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EDITORIAL

Persisting challenges in plasma endocrinology: reference values and endocrine tests

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ABSTRACT

The analysis of plasma hormone concentrations is of fundamental importance for the diagnosis and treatment of endocrine diseases. Although hormone analyses are performed in huge numbers in all hospitals on a daily basis, the interpretation of the resulting plasma hormone concentrations can be difficult. In addition to the effects of the underlying disease, biological and analytical issues affect hormone concentrations. Therefore, adequate reference values and strict standardisation of sampling and analytical procedures are very important for the final interpretation of the results of hormone analysis.

The pretest likelihood of a test profoundly affects the sensitivity and specificity of tests. The pretest likelihood is based on interpretations of the symptoms and physical examination of the patient. Therefore, the experience of the doctor is of paramount importance in establishing an optimal pretest likelihood of the disease. Fortunately, in many cases the diagnosis of the underlying endocrine disorder is straightforward. However, in some patients the interpretation of the results of hormone measurements and endocrine tests may be very difficult. In this respect, it should be realised that many pathophysiological, biological and analytical issues affect hormone concentrations and endocrine tests. Failure to take into account the factors that affect hormone concentrations in addition to the effects of endocrine disorders may lead to great confusion. Therefore, adequate reference values and strict standardisation of sampling and analytical procedures are important for the final interpretation of the results of hormone analyses.

VARIATION IN HORMONE CONCENTRATIONS: PREANALYTICAL FACTORS

Biological variation

Many biological factors influence endogenous hormone secretion in addition to the effects of endocrine disease. These include gender, pulsatile and diurnal variation in hormone secretion, non-endocrine diseases, endocrine effects of non-endocrine drugs, nutritional status and age.

Gender

In addition to the effects of gender on oestrogens and progesterones, there are major effects on other endocrine systems. These include effects on the axis between the growth hormone and insulin-like growth factor-1 (IGF-1), and on leptin.

Pulsatile hormone secretion

Most hormones are secreted in a pulsatile fashion. As a consequence, a considerable variation can be present in hormone concentrations within a single subject. An example of a hormone with a manifold variation in hormone secretion is growth hormone.

Diurnal variation of hormone secretion

Most, if not all, hormones reveal diurnal changes in plasma concentrations. For example, plasma cortisol levels are higher in the early morning, in the afternoon they start to decrease to a nadir around midnight. Reference values for plasma cortisol in healthy subjects will, therefore, depend on clock hours.

Non-endocrine disease

Non-endocrine disease in general has profound effects on all aspects of endocrine regulation. These include changes

in thyroid hormone metabolism in otherwise euthyroid subjects (the so-called euthyroid sick syndrome), in the pituitary-adrenal axis (cortisol levels may increase) and in the pituitary-gonadal axis (decreased testosterone and oestrogen levels). Therefore, in patients with non-endocrine diseases, reference values of plasma hormone concentrations are different from those obtained in healthy subjects. If endocrine diseases are suspected in these subjects, this should be taken into consideration. For instance, the discrimination between the euthyroid sick syndrome and hyperthyroidism/hypothyroidism may sometimes be difficult. In the euthyroid sick syndrome both decreased (free thyroxine) FT_4 levels (in very ill subjects) and increased FT_4 levels (usually during recovery from euthyroid sick syndrome) may be found.[1]

Endocrine effects of non-endocrine drugs
A large number of drugs affect plasma hormone concentrations. In clinical practice it is helpful to consider these effects for every drug unless it has been proven otherwise. These effects of otherwise non-endocrine drugs complicate the interpretation of hormone concentrations. An important example is the diagnostic work-up of pheochromocytoma. These patients are often treated for their hypertension. Many antihypertensive drugs, however, increase plasma catecholamine levels and urinary catecholamine excretion. These drugs may, therefore, result in false-positive tests for pheochromocytoma. A second factor is the analytical interference between a drug and the assay, although in most of the newer methods this factor is of limited importance. Examples are the catecholamine measurement in the presence of methyldopa, sotalol and phenoxybenzamine.

