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Vitamin D - It's Not Just For Rickets Anymore

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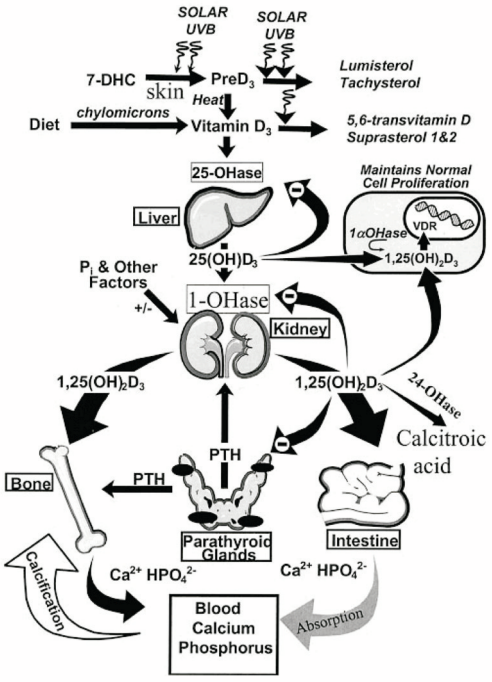
All of us at one point in our careers studied the pathway of vitamin D metabolism and the role it plays in childhood rickets and osteomalacia in adults (see the figure below from Reference 1). Although rickets is one of the most common diseases in children, the prevailing consensus was that rickets is primarily seen in developing countries because in the United States milk was being fortified with vitamin D. Osteoporosis is a major concern in the older population but was still considered an unavoidable parameter of aging. Patients were advised to increase their calcium intake by drinking milk or eating yogurt but compliance is questionable and the bone fracture problem still exists. Things have certainly changed within the last decade as vitamin D receptors were discovered on many tissues in the body. Questions on what are doing there was followed by research to answer the questions, yet much is still unknown. (1)



The dietary sources of vitamin D are somewhat limited and include salmon, mackerel, sardines, cod liver oil, egg yolks, fortified milk, yogurt, and fortified cereal. With the typical American diet, there is little wonder why there is a deficiency of dietary vitamin D in the population. Another source of vitamin D is endogenous production from the cholesterol precursor 7-dehydocholesterol (provitamin D3) that is deposited in the skin. UV-B light in the wavelength of 290-315 nm will convert provitamin D3 to previtamin D3 and hence there is a dilemma. Aren't we suppose to avoid the sun, cover up, and apply sun screen? Excessive UV-B light exposure will not cause vitamin D toxicity because excess vitamin D3 and previtamin D3 are photolyzed to biologically inactive products. So the question remains how to balance UV-B exposure and avoid the hazards of UV-B wavelength.

Vitamin D is hydroxylated in the liver to form 25-hydroxyvitamin D (25OH-D) which is the precursor to the active vitamin D, 1,25 hydroxyvitamin D (1,25OH-D). The second hydroxylation occurs in the kidney and is regulated by parathyroid hormone. The active hormone 1,25OH-D binds to the vitamin D receptor (VDR) in the intestine to increase the absorption of calcium and phosphorus. When vitamin D levels are low only 10-15% of intestinal calcium is absorbed compared to 30-40% under an adequate level of vitamin D. 1,25OH-D also binds to the VDR on the osteoblast to send biochemical signals to the osteoclast which in turn release collengases and hydrochloric acid to dissolve bone matrix and release calcium into the blood. Even though 1,25OH-D is the active hormone, 25OH-D is used to assess the vitamin D status because it is the major circulating prohormone.

The presence of VDR and the 1-alpha-hydroxylase enzyme responsible for the conversion of 25(OH)D to 1,25(OH)D in tissues not associated with bone metabolism has been a revelation. There is growing evidence that vitamin D may be important in cell growth, cell differentiation, and possibly other cell regulatory mechanisms. In vitro studies show that breast (2), ovarian (3) colon, and

prostate cancer are responsive to 1,25(OH)D (4,5,6,7,8,9). This finding also may help explain past epidemiological studies that show increased rates of certain disease states in higher latitudes and thereby relate the finding to sunlight exposure. The higher unexplained disease status in relationship to latitude was largely ignored in the past, given up to some anomaly in the study design, or just thought to be grasping at straws. Research programs now are directing their studies toward the immunomodulatory effects of vitamin D status to not only cancers but also cardiovascular disease, hypertension, multiple sclerosis, diabetes, rheumatoid arthritis, inflammatory bowel disease, systemic lupus erythematosus, and other neuromuscular diseases.

