An introduction to renal biopsy
What should we do when we examine renal biopsies under microscope?

- Checking pathological findings
- Imagining what happened (and is going on)
- Comparing with clinical information
“Pathological findings”

≜ “Assessment of the extent of deviation from normal tissue”

Knowledge of normal tissue is needed for pathological diagnosis!
Outline

1. Anatomy of the kidney
2. Pathological examinations for renal biopsy
3. Basic format of renal biopsy report

Important for accurate diagnosis!
Outline

1. Anatomy of the kidney
2. Pathological examinations for renal biopsy
3. Basic format of renal biopsy report
*Retroperitoneal organ insulted by adipose tissue

*Extends from the 12th thoracic to the 3rd lumbar vertebra (The right is slightly lower)

*Weight
Male: 125-170g, Female: 115-155g

*Length
11-12cm long, 5-7cm wide, 2.5-3cm thick
Kidneys from autopsies
Sakai M. and Kawahara K: Structure, function and materials of the human body, V Kidney Urology, Tokyo, 2005
Sakai M. and Kawahara K: Structure, function and materials of the human body, V Kidney Urology, Tokyo, 2005
Hashiguchi A. In Kidney Biopsy-Atlas and Text, Tokyo Igakusha, Tokyo, 2010
Outline

1. Anatomy of the kidney
2. Pathological examinations for renal biopsy
3. Basic format of renal biopsy report
Pathological examinations for renal biopsy specimens

1. Light microscopy
   HE, PAS, PAPM, MT, EVG…

2. Immunofluorescence
   Immunoglobulins, complements …

3. Electron microscopy
   Details of changes in the basement membrane, cells, electron dense deposit…

4. Enzyme antibody technique
   Immunoglobulins, complements, virus…
Hematoxylin and Eosin staining (HE)

• Checking the entire section
  - Where is the lesion?

• Nuclei are stained dark blue with hematoxylin
  - Are cells abundant or scarce?

• Detailed assessment of the glomeruli is not possible
Periodic acid-Schiff staining (PAS)

- Basement membrane and PAS-positive materials (protein, glycogen...) are easier to detect
  - Indispensable for evaluation of “ptcbm score”
- Inter-facility differences in the staining method are small compared with PASM staining
(Almost) normal

Tubular atrophy

Thickening of the basement membrane
Which do you prefer?
Periodic acid silver-methenamin staining (PASM)

- The basement membrane is visualized clearly
- Good contrast between diffusive lesions or protein to basement membrane
  - Deposits are highlighted clearly
- Suitable for permanent storage without color fading
- Specimens are difficult to prepare, however, no other staining method is superior to PASM staining, while it is stained adequately
Masson-Trichrome staining (MT)

- Three colors: red, blue (or green) and orange
- Collagen fibers are stained blue (or green)
  - Evaluation of fibrosis
- Deposits are stained orange to red
(Almost) normal

Interstitial fibrosis

deposit

deposit

Interstitial fibrosis
Elastica van Gieson staining (EVG)

- Elastic fibers are stained black
- Evaluation of blood vessels
Immunofluorescence (IF)
- Fluorescent antibody technique

• Types and patterns of the immunoglobulins and complements may provide clues to the diagnosis
  - When IF examination is skipped, diagnosis is sometimes impossible

• Using frozen sections, it is easier to prepare and get higher sensitivity than enzyme antibody technique

• Formalin-fixed and paraffin-embedded sections are also available

• A fluorescence microscope is needed!
Membranous glomerulonephritis

IgG

IgG1

IgG2

IgG3

IgG4
IgA nephropathy

Formalin-fixed and paraffin-embedded section → IF

Gd-IgA1: Galactose-deficient IgA1 antibody
Electron microscopy (EM)

- Particularly for detailed evaluation of electron dense deposits, cells, basement membrane…

- Installation of an electron microscope is not simple
  - Requires a wide area ($\geq 3m^2$) and strong support for stability against vibrations

- High cost!
  - A digital camera is needed in addition to the electron microscope
Enzyme antibody technique

- Using formalin-fixed and paraffin-embedded sections, detect immunoglobulins, complements, elucidate tissue-specific and subcellular localization of an antigen within a sample

- Visualized via chromogenic substrates.

