Macrohormones: bigger isn't always better

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Background

- The presence of macrohormones is a type of analytical interference encountered in clinical biochemistry analyses, with potential clinical implications.
- Macrohormones are complexes of monomeric hormone molecules with IgG. Their high molecular weight exceeds the cut-off for glomerular filtration, resulting in their prolonged half-life in the circulation. They are usually immunoreactive, hence leading to high immunoassay results, but biologically relatively inactive.
- We present two cases of unusual macrohormone formation and the laboratory strategies to detect them and perform accurate analyses.

Case 1

A 55 year old male had his thyroid function tests checked as part of investigation for mild chest pain and was started on 50 μg of thyroxine on finding of elevated TSH at 36 mU/L (ref.range 0.35-5) with free T4 at 10 pmol/L (ref.range 9-22). However, he developed flushing and general discomfort whilst on thyroxine, which was discontinued. He was referred to the Endocrinology Clinic for further assessment. Subsequent analyses showed consistently elevated TSH and low normal FT4. Thyroid autoantibodies were not elevated. His TFTs over the years are summarised in Table 1.

Table 1. Changes in the patient's thyroid function test results over a nine-year period.

Date	TSH (0.35 – 5.0 mU/L)	Free T4 (9 – 21 pmol/L)	Total T3 (0.9 – 2.5 nmol/L)
21/05/2018	33.4	8.9	1.5
08/03/2018	30.7	9	
30/07/2012	17.7	9.7	1.6
31/05/2012	18.6	14.1	1.7
30/04/2012	29.65	9.8	
19/03/2012	32.81	10.3	
27/10/2011	17.35	10.7	
17/03/2009	36.08	10	

Since he was clinically euthyroid, the laboratory considered analytical interference as a potential cause of high TSH.

A polyethyleneglycol (PEG) precipitation protocol was implemented as follows:

- One of the samples was divided into two aliquots labelled as 'A' and 'B'. 'A' was measured 6 times 'neat'; 'B' was treated with an equal volume of 25% PEG solution and again measured 6 times.
- Mean TSH value was calculated and was found to be 20.04 mU/L in aliquot 'A', while in 'B' after PEG precipitation that was 3.1 mU/L.
- Percentage recovery was calculated using the following formula:

Recovery (%) = 2 x TSH (after PEG treatment)/TSH (before PEG treatment) x 100.

In this specimen, overall recovery was 30.9%, strongly suggestive of the presence of macro-TSH. The clinicians and the patient were informed of this finding, which was recorded in the patient's medical record to avoid future misinterpretation of TFTs and inadvertent treatment with thyroxine.

Case 2

A 72 year old male, who underwent renal transplantation two years ago due to lithium-induced end stage renal failure, attended his routine appointment in the Renal Clinic. His renal function had been satisfactory since transplantation with eGFR >60ml/min and his intact PTH (iPTH) had consistently been in the range of 26.7-47.8 pmol/l (ref.range 1.6-7.5). He had a recent hospital admission for influenza A virus-associated pneumonia complicated with acute kidney injury, from which he was recovering well. He was currently on maintenance immuno-suppressive therapy.

His blood tests revealed iPTH markedly raised at 506 pmol/l (ARCHITECT Intact PTH, Abbott Diagnostics), confirmed on a repeat sample (526 pmol/L), with normal calcium level. His 25-OH vitamin D was low at 26 nmol/L. The change in his iPTH levels over time is shown in Figure 1.

