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A suspected case of falsely low digoxin and vancomycin concentrations caused by free kappa light chains with PETINIA method

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ABSTRACT

Vancomycin and digoxin are associated with potential toxicity and serum concentrations need to be monitored in certain patients. Previous reports suggested IgM paraproteins could interfere with vancomycin assays, and no paraprotein interference has been reported with digoxin assays. Here we present a suspected case of free-kappa light chains-mediated falsely low digoxin and vancomycin concentrations with Abbott particle-enhanced turbidimetric inhibition immunoassay (PETINIA) method. A 53-year-old patient received multiple doses of vancomycin and digoxin intravenously, but trough vancomycin and random digoxin concentrations repeatedly measured as <1.1 μg/mL and <0.2 ng/mL respectively with Abbott PETINIA method. Results from alternative methods showed concentrations reaching toxic levels and administration of the drugs was immediately terminated. A significantly elevated level of free-kappa light chains, possibly in polymeric form as suggested by protein electrophoresis result, was suspected to be the cause of falsely low results. During the laboratory investigation, absorbance curves revealed increased agglutination in the patient's samples in the latter part of the reaction, suggesting interfering substances led to production of turbidity after reagents were added. Protein-free filtration partially recovered the drugs with Abbott PETINIA. When drug concentrations do not correlate with clinical judgment, clinicians and pharmacists should consult clinical laboratories for investigation of potential interfering substances.

1. Case description

A 53-year-old male had a history of atrial fibrillation, rapid ventricular response, non-ischemic cardiomyopathy, hypertension, chronic obstructive pulmonary disease, dyslipidemia, anemia, pancreatitis, and thrombocytopenia. He presented to the emergency

Abbreviations: Immunoglobulins (Ig), Particle-Enhanced Turbidimetric Inhibition Immunoassay (PETINIA); Kinetic Interaction of Microparticles in a Solution (KIMS), Electrochemiluminescence Immunoassay (ECLIA); Chemiluminescent Microparticle Immunoassay (CMIA), Protein-Free Filtrate (PFF).

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center with a two to four weeks history of abdominal pain, in addition to a 48-h history of nausea, vomiting and pitting lower extremity edema. He was diagnosed with liver cirrhosis secondary to alcohol abuse and was admitted to the intensive care unit.

The patient was receiving numerous medications (Supplemental Table 1). At admission, his creatinine was 2.00 mg/dL with eGFR of 37 mL/min/1.73 m^2. His immunoglobulins (Ig) were measured during his hospital stay, showing a decreased IgG of 483 mg/dL (reference range 550–1650 mg/dL). The IgA and IgM concentrations were within the reference ranges at 166 mg/dL (70–365 mg/dL) and 109 mg/dL (30–263 mg/dL) respectively. The concentration of free kappa light chains was significantly elevated at 399 mg/dL (0.33–1.94 mg/dL) and free lambda light chains was within the reference range at 1.16 mg/dL (0.57–2.63 mg/dL), resulting in an elevated free-kappa-to-lambda ratio of 343.97 (0.26–1.65). Serum protein electrophoresis and immunofixation were interpreted as a small amount of free kappa monoclonal protein (0.1 g/dL) with an additional free kappa band (Supplemental Fig. 1). The patient was subsequently diagnosed with B-cell lymphoma.

Following admission, the patient was found to have an intra-abdominal infection, and pharmacy was consulted for dosing and evaluation of vancomycin therapy. An initial dose of 1750 mg (*c.a.* 21 mg/kg) was administered intravenously over 2 h (Table 1). The first trough level after the initial vancomycin dose was measured as <1.1 μg/mL with a target trough concentration range of 15–20 μg/mL. On day 2 of vancomycin treatment, 1750 mg and 500 mg were administered in the morning and in the evening respectively. However, the pre-dose trough level was again measured as <1.1 μg/mL on day 3. An increased dose of 2500 mg was then administered on the same day. Pre-dose trough level was still measured as <1.1 μg/mL on day 4 despite the dose increase.

