PEARLS OF LABORATORY MEDICINE
www.traineecouncil.org

TITLE: Body Fluids

PRESENTER: Eirini (Irene) Tsilioni

Slide 1:

Hello, my name is Eirini (Irene) Tsilioni. I am a postdoctoral fellow at Tufts University School of Medicine. Welcome to this Pearl of Laboratory M edicine on "**Body Fluids**".

Slide 2:

Most body fluids are ultrafiltrates of blood, and through active transport mechanisms, fluids function to support the delivery and removal of nutrients and metabolic byproducts from surrounding tissue compartments. Pathogenic processes, including infection, malignancy and autoimmune and inflammatory diseases, can disrupt the normal production, circulation and exchange of body fluids, leading to accumulation. Increased volume of fluid in any organ, tissue or joint compartment usually necessitates clinical intervention to actively remove or drain the accumulated fluid. Body fluids can be collected for diagnostic purposes, therapeutic purposes or both.

Slide 3:

There are many types of body fluids including cerebrospinal fluid, pleural fluid, pericardial fluid, peritoneal or ascitic fluid, amniotic fluid, synovial fluid and saliva.

Slide 4:

The cerebrospinal fluid is a clear body fluid that occupies the space between the arachnoid mater (meninges) and the pia mater. It is formed in the choroid plexus by both filtration and active transport. Proteins are largely excluded from the CSF by the blood-CSF barrier. Proteins gaining access to the CSF primarily reach the CSF by transport within pinocytotic vesicles traversing capillary endothelial cells. In normal adults the CSF volume is 125mL to 150mL. Normal adults produce 20mL of CSF per hour. The primary function of CSF is to protect the brain and the spinal cord from injury by acting as a fluid cushion. It is also the medium through which nutrients and the waste products are transported between the brain/spinal cord and the blood. CSF is usually obtained through a lumbar puncture. During the procedure, a needle is inserted usually between the 3rd and 4th lumbar vertebrae and the CSF is collected for testing.

Slide 5:

Some categories of CNS pathologies detected upon CFS analysis include:

Hemorrhage with red blood cells in the CSF which may be secondary to:

- Hypertensive intracerebral hemorrhage into ventricles

- Rupture of a berry aneurysm with bleeding into the subarachnoid space

- Extension of a traumatic haematoma

- Bleeding from vascular malformation

Meningitis is an inflammation of the leptomeninges, usually caused by infection with viruses, bacteria, fungi or protozoa

Malignant tumors may shed cells into the CSF. Primary tumors such as gliomas may spread along the subarachnoid space.

Demyelinating diseases may produce CSF abnormalities by several mechanisms. Products of demyelination may be present in the fluid. Leucocytes from lesional tissue may be present in the fluid. Increased oligoclonal immunoglobulins produced by the site of lesional tissue may be washed into the fluid.

Slide 6:

- The normal CSF protein concentration in adults ranges from 0.15-0.45 g/L (0.015 to 0.045 g/dL). CSF protein concentrations in premature and term neonates normally range between 0.2 and 1.7 g/L (0.02 to 0.17 g/dL). CSF protein can be falsely elevated in the presence of RBCs from subarachnoid hemorrhage or traumatic lumbar punctures. Elevations in the CSF protein concentration occur when inflammation compromises the blood-CSF barrier or in conditions when proteins are synthesized locally. CSF protein elevations may persist for weeks or months following recovery from meningitis and have little utility in assessing cure or the response to therapy. Elevated protein levels may aid in diagnosis of inflammatory conditions such as Guillain Barre Syndrome, where concentrations of over 1g/L are often seen.

Slide 7:

- The glucose concentration in CSF is maintained by both facilitated transport and simple diffusion. Glucose is removed from the CSF by transport across capillaries and arachnoid villi but also utilized by cells lining the ventricular cavities and subarachnoid spaces. As a result, it normally takes hours for the serum glucose to equilibrate with the CSF glucose. The CSF-to-serum glucose ratio is approximately 0.6 in normal individuals. Abnormally low CSF glucose concentrations can occur in bacterial meningitis, mycobacterial and fungal CNS infections and well as malignancies and subarachnoid hemorrhage. Low CSF glucose less than 0.1mmol/L (18.0 mg/dL) are strongly predictive for bacterial meningitis while the CSF glucose concentrations are typically normal during viral CNS infections.

