LifeLabs® Medical Laboratory Services

Inside Diagnostics

THE SKIN BIOPSY: WHEN AND HOW TO DO IT

This is the first of a two-part series on the skin biopsy and discusses clinical indications for analysis. Part 2, in the next issue of Inside Diagnostics, will describe correct collection process for skin biopsy.

Part One: Indications for Skin Biopsy

1. Any eruption which does not follow its expected course or response to treatment

Whenever a skin disease behaves atypically, the diagnosis should be reviewed. The dermatopathologist can often help in this investigation.

2. Vesicular and bullous eruptions

In some cases the diagnosis is clinically obvious, for example acute allergic contact dermatitis, impetigo, or viral infections such as herpes simplex, varicella, or herpes zoster. However, with many vesiculobullous eruptions, the diagnosis can only be confirmed histologically.

3. Confirming the diagnosis in obvious cases

While it is academically desirable that there should be histological confirmation of the diagnosis in all cases, this approach is clearly impractical. Nevertheless, the biopsy has a role to play even in cases where the diagnosis appears clinically "obvious".

4. Chronic ulcers

It is often thought that taking a biopsy from the edge of an ulcer is unwise since it will then tend to enlarge the ulcerating process and delay healing. In practice, this is not so, and any chronic ulcer which fails to heal as expected should be biopsied. Malignancy can be the cause of an ulcer or may develop as a complication of some other ulcerative lesion. Chronic ulcers can be caused by atypical mycobacteria, deep fungi, and leishmania. In these cases, both the pathologist and the microbiologist must search for the causative organism, utilizing the techniques of microscopic examination and cultures, respectively.

5. Tumors and Nevi

Regardless of how clinically obvious the diagnosis may appear to be, every tumor and nevus that is removed from the mucocutaneous surface should be submitted for histological examination. Electrodesiccation of the specimen prior to its removal is contraindicated since this creates a prominent histological artifact that usually negates the possibility of making an accurate microscopic diagnosis.

6.Establishing a diagnosis for unknown lesions

This is perhaps one of the most common (and most obvious) reasons for a skin biopsy.

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LABORATORY TESTING FOR VON WILLEBRAND DISEASE (VWD)

What is Von Willebrand disease?

Von Willebrand disease (VWD) is an inherited bleeding disorder that is caused by either a quantitative deficiency or qualitative dysfunction of Von Willebrand Factor (VWF). VWF is responsible for platelet adhesion and aggregation as well as functioning as a carrier molecule for FVIII (eight). Deficiency or dysfunction of VWF may cause a bleeding disorder by impairing platelet adhesion or by reducing the concentration of FVIII.

What are the components of the VWD screening assay?

There are 3 assays that make up part of the initial VWD screen:

(1) **VWF antigen** is an immunoassay that measures the concentration of VWF protein in the plasma.

(2) **VWF activity** is a functional assay that measures the functional activity of VWF. The commonest VWF assay in use by most laboratories is the VWF Ristocetin cofactor assay that measures the ability of VWF to interact with platelets.

(3) **Factor VIII** is a measure of the cofactor function of the clotting factor VIII. In the context of VWD, Factor VIII activity measures the ability of VWF to bind and maintain the level of FVIII in the circulation.

How is VWD classified?

VWD may be classified into the following subtypes based on the type of deficiency or dysfunction.

- **Type I**
  - Mild-moderate quantitative deficiency of VWF
  - Autosomal dominant trait
  - 60-80% of cases
  - All 3 components of the VWD screen are mildly reduced
- **Type II**
  - Qualitative deficiency of VWF
  - Includes four major subtypes: 2A, 2B, 2M, 2N
  - Generally autosomal dominant trait
  - 10-30% of cases
  - VWF antigen is quantitatively normal but VWF activity is reduced
- **Type III**
  - Quantitative absence of VWF
  - Autosomal recessive trait
  - 1-5% of cases
  - All 3 components of the VWD screen are severely reduced

An Initial Evaluation for VWD or other Bleeding Disorders

(Adapted from: The Diagnosis, Evaluation, and Management of von Willebrand Disease. NIH Publication No. 08-5832, December 2007)

Initial evaluation includes asking the following questions of patients:

Have you or a blood relative ever needed medical attention for a bleeding problem, or been told you have a bleeding disorder or problem:

- During /after surgery?
- With dental procedures, extractions?
- With trauma?
- During childbirth or for heavy menses?
- Ever had bruises with lumps?

