REX

Laboratory Bulletin

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Updates and Information from Rex Pathology Laboratory

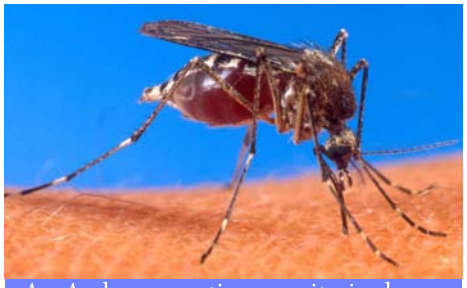
Issue Number 92

West Nile Virus...
The bugs are back in town!

As local backyard grillmeisters can attest, the mosquitoes are back – and ready to resume their role as vectors for a number of pathogenic viruses. Chief among these is West Nile virus (WNV), a flavivirus discussed in detail in an earlier Lab Bulletin. [1] Birds serve as the primary reservoir for the virus. Mosquitoes that feed off of an infected bird and subsequently transmit the virus to another bird spread the disease. Humans and horses are regarded as dead-end hosts, although human-to-human transmission through blood transfusion has been documented. Coincident with mosquito activity, cases generally begin appearing in June and generally peak in August and September. There were 24 documented cases of WNV infection in North Carolina last year, including three in Wake County (two were believed to be acquired by travel outside the county). [2] No cases have been confirmed thus far in 2004 as of this writing. In 2003, the Centers for Disease Control reported a mortality rate of 2.7% (264 deaths of 9862 cases).[4] There were two fatalities reported from North Carolina.

WNV infection in humans has an incubation period of three –14 days. A viremia occurs five – six days prior to the onset of symptoms and may persist for one - two days after clinical illness develops. Many WNV infections are mild and may be clinically inapparent.[3] Approximately 20% develop a mild illness (West Nile fever) characterized by sudden onset of fever and "flu-like" symptoms including headache, myalgias, arthralgias, nausea, malaise, skin rash and lymphadenopathy lasting three - six days.[3] Severe neurologic manifestations of encephalitis, meningitis, muscle weakness, visual disturbances or acute flaccid paralysis occur in a small minority of (generally elderly) patients. These patients have an increased risk of mortality. General laboratory findings are minimal and nonspecific, but may include normal or elevated WBC, lymphocytopenia, anemia, CSF pleocytosis, elevated CSF protein with normal glucose, and hyponatremia (in patients with encephalitis).[3]

The diagnosis should be suspected in all patients with unexplained encephalitis or meningitis, particularly in patients over 50 years of age who present in the summer or early fall with acute onset of symptoms. Local WNV activity or recent travel to an area with known WNV infections should increase suspicions further. Confirmation of the diagnosis is best accomplished by serologic testing. Serum and CSF for WNV are referred to Mayo Medical Laboratories for WNV Antibody determination (MML test code 8416, cost $125) using the ELISA technology. The ELISA test looks for both IgM and IgG antibodies and results are reported as "positive" or "negative". IgM antibodies are generally detectable by the eighth day of clinical illness and persist for one - two months. The presence of IgM antibody suggests acute infection with WNV. If there is a high degree of clinical suspicion of WNV and the IgM antibody is negative, a second specimen should be obtained 14 days after the onset of clinical illness.[5] Detectable CSF IgM antibody suggests active CNS infection (in the absence of a traumatic tap). IgG antibodies are generally detectable by three weeks after the onset of clinical illness and may persist for months. Paired specimens (e.g. separated by one – two weeks) may be helpful in determining if the presence of IgG antibodies indicates recent or remote infection.



An Aedes aegypti mosquito is shown on human skin.[11]

While both assays are relatively (> 95%) sensitive, there are some pre-analytic factors that must be taken into account to improve test performance. The significance of negative results in immunocompromised patients is uncertain.[5] False positive results may occur in patients who have received blood or blood products, vaccinations for other flaviviridae (e.g. yellow fever, Japanese encephalitis, dengue fever), or individuals previously infected with other flaviviridae. A careful medical history (including travel history), selection of patients with appropriate symptoms as described above (cf. screening of asymptomatic patients) coupled with the knowledge of WNV activity in the local community can go a long way in excluding these possibilities and improving the predictive value of a positive result. Use of paired specimens as described above may be helpful in patients

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where the clinical findings don't match the clinical impression.[6] A helpful resource in tracking WNV activity in NC can be found at a web site showing counties where WNV infected birds or humans have been found.[7]