Slide 8:

Typical laboratory findings in bacterial meningitis include:

- The CSF white blood count is usually above 1000/µL, usually with a neutrophilic predominance.

- A CSF protein concentration above 2.5 g/L (0.25 g/dL)

- A CSF glucose concentration below 2.5 mmol/L (45 mg/dL)

While typical laboratory findings in viral meningitis include:

- The CSF white blood count ranges between 250/µL and 2000/µL. The differential shows a predominance of leukocytes.
- The CSF protein concentration is less than 1.5 g/L (0.15 g/dL). It has been estimated that a CSF protein concentration > 2.2 g/L (0.22 g/dL) reduces the probability of viral infection to 1% or less.
- The CSF glucose concentration is usually more than 50% of serum concentration.

Slide 9:

Approximately 10-20 mL of pleural fluid is contained in the pleural cavity, the thin space between parietal and visceral pleura. A pleural effusion occurs when fluid formation exceeds removal resulting in accumulation of excess fluid in the pleural space. Pleural fluid normally originates in the capillaries of the parietal pleura, filtrates into the pleural cavity, and is then absorbed by the parietal lymphatics. Several different forces either promote or oppose fluid filtration. The net movement of fluid from the pleural capillaries to the pleural space depends on the magnitude of these counterbalancing forces. The hydrostatic pressure in the capillary promotes movement of fluid out of the vessel and into the pericapillary space, whereas the colloid osmotic pressure hinders movement of liquid out of the capillary. Likewise, hydrostatic and colloid osmotic pressures in the pericapillary space comprise the opposing forces that act on liquid within the pericapillary region. Thoracentesis, the removal of pleural fluid using a needle or syringe, is performed either for diagnostic or therapeutic purposes.

Slide 10:

Pleural effusions are classified as transudative and exudative. Transudative pleural effusions develop when the systemic factors influencing the formation or the absorption of pleural fluid are altered. Exudative pleural effusions develop when inflammation alters the permeability of the capillaries in the pleural cavity. Many diseases and conditions can lead to the formation of pleural effusion. Differentiating transudates from exudates helps to narrow the differential diagnosis and guide therapy. Here are some examples.

Slide 11:

Thoracentesis is indicated for any patient who has abnormal amounts of fluid accumulated in the pleural space. In instances where the etiology of an effusion can be reasonably concluded from clinical circumstances, for example in congestive heart failure, the procedure may be deferred and the response to therapy observed. Repeat thoracentesis may be required to establish a diagnosis when initial studies fail to do so. Therapeutic thoracentesis may also be indicated for relief of symptoms due to large pleural effusions.

The fluid appearance is a non-specific tool in the evaluation of pleural fluid which can provide information about the etiology of pleural effusion. Most transudates are clear, straw-colored, odorless and non-

viscous fluids. A homogenous bloody appearance narrows the differential diagnosis to malignancy, embolism or trauma. A white-milky appearance indicates chylothorax or pseudochylothorax, a pus appearance indicates empyema and a yellow-green appearance rheumatoid arthritis effusions.

The initial step in the biochemical evaluation of pleural effusions is to determine whether they are transudative or exudative. In clinical practice, Light's Criteria have been widely accepted for this purpose for the past 40 years. According to the Light criteria an exudative effusion is present if one or more of the following conditions are met: (1) pleural fluid protein/serum protein level greater than 0.5, (2) pleural fluid lactic acid dehydrogenase (LDH)/serum LDH level greater than 0.6, (3) pleural fluid LDH level greater than two-thirds the upper normal limit for serum LDH.

Once a pleural effusion is characterized as an exudate, the next challenge is to identify its etiology. Depending on clinical presentation, exudative effusions need additional testing. In general, most exudates have >500x10^6/L leukocytes. Neutrophil predominance may occur in acute inflammatory process such as parapneumonic, tuberculous effusions, pulmonary embolism and viral infections. A pH<7.3 occurs in inflammatory states while a pH below 7.2 in patients with parapneumonic effusion indicates the need for tube drainage. Determination of triglycerides and cholesterol are useful in the diagnosis of chylothorax and pseudochylothorax. Elevated adenosine deaminase (ADA) activity is a sensitive and specific marker for the diagnosis of tuberculous pleural effusions with a sensitivity of 92% and a specificity of 90% at a cut-off point of 40 U/L. Elevated pleural fluid amylase is found in patients with pancreatic diseases, liver cirrhosis and esophageal rupture.

