Parathyroid hormone, calcitonin and vitamin D testing in calcium and bone metabolic disorders

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INTRODUCTION

The test triad parathyroid hormone (PTH), calcitonin and vitamin D is finding increasing use in the diagnosis of calcium and bone metabolic diseases. Healthy bones rely on a constant concentration of calcium in the blood, which is regulated by the concerted action of the three hormones. When the calcium concentration becomes imbalanced, bone density is reduced, weakening the bones and increasing the risk of fractures. Diseases of calcium and bone metabolism include rickets in children, osteomalacia in adults, osteopenia and osteoporosis.

COMPOSITION OF BONE TISSUE

Bone tissue is composed to around 80% of a mineral phase, which comprises mostly calcium phosphate in crystalline form (hydroxyapatite). This is incorporated into the bone matrix and is responsible for the rigidity of the skeleton. The organic bone material consists largely of type I collagen, which provides bone with its elasticity.

On the cellular level, bone tissue can be divided into two main cell types, osteoblasts and osteoclasts, which work closely together to regulate the continuous, dynamic bone remodelling. Osteoblasts are bone-producing cells, which synthesise the proteins necessary for building the extracellular matrix. Osteoclasts on the other hand function osteolytically, releasing protons and proteases responsible for the degradation of bone material.

Both types of cells are regulated by hormones, growth factors and cytokines. In addition to the three main regulators PTH, calcitonin and vitamin D, other regulatory compounds include glucocorticoids, oestrogen, androgens, interleukin-1 and the growth factor IGF-1.

PARATHYROID HORMONE

PTH is a peptide hormone which is synthesised in the parathyroid glands. Its main function is to increase the concentration of calcium in the blood plasma. Intact PTH (iPTH) is the biologically active form and is secreted when the calcium level is low. iPTH indirectly affects the bone matrix by stimulating the formation of osteoclasts, which increases bone resorption. Dysregulation of PTH leads to hyper- or hypothyroidism.

CALCITONIN

Calcitonin (also known as thyrocalcitonin) is produced in the thyroid gland and is the antagonist of PTH. The production and secretion of calcitonin is stimulated by an elevated calcium level and results in a reduction in the calcium concentration in the blood. Calcitonin is indirectly responsible for increasing mineralisation and synthesis of bone matrix. Low calcitonin levels are associated with osteopenia, a preliminary stage of osteoporosis. For this reason, calcitonin is among the substances given in osteoporosis therapy.

VITAMIN D

Vitamin D exists in two forms: vitamin D2 which is acquired by consumption of plant foods such as mushrooms and avocado, and vitamin D3 which is obtained from animal food sources or produced in skin exposed to ultraviolet B radiation. These two forms are bound in the blood to a binding protein and are converted in the liver to the 25-hyroxl forms, 25-OH vitamin D2 and 25-OH vitamin D3, which are storage forms with little biologically activity. In the kidneys the vitamin becomes the biologically active metabolite 1,25-dihydroxy vitamin D (D hormone). D hormone regulates calcium uptake from the intestine, bone mineralisation, differentiation of osteoblasts and synthesis of bone matrix. Vitamin D deficiency results in bone atrophy, and increases the risk for a multitude of other diseases.

HYPERTHYROIDISM

An uncontrolled increase in the level of PTH results in hyperthyroidism (HPT), leading to hypercalcaemia. The excessive calcium results in specific bone changes with a reduction in bone mass (osteopenia or osteoporosis) as well as kidney stones.

HPT can be primary, secondary or tertiary. The primary form may be due to a parathyroid gland adenoma or rarely a carcinoma, or to primary hyperplasia of all four parathyroid glands. The latter may be part of a multiple endocrine neoplasia (MEN). Primary HPT is often asymptomatic, proceeding with a clinically unapparent course for a long time, with the only indication being moderate hypercalcaemia. Secondary HPT is a reactive increase in PTH as a result

of hypocalcaemia caused by kidney, liver or intestinal disease. Tertiary HPT is a reactive parathyroid gland hyperplasia resulting from sudden treatment of secondary HPT, for example renal transplantation, since basal secretion of PTH remains high for a long time.

