**Abstract:**

Aims. Many patients with heart failure and reduced ejection fraction remain at high risk for hospitalization despite evidence-based therapy. Digoxin may decrease hospitalization; however, uncertainty persists concerning its proper administration and effect on mortality. This study investigated whether using dose response concepts to reevaluate the relationship between serum digoxin concentration and key mortality outcomes in patients with reduced ejection fraction in the Digitalis Investigation Group trial would help clarify efficacy and safety.

Methods and Results. Multivariable Cox proportional-hazards modeling and propensity score adjustment assessed the relationship between serum digoxin concentration and key mortality outcomes in patients with reduced ejection fraction in the Digitalis Investigation Group trial. A significant linear association was found between serum digoxin concentration (≥0.5 ng/mL) and all-cause mortality (adjusted hazard ratio 1.25; 95% CI 1.14 to 1.38; P<0.001 per 0.5 ng/mL increase in serum concentration). Based on this relationship, a bidirectional association was found between digoxin therapy and all-cause mortality when compared to placebo. The lowest serum concentrations (0.5-0.7 ng/mL) were
associated with the lowest risk of all-cause mortality (adjusted hazard ratio 0.77, 95% CI 0.67-0.89, \( P<0.001 \)) while high serum concentrations (1.6-2.0 ng/mL) were associated with increased mortality (adjusted hazard ratio 1.33, 95% CI 1.12-1.58, \( P=0.001 \)). Consistent with this finding, lower serum concentrations (0.5 to 0.7 ng/mL) were associated with reduced death from worsening heart failure and a neutral effect on cardiovascular death not due to worsening heart failure.

Conclusion. These findings favor targeting serum concentrations from 0.5-0.7 ng/mL when dosing digoxin in patients with heart failure and reduced ejection fraction.
November 11, 2015

Professor Marco Metra
Editor-in-Chief
European Journal of Heart Failure

Re: Dose Response Characterization of the Association of Serum Digoxin Concentration with Mortality Outcomes in the Digitalis Investigation Group Trial

Dear Dr. Metra:

We respectfully submit our manuscript entitled “Dose Response Characterization of the Association of Serum Digoxin Concentration with Mortality Outcomes in the Digitalis Investigation Group Trial” for publication in the European Journal of Heart Failure.

The landmark Digitalis Investigation Group (DIG) trial demonstrated that digoxin significantly reduced hospitalization due to heart failure. However, uncertainty has persisted concerning the optimal dosing of digoxin to achieve both safety and efficacy.

The goal of our work was to provide novel and compelling evidence to support targeting low serum concentrations to achieve optimal efficacy and safety when treating patients with heart failure and reduced ejection fraction with digoxin. To accomplish this, we revisited the DIG trial dataset to test two novel and related hypotheses.

First, we evaluated whether a classical serum concentration dose response relationship would support a bidirectional relationship of digoxin to mortality. Experimental findings indicate that low digoxin concentrations augment intracellular calcium to produce beneficial inotropic, chronotropic and neurohormonal effects, while higher concentrations increase calcium levels but often provoke arrhythmia. However, whether these basic results would translate into a predictable and clinically meaningful dose response pattern is unknown.

Second, we hypothesized that if a dose response relationship were observed for all-cause mortality, then analysis of cause-specific mortality endpoints would support this relationship by revealing two distinct patterns: substantial reduction in death due to worsening heart failure at low concentrations and clear enhanced risk of cardiovascular death from presumed arrhythmia at high concentrations.

Results of our analysis do provide strong support for both of our hypotheses. In patients treated with digoxin, we did find a significant linear dose response relationship between serum concentration and all-cause mortality. Based on this relationship, there was a bidirectional association between digoxin therapy and all-cause mortality when compared to placebo. The lowest serum concentrations (0.5-0.7 ng/mL) were associated with the lowest risk of all-cause mortality while highest serum concentrations (1.6-2.0 ng/mL) were associated with the greatest risk of mortality.

Consistent with these findings, we found very different relationships between cause-specific mortality endpoints and serum digoxin concentration. Death due to worsening heart failure was
reduced at low serum concentrations while the risk of cardiovascular death due to presumed arrhythmia was increased at higher serum concentrations.