Slide 12:

The pericardial space normally contains 15-50 mL of fluid, which is essentially an ultrafiltrate of plasma. The fluid is thought to originate from the visceral pericardium and serves as lubrication to visceral and parietal layers of the pericardium. Pericardial effusion is an abnormal amount of fluid in the pericardial space. It can be acute or chronic. Effusions can be caused by local or systemic disorders. Pericardial fluid is usually collected for testing through pericardiocentesis.

Slide 13:

Pericardial effusions can be classified as transudative and exudative effusions. A transudative effusion results from systemic alterations in the normal fluid formation or lymphatic drainage such as congestive heart failure, myxoedema and nephrotic syndrome. Exudative effusions reflect inflammatory, infectious, malignant or autoimmune processes within the pericardium such as tuberculosis, empyema and malignant effusions.

Slide 14:

 Pericardiocentesis is indicated in patients with cardiac tamponade. Pericardial fluid may also be analyzed for diagnostic purposes. Cardiac tamponade is a condition in which an accumulation of fluid within the pericardium creates excessive pressure, which then prevents the heart from filling normally with blood.

This can critically decrease the amount of blood that is pumped from the heart, which can be lethal. The removal of the excess fluid reverses this dangerous process. Examples of the need for fluid analysis would be to differentiate whether a fluid collection within the pericardium is due to an infection, spread of cancer, or possibly an autoimmune condition.

- Turbid fluid is indicative of infection or malignancy. Bloody fluid suggests malignant or tuberculous etiology, while a milky appearance results from the presence of chylopericardium.

A total WBC>10,000/μl with neutrophil predominance may be suggestive of bacterial, tuberculous or malignant pericarditis. Also, cytological examination as well as bacteriologic smears and cultures of fluid are the primary laboratory tests used in initial investigations of pericardial effusions of unclear etiology.

Slide 15:

Peritoneal (or ascitic) fluid is a straw colored liquid made in the abdominal cavity which lubricates the surface of tissue that lines the abdominal wall and pelvic cavity. The accumulation of this fluid within the peritoneal cavity is called peritoneal effusions or ascites. The effusion volume is usually >50mL. Transudative peritoneal fluid is produced by visceral capillaries and drained via the diaphragmatic lymphatic system. Exudative peritoneal fluid is rich in protein and cellular debris owing to the increased permeability of capillaries usually as a result of inflammation.

Slide 16:

Increased hydrostatic pressure is associated with portal hypertension and is often caused by cirrhosis, hepatic venous outflow obstruction and constrictive pericarditis. Decreased colloid osmotic pressure occurs secondary to hypoalbuminemia which is characterized as nephrotic syndrome and is associated with several disease conditions such as malnutrition and protein losing enteropathy. Another major cause of peritoneal effusion is malignancy such as adenocarcinoma, epidermoid carcinoma, melanoma and mesothelioma. Infections are often associated with conditions such as tuberculosis. Miscellaneous causes include disease conditions such as chylous ascites.

Slide 17:

Peritoneal fluid is obtained by paracentesis, a procedure in which a needle or a catheter is inserted in the abdominal cavity to remove the fluid that has been collected. Paracentesis is indicated to relieve abdominal pressure from peritoneal effusions, diagnose spontaneous bacterial peritonitis (SBP), metastatic cancer and blood in peritoneal space in trauma.

A cloudy appearance of peritoneal fluid is usually caused by abdominal infection and accompanied by an increased number of neutrophils. Milky peritoneal fluid appearance is characteristic for high triglyceride content. Bloody peritoneal fluid, if not caused by traumatic tap, indicates malignancy, pancreatitis or abdominal trauma. Tea-colored appearance indicates pancreatitis.

The biochemical evaluation of peritoneal effusions begins with identifying the presence or absence of portal hypertension through the use of the serum ascites albumin gradient (SAAG).

- Serum ascites albumin gradient (SAAG) has been proposed as a physiologically based alterative to the traditional classification of ascites into transudates or exudates. SAAG is calculated as the difference between serum and ascitic fluid albumin concentration.

SAAG>11g/L (1.1 g/dL) suggests the presence of portal hypertension while SAAG<11g/L (1.1 g/dL) are not.

