
Diagnostic Performance of a Modified Free-Thyroxin Assay in Subjects with Anomalies in Their Binding Proteins, G. van der Sluijs Veer,1 I. Vermeis,1 and H. A. Bonte2 (1 Dept. of Clin. Chem., SZEEO, Haakabegerstr. 55, NL-7513 ER Enschede, and 2 Dept. of Clin. Chem., Streekziekenhuis Midden-Twente, P.O. Box 546, NL-7550 AM Hengelo (Ov.), The Netherlands)

We tested the very recently introduced simple, direct, one-step "Ria-gnost FT₄" kit (Behringwerke AG, Marburg, F.R.G.). This one-step immunoextraction assay with coated tubes involves a modified [¹²⁵]I/T₄ derivative tracer with an enhanced affinity for the T₄-antibody ("reactive tracer").

All the subjects we tested were clinically healthy and euthyroid. The euthyroid status was confirmed by the finding of a normal thyrotropin (TSH) concentration, i.e., between 0.2 and 3.5 milli-int. units/L (TSH-IRMA; Behringwerke AG). The thyroxin-binding globulin (TBG) concentration was measured with a turbidimetric enzyme immunoassay, Enzymun-TBG test (Boehringer Mannheim GmbH, F.R.G.), with normal reference interval of 10–30 mg/L. Results for FT₄ by Ria-gnost assay were:

<table>
<thead>
<tr>
<th>FT₄, pmol/L (mean SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control subjects</td>
</tr>
<tr>
<td>31</td>
</tr>
<tr>
<td>13.1 (3.7)</td>
</tr>
<tr>
<td>Pregnancy</td>
</tr>
<tr>
<td>22</td>
</tr>
<tr>
<td>8.2 (2.2)*</td>
</tr>
<tr>
<td>Contraceptives</td>
</tr>
<tr>
<td>12</td>
</tr>
<tr>
<td>12.5 (3.9)</td>
</tr>
<tr>
<td>TBG deficiency</td>
</tr>
<tr>
<td>9</td>
</tr>
<tr>
<td>21.8 (9.0)*</td>
</tr>
<tr>
<td>Fam. dysab. hyperthyroxinemia</td>
</tr>
<tr>
<td>9</td>
</tr>
<tr>
<td>27.3 (5.7)*</td>
</tr>
<tr>
<td>Analbuminemia</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>13.4 (0.8)</td>
</tr>
</tbody>
</table>

Manufacturer's ref. values 200 17.0 (3.0)*

*Significantly different (P<0.001) from results for our control subjects.

As shown, the FT₄ concentration measured in our control subjects was significantly lower than the reference value given by the manufacturer. FT₄ concentrations were also significantly lower in women during the third trimester of pregnancy and significantly higher in subjects with familial dysalbuminemic hyperthyroxinemia and TBG deficiency compared with control subjects. The significantly lower FT₄ in women during the third trimester of pregnancy accords with the data given by the manufacturer and might be due to the increased TBG measured in these women (54 ± 7 mg/L). However, most published reports indicate little or no change in FT₄ during pregnancy (1) and support the obvious euthyroid state of these women.

Serum FT₄ concentrations measured in subjects with only slight deviations in serum TBG concentrations, such as women using oral contraceptives (mean TBG concentration 36, SD 4 mg/L), did not deviate significantly from the FT₄ concentrations found in the control group, in accord with the claims of the manufacturer.

However, there is no doubt that the significantly high FT₄ concentrations found in subjects with familial dysalbuminemic hyperthyroxinemia or with TBG deficiency do not reflect the functional state of the thyroid gland. The mean TSH concentrations in these subjects—2.1 (SD 1.1) and 2.1 (SD 0.8) milli-int. units/L, respectively—support the generally accepted view that these subjects are euthyroid (2).

Although the data on subjects with congenital analbuminemia are too few to support a clear conclusion, we, like others (3), find these patients have normal FT₄ values.

The introduction of "sensitive" TSH assays, followed by the new TSH-based diagnostic strategy, has made FT₄ measurement a second-line test in assessing thyroid function, either as an additional test in cases of presumed discrepancy between the clinical status and the TSH value, or as a measurement on which treatment can be based. From our results we conclude that one should be careful in interpreting the Ria-gnost FT₄ in subjects who have anomalies in their thyroxin-binding proteins.

References


For patients suspected of acute myocardial infarction (AMI) at the time of arrival at the hospital, the median time elapsed since the onset of chest pain is 2 h (1). Even with highly sensitive and specific assays for CK-MB, few patients with evolving MI could be recognized by use of CK-MB tests on serum sampled at the time of admission (2).

Results of electrophoretic studies suggest that the ratio of tissue-specific (CK3a) to serum-specific (CK3b) isoforms of CK-MM (CK3) becomes abnormal before values for CK-MB do and so could be used for early recognition of AMI (3). This approach was indirectly incorporated in two newly commercialized test kits, "CheCK-MM" and "ISOFOR-MM" (International Immunoassay Laboratories). CheCK-MM measures CK3a and CK3b by an immunoradiometric (immunoassay) method, and the reference interval is based on (a) the percentage of CK3a, and (b) the difference in mass concentration between CK3a and CK3b. ISOFOR-MM removes CK3a...
by immunoextraction, measures the catalytic activity of CK
before and after the extraction, and uses a reference interval
based on (a) the activity left after immunoextraction and (b)
the percentage of the activity that is removed.