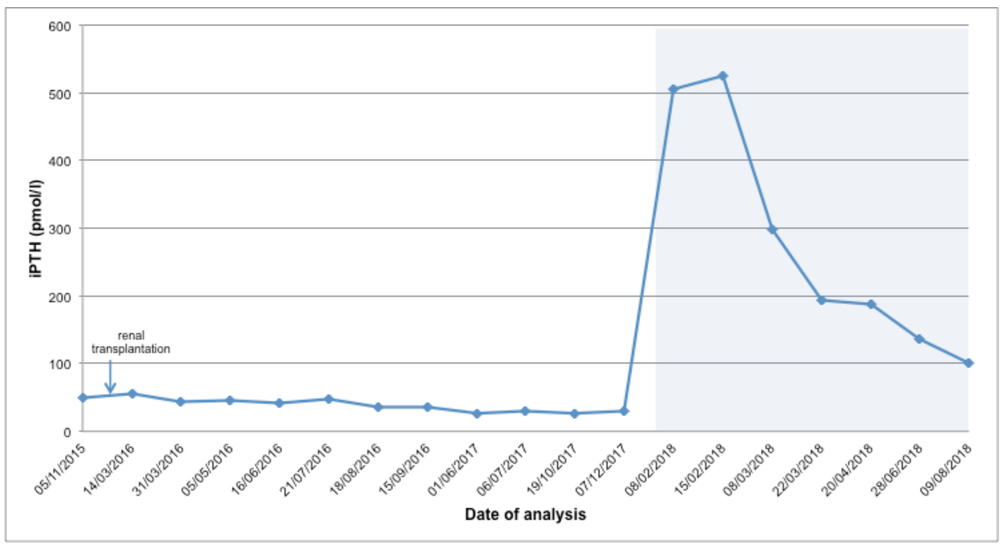

Figure 1. iPTH concentrations over time as measured with the Abbott analytical platform. The shaded area includes iPTH measurements performed in the post-viral period.

He had no clinical signs and symptoms consistent with severely hyperparathyroid state or underlying malignancy. He was not known to have any rheumatic disease. He was looking after a dog for years but had no other exposure to animal tissues.

In order to investigate these unexpectedly high levels, the following action was taken:

- Treatment of plasma with heterophilic blocking reagents – no heterophilic antibodies were detected.
- Analysis of the same sample on two different analytical platforms (Roche and Siemens Diagnostics) – iPTH was measured at 58.7 pmol/l and >263 pmol/l respectively; the discordant results were suggestive of some type of analytical interference.
- Treatment of plasma with polyethyleneglycol (PEG) – another plasma sample received 3 weeks later was subjected to PEG extraction; a reduction of iPTH concentration from 297.9 pmol/l to 61.38 pmol/l was observed, with a recovery rate of 20.6%.
- Serial dilutions of post-PEG plasma – an appropriate reduction of iPTH levels by the corresponding dilution factor was demonstrated (Figure 2).

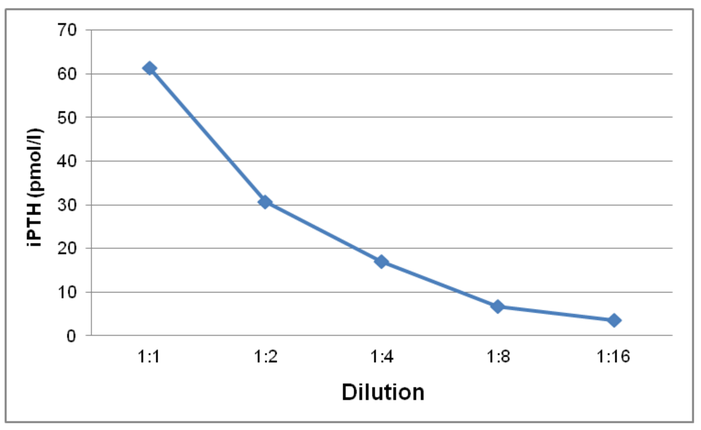

Figure 2. Post-PEG iPTH concentrations following two-fold serial dilutions.

All the above results suggested the presence of macro-PTH. This was likely attributed to anti-influenza antibodies. Of note, antibodies following natural influenza infection could persist at high titres for at least 15 months.

We plan to continue doing sequential PTH analysis to see if it returns to pre-influenza levels.

Conclusions

- Any discrepancy between clinical findings and laboratory results should raise the suspicion of analytical interference.
- Clinical laboratories should have increased awareness of the limitations of the various analytical methods and provide an array of tests for detecting interferences.
- PEG precipitation is a technically straightforward and yet efficient method to identify the presence of macrohormones, with a recovery of monomeric molecule <40% of the initial value usually considered confirmatory.

References

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