Concentration of plasma thyroglobulin and urinary excretion of iodinated material in the diagnosis of thyroid disorders in congenital hypothyroidism

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Abstract. In this paper we describe methods for the early aetiological diagnosis of congenital hypothyroidism, using beside the classical T4, T3 and TSH plasma concentrations, four additional parameters in plasma and urine. The first one is thyroglobulin (Tg). In normal children of more than one year of age and in adults, 5–35 ng/ml plasma is found, in neonates 2–3 weeks old, this level is 10–250 ng/ml. In patients with a stimulated thyroid gland, as in primary congenital hypothyroidism, plasma Tg levels increase. High Tg values are found in iodine deficiency and in organification defects. In the absence of the thyroid gland plasma Tg is undetectable. Low to normal levels are found in cases with hypoplasia of the gland. In patients with a disturbed synthesis of Tg, resulting in Tg deficiency of the gland, plasma Tg levels vary from undetectable to normal. The PBI-T4 plasma difference, which is caused by circulating abnormal iodoproteins is the second parameter. The products of thyroidal breakdown processes of the abnormal iodoproteins are excreted in the urine and used as the third parameter. We found that the excretion of this low molecular weight iodinated material (LOMWIOM) was increased only in Tg-deficient patients.

If the neonate is found to be hypothyroid, thyroid hormone substitution must be given immediately. Blood and urine sampling can be done just before or even directly after starting the therapy. The measurements extended with the determination of the total iodine excretion (fourth parameter) can be carried out within 1 week. With these additional methods it appeared to be possible to distinguish between several types of congenital hypothyroidism in neonates found by screening.

Screening for congenital hypothyroidism has been introduced in many Western countries. The incidence appears to be 1:3600 (DeLange et al. 1981). Congenital primary hypothyroidism may be caused by non-hereditary thyroid dysgenesis, by maternal ingestion of iodine-containing drugs, by maternal iodine excess or deficiency, by amniofoetography, by maternal thyroid antibodies or by various hereditary disorders of thyroid metabolism. To prevent mental retardation in hypothyroid neonates, therapy must be started as soon as possible after recognition. For a firmer basis for this therapy and for the genetic counseling of the family a more precise aetiological diagnosis is required. Scintigraphy with 99mTcO4− allows rapid and simple detection of agenesis or dysgenesis of the thyroid gland, but exposes the patient to unwanted irradiating doses, which might lead to thyroid malignancies in later life (DeGroot & Reilly 1981). In this paper we describe methods for rapid diagnosis of the various types of hypothyroidism which are intended to make the in vivo use of radioactive isotopes unnecessary. Along with the usual parameters like T4, T3 and TSH, four additional parameters (thyroglobulin, PBI minus T4, LOMWIOM, and total iodine) in plasma and urine were used. We measured the plasma concentration of thyroglobulin (Tg), a protein synthesized in the thyroid gland, of which very small quantities escape into the circulation. In Tg-deficient glands other proteins are iodinated. Part of these abnormal iodoproteins leave the gland unaltered, causing a PBI-T4 plasma difference. Another part is broken down in the gland, resulting in the production of iodinated peptides and iodohistidine, which are not dehalogenated and excreted in the urine (Savio et
al. 1973a,b). We compare our results with the results of the more classical laboratory methods, in which radioactive isotopes and invasive procedures (like biopsies) were used.

Material and Methods

Blood

TSH, T4 and Tg in plasma were measured by radioimmunoassay (Odell et al. 1965; Chopra 1972; Van Herle et al. 1973; Ket et al. 1981). Protein bound iodine (PBI) was measured after precipitation of 0.5 ml serum with 4 ml 10% trichloroacetic acid v/v (TCA) and two washings of the precipitate with 4 ml 10% TCA. The iodine content of the precipitate was determined, as described by De Vijlder et al. (1978). Antibodies against thyroid cytoplasma (microsomal antigens) and thyroid colloid (thyroglobulin) were determined by using the indirect Coons immunofluorescence method (Irvin 1966). The calculated inter-assay coefficient of variation for PBI minus T4 was in the normal range (T4: 4–8 µg/100 ml): 8%, in the low ranges (T4 < 3 µg/100 ml): less than 12%.

Urine

The urinary low molecular weight iodinated material (LOMWIOM) was separated from iodine by eluting 5 ml of acidified urine (pH 3) through 18 ml Dowex AG 1 X 2 in 1 mM HCl. Iodide was completely retained and the iodinated material eluted in the 8–20 ml elution volume (see Fig. 1). The iodine content of this pooled fraction and the total iodine concentration of urine was measured (De Vijlder et al. 1978). Creatinine was determined according to Jaffe (De Vries & Van Daatselaar 1956).