- If frozen sections are not obtained, it is possible to evaluate immunoglobulins and complements without a fluorescence microscope, however, it is not easy to prepare
BK virus infection

Intranuclear inclusion body

PML accompanied with transplant kidney
Hemorrhagic necrotic interstitial glomerulonephritis caused by adenovirus infection

Intranuclear inclusion body
Antibody-mediated rejection

C4d positivity on peritubular capillary
Outline

1. Anatomy of the kidney
2. Pathological examinations for renal biopsy
3. Basic format of renal biopsy report
Mayo Clinic/Renal Pathology Society Consensus Report on Pathologic Classification, Diagnosis, and Reporting of GN


**Table 2. Basic format of kidney biopsy report**

1. Specimen type: needle biopsy, wedge, etc.
2. Diagnosis
   - Primary diagnosis
     - Disease process/pathogenic type (e.g., IgA nephropathy, lupus GN, ANCA GN, C3 GN)
     - Pattern of glomerular injury (e.g., mesangial proliferative, membranoproliferative, necrotizing/crescentic, and focal and segmental sclerosing)
     - Histologic scores or grade (e.g., Oxford/MEST for IgA nephropathy and ISN/RPS for lupus nephritis)
     - Additional features (e.g., degree of global glomerulosclerosis, IFTA, vascular sclerosis, clinical modifiers, such as cryoglobulin/clinical HCV, bacterial endocarditis/clinical, staphylococcal cellulitis/clinical)
   - Secondary diagnoses (list; e.g., acute interstitial nephritis and diabetic glomerulosclerosis); these are not felt to be part of the primary disease
3. Comment/narrative
   - Can be used for summarizing/clarifying morphologic basis of primary and/or secondary diagnoses or clinicopathologic correlations, providing prognostic information, discussing differential diagnosis, and providing appropriate references
4. Summary of clinical data
5. Gross description
6. LM description
7. IF/IHC
8. EM
9. Addendum for special studies

MEST, mesangial hypercellularity, endocapillary hypercellularity, segmental sclerosis, interstitial fibrosis/tubular atrophy; ISN/RPS, International Society of Nephrology/Renal Pathology Society; EUVAS, European vasculitis study group; HCV, hepatitis C virus.
<table>
<thead>
<tr>
<th>Pathogenic Type</th>
<th>Specific Disease Entity</th>
<th>Pattern of Injury: Focal or Diffuse</th>
<th>Scores or Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune-complex GN</td>
<td>IgA nephropathy, IgA vasculitis, lupus nephritis, infection-related GN, fibrillary GN with polyclonal Ig deposits</td>
<td>Mesangial, endocapillary, exudative, membranoproliferative, necrotizing, crescentic, sclerosing, or multiple</td>
<td>Oxford/MEST scores for IgA nephropathy ISN/RPS class for lupus nephritis Focal, crescentic, mixed, or sclerosing class (Berden/EUVAS class)</td>
</tr>
<tr>
<td>Pauci-immune GN</td>
<td>MPO-ANCA GN, proteinase 3-ANCA GN, ANCA-negative GN</td>
<td>Necrotizing, crescentic, sclerosing, or multiple</td>
<td></td>
</tr>
<tr>
<td>Anti-GBM GN</td>
<td>Anti-GBM GN</td>
<td>Necrotizing, crescentic, sclerosing, or mixed</td>
<td></td>
</tr>
<tr>
<td>Monoclonal Ig GN</td>
<td>Monoclonal Ig deposition disease, proliferative GN with monoclonal Ig deposits, immunotactoid glomerulopathy, fibrillary GN with monoclonal Ig deposits</td>
<td>Mesangial, endocapillary, exudative, membranoproliferative, necrotizing, crescentic, sclerosing, or multiple</td>
<td></td>
</tr>
<tr>
<td>C3 glomerulopathy</td>
<td>C3 GN, dense deposit disease</td>
<td>Mesangial, endocapillary, exudative, membranoproliferative, necrotizing, crescentic, sclerosing, or multiple</td>
<td></td>
</tr>
</tbody>
</table>

