Digoxin-Like Substance in Term Pregnancy, Newborns, and Renal Failure

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Digoxin-like substance (DLS) is detected in pregnant women near term, newborns, and in patients with renal failure using RIAs for digoxin. We made "digoxin" measurements in such patients using three digoxin RIA kits (Nuclear Medical Laboratories (N), Clinical Assays (C), and Corning Magic (M)). At term mother DLS (μg/l digoxin) was 0–0.19 (N), 0.09–0.24 (C), 0.07–0.24 (M); cord blood DLS was 0.35–0.75 (N), 0.46–0.90 (C), 0.42–0.90 (M); infant DLS was 0.5–1.1 (N), 0.28–0.98 (C) and was not measured by M. DLS was detected at term in mothers and was essentially undetectable by 24 hr postdelivery. Cord levels fell in eight of ten infants to significantly lower levels by the second day of life. DLS in renal hemodialysis patients not receiving digoxin was 0.04–0.41 (N), 0.01–0.34 (C), 0.03–0.40 (M). While postdialysis levels by N tended to be lower than predialysis, they rose by C and M. Dilutions of cord blood yielded similar results by N, nonlinear decreases by C, and more nearly parallel decreases by M. In renal failure patients, dilutions yielded similar results by N and marked nonparallel results by M. Digoxin results obtained with immunoassays may be inaccurate in these patient populations. Digoxin immunoassays must be individually characterized as to the expected results.

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Digoxin assays have been found to produce elevated results in pregnant women near term (*1*), in infants and neonates (*2–5*), and in patients with significantly compromised renal function (*6–8*) when no glycoside has been administered. Displacement of labeled digoxin from antibody by digoxin-like substance in the serum of such patients results in apparent digoxin concentrations. These effects vary from patient to patient and are dependent upon the antibody or assay technology used. We have examined this problem using three different digoxin radioimmunoassay kits.

MATERIALS AND METHODS

Assays

Digoxin kits were obtained from Nuclear Medical Laboratories (NML)*, Corning Medical†, and Clinical Assays (Travenol-Genentech Diagnostics)‡. All three kits are competitive radioimmunoassays employing polyclonal antisera raised in rabbits. The NML and Clinical Assays kits require 50 μl serum samples, the Corning Medical kit 100 μl. Separation of the bound from free fractions is accomplished using charcoal in the NML kit, solid phase magnetic particles in the Corning Medical kit (Magic), and antibody coated tubes in the Clinical Assays kit. Because the interference we investigated resulted in minimal displacement for some patient samples in some assays, we estimated assay sensitivity (least detected dose determined as the mean of 20 replicates of the zero calibrator −2 s.d.), between assay precision, and ability to recover digoxin from digoxin-free serum. In addition, we determined whether addition of one-half and twice the normal assay sample serum volume would result in accurate estimates of digoxin added to serum pools. We examined whether the three assays produced different dose estimates for three digoxin serum pools run in each assay and for 45 cardiac patients with normal renal function who were receiving digoxin. Radioactivity was counted in a 20-well gamma counter (NML). The assay calibration curves were fitted using a nonlinear regression (NML), permitting interpolated dose estimates between the least detected dose and the dose corresponding to the lowest assay calibrator (0.4–0.5 μg/l).

Patients Studied

We measured the apparent digoxin concentration in 28 pregnant women near term, in the cord blood of 23

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newborns, in 19 other neonates 2–7 days of age, and in 60 patients receiving chronic hemodialysis for chronic renal failure, none of whom were receiving digoxin. We measured digoxin concentration in another 30 patients with chronic renal failure who were receiving digoxin. Measurements were made using all three assays in all groups except in the neonates, where available sample precluded using the Corning Medical assay. Twice the usual sample volume was used for measurements in near term pregnant women. We measured apparent digoxin concentration in ten pregnant women immediately pre- and two days postpartum and in the cord blood of their infants and in heel-prick blood obtained from these neonates two days postdelivery using the NML assay. Measurements were made in samples obtained from pregnant women with their consent, in neonatal blood submitted to our laboratory as part of our neonatal hypothyroid screening program, and in renal failure patients using blood obtained during urea kinetics studies. Pre- and postdialysis samples were obtained from renal dialysis patients incidentally to a study of parathormone dynamics to which they consented. Results obtained with all three assays were compared for 45 patients receiving digoxin for cardiac disease, but who had normal renal function (evidenced by normal serum BUN and creatinine concentrations). Portions of this study were approved by our institutions' clinical investigation committee.