We collected the initial sample from each of 125 patients
into tubes containing EDTA and tested for CK-MB with a
commercially available immunochemical kit, "IMPRES-
MB"; an IRMA kit, "QuICK-MB" (International Immunoas-
say Laboratories); and the above-mentioned isoform kits.
Serial specimens for these patients were tested with QuICK-
MB. The value for CK-MB in the initial sample was above
normal in 30 of these patients by the QuICK-MB test and in
22 by the IMPRES-MB test. Fifty-nine of the patients later
exceeded normal CK-MB concentrations in at least one of
the serial samples. The following results were obtained with
the use of the isoform tests, alone and in conjunction with
the CK-MB tests:

| Diagnostic value of the result for | Sensitivity, | Predictive value of |
| the initial sample                | %            | a positive result, % |
| CK-MB test alone:                |              |                    |
| IMPRES-MB                        | 37.3         | 100.0              |
| QuICK-MB                         | 50.8         | 100.0              |
| CK-MB isoform test alone:        |              |                    |
| ISOFOR-MM                        | 64.4         | 95.0               |
| Check-MB                         | 62.7         | 90.2               |
| Combined tests:                  |              |                    |
| IMPRES-MB/ISOFOR-MM              | 79.5         | 95.9               |
| QuICK-MB/Check-MB                | 71.2         | 95.5               |

Calculated as based on 59 patients as AMI and 66 as non-AMI.

Evidently the use of an immunological isoform test al-
lowed recognition of more patients in the early phase of
AMI, as indicated by a normal value for CK-MB at the time
the initial sample was collected, but an abnormal value on
serial testing.

References
1. Turi ZG, Stone PG, Muller JE, et al. Implications for acute
intervention related to time of hospital arrival in acute myocardial
2. Chen I-Wen, Giberl B, Sperling MI, et al. Myoglobin and
creatine phosphokinase-MB (CPK-MB) radioassays for early detec-
tion of acute myocardial infarction (AMI) for myocardial salvage
creatine kinase isozymes early after the onset of acute myocardial

HPLC Determination of Hemoglobin A1c in the
Presence of the Fast Hemoglobin l-Philadelphia, J. S.
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Cation-exchange measurements of glycated hemoglobin
on microcolumns can be falsely increased if "fast" (rapidly
eluted) hemoglobins are present (1). These fast hemoglobins
have an additional negative charge and can co-elute with
Hb A1c. With HPLC one can separate Hb A1c from Hb I-

* "Hb A1c" refers to the total fast hemoglobins by cation-exchange
chromatography, including Hb A1c, Hb A1c, and Hb A1c. Hence Hb
A1c is a specific chromatographic component of the total Hb A1c (2).

Fig. 1. (Top) Densitometric pattern of cellulose acetate hemoglobin
electrophoresis showing approximately 22% Hb l-Philadelphia (arrow);
(middle) cellulose acetate electrophoretogram: top lane Hb AFSC
control; middle three lanes, patient with Hb l (arrow); bottom lane, Hb
AS pattern; (bottom) absorbances from elution of Diamat column: Hb
A1c eluted at 4.5 min (small arrow, 3.6%) and Hb l-Philadelphia eluted
at 5.5 min (large arrow, 19.8%)

Bottom panel: x-axis is time, in minutes, and y-axis is percent of total Hb
Philadelphia, a fast alpha-chain variant (α-16 Lys → Ghu)
(3), thereby permitting quantification of each variant.

A pregnant, non-diabetic, 25-year-old white woman was
evaluated for the presence of a hemoglobinopathy. The
urine was negative for glucose. Total Hb was 118 g/L,
erthrocytes 3.69 × 1012/L, and mean cell volume 92.0 fl.
The blood smear showed slight anisocytosis (S + VI, Coulter
Electronics, Hialeah, FL 33012; and Hematrak, Geometric
Data-SKF, Wayne, PA 19087). Hemoglobin electrophoresis
on cellulose acetate (Helena Labs, Beaumont, TX 77704)
revealed 22% fast variant Hb by densitometry (CDS-200;
Beckman Instruments Corp., Brea, CA 92621; see Figure 1,
top and middle). The sample was later re-assayed by the
same method and the value was reconfirmed as 21% by
densitometry and 19.8% by HPLC (Diamat; Bio-Rad Lab-
atories, Richmond, CA 94804) (4) (Figure 1, bottom). Citi-
trate agar electrophoresis (Helena) had an Hb A pattern. To
rule out diabetes, Hb A1c assay was ordered; the proportion
of Hb A1c by HPLC (Diamat) was 3.6% (normal range, 3.4–
6.1%) (see Figure 1, bottom). One month postpartum, the
woman's Hb A1c by microcolumn cation-exchange was 8.0%,
(Quik-Sep; Isolab, Akron, OH 44321; normal range, 5.5–
8.5%) and the glycated Hb determined by affinity column
chromatography was 4.7% (Glyc-Affin; Isolab; normal range