REX HEALTHCARE

Laboratory Bulletin...

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Rejection of unsatisfactory sputum specimens

Several key parameters have been identified in efforts to maximize the diagnostic yield from sputum cultures. Procurement of adequate sputum samples is an essential first step. To maximize the diagnostic yield of the sputum examination, only samples free of oropharyngeal contamination should be processed. The presence of alveolar macrophages does not alter the bacteriologic findings when substantial numbers of epithelial cells are present, indicating that otherwise adequate samples of sputum can be contaminated with oropharyngeal contents and thereby rendered non-diagnostic.[1]

The presence of squamous epithelial cells in sputum specimens indicates that the specimen is contaminated with saliva and oral flora. Since this oral flora can contain potential pathogens, the processing and reporting of these cultures can be misleading and can result in inappropriate therapy. Screening sputum specimens for squamous epithelial cells has become standard practice and should be used in every laboratory.[2]

In a retrospective study of sputum cultures at Rex Hospital, based on the criteria that if the number of epithelial cells exceeded the number of white blood cells, 214 of 970 specimens would have been considered unacceptable (22%).

As previously proposed in the April 1996 *Laboratory Bulletin*, to prevent the processing of inadequate contaminated sputum specimens, Rex Laboratory will begin rejecting sputum specimens based on screening by Gram stain on March 10, 1997. Sputum specimens will be rejected if the specimen contains greater than 25 epithelial cells per low power microscopic field or if the number of epithelial cells is greater than the number of WBCs. When specimens are rejected, a comment will be entered into the computer, "Specimen unsatisfactory, heavily contaminated with oropharyngeal material." Rejected specimens will be held 24 hours and physicians may contact the clinical microbiologist, Dr. Kleeman, or a pathologist if special circumstances warrant the processing of a rejected specimen.

In addition, the processing of more than 1 properly collected, satisfactory sputum specimen in a 24 hour period is not productive. If more than one sputum is submitted for culture in a 24 hour period, the additional specimen will be rejected.

If there are any questions or comments concerning these procedures, please contact Dr. Kleeman (783-3063) or a pathologist.

Karl T. Kleeman, Ph.D.
John P. Sorge, M.D.

Activated Protein C resistance (factor V Leiden)

[1] From *Principles and Practice of Infectious Diseases* Mandell, Douglas and Bennett, 4th edition, 1995.
[2] "Clinically Relevant, Cost-Effective Clinical Microbiology," Michael L. Wilson, MD, *American Journal of Clinical Pathology*, February, 1997.

Introduction

Activated Protein C (APC) resistance is a hypercoagulable condition that is inherited as an autosomal "dominant" gene and predisposes individuals to deep vein thrombosis (DVT). The phenomenon of resistance to activated Protein C was discovered in 1993 by Dalhback in Sweden. The middle-aged man that was identified by Dalhback had a personal and family history of thrombosis. Approximately one year later, the molecular defect was identified as a point mutation in coagulation factor V, commonly known as factor V 'Leiden' mutation. The abnormality is caused by the substitution of a single amino acid (glutamine for arginine) at position 506 in the coagulation factor V molecule. The mutation explains more than 90% of the APC resistance cases and renders factor V resistant to the natural anticoagulant activated Protein C. This resistance of factor V to the anticoagulant effects of Protein C causes the coagulation cascade to generate excess thrombin predisposing the patient to DVT.

Prevalence

APC resistance is present in 5% of the Caucasian population (rare in Asians and African Blacks) and is found in greater than 20% of all cases of DVT. In cases of DVT where there is a family history, it is found in 50%. It is found in 60% of pregnancy associated thrombosis and approximately 60% of women who experience thrombosis while on oral contraceptives. In APC resistant patients, the risk of recurrent DVT or pulmonary embolus exceeds 30% after eight years. In patients with recurrent DVT, four inherited disorders will explain thrombosis in at least half of these patients (Table 1).

