AN AUDIT OF TOTAL AND FREE THYROXINE MEASUREMENTS IN SCREENING FOR HYPO AND HYPERTHYROIDISM IN PATIENTS USING CHEMILUMINESCENCE IMMUNOASSAY

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ABSTRACT

An audit of 2509 patient specimens analyzed for both total thyroxine (TT4) and free thyroxine (FT4) by the ACS-180 automated chemiluminescence immunoassay analyzer revealed that there were 219 discrepancies (8.7% of the total). A discrepancy was defined as one analyte within its reference range and the other outside. The discrepant results were divided into 4 groups: group A: normal TT4, but decreased FT4, 101 patients (4.0%); group B: increased TT4, normal FT4, 78 patients (3.1%); group C: decreased TT4, normal FT4, 34 patients (1.4%); and group D: normal TT4, increased FT4, 6 patients (0.2%). TSH measurements were available, by a 3rd generation chemiluminescent assay, in 142 of these patients, and were consistent with the FT4 result in 72 patients, with TT4 in 61 cases and with neither in 9 patients. The clinical diagnosis was investigated in a subgroup of 43 endocrine patients. Thirteen of the 20 endocrine patients in group A were diagnosed as hypothyroid, with a measured serum TSH, in 11 of them, of median 14.6, range 1.2 to 46.2 μ U/ml. Eleven of the 19 endocrine patients in group B were on thyroid replacement, with a measured serum TSH, in 7 of them of <0.01 μ U/ml. The audit of current laboratory practice led to a suggestion to replace the current thyroid function screening strategy of measuring both TT4 and FT4 by the combination of FT4 and TSH. The reasons for the discrepancies and the alternative strategies for screening of thyroid function are discussed.

KEY WORDS : Audit, Total thyroxine, Free thyroxine, TSH, Thyroid screening

INTRODUCTION

CMC hospital is a referral center for patients from all over India. During previous thyroid function investigations in other centers they may have had either total thyroxine (TT4) or free thyroxine (FT4) measured and ascertained to be abnormal. So as part of a thorough investigation of thyroid function, all specimens sent for thyroid function testing at CMC are analysed for both total (TT4) and free thyroxine (FT4). Serum thyroid stimulating hormone (TSH) is only measured when separately requested. It was observed that on a number of occasions, the TT4 and FT4 assay results were not consistent with one

another, making the interpretation of the patients' thyroid function very difficult, without the additional measurement of TSH.

Audit in clinical biochemistry may be defined as a process of review and assessment of laboratory performance. The main goal is the improvement of patient care, through improvement of laboratory performance and better use of resources (1). The audit process should lead to definite action to produce a change in practice. It was decided to carry out an audit to determine how well the serum TT4 and FT4 assays agreed with each other when measured on the same patient specimen.

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MATERIALS AND METHODS

All the thyroid function tests were carried out on

the Chiron Diagnostics ACS:180 Plus automated chemiluminescence immunoassay analyzer, Ciba Corning diagnostics Corp., MA, USA. Both FT4 and TT4 are competitive immunoassays using direct chemiluminescent technology. The TSH assay uses a two-site sandwich chemiluminescent immunoassay, and is a 3rd generation assay with a stated sensitivity of 0.011 μ U/ml. All the results of TT4 and FT4 assays requested on the same patient specimens, over a nine-week period, were audited. Specimens where either TT4 or FT4 were outside their reference range, while the other was not, were labeled as discrepant.

RESULTS

The quoted reference ranges for TT4, FT4 and TSH from Chiron are 3.2 – 12.6 μ g/ml [41.3–162.5 nmol/l] for TT4; 0.89 – 1.80 ng/dl [11.5–23.2 pmol/l] for FT4; and 0.35–5.5 μ U/ml for TSH. Our laboratory reference ranges for TT4, FT4 and TSH are as follows: 5.0 – 12.0 μ g/dl [65 – 155 nmol/l]; 0.8–2.0 ng/dl [10.3–25.8 pmol/l]; and 0.3–4.5 μ U/ml.

The ACS-180 analyzer can hold its last 2000 results in its memory and print them as a histogram. The results are split into a series of bands of defined values, with the number of specimens present in each listed. These bands, although not exactly corresponding to either the Chiron reference ranges or to the CMC reference ranges, can give an approximate picture of the spread of all the TT4 and FT4 results during the period of the audit. For TT4 1699 (85.0%) of these 2000 results fell between 4.6 and 12.2 μ g/dl, 125 (6.3%) of results were below this and 176 (8.7%) above it. For FT4, 1674 (83.7%) of 2000 results on the same specimens fell between 0.7 and 2.2 ng/dl, 252 (12.6%) were below this and 74 (3.7%) above it. These results are shown in figure 1. About 6.3% of FT4 results were low while the TT4 was still normal (group A pattern). About 5.0% of TT4 results are above the reference range while the FT4 is still normal (group B pattern).