The next challenge is explaining to the general public the role of vitamin D, especially after they have been told to avoid the sun, use sun screen, and "cover up" for the last thirty years. Fortunately, sensible sun exposure and possible use of inexpensive vitamin D supplements may go a long way helping maintain a better quality of life.

Beginning mid January, 2008, ENH Laboratories will begin offering vitamin D testing as an in-house procedure. The vitamin D assay is based on chemiluminesence and equally measures both vitamin D2 and D3. The new vitamin D method demonstrates excellent correlation to the procedure performed Mayo Medical Laboratories. Total vitamin D will be reported with an interpretation range as follows:

Vitamin D Normal Range: 21-100 ng/mL

Interpretive Range:
< 10ng/mL (deficiency)
10-20 ng/mL (insufficient levels)
40-100 ng/mL (optimal levels)
> 100 ng/mL (possible toxicity)

For questions concerning vitamin D testing at ENH Laboratories contact Robert Rosecrans, Ph.D. at 847-926-5078 or by email rrosecrans@enh.org.

References

1. Holick MF Vitamin D: Importance in the Prevention of Cancers, Type 1 Diabetes, Heart Disease, and Osteoporosis *Am J Clin Nutr* 2004;79:362-71.
2. Bertone-Johnson E, Chen WC, et.al. Plasma 25-Hydroxyvitamin D and 1,25-Dihydroxyvitamin D and Risk of Breast Cancer. *Cancer Epidemiology, Biomarkers, & Prevention* 2005;14(8):1991-97.
3. Twongwer SS, Lee IM, et.al. Plasma 25-Hydroxyvitamin D and 1,25-Dihydroxyvitamin D and Risk of Incident Ovarian Cancer. *Cancer Epidemiology, Biomarkers, & Prevention* 2007;16(4):783-788.
4. Esther JM, Schwartz GG, Dreon DM, and Koo J. Vitamin D and Breast Cancer Risk: The NHANES I Epidemiologic Follow-up Study, 1971-1975 to 1992. *Cancer Epidemiology, Biomarkers, & Prevention* 1999;8:399-406.
5. Schwartz GG, Whitlatch LW, et.al. Human Prostate Cells Synthesize 1,25-Dihydroxyvitamin D3 from 25-Hydroxyvitamin D3. *Cancer Epidemiology, Biomarkers, & Prevention* 1998;7(5):391-5.
6. Tangpricha V, Flanagan JN, et. al. 25-Hydroxyvitamin D-1alpha-hydroxylase in Normal and Malignant Colon Tissue. *Lancet* 2001;357(9269):1673-4.
7. Chen TC, Holick MF, et.al. Evaluation of Vitamin D Analogs as Therapeutic Agents for Prostate Cancer. *Recent Results in Cancer Research* 2003;164:273-88.
8. Dalhoff K, Dancey J, et. al. A Phase II Study of the Vitamin D Analogue Seocalcitol in Patients with Inoperable Hepatocellular Carcinoma. *British J Cancer* 2003;89(2):252-7.

MEDICARE COMPLIANCE INFORMATION

By: James Dohnal, Ph.D., Director of the Core Laboratory, Evanston Hospital

Advance Beneficiary Notice (ABN)

Department of Health & Human Services (DHHS)
Centers for Medicare & Medicaid Services (CMS)
Program Memorandum AB-02-168.
(http://www.cms.hhs.gov/transmittals/downloads/ab02168.pdf)

An ABN is a written notice a physician or supplier gives to a Medicare Beneficiary before services are furnished when the physician or supplier believe that Medicare probably or certainly will not pay for some or all of the services on the basis of the following statutory exclusions including:

a. Medical reasonableness and necessity
b. Research tests
c. Wellness screening
d. Testing without the presence of physical signs/symptoms (screening)

e. Frequency limitations (examples: PAP and PSA testing have frequency limitations)

ABN's are designed for use with Medicare beneficiaries only, including those who are dually-eligible for Medicare and Medicaid. ABNs are not for use with patients who are not Medicare beneficiaries. The purpose of the ABN is to inform a Medicare beneficiary, before he or she receives specified items or services that otherwise might be paid for, that Medicare probably will not pay for them on that particular occasion. The ABN, also, allows the beneficiary to make an informed consumer decision whether or not to receive the items or services for which he or she may have to pay out of pocket or through other insurance.