MEST, mesangial hypercellularity, endocapillary hypercellularity, segmental sclerosis, interstitial fibrosis/tubular atrophy; ISN/RPS, International Society of Nephrology/Renal Pathology Society; EUVAS, European vasculitis study group.

*Some pathologists use the terms immune complex-mediated GN, monoclonal Ig-associated GN, etc. It is up to the discretion of the pathologist to use these terms.

*Multiple patterns include two or more patterns of injury. The patterns should be stated (e.g., focal mesangial proliferative, crescentic, and sclerosing or diffuse necrotizing, crescentic, and sclerosing).
<table>
<thead>
<tr>
<th>Glomerular lesions</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mesangial hypercellularity</td>
<td>&gt;3 Mesangial cells per mesangial area</td>
</tr>
<tr>
<td>Cellular crescent</td>
<td>Extracapillary cell proliferation of more than two cell layers with &gt;50% of the lesion occupied by cells</td>
</tr>
<tr>
<td>Fibrocellular crescent</td>
<td>An extracapillary lesion comprising cells and extracellular matrix, with &lt;50% cells and &lt;90% matrix</td>
</tr>
<tr>
<td>Fibrous crescent</td>
<td>Extracapillary crescents with &gt;90% matrix</td>
</tr>
<tr>
<td>Endocapillary hypercellularity</td>
<td>Hypercellularity caused by an increased no. of cells within glomerular capillary lumina, causing narrowing of the lumina</td>
</tr>
<tr>
<td>Fibrinoid necrosis</td>
<td>Disruption of the GBM with fibrin exudation</td>
</tr>
<tr>
<td>Sclerosis</td>
<td>Obliteration of the capillary lumen by increased extracellular matrix with or without hyalinosis or foam cells</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Patterns of GN</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimal mesangial GN*</td>
<td>Normal glomeruli by LM but mesangial immune deposits by IF</td>
</tr>
<tr>
<td>Mesangial proliferative GN*</td>
<td>Purely mesangial hypercellularity</td>
</tr>
<tr>
<td>Active (proliferative) GN*</td>
<td>Any or all of the following glomerular lesions: endocapillary hypercellularity, karyorrhexis, fibrinoid necrosis, rupture of GBMs, cellular or fibrocellular crescents, subendothelial deposits identifiable by LM, and intraluminal immune aggregates</td>
</tr>
<tr>
<td>Necrotizing GN</td>
<td>Segmental or global fibrinoid necrosis</td>
</tr>
<tr>
<td>Crescentic GN</td>
<td>≥50% Glomeruli with cellular, fibrocellular, or fibrous crescents (with percentage of crescents always noted in the diagnostic line, even when &lt;50%)</td>
</tr>
<tr>
<td>Membranoproliferative GN</td>
<td>Mesangial and/or endocapillary hypercellularity and thickening of capillary walls caused by subendothelial Ig and/or complement factors</td>
</tr>
<tr>
<td>Exudative GN</td>
<td>Neutrophils accounting for &gt;50% of glomerular hypercellularity</td>
</tr>
<tr>
<td>Sclerosing GN*</td>
<td>Any or all of the following glomerular lesions: glomerular sclerosis, fibrous adhesions, and fibrous crescents</td>
</tr>
</tbody>
</table>

ISN/RPS, International Society of Nephrology/Renal Pathology Society.