Over the nine week period of the study, a total of 2509 specimens were analysed for both TT4 and FT4. There were 219 discrepancies in these 2509

Fig. 1. Spread of 2000 FT4 and TT4 serum values from the ACS: 180 memory over the period of the audit

Key : ■ FT4, □ TT4

assays, using our CMC reference ranges, ie 8.7% of all results. These discrepant results were divided into groups: group A: 101 patients (4.0%); group B: 78 patients (3.1%); group C: 34 patients (1.4%); and group D: 6 patients (0.2%). The breakdown of these groups is shown in figure 2 and all the results are shown in table 1. The entire group results were designated A1, B1, C1, or D1. It was not possible to

Fig. 2. Results of 2509 specimens audited for discrepancies between TT4 and FT4

find the diagnosis in all 219 patients with discrepant results, but an investigation was made of a subgroup of endocrine patients. There were 43 of them out of the total of 219 with discrepant results. Their results are shown in table 1, group A3 and B3, and those with concurrent TSH results are shown in group A4

and B4. Results from non-endocrine patients who had concurrent TSH results are shown in group A2, B2, and C2. All results are shown as median and range.

Agreement with the TSH result

Since no diagnosis was readily available for the majority of patients, a comparison of TT4 and FT4

Table 1. Concurrent serum results for TT4, FT4 and TSH in patients with discrepancies between TT4 and FT4 measurement.

Group	Sub Group	Number	Total T4 ($\mu\text{g/dl}$)		Free T4 (ng/dl)		TSH ($\mu\text{IU/ml}$)	
			MEDIAN	RANGE	MEDIAN	RANGE	MEDIAN	RANGE
A	A1	101	5.9	5.0-10.4	0.7	0.4-0.7	-	-
	A2	54	6.1	5.0-10.4	0.7	0.4-0.7	6.1	<0.01-104.0
	A3	20	6.0	5.0-7.8	0.7	0.5-0.7	-	-
	A4	17	6.1	5.0-7.8	0.7	0.5-0.7	9.5	0.01-46.2
B	B1	78	13.3	12.1-18.8	1.5	0.8-2.0	-	-
	B2	40	13.7	12.1-18.8	1.5	0.8-2.0	0.4	<0.01-28.5
	B3	19	13.3	12.1-18.4	1.7	1.3-2.0	-	-
	B4	10	13.7	12.5-18.4	1.9	1.3-2.0	<0.01	<0.01
C	C1	34	4.4	1.8-4.9	1.0	0.8-2.0	-	-
	C2	15	4.4	1.8-4.9	0.9	0.8-1.2	2.8	0.9-40.8
D	D1	6	11.0	10.0-12.0	2.4	2.2-2.9	-	-

A1-D1 = results from all discrepant patients, A2-C2 = results with concurrent TSH from non-Endocrine patients

A3-B3 = all results from Endocrine patients, A4-B4 = results from Endocrine patients with concurrent TSH

Table 2. Agreement between TSH and TT4 and FT4 measurement in patients with discrepant results

Group	TSH result	Number	Agree	Disagree
A2	<low	3	-	TT4, FT4
	< normal >	22	TT4	FT4
	> raised	29	FT4	TT4
A4	<low	1	-	TT4, FT4
	<normal >	5	TT4	FT4
	>raised	11	FT4	TT4
B2	<low	19	TT4	FT4
	<normal >	16	FT4	TT4
	>raised	5	-	TT4, FT4
B4	<low	10	TT4	FT4
	<normal >	-	-	-
	>raised	-	-	-
C2	< low	-	-	-
	< normal >	10	FT4	TT4
	>raised	5	TT4	FT4
C4	<low	-	-	-
	< normal >	3	FT4	-
	> increased	-	-	-
D2	<low	2	FT4	TT4
D4	<low	1	FT4	TT4

Group descriptions as for table 1, Agree means results consistent with TSH result, Disagree means result not consistent with TSH result

result was made in the patients who had a concurrent TSH measurement, to see which was consistent with the TSH. Generally, a raised TSH will be accompanied by a decreased TT4 or FT4, a decreased TSH by a raised TT4 or FT4 and a normal TSH by a normal TT4 or FT4. The data comparing the FT4 and TT4 result with the individual TSH results is summarized in table 2 for both endocrine (A4 to D4) and non- endocrine patients (A2 to D2). In each group the individual TSH result may be low, normal or raised.

