Unexplained Increase in Serum Digoxin: A Case Report

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We describe a patient with unexplained high serum digoxin after cardiac surgery. To control atrial fibrillation in the immediate postoperative period, she was given a brief trial of digoxin (four 0.25-mg doses) over 12 h. Serum digoxin 6 h later was 2.5 μg/L. Two days later, the patient developed ventricular fibrillation, which progressed to cardiac arrest. During or immediately after resuscitation, blood was drawn for a digoxin measurement, and the concentration reported was 9.3 μg/L; this result was verified by repeated analysis. Digoxin decreased rapidly and progressively to near 4.0 μg/L over the next several hours and thereafter decreased slowly to 1.0 μg/L over the next 11 days, despite no digoxin being administered. The unexpectedly high digoxin raised questions about the accuracy of the digoxin measurement, particularly about the possible influence of the digoxin-like immunoreactive factor. Analytical approaches to distinguishing true digoxin from this factor and other artifacts of digoxin measurement were applied to this patient, with unanticipated results.

Indexing Terms: immunofluorometric assay/monitoring therapy/variation, source of/coronary artery surgery/toxicology

Case Report and Background

The patient, a 71-year-old woman, presented to this hospital for valve replacement, coronary artery bypass graft (CABG), and possible repair of ventricular aneurysm. Three months before this admission she was diagnosed as having had a myocardial infarction. At that time, she was noted to have extensive three-vessel disease, a left ventricular (LV) aneurysm, and severe aortic stenosis. Past medical history included atrial fibrillation and a cerebral vascular accident with residual right arm and leg weakness.

One day after admission, the patient underwent successful replacement of her aortic valve, a three-vessel CABG, and an infarctectomy of her LV aneurysm. Postoperatively she was unable to be weaned from the ventilator, developed a new left bundle branch block shown by electrocardiography (ECG), and was inotrope-dependent, first on high-dose dopamine and then on dobutamine. She then developed atrial fibrillation with a heart rate of 160/min and was started (day 0) on digoxin, 0.25 mg intravenously every 4 h for four doses (cumulative dose 1.0 mg). Her first sample for serum digoxin determination, drawn 6 h after the last digoxin dose, was reported as 2.5 μg/L digoxin. No additional digoxin was prescribed but another blood sample was drawn early the following morning (0257 h, day 2) and was reported to contain 1.4 μg/L digoxin. Other medications begun within 24 h of her surgery included heparin, nitroprusside, amrinone, famotidine, and furosemide. Late in the evening of this same day (2330 h), the patient went into ventricular fibrillation and then asystole but was revived after vigorous resuscitation, still in atrial fibrillation. At 0114 h on day 3, 2.5 h after the episode of ventricular fibrillation and asystole, her serum digoxin was 9.3 μg/L, 46 h after her last known digoxin dose. No intervention was initiated in response to the report, but subsequent blood samples were obtained over the next several days for digoxin analyses (Fig. 1).

Throughout the perioperative period, cardiac arrest, and resuscitation, and for 3 weeks following, her renal function remained stable: serum urea nitrogen 6.4-8.6 mmol/L, creatinine 150.3-194.5 μmol/L. Electrolytes were notable for only a slight constant hypernatremia (Na+ ~137 mmol/L) during her hospitalization and potassium in the high normal range because of intravenous replacement. Blood and intravenous fluid replacement were extensive during the surgical procedure, but her fluid input and output thereafter remained stable and appropriate for her renal function. Three weeks after admission the patient was weaned from the ventilator and 2 weeks later administration of digoxin was started again. The patient was discharged to a community hospital.

