Key Clinical Diagnostic Points:
- **Glomerulus** represents a barrier and functions to filter plasma
  - Components include
    - Fenestrated endothelium of glomerular capillary
    - Glomerular basement membrane
    - Podocytes containing negatively-charged slit diaphragms
    - Filtration is limited to molecules that are >68,000 Daltons
  - Function
    - Size and charge determine the “filterability” of a substance from plasma into Bowman’s space
    - Size limit is 68,000 to 70,000 Daltons
      - Albumin is 65,000, but its negative charge precludes filtration
    - From Bowman’s space, the filtrate continues through the tubules
    - For solutes to pass freely through the glomerulus, filtration of a solute is a function of GFR and plasma concentration of the solute:
      - \[ \text{GFR} = K_f \times \text{net filtration pressure} \]
      - \( K_f \) is the permeability coefficient, which is a function of surface area and permeability
      - Net filtration pressure is the sum of Starling’s forces (hydrostatic and oncotic) between plasma and Bowman’s space
        - Hydrostatic pressure in glomerular capillaries has greatest influence on GFR – it is about 60 mmHg
        - Net filtration pressure is typically about 10 mmHg

Figure. Schematic of glomerulus and schematic of the filtration barrier.
• Diagnosis
  • Finding of proteinuria should be interpreted in light of other findings on urinalysis
    • Always examine urine sediment to rule-out inflammation, infection, or hemorrhage, which is associated with proteinuria
    • Proteinuria with an inactive sediment may indicate glomerular disease
• Qualitative methods
  • Dipstick pad
    ▪ Part of most (perhaps all) urine dipsticks
    ▪ Colorimetric method
    ▪ Amino groups of proteins bind to an indicator in filter paper producing a color change
    ▪ Change is graded subjectively to a standard
    ▪ Most sensitive to presence of albumin
    ▪ Range: 30-3,000 mg/dl
      • Graded as negative, trace, and 1+ to 4+ depending on intensity of color change
    ▪ Recent studies suggest this analytical pad is not very good – many false positives and false negatives – and additional testing for proteinuria should be performed when there is a concern
      • False positives
        ▪ Alkaline urine pH (> 7.5)
        ▪ Contamination of urine with quaternary ammonia compounds (eg some cleaners and disinfectants)
        ▪ Prolonged contact with urine
        ▪ Any pigment in urine may absorb into the pad
      • False negatives
        ▪ Very dilute urine
        ▪ Very acidic urine
        ▪ Presence of some abnormal proteins (eg Bence Jones proteins (myeloma proteins))
  • Sulfosalicylic acid
    ▪ More accurate than dipstick
    ▪ Can be used to verify proteinuria
    ▪ 3-5% sulfosalicylic acid solution is mixed with an equal volume of urine
    ▪ Turbidity that results from acid precipitation of protein is evaluated
    ▪ Good for albumin and Bence Jones proteins
    ▪ Range: 5-5,000 mg/dl
    ▪ False positive
      ▪ Radiocontrast agents
      ▪ Certain drugs (eg penicillin, cephalothin, sulfonamide, thymol)
    ▪ False negative
      ▪ Very alkaline urine
      ▪ Very dilute urine
• Quantitative methods
  • Microalbuminuria
    • Recently, an “early diagnosis of renal disease” (ERD) test has become available
      ▪ Measures micro-albuminuria – range of 1 to 30 mg/dl
      ▪ Less than detectable by dipstick
      ▪ May be useful in detecting early renal disease
        ▪ 19% of healthy dogs have micro-albuminuria
        ▪ 36% of dogs seeking veterinary care have micro-albuminuria
        ▪ True with congenital or induced glomerular disease
        ▪ No data (yet) concerning spontaneously occurring non-glomerular renal disease
• Used in human beings for detection of early renal disease due to diabetes mellitus and hypertension (small capillary (glomerular) damage)
• Despite inherent issues, there are indications for determining microalbuminuria including
  • Not overtly proteinuric, but clinical disease likely to be associated with proteinuria
  • Not overtly proteinuric, middle-aged or older
  • When conventional tests for proteinuria are equivocal or conflict
  • Dogs and cats known to be at risk for developing a glomerulopathy
• Verification of significant proteinuria
  • Evaluate urine dipstick in light of urine sediment examination (eg “clean” or “dirty” sediment)
    • As little as 10% whole blood (volume/volume) can result in a positive dipstick reaction
    • Inflammation can result in proteinuria even without hematuria
      • If proteinuria is present with a “quiet” sediment and in a dilute urine, consider doing a urine culture
      • A urinary tract infection can result in very large amounts of protein in urine due to exudation
  • Also, evaluate in light of urine specific gravity and urine pH
    • A “trace” amount of protein in a concentrated urine is probably less significant or even an artifact than if it occurs in very dilute urine
    • Likewise, a “trace” amount of protein in a very alkaline urine pH could be an artifact due to the alkalinity of the sample
  • If proteinuria is present with a “clean” sediment and a bacterial urinary tract infection has been ruled-out, then the degree of proteinuria should be verified and quantitated
    • **Urine protein-to-urine creatinine ratio (UPC)**
      • A spot urine sample can be collected by any method (as long as hemorrhage is not induced)
      • Creatinine concentration (mg/dl) and protein concentration (mg/dl) is determined
      • The result is a unit-less number
        • Normal UPC in dogs is <0.5:1.0 and cats < 0.4:1.0
        • Suspect UPC is 0.4/0.5:1.0 to 1.0:1.0
        • Significant proteinuria occurs when UP:UC is > 1.0:1.0
  • With CKD
    • Relative risk of mortality is 3 times higher when UPC > 1
    • Risk of adverse outcome increased by 1.5-fold for every 1 unit increment of UPC above 1
• How is proteinuria investigated?
What is the clinical significance of renal proteinuria?
• Proteinuria ≠ renal proteinuria
  • Pre-renal
    • Physiologic proteinuria (exercise, stress, fever, seizures, venous congestion, etc)
    • Overload proteinuria (hyperproteinemia, myoglobinemia, and hemoglobinemia)
  • Post-renal – Most common cause
    • Inflammation
    • Infection
    • Hemorrhage
• When renal proteinuria = renal disease
  • Will the kidney disease lead to morbidity or mortality
  • Is the kidney disease a sign of some underlying condition
  • Is therapy indicated to prevent additional renal or systemic injury
  • Types
    • Glomerular
    • Tubular
    • Interstitial