Digoxin (tablet of 125 μg; oral; daily) had been prescribed two months before admission, and the patient has received multiple doses of digoxin intravenously over the first four days following admission (Table 2). However, serum digoxin concentration repeatedly measured as <0.2 ng/mL, while the target therapeutic range is 0.8–2.0 ng/mL. Our laboratory, which uses Abbott particle-enhanced turbidimetric inhibition immunoassay (PETINIA) to measure vancomycin and digoxin, was consulted after multiple lower-than-expected drug levels were reported.

2. Case resolution

The samples in question did not have hemoglobin, bilirubin or triglyceride above the interference cutoffs stated in the package inserts [1,2]. They were also visually inspected, and they were not hemolyzed, icteric, or lipemic. The samples were sent to a reference laboratory, where vancomycin is measured with Roche kinetic interaction of microparticles in solution (KIMS) and digoxin is tested by Roche electrochemiluminescence immunoassay (ECLIA). Serum vancomycin concentrations measured from samples collected on day 2 and 3 of vancomycin treatment were reported as 17.5 μg/mL and 23.5 μg/mL by the KIMS assay (Table 1). As shown in Table 2, digoxin measurements from Roche ELICA assay were 1.4 ng/mL and 3.1 ng/mL for the samples collected on day 4 and 6 of digoxin treatment, reaching toxicity level. The clinical team terminated vancomycin and digoxin administration once notified about the results from the reference laboratory.

We treated the samples with heterophile blocking agent (Scantibodies Laboratory, CA) and ruled out the possibility of heterophilic antibodies or rheumatoid factor [3]. The patient's electronic health record was also reviewed, and we did not identify any possible drug-drug interaction or disease state that could account for the falsely low results. Concentrations of serum vancomycin and digoxin were continually monitored after termination of treatments, and additional investigation was performed in the laboratory with these samples. Protein-free filtrate (PFF) was prepared from these samples using a membrane with a cutoff at 30 kDa. Vancomycin and digoxin concentrations in the neat samples were also measured by alternative methods: chemiluminescent microparticle immunoassay (CMIA) on the Abbott Architect and the Roche KIMS/ELICA assays. Results from these studies showed concentrations of vancomycin and digoxin well above the detection limits (Tables 1 and 2) throughout the observation period. For example, in the sample collected on day 4 following vancomycin initiation (one day after termination of treatment), the PETINIA measured vancomycin as < 1.1 μg/mL in the neat samples and 15.3 μg/mL in the PFF. CMIA and KIMS assays reported toxic levels of 28.8 μg/mL and 23.3 μg/mL respectively in the neat samples.

Similar observations were seen for digoxin (Table 2). For the samples collected on day 6, 7, 8 and 10 since the commencement of intravenous digoxin treatment (day 2, 3, 4 and 6 after the last digoxin dose), all samples were measured as <0.2 ng/mL using the PETINA. However, the samples collected on day 6, 8 and 10 were measured as 3.1, 2.4 and 1.8 ng/mL when analyzed by the ELICA and the samples collected on day 7, 8 and 10 were measured as 3.4, 2.2 and 1.6 ng/mL by the CMIA. Comparable results (3.1, 2.0 and 1.4

Table 1
Doses of vancomycin administered since admission and trough vancomycin concentrations measured by Abbott PETINIA, Abbott CMIA and Roche KIMS assays in serum or protein-free filtrate (PFF) of serum.

Day of treatment since admission	Vancomycin (mg; IV)	Frequency	Serum vancomycin concentration (μg/mL)			
			Abbott PETINIA	PFF Abbott PETINIA	Abbott CMIA	Roche KIMS
Day 1	1750	250 ml/hr over 2 hrs	–	–	–	–
Day 2 a.m.	1750	250 ml/hr over 2 hrs	<1.1	–	–	17.5
Day 2 p.m.	500	100 ml/hr over 1 hr	–	–	–	–
Day 3	2500	220 ml/hr over 2.5 hrs	<1.1	–	–	23.5
Day 4	Discontinued	–	<1.1	15.3	28.8	23.3
Day 5	Discontinued	–	<1.1	7.9	12.6	11.6
Day 7	Discontinued	–	<1.1	4.2	7.0	5.8

Table 2
Doses of digoxin administered since admission and random digoxin concentrations measured pre-and post-termination of digoxin treatment by Abbott PETINIA, Abbott CMIA and Roche ELICA assays in serum or protein-free filtrate (PFF) of serum.

