False low parathyroid hormone values secondary to sample contamination with the tissue plasminogen activator

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Introduction

An 82-year-old Caucasian female with end-stage renal disease secondary to diabetes mellitus and hypertension had undergone maintenance haemodialysis for 6 years. Quarterly parathyroid hormone (PTH) results had ranged between 230 and 480 pg/mL. When PTH increased to 834 pg/mL, 30 mg oral cinacalcet was added to her thrice weekly IV vitamin D and oral phosphate binder regimen. Three months later, PTH had decreased to 142 pg/mL, a surprisingly dramatic decrease of > 80%, higher than the usual decrease of 30–40% following the administration of a calcimimetic [1]. Over the next 7 months, PTH values ranged between 48 and 471 pg/mL. Inconsistency of calcimimetic intake including non-adherence to the recommended 12-h dose interval prior to blood draw was suspected [2]. However, a PTH value of 17 pg/mL made this assumption questionable. Laboratory quality control on precision and accuracy were validated.

Fortuitously, at this time a stability study of PTH measurements was being performed in our laboratory in order to assess any effect of the delay in specimen receipt with centralized laboratory services common in dialysis practice. Left-over plasma samples from the routine monthly blood draw in a nearby dialysis unit were measured...
immediately after draw and 24, 48 and 72 h later. The data revealed very satisfactory consistency of results over time, with only one outlier out of 85 samples. This sample measured at baseline 180 pg/mL, but declined to 78 pg/mL after 24 h, and PTH was no longer detectable after 48 h. In the absence of any explanation the sample was further investigated and identified as coming from the patient described above. On investigating the medical history of this patient, it became apparent that she dialyzed with a tunnelled central venous catheter and intermittently received alteplase (Cathflo® Activase®, Genentech, CA, USA; Actilyse®, Boehringer Ingelheim Pharma, Germany) as a catheter lock to maintain patency. The blood samples had been drawn from the catheter. The patient’s medication history confirmed that the catheter had been locked with alteplase at the end of the previous dialysis treatment prior to the day of the blood draw in question.

We therefore hypothesized that contamination of the blood sample with alteplase was the cause of the decreasing PTH values noted in the specimen in the stability study.

Methods

The leftover plasma samples from routine monthly blood draws of 21 haemodialysis patients with known PTH levels, representing PTH values commonly found in patients on maintenance HD, ranging from 68 to 2340 pg/mL were selected. Each sample (750 µL) was incubated with 25 or 50 µL of alteplase at 4 °C for a total of 48 h to mimic transportation time in a cooled container while in route to the central laboratory. In addition, three samples from healthy volunteers were treated similarly. After 24 and 48 h, each sample was re-tested with the Bayer intact PTH assay using the ADVIA Centaur® system (Bayer, Norwood, MA, USA). The ADVIA Centaur Intact PTH assay is a two-site sandwich immunoassay using direct chemiluminescent technology. A polyclonal goat anti-human PTH labelled with acridinium ester binds in the region of the N-terminal 1–34 AA. The second antibody is a biotinylated polyclonal goat anti-human PTH binding in the 39–84 region. Streptavidin in the solid phase is covalently coupled to paramagnetic latex particles as the capture mechanism.

To confirm that such an effect was not specific to the Bayer assay but rather a more general phenomenon, the study was repeated measuring PTH values in parallel with both the Bayer assay as outlined above and the Roche PTH assay (Roche Diagnostics, Indianapolis, IN, USA).

The Roche PTH assay is an electrochemiluminescence immunoassay also based on a sandwich test principle utilizing, however, different antibodies and detection systems. A biotinylated monoclonal PTH-specific antibody reacts with the N-terminal fragment (1–37) and another monoclonal antibody labelled with a ruthenium complex reacts with the C-terminal fragment (38–84). The respective epitopes recognized by the antibodies correspond to the amino acid regions 26–32 and 55–64. Streptavidin-coated microparticles bind the complex to the solid phase via interaction of biotin and streptavidin.

Pooled samples from haemodialysis patients with PTH levels in the ranges of 100–300 pg/dL, 300–600 pg/dL, 600–1200 pg/dL and >1200 pg/dL were incubated with various amounts of tPA including 0 (control) 6.5, 12.5 and 25 µL and measured with both PTH assays at different time points (1, 3, 6, 12 and 24 h) after addition of alteplase to further study the kinetics of this process and to test for dose dependence.

Results

Twenty-one samples of left-over plasma from patients with varying PTH baseline values ranging from <100 pg/mL to levels >2000 pg/mL were incubated at 4 °C with 25 or 50 µL alteplase. A dramatic decline to 2.5–33.5% of the baseline PTH levels was noted after 24 h (Figure 1) with a further decline to <1–15% after 48 h (data not shown). The degree of decline was not significantly enhanced with the higher dose of 50 µL alteplase at the times measured (data not shown). A similar effect was noted in the three healthy control subjects, where initial PTH baseline values of 17, 38 and 39 pg/mL all decreased to <2.5 pg/mL after 24 h incubation, equivalent to a 93–98% reduction from the baseline value.