In addition, the ABN allows the beneficiary to better participate in his/her own health care treatment decisions by making informed consumer decisions. If the physician or supplier expects payment for the items or services to be denied by Medicare, the physician or supplier must advise the beneficiary before items or services are furnished that in their opinion the beneficiary will be personally and fully responsible for payment. To be "personally and fully responsible for payment" means that the beneficiary will be liable to make payment "out-of-pocket," through other insurance coverage (e.g. employer group health plan coverage), or through Medicaid or other Federal or non-Federal payment source. The physician or supplier must issue notices each time and as soon as they make the assessment that Medicare payment probably or certainly will not be made.

To be acceptable, an ABN must be on the approved Form CMC-R-131, must clearly identify the particular item or service, must state that the physician or supplier believes Medicare is likely (or certain) to deny payment for the particular item or service, and must give the physician's or supplier's reason(s) for their belief that Medicare is likely (or certain) to deny payment for the item or service.

A physician or supplier should not give an ABN to a beneficiary unless the physician or supplier has some genuine doubt regarding the likelihood of Medicare payment. Giving ABNs for all claims or items or services (i.e. "blanket ABNs") is not an acceptable practice. Notice must be given to a beneficiary on the basis of a genuine judgment about the likelihood of Medicare payment for that individual's claim.

A physician or supplier is prohibited from obtaining beneficiary signatures on blank ABNs and then completing the ABNs later. An ABN, to be effective, must be completed before the delivery to the beneficiary.

The CMS information brochure "What Doctors Need to Know about the Advance Beneficiary Notice (ABN) is located at: http://www.cms.hhs.gov/MLNProducts/downloads/MLNCatalog.pdf

ABN Instructions

Patient Name

The physician or supplier enters the name of the patient, not substituting the name of an authorized representative.

Medicare Health Insurance Claim Number (HICN)

The physician or supplier enters the patient's Medicare HICN. Do not invalidate an ABN solely on the lack of a Medicare HICN unless the beneficiary recipient of an ABN alleges that the ABN was signed by someone else of the same name and you cannot resolve the matter with certainty.

Medicare does not pay for these tests for your condition

The physician or supplier specifies the laboratory tests for which he/she/it expects Medicare will not pay in the box. The laboratory tests at issue must be described in sufficient detail so that the patient can understand what laboratory tests may not be furnished. The use of standard laboratory test descriptions is permitted. HCPCS codes by themselves are not acceptable as descriptions.

Estimated Cost

The physician or supplier may provide the patient with an estimated cost of the items and/or services. The patient may ask about the cost and jot down an amount in this space. The physician or supplier should respond to such inquiries to the best of their ability. The lack of an amount does not invalidate the ABN: an ABN will not be considered to be defective on that basis. In the case of an ABN which includes multiple items and/or services, it is permissible for the physician or supplier to give

estimated amounts for the individual items and/or services rather than an aggregate estimate of costs.

Options 1 and 2 Boxes

The patient must personally select an option.

Date

The patient or his or her authorized representative should enter the date on which he or she signed the ABN. If the date is filled in by the physician or supplier and the beneficiary or his or her authorized representative does not dispute the date, you should accept that date. Do not reject ABNs simply because the date is typed or printed.

Signature

The beneficiary himself or herself may sign an ABN. In the case of a beneficiary who is incapable or incompetent, his or her "authorized representative", may sign an ABN. The beneficiary retains the patient's copy of the signed and dated ABN and the physician or supplier retains the original ABN. These copies will be relevant in case of any future appeal.

Diagnosis Codes (ICD-9)

Medicare regulations (Program Memorandums B-03-045, B-03-046, AB-03-091) require the ordering physician to provide the laboratory with accurate and encounter specific ICD-9 codes when ordering laboratory testing. Medicare uses this information to determine if the testing is eligible for reimbursement. Medicare has instructed the laboratory to contact the ordering physician if this information is not provided. A physician that does not provide this information on a regular basis is considered by Medicare to be in violation of Medicare regulations.

The most current information from the Center for Medicare & Medicaid Services (CMS) regarding National Coverage Determinations (NCD) can be found on the CMS website at: http://www.cms.hhs.gov/center/clinical.asp

ENHLS Policy Regarding the Billing of Medicare

All ENHLS staff members must comply with all Medicare regulations at all times. Policies have been written with the goal of complete compliance and are reviewed regularly to ensure that they are compliant with the most current Federal regulations. Staff members are provided training in Medicare regulations and are clearly instructed to follow policy. They are encouraged to bring to the attention of management any policy or procedure that they think may be in conflict with the Medicare regulations. Their performance is monitored regularly and deviation from policy is addressed with additional training. Repeated deviation from policy results in disciplinary action up to and including termination. Any deviation from policy that might represent a deliberate attempt to violate the Medicare regulations is reported to the Medicare program.