HYPOPARATHYROIDISM

A deficiency in PTH results in hypoparathyroidism. This usually occurs following a throat operation. It is generally iatrogenic, e.g. due to inadvertent removal of parathyroid glands during thyroidectomy, following radioiodine therapy or in cases of malignant parathyroid gland tumours. Other causes are kidney insufficiency, genetic mutations, oversupply of vitamin D, autoimmune diseases, autoimmune polyglandular syndrome, long-standing and severe magnesium deficiency and DiGeorge syndrome (due to underdevelopment of the parathyroid glands). Symptoms of hypoparathyroidism are hypocalcaemia with cramps and heart failure.

HYPOVITAMINOSIS D

Vitamin D deficiency is mainly caused by insufficient exposure to UV light, for example, due to a predominantly indoor lifestyle, excessive use of sunscreens, dark skin, complete covering of the skin with clothes, a vitamin D deficient diet or medical conditions that affect the vitamin D supply in the body.

Vitamin D deficiency has manifold health consequences. Even a slight deficiency is sufficient to cause a secondary increase in PTH and an elevated osteolysis rate. Severe vitamin D deficiency causes rickets or osteomalacia, characterised by defective bone growth and matrix mineralisation. Vitamin D deficiency is also one of the most important risk factors for senile osteoporosis.

Hypovitaminosis D is, furthermore, a risk factor for numerous other diseases. These include autoimmune diseases, infections, cardiovascular and musculoskletal diseases, malignant tumours, cognitive disorders, Parkinson's disease, dementia and mental illnesses. Vitamin D deficiency is generally treated with supplementation.

ROLE OF SEROLOGICAL DIAGNOSTICS

The serological determination of iPTH, calcitonin and vitamin D plays an important role in the diagnosis of bone metabolic diseases and related conditions. It supports other diagnostic methods such as case history, physical examination, radiographic analysis, bone biopsy and other laboratory tests, including serum calcium, phosphate, alkaline phosphatase and urinary calcium.

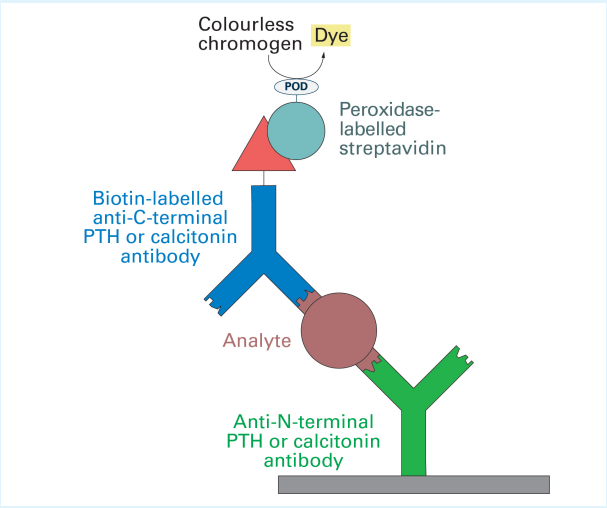
iPTH determination is an essential component in the diagnosis of functional disorders of the parathyroid gland. It is the most important serological test for the diagnosis of primary HPT, and it supports clarification of the secondary and tertiary forms.

Calcitonin, in addition to its application in bone disease diagnostics, is also the most specific and sensitive biochemical marker for diagnosing and monitoring medullar thyroid carcinoma (C-cell carcinoma). The serum calcitonin level is strongly increased in this type of cancer. After successful total thyroidectomy, calcitonin is no longer detectable. Increasing concentrations of calcitonin after therapy indicate relapse or metastasis. Hyperparathyroidism is present in 20-30% of patients with medullar thyroid carcinoma.

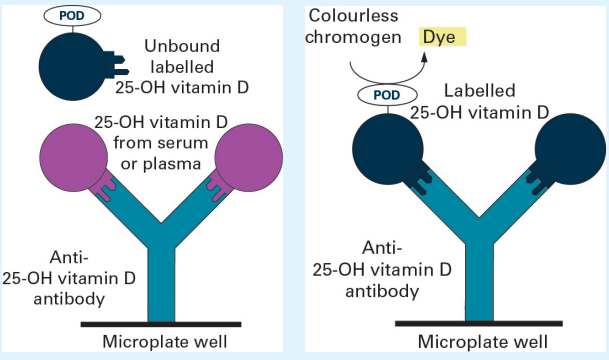
Measurement of the vitamin D concentration allows identification of a vitamin D deficiency or overdose. The serum level of 25-OH vitamin D, which of all vitamin D metabolites in the blood shows the highest concentration, is the best indicator of the vitamin D supply in the body. Regular monitoring of the vitamin D level in patients under treatment is also important due to the widely differing responses. For example, some individuals require long periods of supplementation lasting years or even decades. ➔