Thyroid gland

In 9 patients with a huge and persistent goitre causing mechanical complaints and with noduli in the gland, thyroidectomy was performed. The presence of thyroglobulin and abnormal iodoproteins in the excised glands was determined after homogenizing the gland in 0.01 M sodiumphosphate, 0.15 M NaCl and 0.02% sodiumazide pH 6.8 at 0°C. The homogenate was centrifuged (1 h, 4°C, 105 000 × g). The supernatant was chromatographed on Biogel P300 (Biorad) or Ultrogel AcA 34 (LKB) columns (dimension 2.6 × 36 cm). The concentration of thyroglobulin was determined by double immunodiffusion techniques (Munoz 1971), Tg-radioimmunoassay (Van Herle et al. 1973; Ket et al. 1981) or rocket immunoelectrophoresis (Laurell 1966), in the 105 000 × g supernatant or in the column eluates. The presence and concentration of iodinated albumin in the gland was determined by double-immunodiffusion techniques (Munoz 1971) or quantitative immunoprecipitation. The glands of normal goats were used for comparison.

Radioactive studies

Thyroid imaging studies were carried out with 0.5–1 mCi 99mTcO4−, using a gamma camera (Searle) equipped with a pin-hole collimator. Thyroidal uptake of orally administered radioiodine was determined by epitrothyroid counting with a Siemens Nucleopan 1K. The perchlorate discharge test for detecting impaired organic binding of iodine was performed by administrating KCIO4 or KCNS

![Fig. 1.](#) Gel chromatography pattern on Dowex AG 1 X2 of urine of an adult patient with Tg deficiency, containing in vivo 125I-labelled LOMWIOM. The 125I-labelling was performed before thyroidectomy.

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Fig. 2.
Gel chromatography patterns on Biogel P 300 or Ultrogel ACA 34 of 105 000 × g thyroid supernatants. a and b: of a normal goat on Biogel and Ultrogel, respectively, c: of a patient with a thyroglobulin deficiency (0.02–0.05 mg Tg/g tissue) on Ultrogel, d: of a patient with a thyroglobulin deficiency (17 mg Tg/g tissue) on Biogel.
(10 mg/kg or 150 mg/m² surface area) 60–120 min after a tracer dose of radioiodine. A discharge of more than 15% of the radioiodine taken up by the gland was indicative of an organification defect. To exclude a dehalogenase defect 125I-labelled diiodotyrosine was administered iv. In normal subjects less than 8% of the given dose is found in the urine after 24 h.

Subjects
The subjects were divided into three groups.

I. Controls. Venous blood samples for Tg measurements were taken 3 weeks after birth from 44 normal pre-term infants and from 9 normal term infants. Neonates with congenital malformations or sepsis, suffering from IRDS or from mothers with diabetes mellitus were excluded. Urine of 32 normal children and adults, 5 patients with Graves' disease, 25 patients with Hashimoto's disease and 22 adult patients with thyroid carcinoma were investigated for the presence of LOMWIOM.

II. Twenty-four hypothyroid patients with known deficiencies were studied, either before thyroid treatment, or 6 weeks after discontinuing therapy.

A. Nine patients, aged 0–8 years, with a dysgenesis of the thyroid gland. Agenesis of the gland was found in 6 patients (including 2 neonates), 3 patients had ectopic glands, as shown by scintigraphy.

B. Nine patients, aged 2–10 years (from 4 families) hypothyroid and goitreous from birth had a hereditary defect in the synthesis of thyroglobulin. The radioiodine uptake by the gland was rapid and high (60–80% of the administered dose within 2–4 h). The perchlorate discharge test was negative. A dehalogenase defect could be ruled out. In the thyroid gland of 5 of these patients only traces of Tg-antigens could be detected (0.02–0.05 mg Tg/g tissue, normal 60–80 mg/g tissue). In the thyroid gland of 4 patients (of 2 families) of this group thyroglobulin was present in concentrations of 1.7–17 mg/g tissue. In all the goitres and in the plasma of these patients other iodinated proteins than thyroglobulin, amongst which iodoalbumin, were found. In Fig. 2 examples of the various patients are shown. Since it was impossible to investigate a normal human thyroid gland for comparison, we used a thyroid gland of a normal goat, Fig. 2a-b.

C. Two goitrous children, aged 14 and 15 years, who were found to be iodine-deficient. In both patients the urinary excretion of total iodine was 20 μg/g creatinine. The mean excretion of normal Dutch children living in the region of Amsterdam at that age is 100–200 μg/g creatinine.