RESULTS

The least detected digoxin concentration was found to be 0.03 μg/l NML, 0.08 μg/l Clinical Assays, and 0.11 μg/l Corning (mean + 2 s.d.). Between-assay precision and dose estimates for three serum pools are shown in Table 1. Recoveries of digoxin added to serum samples for the three assays are shown in Tables 2–4. Dose estimates obtained for one-half and twice the usual assay serum volumes were appropriate (Fig. 1). Results obtained with each assay in 45 patients receiving digoxin are shown in Table 5 and were not significantly different from one assay to another. Correlation coefficients (Pearson's r) were 0.96–0.98 for each assay compared with the other two. We did note, however, results in patients with digoxin concentrations less than ~1.0 μg/l were lower by NML and Clinical Assays then by Corning Medical. Differences above 1.0 μg/l were not apparent (Table 1). Apparent digoxin concentrations measured in each group of patients are shown in Table 5. Correlations between assay results were poor except for renal failure patients receiving digoxin (Table 6). Results obtained using one-half or twice the usual sample volume in cord blood and in patients with renal failure are shown in Figs. 2 and 3. Results for ten mothers and their infants are shown in Fig. 4. Pre- and postdialysis results in patients with chronic renal failure receiving digoxin and receiving no digoxin are shown in Figs. 5 and 6.

TABLE 1
Digoxin Assays—Precision

	CL4	BIO2	BIO3
	Means		
NML	(Based on means of replicates in 39 assays)		
Mean (μg/l)	0.7 + 0.1*	2.1 + 0.1	3.0 + 0.15
CV (%)	11	5.8	5.6
Corning	A = 5, N = 32†	A = 8, N = 65	A = 8, N = 66
Magic			
Mean (μg/l)	0.94 + 0.09	2.0 + 0.13	3.1 + 0.19
CV (%)	9.1	6.6	6.0
Clinical	A = 8, N = 28	A = 11, N = 66	A = 11, N = 66
Assays			
Mean (μg/l)	0.71 + 0.08	1.9 + 0.18	3.1 + 0.28
CV (%)	10.9	9.2	9.3

*+ 1 s.d.
†A = number of assays, N = total number of replicates. Standard deviation calculated using the means of replicates for each assay.

TABLE 2
Digoxin Assays—Recoveries: NML

	Normal (4)*	Cord blood (4)	Renal failure (4)
Digoxin (μg/l)	0	0.55–0.63	0.09–0.21
Recoveries (%) added:			
0.5 μg/l	84 (78–90)†	167 (160–176)	132 (126–140)
1.0 μg/l	106 (100–112)	167 (160–175)	129 (124–131)
2.0 μg/l	110 (108–116)	137 (132–144)	116 (114–117)

*( ) = Number of serum samples studied (Tables 2–4).
† Mean, range (Tables 2–4).

DISCUSSION

The measurement of digoxin by immunoassay is complicated by the presence in serum of digoxin-like substance(s) which is recognized by the antibodies used. This substance is present in normal serum in low concentrations (*9*) and has been described in the serum of pregnant women (*1*), neonates and infants (*2–5*), and

TABLE 3
Digoxin Assays—Recoveries: Corning Magic

	Normal (1)	Cord blood (5)	Renal failure (4)
Digoxin (μg/l)	0	0.47–0.57	0.05–0.15
Recoveries (%) added:			
0.5 μg/l	95 (90–100)	132 (112–166)	108 (102–114)
1.0 μg/l	96 (93–100)	151 (135–164)	113 (106–119)
2.0 μg/l	106 (101–112)	135 (122–144)	116 (113–123)

TABLE 4
Digoxin Assays—Recoveries: Clinical Assays

	Normal (1)	Cord blood (5)	Renal failure (4)
Digoxin (μg/l)	0	0.42–054	0.08–0.13
Recoveries (%) added:			
0.5 μg/l	79 (78–80)	105 (86–116)	81 (74–86)
1.0 μg/l	94 (92–97)	97 (88–106)	90 (78–97)
2.0 μg/l	93 (93–94)	90 (78–104)	94 (91–99)

patients with compromised renal function (*6–8*). Digoxin results obtained using different immunoassays in patients with renal failure but receiving digoxin have been noted to be discrepant (*10*). In addition, this substance is measured in serum from seriously ill patients not receiving digoxin (*11–14*) and may be associated with abnormal or unusual serum proteins (*12*). Digoxin-like substance is increased by salt loading (*15*) or volume expansion (*16*) and is present in experimental hypertension (*17,18*). Increased concentrations of digoxin-like substance have been found in pre-eclamptic women (*19*).