Renal biopsy
• Indications
  • Renal biopsy is most useful with
    • Nephrotic syndrome/glomerular disease
    • Mass lesions/neoplasia
    • Acute renal failure (for diagnosis and prognosis)
    • Patients with proteinuria
    • Cats with feline infectious peritonitis (diagnosis)
    • Suspected familial or congenital renal disease
    • Perinephric cysts (fine needle aspiration only)
  • Investigation
  • Renal biopsy may be useful with
    • Infectious renal disease (fine needle aspiration of tissue or pelvic urine)
    • Culture of pelvic urine
    • Slowly progressive tubulointerstitial disease
    • Patients with undiagnosed renal hematuria
  • Renal biopsy is not helpful or should not be performed with
    • Chronic renal failure (unless associated with neoplasia)
    • Polycystic kidney disease
• Techniques – understand general information
• Fine needle aspiration
  • Advantages
    • Fine needle aspiration is good for collection of fluid, urine, or small amounts of cells
    • It is easy and relatively safe
    • It is minimally invasive
  • Disadvantages
    • Collection of small amount of tissue
    • Seeding of neoplastic cells or infectious agents along needle tract
    • Leakage of fluid from cystic structure
    • Damage to renal vasculature or other abdominal organs
  • Technique
    • 22-G, 1 ½ inch needle or spinal needle is used
• Needle attached to syringe (size dependent on sample to be collected) – usually a 6-ml syringe, but larger size may be used to collect a large volume of urine
• Clip and aseptically prepare the abdominal wall at site of insertion (ventral abdomen or pericostal space)
• Needle is inserted into area of interest in kidney by palpation or by ultrasound-guidance
• Using ultrasound or fluoroscopy, the needle can be inserted into the renal pelvis for aspiration of urine
• Cellular material is injected onto glass microscopic slides for cytologic examination; fluid is transferred to glass microscopic slides, injected into sterile tubes, or transferred to culturettes