D. Four goitrous hypothyroid patients, aged 2–15 years, had an organification defect. In 2 of these patients a discharge of 90% of the thyroidal radioiodine by potassium perchlorate was observed. The other 2 patients had a Pendred syndrome, since they were deaf and showed a discharge of 25% of the radioiodine after potassium perchlorate administration. In the thyroid gland of one of the Pendred patients, 33 mg thyroglobulin/g tissue was present, with an iodination degree of 0.03% (normal 0.1–1.0%).

III. Twenty-two neonates, the main object of our study, were found to be hypothyroid by screening, but without an aetiological diagnosis. Blood and urine, taken before the start of therapy, were sent to us for further diagnostic investigation with the above mentioned methods. This sampling did not delay the start of the therapy.

Results
In plasma of normal humans, older than one year, we found concentrations of 5–35 ng thyroglobulin (Tg)/ml. In 2–3 weeks old normal neonates, serum Tg levels of 10–250 ng/ml were observed. There was no significant difference (P > 0.05, test of Wilcoxon) between serum Tg values of pre-term and term neonates in this age group.

In order to investigate whether plasma Tg concentrations could be used for the diagnosis of thyroid diseases, we measured the plasma Tg levels in patients with various known thyroid disorders (Group II). The results are summarized in Table 1.

In 6 athyroid children (Group II A), plasma Tg was undetectable: in 3 patients with ectopic glands, normal Tg levels of 14–28 ng/ml were found. Apparently these values reached normal levels by high TSH stimulation.

Plasma Tg was also undetectable in the 5 patients of Group II B, whose thyroid glands contained only 0.02–0.05 mg Tg/g tissue (normal 60–80 mg/g tissue). Plasma Tg levels were in the normal range (7–35 ng/ml) in 4 patients who had only 1.7–17 mg Tg/g thyroid tissue.

Increased plasma Tg concentrations (both 52 ng/ml) were found in the 2 iodine-deficient patients (Group II C).

Very high Tg concentrations (800–2060 ng/ml) could be observed in the 4 patients of Group II D, who had a defect in organification of iodide.

In all hypothyroid patients with goitre and Tg-deficiency (Group II B) other iodinated proteins were present in the thyroid gland (Fig. 2) and in the circulation: this was shown by a PBI-T₄ difference. Moreover they all excreted increased
amounts of low molecular weight iodinated material (LOMWIOM) in the urine (Table 1).

In normal individuals, neonates and adults, and in patients suffering from other thyroid diseases, such as Graves' disease, Hashimoto's disease and thyroid carcinoma, the excretion of LOMWIOM was less than 5% of the total I-excretion or less than 5 µg I/g creatinine (in adults). This was also found in hypothyroid goitrous patients with a hereditary defect in the synthesis of thyroid hormone other than Tg synthesis defects. From these observations it can be concluded that when the usual measurements of T₄, T₃ and TSH are extended with plasma Tg levels, plasma PBI-T₄ differences, the urinary excretion of LOMWIOM and the total urinary excretion of iodine, a more exact aetiological diagnosis in congenital hypothyroidism will be possible.

For that reason we examined blood and urine of 22 hypothyroid neonates without an aetiological diagnosis, aged 3–5 weeks using these four parameters. They all had high TSH and low T₄ levels. The results are summarized in Table 2.

In one patient neither Tg, nor excretion of LOMWIOM could be detected.

In 6 patients the plasma Tg levels varied between low and normal and the excretion of LOMWIOM was normal.

In 5 patients a PBI-T₄ difference could be observed and increased amounts of LOMWIOM were found in the urine. In one patient from this group Tg was undetectable. The excretion of LOMWIOM expressed as percentage of total iodine, was only slightly increased in this patient. This was caused by a much higher total iodine excretion than normal. When expressed as µg/g creatinine, the LOMWIOM excretion was high. In the other 4 patients of this group plasma Tg levels of 20–89 ng/ml were found.

In one patient, with a low total iodine excretion, indicating an iodine-deficiency, an increased plasma Tg concentration was found.

In 10 patients very high plasma Tg levels were found, whereas the LOMWIOM excretion was normal. In some of these patients a PBI-T₄ difference of up to 3 µg was observed. It is possible that this difference is caused by circulating Tg, since 2100 ng/ml of normally iodinated Tg (0.1–1.0%) would give a PBI-T₄ difference of 0.2–2.0 µg I/100 ml.