The concentrations of digoxin-like substance found in these groups of patients are, with the exception of newborns, generally <0.5 μg/l. For this reason, differences in assay response to this material may be in part related to assay sensitivity, accuracy, or precision. We examined the problem of digoxin-like substance using three commercial digoxin kits which we first carefully characterized. We did find minor differences in assay performance, but all three kits we chose had similar sensitivities, were precise, and produced similar results in patients receiving therapeutic amounts of digoxin but who had normal renal function. We increased assay sensitivity by doubling the sample volume, permitting estimation of 0.05–0.1 μg/l of digoxin in serum. We demonstrated that each assay produced appropriate results when either one-half or twice the usual serum sample volume was assayed. Each assay was able to satisfactorily recover digoxin added to serum.

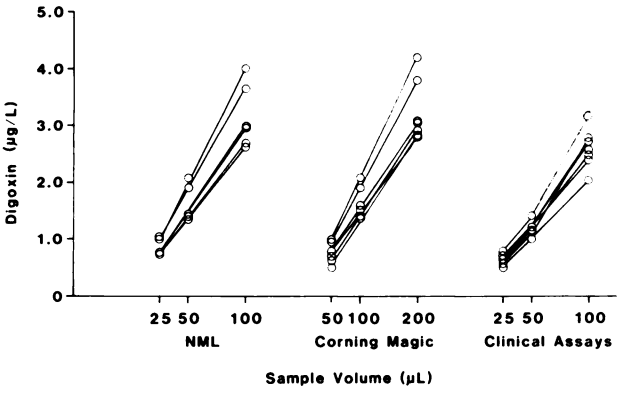

FIGURE 1
Digoxin measured using usual, one-half, and twice the usual assay serum volumes for patients receiving digoxin

TABLE 5
Apparent Digoxin Concentrations Measured in Patient Serum Samples

	Digoxin (μg/l)		
Patients	NML	Magic	Clinical assays
Cardiac (45)*	0–4.3†	0–4.4	0–4.0
	1.2 + 1.1‡	1.5 + 0.97	1.3 + 1.0
Pregnancy (28)	0–0.19	0.09–0.24	0.07–0.24
	0.14 + 0.04	0.16 + 0.03	0.14 + 0.04
Cord blood (23)	0.35–0.77	0.42–0.86	0.45–0.86
	0.58 + 0.11	0.65 + 0.11	0.69 + 0.11
Neonates (19)	0.22–1.20	—	0.04–0.98
	0.72 + 0.24	—	0.47 + 0.20
Renal failure:			
Digoxin (30)	0–4.1	0.23–4.3	0.16–4.0
	1.23 + 0.88	1.29 + 0.83	1.07 + 0.78
No digoxin (60)	0.04–0.80	0.02–0.80	0–0.60
	0.20 + 0.14	0.19 + 0.15	0.10 + 0.12

*( ) = Number of patients studied.
† Observed range of results.
‡ Mean + 1 s.d.

In agreement with others, we found digoxin-like substance in women in the last trimester of pregnancy (*1*), in newborns and neonates (*2–5*), and in patients with renal failure (*6–8*). Using the three assays, results in pregnancy women were <0.3 μg/l.

