TO THE POINT CLASS LECTURES

CLINICAL PATHOLOGY (PATH 404) PART V

Top Stories

- **Transudate and Exudate**
- **Bone Marrow Examination**
- **Total Leukocyte Count (TLC)**
- **Differential Leukocyte Count (DLC)**
- **Kidney Function Tests**
- **Urinalysis**
  - Physical Examination (*Continued…*)

Delivered by:

Dr. Farzana Rizvi
Dr. Ayesha Khatoon

Department of Veterinary Pathology
Faculty of Veterinary Science
University of Agriculture
Faisalabad

Presented by:

MUHAMMAD SAJJAD HUSSAIN

Up to date: 13 Dec 2010, Monday

Transudate and Exudate

Transudate:

It is non-inflammatory fluid.
It can be seen in the following conditions:

i) Hypoproteinemia	ii) Venous stasis	iii) Congestive heart failure
iv) Parasitism	v) Chronic liver infection	vi) Hydroperitoneum
vii) Hydropericardium	viii) Hydrothorax	

Exudate

It is inflammatory in origin and may be due to bacterial, viral, parasitic or fungal infection.
Nature of the fluid varies. It may be:
i) Serous ii) Purulent iii) Fibrinous iv) Hemorrhagic

Collection of Fluid

- Take a syringe and aspirate 10 ml of fluid from any cavity in aseptic conditions.
- Take one part of this fluid for determination of specific gravity, one part for smear preparation (cytological examination) and one part for microbiological examination.
- If fluid is watery, then centrifuge it and go for cytological examination.
- If fluid is concentrated, then take sediment after centrifugation and go for cytological examination.

Physical Examination

i) **Color of the fluid**:
Light to dark yellow -------- RBCs, red to reddish brown coloured plasma / serum in fluid.
White to creamy color and thick consistency ---------- WBCs are present in the fluid.
Yellow, orange or red ---------- Bilirubin is present in the fluid.

ii) **Determination of turbidity**:
If red turbidity ---------- RBCs present ----- on centrifugation, red button formed at the bottom of the test tube.
Light yellow turbidity ---------- is due to presence of WBCs.
Colourless supernatant when placed in the refrigerator ---- Fat and adispose tissue present.
[Fat droplets can be seen under microscope after staining tissue slide with Sudan 3 or 4]

iii) **Coagulation test**:
Trasudate rarely clot or partially clot, but on the other hand, Exudate clot quickly, that's why you must add anticoagulant in it.
If fluid is purulent or contaminated with bacteria, then it may partially clot.

iv) **Odour of fluid**:
Normally fluid is odourless. In putrefaction, it may give fat-rancid smell.

v) **Specific gravity**:
Instrument used for determination of specific gravity: Refractometer
Transudate: refractometer value is less than 1.017
Exudate: refractometer value is more than 1.017

Chemical Examination

i) **Determination of protein concentration**:
It is also determined by refractometer.
Transudate: less than 3 g/dL
Exudate: more than 3 g/dL

ii) **Determination of fibrinogen**:
Fibrinogen clots at 56 C. Take the fluid sample and determined its protein concentration. Then place it at 56 C for 10 minutes in a bath. Again determine the protein concentration.

iii) **Determination of urea nitrogen**:
It is usually performed for a fluid collected from abdominal cavity. This fluid may be due to rupture of urinary bladder or as a result of ascitis, but the urea nitrogen concentration confirms the origin of the fluid.
You may perform glucose and bilirubin tests by kits available in the market.

iv) **Benedict test**:
It confirms the presence of erytrocytes in the fluid.

Cytological Examination

Take the fluid and dilute it in normal saline. Count total nucleated cells by using hemocytometer. Determine total cell count and conclude as:
Transudate: < 100 cells /dL
Exudate: > 50,000 cells /dL
Prepare the slide and stain it with Wright & Geimsa stain and observe under microscope.