Table 1.
Results of plasma determinations of PBI-T₄, TSH and Tg and of the urinary excretion of low molecular weight iodinated material (LOMWIOM) in the various hypothyroid patients, all more than one year of age, with proven thyroid disorders or iodine deficiency.

<table>
<thead>
<tr>
<th>Patients</th>
<th>PBI-T₄ µg/100 ml</th>
<th>TSH mU/l</th>
<th>Tg ng/ml</th>
<th>LOMWIOM % of total I</th>
</tr>
</thead>
<tbody>
<tr>
<td>A athyroidism (n = 6)</td>
<td>± 0.5</td>
<td>&gt; 100</td>
<td>&lt; 3</td>
<td>n.d.</td>
</tr>
<tr>
<td>ectopia (n = 5)</td>
<td>± 0.5</td>
<td>&gt; 40</td>
<td>14–28</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>B Tg-deficiency (n = 5)</td>
<td>0.3–9.0</td>
<td>60–300</td>
<td>&lt; 3</td>
<td>9–53</td>
</tr>
<tr>
<td>(n = 4)</td>
<td>1.6–5.0</td>
<td>6–66</td>
<td>7–35</td>
<td>7–30</td>
</tr>
<tr>
<td>C I-deficiency (n = 2)</td>
<td>± 5</td>
<td>&lt; 5</td>
<td>52</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>D organification defect (n = 4)</td>
<td>1.0–2.0</td>
<td>8–500</td>
<td>800–2060</td>
<td>&lt; 5</td>
</tr>
<tr>
<td>Normal values children/adults (n = 32)</td>
<td>± 5</td>
<td>&lt; 5</td>
<td>5–35</td>
<td>&lt; 5</td>
</tr>
</tbody>
</table>
(n.d. = not detectable).
Table 2. Results of plasma determinations of PBI-T₄, TSH and Tg and of the urinary excretion of low molecular weight iodinated material (LOMWIOM) of 22 hypothyroid newborns, found by screening on CHT. The investigations were performed at the age of 3–5 weeks.

<table>
<thead>
<tr>
<th>Hypothyroid newborns</th>
<th>PBI-T₄ μg/100 ml</th>
<th>TSH mU/l</th>
<th>Tg ng/ml</th>
<th>LOMWIOM % of total I</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (n = 1)</td>
<td>± 0.5</td>
<td>&gt; 50</td>
<td>&lt; 3</td>
<td>n.d.</td>
<td>athyroidism</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>± 0.5</td>
<td>&gt; 50</td>
<td>9–127</td>
<td>&lt; 5</td>
<td>hypoplasia/ectopia</td>
</tr>
<tr>
<td>B (n = 1)</td>
<td>0.8</td>
<td>&gt; 50</td>
<td>&lt; 3</td>
<td>6¹</td>
<td>Tg-deficiency</td>
</tr>
<tr>
<td>(n = 4)</td>
<td>4–6</td>
<td>&gt; 50</td>
<td>20–89</td>
<td>10–18</td>
<td></td>
</tr>
<tr>
<td>C (n = 1)</td>
<td>n.d.</td>
<td>&gt; 50</td>
<td>1122</td>
<td>&lt; 5</td>
<td>I-deficiency</td>
</tr>
<tr>
<td>D (n = 10)</td>
<td>0.5–3.0</td>
<td>&gt; 50</td>
<td>300–2100</td>
<td>&lt; 5</td>
<td>inborn error other than Tg-deficiency</td>
</tr>
</tbody>
</table>

Newborns:
about 3 weeks of age (n = 53)
0.5   < 25   10–250  < 5

(n.d. = not detectable). ¹ expressed per g creatinine: 50 μg (normal 4–30 μg/g creatinine).

Discussion

Tg is synthesized only in the thyroid gland and very small quantities leave the gland by an unknown pathway. TSH stimulates Tg secretion into the circulation (Van Herle et al. 1979). In the group of patients with the known defects, plasma Tg was undetectable, when there was no thyroid tissue present. Low to normal plasma Tg levels, due to TSH stimulation, were observed in patients with ectopic tissue. In hypothyroid patients with very low amounts of Tg in their glands (<0.05 mg/g tissue, normal 60–80 mg/g tissue) plasma Tg levels were undetectable. Low to normal Tg plasma values were observed in patients with diminished Tg levels in the gland (1.7–17 mg/g tissue). In the latter patients with a Tg-deficiency, proteins other than thyroglobulin are iodinated in the thyroid gland. For a part iodoproteins leave the gland unaltered (De Vijlder et al. 1978; Gons 1981), causing a PBI-T₄ difference in plasma, and for another part they are taken up by endocytosis from the colloid in the thyroid cell and proteolysed by lysosomal enzymes, producing iodinated peptides and iodohistidine (LOMWIOM) (Gons 1981; Savoie et al. 1973a,b). These products will not be dehalogenated and are excreted in the urine via the circulation.