Three other laboratory tests deserve brief menton. WNV has been successfully cultured, but this is of little practical benefit, in part because the viremia is largely over by the time most patients present for medical attention with symptoms. Reverse-transcriptase polymerase chain reaction (RT-PCR) can detect the viral RNA in very early phases of illness, and has been used primarily to test blood or other body tissue prior to transfusion/transplantation. RT-PCR testing of serum in suspected acute infections is of limited utility as only 10% of samples will be positive, those collected very early after the onset of symptoms.[8] RT-PCR testing of CSF may be helpful in evaluating patients suspected of having CNS infection with WNV. This test is available through Mayo Medical Laboratories (MML test code 91283, cost $216) although the test is actually performed at Focus Technologies (formerly known as Microbiology Reference Laboratory). Finally, there is the West Nile Virus Plaque Reduction Neutralization Test (PRNT). This test (MML test code 91333, cost $275) is useful primarily in helping to determine if an ELISA positive result is due to WNV or some other type of flavivirus. It is useful in for epidemiologic studies, but has several limitations. It does not distinguish between IgM and IgG, it is cumbersome and laborious – requiring active cell cultures and careful titration of the test virus, and it may take up to 30 days to complete.[9] For these reasons, it is not recommended for use on a routine basis.[6,7] As noted above, the ELISA serologic test for WNV performs well if used appropriately and correlated with available clinical and epidemiologic information.

There is no specific treatment for WNV infection, but supportive measures (IV fluids, ventilatory assistance, and prevention of secondary bacterial infection) can be helpful in severely ill patients. The best disease management is prevention - both by mosquito control efforts and minimizing exposure to mosquitoes. The latter can be facilitated by use of long sleeved shirts, long pants and DEET (N,N-diethyl-m-toluamide) and staying indoors at dawn and dusk (if possible). There are several web sites that may be of interest to physicians and patients interested in learning more about the subject.[2-4,7,10]

Flame on!
John D. Benson, MD

References:

1. *Sorge JP. West Nile Virus. Rex Healthcare Lab Bulletin. Issue 73, Oct. 2002.*
2. *http://www.deh.enr.state.nc.us/phpm/wnv/Data_and_Maps/Human/human.html*
3. *http://slph.state.nc.us/VirologySerology/Arbovirus/cdc_WestNilesFactSheet.pdf*
4. *http://www.cdc.gov/ncidod/dvbid/westnile/surv&controlCaseCount03_detailed.htm*
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6. *Thomas Smith, PhD, Mayo Medical Laboratories; personal communication 6/22/2004.*
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10. *http://www.epi.state.nc.us/epi/arbovirus/wnv.html*
11. *Judy McBride, Better Mosquito, Tick Repellents in the Wind? USDA Research Service article, January 3, 2002 - (Mosquito photo) http://www.ars.usda.gov/is/pr/2002/020103.htm*

Vaginal Lubricants Do Not Affect the Quality of Surepath™ PAP Specimens

Dave Shepard preparing liquid media pap smear slides.

The use of gel lubricants can help alleviate discomfort associated with vaginal speculum examinations. Some clinicians and pathologists have speculated that if the lubricant material inadvertently contaminates the Pap specimen, a less than optimal or unsatisfactory result might ensue. Theoretically the lubricant could interfere with a conventional smear if it were smeared on the slide and obscured visualization of the cells or prevented their adherence to the slide. For the newer, liquid-based Pap techniques, the presence of the lubricant in the specimen container could lead to lack of cell adherence.

Recent studies of the effects of lubricant on conventional Pap smears indicate that the use of a small amount of water-soluble gel lubricant on the outer aspect of the speculum does not adversely affect cervical cytology results[1] but that the use of a large amount of lubricant or inadvertent smearing of the lubricant on the slide can obscure cellular detail[2]. This latter study also noted that lubricants interfered with specimen quality if the ThinPrep® Pap Test is used but did not affect the results of SurePath™ Pap specimens. The lubricant produced a flocculent material which adhered to the filter membrane during ThinPrep® processing and prevented cells from adhering to the ThinPrep® slide. Since the SurePath™ Pap method uses a density gradient which excludes blood, mucus, and extraneous material such as lubricants, their preparations were unaffected by the presence of gel lubricant in the specimen container. At Rex we use the SurePath™ method and have been pleased with the uniform quality of the specimens. Anecdotally, we have attempted to spike SurePath™ specimens with large amounts of a variety of potential lubricants including KY Jelly, petroleum jelly and spermicides. None of these materials survived the density gradient and thus none adversely affected Pap specimen quality.

Keith V. Nance, MD

References:

1. *Amies AM, et al. The Effect of Vaginal Speculum Lubrication on the Rate of Unsatisfactory Cervical Cytology Diagnosis. Obstet. Gynecol 2002;100:889-892.*

Revisions to Surgical Pathology Cancer Synoptic Reports

Dr. John Sorge and I introduced synoptic-style surgical pathology reports for oncologic resections at Rex 19 years ago, in collaboration with the Depts. of Surgery, Obstetrics & Gynecology, Radiation Oncology, and local medical oncologists. Our intention was to provide standardization, assure inclusion of information critical to patient management, and provide a relatively short, readable but complete report. Over the years Dr. Sorge has spent considerable time and effort maintaining and revising the synoptic templates, including through several different anatomic pathology information systems (beginning with the one created by the venerable Dr. Albert Chasson.) The revisions reflected changes in disease classification (e.g. Van Nuys Prognostic Index for mammary intraductal carcinoma) or management (e.g. Her-2-neu over-expression in mammary carcinoma) and were incorporated with the support of the Rex pathologists and appropriate members of the medical staff.