While the concentration of digoxin-like substance was generally low in renal failure, several patients with apparent digoxin concentrations of 0.6–0.8 μg/l were encountered. We noticed only minor differences in apparent digoxin concentrations in serum samples obtained from renal failure patients receiving digoxin. Concentrations in neonates and infants were higher, to 1.0–1.2 μg/l. The apparent concentrations of digoxin-like substance varied depending upon which assay was used. Although dilutional parallelism and good recoveries have been reported (*1,4*), we found generally appropriate results with different sample volumes of cord blood using the Corning and the Clinical Assays kits, but not the NML kit. None of the assays produced appropriate recovery results from serum from patients

TABLE 6
Digoxin Assays: Correlations Between Assays for Various Patient Groups

	Correlation coefficient (r)		
Patients	NML vs. CA*	NML vs. Magic	CA vs. Magic
Pregnancy	0.59	0.27	0.46
Cord blood	0.48	0.61	0.74
Neonates	0.17	—	—
Renal failure:			
Digoxin	0.92	0.95	0.98
No digoxin	0.68	0.82	0.85

*CA = Clinical Assays.

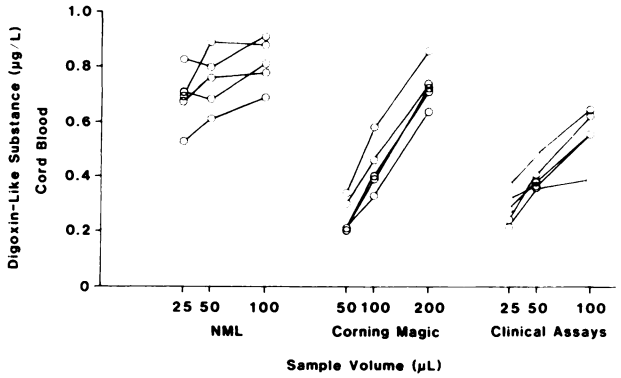

FIGURE 2
Apparent digoxin measured using usual, one-half, and twice the usual assay serum volumes for cord blood

with renal failure (initial concentrations in the serum samples we studied were quite low using the Clinical Assays kit).

In a series of mothers and their infants, maternal concentrations of digoxin-like substance fell to nondetectable levels in the first postpartum day while results in the infants were more variable. Although concentrations of digoxin-like substance fell by the second day of life in most neonates, it rose in two. Digoxin-like substance may be detected in infants several months of age (*4*).

As expected for a protein-bound hapten, digoxin concentration was generally little changed by hemodialysis. It fell in some patients. Renal hemodialysis had no consistent effect upon the measured concentrations of digoxin-like substance. Concentrations tended to decline as estimated by the NML kit and to either rise or fall as estimated by the Clinical Assays or Corning Medical kits. There was little agreement in the predialysis concentrations as measured by the three assays.

Valdes and associates (*20*) have recently shown that digoxin-like substance in serum is water soluble, heat

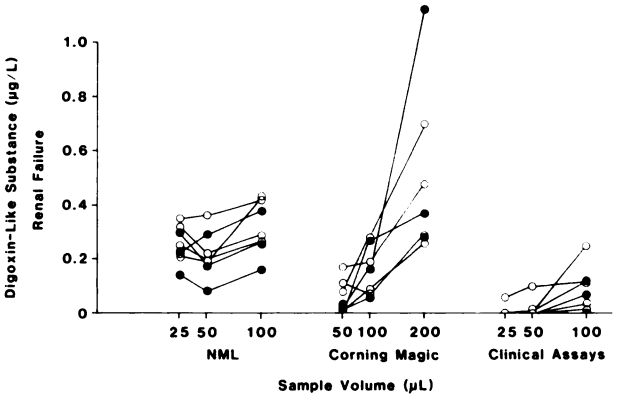

FIGURE 3
Apparent digoxin measured using usual, one-half, and twice the usual assay serum volumes for patients with renal failure, (●) Postdialysis; (○) Predialysis

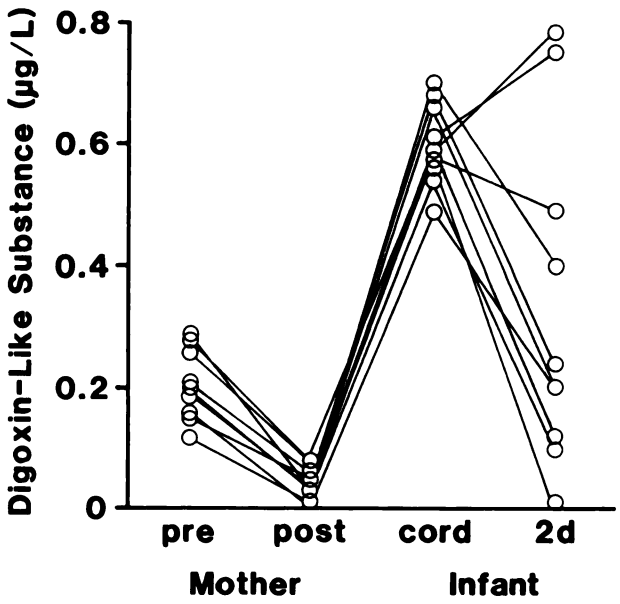

FIGURE 4
Apparent digoxin measured in mothers pre- and postdelivery and their infants (NML assay)