The second set of experiments comparing the PTH results with the two different PTH assays and incubation with various amounts of alteplase revealed that indeed the PTH results declined with both assays, resulting in a dramatic decline of the PTH results over time independent of the assay used. When comparing the control samples without any alteplase, it was evident that both assays performed very well, with consistent values over the 48 h evaluation time, the variation being well below the 12.5% allowable error for accuracy limits.

To assess the influence of alteplase incubation we defined any change >12.5% being significant to differentiate from the inherent test variation. With this measure in mind, decreased PTH values occurred as early as 1 h of incubation in some samples spiked with the higher amounts of alteplase (12.5 and 25 µL). After 3 h, all samples incubated with 12.5 or 25 µL showed a significant decline of their PTH measures by 17–53% of their baseline value in both assays. After 6 h, all the samples showed PTH values decreased by 14–72%, independent of the alteplase amount. After 12 h, the PTH values had decreased by 35–88% compared to their baseline measure; after 24 h the change was between 70 and 96%. PTH was barely detectable after 48 h with both assays measuring only 1–9% of the baseline PTH value prior to alteplase incubation. The exact percentages...
of decrease in PTH values are shown in Table 1 for both the Bayer and Roche assays. While the trend of decline was very similar in both assays, the decrease of PTH analysed with the Bayer assay appeared to be slightly accelerated, resulting in larger declines with time. The similar course of the gradual PTH decline over time in all pooled samples demonstrates that the effect is independent of the amount of substrate present but rather, as one would expect with an enzymatic reaction, depends on the amount of the alteplase present in both assays in a time-dependent manner (Figure 2).

**Discussion**

Thirty-two to seventy-five percent of errors in laboratory medicine are accounted for by pre-analytical specimen errors. The majority of such errors occur due to improper
handling of the specimen, including failure to centrifuge a specimen, haemolyzed or clotted samples, or quantity not sufficient [3], very similar to nationally reported data in annual CAP surveys [4]. In patients suffering from ESRD undergoing maintenance haemodialysis or peritoneal dialysis, the reasons for rejecting specimens are very comparable (internal data on file). In addition to these causes, blood samples drawn from a central catheter can have additional issues.

A significant number of patients undergoing HD in the USA are treated at some time with a tunnelled catheter as access [5,6]. In order to prevent the two most significant complications in these patients, infections and clotting, a variety of catheter lock solutions are used. These include solutions containing anticoagulants such as heparin and citemate or antibiotics such as gentamicin or vancomycin [7,8].

There are various mechanisms by which catheter lock solutions can affect laboratory results if not completely removed from the catheter. Dilution of the sample is an obvious cause and can often be verified by assessing the sample and the simultaneous decrease in other analytes. In other instances, the result may be falsified by the solution binding the anlyte, thereby preventing its recovery, such as citrate binding calcium. Spurious elevation of phosphate results through contamination with heparin and alteplase has been reported through the addition of phosphate contained in the solution or by interference with the assay [9,10]. Spurious hypernatraemia has also been described secondary to trisodium citrate contamination by a catheter lock solution [11].

Our study describes a previously unreported mechanism, whereby PTH results can be falsely decreased due to the contamination of the pre-analytical specimen with small amounts of alteplase. The incubation of plasma samples occurring during transit of a sample to the laboratory even at 4°C results in a time-dependent digestion of PTH into fragments that are no longer detectable by the PTH assay. This effect was noted both in patients undergoing haemodialysis and in healthy control subjects indicating that it is independent of the uraemic state.

This effect was reconfirmed with two different PTH assays available for automated platforms. Variations in the PTH result dependent on the assay utilized are well documented, and the difference between the two assays seen in our studies correlates well with published data [12]. The results, however, show that both assays are similarly affected by the addition of alteplase when analysing both the kinetics of the effect and the dose dependence. Both assays studied here are based on a sandwich immunoassay, one using chemiluminescence and the second one electrochemiluminescence. The effect is seen with both assays even though they use completely different antibodies with affinity to different epitopes and different detection systems. Although the only substrate of alteplase under physiological conditions is plasminogen [13], these experiments demonstrate that contamination with alteplase can lead to digestion of PTH with prolonged 'incubation' in vitro and consequent extreme variability in results. Alteplase activates the plasminogen pro-enzyme by splitting a single valine–arginine peptide bond. The PTH protein contains one such bond in the area of AA 19–20. It is possible that the labelled polyclonal antibody in the Bayer assay which binds in the area of AA 1–34—a position which includes the valine–arginine bond—will no longer be captured, and therefore, the recovery of the PTH fragment may be hindered resulting in tremendously decreased results. In the case of the Roche assay, neither of the monoclonal antibodies binds in the critical region of the AA19-20 of the PTH molecule. However, the assay is nonetheless affected implying an as yet unidentified pathway of alteplase interference with this assay. As PTH results often vary secondary to clinical-physiological causes, one needs to be aware of externally induced variability due to a contaminated sample. Only a strict protocol when sampling blood from a catheter to avoid any contamination and interference with assays will prevent such errors.

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Conflict of interest statement. None declared.

References


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