Table 1. **ESTIMATED PREVALENCE OF INHERITED THROMBOTIC DISORDERS**

Disorder	***Prevalence***
Antithrombin III deficiency	1 - 4 %
Protein C deficiency	5 - 6 %
Protein S deficiency	5 - 6 %
APC resistance (factor V Leiden)	20 - 60 %

Individuals with APC resistance have a sevenfold increased risk of venous thrombosis. The inheritance is common and a moderate risk factor for thrombosis. When combined with Protein C, Protein S or antithrombin III deficiency, the risk for thrombosis is markedly increased.

Lab testing

The recommended method of diagnosing APC resistance is to test the patient while off anticoagulants. The measurement of APC resistance can be done in two ways: by a modified activated partial thromboplastin time (aPTT) or DNA analysis. The modified aPTT method compares the aPTT of patient's plasma with and without added activated Protein C. The aPTT result (in seconds) from the sample with added APC is divided by that obtained in the absence of exogenous APC to yield the APC ratio. An APC ratio between 2.4 and 4.0 is normal. Those with a ratio of less than 2.4 (when the baseline aPTT is normal) should be verified by another independently collected sample.

Lab testing while on anticoagulants (coumadin and heparin)

The FDA has recently approved a modification of the coagulation-based APC test for patients while receiving heparin or coumadin. The modified method adds exogenous factor V deficient substrate to the plasma. It will soon be commercially available for

routine use.
The DNA test for factor V Leiden can also be done while the patient is on anticoagulants. DNA-based testing is more expensive but may help to confirm or exclude hereditary APC resistance; however, not all persons with hereditary APC resistance have a demonstrable genetic defect. These patients without the factor V Leiden defect have an abnormal coagulation-based APC ratio but a different point mutation in the factor V molecule.

Management decisions
Although DVT increases with age, most physicians would not subject an APC resistant patient to life-long anticoagulation therapy after the first DVT. Asymptomatic persons with APC resistance should receive prophylactic intervention only when clinical thrombosis risk factors are present (for example, consideration of perioperative short-term anticoagulation or other measures). It is prudent to give prophylactic anticoagulation during surgery and pregnancy if a patient has had a previous thrombosis. Birth control pills should be avoided. Those patients with APC resistance and a second hypercoagulable risk factor such as Protein C deficiency should receive anticoagulation therapy. The recommended therapeutic range for coumadin anticoagulation for APC resistance is a prothrombin time with an INR (international normalized ratio) of 2.0 to 3.0. In all patients it is essential to balance the protective effect of anticoagulant therapy against the risk of inducing bleeding.

Future considerations
Should patients be screened preoperatively for hypercoagulable states when a high risk procedure is planned (e.g. joint replacement surgery)? Should patients with a family history of thrombosis or obesity be tested prior to prescribing birth control pills? Would subtherapeutic coumadin therapy (prothrombin time with a INR <2.0) decrease the incidence of a second DVT and avoid the undesirable bleeding complications in patients with APC resistance? Currently there is no data to answer these questions. The importance of a detailed medical history in evaluating patients with thrombosis cannot be overemphasized. A consultation with specialists in hematology or vascular medicine may be helpful in treating individuals with APC resistance and in identifying and counseling family members.

Stephen V. Chiavetta, M.D.

References

[1] Simioni P, Orabdibu P, et. al. "The Risk of Recurrent Venous Thromboembolism in Patients with an Arg^{506}-Gln Mutation in the Gene for Factor V (Factor V Leiden)." *N Engl J Med* 1997:336:399-403.
[2] Nichols W and Heit J, "Activated Protein C Resistance and Thrombosis," *Mayo Clinic Proceedings* 1996; 71: 897-898.
[3] Rogers G, "Activated Protein C Resistance and Inherited Thrombosis (editorial)," *Am. J of Clinical Pathology* 1996; March, page 261- 262.
[4] Keeling D, (Hematologist @ Churchill Hospital, Oxford) "Resistance to Activated Protein C Due to Factor V R506Q (Factor V Leiden)," unpublished on the internet...jr2.ox.ac.uk/Bandolier/bandopubs/keeling.htm.
[5] DiGiuseppe J A, "Laboratory Evaluation of Hypercoagulable States," *Clinical Laboratory News*, Oct. 1995, p.8 - 13.
[6] Stylianos K Quehenberger P et. al., "Improved Characteristics of APC-Resistance Assay," *Am J of Clinical Pathology*, 1996 Vol 106 No. 5 p. 588-593.
[7] Rosendaal F R Koster J P Vandenbroucke J P and Reitsma P H, "High Risk of Thrombosis in Patients Homozygous for Factor V Leiden (Activated Protein C Resistance)," *Blood*, 1995; 85:6 p 1504 - 1508.