Digoxin Toxicity

Among the elderly, ~5–15% of individuals taking digoxin will develop signs of digitalis toxicity (1, 2). These statistics reflect the narrow therapeutic range of digoxin and the multiple mechanisms that can lead to intoxication: overdose, drug interactions, hypokalemia, hypomagnesemia, volume depletion, renal insufficiency, and chronic disease states (e.g., hypothyroidism). Most of these mechanisms act via high concentrations of serum digoxin. Because digoxin is predominantly excreted in the urine, a reduction in renal clearance would prolong the half-life of digoxin (normally, ~1.7 days). Hy-
Digoxin-Like Immunoreactive Factors

Apparent concentrations of serum digoxin may be increased by the presence of endogenous cross-reacting substances. The first description of these endogenous interferences in a digoxin RIA involved a patient reminiscent of the patient described here (7). In that report, despite the discontinuation of digoxin therapy, the digoxin in the patient’s serum increased for 9 days. Afterwards, many digoxin-free individuals with renal insufficiency were found to manifest detectable digoxin-like activity as measured by RIA (8). The term coined for this cross-reacting species was digoxin-like immuno-reactive factor (DLIF). Other populations with increased DLIF include neonates (9), pregnant women (10), and patients with liver disease (11); suggestions of increases have been reported in patients with subarachnoid hemorrhage (12), congestive heart failure (13), insulin-dependent diabetes (14), stress (15, 16), and spironolactone therapy (17). The chemical basis for this DLIF activity is still controversial (18).

Experimental Differentiation Among Possible Causes of Increased Digoxin

Possible explanations for the increased digoxin in this patient include the following:

1. The values are correct and reflect the administration of substantial additional digoxin.
2. The values are correct and represent a redistribution of digoxin from tissue stores to blood.
3. The values are artifactual and represent a cross-reacting species, i.e., DLIF.
4. The values are artifactual and represent a serum component that interfered with the assay used.

Strategies to distinguish between true and artifactual digoxin measurements have taken advantage of either (a) the weaker and unique digoxin–antibody binding characteristics of the interfering substance or (b) the physical state of the interfering substance in serum (highly protein-bound) as contrasted with true digoxin.

Non-specific interferences typically reduce the binding affinity of the antigen–antibody complex nonspecifically. In a competitive binding assay (e.g., RIA), this results in less label bound and an artificially increased assay result. Frequently, a serum containing such a substance will show variable reproducibility and non-linear dilution characteristics, and may artifactualy increase RIA results for other compounds.

A cross-reacting substance typically binds to or near the digoxin binding site on the antibody and competes with digoxin for it. Frequently, cross-reacting species will produce nonlinear dilutions, e.g., as seen even with digoxigenin, or the mono- and bis-digitoxoside of digoxin (19). Additionally, each different digoxin antisemur will show a reproducible but unique amount of cross-reactivity with a given cross-reactant. In most instances, cross-reacting species will be reported as a different concentration of digoxin when reassayed by another digoxin assay (20).

Because the binding of the DLIF is weaker than that

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\text{Serum Digoxin Concentration (ng/mL)}
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\text{Time Post Operation (days)}
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Fig. 1. Serum digoxin concentrations measured by an immunofluorometric assay on an aca in sequential postoperative specimens of the patient.

Inset: Digoxin concentrations remeasured by the NEN digoxin RIA and plotted vs the aca results.

potthyroidism may also decrease the renal clearance of digoxin (3). Volume contraction leads to higher steady-state concentrations.

Drugs can potentially modify the absorption, distribution, metabolism, and elimination of digoxin. Anticholinergic medications may slow intestinal peristalsis and thereby increase absorption of the drug (4). Drugs that are nephrotoxic or impede renal clearance will affect the concentration of digoxin. However, such drugs only delay digoxin’s removal from the body; they do not increase circulating concentrations in the absence of continued digoxin administration. Most digoxin passes from the body unaltered, with <5% being actively metabolized. Decreased digoxin metabolism is not known to increase serum digoxin significantly. Digoxin has an extremely wide apparent volume of distribution (~500–700 L), with large body stores in fat and muscle (1, 3). Lipophilic drugs may displace digoxin from storage sites and increase serum concentration. The most widely studied is quinidine, which has caused the serum digoxin concentrations to double (5).

