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Measurement of Free Triiodothyronine in Intensive Care Patients – Comparison of Two Routine Methods

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Summary: One hundred sera from intensive care patients, and 93 sera from endocrinological outpatients were used for a comparison between two automated assays for free triiodothyronine (Enzymun Test, and Elecsys 2010, both Boehringer Mannheim, Mannheim, Germany). In outpatients a good correlation between both methods was found (r=0.932). In contrast, comparability between the two assays was poor in intensive care patients (r=0.75, after exclusion of two outliers); significantly more values in the hypothyroid range were found with the Elecsys 2010 assay (n=83, compared with n=33 with the Enzymun Test; χ^2 test p=0.001). We conclude that routine measurement of free triiodothyronine which has the theoretical advantage of quantifying the biologically active fraction of thyroid hormones may have methodological limitations in severely ill patients.

Introduction

Measurement of free thyroid hormones has become part of clinical laboratory routine in recent years. Determination of free triiodothyronine (fT3) provides a special challenge since the binding to the transport proteins is comparatively weak and the concentration of the free hormone is only 0.01% of the total thyroxine concentration. There is no reference method established neither for free nor for total triiodothyronine (T3). Triiodothyronine is the biologically active thyroid hormone. It is formed predominantly by intracellular deiodination of thyroxine in the target tissues. In severely ill patients substantial discrepancies have been reported for free thyroid hormone levels determined either by routine methods or by candidate reference methods such as equilibrium dialysis and ultracentrifugation (1–3). Routine monitoring of low thyroid hormone concentrations in intensive care patients may become increasingly relevant, although the biological importance of alterations in thyroid function and peripheral thyroid hormone metabolism in severe illness is still a matter of debate (4). However, a possible benefit of hormone treatment in subgroups of intensive care patients has been reported (5,6).

The aim of this study was to compare free thyroid hormone concentrations in sera from intensive care patients as determined by two assays run on automated analyzers used in our laboratory.

Patients and Methods

One hundred sera from intensive care patients (medical intensive care unit n=29; cardiosurgical intensive care unit n=31; neurosurgical intensive care unit n=20; surgical intensive care unit n=20) consecutively sent for thyroid hormone determination were analysed. Thyrotropin (thyroidea stimulating hormone), free thyroxine (fT4), and free triiodothyronine (fT3) were measured on two automated analyzers of the same manufacturer (Boehringer Mannheim, Germany). Additionally, 93 sera from endocrinological outpatients were included in the investigation. For method comparison simple regression analysis and *Pearson*'s correlation coefficient were determined. The following assays were applied:

Enzymun tests. Assays for thyroid hormones: One-site immunoenzymometric assays (competition principle) with streptavidin-coated tubes and photometrical quantification; in a first incubation the sample is incubated with peroxidase-conjugated hormone-antibodies; in a second step biotinylated triiodothyronine (or thyroxine) is added, that binds to the streptavidin-coated tubes; free conjugated antibodies are captured by this bound triiodothyronine (or thyroxine), while unbound reagents and the sample are then removed by aspiration; addition of a chromogenic substrate generates a colour reaction which is quantified. Thyrotropin assay: Sandwich ELISA with thyrotropin-antibody coated tubes, quantification of an enzymatic colour reaction.

Elecsys 2010 tests. Assays for thyroid hormones: Essentially the same principle as in the Enzymun assay; ferromagnetic microbeads are used as solide phase; antibodies are conjugated with a ruthenium complex. The separation of bound and unbound antibodies and of the sample is achieved by magnetic retention of the beads in the measuring cell; an electrical voltage induces electrochemiluminescence of the ruthenium-complex, which is photomultiplied and quantified. Thyrotropin assay: Sandwich ELISA. According to the manufacturer's information all antibodies used in the Enzymun and Elecsys assays are identical (except for the conjugate).

