HPV TYPING AS AN ADJUNCT TO THE ASCUS PAP SMEAR

Human Papilloma Virus (HPV), in addition to being the causative agent for common warts and genital warts, is closely linked to the risk of developing cervical cancer. It is estimated that greater than 90% of cervical carcinomas are associated with HPV infection. At least 20 million Americans harbor genital infections with this sexually transmitted virus. The great majority of those infected are in the reproductive age group. There are numerous HPV subtypes that have been associated with cervical infection. In addition, recent studies have grouped many of these subtypes into low, intermediate, and high-risk groups for their association with cervical cancer. Certainly not everyone with the high-risk subtypes develop cervical cancer as there are thought to be numerous cofactors involved in the development of the disease. These include tobacco use, coexistent herpes virus infections, and, possibly, dietary factors. Recently, oral contraceptive use (The Lancet 2002;359;1085-1092.) and multiparity (The Lancet 2002;359;1093-1101.) have been established as cofactors associated with the development of cervical cancer in high-risk HPV positive women. It should also be noted that in many women HPV infections are cleared over time by the individual’s immune response.

The Pap smear, which has been used since the 1940’s, has led to a 70% decrease in deaths due to cervical cancer. HPV cytopathic changes can often be well visualized on the Pap smear and thus the Bethesda system for Pap smear reporting includes the designation, HPV Effect, under the category of epithelial cell abnormalities. In the past the presence of HPV effect on Pap smears was often reported synonymously as koilocytotic atypia or condylomatous atypia. Unfortunately the Pap smear changes that allow diagnosis of HPV infection are not present in all cases and, when present, do not allow for stratification into low or high-risk for the development of cervical cancer. In addition there is the problem of what to do with cases of Pap smears with a diagnosis of Atypical Squamous Cells of Uncertain Significance (ASCUS).

Within the past several years three new tests, each touted as either adjuncts to or replacements for the traditional Pap smear, have received FDA approval. The first category includes two of these tests, Thinprep and Surepath (Autocyte Prep) which are each liquid-based Pap smears that produce a thin layer or monolayer preparation. This methodology utilizes a liquid based medium for the preservation and transportation of Pap specimens as opposed to the traditional technique where the specimen is simply smeared on a glass slide. Several studies have shown an increased sensitivity for the detection of cervical dysplasia when the thin layer method is used. In addition, the thin layer fluid specimen can be retained for possible additional studies including HPV typing. The second type of test to receive FDA approval, as an adjunct to the traditional Pap specimen, is the Digene HPV Hybrid Capture II DNA Assay for HPV typing and subsequent stratification into low and high-risk types.

HPV typing by the DNA assay can be performed either from a cervico-vaginal sample collected into a Digene specimen transport kit or can be performed on leftover sample from a ThinPrep or Surepath Pap Test. It is essential that the results of the DNA assay be interpreted in conjunction with other clinical
information such as the presence or absence of cervical dysplasia in a patient, the grade of any cervical dysplasia and any other clinical findings. Very low levels of HPV infection can cause false negative results by the DNA Assay. At Rex Healthcare, samples for HPV DNA typing are forwarded either to Mayo Medical Laboratories (ThinPrep Specimens) or to LabCorp (Surepath Specimens).

The American Society of Cytopathology currently does not recommend primary, population-based, screening with the HPV Assay due to concerns regarding specificity. However, HPV testing has been shown to be a useful secondary (adjunctive) test following an indeterminate (ASCUS) cytology result as a means to help triage patients that need colposcopy versus those that can be followed by repeat Pap testing. (JAMA. 1999;281(17): 1605-1610. J Nat Cancer Inst 2001;93:293-299) For patients receiving an ASCUS diagnosis on a traditional Pap smear, a useful strategy would include a repeat Pap test using a thin layer specimen. If the follow-up thin layer specimen also were to yield equivocal results then HPV typing using the Digene assay could be performed off of the residual thin layer specimen. Women with positive High Risk HPV typing results would then be triaged to colposcopy. Patients with no evidence of oncogenic (high risk) HPV could return to a traditional one-year repeat Pap interval. In summary, the authors of the above-cited studies propose the following as the most efficient and cost effective means for cervical cancer screening:

Use liquid-based (thin layer, monolayer) Pap testing for routine screening.
Reflexively test all ASCUS samples for HPV.
Proceed directly to colposcopic examination for all patients that have Low Grade Squamous Intraepithelial Lesion or higher on their screening Pap test and for those that have an ASCUS Pap test with High Risk (oncogenic) HPV detected on the Hybrid Capture Assay.
Patients with an ASCUS Pap test but no evidence of High-Risk (oncogenic) HPV can return for a repeat Pap test in one year.

Digene (the manufacturer of the Hybrid Capture HPV Assay) has determined that HPV typing performed on a liquid-based cytology sample must be done within three weeks of specimen collection. If you would like to retrospectively add on HPV testing to a specimen that has been diagnosed as ASCUS please send your request by fax to the cytology department at 784-3362. Alternatively you can prospectively request that HPV typing be performed reflexively on a liquid-based Pap specimen by so indicating on the Pap smear requisition. This can currently be accomplished by writing in the statement: “HPV Type if ASCUS”. Soon all cytology requisitions will include a check box to request this reflex test. If you have any further questions please contact Dr. Keith V. Nance (Medical Director of Cytology) at 784-3286

Keith V. Nance, MD

FINE NEEDLE ASPIRATION BIOPSY

Fine needle aspiration (FNA) biopsy of palpable, superficial lesions has been utilized extensively in European countries for over 30 years. It has only been in the past 20 years that the procedure has become widely utilized in the United States. The technique has rapidly gained acceptance because it is fast, inexpensive, convenient, leaves no scar, and is relatively painless. The equipment required is minimal and includes a 22 or 23 gauge needle, a 20 cc syringe, and a syringe holder which allows for one handed operation. For best results the operator should be experienced in performing the procedure and an experienced pathologist should interpret the results. Currently, fine needle aspiration biopsy is the recommended first step in the evaluation of thyroid and salivary gland lesions. It can also be quite
helpful in appropriately triaging breast lesions and enlarged lymph nodes. The technique is especially useful in the diagnosis of recurrent or metastatic carcinoma. For more information on fine needle aspiration biopsy including instructions on how to perform the procedure, or to schedule a patient to have the procedure performed by a pathologist, please contact the Department of Cytology at 784-3040 or Keith V. Nance, MD at 784-3286.

