VOLUME - VIII
ISSUE - XLVII
SEP / OCT 2011

The Crux

BIMONTHLY FORUM FOR THE LABORATARIANS

CONTENTS

Editorial

Have you ever heard of an elderly gentleman taking a long haul flight and being advised to pop in an acetyl salicylic acid tablet before boarding the aircraft? Have you ever heard of a lady on oral contraceptive pills getting swollen intestines? No! Well these are common. The reason for these actions and conditions is deep vein thrombosis in the first one and mesenteric vein thrombosis in the second one. Deep vein thrombosis commonly affects the leg veins (such as the femoral vein or the popliteal vein) or the deep veins of the pelvis. Occasionally the veins of the arm are affected (if spontaneous, this is known as Paget-Schrötter disease). A DVT can occur without symptoms, but in many cases the affected extremity will be painful, swollen, red, and warm, and the superficial veins may be engorged. The most serious complication of a DVT is that the clot could dislodge and travel to the lungs, which is called a pulmonary embolism (PE). DVT is a medical emergency, so, all limb swellings, however trivial, should be regarded as a DVT until proven otherwise. Untreated lower extremity DVT has a 3% PE-related mortality rate. Deaths associated with upper extremity DVT are extremely rare. A late complication of DVT is the post-thrombotic syndrome, which can manifest itself as edema, pain or discomfort and skin problems.

According to Virchow's triad, venous thrombosis occurs via three mechanisms: decreased flow rate of the blood, damage to the blood vessel wall and an increased tendency of the blood to clot (hypercoagulability). Several medical conditions can lead to DVT, such as compression of the veins, physical trauma, cancer, infections, certain inflammatory diseases and specific conditions such as stroke, heart failure or nephrotic syndrome. There are several factors which can increase a person's risk for DVT, including surgery, hospitalization, immobilization (such as when orthopedic casts are used, or during long-haul flights, leading to traveller's thrombosis), smoking, obesity, age, certain drugs (such as estrogen, or erythropoietin) and inborn tendencies to form clots known as thrombophilia (for example, in carriers of factor V Leiden). Women have an increased risk during pregnancy, if they are on oral contraceptives, and in the postnatal period, due to increased estrogen levels. The **"DISEASE DIAGNOSIS"** segment of this issue delves deep into the clinico-diagnostic aspects of "DVT".

We do take various body fluids under **"INTERPRETATION"** portion. This issue talks about "Urine Calcium: Laboratory Measurement and Clinical Utility. Well, when urinary calcium is ordered and in which clinical conditions are the values altered! Answers to these and all related questions are available within the covers of this issue.

The overflow from the previous issue on "Quality control in the new environment: automated hematology" is presented under **"TROUBLE SHOOTING"** and is concluded here.

Nothing is brought forward from anywhere in **"BOUQUET"**. It is unique as it is new. Read ON…..

DISEASE DIAGNOSIS

Deep Venous Thrombosis and Thrombophlebitis

Introduction: Background: Deep venous thrombosis (DVT) and its sequela, pulmonary embolism (PE), are the leading causes of preventable in-hospital mortality internationally. Although PE itself should be discussed separately, it occurs primarily as a complication of DVT. The earliest known reference to peripheral venous disease is found on the Ebers papyrus, which dates from 1550 BC and documents the potentially fatal hemorrhage that may ensue from surgery on varicose veins. In 1644, Schenk first observed venous thrombosis when he described an occlusion in the inferior vena cava. In 1846, Virchow recognized the association between venous thrombosis in the legs and PE. Heparin was introduced into clinical practice in 1937. Over the last 25 years, the pathophysiology of DVT has become much better understood, and considerable progress has been made in its diagnosis and treatment.

Pathophysiology: The Virchow triad of venous stasis, vessel wall injury, and hypercoagulable state is still considered the primary mechanism for the development of venous thrombosis. The relative importance of each factor remains a topic of debate, however. The formation, propagation, and dissolution of venous thrombi represent a balance between thrombogenesis and the body's protective mechanisms, specifically the circulating inhibitors of coagulation and the fibrinolytic system. In practical terms, the development of venous thrombosis is best understood as the activation of coagulation in areas of reduced blood flow. This explains why the most successful prophylactic regimens are anticoagulation and minimization of venous stasis. DVT of the lower extremity usually begins in the deep veins of the calf around the valve cusps or within the soleal plexus. A minority of cases arise primarily in the ileofemoral system as a result of direct vessel wall injury, such as from hip surgery or intravenous catheters. The vast majority of calf vein thrombi dissolve completely without therapy. Approximately 20% propagate proximally. Propagation usually occurs before embolization. The process of adherence and organization of a venous thrombus does not begin until 5-10 days after thrombus formation. Until this process has been established fully, the nonadherent disorganized thrombus may propagate and/or embolize. Not all venous thrombi pose equal embolic risk. Studies have shown that isolated calf vein thrombi carry a limited risk of PE. Furthermore, studies have suggested that isolated calf vein thrombi are smaller and do not cause significant morbidity or mortality if they embolize. Contradictory evidence from several other studies has indicated that isolated calf vein thrombi do embolize, suggesting that proximal propagation may occur rapidly and that fatal PE arising from isolated calf vein DVT is a significant risk. The current diagnostic and therapeutic management of DVT is strongly influenced by the different risks assigned to proximal and calf vein thrombi. The propagation and organization of the venous thrombus usually result in destruction of venous valves and produce varying degrees of venous outflow obstruction. Spontaneous lysis and complete recanalization of established proximal DVT occurs in fewer than 10% of patients, even with anticoagulation. These factors are the most important pathogenic mechanisms in the development of chronic venous insufficiency.

Frequency: The exact incidence of DVT is unknown because most studies are limited by the inherent inaccuracy of clinical diagnosis. More importantly, most DVT is occult and usually resolves spontaneously without complication. Existing data that probably underestimate the true incidence of DVT suggest that about 80 cases per 100,000 population occur annually. Approximately 1 person in 20 will develop a DVT in the course of his or her lifetime. In hospitalized patients, the incidence of venous thrombosis is considerably higher and varies from 20-70%. Venous ulceration and venous insufficiency of the lower leg, which are long-term complications of DVT, affect 0.5% of the entire population.

