REX HEALTHCARE

# Laboratory Bulletin...

*Updates and Information from Rex Healthcare and Rex Outreach*

*February 2000* *Issue Number 45*

**ATYPICAL SQUAMOUS CELLS OF UNDETERMINED SIGNIFICANCE**

The Bethesda System for Cervicovaginal Cytology nomenclature introduced the concept of atypical squamous cells of undetermined significance(ASCUS). According to the Bethesda System, the ASCUS diagnosis should be restricted to cellular changes that exceed those attributed to benign reactive processes but fall short of changes of dysplasia. This statement could be construed as a very large waste basket with all sorts of cytologic changes dumped into it. However, there are essential criteria for ASCUS diagnosis that include increased nuclear size generally involving mature squamous cells, in which the nucleus must be 2 ½ - 3 times that of the normal intermediate cell nucleus. The nucleus of a metaplastic cell on the other hand must be at least 1½ times that of a normal metaplastic cell. In addition, there are changes in the nuclear cytoplasmic ratio, nuclear shape and the chromatin pattern. In some laboratories, specimens are down graded from low grade squamous intraepithelial lesion to ASCUS when there are less than five cells per specimen showing these alterations. In a mature squamous cell, the differential diagnosis is between reactive/repair reactions and low grade squamous epithelial lesion, whereas the changes in metaplastic cells is between reactive/repair reaction and high grade squamous epithelial lesion. Even with these stated criteria, the diagnosis of ASCUS remains one of the most controversial areas of the Bethesda System.

For practical purposes, what does the ASCUS diagnosis mean in the Rex Cyto-pathology Laboratory? The national average for ASCUS diagnosis that are confirmed as dysplasia by surgical pathology biopsy material is on the order of 30-50%. In other words, 1 in every 2 or 3 ASCUS diagnosis are followed up by squamous epithelial neoplasia(SIL) in the biopsy material. In the past year, the Rex Cytopathology Laboratory rate of confirmation of ASCUS diagnosis to surgical biopsy proven dysplasia has been in the order of 75%. In other words, every 3 of 4 ASCUS diagnosis are confirmed as dysplasia by surgical biopsy material. One could argue that we should just call more ASCUS cases outright dysplasia, but there are criteria to be followed and individual cases must show the changes characteristic of the diagnostic categories.

The question arises, " What should a physician do upon receipt of a Pap smear interpretation of ASCUS?". For those ASCUS cases where low grade dysplasia is questioned, we would recommend conservative follow up at a 3-6 month interval by using a repeat Pap smear. We would further recommend that due to its increased sensitivity, the repeat Pap smear should be performed by the thin layer methodology. If the ASCUS diagnosis raises the question of atypical squamous metaplasia vs high grade squamous intraepithelial lesion, we would highly recommend biopsy confirmation rather than repeat Pap smear testing.

*John P. Sorge, M.D.*

**Preterm Labor Risk and Fetal**

**Introduction:** Fetal fibronectin (fFN) is a glycoprotein that reflects the growth of the placenta. It is elevated in cervicovaginal secretions during the first 24 weeks of

**Fibronectin**

pregnancy but diminishes between 24 and 34 weeks. The presence of elevated fFN in the cervicovaginal secretions of pregnant women between 24 and 34 weeks gestation is associated with preterm delivery. When compared to other risk factors for preterm labor such as uterine activity, cervical dilatation, vaginal bleeding and infection, it is the best predictor of preterm labor. Approximately 83% of symptomatic women who deliver prematurely have positive fFN within two weeks prior to delivery.

**Clinical Value:** If a women is having contractions and the fFN is negative, then 99% of the time she will not deliver with the next two weeks. The negative predictive value of the test is extremely high. The negative test adds significant value in deciding whether a patient should be admitted to a hospital or discharged to rest at home. While the reliability of a positive test does not approach that of a negative test, a positive fFN is more predictive of delivery within a week that any other clinical variable. One study has shown significantly reduced preterm labor admissions, length of stay and use of tocolytic agents.

**Method of Collection:** The Adeza Biomedical Specimen Collection Kit is the only acceptable specimen collection system that can be used for this assay. The collection kits are stored in the Birthing Center Medicine Room. Collection of the specimen is contraindicated in advanced cervical dilation (>3 cm), rupture of amniotic membranes, cervical cerclage or gross vaginal bleeding. Typically delivery is imminent when dilation is > 3 cm. Vaginal bleeding is associated with obstetrical or medical problems. Specimens should not be obtained prior to digital cervical examination or vaginal probe ultrasound examination as manipulation of the cervix may cause the release of fFN. The swab should not be contaminated with lubricants, creams or soaps. Specimens should not be collected if the patient had sexual intercourse within the last 24 hours. Finally, since cellular debris may interfere with sample preparation, specimens should be collected prior to collection of culture specimens.