The College of American Pathologists began developing guidelines for surgical pathology reporting several years ago. Many of the original guidelines included data elements that were of debatable clinical significance (e.g. "pushing" vs. "infiltrative" tumor margins, character of "host lymphocytic response" to tumor). Not surprisingly, many pathologists were reluctant to adopt them. The College's position was the guidelines were simply that, not standards, which had to be incorporated into pathology reports.

A year ago the American College of Surgeons Commission on Cancer (ACS CoC) decided to adopt the CAP guidelines as "essential data elements" that must be included in pathology reports for accreditation by the ACS CoC as a cancer treatment center. After a hue and cry in the pathology community, the CAP and ACS delayed implementation and restudied the issue.[1] The CAP revised the guidelines and trimmed away data elements that were not considered "essential" in creating the new cancer protocols. The ACS moved back the implementation date from January 2004 to July 2004. Reports will not be formally checked against the protocols until July 2005, at which time reports from the preceding 12 months will be subject to review. The ACS CoC also indicated that the protocols would apply only to oncologic surgical resections only (cf. diagnostic biopsies or needle aspirations). Finally both the CAP and ACS agree that pathologists are free to incorporate the "essential data elements" into whatever format they choose. There is no universal pathology report format.

For the past two months, Dr. Sorge and Dr. Steve Chiavetta have been comparing the revised CAP protocols with the Rex synoptic templates.[2] In order to make the transition as easy as possible, any new "required" data elements will be added to our current templates. For the most part, very few changes are necessary. Some of the changes don't appear sensible to us, but we will do our best to comply with the new protocols to assist with Cancer Center accreditation. In addition there are certain data elements not required by the new protocols, but which we will continue to provide for the reasons stated above.

Surgeons may also be affected the new protocols. In particular, the ACS CoC is asking for more detailed tumor location than is often provided in our current reports. The more common examples would be breast "lumpectomies" (what quadrant is the tumor in?) and colon segmental resections (without obvious landmarks). Accordingly, it would be helpful for (all of) us if this information (anatomic tumor location) could be included in excisional biopsies or subtotal excisions of any organ for neoplastic disease.

We plan to roll out the revised templates sometime in July. Comments or concerns are welcome and should be directed to Dr. Sorge or Dr. Chiavetta.

John D. Benson, MD

Reference

1. *Paxton A. Cancer protocols: leaner, later, more lenient. CAP Today 18(6), 58-66, June 2004.*
2. *http://www.cap.org/apps/docs/cancer_protocols/protocols_index.html*

Dr. Chiavetta and Dr. Sorge discussing surgical pathology synoptic reporting.

Instructions for Outpatient Glucose Tolerance Testing

Enclosed in this issue of the Bulletin is a set of instructions for Outpatient Glucose Tolerance Testing at Rex.

Feel free to reproduce this for use in your office. Originals can be obtained by contacting Kori Horsely (Rex Outreach) at (919) 784-4340.

D-Dimer Assay Change

Rex Hospital Laboratory has improved the method of assaying plasma for the D-Dimer. The clinical usefulness of a D-Dimer result is in its negative predictive value. A negative result is very helpful in ruling out thrombosis. A positive result is not helpful in making the diagnosis of thrombosis. Positive results are seen in many hospitalized patients including the elderly, the pregnant, those with cancer or burns, and those in the postpartum or postoperative period. These patients have a hypercoagulable state and subclinical fibrinolysis. A negative D-Dimer test in these patients is uncommon. The SimpliRED test is a manual qualitative method we have used for years. The laboratory has changed to an automated quantitative method for reporting the D-Dimer. The quantitative method is more reproducible because it eliminates subjective interpretation by the technologist.

A value less than 1.0 g/ml is considered negative for the D-Dimer. Although the literature[1] has reported a value of less than 0.5g/ml, a chart review of 50 patients at Rex Hospital showed numeric values less than 1.0 g/ml as the most useful for ruling out thrombosis. The D-Dimer is available 24 hours a day and requires blood anticoagulated with sodium citrate. The reference range is 0.0 to 0.99 g/ml. A text footnote is attached to the numeric result of the D-Dimer that interprets the numeric result as positive if equal to or greater than 1.0 g/ml.

Stephen V. Chiavetta, MD

Reference:
1. *Oger, E., Leroyer, C., et. al., "Evaluation of a New Rapid and Quantitative D-Dimer Test in Patients with Suspected Pulmonary Embolus", Am. Journal of Respiratory and Critical Care Medicine, Vol. 158, 1998, p 65-70.*
2. *Chiavetta, S., "D-Dimer Case Study", Rex Laboratory Bulletin, Issue 81, June 2003 page 3 – 4.*

Fabrianne Saunders operating a Coag analyzer.

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Pictured right:
Jimmy Ashley

Pictured left:
Steve Pittman

Pictured left to right: Jeff Eason, Steve Pittman, Baxter Walker, Roy Ford, Horace Walker, Delores Smith, Jimmy Ashley, Pat Wells, Harold Desanty, Alan Wood and Vann Marshall.

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