Mortality/Morbidity: Death from deep venous thrombosis (DVT) is attributed to massive pulmonary embolism (PE), which causes numerous deaths annually internationally. PE is the leading cause of preventable in-hospital mortality. The age-standardized incidence of first-time venous thromboembolism (VTE) is 1.92 per 1000 person-years. The incidence of VTE is higher in men than in women and increases with age in both sexes. In a study that evaluated ovar a thousand acutely ill, immobilized admitted general medical patients, multiple logistic regression analysis found the following factors to be significantly and independently associated with an increased risk for VTE, most of which were asymptomatic and diagnosed by venography of both lower extremities: Presence of an acute infectious disease, Age >75 years, Cancer, History of prior VTE: The principal long-term morbidity from DVT is the post-thrombotic syndrome (PTS), which complicates about a quarter of cases of symptomatic proximal DVT; most cases develop within 2 years afterward. Race: From a demographic viewpoint, Asian and Hispanic populations have a lower risk of VTE, whereas Caucasians and African Americans have a higher risk (2.5-4 times higher). Sex: The male-to-female ratio is 1.2:1, indicating that males have a higher risk of DVT than females. Age: Deep venous thrombosis (DVT) usually affects individuals older than 40 years.

Clinical History: The signs and symptoms of DVT are related to the degree of obstruction to venous outflow and inflammation of the vessel wall. The bedside diagnosis of venous thrombosis is insensitive and inaccurate. Many thrombi do not produce significant obstruction to venous flow; venous collaterals may develop rapidly, and venous wall inflammation may be minimal. Conversely, many nonthrombotic conditions produce signs and symptoms suggestive of DVT. Studies have repeatedly documented this inherent difficulty of the clinical diagnosis of lower extremity DVT. Many patients are asymptomatic; however, the history may include the following: Edema, principally unilateral, is the most specific symptom. Massive edema with cyanosis and ischemia (phlegmasia cerulea dolens) is rare. Leg pain occurs in 50% of patients, but this is entirely nonspecific. Pain can occur on dorsiflexion of the foot (Homans sign). Tenderness occurs in 75% of patients but is also found in 50% of patients without objectively confirmed DVT. Clinical signs and symptoms of PE as the primary manifestation occur in 10% of patients with confirmed DVT. The pain and tenderness associated with DVT does not usually correlate with the size, location, or extent of the thrombus. Warmth or erythema of skin can be present over the area of thrombosis.

Physical: No single physical finding or combination of symptoms and signs is sufficiently accurate to establish the diagnosis of DVT. The following is a list outlining the most sensitive and specific physical findings in DVT: Edema, principally unilateral, Tenderness, if present, is usually confined to the calf muscles or along the course of the deep veins in the medial thigh. Pain and/or tenderness away from these areas is not consistent with venous thrombosis and usually indicates another diagnosis. **Homans sign:** Discomfort in the calf muscles on forced dorsiflexion of the foot with the knee straight has been a time-honored sign of DVT. However, Homans sign is neither sensitive nor specific: it is present in less than one third of patients with confirmed DVT, and is found in more than 50% of patients without DVT. Venous distension and prominence of the subcutaneous veins. Superficial thrombophlebitis is characterized by the finding of a palpable, indurated, cordlike, tender, subcutaneous venous segment. Forty percent of patients with superficial thrombophlebitis without coexisting varicose veins and with no other obvious etiology (eg, intravenous catheters, intravenous drug abuse, soft tissue injury) have an associated DVT. Patients with superficial thrombophlebitis extending to the saphenofemoral junction are also at higher risk for associated DVT. Further diagnostic studies for DVT are required in these particular subgroups of patients. Fever: Patients may have a fever, usually low grade. High fever is usually indicative of an infectious process such as cellulitis or lymphangitis. **Phlegmasia cerulea dolens:** Patients with venous thrombosis may have variable discoloration of the lower extremity. The most common abnormal hue is reddish purple from venous engorgement and obstruction. In rare cases, the leg is cyanotic from massive ileofemoral venous obstruction. This ischemic form of venous occlusion was originally described as phlegmasia cerulea dolens or painful blue inflammation. The leg is usually markedly edematous, painful, and cyanotic. Petechiae are often present. **Phlegmasia alba dolens:** Painful white inflammation was originally used to describe massive ileofemoral venous thrombosis and associated arterial spasm. The affected extremity is often pale with poor or even absent distal pulses. The physical findings may suggest acute arterial occlusion, but the presence of swelling, petechiae, and distended superficial veins point to this condition.

Clinical findings of PE: These findings are the primary manifestation in about 10% of patients with DVT. In patients with angiographically proven PE, DVT is found in 45-70%. In the vast majority of these patients, DVT is clinically silent.

Causes: The clinical evaluation of patients with suspected DVT is facilitated by an assessment of risk factors. The diagnosis of DVT is confirmed in only 20-30% of ED patients with clinically suspected DVT. The prevalence of DVT in the ED patient population correlates with the number of risk factors present. In patients with no identified risk factors, DVT is confirmed in only 11%. In patients with 3 risk factors, the number rises to 50%. The following risk factors for DVT have been identified in many different epidemiologic studies: General, Age, Immobilization longer than 3 days, Pregnancy and the postpartum period, Major surgery in previous 4 weeks, Long plane or car trips (>4 h) in previous 4 weeks,Medical, Cancer, Previous DVT, Stroke, Acute myocardial infarction (AMI), Congestive heart failure (CHF), Sepsis, Nephrotic syndrome, Ulcerative colitis,Trauma, Multiple trauma, CNS/spinal cord injury, Burns, Lower extremity fractures, Vasculitis, Systemic lupus erythematosus (SLE) and the lupus anticoagulant, Behçet syndrome, Homocystinuria,Hematologic, Polycythemia rubra vera, Thrombocytosis, Inherited disorders of coagulation/fibrinolysis, Antithrombin III deficiency, Protein C deficiency, Protein S deficiency, Prothrombin 20210A mutation, Factor V Leiden, Dysfibrinogenemias and disorders of plasminogen activation, Drugs/medications, Intravenous drug abuse, Oral contraceptives, Estrogens, Heparin-induced thrombocytopenia. The diagnosis of DVT is complicated by the interplay between risk factors, the nonspecific nature of the physical findings, and the frequent discordance between the clinical assessment and the results of objective testing. For example, patients deemed to be at high risk for DVT may have a negative finding on duplex ultrasonographic study. However, the probability of DVT in those patients is still greater than 20%, given the known sensitivity, specificity, and negative likelihood ratio of duplex ultrasonography. Having an objective method to determine pretest probability would simplify clinical management. The Wells clinical prediction guide quantifies the pretest probability of DVT. The model enables physicians to reliably stratify their patients into high-, moderate-, or low-risk categories. Combining this with the results of objective testing greatly simplifies the clinical workup of patients with suspected DVT. The Wells clinical prediction guide incorporates risk factors, clinical signs, and the presence or absence of alternative diagnoses.