The manifestations of digoxin intoxication can include cardiac, gastrointestinal, and neurological symptoms. Cardiotoxicity can result in premature ventricular depolarization, junctional tachycardias, and atrial–ventricular (AV) nodal block. These changes are not limited to excess digoxin. Other arrhythmias, considered more diagnostic of digitalis toxicity, include paroxysmal atrial tachycardia with AV block, bidirectional ventricular tachycardias, and regularization of the rhythm in atrial fibrillation (4, 6). Gastrointestinal symptoms include nausea, vomiting, diarrhea, and anorexia (4, 6). Headaches, dizziness, trigeminal neuralgia, visual and auditory hallucinations represent neurological manifestations (4, 6).

An apparent excess of serum digoxin may result from improper sampling. Peak concentrations of the drug are seen 2–3 h after oral ingestion, with a maximal therapeutic effect apparent in 4–6 h (1, 3). After intravenous administration, the maximal effect is seen in 1–3 h (1, 3).
of digoxin, the longer the binding reaction, the more the equilibrium will favor digoxin binding (21). Hence, if the assay incubation period is prolonged (22) or if the incubation temperature is increased (23), there is a resulting decrease of the influence of DLIF. Increasing the incubation time even from 30 to 60 min reduces DLIF by half (22). Both techniques have been used successfully (22–25).

DLIF is highly protein bound, whereas digoxin is >90% unbound at 4°C. Therefore, ultrafiltration (26) or extraction columns (27) can remove DLIF. Recoveries of true digoxin in these processes have been >90%, allowing for specimen clean-up before assay with minimal impact on the true digoxin value.

Finally, other analytical methods exist to determine more rigorously the serum digoxin concentrations (28, 29). They generally include an extraction step that is followed by a chromatography step (typically HPLC) with quantitative assay of the eluted fractions; the most unambiguous assay of the eluate is mass spectroscopy. Standards indicate the chromatographic location of true digoxin. Such techniques have successfully allowed for the concurrent quantitation of digoxin and its metabolites, which are highly cross-reactive in many digoxin RIAs (19).

Materials and Methods
Specimens were originally assayed for digoxin on an automated analyzer (acea; Dupont Diagnostic Systems, Wilmington, DE) according to the manufacturer’s protocol. The upper limit of the assay range is 4.0 μg/L. The inter- and intraassay variability of the method for a control specimen of 2.25 μg/L were 3.1 and 2.6%, respectively. The specimen that was measured as 9.3 μg/L was reassayed multiple times by the laboratory.

Potential contribution of nonspecific assay interferences. Specimens from the patient before and after her cardiac arrest, representing a wide range of apparent digoxin concentrations, were reassayed in duplicate with a testosterone RIA (RLS 125I-testosterone kit; ICN Biomedical Inc., Diagnostics Division, Carson, CA) according to the manufacturer’s protocol.

Potential contribution of DLIF. Specimens from the patient that were originally assayed by the acea method were reassayed in duplicate with a 125I-digoxin RIA (Dupont–NEN, Boston, MA), which uses a different antisem, by the manufacturer’s method (30-min incubation at room temperature). The inter- and intraassay variability were <5%. We then reassayed the same series of specimens with the NEN digoxin RIA but with a 37°C, 90-min incubation. Standards and unknowns were processed in the same way.

Also, we ultrafiltered the same series of specimens before assaying with the NEN digoxin RIA (manufacturer’s protocol). Specifically, 0.5 mL of serum was introduced into a Centrifree micropartition system (Amicon, Beverly, MA), in which the sample tube is divided by a 30 000 exclusion membrane. The sample is centrifuged and the ultrafiltrate, which passes through the membrane, is captured in a cup. Typically, for 0.5 mL of serum introduced, centrifugation at 2500g for 20 min at 4°C produces 0.25 mL of filtrate. We aliquoted the filtrate for assay by the NEN digoxin assay (manufacturer’s protocol).