• Percutaneous biopsy
  • Allows for rapid, minimally invasive collection of larger quantity of renal tissue
  • But sample size is smaller than that obtained with wedge biopsy and direct control of hemorrhage is not possible
  • Biopsy is obtained using a Tru-Cut type of instrument
    • Inner rod has a small specimen notch
    • Outer cover has an angled sharp edge used to cut the core of tissue from surrounding tissue
  • Technique
    • Biopsy instrument is inserted by palpation, ultrasonographic guidance, through a “key-hole” approach, or at laparotomy into the kidney; it should be inserted into the cortex only as damage to the arcuate artery will result in substantial loss of renal tissue
    • Biopsy instrument is inserted so that it is in contact with renal capsule or so that it is inserted a small way into the renal parenchyma
    • The inner rod containing the specimen notch is rapidly inserted into the renal tissue so that the specimen notch is allowed to fill with tissue
    • The outer cutting sheath is rapidly advanced in order to cut the tissue contained in the specimen notch from the surrounding parenchyma
    • Biopsy instrument is removed
  • NOTE: This technique describes use of a Tru-Cut or Tru-Cut-like biopsy instrument. There are many different types of biopsy instruments available and the required technique may be different from this description

• Pre-biopsy considerations
  • Good physical examination and determination of metabolic status is important
  • Do not perform renal biopsy in patients with a coagulopathy
  • Do not perform renal biopsy in hypertensive patients

• Post-biopsy considerations
  • After biopsy, allow site to clot
  • Hemorrhage is the most common complication of renal biopsy
    • Microscopic hematuria occurs in almost all cases and lasts for up to 3 days
    • Gross hematuria occurs in approximately 3% of cases
    • Approximately 4% of cases develop hydronephrosis due to clot formation and ureteral/pelvic obstruction
    • Post-biopsy fluid administration helps to decrease risk of clot formation
  • Antibiotics are not usually necessary following renal biopsy

• Complications
  • Because biopsy disrupts blood flow distal to site, infarction of renal tissue supplied by the damaged artery occurs
  • Clot formation and hydronephrosis may occur

• Surgical biopsy
  • Renal biopsy may be obtained using a biopsy instrument or by wedge biopsy at laparotomy
  • In general, it is best to collect at least 2 cortical cores if each is > 10 mm long. When the cores are shorter than 10 mm each, 3 cores usually are required. Needle biopsy cores do not need to be cut
into smaller pieces except as needed to subdivide them appropriately separate evaluations (figure 23.4); however, a portion of a wedge biopsy specimen must be carefully cut into pieces that are no greater than 1-2 mm in any dimension before placement in the fixative (e.g., 3% glutaraldehyde in phosphate buffer) for electron microscopy.