Table 1. Wells Clinical Score for DVT

Clinical Parameter Score	Score
Active cancer (treatment ongoing, or within 6 mo or palliative)	+1
Paralysis or recent plaster immobilization of the lower extremities	+1
Recently bedridden for >3 d or major surgery <4 wk	+1
Localized tenderness along the distribution of the deep venous system	+1
Entire leg swelling	+1
Calf swelling >3 cm compared with the asymptomatic leg	+1
Pitting edema (greater in the symptomatic leg)	+1
Previous DVT documented	+1
Collateral superficial veins (nonvaricose)	+1
Alternative diagnosis (as likely or greater than that of DVT)	-2
Total of Above Score	
High probability	>3
Moderate probability	1 or 2
Low probability	≤0

Differential Diagnoses: Cellulitis, Pulmonary Embolism, Thrombophlebitis, Septic, Thrombophlebitis, Superficial, Other Problems to Be Considered, In approximately 70% of patients with clinically suspected DVT, alternate diagnoses are ultimately found, as follows: Achilles tendonitis, Arterial insufficiency, Arthritis, Asymmetric peripheral edema secondary to CHF, liver disease, renal failure or nephrotic syndrome, Cellulitis lymphangitis, Extrinsic compression of iliac vein secondary to tumor, hematoma or abscess, Hematoma, Lymphedema, Muscle or soft tissue injury, Neurogenic pain, Postphlebitic syndrome, Prolonged immobilization or limb paralysis, Ruptured Baker cyst, Stress fractures or other bony lesions, Superficial thrombophlebitis, Varicose veins.

Workup: Laboratory Studies: D-dimer testing: The D-dimer test has an important role in the diagnostic approach to deep venous thrombosis (DVT). D-dimer fibrin fragments are present in fresh fibrin clot and in fibrin degradation products of cross-linked fibrin. Monoclonal antibodies specific for the D-dimer fragment are used to differentiate fibrin-specific clot from non–cross-linked fibrin and from fibrinogen. These specific attributes of the D-dimer antibodies account for their high sensitivity for venous thromboembolism. D-dimer level may be elevated in any medical condition where clots form. D-dimer level is elevated in trauma, recent surgery, hemorrhage, cancer, and sepsis. Many of these conditions are associated with higher risk for DVT. The D-dimer assays have low specificity for DVT; therefore, they should only be used to rule out DVT, not to confirm the diagnosis of DVT. D-dimer levels remain elevated in DVT for about 7 days. Patients presenting late in the course, after clot organization and adherence have occurred, may have low levels of D-dimer. Similarly, patients with isolated calf vein DVT may have a small clot burden and low levels of D-dimer that are below the analytic cut-off value of the assay. This accounts for the reduced sensitivity of the D-dimer assay in the setting of confirmed DVT. Many different D-dimer assays are available, with varying sensitivities and specificities. The assays are not standardized. They incorporate different monoclonal antibodies to the D-dimer fragment. Results may be reported quantitatively or qualitatively. Different units may be used; some assay results are reported as fibrinogen equivalent units (FEU) and others in nanograms per milliliter (ng/mL). The results of one assay cannot be extrapolated to another. Most studies have confirmed the clinical utility of D-dimer testing, and most clinical algorithms incorporate their use. Physicians should know their hospital's D-dimer assay. All D-dimer assays have been evaluated in various validation studies that determine the assay's sensitivity, specificity, and negative predictive value (NPV). Unfortunately, fewer management studies have been conducted to determine the safety of withholding anticoagulant therapy on the basis of a negative test result. Furthermore, the NPV of a specific assay falls as the pretest probability of the study population at risk for DVT increases. An assay with a sensitivity of 80% has an NPV of 97.6% in a low-risk patient. However, the NPV of the same assay is only 33% in high-risk patients with a pretest probability of 90% for DVT. Traditional enzyme-linked immunosorbent assays (ELISAs), although accurate, are time-consuming and not practical for use in the ED. A rapid ELISA assay (VIDAS) with high sensitivity has also been developed. Qualitative rapid ICRT formats have also been devised. Negative multiple technology system results of D-dimer assay essentially rule out DVT. All patients with a negative D-dimer result do not require further diagnostic testing with ultrasonography. The older qualitative latex agglutination assay is not so accurate and should not be used for making treatment decisions in patients with suspected DVT. Newer latex-enhanced immunoturbidimetric and immunofiltration assays have high sensitivity and are available. A rapid qualitative RBC agglutination assay is available. It is sensitive for proximal vein DVT but less so for calf vein DVT. Current evidence strongly supports the use of a D-dimer assay in the clinical algorithm of suspected DVT. A negative D-dimer assay result rules out DVT in patients with low-to-moderate risk (Wells DVT score <2). **A negative result also obviates surveillance and serial testing in patients with moderate-to-high risk and negative ultrasonographic findings. *D-dimer results should be used as follows:*** A negative D-dimer assay result rules out DVT in patients with low-to-moderate risk and a Wells DVT score less than 2. All patients with a positive D-dimer assay result and all patients with a moderate-to-high risk of DVT (Wells DVT score >2) require a diagnostic study (duplex ultrasonography).

Other blood tests: Protein S, protein C, antithrombin III, factor V Leiden, prothrombin 20210A mutation, antiphospholipid antibodies, and homocysteine levels can be measured. Deficiencies of these factors or the presence of these abnormalities all produce a hypercoagulable state. These are rare causes of DVT. Laboratory investigations for these abnormalities are primarily indicated when DVT is diagnosed in patients younger than 50 years, when there is a confirmed family history of a hypercoagulable state or a familial deficiency, when venous thrombosis is detected in unusual sites, and in the clinical setting of warfarin-induced skin necrosis.

