Guideline for the Appropriate Ordering of Serum Tests for 25-hydroxy Vitamin D and 1,25-dihydroxy Vitamin D (CLP026) June 2010

1. Purpose

The significance of vitamin D in maintaining good health has received a great deal of attention in recent years. In addition to its role in skeletal development, the maintenance of bone mass and prevention of fractures, higher levels of vitamin D have recently been associated with significant disease prevention. This association includes decreased risk of certain cancers, diabetes mellitus, multiple sclerosis, autoimmune diseases, cardiovascular disease, Crohn's disease and other inflammatory bowel diseases, as well as lowered mortality in patients with chronic kidney disease. These findings appear to be responsible, at least in part, for the recent dramatic increase in demand for vitamin D testing.

The purpose of this Guideline is to clarify when testing for 25-hydroxy vitamin D and for 1,25-dihydroxy vitamin D is indicated and more importantly when such testing is not indicated.

Readers are reminded that OAML Guidelines cannot be applied to every clinical situation, nor can they serve as a substitute for sound clinical judgement.

2. Background

Vitamin D is a commonly used term for a group of secosteroids that act as precursors to biologically active 1,25-dihydroxy vitamin D. It refers to both the D_2 and D_3 forms of these compounds. Definitions of the specific products of this metabolic pathway are described in the appendix of this document (see page five).

Vitamin D_2 /D_3 is metabolized in the liver to produce 25-hydroxy vitamin D_2 /D_3. These compounds then undergo further hydroxylation, primarily in the kidneys, to produce 1,25-dihydroxy vitamin D. Active vitamin D is transported in the serum bound to vitamin D binding protein (VDBP). It can bind to vitamin D receptors in target tissues, where it plays a central role in regulating calcium homeostasis and in promoting cellular differentiation.

Serum 25-hydroxy vitamin D is the analyte of choice for assessment of a patient's vitamin D level. It is preferred because it reflects precursor levels of vitamin D derived from cutaneous metabolism as well as from dietary intake. In addition, when compared to 1,25-dihydroxy vitamin D, its concentration is an order of magnitude higher, is less subject to physiological variation, has a longer half-life, and correlates well with bone mineral density. Current 25-hydroxy vitamin D assays measure both the D_2 and D_3 forms of the vitamin equally well (i.e. in equimolar amounts) and are accurate and reproducible (CV's less than 15%).

3. Vitamin D Supplementation

The average Canadian's exposure to sunlight is generally inadequate to maintain vitamin D sufficiency. This is especially true in winter, in people with greater degrees of skin pigmentation, in those who choose to cover much of their skin when outdoors, in those using sun blocking agents and individuals living at higher geographical latitudes. Despite fortification of some foods (e.g. milk) with vitamin D, many Canadians are still unable to maintain their serum 25-hydroxy vitamin D levels in the "sufficient" range, defined as 75 – 250 nmol/L.

The difficulty in sustaining serum vitamin D levels is evident from a database review of 25-hydroxy vitamin D results conducted by one Ontario community laboratory (2009 data, n=54,456).[1] The review showed that:

- 45-58% of patient results were in the "insufficient" range (25-74 nmol/L)
- 38-54% were "sufficient" (75 – 250 nmol/L)
- less than 5% were "deficient" (<25 nmol/L)
- less than 0.13% were "high" (>250 nmol/L)

The ranges of percentages for vitamin D for each observed category reflect seasonal variations.

Several American and Canadian health agencies now recommend a minimum level of daily dietary or supplemental vitamin D intake of:

- 200 IU for adults 19-50 years of age,
- 400 IU for adults between 50 and 70 years of age,
- 600 IU beyond 70 years of age, to a maximum of 2000 IU/day.[2,3]

Evidence is accumulating to suggest that vitamin D intake should be in the range of 800 - 1000 IU per day, and up to 2000 IU per day for pregnant or lactating women.[4] The Canadian Cancer Society recommends supplementation of 1000 IU per day during the fall and winter, and year-round for older people and for those with darker skin pigmentation. It has recently been suggested that a daily dose of as much as 10,000 IU/day may be required in some morbidly obese patients.[5]

Others have suggested that although the requirements noted above are probably adequate to maintain the calcium and phosphorus regulating functions of vitamin D, far higher doses may be required to achieve the disease-risk reductions outlined in Section 1 of this Guideline. [6] Consensus on the appropriate dosage and duration of supplementation has yet to be reached.

Vitamin D toxicity is rarely seen, but may occur following prolonged consumption of very high doses (> 10,000 IU/day) of vitamin D.[5] Toxicity is manifested by pain, muscle weakness, vomiting and confusion. Hypercalcemia, hypercalcuria and hyperphosphatemia may also be present.

Caution: Vitamin D supplementation may provoke hypercalcemia in patients with certain clinical conditions. These include:

- Parathyroid disease [7]
- Granulomatous disease [5]
- Sarcoidosis [5]
- Lymphoma [5]
- Kidney disease [7, 8]

One must be aware that the potential toxicity of certain medications such as digoxin and thiazide diuretics may be increased secondary to a resulting hypercalcemia. Administration of vitamin D in

these patients must be supervised by a physician and vitamin D and calcium testing may be indicated. [8,9]

Vigilance is especially advised when vitamin D supplementation is provided to pregnant women with the above clinical conditions.