- **When performing a renal biopsy, the core of tissue is divided for histopathology (light microscopy = LM), immunofluorescence (IF), and electron microscopy (EM)**

- **Conservative approach**
  - Serially monitor urinalysis, UPC, and renal function
  - Patients with stable or improving mild proteinuria (UPC < 2)
  - If severe or progressive proteinuria – investigate further
    - Identify and treat inciting disorder
    - Limit proteinuria
      - Limits albumin loss and consequences of hypoalbuminemia
    - Renoprotective
      - Proteinuria is nephrotoxic
      - Activates fibrosis and inflammatory pathways

**General clinical signs of glomerular disease**
- Vary with severity of disease and underlying cause, if any
- Azotemia may or may not be present and is unassociated with the degree of proteinuria and hypoalbuminemia
- Mild to moderate proteinuria results in serum albumin concentrations >1.5 g/dl, but < 2.5-3.0 g/dl
  - At this level, clinical signs often include polyuria, weight loss, and lethargy
  - With severe or heavy proteinuria, serum albumin is < 1.5 g/dl, and clinical signs are more severe
- In addition to aforementioned signs
  - Muscle wasting
  - Edema/ascites
Nephrotic syndrome
- Occurs with severe proteinuria and is characterized by proteinuria, marked hypoalbuminemia, hypercholesterolemia, hyperlipidemia, and edema

Therapy
- Treatment is often frustrating and biologic course is variable
- Goals of therapy are similar to those for CKD with additional goal of increasing serum albumin concentration and minimizing likelihood of nephrotic syndrome
- Treatment includes:

Treat the underlying cause, if it can be identified
- Two major glomerulopathies
  - Glomerulonephritis
    - Glomerulonephritis (GN) is better termed glomerulopathy
      - “-itis” implies inflammation, which typically occurs, but is not always present depending on cause of the glomerular disease (eg congenital renal disease, glomerulosclerosis)
    - Many causes that have been described, primarily in dogs:

<table>
<thead>
<tr>
<th>Familial</th>
<th>Neoplastic</th>
<th>Infectious</th>
<th>Inflammatory</th>
<th>Miscellaneous</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doberman pinscher, Samoyeds (X-linked dominant), Bull terrier (autosomal dominant), soft-coated Wheaton Terrier, Greyhound, Burmese mountain dog, Rottweiler, English Cocker spaniel (autosomal dominant), Norwegian Elkhound, Brittany spaniel (autosomal dominant), Deficiency of C3 results in recurrent bacterial urinary tract infections and membranoproliferative GN</td>
<td>Lymphosarcoma, mastocytosis, hemangiosarcoma, adenocarcinoma</td>
<td>Bacterial endocarditis, infectious canine hepatitis, brucellosis, dirofilariasis, ehrlichiosis, systemic fungal or bacterial infection, feline infectious peritonitis, feline leukemia virus</td>
<td>Systemic lupus erythematosus, chronic pancreatitis, chronic pyoderma, chronic otitis externa, polyarthritis</td>
<td>Hyperadrenocorticism, diabetes mellitus, chronic glucocorticoid treatment, systemic arterial hypertension, idiopathic</td>
</tr>
</tbody>
</table>

- Any process resulting in antigenic stimulation may result in GN
- In most cases, underlying cause(s) is/are not identified; therefore, most are classified as idiopathic
- Glomerulopathies may be immune-mediated or non-immune-mediated:
- Immune-mediated GN:
  - Accounts for @ 48% of renal proteinuria in dogs; amount in cats...?
  - Etiopathogenesis is related to presence of immune complexes in glomerular capillary walls
  - Histologically, GN can be classified as:
    - Proliferative:
      - Mesangial and epithelial cell proliferation and infiltration (primarily neutrophils)
      - Cellular proliferation compresses glomerular capillaries resulting in decreased blood flow and GFR
    - Membranous:
      - Thickening of basement membrane due to subepithelial deposition of immune complexes
      - Membranoproliferative:
        - Combination of membranous and proliferative GN
    - Immunofluorescence can be used to document the immune-mediated nature; however, few labs do this technique and even fewer do it well
• Prognosis is thought to be related to histologic type with membranous having a better prognosis than proliferative or membranoproliferative.