Imaging Studies: Because of the inherent inaccuracy of clinical diagnosis that is based on the history, the physical examination, and the assessment of risk factors, D-dimer testing and a determination of pretest probability (eg, Wells DVT score) should be used to identify those patients who require further objective diagnostic testing. Diagnosing DVT and committing patients to the risks of anticoagulation therapy without confirmatory objective testing is unacceptable. The criterion standard for evaluating patients with suspected DVT has been

contrast venography. For many reasons, including allergic reactions, contrast-induced DVT, technical difficulties, inadequate studies, interobserver variability, and lack of availability, venography is either contraindicated or nondiagnostic in as many as 20-25% of patients. As a result, noninvasive studies have essentially replaced venography as the initial diagnostic test of choice. **Duplex ultrasonography:** Technological advances in ultrasonography have permitted the combination of real-time ultrasonographic imaging with Doppler flow studies (duplex ultrasonography). The major ultrasonographic criterion for detecting venous thrombosis is failure to compress the vascular lumen, presumably because of the presence of occluding thrombus. The absence of the normal phasic Doppler signals arising from the changes to venous flow provides indirect evidence of venous occlusion. Many studies have confirmed the diagnostic sensitivity and specificity of duplex ultrasonography for proximal vein thrombosis. Sensitivity of duplex ultrasonography for proximal vein DVT is 97% (95% confidence interval [CI], 96-98%) but only 73% for calf vein DVT (95% CI, 54-93%). The NPV for proximal vein DVT is 99%. Overall specificity is 95%. Duplex ultrasonography is also helpful to differentiate venous thrombosis from hematoma, Baker cyst, abscess, and other causes of leg pain and edema. The primary disadvantage of duplex ultrasonography is its inherent inaccuracy in the diagnosis of calf vein thrombosis. Venous thrombi proximal to the inguinal ligament are also difficult to visualize. Nonoccluding thrombi may be difficult to detect. In patients with suspected acute recurrent DVT, duplex ultrasonography may not be able to differentiate between old and new clots. Diagnostic accuracy varies depending on local expertise. **Impedance plethysmography:** In some countries, impedance plethysmography (IPG) has been the initial noninvasive diagnostic test of choice. Plethysmography is derived from the Greek word meaning "to increase." This procedure is based on recording changes in blood volume of an extremity, which are directly related to venous outflow. Several different techniques can be used to measure these changes, including electrical impedance. In the setting of proximal vein thrombosis, venous outflow from the lower extremity is slowed and the blood volume or venous capacitance is increased. Standardized graphs are used to discriminate normal IPG study results from abnormal results. In many studies, IPG has been shown to be sensitive and specific for proximal vein thrombosis. It is insensitive for calf vein thrombosis, nonoccluding proximal vein thrombus, and ileofemoral vein thrombosis above the inguinal ligament. IPG cannot distinguish between thrombotic occlusion and extravascular compression of the vein. False-positive results occur in the setting of significant CHF and raised central venous pressure as well as in severe arterial insufficiency. **MRI:** MRI has increasingly been investigated for evaluation of suspected DVT. In limited studies, the accuracy approaches that of the criterion standard, contrast venography. MRI is the diagnostic test of choice for suspected iliac vein or inferior vena caval thrombosis when CT venography is contraindicated or technically inadequate. In the second and third trimester of pregnancy, MRI is more accurate than duplex ultrasonography because the gravid uterus alters Doppler venous flow characteristics. In suspected calf vein thrombosis, MRI is more sensitive than any other noninvasive study. Expense, lack of general availability, and technical issues limit its use. **Nuclear medicine imaging studies:** Nuclear medicine studies with I^{125} -labeled fibrinogen are no longer recommended for patients in the ED. They are relatively insensitive for proximal vein thrombosis and take longer than 24 hours to obtain results. I^{125} -labeled fibrinogen is no longer available in most nations. With the introduction of multidetector CT technology, CT venography has been incorporated into CT angiographic studies of the chest as part of the diagnostic evaluation for suspected PE. CT venography of the lower extremities is performed after scanning of the chest has been completed. Scanning usually begins at the level of the iliac crests and continues caudally down to the popliteal fossa. In the Prospective Investigation of Pulmonary Embolism Diagnosis II (PIOPED II) study reported by Stein et al, the addition of CT venography to CT angiography of the chest increased the diagnostic sensitivity for venous thromboembolic disease than CT angiography alone. A number of small studies have compared CT venography alone to duplex ultrasonography alone for the diagnosis of lower extremity DVT. Similar high sensitivities for ultrasonography and CT have been reported, but no large trials comparing the two have yet been performed. The primary utility of CT venography is for the diagnosis of ileofemoral DVT. Ultrasonography is limited to the diagnosis of DVT in the venous system distal to the inguinal ligament. The iliac veins cannot usually be visualized by ultrasonography, and a different diagnostic modality must be used. Herein lies the value of CT venography where venous occlusion proximal to the inguinal ligament may be detected. The diagnosis of ileofemoral DVT should be considered if the ultrasonographic examination reveals thrombus extending into the superficial femoral vein at the inguinal ligament. A CT venogram should be obtained to assess for proximal thrombus and ileofemoral DVT. The major problems with CT venography are technical issues with inadequately visualized veins, artifactual interference from metal implants such as hip and knee arthroplasties, and contraindications to the administration of contrast dye.

