

Lab Focus

March 2001—periodic insert to 'Scope from Fairview Clinical Laboratories

Sound Bites . . .

Rapamycin Assay Now Available

The Drug Analysis Laboratory at Fairview-University Medical Center (F-UMC) now offers Rapamycin (Rapamune®, sirolimus) testing.

The FDA has approved the immunosuppressive drug, rapamycin, administered in combination with cyclosporine or tacrolimus and other immunosuppressive agents, for use in transplant patients.

Transplant patients are discharged from the hospital with kits to send future blood collections to F-UMC for analysis of cyclosporine or tacrolimus in combination with rapamycin.

Contact Cindy Johnson at 612-273-5059 or Michael Tsai, PhD at 612-626-3629 with questions.

Turnaround Time: Assayed twice per week, results are reported the next day.

Collection: 3 mL, 1.2 mL minimum in purple (EDTA) tube. Collect trough level prior to next dose.

Methodology: Solvent extraction; high performance liquid chromatography (HPLC).

Free Protein S Replaces Total Protein S

Effective immediately, The F-UMC Special Coagulation Laboratory will report free Protein S, the active part of the Protein S molecule instead of total Protein S. Total Protein S will continue to be available upon request.

Reference Ranges:

Free Protein S

Males: 75-135% of normal

Females: 65-125% of normal

Total Protein S

Males: 75-135% of normal

Females: 65-125% of normal

Agnes Aysola, MD, F-UMC

Changes to Lupus Inhibitor Testing

The F-UMC Special Coagulation Laboratory has added a second clotting test, DRVVT (Dilute Russell's Viper Venom Time), to the Lupus Inhibitor protocol to be consistent with recommended practice.

Previously PTT 1:2 mixing studies and Platelet Neutralization were performed as indicated. Effective immediately, if the aPTT is normal, the mixing study will no longer be performed. The occurrence of lupus inhibitors in patients with normal aPTT's is extremely rare and clinically insignificant.

The **DRVVT Screen**, performed on every patient, includes the ratio of the patient screen to normal standard reference plasma. If this ratio is 1.20 or greater, the **DRVVT mixing study** is performed on a 1:2 mixture of patient and normal plasma to rule out factor 2, 5, or 10 deficiency. This DRVTT mixing study is again compared to a standard reference plasma and the ratio determined.

If the ratio of this mixing study is 1.20 or greater, a **DRVVT Confirm** is performed on the patient and the standard reference plasma. The normalized ratio of patient ratio/standard ratio is determined. If this is 1.20 or greater, the patient has a lupus inhibitor.

Agnes Aysola, MD, F-UMC

Human Papillomavirus Testing as an Aid in the Early Detection of Cervical Carcinoma

Reprinted from the Dec. 2000 issue of 'Scope.

Introduction

Fairview-University Medical Center is pleased to offer new testing to aid in the management of patients with the Papanicolaou (Pap) smear diagnosis known as ASCUS (atypical squamous cells of undetermined significance). The management of patients with ASCUS is

“one of the leading causes of headaches” in gynecological medicine – as well as a huge cost burden.¹ In helping the clinician identify which patients with ASCUS need immediate colposcopy and which may be followed conservatively, human papillomavirus (HPV) testing can bring a new level of clarity to an often unclear diagnosis. HPV testing may also decrease repeat Pap examinations, diminish patient anxiety, minimize the number of high-risk cases lost to follow-up, and decrease the average cost of working up an abnormal Pap smear – in short, it may allow clinicians to provide better patient care.¹

Background

Carcinoma of the cervix is one of the most common malignancies in women, accounting for 15,700 new cases (6 percent of all cancers) and 4,900 deaths in the United States each year. However, since the implementation of the Pap smear in the 1950s, the number of deaths due to cervical carcinoma has decreased by over 70%.² Clearly, the Pap smear is an excellent screening tool for detecting high grade cervical lesions and preventing cervical carcinoma.

For most Pap smear diagnoses, treatment is relatively clear-cut, consisting of either routine follow-up, or colposcopy with biopsy. In contrast, the diagnosis of ASCUS can pose a management dilemma. Most patients with ASCUS have no significant pathologic abnormalities, but an estimated 5 to 10% of patients harbor high-grade dysplasia or carcinoma.³

Efficient triage of ASCUS cases is critical – yet clinicians have had no way to determine which cases of ASCUS will regress without treatment, and which will progress to dysplasia or carcinoma. The annual cost of managing patients with ASCUS is estimated at \$3.6 billion to \$6 billion, much of which may be needless expense.¹ Worse still are the costs to the patient: emotional strain, unnecessary clinic visits and procedures, and loss to follow-up.