• Non-immune-mediated:
  • Glomerular disease may occur due to developmental abnormalities in glomerular structure and function
  • Glomerular disease (sclerosis characterized by glomerular thickening and mesangial expansion) has been reported to occur with:
    • Glucocorticoid excess (endogenous or exogenous)
    • Systemic arterial hypertension
    • Diabetes mellitus
    • Renal failure
  • Immunofluorescence studies would be negative

• Amyloidosis
  • Amyloid is a beta-pleated sheet of serum amyloid A protein
  • Deposits in and around glomerulus, usually beginning in the tubulointerstitial area
  • Amyloidosis is uniformly progressive in nature and animals invariably develop chronic renal failure
  • It may occur:
    • Primary familial disease
      • Primarily described in Shar pei dogs Abyssinian cats
      • In these animals, amyloid may be deposited primarily in the medulla and not glomerulus
      • Proteinuria, therefore, may not be present
      • Amyloidosis develops typically in animals under 5-6 years of age
      • Shar pei dogs with amyloidosis often develop an arthropathy associated with fever and joint pain (called Shar pei fever, Shar pei swollen hock syndrome, Mediterranean Fever)
      • Has also been described in Siamese cats, Oriental shorthair cats, Walker hound dogs, Beagle dogs, and Collies
    • Secondary to chronic inflammatory diseases (eg infections, inflammatory organ disease, or cancer)
  • Clinical signs of amyloidosis include:
    • Proteinuria
    • Edema (depending on degree of proteinuria and hypoalbuminemia)
    • Chronic renal failure
    • Systemic arterial hypertension (and its related clinical signs – see Chronic Renal Failure lectures)
    • Inappetence and weight loss
    • Fever (depending on cause)
    • Arthropathy (depending on cause) – especially in Shar Pei breed
    • Other signs related to inciting cause of secondary amyloidosis
  • Diagnosis:
    • Differentiated from other glomerulopathies by renal biopsy
    • On light microscopy, amyloid appears as an eosinophilic substance in the mesangium and/or interstitium
    • Congo red stain using polarized light confirms the presence of amyloid (Congo red gives an “apple green” color when viewed under polarized light)
    • Amyloid may occur outside of the glomeruli particularly in the medulla in cats; therefore, it may be missed with a renal cortical biopsy

Feed a protein-restricted diet. Studies have shown that dietary protein restriction decreases the degree of proteinuria and increases serum creatinine concentration. Supplementing dietary protein actually makes the situation worse.

Decrease sodium intake. This can usually be accomplished by feeding a low protein, renal failure diet. Dietary salt restriction aids in decreasing fluid retention.
Administer an angiotensin-converting enzyme inhibitor

Enalapril is the only ACE inhibitor that has been evaluated in dogs with proteinuria although other ACE inhibitors have been evaluated in dogs with induced diabetes mellitus (lisinopril) and in human beings (captopril, ramipril, etc). Benazepril has been shown to reduce proteinuria in cats. Benazepril is excreted more through biliary system than urinary system (although it is renally excreted as well) when compared with enalapril; therefore, it may be safer to use in animals with renal azotemia. Enalapril has been shown in a controlled study to decrease proteinuria, increase serum albumin concentration, and prolong survival in dogs with GN

**Enalapril in dogs or benazepril may be tried**

There is a potential to worsen azotemia if present; therefore, start with a lower dose in azotemic animals and monitor BUN and creatinine

Indicated when UPC is > 1-2 in IRIS stage 1 or > 0.4 (cats) and 0.5 (dogs) in IRIS stage 2-4

Administer anti-inflammatory drugs to decrease platelet aggregation

Platelet activation and intraglomerular thrombosis occurs with glomerular disease

**Aspirin is usually administered at 0.25-0.5 mg/kg PO q12-24hr in dogs or ½-1 baby aspirin q3d in cats.** Efficacy is not proven in dogs, but it is in human beings.

Consider omega-3 fatty acids

Theoretically, diets containing higher levels of omega-3 fatty acids may decrease inflammation. The omega-3 fatty acid becomes incorporated into plasma lipid membranes instead of arachidonic acid. The prostaglandins, thromboxanes, and leukotrienes produced from metabolism of omega-3 fatty acids tend to promote less inflammation and coagulation.