Summary-Which test is best? When directly compared, duplex ultrasonography has superior sensitivity and specificity over IPG. Guidelines recommend duplex Doppler compression ultrasonography as the most appropriate study in suspected lower extremity DVT. The guidelines note that, in addition to lower accuracy than compression ultrasonography, plethysmography requires meticulous technique and has a sensitivity of only 20-30% for calf vein thrombosis. Controversy still exists over the use of noninvasive studies such as duplex ultrasonography for the diagnosis of suspected calf vein DVT. Recognizing that duplex ultrasonography is relatively insensitive for calf vein thrombosis only matters if the clinician is inclined to place patients with calf vein DVT on anticoagulation therapy. If the clinical algorithm for calf vein thrombosis recommends clinical surveillance and serial studies to detect proximal extension, the lack of sensitivity of the noninvasive study for calf vein thrombosis is irrelevant. Reports on the use of noninvasive studies for DVT in asymptomatic hospitalized patients should not be used to determine the optimal evaluation of ED patients with suspected DVT who are usually ambulatory and symptomatic. A number of authors have incorrectly recommended the routine use of contrast venography rather than a noninvasive study for suspected DVT on the basis of the low sensitivity that has been reported in studies of hospitalized patients after hip surgery. In ambulatory outpatients with suspected DVT, the sensitivity of duplex ultrasonography for proximal vein thrombosis is 97%, and it remains the initial diagnostic test of choice. CT venography is the best diagnostic modality for suspected ileofemoral DVT. Simplified clinical management strategy for patients with suspected DVT: Using the pretest probability score calculated from the Wells DVT score, patients are stratified into 2 risk groups: DVT unlikely (DVT score <2) or DVT likely (DVT score >2). Using a sensitive D-dimer assay such as the VIDAS rapid ELISA, the D-dimer results should be used as follows: A negative D-dimer result rules out DVT in the unlikely group (low-to-moderate risk of DVT) with a Wells DVT score less than 2. All patients with a positive D-dimer result and all patients in the likely group (moderate-to-high risk of DVT) with a Wells DVT score of 2 or greater require a diagnostic study (ie, duplex ultrasonography). The results from duplex ultrasonography are incorporated as follows: If the patient is scored as likely and the duplex ultrasonographic findings are positive, treat for DVT. If the duplex study result is negative and the patient is scored as unlikely to have DVT, DVT is ruled out even if the D-dimer assay is positive. When discordance exists between the pretest probability and the duplex ultrasonographic study result, further evaluation is required. If the patient is scored as likely to have DVT (DVT score >2) but the ultrasonographic findings are negative, the patient still has a significant probability of DVT. Some authors recommend venography to rule out a calf vein DVT that ultrasonography did not detect. Most recommend surveillance with repeat clinical evaluation and ultrasonography in 1 week. Others use the results of the D-dimer assay to guide management. A negative D-dimer assay in combination with negative ultrasonographic findings rules out DVT. A positive D-dimer assay in this group mandates surveillance and repeat ultrasonography in 1 week. If the patient is scored as unlikely to have DVT (DVT score <2) but the ultrasonographic findings are positive, some authors recommend a second confirmatory study such as venography before treating for DVT and committing the patient to the risks of anticoagulation. Most, however, treat the patient for DVT. If the patient is scored as likely to have DVT (DVT score >2) but had a positive D-dimer assay result and the ultrasonographic findings are negative, repeat clinical evaluation and ultrasonography in 1 week is recommended. The DVT score was developed in a specific subgroup of patients. Excluded from the model were patients with suspected coexistent PE and patients already taking anticoagulants. Therefore, the evaluation and subsequent treatment of these excluded subgroups must be individualized.

INTERPRETATION

Urine Calcium: Laboratory Measurement and Clinical Utility

Introduction: Urine calcium measurement is a commonly ordered test in clinical laboratories. Unlike other urine markers, the utility of urine calcium is less clear to many laboratorians and physicians. Urine calcium can be used to assess parathyroid disease and familial hypocalciuric hypercalcemia (FHH). Although not predictive of stone formation, urine calcium is frequently elevated in patients with lithiasis. The primary clinical value of urine calcium measurement is to aid in the differential diagnoses of patients and direct optimal treatment options for patients with abnormal serum calcium. Because calcium is required for muscle contraction and nerve signaling, the serum concentration of calcium has obvious significance. Serum calcium also reflects parathyroid hormone (PTH) function and vitamin D status. Although the clinical importance of these 2 hormones and the resulting concentration of calcium in the serum is widely known, the role of urine calcium testing is not often discussed and is less obvious. The average adult store of calcium is approximately 1–2 kg. The vast majority (99%) resides in the skeleton. Only a fraction of the stored calcium is present in extracellular fluid and available for use in the form of ionized calcium. Ionized calcium is tightly regulated by PTH. Adult calcium plasma concentrations are normally between 8.5–10.5 mg/dL (2.2–2.6 mmol/L). Most of this circulating calcium is bound to albumin. Because of this, changes in serum protein concentrations can affect total blood calcium concentrations. Calcium enters the extracellular fluid through absorption from the gut and resorption from bone. It is removed through secretion into the gastrointestinal tract and urine as well as losses in sweat and deposition in bone. The recommended dietary allowance (RDA) for calcium varies with age and, for adults, with gender. Recommended dietary allowance values for adults start at 1000 mg per day. Urine calcium levels will reflect dietary intake. In an average adult urine sample collected over 24 hours, 100–250 mg of calcium (15–20 mmol) is expected. For those on low-calcium diets 50–150 mg/day is expected, while those on a calcium-free diet will have 5–40 mg/day. It is also important to note that calcium excretion (CE) is heavily influenced by sodium excretion. Low-sodium diets tend to decrease CE and vice versa. Although a 24-hour collection is best, random urine calcium measurement can be performed and is expressed in relation to creatinine. A normal reference interval for the urine calcium (mg/dL):urine creatinine (mg/dL) ratio is <0.14. Values exceeding 0.20 are found in patients with hypercalciuria. In children, the calcium:creatinine ratio decreases steadily with time until approximately age 6. It is important to note this fact since most children will be falsely flagged as hypercalciuric using adult cut-offs. Elevated urine calcium (>300 mg/24 hr) is often a sign of an overactive parathyroid gland. Parathyroid hormone is produced in response to serum calcium levels. Parathyroid calcium-sensing receptors (CASRs) stimulate increased PTH release in the presence of decreased serum calcium levels. Parathyroid hormone then works to increase serum calcium levels. The increases in serum calcium are achieved via increased renal tubule reabsorption of calcium and simultaneous decreases in phosphorus reabsorption. The serum concentration of phosphorus should be very similar to that of calcium since both are held in equilibrium to each other; as 1 goes up, the other tends to go down. Parathyroid hormone also causes reabsorption of calcium from bone as well as increased synthesis of 1,25-dihydroxy vitamin D, which stimulates calcium absorption from the gut. All of these actions lead to increased serum calcium. Hyperparathyroidism results in excessive uptake and increased concentrations of calcium in serum leading to hypercalcemia and hypophosphatemia. This is then reflected in the urine as hypercalciuria and hyperphosphaturia. Thus, urine calcium levels are often increased in the setting of hyperparathyroidism. However, one-third of hyperparathyroid patients have normal urine calcium, so this test is not reliable in differentiating or diagnosing hyperparathyroidism.