Day of treatment since admission	Digoxin (μg)	Frequency	Serum digoxin concentration (ng/mL)			
			Abbott PETINIA	PFF Abbott PETINIA	Abbott CMIA	Roche ELICA
Day 1	250; IV	Once Over 5 min	<0.2	–	–	–
Day 3	62.5; IV	Once Over 5 min	–	–	–	–
Day 4	500 and 250; IV	Once at each dose Over 5 min	<0.2	–	–	1.4
Day 6	Discontinued	–	<0.2	–	–	3.1
Day 7	Discontinued	–	<0.2	3.1	3.4	–
Day 8	Discontinued	–	<0.2	2.0	2.2	2.4
Day 10	Discontinued	–	<0.2	1.4	1.6	1.8

ng/mL) were observed for the PFF of the same samples.

For each drug the reaction absorbance curves were checked to determine whether assay interferences were present for Abbott PETINIA (Figs. 1 and 2). Comparing with the reaction curves from control patients whose results were undetectable (<1.1 μg/mL for vancomycin and <0.2 ng/mL for digoxin), it was clear that increased agglutination/turbidity in this patient's samples occurred in the latter part of the reaction process, upon addition of the reactive reagents. The absorbance in the neat sample of this patient reached 2.7 at the end of the measurement window (read point 25), compared to an absorbance of 1.6 in the control patient (vancomycin of <1.1 μg/mL) (Fig. 1). Similarly, the absorbance in this patient reached 1.5 for digoxin measurement, compared to 1.1 in the control patient (digoxin of <0.2 ng/mL) (Fig. 2). Since the rate of agglutination reaction is inversely related to the concentrations of the drugs, this increased agglutination resulted in an undetectable reading. Protein free filtration seemed to decrease agglutination in the second half of the reaction process, possibly by removing the interfering substances (Figs. 1C and 2C).

3. Discussion

The Abbott PETINIA methods are based on competition between drug in the sample and drug coated onto a microparticle for drug-specific antibody. The drug-coated microparticle reagent is rapidly agglutinated upon the addition of the anti-drug antibody. When a sample containing the drug is added, the agglutination reaction is partially inhibited, slowing down the rate of absorbance change. Therefore greater agglutination/turbidity represents lower serum drug concentrations, and vice versa [2].

In this patient, multiple doses of vancomycin and digoxin were administered intravenously. After the initial PETINIA results showed undetectable drug levels, the clinical team made sure that the medications were administered properly. However, the two drugs remained undetectable by the Abbott PETINIA throughout the investigation period. Results from assays other than the PETINIA, on the other hand, matched the clinical picture. A likely etiology of the false results could be free kappa monoclonal protein. Paraproteins have been shown to cause increased turbidity in specimens during a test reaction, and therefore alter reaction kinetics and lead to false results [4–6]. It has also been suggested that paraproteins can bind to the analyte itself occasionally, or a component of the assay system, and subsequently cause false results [4,6]. In contrast to previous reports that suggested IgM paraproteins as the root cause of interference for vancomycin assays [5,7,8], the patient in our report did not have elevated IgM, or an IgM paraprotein based on the immunofixation result (Supplemental Fig. 1). Instead, the patient had a very high concentration of free kappa light chains, and there seemed to be more than one form of free kappa in this patient's sample (Supplemental Fig. 1).

It has been previously shown that free kappa light chains in some patients can exist as a mixture of monomeric, dimetric and even tetrameric forms [9,10]. It is known that when IgM forms polymers, its solubility decreases, hence sample turbidity is increased [6]. If the same holds true for free kappa, this could be one possible mechanism by which polymeric free kappa light chains lead to production

