedures [8].

**Light microscopy (Figure 1A–D)**

Routine stains (paraffin sections)

- **Haematoxylin and eosin (HE)**
- **Periodic acid–Schiff’s (PAS)**
- **Fibrous tissue stain (i.e. Sirius red, Trichrom, Ladewig, etc.)**
- **Silver stain**
- **Protein stain**
- **Optional stains**
  - **Kossa stain (calcifications)**
  - **Congo red stain (amyloid)**

It is useful to apply these different stains because the information obtained is complementary. For first hand information in routine diagnostic evaluation, the HE stain is appropriate (Figure 1A). It provides a first impression of the composition of the tissue (i.e. renal cortex vs medulla, number of glomeruli, cellular infiltration, etc.). To analyse the glomerulus, the PAS stain is most useful, since it delineates in great detail glomerular cells, mesangial matrix and potential expansion, as well as potential modifications of the composition of the matrix, changes of the GBM, i.e. thickening, irregularities, doubling, rupture and finally fibrinoid necrosis of the glomerular tuft (Figure 1B). A further advantage of the PAS stain is that alterations of the vessels, particularly arterial hyalinosis and fibrinoid necrosis, are easy to detect. Immune deposits can best be visualized by protein stains (Figure 1C) such as the acid fuchsin–Orange G stain (SFOG). Often immunodeposits can already be seen or suspected by light microscopy, but definite information requires specific stains or additional investigations, i.e. immunohistochemistry or electron microscopy.

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**Table 1. Fixation and subsequent processing of kidney biopsies**

<table>
<thead>
<tr>
<th>Light microscopy</th>
<th>Immuno-fluorescence</th>
<th>Immunohisto-chemistry</th>
<th>Electron microscopy</th>
</tr>
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<tbody>
<tr>
<td>PFA (30 min)</td>
<td>NaCl</td>
<td>PFA</td>
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<td>Routine processing</td>
<td>Cryosections</td>
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<tr>
<td>(4h)</td>
<td>(1 day)</td>
<td>(1 day)</td>
<td>(1 week)</td>
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</tbody>
</table>

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The main advantage of the silver stain (Figure 1D) is that it permits the detection of changes of the GBM, i.e. so-called reduplication or ‘spikes’ which develop as a result of overshooting glomerular production of basement membrane material encircling immunodeposits. In order to evaluate the extent of fibrosis in the glomerulus or tubular interstitium, fibrous tissue stains such as Trichrom, Sirius red or Ladewig stains are indispensable [9]. For specific questions, additional staining techniques are available, for instance the Congo red stain for visualization of amyloidosis, or the Kossa stain to detect calcification.

What are immunohistochemistry and immunofluorescence, and what information can be obtained from them?

In order to probe the tissue with antibodies, two techniques are available: immunofluorescence...
(Figure 1E) uses labelled antisera or antibodies (which require native tissue without fixation) and immunohistochemistry (which can be done with formalin-fixed tissue, whilst more aggressive fixatives destroy the epitopes and preclude immunohistochemical investigations) (Figure 1F).

The workhorses for immunofluorescence/immunohistochemistry are antisera or monoclonal antibodies against immunoglobulins (IgA, IgG and IgM) and components of the classical or alternative complement pathway (C1q, C3c and C4) as well as \( \kappa \)- and \( \lambda \)-light chains, albumin and fibrinogen, although for research purposes many others are available. The pathologist should not only report whether the reaction is positive, but should also comment on the pattern of staining, e.g. mesangial vs capillary staining pattern, linear (or pseudolinear) vs granular staining. If possible, he should also describe where the deposits are located, e.g. in a subendothelial, intramembranous or subepithelial position.

For specific questions, antibodies against amyloid subunits (AA or AL amyloid, transthyretin, etc.) or antibodies against viruses (cytomegalovirus, polyomavirus and adenosivirus) are available. In renal transplant biopsies, immunostaining for the C4d fragment of the complement pathway has become extremely popular. Although this is still somewhat controversial, it appears to be helpful in the diagnosis of acute humoral rejection [10,11].

When is electron microscopy necessary?