Keith V. Nance, MD

THE MCV AND HEMOGLOBINOPATHIES

The automated CBC routinely measures mean red cell volume (MCV), a parameter which may suggest an underlying red cell disorder. The MCV is very reproducible over time and does not fluctuate with the hemoglobin level except in a brisk reticulocytosis. Most physicians use the MCV as a starting point in the evaluation of anemia. An elevated MCV is frequently associated with Vitamin B₁₂ or folic acid deficiency, refractory anemia (myelodysplasia), liver disease or a marked reticulocytosis. A low MCV is commonly associated with thalassemia, iron deficiency and anemia of chronic disease. A low MCV is normal in the pediatric age group (<10 y/o). Except for thalassemia, the majority of hemoglobinopathies have normal MCV values.

In the U.S. population the most common cause for a low MCV is iron deficiency. A serum ferritin and iron package (serum iron, TIBC and % saturation) aid in differentiating iron-deficiency from thalassemia. An elevated serum ferritin, low serum iron, low % saturation and elevated TIBC characterize iron deficiency. However, chronic inflammation or liver disease can elevate the serum ferritin and potentially be misleading. Both α (alpha) and β(beta) thalassemia have low MCV values and normal serum ferritin levels. Free erythrocyte protoporphyrin (FEP) is a valuable reference test to help in the differential diagnosis in some cases. FEP is elevated in lead poisoning, iron deficiency, and chronic disease but is normal in α and β-thalassemia. An elevated FEP rules out thalassemia.

Alpha-thalassemia results in decreased synthesis of the α chains in the hemoglobin molecule. The hemoglobin molecule is made of two alpha and two beta chains (α₂β₂) attached to heme. The majority of the U.S. population with α-thalassemia have slight microcytosis and no or very mild anemia. Moderate or severe anemia is seen primarily in the pediatric age group and results in hemoglobin Bart’s (γ₄) icterus and splenomegaly. The most severe form of α-thalassemia results in fetal death with hydrops.

Because there is no definitive test for α-thalassemia, it is often a diagnosis of exclusion. When the hemoglobin electrophoresis and iron studies are normal, and there is no other explanation for a microcytosis the diagnosis of α-thalassemia is strongly suggested. Genetic studies and reference tests such as globin chain analysis may be done but ordinarily are of little clinical value in an individual with a normal hemoglobin and microcytosis. The recognition of α-thalassemia is also important when the disorder is in combination with another hemoglobinopathy or other disease state. For instance, sickle cell anemia and sickle cell trait (HgbSS and HgbAS) have normal MCVs. If a sickle cell trait and α-thalassemia are present in the same individual (double heterozygote), then the red cells will be microcytic. In this situation, the hemoglobin electrophoresis will show approximately 25% hemoglobin S and 75% hemoglobin A instead of the usual 40% hemoglobin S and 60% hemoglobin A. A similar phenomenon is observed in hemoglobin E seen in the Indian/Asian population. Coexisting iron deficiency and sickle cell trait may result in a decrease in hemoglobin S concentration.
Both β-thalassemia minor and major have low MCVs. Thalassemia major is a disease of the pediatric population and is characterized by severe growth retardation, skeletal abnormalities hepatosplenomegaly and jaundice. There is a severe microcytic anemia with target cells, schistocytes and poikilocytosis. The hemoglobin electrophoresis shows a minor (or absent) hemoglobin A and a prominent hemoglobin F band. Hemoglobin A₂ may not be increased. When the concentration of F is very high (40 to 100%), A₂ is normal; when F is only moderately increased A₂ may be in the range of 3.5 –7.0 percent.

β-thalassemia minor is not uncommon in the U.S. and is seen primarily in individuals of African, Indian, Chinese, Greek and Italian ancestry. Anemia is usually mild or absent. Basophilic stippling is observed in about half the cases. On electrophoresis, hemoglobin A₂ is in the range of 3.5 – 7.0 percent. Hemoglobin F is often normal, but in half the cases is mildly elevated (2 to 4%). β-thalassemia minor often presents as a mild microcytic anemia but can be more severe. When β-thalassemia minor is present in combination with another hemoglobinopathy, the anemia is more severe. For example sickle cell trait (HgbAS), a harmless condition without microcytosis, in combination with β-thalassemia minor may produce a mild microcytic anemia or a very severe sickling disorder. In those with mild disease, the electrophoresis shows a small hemoglobin A band (15 to 30%). The more severe form of S-β-thalassemia may have complete absence of A (lack of β chain synthesis) with high concentration of S and lesser amounts of F and A₂.

Hemoglobin electrophoresis is available routinely at Rex Hospital Laboratory and is accompanied by an interpretation by a pathologist. The clinical history and suspected diagnosis are helpful and should be communicated to the pathologist to assist in correct interpretation.

Stephen V. Chiavetta, MD

References:


Antibiogram

The Rex Laboratory 2001 Antibiogram is included as an insert in this bulletin. We hope you find this information helpful in managing bacterial infections.

REX Healthcare Microbiology Laboratory