Potential contribution of digoxin. In addition to the maneuvers used to eliminate contributions of DLIF, we fractionated an extract of the patient’s serum by HPLC and used the NEN digoxin assay to determine the digoxin content in sequential fractions of the eluate. Specifically, 0.5 mL of the patient’s specimen (having an original value of 4.0 μg/L of apparent digoxin by the NEN RIA) was extracted with 2.5 mL of methanol/chloroform (30/70, by vol). After 2 min of vortex-mixing, the sample was centrifuged for 10 min at 2500g. The organic phase was removed and reduced in volume to ~0.5 mL (by then, predominantly methanol), diluted with 9.5 mL of distilled water, and loaded onto a short C18 reversed-phase HPLC column (7.5 cm × 4.6 mm, 3-μm particles; Supelco, Bellefonte, PA). After loading the specimen on the short column, we connected the short column to an analytical column (15.0 cm × 4.6 mm, 5-μm particles; Vydac, Hesperia, CA) and eluted the columns with an ethanol/water/trifluoroacetic acid (0.1 g/L) gradient (see Fig. 3 for details). A serum-based digoxin standard of the same digoxin concentration (4.0 μg/L) was processed identically to assess the recovery efficiency of the extraction–HPLC procedure. Fractions (1.0 mL) of the eluate were collected, divided equally between two tubes, dried thoroughly, and assayed by NEN digoxin RIA in the tubes. A high concentration (100 μmol/L) of digoxin was chromatographed on HPLC, using the same elution gradient, and its absorbance was monitored by a diode-array spectrophotometer to establish the elution time of digoxin.

Inhibition of [Na,K]ATPase. To assay the inhibition of [Na,K]ATPase, we used a procedure that monitors ATP hydrolysis (15), but modified the preincubation step by carrying it out for 2.5 h in the absence of potassium. The assay uses canine renal [Na,K]ATPase (Sigma Chemical Co., St. Louis, MO). The specimen drawn closest to the time of cardiac arrest and previously reported to contain 9.3 μg/L digoxin was assayed in duplicate for its ability to inhibit [Na,K]ATPase activity. For comparison, we also assayed a digoxin standard (8.0 μg/L) in duplicate. Ouabain (0.1 mmol/L) was used to determine complete inhibition of ouabain-sensitive activity.

Results
Results for all of the original digoxin measurements are provided in Fig. 1. The specimen originally analyzed for digoxin by the acea and giving a result of 9.3 μg/L was reassayed by the acea: Values ranged from 7.0 to 10.0 μg/L. Thereafter, the specimens were reassayed by another digoxin RIA (NEN). The results were very similar to the originals with correlation between the two assays being highly significant (Fig. 1, r = 0.90, P = 0.000002). By either set of results, the biological elimination half-life was 5.5 days. In contrast, when we assayed several of these same specimens for testosterone by RIA, the
testosterone results showed no correlation with either digoxin assay (NEN digoxin vs testosterone: $r = 0.19$, $P = 0.72$).

Dilutions of one of the high-digoxin specimens taken from day 3 showed responses that paralleled those of true digoxin almost identically ($r = 0.998$, $P = 0.002$).

Reassay of the original specimens by the second digoxin RIA, but modified by increasing both temperature and incubation time, showed results very similar to the unmodified assay. Values for paired $t$-tests of the two sets of results were not significantly different. Correlation between the sets of results was highly significant ($r = 0.97$, $P = 1.5 \times 10^{-9}$, Fig. 2A). Likewise, ultrafiltration of the individual sera before the digoxin RIA produced results that were well correlated with values from untreated sera ($r = 0.98$, $P = 9.7 \times 10^{-10}$, Fig. 2B).