In a chronic CKD model, dogs consuming a diet with an omega-6-to-omega-3 fatty acid ratio of 5:1 maintained GFR longer, survived longer, and had less inflammatory prostaglandin excretion than when dogs consumed diets containing higher levels of omega-6 fatty acids. There is no data in dogs with proteinuria. Furthermore, cats with chronic renal failure do not appear to respond to omega-3 fatty acid supplementation. Most renal failure diets contain an omega-6-to-omega-3 fatty acid ratio of 5:1. Supplementation of omega-3 fatty acid should be done to achieve a ratio of omega-6-to-omega-3 fatty acids of somewhere between 1:1 to 5:1. Therefore, the amount of omega-3 fatty acid supplementation must be done based on the fat content of the diet and type of fat in the diet

Omega-3 fatty acids can be supplemented with diet with a starting dose of 300 mg of EPA + DHA per 10 lbs per day. Remember, EPA is the 20-carbon long-chain fatty acid and DHA is the 22-carbon long-chain fatty acid.

Consider immunosuppressive drugs

Administration of immunosuppressive drugs to dogs with proteinuria is controversial. None have been shown to be effective in controlled studies, although there are sporadic case reports of response. However, biopsies show that 48.7% of glomerular disease in dogs have an immune-mediated basis

In human beings, glucocorticoids are often administered. Studies in dogs have shown that in most cases of proteinuria, glucocorticoid administration is not beneficial and is often associated with a worsening of the proteinuria. Glucocorticoids appear to promote glomerulosclerosis and intraglomerular hypertension. Therefore, glucocorticoids are not recommended unless the proteinuria is secondary to glucocorticoid-responsive systemic disease.

Cyclosporine was not found to be effective in dogs with idiopathic GN in a controlled, blinded study. Therefore, it cannot be recommended at this time.

Other immunosuppressive drugs that may show benefit, but that have not been evaluated in placebo-controlled, blinded studies are azathioprine, cyclophosphamide, and chlorambucil. The most promising are mycophenolate and azathioprine + chlorambucil
Most immunosuppressive drugs are also cytotoxic; therefore, their administration may be associated with worsening azotemia. The decision to use immunosuppressive therapy should be based on the likelihood of an immune-mediated cause of proteinuria, the patient’s overall condition, and the ability to monitor the patient.

Consider diuretics to decrease sodium retention and edema/ascites
In human beings with nephrotic syndrome, diuretics are often used to decrease ascites/edema. Commonly a combination of a loop diuretic (such as furosemide) and a thiazide diuretic (such as chlorothiazide) are used. These diuretics promote natriuresis thereby decreasing sodium and fluid retention.

Furosemide is often used in veterinary medicine to decrease fluid retention and should be considered in dogs or cats that have nephrotic syndrome. Combination diuretic therapy may be considered in animals that are refractory to single agent therapy.

Treatment targets
- Ideal goal: reduce UPC to < 0.5 in dogs and 0.4 in cats
- Realistic goal: reduce UPC by at least 50%

Additional therapies
- If goal is not achieved:
  - Increase dosage of ACE-I and monitor
  - Angiotensin receptor blocking (ARB) agent
    - Some ATII escapes ACE inhibition
    - ARB block ATII interaction at receptor
    - Works synergistically when combined with an ACE-I as it blocks ATII that escapes ACE-I inhibition at receptor
    - Same tendency for complications as with ACE-I
  - Immunosuppression, if not done
  - Doxycycline
    - Loose information of decreasing proteinuria in humans
    - Metalloproteinase inhibitor – anti-inflammatory
    - Used with tick-borne disease-associated glomerular disease

Prognosis
- Generally poor
- Most patients dead within 1-2 months of diagnosis
- However, can be stable for long time and may resolve with therapy