We believe that LOMWIOM originates mainly from breakdown of abnormal iodoproteins, although thyroglobulin itself may contribute to the production. For that reason, healthy individuals excrete this material in amounts of less than 5% of the total iodine excretion or less than 5 μg I/g creatinine.

In patients with other thyroid defects, such as Graves’ disease, Hashimoto’s disease and thyroid carcinoma, in which a PBI-T₄ difference is usually present (Stanbury & Janssen 1962; Owen & Conahy 1956; Robbins et al. 1955), the excretion of LOMWIOM was in the normal range.

Increased urinary excretion of LOMWIOM was only found in patients with a Tg-deficiency. This deficiency of Tg may be caused by a defect in the synthesis of Tg. In Tg extracted from the goitre of one patient, we found a ratio iodotyrosines/iodothyronines of 5 (normal 1–3), while this Tg was normally iodinated (0.2%). This indicates a coupling defect, probably caused by a structural alteration in the protein.

Very high plasma Tg levels, with non-elevated urinary excretion of LOMWIOM were found in 4 patients with a proven organification defect. High Tg levels were found in two patients with iodine-deficiency. The finding of increased plasma Tg levels in patients with an organification defect or iodine-deficiency points to a TSH-effect on the leakage of Tg into the circulation. The iodination degree of Tg may be an additional factor since it has been reported that low iodinated Tg will leave...

In neonates, high cord plasma Tg levels are found, which decrease with gestational age (Ket et al. 1981). These high levels are independent of TSH (Ket et al. 1981). The TSH surge, however, occurring 1 h after birth, gives an extra Tg increase 6–96 h later (Pezzino et al. 1981). This TSH dependent Tg increase normalizes in 2–3 weeks and this moment fits well with the time of investigation (3–5 weeks) of hypothyroid neonates, who are found by screening.

From this results, obtained in the hypothyroid neonates of Group III (Table 2), we can draw the following conclusions:

1 patient of Group A will be athyroid. This was later confirmed by scintigraphy,
6 patients of Group A may have dysplasia of the gland since, notwithstanding high TSH stimulation, low to normal plasma Tg concentrations were found and < 5% of the total iodine excretion was LOMWIOM,
5 patients of Group B will have a Tg-deficiency and metabolism of abnormal iodoproteins in the gland.
This type of defect may also have been present in the 3 infants reported by Black et al. (1982) without detectable plasma Tg but with circulating abnormal iodoproteins and a normally positioned thyroid gland.
1 patient of Group C with a high plasma Tg level and normal LOMWIOM excretion appeared to have a too low iodine excretion, proving iodine deficiency,
in the 10 patients of Group D with high plasma Tg levels and normal LOMWIOM excretion in urine, a number of diagnoses is possible, namely: inborn errors of thyroid metabolism, other than Tg-deficiencies, such as organification defects, the rare occurring trapping defect and dehalogenase defect. This last defect can be ruled out by measurement of the iodotyrosine excretion in the urine.

We conclude that, using the above mentioned methods, which can be carried out within 1 week, a reliable diagnosis can be made in cases of athyroidism, ectopia or hypoplasia of the thyroid gland and various hereditary defects in the synthesis of thyroglobulin. Exogenous causes of hypothyroidism, such as deficiency or excess of iodide, or intake of iodinated drugs can also be detected. A more tentative diagnosis is possible in cases of hereditary defects concerning organization or dehalogenation. In the latter, the investigation can be extended with the measurement of iodotyrosines in serum (Meinhold et al. 1980) or urine without any need of radioactive isotopes in vivo (Kok et al., unpublished results).

To discriminate organization defects from transient hypothyroidism therapy could be started with T3. If T4 rises spontaneously after some time, substitution therapy can be stopped.

An aetiological diagnosis is important for the choice of treatment and for genetic counseling purposes. Some children need no treatment at all (some transient forms). Others need only supplemental iodide. These should be differentiated from the infants which need a life long thyroxine treatment. Since the administration of thyroid hormones can be started directly after or even before taking blood and urine samples, there is no delay in treatment.

We recommend the use of these relatively easy methods, especially in hypothyroid neonates. The results, together with the patient's history and physical examination, can lead to an aetiological diagnosis, without the in vivo use of radioactive isotopes. Moreover in most cases hereditary defects can be discriminated from non-hereditary causes of congenital hypothyroidism.

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References


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