Extraction of a specimen from day 3, when the highest digoxin concentrations were seen (original concentration 4.0 $\mu$g/L), followed by HPLC fractionation and digoxin RIA of dried eluate, showed a pattern of activity strikingly similar to that of a similarly processed and analyzed 4.0 $\mu$g/L digoxin standard (Fig. 3). The exact location of digoxin in the chromatogram was indepen-

dently determined by introducing 100 $\mu$L of a 100 $\mu$mol/L solution of digoxin onto the HPLC system and monitoring the absorbance. The peak eluted at 23 min, which is identical to the fraction collected at 24 min, given the $\sim$1 min delay between the ultraviolet detector and the fraction collector.

Both the selected patient's specimen and the digoxin standard with a similar RIA digoxin concentration showed comparable inhibition of [Na,K]ATPase activity: 88% inhibition vs 81%, respectively.

**Discussion**

Biochemical Differentiation Between Digoxin and Nondigoxin Assay Interferences

The validity of immunoassays for measurement of digoxin has been shown to be compromised in certain clinical populations by the presence of a cross-reacting factor or factors (DLIF) (18). Its presence and cross-reactivity are sufficiently great that it can completely confound digoxin results measured in neonates and can modestly increase digoxin results in pregnant women, some patients in renal failure, and some patients with liver disease (8–11). Even in these cases, though, the highest concentration reported for a patient by the NEN digoxin RIA has been 2.0 $\mu$g/L in a neonate (30). Typically, $\leq$0.5 $\mu$g/L digoxin is present in the serum of pregnant women or patients in renal or liver failure (8–10). Because of the confusion surrounding the actual digoxin value in this patient, we used several techniques capable of differentiating real from factitious digoxin (22–25, 27–29).

Reassay of specimens with two different antibodies gave highly similar, highly correlated results. Likewise, dilutions of a single specimen during the period of high digoxin concentrations and significant increases in digoxin results showed a linear pattern. Reassay of several specimens in an unrelated steroid assay (testosterone) showed no correlation with digoxin concentrations and no increase. These results are consistent with the activity arising from true digoxin, making nonspecific assay interferences highly unlikely. The half-life of
elimination for the activity in this patient would be close to that predicted for digoxin in a patient with this degree of renal impairment.

Use of prolonged incubation times and increased incubation temperatures produce profound reductions in DLIF measurements (22). However, these procedures produced no significant reductions (2.6 ± 0.5 vs 2.3 ± 0.5 µg/L) in this patient's specimens. Likewise, preultrafiltration of specimen, which has eliminated DLIF quantitatively in other studies (22), produced no significant reduction in this patient's apparent digoxin content (2.6 ± 0.5 vs 2.4 ± 0.5 µg/L). Again, these results argue strongly against DLIF or any other cross-reactant and strongly for digoxin giving rise to the activity measured.

Finally we carried out HPLC fractionation of a true digoxin standard and of the patient's specimen at high apparent digoxin concentration. Because of uncertainty about the efficiency of extraction and recovery through the system, we concentrated the specimens 2.5 times. The recovery was excellent, however, which thus placed many of the specimens beyond the highest standard (4.0 µg/L) so that the results were quantitatively unreliable. The elution pattern of the digoxin standard demonstrated that most of the activity eluted at 24 min, with another sizable peak of activity at 19 min and a shoulder at 16 min. The identity of this earlier-eluting substance is unknown, but is related to digoxin because of its ability to be measured by the digoxin RIA. The patient's specimen showed virtually the same pattern (but with no shoulder at 16 min) and magnitudes. Likewise, the peak specimen from this patient inhibited renal [Na,K]ATPase as much as did a digoxin standard of comparable concentration. These results strongly support the view that the activity measured in the patient's specimen was indeed digoxin. Taken in total, the data leave little doubt that all of the apparent digoxin was digoxin.

Drug Interactions and Physiological Changes: Possible Explanation of Increased Digoxin?