Nutritional status
This is an important modulator of endocrine regulation. Both low (e.g. anorexia nervosa) and high body mass index profoundly affect hormone secretion. For instance, growth hormone secretion is increased in anorexia nervosa, but decreased in obesity. This effect of nutritional status affects the proper interpretation of endocrine tests aimed at diagnosing insufficient or excessive growth hormone secretion.

Age
The endocrinology of ageing is characterised by decreased plasma levels of several important hormones such as growth hormone, IGF-1 and sex steroids in both sexes. Therefore, age-adjusted reference values are required for such hormones.

Variation due to logistical factors
In addition to biological factors, logistical issues of sample collection, sample handling and storage may affect the final results of hormone measurements. These include the kind of tubes used to collect samples, temperature of the tubes, immediate versus delayed plasma collection, and duration and temperature of storage.

VARIATION IN HORMONE CONCENTRATIONS: ANALYTICAL FACTORS

The description of the radioimmune assay by Yalow and Bersow in 1959 has revolutionised the analysis of hormone analysis. Prior to their Nobel Prize winning discovery, the measurement of hormone concentrations by bioassays was very cumbersome. In the past forty years a continuing evolution of hormone assays has occurred. In recent years this has resulted in the widespread implementation of different kinds of robotic assays. Initially, clinical endocrinologists were able to base their career on the implementation of new (radioimmune) assays. Subsequently, hormone analysis was taken over by clinical chemists, at least in the Netherlands. Finally, with the introduction of robotic assays, there is a great danger that experience with the problems of the analysis of hormone concentrations is also waning not only in internists, but also in clinical chemists. In this respect there are several major problems. Each robot allows only a limited number of hormone assays, thus limiting the choice between the available assays. Frequently, choices for less optimal assays are based merely on the available types of robots. Another problem is that reference values are usually not determined within each laboratory, but derived from the description by the commercial producers of the assays. Importantly, it appears that different laboratories using the same commercial assays may yield different results for the same plasma samples. Moreover, continuous quality control within each laboratory remains necessary to ascertain that the intralaboratory variation remains within acceptable limits. Problematic assays with respect to large intralaboratory and interlaboratory variation are those of IGF-1 and of urinary cortisol. Within the Netherlands the LWBA, the national work group for bindings analysis, focuses on quality control of analytical variation of hormone analysis between and within laboratories.

PLASMA HORMONE CONCENTRATIONS AND DIAGNOSTIC TESTS OF ENDOCRINE DISEASES

The endocrinology department of the Academic Medical Centre of Amsterdam University is to be praised for their continuing efforts to evaluate the reference values for endocrine tests. By evaluation of a balanced group of healthy volunteers with respect to sex and age, they report

the normal reference values for plasma aldosterone concentration and plasma renin activity.[2] These values were determined in plasma and urine samples obtained during a strict protocol, limiting preanalytical variation as much as possible. The next step is to evaluate the sensitivity and specificity of these tests in patients with hypertension with and without primary hyperaldosteronism.

In the other publication,[3] the same group evaluated the value of the thyrotropin-releasing hormone (TRH) test in differentiating idiopathic hyperprolactinaemia and prolactinoma in 92 consecutive patients with hyperprolactinaemia. In a previous study the reference values of this test were established in healthy subjects. Remarkably, hyperprolactinaemia was not confirmed in 17% of the patients, which may be due to interlaboratory analytical variation and/or preanalytical biological variation. In addition, they show that the TRH test can be omitted in the evaluation of hyperprolactinaemia.[3] Previously, the TRH test was also omitted in the evaluation of thyroid dysfunction, because the thyroid-stimulating hormone (TSH) response is directly related to baseline TSH levels measured by ultrasensitive TSH assays. One of the few indications remaining for TRH testing in clinical endocrinology may be the follow-up of patients with cured acromegaly. During prolonged follow-up, recurrence of growth overproduction occurs in ~ 19% of the patients and in some of them the persistent, paradoxical response of growth hormone to TRH predicts recurrence of growth hormone overproduction.[4]

