A Physician's Office-based Digoxin Assay (Seralyzer) Evaluated for Interference by Endogenous Digoxin-like Immunoreactive Factors*

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ABSTRACT

A digoxin test for a physician's office based-chemistry analyzer (Ames Seralyzer) was evaluated for possible interference by digoxin-like immunoreactive factors (DLIF). Sera from patients likely to have high concentrations of DLIF (renal and hepatic patients, pregnant women, and neonates) as well as from normal patients and umbilical cord blood were analysed by the Seralyzer digoxin immunoassay and by a fluorescence polarization digoxin immunoassay (Abbott TDx) known to detect DLIF. For all patients who were not taking digoxin (n = 85) only four patients (4.7 percent) measured apparent digoxin values >0.2 ng per mL by the Seralyzer compared to 64 (75 percent) by the TDx analyzer. Measurements of DLIF from adrenal extracts demonstrated a 17-fold greater potency for detection of DLIF by the TDx (2.9 ng per mL) compared to the Seralyzer technique (0.18 ng per mL). However, recovery data suggest that the presence of digoxin reduces the potency of DLIF interference as a function of increasing digoxin concentrations especially for the TDx assay. This diminished DLIF crossreactivity in the presence of digoxin is one explanation for the comparable correlation observed for both non-renal and renal failure patients taking digoxin when measured by these two immunoassays.

Introduction

Digoxin-like immunoreactive factors (DLIF) are endogenous factors that crossreact with anti-digoxin antibodies. These factors are found in human plasma and have been reported to interfere with

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able to DLIF-antibody binding were utilized. A new physician's office based chemistry analyzer digoxin assay (Ames Seralyzer) was evaluated by the current authors to determine the extent to which DLIF was detected by that assay in these patient groups. The degree to which DLIF extracted from adrenal tissues interferes with the measurement of digoxin was also determined.

Materials and Methods

Assays

Patient specimens were analyzed by the Ames Seralyzer digoxin assay in duplicate measurements and the Abbott TDx Digoxin II assay (unmodified), as single measurements. The detection of DLIF by this latter assay has been described previously. The Seralyzer utilizes an enzyme immunometric assay as depicted in figure 1. The dry solid capture phase contains digitoxigenin which is attached to a cross-linked, beaded polyacrylamide gel via a spacer arm. The sample containing digoxin is added to a solution containing an excess of an anti-digoxin Fab':β-galactosidase monon conjugate and the binding allowed to reach equilibrium. The free conjugate is separated from the bound species by mixing the solution with the solid phase. The solid phase is separated by filtration and the enzyme activity of the filtrate determined on a reagent strip. The analyzer displays the digoxin concentration determined from a calibration line. The initial specimens mixing/extraction step was performed by a prototype mixing instrument provided by an outside group. *

Enzyme Immunometric Assay

![Enzyme Immunometric Assay Diagram]

Figure 1. Schematic description of Seralyzer® digoxin immunometric assay.

Patients

Normal (control) patients were defined as those with serum creatinine less than 1.5 mg per dL, BUN less than 20 mg per dL, serum total bilirubin less than 1.5 mg per dL, and serum alkaline phosphatase less than 110 mU per mL.

Patients in renal failure were defined as those with serum creatinine greater than 2.0 mg per dL. Hepatic failure patients were defined as those with serum total bilirubin greater than 2.0 mg per dL. These two groups were divided into those on oral or intravenous digoxin therapy and those not on digoxin therapy.

In addition, pools were prepared from plasma drawn from neonates (two to 10 days of age). Specimens were also obtained from women in third trimester of pregnancy and from umbilical cord bloods. None of these groups were on

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digoxin therapy. In all cases, serum was separated from the cells and stored at −70°C until analysis.

**DLIF and Digoxin Recovery Studies**

A pool of normal human serum obtained from laboratory personnel was used to prepare samples spiked with 4.0, 2.0, 1.0, 0.5 ng digoxin per mL or no digoxin.¹ Bovine adrenal extracts used were prepared by the method of Valdes et al.¹¹ The undiluted adrenal DLIF stock (called dilution factor 1.0) was then diluted to 0.5 and 0.25 times the original stock concentration. Combinations of digoxin and DLIF concentrations in control serum were prepared as indicated in the forthcoming section. Dilutions of DLIF stock were also prepared in buffer from the respective manufacturer’s assay kits for the immunoreactive potency comparison studies.

**Statistical Analysis**

All data reduction was performed by the instruments as programmed by the manufacturer using digoxin as standards. Statistical analysis and plots of the data were performed by RS-1 on an IBM-XT.

¹ Sigma Chemical, St. Louis, MO.

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**Results**

**Patients Not on Digoxin**

In all patient groups, (normal controls, patients in renal failure, patients in hepatic failure, women in third trimester of pregnancy, neonates, and also for umbilical cord bloods), the apparent digoxin results (DLIF in digoxin equivalents) were lower by the Seralyzer assay than by the TDx assay (table I and figure 2). In the case of normal controls (n = 21) only one subject measured DLIF greater than 0.1 ng per mL (actual measurement was 0.31 ng per mL) by the Seralyzer; all remaining 20 subjects measured zero (undetectable) by this assay. In contrast, 15 controls had detectable DLIF values (>0.1 ng per mL) by the TDx assay.