After differentiation of ascites into two broad categories, specific biochemical analyses can be useful for further evaluation of ascites etiology. Total protein greater than 25 g/L (2.5 g/dL) helps differentiate some causes of high gradient ascites. Polymorphonuclear (PMN) leukocyte count > 250 X 10^6/L is used to make a presumptive SBP diagnosis. Low glucose has been reported in tuberculous peritonitis, carcinomatosis and SBP due to leukocyte or bacterial consumption. Amylase activity higher than 2000 U/L is useful in identifying pancreatic ascites, gut perforation and ruptured pseudocyst. Chylous ascites can be differentiated from pseudochylous effusions by its high triglyceride concentration. Urea or creatinine concentrations higher than serum indicate urinary bladder rupture. Determination of ADA has proven to be a rapid diagnostic tool in peritoneal tuberculosis with a sensitivity of 100% and a specificity of 97% at a cut-off value of 39 U/L.

Slide 18: The amniotic fluid is a clear, watery, slightly yellowish liquid that surrounds the fetus during pregnancy and is contained in the amniotic sac. The amniotic sac has an inner and outer membrane. The inner membrane, which is called the amnion, contains the amniotic fluid and the fetus. Amniotic fluid has numerous functions that include:

- Providing a protective cushion for the fetus
- Allowing fetal movement
- Stabilizing the temperature and permitting proper lung development

The volume of amniotic fluid increases as the fetus develops, to a maximum of 800mL at approximately 34 weeks of gestation and decreases to around 600mL at full term of 40 weeks.

Slide 19:

Amniotic fluid is obtained by amniocentesis to diagnose fetal chromosomal anomalies after other tests such as ultrasound or biomarkers have determined a significant likelihood that the fetus will be affected with a chromosomal problem. Other diagnostic tests performed using amniocentesis determine fetal lung maturity and evaluate alloimmunization. Therapeutic indications for amniocentesis include the direct delivery of medications to the unborn fetus and to release intrauterine pressure in the presence of polyhydramnios.

Approximately 10mL of the amniotic fluid is obtained by amniocentesis under ultrasound guidance. A long thin needle is inserted through the uterus into the amniotic sac taking care not to contaminate with blood. Amniocentesis is performed safely after the 14th week of gestation.

Slide 20:

An excessive amount of amniotic fluid is called polyhydramnios which indicates fetal distress is often associated with neural tube disorders.

A decreased amount of amniotic fluid is called oligohydramnios and is associated with placental dysfunction, ruptured membranes and fetal abnormalities.

Erythroblastosis fetalis is a hemolytic disease of the fetus and is caused by maternal antibodies directed against antigens on fetal erythrocytes. One of the consequences of erythroblastosis fetalis is the increase in bilirubin amniotic fluid concentration.

Slide 21:

Amniotic fluid is colorless in appearance, but it is also seen in slight yellow color. Blood streaked amniotic fluid indicates traumatic tap, abdominal trauma and intra-amniotic hemorrhage while yellow fluid indicates Rh Disease. A dark green appearance indicates meconium and a dark red-brown fetal death.

The amniotic fluid Bilirubin is an indirect method for assessing the level of anemia in the fetus. Normal levels very low   (2.7 to 3.1 μmol/L or 0.16-0.18 mg/dL) peaking at around 19 to 22 weeks.

Slide 22:

Synovial fluid is a colorless to light yellow highly viscous fluid which does not clot. It is found in joint cavities and it is formed as an ultrafiltrate of plasma across the synovial membranes. Its function is to supply nutrients to cartilage, act as a lubricant to joint surfaces and to carry away waste products. Normal fluid volume in the knee joint is 3-4mL. Synovial fluid is collected for testing through arthrocentesis.

Slide 23:

Increased volume of synovial fluid may be the result of a variety of pathological processes. Such synovial fluids are often classified pathologically into four groups:

- Non-inflammatory, such as osteoarthritis and neuropathy
- Inflammatory such as rheumatoid arthritis
- Septic such as bacterial or fungal infection and
- Hemorrhagic such as hemophilia and trauma.

Slide 24:

Examination of synovial fluid provides important diagnostic information in joint disease. The most common site for collection of synovial fluid is the knee. Arthrocentesis can be performed either diagnostically for the evaluation of suspected septic arthritis, crystal induced arthritis and unexplained arthritis with synovial effusion or therapeutically for pain relief, drainage of effusion or injection of medications.

Slide 25:

Routine synovial fluid analysis should include gross inspection of the synovial fluid to evaluate volume, viscosity, clarity, and color as well as microscopic assessment for Gram stain, cell count, and crystal detection. Normal fluid is less than 3.5 mL volume, highly viscous, clear, and colorless to light yellow. .