Reference values, as given by the manufacturer are as follows: TSH Elecsys, 0.27–4.2 mU/l; TSH Enzymun, 0.25–3.1 mU/l. fT3 Elecsys, 4.3–8.3 pmol/l; fT3 Enzymun 5.4–9.3 pmol/l. fT4 Elecsys and fT4 Enzymun, both 11.8–24.6 pmol/l (n=695 with the 5^{th}-95^{th} percentile for Enzymun assays; n=503 with the 2.5^{th}–97.5^{th} percentile for Elecsys assays).

Inter-assay imprecision of the Elecsys fT3 assay was tested over fifteen series on two different analyzer machines using aliquoted unpooled serum from one intensive care patient.

The manufacturers recommendations for calibration were followed closely, quality controls with control materials at two different levels were performed in each run.

Results

We found a good correlation for thyrotropin and for free thyroxine between the two assay systems in both outpatients and intensive care patients ((for intensive care patients: r = 0.996, y = 1.13 x + 0.06 (TSH), and r = 0.932, y = 0.91 x + 0.15 (fT4); x: Elecsys, y: Enzymun)). In contrast the correlation between the two fT3 assays was poor in intensive care patients (fig. 1a)) (r = 0,36 for all values, r = 0.75 after exclusion of two obvious outliers; y = 0.96 x + 2.21; x: Elecsys, y: Enzymun; note different reference values); moreover the value distribution of free triiodothyronine among intensive care patients differed considerably (*Elecsys fT3*: low n = 33, normal n = 61, high n = 5; median 2.8 pmol/l, 25th percentile 2.2 pmol/l, 75th percentile 3.4 pmol/l. *Enzymun fT3*: low n = 83, normal n = 13, high n = 3; median, 5.8 pmol/l, 25th percentile 4.9 pmol/l, 75th percentile 7.2 pmol/l). A χ^2 test (low fT3 values versus normal values between both systems was significant (p = 0.001). In contrast, in outpatients the correlation of fT3 values was good (r = 0.973), with only one misclassification between the two assays (fig. 1b)).

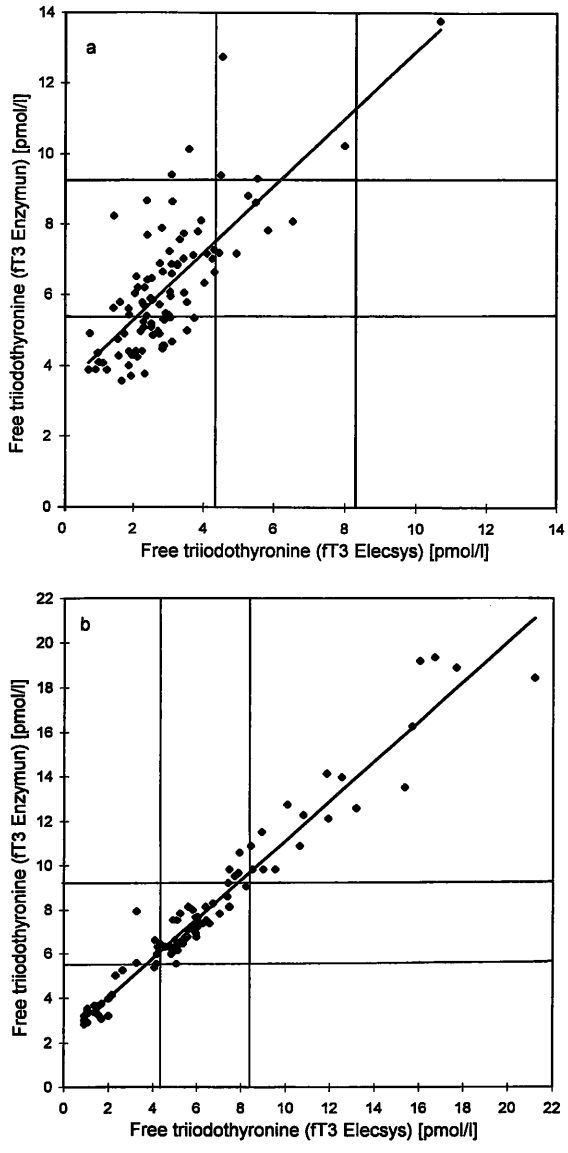

Fig. 1 a) Free triiodothyronine (pmol/l): method comparison between the Elecsys 2010 (x) and Enzymun assay (y) in 100 intensive care patients (after exclusion of two obvious outliers; lines indicating the respective reference range).
y = 0.96 x + 3.39, r = 0.75