Medicare reimbursement for tests performed on Medicare beneficiaries will be equal or less than the amount listed in the Medicare laboratory fee schedule.

Professional Courtesy

The Office of the Inspector General (OIG) interpret a discount on laboratory testing for physicians, their office staff and family members as a potential kickback to the physician. For this reason;

ENHLS DOES NOT OFFER PROFESSIONAL COURTESY, ON OUR LABORATORY SERVICES TO PHYSICIANS OR THEIR EMPLOYEES OR FAMILY MEMBERS.

Testing Supplies

A laboratory may provide a physician with supplies that are DIRECTLY related to the testing that is submitted to the laboratory in amounts PROPORTIONAL to the volume of testing performed. Any supplies outside of these limits are considered a kickback to the physician and therefore fraud. Additionally, multi-purpose supplies outside of these limits supplied are considered a kickback. For example, a fax machine supplied by the laboratory for faxing results is considered a kickback because the client could use the fax machine for non-laboratory purposes. Other items included in this category include latex gloves, laboratory coats and medical waste disposal.

Test Profiles

Federal law requires that each test billed to Medicare meet Medicare's medical necessity guidelines. Medicare believes that the use of profiles greatly increases the likelihood that medically unnecessary tests will be billed to Medicare. Therefore, the use of profiles is strongly discouraged. When ordering tests. Please use the following guidelines.

a. Order tests on an individual basis determined by the patient's medical condition.
b. Limit orders to only those tests that are directly related to the condition being treated.
c. Limit profiles to the AMA CPT Code Committee/Medicare listed below.
d. ENHLS does not offer customized profiles other then the ones recognized by the AMA and Medicare.

Effective January 01, 2008

Test Code	Profile Name (CPT/Medicare Fee)	Components
4255	Acute Hepatitis Panel 80074/$66.54	Hepatitis A IgM Ab (86709), Hepatitis B core IgM Ab (86705), Hepatitis B surface Ag (87340), Hepatitis C Ab (86803)
5005	Basic Metabolic Panel 80048/$11.83	Calcium (82310), Carbon dioxide (82374), Chloride (82435), Creatinine (82565), Glucose (82947), Potassium (84132), Sodium (84295), BUN (84520)
9541	Basic Metabolic Panel with Ionized Calcium 80047/$11.83	Ionized Calcium (82330), Carbon dioxide (82374), Chloride (82435), Creatinine (82565), Glucose (82947), Potassium (84132), Sodium (84295), BUN 84520
5130	Cardiac Risk 80061/$18.72	Cholesterol total (82465), HDL (83718), Triglycerides (84478), Relative risk, Calculated LDL
5006	Comprehensive Metabolic Panel 80053/$14.77	Albumin (82040), Bilirubin total (82247), Calcium (82310), Carbon dioxide (82374), Chloride (82435), Creatinine (82565), Glucose (82947), Alkaline Phosphate (84075), Potassium (84132), Protein total (84155), Sodium (84295), ALT/SGPT (84460), AST/SGOT (84450), BUN (84520)
21	Electrolytes 80051/$9.80	Carbon dioxide (82374), Chloride (82435), Potassium (84132), Sodium (84295)
5004	Hepatic Functional Panel 80076/$11.42	Albumin (82040), Bilirubin total (82247), Bilirubin direct (82248), Alkaline phosphate (84075), Protein total (84155), ALT/SGPT (84460), AST/SGOT (84450)
5011	Renal Function Panel 80069/$12.13	Albumin (82040), Calcium (82310), Carbon dioxide (82374), Chloride (82435), Creatinine (82565), Glucose (82947), Phosphorus inorganic (84100), Potassium (84132), Sodium (84295), BUN (84520)

Although approved by the AMA CPT Code Committee, Medicare generally denies the panels listed below.

5901	General Health 80050	Comprehensive Metabolic panel (80053), CBC w/Auto Diff/Platelet (85027), TSH (84443)
9002	Obstetric Panel 80055	CBC w/Auto diff/Platelet (85027), Hepatitis B Surface Ag (87340), Rubella (86762), RPR (86592), Antibody screen (86850), ABO (86900)/Rh (86901)