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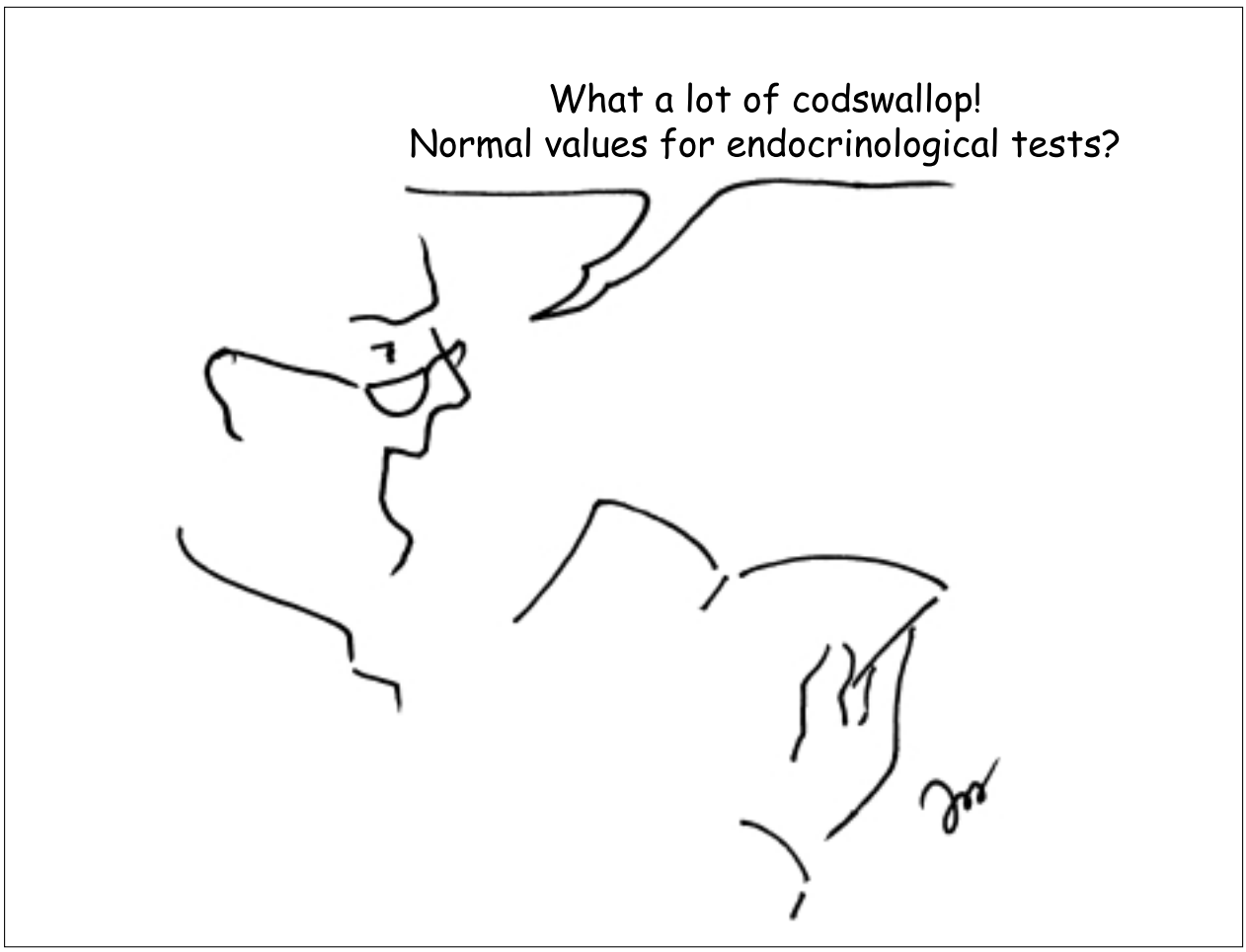

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