* Rule out liver and kidney disorders, blood and bone marrow disorders, high or low platelet counts and use of anti-platelet agents, NSAIDs and anticoagulants

IF ALL NEGATIVE → **No evaluation, usual care**

IF ANY POSITIVE →

Suggested questions for screening persons with a bleeding disorder:

1. Do you have a blood relative who has a bleeding disorder, such as von Willebrand disease or hemophilia?
2. Have you ever had prolonged bleeding from trivial wounds, lasting more than 15 minutes or recurring spontaneously during the7 days after the wound?
3. Have you ever had heavy, prolonged, or recurrent bleeding after surgical procedures, such as tonsillectomy?
4. Have you ever had bruising, with minimal or no apparent trauma, especially if you could feel a lump under the bruise?
5. Have you ever had a spontaneous nosebleed that required more than 10 minutes to stop or needed medical attention?
6. Have you ever had heavy, prolonged, or recurrent bleeding after dental extractions that required medical attention?
7. Have you ever had blood in your stool, unexplained by a specific anatomic lesion (such as an ulcer in the stomach, or a polyp in the colon) that required medical attention?
8. Have you ever had anemia requiring treatment or received a blood transfusion?
9. For women, have you ever had heavy menses, characterized by the presence of clots greater than an inch in diameter and/or changing a pad or tampon more than hourly, or resulting in anemia or low iron level?

Initial hemostasis evaluation:

- CBC and platelet count
- PTT, PT/INR, fibrinogen, thrombin time
- If bleeding history strong consider performing initial VWD assays

IF ANY POSITIVE →

PTT either normal OR Isolated prolonged PTT that corrects in 1:1 mixing study

Initial VWD screening assays:

VWF:Ag
VWF:RCoF
FVIII

1 OR MORE TESTS ABNORMAL →

Repeat to confirm* & Referral to haematologist for confirmation of diagnosis and sub-typing studies

* false positives are a concern

TOP FIVE things to remember while ordering and interpreting VWD studies

5. Blood draw should be atraumatic as this limits activation of clotting factors and thus minimizes falsely high or low values. Undue stress, including crying in children or anxiety in adults, exercise, acute or chronic inflammatory conditions or pregnancy may falsely elevate VWF levels therefore repeated testing may be required to make a diagnosis of low VWF.

4. Repeated freeze thawing of plasma samples during sample transportation or testing can cause spuriously low VWF and FVIII due to loss of these proteins due to cryoprecipitation – an important cause of "false positive" diagnoses.

3. The analytic variability of the VWF ristocetin cofactor assay is high and is a common cause of non diagnostic results on VWD screens.

2. The ABO blood group significantly influences plasma VWF concentrations. Blood type O has approximately 25% lower concentration of VWF than the other blood types.

1. **The most IMPORTANT screening tool is the bleeding history.** The diagnosis of VWD is complex and an individualized approach to diagnosis should be taken. Always correlate the laboratory testing with the clinical (pre-test) probability of a bleeding disorder (see figure for suggested questions to ask on a bleeding history). If clinical probability and VWD screen results are discordant consider the biological, pre-analytical and analytical factors that can cause variability in test results (reasons 2 to 5). Always confirm abnormal or unexpected findings with repeat testing.

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VITAMIN D TESTING IN PRIMARY CARE ENVIRONMENT

The role and significance of Vitamin D in maintaining good health has recently received a great deal of attention. In addition to its role in skeletal development and maintenance, higher levels of vitamin D have been associated with decreased risk of certain cancers, diabetes mellitus, multiple sclerosis, autoimmune disease, cardiovascular disease, Crohn's disease and other inflammatory bowel diseases and lowered mortality in patients with chronic kidney disease. Although the definitive mechanism of action in prevention of progression of many of these diseases has not been established, study results have been published and a dramatic increase in demand for measurement of vitamin D has resulted.

Physiology

Vitamin D_2 and D_3 from the diet and skin are metabolized in the liver to produce 25-hydroxyvitamin D_3 (25[OH] D_3), which undergoes further metabolism in the kidney to produce the biologically active form, 1,25-dihydroxyvitamin D_3 ($1,25[OH]_2D_3$). The latter binds to the vitamin D receptor and plays a central role in regulating calcium homeostasis and promoting cellular differentiation.