Calcium Crystals and Stones: Calcium is a common ingredient in urine stones and crystals. Calcium oxalate ($Ca[COO]_2$) crystals are the most frequently observed crystals in urine, and 75% of renal calculi have calcium oxalate as a component. Calcium oxalate crystals can form at any pH and have various microscopic morphologies. It is estimated that about half of the oxalate in urine comes from ascorbic acid (vitamin C), which is a precursor to oxalate. Calcium oxalate crystals are also associated with ethylene glycol ingestion, another oxalate precursor. Calcium carbonate ($CaCO_3$), the main component of marine shells and egg shells, can be found as small granular crystals in alkaline urine. Calcium carbonate crystals are not common in urine but when present can be mistaken for bacteria. To help discriminate these 2, acetic acid can be added to the sample, causing the crystals to release CO_2 which appears as effervescence. Calcium phosphate ($CaHPO_4$ or $Ca[H_2PO_4]_2$) crystals can have different morphologies depending on their state of hydration and can be present in the urine sediment of neutral or slightly alkaline or acidic urine. Although not all patients with calcium crystals present in urine will suffer from kidney stones, renal calculi can be caused by calcium oxalate, $CaCO_3$, or $CaHPO_4$. Acidification of urine helps prevent calcium from precipitating as salts and thus prevents falsely decreased measurements of urine calcium. Because of the possible interference of crystals, acidification of urine to pH <2 or pH 4–5 is recommended by many manufacturers of urine calcium reagents. However, adding acid to urine specimens presents some risk to technologists; it will dilute the specimen (although usually only to a minor degree), and it is time-consuming and often requires training and monitoring at collection sites. A recent study has questioned the need to acidify urine.

Hypocalciuria: Hypocalciuria is often mistakenly due to incomplete collection (a random sample thought to be an aliquot from a 24-hour specimen). Some drugs can decrease urine calcium, including thiazide diuretics, benzothiadiazide diuretics (like chlorthalidone), and estrogen. Decreased urine calcium is also seen in hypoparathyroidism, pseudohypoparathyroidism (a lack of response to PTH rather than decreased secretion of PTH), rickets, hypothyroidism, steatorrhea, and nephrosis. Another cause of low urine calcium is familial hypocalciuric hypercalcemia (FHH), also known as familial benign hypercalcemia. This disease is often initially misdiagnosed as hyperparathyroidism, but unlike hyperparathyroidism, FHH will not resolve with parathyroidectomy. Familial hypocalciuric hypercalcemia is now known to be caused by various autosomal-dominant loss-of-function mutations in the gene coding for the CASR. The CASR responds to serum calcium and mediates feedback inhibition of PTH release. A loss-of-function mutation results in a rising of the calcium threshold that triggers reduction of PTH secretion. Serum concentration of calcium is thus maintained at higher levels since more calcium is needed to trigger negative feedback. This results in hypercalcemia, and since more calcium is sequestered in the serum, hypocalciuria often results. It is important to note that hyperparathyroidism and FHH can show elevated serum PTH levels. There are also known cases in which FHH patients have hypercalciuria.

Hypercalciuria: Any disease causing increases in serum calcium can lead to increases in urine calcium. In addition to hyperparathyroidism, other diseases include multiple myeloma (or any osteolytic neoplasm), osteoporosis, vitamin D overdose, renal tubular acidosis, hyperthyroidism, Paget's disease, and sarcoidosis. Drugs containing calcium (such as some antacids) and calcium supplements can lead to direct increases in urine calcium. The diuretic spironolactone can also cause increases in urine calcium since it is given as a calcium salt and appears to decrease tubule reabsorption of calcium. Androgens such as nandrolone and treatment with growth hormone can also cause increases in urine calcium. Acetazolamide and systemic corticosteroids are also associated with increased CE. Patients who have alterations in serum calcium levels are often asymptomatic at the time their abnormal calcium levels are discovered by the clinical laboratory. However, patients with abnormal calcium levels can present with severe signs and symptoms, such as tetany and seizure. Other clinical symptoms suggestive of alteration in calcium metabolism may include perioral and peripheral paresthesias, carpal and pedal spasms, muscle aches, depression, anxiety, fatigue, constipation, abdominal pain, polyuria, and polydypsia. Irritability and lethargy may be the only presenting symptoms in an infant. Maternal hypercalcemia can result in an increased risk of spontaneous abortions, fetal demise, and neonatal hypocalcemia. Maternal hypocalcemia can cause neonatal hyperparathyroidism with abnormal serum calcium levels and osteitis fibrosa cystica. Unrecognized hypoparathyroidism and FHH in women who were diagnosed after the birth of their infants have been reported as well. Although not ordered on a frequent basis by primary care clinicians, urinary calcium determination may have a significant impact on the diagnosis and treatment of some diseases. In the initial investigation of identifying the cause of abnormal calcium metabolism, a 24-hour urine collection for calcium, urine volume, and creatinine should be performed along with blood testing. The 2

methods most commonly used by clinicians to determine abnormality in renal excretion of calcium are measurement of 24-hour urine CE or calculation of the 24-hour urine calcium/creatinine excretion ratio (CR). Recent research, however, seems to indicate that these tests may not adequately identify hyperparathyroid patients. Instead, the calculation of the calcium/creatinine clearance ratio (CCCR), also known as the fractional excretion of calcium (FEca), may be a better method by which to identify the cause of abnormal calcium determinations of plasma calcium and creatinine along with the 24-hour renal excretions of calcium and creatinine and applying the following formula: (24-hour U-calcium/P-total calcium)/(24-hour U-creatinine/P-creatinine). A fasting urine calcium may also be useful in uncovering calcium overdose (increased absorption from the gut). If a 2-hour urine collection is obtained after a 14-hour fast, the urine calcium:creatinine ratio should fall to <0.15. If it is >0.15, metabolic/nephrogenic hypercalciuria is suspected.