The single most important risk factor for the development of cervical carcinoma is long-term HPV infection. Infection with high-oncogenic-risk HPV (types 16, 18, 31, 33, 35, 39, 45, 51, 52, and 56, 58, 59

and 60) is known to cause cervical dysplasia and carcinoma, whereas infection with low-risk HPV (types 6, 11, 13, 42, 43, and 44) is associated with benign cervical lesions, such as condyloma acuminatum, but rarely, if ever, cervical carcinoma. For these reasons, HPV DNA testing can be very useful in the context of ASCUS. In a recent large-scale study conducted by the National Cancer Institute, HPV DNA testing identified about 96 percent of the cases of ASCUS that were found to harbor moderate to severe dysplasia on biopsy.⁴ Approximately 5 percent of the patients with ASCUS diagnoses were found to have underlying dysplasia or invasive carcinoma; HPV testing detected virtually all of these high-risk patients.⁴

HPV DNA typing has been shown to be extremely sensitive in identifying which patients with ASCUS harbor high-grade dysplasia or carcinoma. The presence of HPV alone is not absolutely predictive of dysplasia or carcinoma due to the high infectivity – and high clearance – of the virus. However, the transient sort of HPV infection is thought to involve primarily low-risk DNA types, whereas the persistent sort of HPV infection involves the carcinogenic, high-risk DNA types. Knowledge of the patient's HPV DNA type enables clinicians to identify those patients at increased risk for developing high-grade lesions, yet not overtreat unaffected patients.

The Test

The Molecular Diagnostics Laboratory at Fairview-University Medical Center performs HPV testing using a two-tiered approach. First, extracted specimen DNA is amplified using the polymerase chain reaction (PCR) to determine whether HPV DNA is present. Then, the PCR products are digested with restriction enzymes, and electrophoresed on polyacrylamide gels to identify the specific type(s) of HPV DNA present. Using this method, HPV testing may be performed on a wide variety of specimen types, including: Autocyte™ Pap smear specimens; cytobrush residua following routine Pap smear procurement; fresh or frozen tissue specimens; archival tissue samples, including paraffin-embedded biopsies; and fixed and stained Pap smears. The average turnaround time is five to ten days.

We have chosen to perform HPV testing using a PCR-based method, rather than a

hybrid capture method (such as that marketed by Digene corporation) for a several reasons. Foremost, PCR-based HPV testing identifies the specific type of HPV DNA present, whereas hybrid capture testing detects only the presence of low-risk or high-risk groups. The higher resolution of the PCR-based method may heighten test specificity, and the added knowledge of specific HPV type may be clinically useful. The sensitivity of the PCR-based method is also superior, showing a ten-fold increase over that of hybrid capture methods.

In addition, PCR-based HPV testing may be performed on a the wide variety of specimen types listed above, whereas hybrid capture methods are limited to thin-preparation Pap smear specimens.

PCR testing for HPV DNA can detect as few as 500 viral particles per specimen. The negative predictive value of this test is estimated to be greater than 99.9%: **if a patient's HPV test result is negative for high-risk HPV DNA, the chance she harbors high-grade dysplasia or carcinoma is less than 0.1%.**

In addition, PCR testing is highly specific for the detection of HPV. The natural course of high-risk HPV infection involves an indeterminate period of latent infection, frequently followed by dysplasia and then carcinoma. Consequently, some patients in the latent infection period may test positive for HPV but show no current abnormalities on biopsy. Large-scale evaluation will clarify the number of patients with high-risk HPV infection who later develop dysplasia or carcinoma; presently, studies suggest that this percentage will be very high. Accordingly, **patients with high-risk HPV infection should be followed more closely than HPV-negative patients, as abnormalities are more likely to develop**

Interpreting Results

The greatest usefulness of HPV testing is in those cases in which the screening or diagnostic Pap smear diagnosis is equivocal – particularly in cases of ASCUS. In addition, testing may be ordered on specimens other than Pap smears – such as biopsy specimens – to help interpret equivocal results.

A useful patient management algorithm integrating Pap smear diagnoses and HPV results is shown in figure 1. The absence of HPV (or the presence of low-risk HPV

types) is a very reliable predictor of the absence of dysplasia in the setting of ASCUS. A negative HPV result in this setting allows the clinician to follow the patient with repeat Pap screening. A positive HPV result may indicate that the patient is at high risk for harboring dysplasia or malignancy; colposcopy with directed biopsy is suggested in this group of patients.

An added benefit of performing HPV testing on the same specimen collected for Pap screening is that **clinicians may make an informed triage decision based on the initial Pap smear specimen, without calling the patient back into clinic for additional specimen collection.**

Conclusion

HPV testing is a valuable aid in the management of patients with ASCUS. By providing a new degree of certainty to an often uncertain diagnosis, HPV testing can reduce unnecessary clinic visits and procedures, alleviate patient anxiety, and minimize loss of high-risk patients to follow-up. Furthermore, tailoring management to the individual patient can significantly reduce the cost of working up an ASCUS diagnosis. In our limited arsenal against cervical cancer, HPV testing is a helpful, new weapon.

Contacts

For more information, please contact Klint Kjeldahl, cytology supervisor, at 612-273-4136, or David Olson, molecular diagnostics laboratory supervisor, at 612-273-8445, or visit our website at www.fairview.org/hpv.

References:

1. Titus K. HPV test: antidote to ASCUS headache? CAP today, September 1996, pp. 1, 22, 52-58.
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