Differential Cell Count:

Purulent exudate:	Predominant cells are neutrophils, macrophages and mesothelial cells.
Chronic exudate:	Lymphocytes
Parasitic exudate:	Eosinophils
Transudate:	Endothelial cells (predominant) but few RBCs & lymphocytes may be.
Tumor growth:	Unidentified cells with giant nucleus.

Microbiological Examination

Collect the fluid in aseptic conditions. Prepare the slide and stain with methylene blue and Periodic Acid Schiff (PAS). Observe it under microscope for cocci, bacilli (present, in pairs, colonies, chains or singly).

Bone Marrow Examination

Bone marrow:
Bone marrow is the flexible tissue found in the interior of bones.

Types of bone marrow:

It is of two types:

i) **Red Marrow**:
It consisting mainly of hematopoietic tissue which is responsible for production of erythrocytes, platelets, granulocytes, agranulocytes.
ii) **Yellow Marrow**:

It consisting mainly of fat cells, fibroblasts, osteoblasts, osteoclasts, macrophages and endothelial cells.

Bone marrow present in the flat bones, cranial bones, pelvis, ribs, short bones esp. vertebrae and at the ends of the long bones.

Aspiration Sites

In dogs, cats and lab animals;

i) Iliac Crest ii) Proximal end of Femur (through trochanteric fossa)

In horse, cattle, sheep and goat:

i) Tuber coxae ii) Ribs iii) Vertebral spine iv) Iliac Crest
iv) Sternum (not preferable)

Needles for Aspiration:

In dogs, cats:

18 gauge ---------- Length: ¾ inch – 1 ½ inch

In horse, cattle:

16-18 gauge ----- Length: 1 ½ inch – 2 ½ inch

Examination of Bone Marrow Smears

Bone marrow smears can be observed for:

i) Total cell count:
Its not preferable as if bone marrow is diluted with blood then its value may greatly differ.
ii) Degree of cellularity in bone marrow:
It may be normal, increased or decreased.
iii) Differential leukocytic count (DLC):
iv) Myeloid-erythroid ratio (ME ratio):

$$= \frac{\text{No. of cells of myeloid series in the bone marrow}}{\text{No. of erythroblasts (nucleated) in the bone marrow}}$$

There is no nucleus in the mature erythrocyte and reticulocytes.

ME ratio increases in certain conditions like:

i) Myeloid hyperplasia
ii) Erythroid hypoplasia
iii) Leukocytosis
iv) Granulocytic leukemia
v) Lymphosarcoma
vi) Chronic interstitial nephritis

ME ratio decreases in certain conditions such as:

i) Myeloid hypoplasia
ii) Erythremic/eryhtroid hypoplasia

Total Leukocyte Count (TLC)

Apparatus:

Hemocytometer contains chamber, pipette, microscope, blood, diluting fluid (glacial acetic acid).

Dilation Fluid:

Gentian violet: 2 ml, distilled water: 100 ml, glacial acetic acid 2 ml.

Procedure:

Suck sample upto mark 0.5. Then Suck dilution fluid upto mark 11. Mix the two fluids while keeping pipette at horizontal position. Discard first 2-3 drops. Then place a medium size drop on a chamber at a side of cover slip. Let the leukocyte settle for 2-3 minutes and count leukocyte in four corners square at 10 X.