It is certainly not necessary to perform electron microscopy on every kidney biopsy but, because the result of the pathological investigation cannot be predicted, it is wise to preserve the material in such a fashion that ultimately electron microscopy is still possible. Electron microscopy requires particular fixation and handling of the material. It is therefore time-consuming and not universally available. If electron microscopy is envisaged at the time of a biopsy, e.g. for Alport’s disease or thin basement disease, a portion of the material should be fixed with glutaraldehyde. Alternatively, formalin-fixed tissue can be used subsequently for electron microscopy.

For some renal diseases, the definite diagnosis requires electron microscopy, such as Alport’s disease, thin basement disease, immunotactoid disease, minimal change nephropathy.

What information is provided by electron microscopy?

Electron microscopy permits assessment of the following:

- Changes in cell structure (i.e. podocyte foot process fusion or podocyte vacuolization)
- Necrosis or apoptosis of cells
- Changes of glomerular basement membrane (i.e. thickening, thinning, splicing, irregularities)
- Localization of immunoglobulin deposits (i.e. mesangial, subendothelial or subepithelial)
- In some renal diseases, such as lupus nephritis, specific morphological changes can be detected by electron microscopy, e.g. fingerprints or tubuloreticular structures.

How should the pathologist report the findings in a renal biopsy?

It is useful for communication between the clinician and the pathologist to adhere to a standard format in the report. In our opinion, the final report of a kidney biopsy should include information on:

- The adequacy of the specimen (number of glomeruli and arteries)—(although this will sometimes be painful for the clinician who performed the biopsy)
- A description of the morphological changes in a systematic fashion for each of the compartments of interest (glomeruli, tubules, interstitium, vessels, see Table 2)
- The results of immunofluorescence/immunohistochemical studies.
- The results of the electron microscopy (this is more time-consuming and will usually require a separate report later on)

Table 2. Systematic analysis of a kidney biopsy

| Glomeruli | Focal–diffuse Segmental–global Number and size Cellularity (which cells?) Deposits Mesangial matrix Fibrinoid necrosis Crescents |
|-----------|-------------------------------------------------|-------------------------------------------------|-----------------|-----------------|------------------------------|-----------------|------------------------------|
| Tubulointerstitium | Tubular atrophy and/or dilatation Necrosis of tubular epithelial cells Interstitial inflammation (mononuclear cells, granulocytes, eosinophils) Interstitial fibrosis Electron microscopy Additional features (calcification, giant cells, etc.) |
| Vessels | Wall thickening Hyalinosis Fibrinoid necrosis Inflammation of the vessel wall or the endothelium (endothelialitis) |

It is useful to give two different types of diagnoses: first, a descriptive diagnosis (e.g. mesangiproliferative glomerulonephritis) and then the final diagnosis (including the results of immunofluorescence/immunohistochemical, electron microscopic studies.
as well as clinical information), e.g. IgA glomerulonephritis.

It is hoped that adherence to such a systematic procedure will improve the results and heighten the impact of pathology on clinical decision making.

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Why is homocysteine elevated in renal failure and what can be expected from homocysteine-lowering?

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Patients with chronic kidney disease, especially end-stage renal disease (ESRD), exhibit many abnormalities in protein and amino acid metabolism. One of these alterations involves an increased plasma concentration of the sulphur-containing amino acid homocysteine. Hyperhomocysteinaemia has attracted a lot of attention in renal patients, not only because of its close relationship with renal function, but also because it has been implicated as an independent cardiovascular risk factor in these patients [1–3], although some recent studies have found no significant or even an inverse association between plasma homocysteine level and cardiovascular events and mortality in ESRD patients [4–6]. These discordant findings may have been caused by strong confounders which are associated with low homocysteine levels and increased mortality, such as protein energy malnutrition and/or inflammation [7].

Homocysteine and renal function

Plasma homocysteine is strongly correlated with (estimates of) glomerular filtration rate (GFR). Hyperhomocysteinaemia, defined as a plasma total homocysteine level of 12 μmol/l, occurs already at a GFR of about 60 ml/min and when ESRD has been