Use in FHH and Primary Hyperparathyroidism: The prognosis and treatments differ significantly between FHH and primary hyperparathyroidism (PH). Familial hypocalciuric hypercalcemia is typically a benign disease requiring no treatment, whereas surgical intervention is required to treat PH in order to prevent long-term complications of hypercalcemia. Can urine calcium measurement help differentiate PH from FHH? Guidelines have been suggested stating that a CCCR of <0.010 implicates FHH, whereas a CCCR of >0.020 is highly suspicious of PH. Christensen and colleagues compared CCCR measurement with CE and CR in 54 patients with FHH (all with mutations in the CASR gene), and 97 hypercalcemic patients with histological confirmation of PH. They found the CCCR measurement was marginally better than CE or CR at differentiating the 2 diseases. At a cut-off point of <0.020, the CCCR index in their population included 98% of all patients with FHH but still included 35% of patients with PH. Although this cut-off still included some PH patients, the CCCR misclassified fewer PH patients than CR or CE. The authors concluded CCCR might be useful as an initial screening test for FHH, followed by CASR gene analysis for patients <0.020 to rule in/out FHH. Urinary calcium measurements may also play a role in identifying certain patients with osteoporosis who form kidney stones. Patients at risk for stone formation typically follow different treatment options for their osteoporosis. Researchers determined that measuring urinary CE in osteoporotic patients may help identify those patients with idiopathic hypercalciuria and calcium nephrolithiasis. As pointed out in their study, there are data to support the association between low bone density in nephrolithiasic patients with hypercalciuria but not in those without hypercalciuria. More importantly, the author points out a number of retrospective and prospective studies showing thiazides (which decrease urine calcium), have been associated with a reduction in fracture incidence, and an increase in bone density. Researchers suggest the majority of the patients in their study may suffer from a diet-independent form of hypercalciuria similar to that seen in patients with kidney stones and low bone density. They conclude that urinary CE should be measured in osteoporotic patients in order to identify this group of patients.

Use in Assessing Stones: In general, patients with calcium urolithiasis excrete more lithogenic substances (calcium and oxalate) in urine than non-stone formers. They also typically secrete less of the stone-inhibitory substances citrate and magnesium. Yet measurement of urine calcium is not considered a good predictive measurement for stone formation. Calcium-based risk markers for urolithiasis have been studied and include ratios such as the calcium/magnesium ratio, the calcium/citrate ratio, and the (calcium* oxalate)/(magnesium* citrate) ratio. Since stone formation is multi-factorial and inherently variable, currently neither calcium nor any other marker for urolithiasis risk is well accepted. Although urine calcium is not a decisive marker for stone formation, urine calcium measurement may play a role in identifying certain patients who form kidney stones due to the presence of systemic disease (particularly PH). Investigators compared laboratory features and outcomes of treatment in urinary stone-forming patients with hyperparathyroidism to those without systemic disease. They concluded that the hypercalciuria in PH patients who formed stones was greater than the hypercalciuria of the stone-formers without systemic disease. Thus, measurement of the CCCR might help discriminate stone-formers with PH from those with other disorders.

BOUQUET

In Lighter Vein

An older, white-haired man walked into a jewelry store one Friday evening with a beautiful young gal at his side. He told the jeweler he was looking for a special ring for his new girlfriend. The jeweler looked through his stock and brought out a $5,000 ring. The old man said, 'No, I'd like to see something more special.'
At that statement, the jeweler went to his special stock and brought another ring over… 'Here's a stunning ring at only $40,000' the jeweler said. The young lady's eyes sparkled and her whole body trembled with excitement. The old man seeing this said, 'We'll take it..'
The jeweler asked how payment would be made and the old man stated, 'by check. I know you need to make sure my check is good, so I'll write it now and you can call the bank Monday to verify the funds and I'll pick the ring up Monday afternoon,' he said.
Monday morning, the jeweler phoned the old man... 'There's no money in that account.'
'I know,' said the old man, 'But let me tell you about my weekend!'

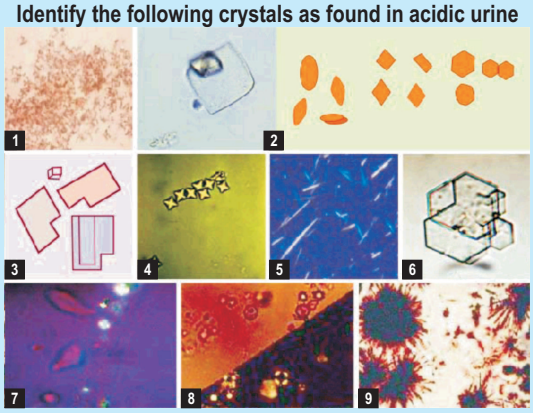
An army Major is visiting sick soldiers. He goes up to one private and asks:
"What's your problem, soldier?" "Chronic syphilis, sir"
"What treatment are you getting?" "Five minutes with the wire brush each day."
"What's your ambition?" "To get back to the front sir"
"Good man," says the Major.
He goes to the next bed.
What's your problem, soldier?" "Chronic piles, sir"
"What treatment are you getting?" "Five minutes with the wire brush each day."
"What's your ambition?" "To get back to the front sir"
"Good man," says the Major..
He goes to the next bed.
What's your problem, soldier?" "Chronic gum disease, sir"
"What treatment are you getting?" "Five minutes with the wire brush each day."
"What's your ambition?" "To get the wire brush before the other two sir!!"….

Wisdom Whispers

- "Sorrow dwells on the confines of pleasure."
- "A deaf auditor makes a crazy answerer."
- "Fortune does not stand waiting at any one's door."
- "Repentance for silence is better than repentance for speaking."
- "Once burned by milk you will blow on cold water."
- "A house can't be built for the rainy season that is past."
- "A cat may go to a monastery, but she still remains a cat."
- "Chastise the good man, he will grow better; chastise the bad, and he will grow worse."
- "Better good sale, than good ale."
- "He that hides is no better than he that steals."

Brain Teasers

Identify the following crystals as found in acidic urine



Answers: Crystals etc.: Usually found in acidic urine: (1) Amorphous urates, (2) Uric acid, (3) Cholesterol, (4) Calcium oxalate crystal (5) Sodium urate, (6) Cystine crystal (7) Fat droplets as seen in polarising light (8) Leucin spheres, and (9) Tyrosine needles.