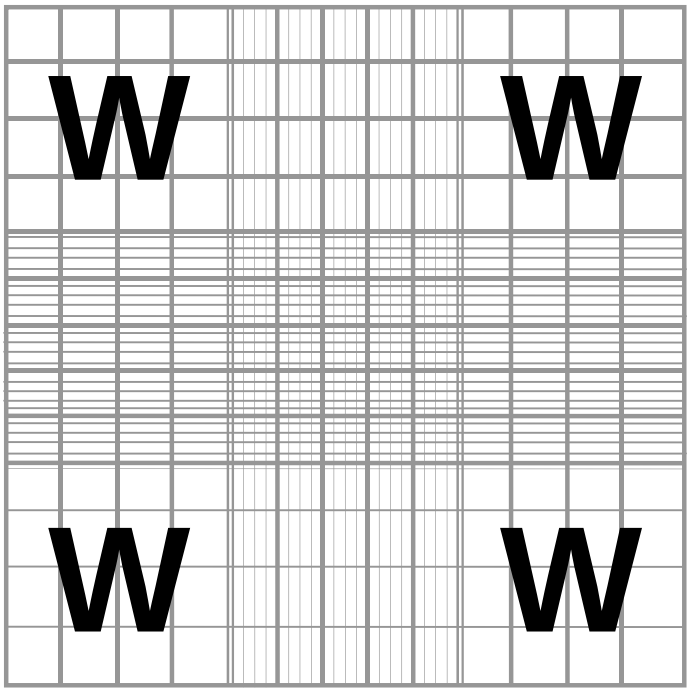
$X \times 50/\mu l$

Area = ¼ × ¼ = 1/16 = Volume = A × H = 1/16 × 1/10 = 1/160

X/64 × 1/1/160 = X/64 × 160 = X/4 × 10 × 20 (dilution factor)

TLC = X/4 × 10 × 20 (or)

TLC = X × 50



Differential Leukocyte Count (DLC)

- For better results take fresh blood or take blood in which EDTA is added. Smear must be made in first 15 minutes of sample collection.
- Take 2 glass slides. Pour a drop of blood on one side. Hold another glass slide and place it against the surface of that slide, move the second glass slide in forward direction by keeping at an angle of 30^o.
- Ideal smear is that which is thick at its start and is then at its end. The smear should be smooth. It should not be torn. Dry the smear then cover the blood smear with Wright Geimsa stain and allow it to stand for 1-3 minutes. Then add an equal amount of buffered distilled water. And again allow it to stand for 3-5 minutes. Then wash it with water and let it dry.
- Then examine the slide under microscope at 100 X by adding a drop of oil. Leukocytes are identified until 100 cells are counted.

Draw shapes of neutrophils, eosinophils, basophils, monocytes, and lymphocytes.

Kidney Function Tests

Kidney function tests are categorized into two sub-divisions:

i) Direct evaluation
ii) Indirect evaluation

Direct evaluation

It includes:

Determination of urea (BUN)
Determination of creatinine

Indirect evaluation

It is also synonymously called "urinalysis". It includes:

Physical examination of urine
Chemical examination of urine
Microscopic examination of urine

Urinalysis

Urinalysis is very useful tool to evaluate healthy and diseased/sick individuals. By urinalysis, valuable information can be obtained particularly about the urinary system and in general about the other organs/systems of the body in minutes. In diseases not yet diagnosed, urinalysis might be helpful diagnosis.

Collection of Urine Samples

a) Timing of Collection

Early morning collection.
Fresh samples.
Do not collect early stream of urine because it contains cellular debris, bacteria. Urea splitting bacteria are present which convert urea into NH_3 makes the urine alkaline and there will be cats, crystals, and cells in urine.

b) Container

Preferably plastic disposable container/glass container, opaque or dark in colour because sunlight degrades urobilinogen, bilirubin. Container should be sterilized.

c) Collection Methods

i) **Clean catch method (Voided urine collection)**
Urine is directly collected from the body as the animal is voiding by avoiding first few drops. This is preferred method of urine collection in large animals.
ii) **Catheterization**
Sterilized catheter is passed into the urinary bladder to collect the urine. Traumatic catheterization may lead to sloughing of transitional epithelial cells from the urethra and cause iatrogenic hemorrhages.
iii) **Cystocentesis**

In small domestic animals (dos, cat) urine is collected with a needle and syringe directly from the urinary bladder.