stable, and neutral in molecular charge, with a molecular weight of about 200 daltons. This substance is noncovalently bound to serum proteins. Immunoassays measure the unbound and weakly protein-bound portions. In normal subjects, this represents less than 10% of the total, but a greater portion in pregnant women, neonates, and patients with renal compromise. Altered protein binding appears to be important in the detection of digoxin-like substance by digoxin assays (*20*).

Apparently, different antibodies recognize digoxin-like substances differently. It is not clear whether multiple substances exist. If so, differing proportions of digoxin-like substances may play a role in the apparent concentration differences obtained with different assays. In view of the findings of Valdes et al. (*20*), it is interesting that we found different results with the three assays we studied in samples obtained from patients with renal failure pre- and postdialysis.

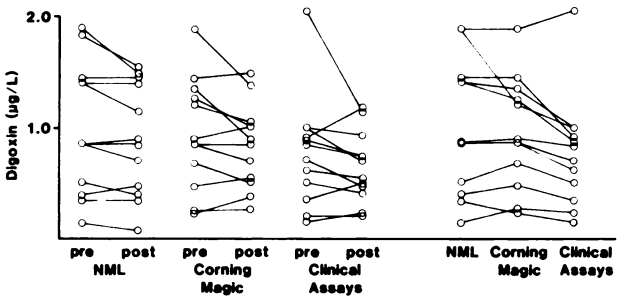

FIGURE 5
Digoxin measured pre- and postdialysis in renal failure patients receiving digoxin in various doses. Predialysis values for the three assays are related in right side of figure

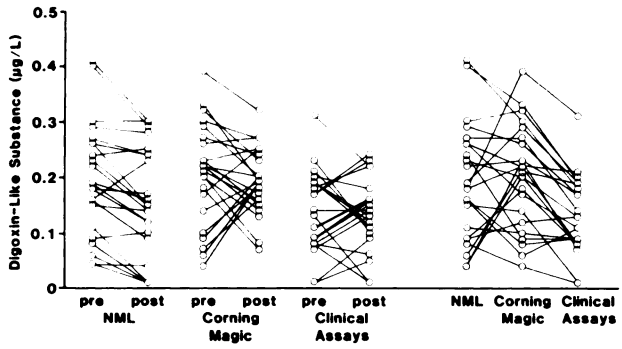

FIGURE 6
Apparent digoxin measured pre- and postdialysis in renal failure patients not receiving digoxin. Predialysis values for the three assays are related in right side of figure

It is as yet not clear what role, if any, endogenous serum protein binding of the labeled digoxin analogs used in various immunoassays may play in the differing estimates of digoxin-like substance. Variable results have been obtained with tritiated (*10*) and iodine-analog (*4,5*) labeled digoxin, as well as enzyme (*7,11*) and fluorecent (*3,8*) labels.

Until the nature of digoxin-like substance(s) and its effect upon immunoassays for the measurement of digoxin concentration are better understood, we suggest it is important to characterize the interference to be expected in any digoxin assay used in the clinical laboratory. Failure to demonstrate dilutional parallelism or quantitative recovery of digoxin added to serum may disclose the presence of interference, but appropriate results do not exclude it. In general, interference from digoxin-like substance was potentially significant in newborns and neonates (incremental increases exceed the assay imprecision), but not in mothers nor in renal failure patients for the assays we studied. While some antisera used in commercial digoxin assays appear to be more free of interference from digoxin-like substance than others (*21*), none may be assumed to reliably measure digoxin concentration in the groups of patients we studied.

FOOTNOTES

*Nuclear Medical Laboratories, Irving, TX.

†Corning Medical, Med Field, MA.

‡Clinical Assays (Travenol-Genetech Diagnostics), Cambridge, MA.

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