Non-inflammatory fluid is more than 3.5 mL volume, highly viscous, clear and yellow. Inflammatory and septic fluids are cloudy and yellow/green. Hemorrhagic fluid is cloudy with low viscosity and red, brown or xanthochromic. The cell count and differential help to distinguish the non-inflammatory from the inflammatory fluid. A WBC count greater than 50,000/μL is indicative of septic arthritis. A cell count in the range of 2000 WBC/μL is a proposed cutoff value to distinguish inflammatory versus non-inflammatory arthritis. Gram's stain and culture of synovial fluid are the cornerstone of the diagnosis of septic arthritis. The sensitivity of culture has been estimated to be 90% for bacterial arthritis. The specimen should be examined under a polarizing light microscope for monosodium urate and calcium pyrophosphate crystals. Even if crystals are found, the specimen should still be analyzed for Gram's stain and culture, since it is possible to have coexisting crystalline and septic arthritis.

Slide 26:

Saliva is a mixture of oral fluids including salivary gland secretions, cellular material and food debris.

Saliva contains molecules normally found in serum and that reach the saliva by several mechanisms including intra-cellular routes such as passive diffusion, while extracellular routes include ultra-filtration at tight junctions between the cells.

Slide 27:

Saliva may be affected directly by systemic diseases or may reflect changes in serum concentrations of certain analytes.

The advantages of using saliva include ease of collection, particularly when such collection requires supervision, and storage. Saliva collections are also non-invasive and presumably lower stress procedures which may be of use in pediatrics and in the measurement of stress affected hormones such as cortisol.

Disadvantages of saliva analysis include the low levels of analytes present compared to serum, contamination from oral cavity before collection, and viscosity of the fluid.

Slide 28:

Saliva is a useful biological fluid for assaying antibodies detection including Helicobacter pylori, Lyme disease, mumps and measles, as well as HIV-1 antibodies.

Saliva can be also used to quantify the levels of several hormones such as steroids. Salivary hormones may represent the free or non-protein-bound hormone levels. Cortisol, for example, correlate well with serum concentration and may represent 10% of the unbound plasma concentrations. However, cortisol may undergo metabolism in the salivary gland to cortisone which may have implications for the specificity of the analytical process.

Salivary testosterone also correlates well with serum concentration and may be a useful test in research on male hypogonadism or in sports medicine.

Saliva can be also used to detect and/or monitor several drugs. The presence of a drug in saliva is influenced by the physico-chemical characteristics of the drug molecule. Passive diffusion of small non-ionized molecules is the major mechanism by which a drug will appear in saliva. Since, binding proteins do not cross the membrane due to their size, only the unbound fraction of the drug in serum is available for diffusion into saliva.

Slide 29: References

1. Johnson M, Rohlfs EM, Lawrence MS, Protein.In:Burtis CA, Ashwood ER, editors. Teitz Fundamentals of Clinical Chemistry. Saunders 5th Ed, 2000:341-342
2. Porcel JM, Light RW. Pleural effusions. Dis Mon. 2013;59:29-57
3. Light RW. Pleural effusions. Med Clin North Am. 2011;95:1055-70
4. Light RW. Pleural effusions. N. Engl. J Med 2002; 346:1971-7
5. Burgess LJ, Reuter H, Talijaard JJF, and Doubell AF. Role of biochemical tests in the diagnosis of large pericardial effusions. Chest 2002; 121;495-9
6. Jenkinson F, Murphy MJ. Biochemical analysis of pleural and ascitic fluid: effect of sample timing on interpretation of results. Ann Clin Biochem. 2007;44:471-473
7. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 4th Edition Eds. C.A. Burtis, E.R.. Ashwood and D.E. Bruns 2006; 2153-2206
8. Kaufman E. The diagnostic applications of saliva-A review. Crit Rev Oral Biol Med 2002; 13:197-212

Slide 30: Disclosures

Upon Pearl submission, the presenter completed the Clinical Chemistry disclosure form. Disclosures and/or potential conflicts of interest:

- Employment or Leadership:
- Consultant or Advisory Role:
- Stock Ownership:
- Honoraria:
- Research Funding:
- Expert Testimony:
- Patents:

This slide will be completed by CCJ Staff based on disclosure form completed electronically in the submission site.

Slide 31: Thank You from www.TraineeCouncil.org

Thank you for joining me on this Pearl of Laboratory Medicine on "**BODY FLUIDS**"