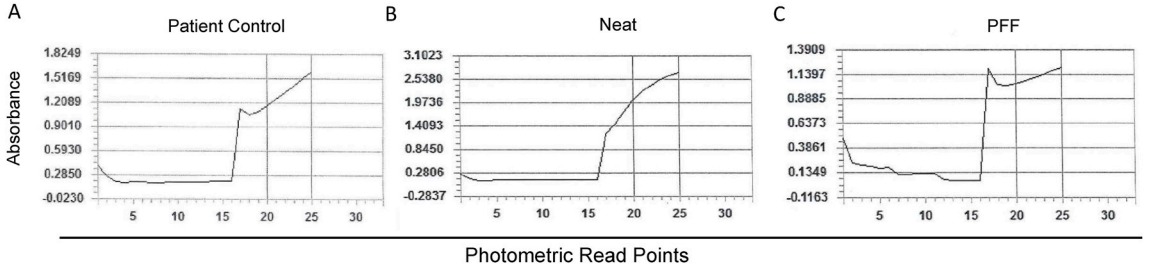

Fig. 1. Reaction absorbance curves of the Abbott PETINIA vancomycin assay. Measurements are taken between 20 and 25 read points. A, Control patient with result of <1.1 μg/mL. B, Patient sample from day 4 of vancomycin treatment, measured as <1.1 μg/mL. C, Patient sample after protein-free filtration (PFF) from day 4 of vancomycin treatment, measured as 15.4 μg/mL.

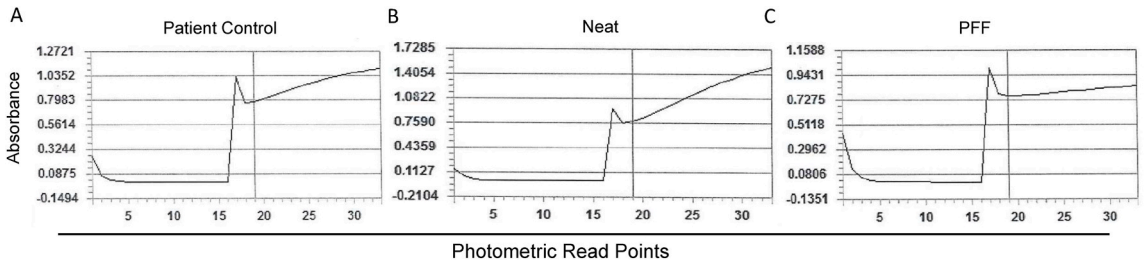

Fig. 2. Reaction absorbance curves of the Abbott PETINIA digoxin assay. Measurements are taken between 19 and 33 read points. A, Normal control patient with result of <0.2 ng/mL. B, Patient sample from day 7 of digoxin treatment, measured as <0.2 ng/mL. C, Patient sample after protein-free filtration (PFF) from day 7 of digoxin treatment, measured as 3.1 ng/mL.

of turbidity and therefore false results. The presence of polymeric forms of free kappa could also explain why vancomycin and digoxin were at least partially recovered in PFF. The monomeric free kappa is only 22.5 kDa and therefore it should not have been removed by PFF and results should stay unchanged after PFF. The dimer or tetramer of free kappa, on the other hand, should have been filtered by the membrane with a 30 kDa cutoff. Further studies are needed to investigate the correlation of different molecular species of free kappa light chains and their solubility.

Roche KIMS, which was able to detect vancomycin in the questioned samples, was designed in a similar way as Abbott PETINIA. Both methods were based on competition between drug in the sample and drug coated onto microparticles [2,11]. We suspect that the difference in results from the two methods was due to absorbance wavelengths used in the two assays and the baseline subtraction step in KIMS. The Abbott PETINIA measures at 700 nm without baseline subtraction, while the Roche KIMS performs measurement at 600 nm and subtracts the baseline at 800 nm. Unfortunately, we did not have access to the absorbance traces of KIMS from the reference laboratory to investigate.

Vancomycin and digoxin are associated with potential toxicity and the serum levels need to be monitored in certain patients [5]. If therapeutic drug monitoring is indicated, clinicians and pharmacists should be aware of the potential assay interferences, including the one identified in this study. For laboratorians, having the tools to detect and potentially remove the interferences is key to ensure prompt and accurate laboratory results. Here reanalyzing the sample using an alternative method, ultrafiltration to remove bigger molecules, checking the reaction kinetics all helped us solve the false results. It should be noted that when interference is detected for one analyte, it is possible that other assays using the same analytical method can be affected and therefore all should be investigated [12].

4. Conclusion

This report describes a case which a highly elevated level of free kappa light chain, possibly in polymeric form, was suspected to be the cause of falsely low results using the Abbott vancomycin and digoxin PETINIA methods.

Funding

None.

Declaration of competing interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plabm.2022.e00277.

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