**Laboratory Details:** The Adeza collection tubes must be tightly capped and refrigerated. The test is performed at Wake Med at a charge of $212.00. The specimen will be run the same day if received prior to 9:00 AM. The majority of the time the results are available the next day. Specimens are transported to Wake by cab. The assay is available 7 days a week. Levels greater than 0.050 ug/ml are considered elevated and suggest preterm labor.

References:
1. Joffe, Gary MD, Jacques, Debbie MD, et. Al. “Impact of the fetal fibronectin assay on admission for preterm labor,” *Am. Journal of Obstetrics and Gynecology*, March ’99 p581-586.

2. The American College of Obstetricians and Gynecologists, Committee on Obstetric Practice Number 187, September 1997.

3. Paxton, Anne, “For fetal fibronectin test, the time is right”, College of American Pathologist – *CAP Today*, November 1998, pages 1-6.

*Stephen V. Chiavetta, MD*

***(Yet Another) Change in Glyco-hemoglobin***

A while back we wrote about the importance of glycohemoglobin (or hemoglobin A1c) measurement in diabetic patients to assess average blood glucose levels in the preceding 2-3 month period. [1] Hemoglobin A1c is the subfraction of glycohemoglobins that has been studied most widely. Ion exchange chromatography

was used in many of the earlier studies to measure Hb A1c and was chosen as the reference method for the Diabetes Control and Complication trial. Our original article discussed some of the problems with earlier ion exchange chromatographic methods and indicated our preference for a different method. Time moves forward and technological advances continue. Lest our patrons grow complacent, we've made another change in our methodology for measuring Hb A1c.

Effective immediately, the Laboratory will measure hemoglobin A1c directly using ion exchange high performance liquid chromatography (HPLC). This method will replace the previously used boronate affinity fluorescence polarization assay (Abbott IMx®). The Bio-Rad Variant II® separates hemoglobins based ionic interactions with chromatography cartridge matrix. The separated hemoglobins are measured photometrically by their absorbance. This method represents an advance over previous ion-exchange methods in that variant hemoglobins (e.g. Hb F and S) and labile A1c fractions are separated from A1c and do not interfere with the result. Indeed abnormal hemoglobins can be detected with this method. As the assay is automated, precision should improve (coefficient of variation between 1.68 – 2.14% in range of most patient Hb A1c levels). [2]

Patients with hemoglobinopathies can produce problems in interpretation of A1c results. With the new method, patients heterozygous for Hb S (sickle cell trait) or Hb C have valid A1c results (peak areas for these hemoglobins not included in calculation of total hemoglobin). Thus their results can be correlated directly with literature recommendations for A1c levels. Patients homozygous for Hb S (sickle cell disease), C (hemoglobin C disease), or with hemoglobin SC disease have no hemoglobin A present. Therefore this test is not valid for such patients. Patients with hemoglobinopathies other than sickle cell trait or Hb C trait have not been studied sufficiently with this method. Accordingly, any patients with hemoglobinopathies other than sickle cell trait or Hb C trait will have their specimen forwarded to Mayo Medical Laboratory for total glycated hemoglobin determination.

The reference range will be modified to 3.9% - 6.1% Hb A1c. **The American Diabetes Association recommends the therapeutic goal of Hb A1c levels less than 7%.** Nathan *et al* studied 21 diabetic patients who performed an average of 4 blood glucose self-determinations over a 2 month period and compared it with Hb A1c measured by ion exchange HPLC at the end of the study period.[3] Using linear regression analysis, they derived the following relationship between the measured % Hb A1c and the calculated mean blood glucose (MBG):

$$MBG = 33.3\ (\%Hb\ A1c) - 86\ .$$

Representative values are shown in a tabular fashion on next page.

**Mean Blood Glucose as a Function of %Hb A1c[2,3]**

| Mean Blood Glucose (mg/dL) | %Hb A1c |
|---|---|
| 360 | 14 |
| 330 | 13 |
| 300 | 12 |

| | |
|---|---|
| 270 | 11 |
| 240 | 10 |
| 210 | 9 |
| 180 | 8 |
| 150 | 7 |
| 120 | 6 |
| 90 | 5 |

The test can be ordered in the Hospital Information System as "hemoglobin A1c", "A1c" or "glycohemoglobin". The assay is performed daily, Monday – Friday.

*John D. Benson, MD*
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1. Benson JD, Brown D. Change in glycohemoglobin reporting. *Rex Healthcare Laboratory Bulletin*, Issue 34, Aug/Sep 1998.
2. BioRad Diagnostics Group. Variant® II Hemoglobin A1c Program Instruction Manual 702:001-1, June 1999.
3. Nathan D *et al*. The clinical information value of the glycosylated hemoglobin assay. *N Engl J Med* 310:341-6, 1984.


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