25-hydroxyvitamin D is the major circulating metabolite of vitamin D and reflects precursor levels of vitamin D derived from cutaneous metabolism and dietary intake. In addition, 25-hydroxyvitamin D is less subject to physiological factors, has a longer half life than 1,25-dihydroxyvitamin D (calcitriol) and correlates with bone mineral density. It is therefore the analyte of choice when assessment of patient vitamin D status is necessary.[1]

Supplementation

Adequate vitamin D levels may not be achieved though diet alone and sufficient exposure to sunlight is generally not adequate for vitamin D production depending on the season, skin pigmentation and geographical latitude. There is a concern that low vitamin D levels in older adults contributes to bone loss and resulting fractures.

A database review completed in 2009 at LifeLabs illustrated the seasonal distribution of vitamin D concentrations in the population. This data review indicated that 38-53% of patients maintained vitamin D values within the sufficient range (75-250 nmol/L) with lower concentrations measured in the winter months. However, 45-58% of individuals demonstrated vitamin D values in the insufficient range (25-74 nmol/L) while 0.7-4.0% fell into the deficient range (< 25 nmol/L).

Health Canada recommends a daily dietary or supplemental source of 200 IU for adults 19-50 years with increasing doses after age 50 (400 IU) and 70 years (600 IU) to a maximum of 2000 IU/day.[2] Evidence is accumulating to indicate dietary intake should be in the range of 800-1000 IU per day and up to 2000 IU/day for pregnant or lactating women.[3] The Canadian Cancer Society recommends 1000 IU during the

fall and winter and year round if older or for those with darker skin pigmentation. Some suggest that much higher doses (2000-3000 IU/day) may be required to achieve the disease risk reductions noted above. There is still however, much controversy on the appropriate dosage and duration of supplementation.

Vitamin D toxicity is rarely seen but may become evident following prolonged consumption of very high doses of vitamin D. Toxicity is manifested by pain, muscle weakness, vomiting and confusion. Hypercalcemia, hypercalcuria and hyperphosphatemia may be observed.

As vitamin D supplementation may result in hypercalcemia in some patients, blood calcium levels should be monitored in those with renal disease, parathyroidism, sarcoidosis, lymphoma or granulomatous disease.

Vitamin D Testing

In February 2010, the Ontario Health Technology Advisory Committee (OHTAC) published an evidence based analysis of the clinical utility of vitamin D testing.[4] As the literature and ON data have shown, there is a relatively small number of patients who are deficient but a significant number with insufficient concentrations of vitamin D. As supplementation is generally safe, it is reasonable to supplement asymptomatic, at-risk, individuals without baseline or follow up testing. This may include elderly or infirmed patients or those who are believed to receive inadequate sun exposure. The OHTAC recommends following Health Canada guidelines for supplementation of Vitamin D, but does not recommend routine vitamin D testing in the healthy population.[4]

As recommended by OHTAC for 25-Hydroxy vitamin D testing is however, recommended for those with clinical symptoms, those at increased risk or patients with the following conditions: [4, 5]

- Renal or liver disease
- Osteomalacia, osteopenia, osteoporosis or rickets
- Malabsorption syndromes
- Hypo- or hypercalcemia
- Hypo- or hyperparathyroidism
- Unexplained increased serum ALP or phosphate

Quantitation of 25-hydroxy vitamin D may be helpful for those receiving medications that affect vitamin D metabolism or for individuals receiving high doses (> 2000 IU daily) for an extended period (>6 months). Consultation with a specialist should be considered for patients with unexplained bone pain, unusual fractures or other evidence suggesting metabolic bone disease.

Quantitation of 1, 25 dihydroxy vitamin D is rarely required but may be helpful when renal α1-hydroxylase deficiency or receptor defects are suspected and with complex clinical cases.[5]

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LAST CHANCE TO PARTICIPATE IN THE LIFELABS *CLIENT SURVEY*

Thanks for your feedback! We are pleased to announce we have received good feedback from physicians and other clients that have taken a few minutes to fill out our *Client Survey*. We plan to close the survey at the end of summer—so this is your last chance to let us hear your views.

The LifeLabs survey is focused on assessment of the quality of our Medical-Scientific services. You can access the survey online through the following link: http://www.surveymonkey.com/s/Lifelabs_Client_Satisfaction_Survey

The survey should take less than five minutes of your time and is completely confidential. The results will help us understand how we are doing today and where we need to make improvements.

Thanks in advance for participating in the survey. We look forward to receiving your feedback and will report back the findings in a later issue of *Inside Diagnostics*.

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