In group A1, table 1 (all patients), 101 patient samples have a FT4 below the reference range while the median TT4 is at the low end of normal. Sixty-five of the 101 results for FT4 had a value of 0.7 ng/dl. It is possible that the FT4 lower limit of the normal range is actually 0.7 and not 0.8 ng/dl. This FT4 value of 0.7 ng/ml was accompanied by a high TSH in 15 patients, if this FT4 was classed as normal then the high TSH and low normal FT4 were inconsistent, or would have to be interpreted as subclinical hypothyroidism. On the other hand 12 normal TSH values were accompanied by a FT4 of 0.7 ng/dl and these would now have been classified as consistent with one another.

In group A2, table 2, (non-endocrine), 53.7% of the TSH results were above the reference range, consistent with the low FT4, while 40.7% were normal, consistent with the normal TT4, and only 5.6% were under the reference range, consistent with neither TT4 or FT4. In group B1, table 1 (all patients), the range of TT4 values was from 12.1 to 18.8 mg/dl, while the median FT4 was towards the upper end of the reference range with 50% of values >1.5 ng/dl. In group B2, table 2 (non-Endocrine), the TSH is lower than its reference range in 47.5% of patients, normal in 40% and raised in 12.5%. In group C1 (all patients), table 1, all the TT4 values are low and about 50 % of the FT4 values are low normal. In group C2, table 2 (non-Endocrine), 10 of the TSH results were normal, and 5 elevated. In group D2, table 2 there were two low TSH results (0.01 μ U/ml or less).

Endocrine patients

Of the forty-three total endocrine patients, twenty

fell into group A (A3), of these 13 were diagnosed as hypothyroid and 5 were on thyroxine replacement, 50-150 μ g/day. Eleven of these thirteen had concurrent TSH measurements (median 14.6, range 1.2 to 46.2 μ U/ml). Totally 13 of the 20 patients had a FT4 value of 0.7 ng/ml. TSH (table 2, group A4) was raised in 11 patients, normal in 5 and low in one patient. Three other patients in group A 3 were receiving anti thyroid treatment with carbimazole. One of them had a suppressed TSH (<0.01 μ U/ml) and the others had a normal TSH. The other patients included one patient with Conn's syndrome, one following thyroid surgery, one with obesity and one with diabetes plus renal failure.

Nineteen of the patients fell into group B (B3). Eleven of them were on thyroid replacement, ten of these on a dose between 100 to 250 μ g/day, and one on 400 μ g/day. Seven of these 11 had concurrent TSH results, (Group B4, all <0.01 μ U/ml). Here a suppressed TSH was consistent with a raised TT4. Three other patients were on follow up after receiving I¹³¹. Two others were being treated with carbimazole, one had Graves disease, one was pregnant euthyroid, and one had a TSH producing pituitary adenoma, (TSH 13.0 μ U/ml). Three endocrine patients fell into group C (C3), one had pulmonary TB, one treated T3 toxicosis and the other was on replacement therapy with thyroxine (no TSH result available). Table 2, group C4, shows that the other two had a normal TSH. One endocrine patient came into group D, here the diagnosis was treated Graves disease and the TSH was <0.01 μ U/ml.

DISCUSSION

The results of TT4 and FT4 measurements in the same patient are in agreement in 91.3% of the specimens analyzed, but problems of interpretation arise in the other 8.7% of patients. Figure 1 showing the Chiron data summary, indicates that the FT4 and TT4 reference range do not completely overlap and this is reflected in the observed discrepancy rate of 4.0 % in group A1 and 3.1 % in group B1. Slightly lowering the lower limit of the FT4 range to 0.7 ng/ml will improve the agreement between TT4 and FT4 but

not improve the agreement between FT4 and TSH results. About 50% of results in group A1 and B1 have one analyte above or below its reference range and the other at the upper or lower end, while the other 50% of results are well within the respective reference ranges. So slight adjustments to either reference range will not produce agreement in at least 50% of the discrepancies.