**DLIF Recovery Studies**

Adrenal DLIF extract (bovine) was lyophilized and diluted in buffer and in serum. Two-fold serial dilutions were measured by the Seralyzer and TDx digoxin assays. Measurements were made exactly as in samples taken for routine measurements. In figure 3 is shown the relative reactivity (potency) of this DLIF extract for both of these assays. The Seralyzer results show a 17-fold reduced reactivity at the highest concen-

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**TABLE I**

Detection of Digoxin-Like Immunoreactive Factors in Serum from Patients not Taking Digoxin

<table>
<thead>
<tr>
<th></th>
<th>Seralyzer®</th>
<th>TDx®</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of Patients with Values</td>
<td>Number of Patients with Values</td>
</tr>
<tr>
<td></td>
<td>&gt;0.1</td>
<td>&gt;0.2</td>
</tr>
<tr>
<td>Normals (n = 21)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Renal (n = 18)</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Hepatic (n = 10)</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Pregnancy (n = 20)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Neonates (n = 16)</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Cord Bloods (n = 21)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Apparent digoxin concentration are in ng/mL.
tration of DLIF tested. The simultaneous addition of digoxin and DLIF, each at various concentrations, to a control sera demonstrates the net effect of crossreactive potency of DLIF in the presence of several concentrations of digoxin (the principal competing ligand). From the slopes of the curves (measured digoxin vs. added digoxin and DLIF) for each digoxin concentration in figure 4, it is clear that the DLIF interference is additive relative to digoxin when measured on the TDx, whereas the addition of DLIF has practically no effect on the Seralyzer measurement over a wide range of both digoxin and DLIF concentrations.

Patients on Digoxin

A group of 15 adult patients (controls) not pregnant or in renal or hepatic failure but taking digoxin were analyzed by both assays. The correlation of these results along with 20 patients in renal and/or hepatic failure also taking digoxin are shown in Figure 5. The correlation parameters for both groups were essentially identical (no normal patients: Seralyzer = 1.01 TDx – 0.03 ng per mL, \(r^2 = 0.850\), SD of regression 0.158 ng per mL; and renal and hepatic failure patients: Seralyzer = 1.027 TDx + 0.094 ng per mL, \(r^2 = 0.974\), SD of regression 0.175 ng per mL).

Discussion

In this study investigation was made of the detecton of endogenous digoxin-like
immunoreactive factors (DLIF) by a new physician's office based digoxin immun­
 assay. The Seralyzer digoxin assay reacted very little, if at all, with DLIF in
all three types of experiments: direct measurement of patients known to have 
elevated DLIF in serum, spikings with DLIF from adrenal source, and spikings 
serum with a combination of digoxin and DLIF. There was little to no apparent 
digoxin (DLIF) measured by the Seral­
 lyzer assay in patients not receiving 
digoxin. Less than 4.7 percent of the 
patients not taking digoxin showed 
apparent digoxin values greater than 0.2 ng per mL. This percentage was signifi­
cantly lower compared to 75 percent of 
these same patients whose apparent 
digoxin concentrations were greater than 
0.2 ng per mL when assayed by the TDx 
digoxin assay. These data are consistent 
to those reported for the TDx II assay 
(unmodified).8

The DLIF from bovine adrenal 
extracts was added into the reagent 
buffers used in these two assays and into 
control serum (DLIF-free as measured 
by each assay). The relative reactivities 
of these preparations in these two assays

![Figure 4](image)

**Figure 4.** Combined spiking of bovine adrenal DLIF and digoxin into serum at the digoxin concentrations indicated in ng per mL.

![Figure 5](image)

**Figure 5.** Correlation of patients on digoxin either in renal or hepatic failure (closed symbols, solid line) or without these diseases (open symbols, dashed line) by Sera­
lyzer and TDx digoxin assays. Correlation param­
eters are given in text.
demonstrate that DLIF isolated from the adrenal behaves immunoreactively like the DLIF detected in serum in that adrenal DLIF does not react in assays that do not detect DLIF in serum specimens. This is consistent with the recent hypothesis\(^3,11\) suggesting that the adrenal gland is likely a source of DLIF found in plasma.

Spiking experiments with a combination of digoxin and DLIF showed no appreciable interference of digoxin measurements over a wide range of DLIF concentration for the Seralyzer assay. However, the same experiments consistently demonstrated a substantial concentration-dependent interference for the TDx digoxin assay over a wide range of both digoxin and DLIF concentrations. At the lower digoxin concentrations (0.5 or 1.0 ng per mL), the effects of DLIF are worse in that the interference is quantitatively additive, whereas at the higher digoxin concentrations (e.g., 4.0 ng per mL), the reduced slope of the interference curve (figure 4) indicates proportionately less interference from DLIF at the higher digoxin concentrations. This behavior would be expected for a crossreaction model system with nonparallel displacement of the primary ligand (digoxin) by the interfering ligand (DLIF) as observed in figure 3.

There are two possible explanations for the better performance of the Seralyzer assay with respect to DLIF. The first is that the antibody may be more specific. Enhancing antibody specificity for digoxin compared to DLIF has been recently demonstrated to decrease very significantly detection of DLIF in serum.\(^6\) The second possible explanation is that during the separation of the free antibody-conjugate from that bound to digoxin, the high affinity ligand (digitoxigenin) on the solid phase may effectively strip the lower affinity DLIF from the antibody-conjugate, in effect not detecting the presence of DLIF in the assay.

The correlation parameters comparing the Seralyzer and TDx digoxin measurements for patients on digoxin therapy were not different between patients in renal failure and those not in renal failure. This suggests that low concentrations of DLIF, such as those typically detected in patients with renal failure, may not lead to appreciable interference in the presence of digoxin by the TDx assay. However, in some patients with renal and/or hepatic failure and for those patients in groups which may exhibit higher apparent digoxin concentrations owing to DLIF crossreactivity (e.g., third trimester of pregnancy and neonates, cf. figure 2), an assay which has low specificity for DLIF will likely give more reliable assay results for digoxin.

Acknowledgments

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References