Catheterization and Cystocentesis provide high quality uncontaminated urine specimens but are associated with tissue trauma of varying degree.

d) Best Time of Analysis

Fresh urine sample is preferred for analysis. Ideally, the urinalysis should be performed within 30 minutes of sample collection. If time is not sufficient then store samples in refrigerator. Refrigeration may be maximum up to 12 hours. It slightly increases specific gravity and interferes with tests using enzymes for reaction. Freezing also damages the cells.

e) Chemical Preservation

Only preferable for physical examination but nor for chemical examination of urine.

- Thymol
- Toluene
- Formalin
- Boric acid
- Metaphosphoric acid
- Chloroform

Physical Examination

Gross (physical) examination is associated with; (of urine)

a) Color b) Volume c) Odour d) Transparency e) Specific gravity

a) Colour

- **Yellow to amber**: Normal color
  It may be due to presence of urochrome and urobilin.
- **Colorless to pale yellow**:
  It may be seen in: polyurea (due to diabetes inspidus), salty feeding by the animals, increased water intake by the animals, administration of fluids, end stage of renal disease, and pyometra. Specific gravity will be low.
- **Dark yellow to yellow brown**:
  It may be seen in: acute nephritis, prolonged dehydration, diarrhea, fever and decreased water intake.
- **Yellow brown to greenish yellow**:
  It may be seen when urobilin and urochrome increased. It is due to excessive degradation of haeme (excessive hemolysis).
- **Red brown to dark brown**:
  It may be translucent or cloudy.
  In hematuria: red cloudy urine becomes clean and transparent after centrifugation.
  In hemoglobinurea: translucent red urine remains as such after centrifugation.
  Due to presence of porphyrin – urine gets burgun color (pinkish and brownish tinge).
- **Brownish black**:
  It is seen in: hemoglobinurea, when urine is kept for long time at room temperature, when bile pigments are present in the urine.
  Voided urine of horse gives yellowish color and it turns to deep yellow to brown black due to pyrocatechin.

Excessive myoglobin in the urea may be seen in: azaurea, carbolic acid toxicity (accidently taken by the animal).

In methemoglobinurea, urine color may be reddish brown.

- **Green color**:
  It may be seen in: poisoning due to phenothiazine, presence of biliverdin in the urine and when methylene blue is used as urinary antiseptic.
  In case of acriflavin poisoning, urine color may gets pinkish green color.

b) Volume

It depends on the several factors;

- Diet
- Fluid intake
- Climate effect (temperature and humidity)
- Size and weight of animal
- Exercise by the animal
- Amount of urine for individual animal specie.

Normal dog produces 0.9 liter /day (range = 0.5-2.0)

Volume of urine is inversely proportional to the body weight of the animal and to the specific gravity of the urine with exception in case of diabetes mellitus.

In case of diabetes mellitus: volume of urine increases along with the increase in specific gravity due to glucose urea.

►Increased volume of urine◄

It may be associated with;

a) **Pathological conditions**
   - ► Progressive renal failure
   - ► Glucose urea in diabetes mellitus
   - ► Renal amyloidosis
   - ► Chronic pyelonephritis
   - ► Pyometra (develop polydyspia)
   - ► All types of generalized liver diseases
   - ► Presence of serous effusions in the body cavities

b) **Non-pathological conditions**
   - ► Increased water intake
   - ► Administration of fluids and diuretics

►Decreased volume of urine◄

It may be associated with:

a) **Physiological conditions**:
   - ► Decreased water intake
   - ► Decreased environmental temperature
   - ► Hyperventilation and exercise
   - ► Dehydration

b) **Pathological conditions**:
   - ► Fever due to any cause
   - ► Acute and chronic renal failure
   - ► Edema development
   - ► Marked decrease in blood pressure (BP)
   - ► Obstruction in urinary passage

Assignment: Physiology of a Nephron???

---------- Up to date: 13 Dec 2010 - Monday