Having reached this conclusion, we must evaluate the possible mechanisms for this dramatic and sustained increase in digoxin concentration and also examine any clinical signs and symptoms that corroborate our hypothesis. The patient was originally prescribed a total of just 1.0 mg of digoxin, which was discontinued 2 days before the peak concentrations were measured. There is no possibility that the patient surreptitiously took oral medications; she was intubated and obtunded. There were no changes in electrolytes that could account for changes in digoxin concentration. Serum potassium remained constant throughout her hospitalization. Renal function was reduced in this patient (serum creatinine, 150.3–194.5 µmol/L), which, in the absence of additional digoxin, would prolong its elimination. The calculated half-life was 5.5 days, within the range seen for patients of her age and renal status. The patient became mildly acidemic for <20 min at the time of her cardiac arrest.

The effects of acidemia of this magnitude and duration on tissue stores are unknown. However, this does not explain the increased digoxin for days after.

As previously mentioned, some drugs may increase serum digoxin concentrations through various mechanisms. Those drugs that would interfere with renal clearance or metabolism would only prolong the half-life, leading to an increase in steady-state values, and would not produce the pattern seen in Fig. 1. Similarly, the patient was not taking oral digoxin, so bioavailability and absorption are not factors. This patient was not volume-contracted; if anything, she was somewhat volume-expanded on day 3 as a consequence of the fluid gained during the extensive cardiac surgery. Excess blood volume would lead to decreased digoxin, not increased. Verapamil and other calcium-channel blockers increase digoxin by as much as twofold via an unknown mechanism; however, although the patient was prescribed famotidine, this could not account for all of the increase in serum digoxin found here (31). The only drug interaction that could theoretically increase the serum concentrations in this patient would be something that displaced massive amounts of digoxin from peripheral storage sites. Quinidine and theophylline are the prototypical drugs of this type, but the patient took neither during her current hospitalization. By process of elimination and on the basis of the pattern in Fig. 1, the only conclusion supported by the history and data remains that during day 3, this patient received additional digoxin, ~2–3 mg.

Evidence of Digoxin Toxicity

Physiological evidence for digoxin intoxication was sought retrospectively by reviewing the patient's ECG records. Only one tracing from the morning of day 5 showed evidence of such possible toxicity. There was clear regularization of the patient's rhythm in atrial fibrillation. Although this type of rhythm is not entirely specific for digoxin intoxication, it is very suggestive and reflects an increase in the discharge of junctional pacemakers, leading to a nonparoxysmal, automatic, AV junctional tachycardia (3, 5, 6). The patient's digoxin concentration was ~4.0 µg/L at this time.

Although ventricular fibrillation is unlikely to be the first sign of digoxin intoxication, this patient's cardiac arrest on the night of the second postoperative day could possibly be the result of excess digoxin administered just before the event. The exact temporal and hence causal linkage between this patient's cardiac arrest and the toxic digoxin concentrations may never be known; it is also possible that the toxic concentrations recorded were the result of digoxin being administered inadvertently during resuscitation efforts. What remains unexplained is that, despite the extremely toxic concentrations of digoxin, there was no evidence of AV nodal block throughout this period. The patient never became bradycardic. Even during the documented episode of regularization of ventricular rhythm, the rate was 82 beats/min.
Concluding Comment

This case presented a dilemma to both the physicians and the clinical laboratory. An extraordinary digoxin concentration was measured but was originally thought to reflect a problem with the digoxin assay. Had there been more clinical evidence for digoxin toxicity, there is no doubt that therapy with digoxin-specific Fab antibody fragments would have been instituted (6, 32). However, the patient was not overtly toxic after her cardiac arrest, and anti-digoxin therapy was omitted. In retrospect, the clinical chemistry laboratory could have greatly clarified the result had it implemented ultrafiltration or increased incubation time and temperature to eliminate the contribution of DLIF. Such procedures would have lent considerable credence to a true digoxin intoxication and could have been available in a matter of minutes. Such approaches may be very useful in complicated cases such as this one.

References