*Except for the first two patterns, multiple patterns can occur together in a single specimen (derived from the ISN/RPS lupus classification).

bThe term crescentic GN is used when crescents are present in at least 50% of glomeruli, and applies to immune-complex GN/C3 glomerulopathy. This does not apply to ANCA GN and anti-GBM GN, where less than 50% of the glomeruli may be involved by crescents.
Table 5. Guidelines for LM

<table>
<thead>
<tr>
<th>Glomeruli</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of glomeruli, including no. of globally and segmentally sclerosed and ischemic glomeruli</td>
</tr>
<tr>
<td>Focal versus diffuse and segmental versus global findings</td>
</tr>
<tr>
<td>Hypercellularity: mesangial, endocapillary, or exudative</td>
</tr>
<tr>
<td>Crescents: no./percentage, type (cellular, fibrocellular, or fibrous), and size (segmental or circumferential)</td>
</tr>
<tr>
<td>Fibrinoid necrosis and karyorrhexis</td>
</tr>
<tr>
<td>Wire loops, pseudo-(hyaline) microthrombi, and fibrin thrombi</td>
</tr>
<tr>
<td>Mesangial matrix expansion and presence of mesangiolysis</td>
</tr>
<tr>
<td>GBM thickening/thinning, double-contour formation, and other GBM abnormalities (e.g., spikes)</td>
</tr>
<tr>
<td>Disruption of GBM</td>
</tr>
<tr>
<td>Disruption of Bowman’s capsule</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tubules and interstitium</th>
</tr>
</thead>
<tbody>
<tr>
<td>Interstitial inflammation: type of infiltrate and location</td>
</tr>
<tr>
<td>Casts, crystals, and cysts</td>
</tr>
<tr>
<td>Acute tubular injury</td>
</tr>
<tr>
<td>Tubular basement abnormalities</td>
</tr>
<tr>
<td>IFTA: absent, mild, moderate, or severe</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Vessels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arteritis, emboli, and thrombosis</td>
</tr>
<tr>
<td>Arteriosclerosis and arteriolosclerosis: absent, mild, moderate, or severe</td>
</tr>
<tr>
<td>No. of glomeruli, including no. of globally sclerosed glomeruli or with other evident lesions</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Intensity of staining: negative, ±, 1+, 2+, and 3+</td>
</tr>
<tr>
<td>Staining pattern: granular, linear, semilinear, smudgy, and linear accentuation</td>
</tr>
<tr>
<td>Location: focal or diffuse; segmental or global; and mesangial, glomerular capillary wall, or both</td>
</tr>
<tr>
<td>Interstitial and tubular basement membrane staining: if present</td>
</tr>
<tr>
<td>Segmental trapping of C3 and IgM is common in areas of segmental sclerosis or scarring: segmental glomerular tuft or coarse segmental staining</td>
</tr>
<tr>
<td>Internal controls: albumin along TBM and GBM, C3 in vessels, and polyclonal IgA casts in tubules</td>
</tr>
</tbody>
</table>

TBM, tubular basement membrane.
| No. of glomeruli studied by EM, including no. globally sclerosed or with other evident lesions |
| Glomerular deposits: location, type, quantity, size, and substructure |
| GBM: architecture, thin/thick, duplication, ischemic changes, and rupture |
| Endothelium: fenestrations, swelling, and presence of tubuloreticular inclusions |
| Mesangial matrix: normal/increased and mesangiolysis |
| Mesangial cellularity: normal/increased |
| Podocytes: preserved or effaced (%), protein reabsorption granules, and microvillus change |
| Leukocytes/platelets/fibrin in capillary lumen/Bowman’s space |
| Tubular epithelial and basement membrane abnormalities when present |
Table 3. Some examples of GN diagnoses