▼FIGURE 1: ELISA microplate

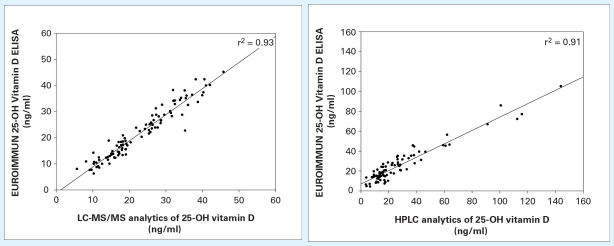
▼FIGURE 2: Principle of the iPTH and calcitonin ELISAs



▼FIGURE 3: Principle of the vitamin D ELISA **A**. Binding of vitamin D in sample ® no colour reaction **B.** No vitamin D present in sample ® colour reaction



▼FIGURE 4: Comparison of vitamin D ELISA with A. HPLC and B. LC-MS/MS



There are various methods for measuring iPTH, calcitonin and vitamin D in patient serum. Microplate ELISA (Figure 1) is usually favoured in a routine setting due to its speed, ease of use and automatability.

IPTH AND CALCITONIN ELISAS

ELISAs for determination of iPTH and calcitonin are based on a sandwich principle (Figure 2). The metabolite to be measured is sandwiched between an anti-N-terminal antibody coated onto the microplates and an anti-C-terminal antibody in the liquid phase. The complex is then detected by means of a chromagen/substrate reaction. The intensity of the colour formed is proportional to the concentration of the metabolite in the serum. The iPTH/calcitonin determination takes less than four hours. Ready-to-use reagents and break-off ELISA wells enhance the convenience of the ELISA analysis.

The performance of the EUROIMMUN iPTH and calcitonin ELISAs has been assessed in various studies. In samples from healthy blood donors the assays yielded values in the expected normal range. The ELISAs showed, moreover, an excellent correlation with other tests, such as other commercial enzyme immunoassays (iPTH) or radioimmunoassay (calcitonin).

VITAMIN D ELISA

The 25-OH vitamin D ELISA works on a competitive principle (Figure 3 A and B). Labelled 25-OH vitamin D competes with unlabelled metabolite from the patient serum for binding sites on an anti-25-OH vitamin D antibody coated onto the microplates. Following a chromagen/substrate reaction, the colour intensity obtained is inversely proportional to the 25-OH vitamin D concentration in the serum.

The monoclonal antibody used in the EUROIMMUN 25-OH vitamin D ELISA is equally specific for both D2 and D3 forms of the vitamin. This is critical since either D2 or D3 may be used for treatment. The ELISA procedure is quick, simple, cost-effective, taking less than 3 hours owing to enhanced analyte release and incubation in a single step and predominantly ready-to-use reagents.

The EUROIMMUN 25-OH vitamin D ELISA shows a high correlation with the reference methods HPLC and LB-MS/MS (Figure 4 A and B). These chromatography-based methods provide the most accurate measurement of vitamin D, but are largely unsuitable for use in a routine setting as they are generally time-consuming and labour-intensive, requiring costly equipment and large sample volumes.

A dedicated external vitamin D quality assurance scheme, DEQAS, allows laboratories to assess their performance at regular intervals and ensure high quality standards in vitamin D determination. In DEQAS schemes, the 25-OH vitamin D ELISA from EUROIMMUN has consistently performed well, achieving all performance targets since its introduction in April 2011. The 25-OH vitamin D ELISA is, moreover, FDA registered.

PERSPECTIVES

Bone metabolic diseases are a major health problem, causing bone fractures, skeletal pain and deformities. As the population ages, diseases caused by low bone mass are set to increase in prevalence, placing more people at risk of fractures. Serological testing for iPTH, calcitonin and vitamin D aids identification of underlying imbalances in calcium regulation that contribute to bone disease. Thus, corrective or therapeutic measures can be targeted to the causative disorder, helping to minimise the risk of severe long-term health effects.