b) Free triiodothyronine (pmol/l): method comparison between the Elecsys 2010 (x) and Enzymun assay (y) in 93 endocrinological outpatients (lines indicating the respective reference range).
y = 0.89 x + 2.24, r = 0.932
(For values below 7.7 pmol/l (on the Elecsys system) a nearly identical correlation and equation was found (r = 0.942, and y = 0.84 + 1.57)).

In the intensive care patient group each one outlier of free triiodothyronine measurement was identified and reproduced on both systems compared :

Patient E. W. (82 years, male), multiple organ failure due to septicaemia after cardiac surgery requiring haemofiltration: fT3 Elecsys *19.0* pmol/l, fT3 Enzymun 5.6 pmol/l, T3 Elecsys 1.2 nmol/l (reference range 1.2−3.1 nmol/l), T3 Enzymun 1.4 nmol/l (reference range 1.2−2.7 nmol/l).

Patient G. B. (62 years, female), lysis therapy for myocardial infarction: fT3 Elecsys 2.1 pmol/l, fT3 Enzymun *17.5* pmol/l, T3 Elecsys, 0.7, T3 Enzymun 0.6 nmol/l.

The interassay coeffecient of variation of the Elecsys 2010 fT3 assay was 8.1% at 3.4 pmol/l, and 20.9% at 1.1 pmol/l.

Discussion

The comparability of the two automated free triiodothyronine assays was poor in intensive care patients but good in outpatients. In the group of intensive care patients the frequency of subnormal free triiodothyronine values was significantly different depending on which method of the same manufacturer was used. In the absence of an established reference method, however, it is not possible to decide whether the Enzymun test in fact overestimates free triiodothyronine concentrations or the Elecsys assay underestimates this analyte. The clinical identification of the thyroid status in intensive care patients is difficult and often misleading.

Still undefined factors of endogenous or exogenous origin (e. g. common drugs like plasma expanders, or increased levels of free fatty acids in patients on heparin treatment) may be responsible for interferences with assays of free thyroid hormones in intensive care patients. At least three mechanisms may be involved: The bound to free (B/F) equilibrium or the avidity of hormone bind-

ing may be altered both in vivo and ex vivo; the binding characteristics of analytical antibodies may be altered; and though signal generation takes place in a sample-free medium in both assays substances that interfere with the signal generation (enzymatic colour reaction and electrochemiluminescence) may be bound to the assay solid phase. It is possible that the two tests compared here differ in their sensitivity to these putative interfering factors.

Outliers of free triiodothyronine results occured in both systems among intensive care patients (n=2). These results were reproducible. Thus spurious results have to be encountered in the percent range in intensive care patients.

In the hypothyroid range, precision of the Elecsys fT3 assay is limited with a functional sensitivity (coefficient of variation ≤ 20%) of about 1.1 pmol/l as evidenced by inter-assay imprecision study.

In summary, our results indicate that free triiodothyronine assays may be more prone to still undefined interfering factors in sera of intensive care patients than other thyroid hormone assays and that as a consequence routine measurement of free triiodothyronine may have substantial methodological limitations in hospitalized patients. Further clinically based evaluation in intensive care patients seems necessary for each test if clinical decisions like hormone substitution are based on free triiodothyronine test results in the low measuring range. At present the only clear indication for triiodothyronine measurement is the determination of isolated triiodothyronine-hyperthyroidism, and the monitoring of triiodothyronine-substitution therapy.

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