(1) IgA nephropathy
   Primary diagnosis: IgA nephropathy
   Pattern of injury: diffuse mesangial and focal segmental endocapillary proliferative and
   sclerosing GN
   Score/grade: Oxford classification: M1 E1 S1 T1
   Additional features: focal global glomerulosclerosis (20%), moderate tubular atrophy and
   interstitial fibrosis (30%), mild arteriosclerosis and hyaline arteriolosclerosis
   Secondary diagnoses: diabetic nephropathy, mild

(2) Lupus nephritis
   Primary diagnosis: (1) lupus nephritis and (2) thrombotic microangiopathy
   Pattern of injury: diffuse proliferative and sclerosing GN with focal (10%) cellular crescents
   Score/grade: ISN/RPS class IV-G (A/C)
   Additional features: thrombotic microangiopathy associated with antiphospholipid
   antibodies/clinical, focal global glomerulosclerosis (10%), mild tubular atrophy and
   interstitial fibrosis (10%), moderate arteriosclerosis, and moderate hyaline arteriolosclerosis

(3) Hepatitis C–associated immune–complex GN
   Primary diagnosis: immune-complex GN
   Pattern of injury: membranoproliferative GN
   Additional features: with features of cryoglobulinemic GN (hepatitis C/clinical), focal
   global glomerulosclerosis (20%), moderate tubular atrophy and interstitial fibrosis (30%),
   moderate arteriosclerosis, and moderate hyaline arteriolosclerosis

(4) Infection-related GN
   Primary diagnosis: IgA–dominant infection–related GN
   Pattern of injury: diffuse exudative GN
   Additional features: associated with S. aureus cellulitis infection/clinical, focal global
   glomerulosclerosis (30%), moderate tubular atrophy and interstitial fibrosis (30%), moderate
   arteriosclerosis, and moderate hyaline arteriolosclerosis
   Secondary diagnoses: diabetic nephropathy, moderate
(5) ANCA GN

Primary diagnosis: proteinase 3-ANCA GN
Pattern of injury: necrotizing and crescentic GN
Prognostic class: focal (≥50% normal glomeruli)
Additional features: clinicopathologic features of granulomatosis with polyangiitis (proteinase
3 and cytoplasmic ANCA/clinical), focal global glomerulosclerosis (10%), mild tubular
atrophy and interstitial fibrosis (10%), mild arteriosclerosis, and moderate hyaline
arteriolosclerosis

(6) Anti-GBM GN

Primary diagnosis: anti-GBM GN
Pattern of injury: necrotizing and crescentic GN, severe
Additional features: clinicopathologic features of Goodpasture syndrome (anti-GBM
antibody/clinical), focal global glomerulosclerosis (40%), moderate tubular atrophy and
interstitial fibrosis (40%), mild arteriosclerosis, and moderate hyaline arteriolosclerosis

(7) Monoclonal Ig GN

Primary diagnosis: monoclonal Ig GN
Pattern of injury: membranoproliferative GN with intracapillary hyaline thrombi
(pseudothrombi)
Additional features: IgM κ-staining of glomerular intracapillary deposits consistent with type 1
cryoglobulins (Waldenström macroglobulinemia/type 1 cryoglobulins/clinical), focal global
glomerulosclerosis (30%), moderate tubular atrophy and interstitial fibrosis (40%), moderate
arteriosclerosis, and moderate hyaline arteriolosclerosis

(8) C3 glomerulopathy

Primary diagnosis: C3 GN
Pattern of injury: membranoproliferative GN
Additional features: focal global glomerulosclerosis (20%), mild tubular atrophy and interstitial
fibrosis (20%), mild arteriosclerosis, and moderate hyaline arteriolosclerosis

ISN/RPS, International Society of Nephrology/Renal Pathology Society; A/C, active/chronic.
*If MPO/PR3 titers are not known, it is acceptable to label as ANCA GN.
Standardized classification and reporting of glomerulonephritis

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FIGURE 1: Overview of standardized classification and reporting of GN.