There are a number of different reasons for a discrepancy between the TT4 and FT4 results in the same patient. The first is the presence of a protein binding abnormality in euthyroid subjects. This could be TBG excess, familial dysalbuminaemic hyperthyroxinaemia, or transthyretin-associated hyperthyroxinaemia (2). It could be responsible for a group B type of discrepancy, of a raised TT4 but a normal FT4, seen in 3.1% of all patients. A decreased TT4 in the presence of a normal FT4 in euthyroid subjects could be seen in TBG deficiency, and this group C pattern was found in 1.4% of all patient results. Serum albumin measurements were not available for the subjects investigated in this audit, so binding protein abnormalities could not be excluded as a cause of discrepant results. A prospective study to investigate the incidence of binding protein abnormalities could be done in a group of patients with a defined clinical diagnosis and discrepant TT4 and FT4 results, by measuring their serum albumin, thyroxine binding globulin (TBG) and transthyretin-associated BP.

The medical history in the subgroup of endocrine patients revealed they were patients on anti-thyroid drugs, patients who had I¹³¹ therapy, or hypothyroid patients either on replacement therapy or not. None of them were receiving anti-convulsant therapy, beta-blockers, oestrogen containing preparations or amiodarone, which are among the list of drugs known to displace thyroxine from plasma protein binding sites or inhibit conversion of T4 to T3 (3). This excludes concurrent drug therapy as a cause of the discrepancies found, in these Endocrine patients but not in the non-endocrine patients, for whom there was no drug history available.

In non-thyroidal illness (NTI) abnormalities in thyroid function tests are frequently found with a

tendency for TT4 to decrease and FT4 to increase (4). Again NTI was excluded only in our Endocrine patients not in the non-endocrine ones.

In discussing which are the best combinations of tests to screen for thyroid disorders, the greater susceptibility of FT4 assays to assay interference should be mentioned (5). Interfering factors include the presence in the patients' serum of autoantibodies to T4 and T3, heterophilic antibodies from intimate contact with animals, and human anti-mouse antibodies [HAMA] (6), any of which may cause an incorrect FT4 result to be obtained.

In investigation of thyroid disorders, the American Thyroid Association recommends a strategy of using both TSH and an estimate or direct measure of FT4 (7). The consensus statement in the UK (8) recommends the use of a combination of serum TSH and serum thyroxine to confirm the diagnosis of hypo or hyperthyroidism. Many hospitals in the UK use TSH alone as a first line test with FT4 as a second line test when the TSH is abnormal. Hay and Klee (9) recommend a third generation assay with a lower limit of sensitivity of 0.01 $\mu\text{IU/ml}$ for this purpose. However measurement of TSH alone may be misleading in the following circumstances: hypopituitarism, thyroid hormone resistance and TSH secreting tumours, as well as NTI. Davey et al (10) have compared the measurement of TSH and FT4 in 1000 patient specimens and conclude that an algorithm for thyroid function testing based on serum TSH alone, is a reliable indicator of thyroid function. Thus the literature suggests that any screen of thyroid function should include a measurement of TSH. In our subgroup of Endocrine patients with treated hyperthyroidism, TSH measurement was not always available because the treatment had been going on for less than 3 months. In this case the TSH may be still be suppressed while the TT4 or FT4 have normalized.

The purpose of an audit in clinical biochemistry is to review the current laboratory practice, to establish drawbacks in that current practice, set a standard that may improve performance, implement it and then review the change achieved by the implementation

(1). The standard that may improve diagnostic performance in screening for thyroid disorders is to include a third generation TSH serum measurement with either the FT4 or TT4 measurement. In choosing between TT4 and FT4 assays it is known that using FT4 will eliminate abnormalities due to an excess of thyroxine binding protein. There also seems to be a better agreement between FT4 and TSH than TT4 and TSH. Therefore the proposal is that the routine screening test for thyroid function should be changed to FT4 and a 3rd generation TSH assay. Since the FT4 and TSH result agreed in 72 out of 142 of the discrepancies (table 2) this would reduce the observed discrepancy rate by about 50%.

Regardless of the strategy chosen there will still

be discrepancies between the various test results, which can only be resolved only by further investigations. The investigation and treatment of known thyroid abnormalities may also require further investigation including TT4, T3, thyroglobulin and antithyroid antibody titres, at the discretion of the treating physician. In order to implement the change in screening strategy suggested ideally it would be necessary to confirm the reference ranges for FT4 and TSH as measured by our current methodology with an optimum number of 125 normal subjects. This is a large but necessary expense for any hospital to make when there is uncertainty about the validity of the existing ranges. A further audit after a 6-month period would be necessary to review the change achieved by implementation of the new procedure.

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