TROUBLESHOOTING

QUALITY CONTROL IN THE NEW ENVIRONMENT: AUTOMATED HEMATOLOGY

(Contd. from previous issue)

Clinical quality control: Costs can also be cut in hematology if we eliminate or identify as far as possible the sources of results variation that occur before the specimen is analyzed. This really has more to do with quality assurance than quality control but should not be overlooked in our efforts to improve quality assurance and control costs. The causes of preanalytic variation range from patient-specific changes, resulting from physiologic, environmental, or pathologic conditions, to a multitude of variables during the process of obtaining and transporting the specimen to the analytic site. Specimen collection variables include choice of needles, collection systems, containers, anticoagulants, and transport conditions. Systematic variation is associated with specimen source-- earstick versus fingerstick, capillary versus venous--as well as specimen preparation. QC for new analyte additions: At first glance, new automated hematology instruments that offer additional analytes would seem to increase rather than save costs. After all, if you have to perform quality control on a parameter that did not exist before, it will cost you money. While that is true, new instruments do make cost savings possible in a number of ways--the much smaller amounts of reagents, for example, and the elimination of labor-intensive manual differentials. Let's look briefly at some of the latest analyte additions. Automated platelet counting: Automation of the platelet count and its incorporation into the routine hemogram are increasingly common on newer multiparameter instruments. These instruments are increasingly precise, although evaluation of specimens with moderate to severe thrombocytopenia is still an area of concern. Quality control for automated platelet counting has been simplified by stabilized whole blood control material. However, instruments using different technologies may perceive the stabilized or fixed platelets differently from fresh patient samples. Among the trilevel controls that many manufacturers offer, those with platelet counts within the normal and low ranges are most important for quality control monitoring. Smaller amounts of reagents and less labor account for the major cost savings from automated platelet counting. Red cell, platelet histograms, and related parameters: Another recent addition to hematology instrumentation is the graphic display of red cells and platelet volume histograms with related indices such as the red cell distribution width, the red cell morphology index, and the mean platelet volume. Used in conjunction with the MCV, these new parameters hold promise in the differential diagnosis of anemia and in identifying specimens that require careful review. In particular, the RDW is of use in the differential diagnosis of iron deficiency and beta thalassemia minor. An elevated RDW has been noted in association with hemoglobinopathies. Platelet histograms and the MPV offer information beyond a mere count of platelets in the blood specimen. For example, certain disease states such as chronic myelogenous leukemia are associated with platelets of abnormal size. Quality control is a problem in this group of parameters: Interpretation of the MPV is complicated by the fact that it varies inversely with the platelet count in normal individuals. Furthermore, Threatte et al have demonstrated significant changes in MPV resulting from use of different anticoagulants and the temperature at which the analysis is performed. Matrix problems with stabilized control materials also appear to be significant. These various studies highlighting MPV difficulties underscore the need for each laboratory to confirm its reference range with regard to instrument, reagent system, and type of anticoagulant used. It is possible that stabilized control material may be limited to providing comparison data only for a similar instrument-reagent combination. MPV may be best controlled by monitoring groups of patient specimens with similar platelet counts analogous to Bull's algorithm for the red cell indices. The same may be true of the red cell histogram and related parameters. It is unlikely that different levels of manufactured control material will show significant differences in the RDW and red cell histogram distributions since that might be costly to produce. Automated white cell differential: The manual 100-cell differential is a rather imprecise test, a fact that poses serious limitations to the clinical usefulness of the test. This conclusion is supported by recent clinical studies that question the inherent value of routine manual differential counts, both in the inpatient and outpatient settings. These studies and others, prompted at least in part by medical cost containment, lend support and impetus not only for reducing the number of manual differential counts performed but also for using the alternatives provided in the various automated methodologies. In general, automated differential instrumentation can be classified as either image analysis or flow analysis systems. Image analysis systems try to replicate the classic manual differential, although cell identification criteria are standardized and test precision is increased. Quality control procedures for these systems are relatively simple: The control material (stained slides) is not consumed by the analysis and can be used over and over to check the system. Image analysis systems are expensive and limited, however. They are also dedicated instruments and do not provide data on such other parameters as hemoglobin, hematocrit, platelet count, and total white cell count. For these reasons, they are not really suited to small or even medium size hospitals. Flow analysis systems, on the other hand, do provide additional hematologic data, and the required hardware and software can sometimes be added to present laboratory instrumentation via upgrades. These systems therefore offer many institutions a practical alternative to the labor-intensive manual differential. Various studies have estimated that 60 to 80 per cent of manual differentials could be replaced by such automated differentials without compromising patient care. Quality control for flow analytic systems is still in the formative stage. One possibility is to compare automated results with values obtained from the same patient specimens done manually. This is both expensive and tedious, however, since 400- to 500-cell differentials would be required on several samples to achieve meaningful precision for comparison purposes. The limited stability of white cell populations in the fresh state also raises an obstacle to periodic replicate analysis of patient specimens as control material for differentials. Stabilized quality control material for flow analytic systems is a relatively recent development and is still not perfected. An ideal control material compatible with all instruments may be extremely difficult if not impossible to produce. If this is the case, then interlaboratory comparison would be limited to similar instrument-reagent combinations. It is our opinion that the optimal quality assurance system for this area will involve the use of stabilized control materials; limited comparison analysis of patient samples using extended (400-cell) manual differentials coupled with clinical criteria to eliminate unnecessary manual differentials; and laboratory criteria, including instrument flage, review of histograms, and action limits to determine which patient specimen requires careful morphologic review of the blood smear. By eliminating unnecessary manual differentials and concentrating on those specimens that are abnormal or potentially abnormal, it is possible not only to achieve cost savings but also to increase the medical usefulness of this laboratory test.

TULIP NEWS

Printed and published by D.G. Tripathi, Edited by Dr. Ramnik Sood, M.D. (Path.) for and on behalf of Tulip Diagnostics Private Ltd., Gitanjali, Tulip Block, Dr. Antonio Do Rego Bagh, Alto Santacruz, Bambolim Complex Post Office, Goa - 403 202, INDIA.
Fax: (0832) 2458544. E-mail: sales@tulipgroup.com Website: www.tulipgroup.com