study has shown that some patients with AIDS develop an HIV-ANCA.⁹ It may be that both the presenting and terminal pathological processes in our patient were immunologically mediated, with ANCA possibly playing a part.

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Spuriously high free thyroxine with the Amerlite MAB FT₄ assay

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Abstract

In the Amerlite MAB FT₄ assay, as stated by the manufacturer, FT₄ values should be normal in patients with anti-thyroid hormone autoantibodies. The case of a 69 year old woman with a spuriously high FT₄ using the Amerlite MAB FT₄ assay is reported. Laboratory investigations showed that her spurious FT₄ result was likely to have been caused by anti-thyroid hormone autoantibodies.

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It is well documented that anti-thyroid hormone autoantibodies can interfere with the measurement of free thyroxine (FT₄) and free tri-iodothyronine (FT₃).¹ In the non-isotopic Amerlite MAB FT₄ assay, FT₄ in the sample competes with a cross-reactant T₃, which is chemically bound to the well surface, for binding to a horseradish peroxidase labelled mouse monoclonal anti-T₄ antibody. The horseradish peroxidase activity in the bound conjugate is measured by an enhanced luminescence reaction. As stated by the manufacturer, FT₄ values should be normal in patients with anti-thyroid hormone autoantibodies using this assay (Amerlite MAB FT₄ assay kit insert, Kodak Clinical Diagnostics Ltd; Amersham UK).

Case report

A 69 year old woman with non-insulin dependent diabetes mellitus and primary

biliary cirrhosis had thyroid function tests performed as she had complained of tiredness and was known to have high titres of anti-thyroid microsomal autoantibodies (1/800) and anti-thyroglobulin autoantibodies (1/1600). She also had antimitochondrial IgG (greater than 1/640) and rheumatoid factor (1/40). Her serum thyroid stimulating hormone (Amerlite immunometric assay) was 3.0 mU/l (reference range: 0.15-3.2 mU/l) and her serum total T₃ (Amerlex-M T₃ radioimmunoassay) was 1.6 nmol/l (0.8-2.5 nmol/l), but her serum FT₄ (Amerlite MAB FT₄ assay) was unexpectedly and inappropriately high: 92 pmol/l (10-27 pmol/l). This spuriously high FT₄ was found on assaying subsequent serum samples from the patient over a period of one year. Her serum albumin was 35 g/l, total protein was 68 g/l, and serum protein electrophoresis was normal. The patient was receiving the following medication during the period concerned: tolbutamide, metformin, isosorbide mononitrate, glyceryl trinitrate, diltiazem, cimetidine, digoxin, spironolactone, calciferol, hydroxyapatite and bisacodyl.

Methods and results

FT₄ was measured in the patient's serum using the Boehringer Mannheim assay, which is a competitive immunoassay, and yielded a normal FT₄ value of 15 pmol/l (reference range: 10-26 pmol/l), confirming that the FT₄ value obtained with the Amerlite MAB FT₄ assay was indeed spuriously high.

Mouse IgG up to 2.5 g/l (final concentra-

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Percentage radioactivity in the pellets

Label	Percentage radioactivity in pellet		
	Control (n = 20) (mean $\pm$ 1SD)	Patient	Number of SDs from mean
$^{125}\text{I-T}_4$	5.97 $\pm$ 1.107	9.7	3.37
$^{125}\text{I-T}_3$	4.53 $\pm$ 0.63	7.7	5.03

tion) was added to the patient's serum but this did not reduce the positive interference in a subsequent FT_4 measurement. Hence the spuriously high FT_4 was not likely to be attributable to the presence of heterophilic antibodies in the patient's serum.

The assay wells were pre-incubated with 25 μl of patient's serum, sera from four patients with normal free T_4 concentrations as controls, and zero standard for 30 minutes at 37°C. The wells were washed. Horseradish peroxidase-labelled anti- T_4 antibody (100 μl) was added to the wells and a further incubation of 30 minutes at 37°C was carried out, followed by washing. The signal reagent (250 μl) was then dispensed immediately into the wells and the signal intensity measured using the Amerlite Analyser.

The patient's sample gave a signal intensity which was 44% of the zero standard compared with that of the four controls which gave signal intensities ranging between 71% and 81%. This indicates the presence of substances in the patient's serum which bind to the modified well surface and therefore inhibit binding of the conjugate to the well surface.

An experiment to ascertain whether anti-thyroid hormone autoantibodies were present in the patient's serum was performed. The patient's serum and sera from patients with normal thyroid function acting as controls (50 μl) were incubated with $^{125}\text{I-T}_4$ and $^{125}\text{I-T}_3$ (250 μl , containing about 25 000 counts per minute) at room temperature for 24 hours. Precipitation with polyethylene glycol (20% w/v, 150 μl) was then carried out (10 minutes at room temperature). The radioactivity in the pellets after centrifugation was expressed as a percentage of the total counts added (table). Autoantibodies were considered to be present when the percentage radioactivity in the pellet was more than + 3 standard deviations from the mean of the controls. The results obtained implied that the patient probably had autoantibodies to T_4 and T_3 .

FT_4 in the patient's serum was also measured using the Amerlex MAB FT_4 assay which has been reported reliably to measure FT_4 in the presence of high concentrations of

anti-thyroid hormone autoantibodies.² This one-step radioimmunoassay is based on competition between free T_4 in the sample with an excess of a cross-reactant T_3 , which is chemically coupled to magnetisable polymer particles for binding to ^{125}I -labelled mouse monoclonal anti-thyroxine antibody. The Amerlex MAB FT_4 method should, theoretically, be less susceptible to interference by anti-thyroid hormone autoantibodies as the cross-reactant T_3 is chemically coupled to magnetisable polymer particles and would offer a much larger surface area for binding by the labelled antibody/anti-thyroid hormone autoantibodies than in the Amerlite MAB FT_4 assay. The serum FT_4 obtained by this method was still high: 55 pmol/l (reference range: 11–24 pmol/l), although as anticipated the interference was less than that obtained with the Amerlite MAB FT_4 assay. This finding indicates that this procedure is also susceptible to interference by anti-thyroid hormone autoantibodies.

To exclude possible interference from some of the patient's other autoantibodies positive control antigens against mitochondrial, rheumatoid factor, thyroid microsomal and thyroglobulin antibodies were separately added to the patient's serum before the FT_4 assay. There was no reduction in the FT_4 concentration after correcting for dilution with any of the above positive control antigens. Hence her autoantibodies to the above control antigens were unlikely to be the cause of her spuriously high FT_4 .

Conclusion

The above experimental results suggest that the spuriously high FT_4 obtained from this patient was due to the presence of anti-thyroid hormone autoantibodies which recognise the T_3 on the modified well surface. Anti-thyroid hormone autoantibodies may interfere with the measurement of FT_4 in the Amerlite MAB FT_4 assay, as well as the Amerlex MAB FT_4 assay.

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