4. Vitamin D Testing

(a) 25-hydroxy vitamin D (D_2 or D_3)

In February 2010, the Ontario Health Technology Advisory Committee (OHTAC) published an evidence-based analysis of the clinical utility of vitamin D testing.[10] As the literature and the Ontario laboratory data presented in this Guideline have shown, there are a relatively small number of patients who are truly vitamin D deficient, but there are a significant number of patients with insufficient serum concentrations of vitamin D.[1,10] Michael Holick, a recognized expert on the topic, has stated "with the recognition of wide spread deficiency/insufficiency in children and adults there is no need to measure everybody's blood 25 hydroxy vitamin D". He further advocates a vitamin D supplementation program for all children and adults. [6]

The OHTAC recommendations for vitamin D testing support Holick's conclusion and specify that:

- Routine vitamin D testing is not warranted in the average risk population
- Health Canada guidelines for vitamin D intake and supplementation should be followed for asymptomatic, at-risk individuals such as elderly or infirm patients, or those who are believed to receive inadequate sun exposure. Baseline and/or follow-up vitamin D testing is unnecessary in this group.[11]

OHTAC did recommend that measurement of serum 25-hydroxy vitamin D may be of clinical value in individuals with conditions in which vitamin D is implicated such as:

- Significant renal or liver disease
- Osteomalacia, osteopenia or osteoporosis
- Possible cases of rickets
- Malabsorption syndromes
- Hypo or hypercalcemia/hyperphosphatemia
- Hypo or hyperparathyroidism.[11]

Clinical situations in addition to those identified by OHTAC and in section three for which measurement of serum 25-hydroxy vitamin D may be of value include:

- Patients on medications that affect vitamin D metabolism such as phenobarbitol, carbamazepine, phenytoin and valproate [8,12]
- Unexplained increased levels of serum alkaline phosphatase
- Patients taking high doses of vitamin D (> 2000 IU daily) for extended periods of time (>6 months), and who are exhibiting symptoms suggestive of vitamin D toxicosis (hypervitaminosis D).

Note: Vitamin D levels in infants (<1 year) could be misleading due to the presence of relatively high levels of biologically less active C-3 epimers of vitamin D. If a vitamin D test is required on an infant, the Laboratory Director of your laboratory should be contacted before ordering.

(b) 1,25-dihydroxy vitamin D

Measurement of serum 1,25-dihydroxy vitamin D levels should be limited to patients suspected of having renal 1α-hydroxylase deficiency. This is most often seen in cases of significant chronic renal failure (i.e. stage 4 or 5 chronic kidney disease). The test may also be useful in resolving unexplained hyperparathyroidism in patients with seemingly adequate serum levels of 25-hydroxy vitamin D, lymphoma, sarcoidosis, or in cases of suspected vitamin D receptor defects.[13]

Other than in very complex cases (e.g. hypercalcemia with reportedly low endogenous levels of 25-hydroxy vitamin D), there are rarely indications for ordering both 25-hydroxy vitamin D and 1,25-dihydroxy vitamin D on the same specimen at the same time.

Consultation with a specialist should be considered for patients with unexplained bone pain, unusual fractures, hypercalcemia of unexplained origin or other evidence suggestive of metabolic bone disease.

5. Summary

- Measurement of serum 25-hydroxy vitamin D levels within a healthy population has little diagnostic value, since supplementation is generally indicated regardless of the outcome of the 25-hydroxy vitamin D test. Asymptomatic patients at risk for vitamin D deficiency should therefore be put on vitamin D supplements, as per the latest Health Canada guideline without any need to measure serum levels of vitamin D.
- Measurement of serum levels of 25-hydroxy vitamin D should be reserved for patients with the clinical conditions identified above in Section 3 and 4a.
- Serum 25-hydroxy vitamin D is the test of choice for determining vitamin D level in the infrequent occasions when clinically indicated.
- Measurement of serum levels of 1,25-dihydroxy vitamin D is rarely indicated and is to be reserved specifically for patients with the clinical conditions identified above in section 4b.

6. References

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Appendix

Definitions

- 25-hydroxy vitamin D - refers to either of the hydroxylated forms of vitamins D_2 or D_3
- 1,25-dihydroxy vitamin D - refers to either the di-hydroxylated (at positions 1 and 25) forms of vitamins D_2 or D_3
- Vitamin D_2 (ergocalciferol) - a plant/invertebrate/fungus-derived source of vitamin D; often used for oral Vitamin D supplementation
- 25-hydroxy vitamin D_2 - the hydroxylated product of Vitamin D_2
- 1,25-dihydroxy vitamin D_2 - the physiologically active form of vitamin D_2
- 25-hydroxy vitamin D_3 (calcidiol or calcifediol) - a product of the hydroxylation of Vitamin D_3 in various body tissues, primarily the liver
- Vitamin D_3 (cholecalciferol) - synthesized naturally from 7-dehydrocholesterol in skin upon exposure to sunlight and can also be absorbed from food containing vitamin D_3
- 1,25-dihydroxy vitamin D_3 (calcitriol) - the physiologically active form of vitamin D_3, which is a product of the enzymatic hydroxylation of calcidiol by 1α-hydroxylase that takes place primarily in the kidneys
- 24,25-dihydroxy vitamin D_3 - an inactive form of dihydroxylated vitamin D_3, produced in the kidneys when the 1α-hydroxylase is not active.
- 1-hydroxy vitamin Dз (alfacalcidiol) - a synthetically-produced vitamin D that has been hydroxylated at position 1. Administration of this compound to patients allows the bypass of the renal hydroxylation step and production of calcitriol in patients with 1-α hydroxylase deficiency e.g. in severe chronic renal failure.
- Vitamin D binding protein (VDBP) - the carrier protein for 1,25-dihydroxy vitamin D (D_2 or D_3) in serum
- Vitamin D receptor (VDR) - the tissue receptor for 1,25-dihydroxy vitamin D (D_2 or D_3), found in the nuclei of vitamin D target cells.

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