ASCP

Rex Laboratory will be hosting an ASCP teleconference on March 26 at 2PM in the Laboratory Conference Room. The title is "Does Your Phlebotomy Service Have

phlebotomy teleconference scheduled for March 26

What It Takes to Satisfy Patients?" It will be presented by Jane C. Dale, M.D., who is from the Department of Laboratory Medicine and Pathology at the Mayo Clinic. Dr. Dale plans to cover many of the factors known to be correlated with patient satisfaction, including business, patient, and health care provider characteristics. Results from several national surveys of patient satisfaction with phlebotomy are presented and opportunities for improvement are suggested. The teleconference should last approximately 1 1/4 hours and carries 1.0 CME/CMLE credit hours. There is no charge for attendance. If you or someone from your office is interested in attending this teleconference, please contact Karen Sanderson at 783-3396.

Karen Sanderson, MT(ASCP),SC

Diarrhea in hospitalized patients

It has been reported and verified in several studies that the yield for stool O&P examination and for stool cultures on hospitalized patients beyond three days after admission is very low.[1] It is now standard procedure for hospital laboratories to reject stool specimens collected after the third day of hospitalization.[2]

Clostridium difficile is now considered the primary cause of diarrhea in hospitalized patients. As a result of antibiotic or other drug treatment, these organisms can overgrow the normal flora of the intestine and produce a toxin which induces the diarrhea. Clinical data associated with *C. difficile* infection include a hospital stay longer than 15 days, onset of diarrhea more than 6 days after the initiation of antimicrobial therapy, use of a cephalosporin, semiformed (not watery) stools, and the presence of fecal leukocytes.[2] The toxin can be detected by an EIA procedure performed by the laboratory. It is recommend that two stools be submitted, collected on successive days to provide adequate sensitivity for the detection of the toxin. Since diarrhea is an important clinical finding, fully formed stools are not productive.

As of April 1, 1997, Rex Laboratory will no longer accept stools for O&P examination or for routine stool culture after the third day of hospitalization. If submitted, these specimens will be held for 24 hours. If special circumstances warrant the processing of these specimens, Dr. Kleeman, the clinical microbiologist, or one of the pathologists must be notified.

If you have any questions or comments concerning these procedures, contact Dr. Kleeman at 783-3063 or one of the pathologists.

Karl T. Kleeman, Ph.D.

[1] "Inappropriate testing for diarrheal diseases in the hospital," Siegel DL, Edelstein PH, Nachamkin I.; *JAMA*, 1990; 263;979-982.

[2] "Clinically Relevant, Cost-Effective Clinical Microbiology," Michael L Wilson, MD; *American Journal of Clinical Pathology*; February, 1997; 107; 154-167.

For further information, call the Laboratory (783-3040). Telephone extensions are: Dr. Benson (3059), Dr. Brainard (3056), Dr. Carter (3058), Dr. Chiavetta (3040), Dr. Kanich (3057), Dr. Kleeman (3063), Dr. Nance (3286), Dr. Sorge (3062), Barbara Wetherbee (Director 3055), Robin Ivosic (Core Lab Manager 3053), Linda Lompa (Blood Services Manager 785-4770), Kimberly Skelding (Customer Services Manager 3318), Rex Outreach (783-3040), Karen Sanderson (Lab Compliance Specialist 3396).