Case Study – Cryoglobulinemia

By: Ben Witt, M.D., Department of Pathology, 2nd year resident

GG is a 66 year old male who experienced recurrent, intractable nosebleeds during the winter of 2006. Concomitantly he developed multiple painful, expanding purpuric skin lesions (ranging from 0.5 to 3 cm in size). He had no contributory past medical history. A skin biopsy was performed in May 2006, which demonstrated PAS positive material occluding blood vessels in the superficial dermis. The absence of vasculitis made an essential (or monoclonal) cryoglobulinemia the most likely disease process. The suspicions of the dermatopathologist were confirmed shortly thereafter when a blood cryoglobulin quantitation of 2% (abnormal >1%) was observed. Furthermore, the cryoprecipitate contained a monoclonal IgM lambda protein. This finding directed the downstream laboratory workup towards the identification of entities that are

associated with essential cryoglobulinemia including plasma cell dyscrasias, macroglobulinemias, and lymphomas. Accordingly, the patient was evaluated by serum protein electrophoresis (SPEP). The SPEP revealed normal serum protein values. Additionally, the patient demonstrated no significant adenopathy or abnormalities on thoracic and abdominal imaging. Such negative screenings were somewhat of a mystery given the circulating monoclonal cryoglobulin.

Nonetheless, laboratory persistence prevailed when immunofixation (IF) performed on the aforementioned SPEP specimen demonstrated two faint monoclonal bands, an IgM lambda and an IgG lambda, that were missed by the quantitative screening. Appropriate diagnostic studies, including a bone marrow biopsy and flow cytometry uncovered the CD19+CD20+, lambda marginal zone lymphoma with significant marrow involvement that was afflicting our patient, GG. The patient was then admitted for emergent plasmapheresis and began chemotherapy. He has had improvement of his skin lesions and is tolerating the regimen.

Cryoglobulins are immunoglobulins that precipitate as serum is cooled below core body temperatures. By definition, these proteins undergo resolubilization upon rewarming. The prevalence of cryoglobulinemia is 1 per 100,000, with a female to male ratio of 3:2, and a mean age at presentation of 42-59 years. Their classification according to composition was delineated by Brouet in 1974. Type I (essential) cryoglobulins consist of a single component, almost invariably a monoclonal immunoglobulin (usually IgM or IgG). Type II (mixed monoclonal) cryoglobulins consist of a monoclonal IgM plus a polyclonal IgG. Both types I and II are associated with multiple myeloma, Waldenstrom's macroglobulinemia, and various lymphomas. Type II is also linked to hepatitis C and numerous rheumatologic disease states. Type III (mixed polyclonal) cryoglobulins are composed of polyclonal IgM and polyclonal IgG proteins. These cryoglobulins are associated with a variety of disorders such as connective tissue diseases and chronic infections in which immune complexes may occur. Isolated cryoglobulins may be quantitated by reference to the volume of serum from which they are isolated as a percentage (cryocrit), or as an absolute value determined by various protein assays. In general, a cryocrit greater than 1% is considered a positive test. After repeated washing in cold saline, purified cryoglobulins may be further analyzed by IF.

Two salient points should be emphasized in reviewing the current case. One is the affect a circulating cryoglobulin can have on other lab results, and another is that SPEP alone is not a sufficient screen for a monoclonal protein. First, care is needed in processing of whole blood or serum specimens when cryoglobulinemia is in the differential diagnosis. Significant delay in processing, or exposure to cold may allow for a previously unknown cryoglobulin to form small aggregates with circulating factors including serum proteins, red blood cells, white blood cells, and platelets; thereby artifactually altering their determinations. Second, SPEP is typically performed if multiple myeloma, Waldenstrom's macroglobulinemia, primary amyloidosis, or a related disorder is suspected. Immunofixation with IgG, IgA, IgM, kappa, and lambda (and IgD and IgE if needed) is done when a sharp peak or band is found. However, if no monoclonal protein is observed on SPEP, and there is a low likelihood of disease, IF testing with only kappa and lambda is warranted. Furthermore, if the clinical index of suspicion for disease is high and no monoclonal protein is observed on SPEP, immunofixation with IgG, IgA, IgM, IgD, IgE, kappa, and lambda should follow as SPEP alone may miss a small monoclonal protein in the presence of normal background immunoglobulins.

References:

1. Kyle R. Sequence of Testing for Monoclonal Gammopathies, Serum and Urine Assays. *Arch Pathol Lab Med*., Vol 123, 1999: 114-118.
2. Choudry I, Check I, Perkins J. Severe Cryoglobulinemia Causing Spurious Laboratory Results and Necessitating High Temperature Plasma Exchange. Department of Pathology and Laboratory Medicine, ENH, Evanston, IL.
3. Gorevic P. Cryopathies: Cryoglobulins and Cryofibrogenemia. *Samter's Immunologic Diseases*. 6th Edition, volume 2, pp 1002 -1015.

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