Our findings provide critical support for a bidirectional relationship of digoxin to mortality and for targeting specific low serum concentrations in patients treated with digoxin. when dosing digoxin in patients with heart failure and reduced ejection fraction.

All authors have read and approved the submission of the manuscript. The manuscript has not been published and is not being considered for publication elsewhere. None of the paper’s contents have been previously published. Conflict of interest information is provided following the acknowledgement section in the manuscript. I will serve personally as the corresponding author for this manuscript. My contact information is provided on the manuscript title page.

I will serve as the corresponding author for this paper; my complete contact information is provided below. Thank you for the opportunity to submit our work for consideration of acceptance in European Journal of Heart Failure. We look forward to receiving the reviewers’ and editor’s comments.

Sincerely,

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Dose Response Characterization of the Association of Serum Digoxin Concentration with Mortality Outcomes in the Digitalis Investigation Group Trial

Adams: Association of Serum Digoxin Level With Outcomes


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ABSTRACT

Aims. Many patients with heart failure and reduced ejection fraction remain at high risk for hospitalization despite evidence-based therapy. Digoxin may decrease hospitalization; however, uncertainty persists concerning its proper administration and effect on mortality. This study investigated whether using dose response concepts to reevaluate the relationship between serum digoxin concentration and key mortality outcomes in patients with reduced ejection fraction in the Digitalis Investigation Group trial would help clarify efficacy and safety.

Methods and Results. Multivariable Cox proportional-hazards modeling and propensity score adjustment assessed the relationship between serum digoxin concentration (≥0.5 ng/mL) as a continuous variable and mortality outcomes. In patients treated with digoxin, a significant linear association was found between serum concentration and all-cause mortality (adjusted hazard ratio 1.25; 95% CI 1.14 to1.38; P<0.001 per 0.5 ng/mL increase in serum concentration). Based on this relationship, a bidirectional association was found between digoxin therapy and all-cause mortality when compared to placebo. The lowest serum concentrations (0.5-0.7 ng/mL) were associated with the lowest risk of all-cause mortality (adjusted hazard ratio 0.77, 95% CI 0.67-0.89, P<0.001) while high serum concentrations (1.6-2.0 ng/mL) were associated with increased mortality (adjusted hazard ratio 1.33, 95% CI 1.12-1.58, P=0.001). Consistent with this finding, lower serum concentrations (0.5 to 0.7 ng/mL) were associated with reduced death from worsening heart failure and a neutral effect on cardiovascular death not due to worsening heart failure.

Conclusion. These findings favor targeting serum concentrations from 0.5-0.7 ng/mL when dosing digoxin in patients with heart failure and reduced ejection fraction.
Key Words: heart failure, drugs, digoxin, morbidity, mortality
INTRODUCTION

Despite major advances in evidence-based therapy, digoxin continues to be commonly utilized in patients with heart failure, and the growing public health importance of recurrent hospitalization suggests this drug may be more frequently utilized in the future.\textsuperscript{1,2} However, prescribers of digoxin still face genuine uncertainty concerning its optimal dosing to achieve both safety and efficacy.\textsuperscript{3,4} Digoxin significantly reduced hospitalizations due to heart failure in the landmark Digitalis Investigation Group (DIG) trial, but it had a neutral effect on all-cause mortality. Moreover, there was a suggestion that the lack of benefit on mortality was associated with different effects on cause-specific mortality.\textsuperscript{4} There was a trend for digoxin to reduce death due to worsening heart failure while increasing risk of death due to presumed arrhythmia;\textsuperscript{4} consistent with an unpredictable influence on mortality in individual patients.\textsuperscript{5-8}

There has been longstanding interest in whether targeting serum concentration could address this variable effect of digoxin on mortality and guide dosing to optimize clinical benefit. Previous important post-hoc categorical analyses of the DIG Trial suggest high serum digoxin concentrations associate with increased mortality and low concentrations with reduced mortality.\textsuperscript{9-11} Although clearly informative, these results have not been widely adopted. Major heart failure guidelines do not consistently recommend specific lower serum concentration targets.\textsuperscript{12-14} Strikingly, several recent retrospective analyses evaluating the association between digoxin and mortality in atrial fibrillation and heart failure have been reported despite not being able to account for the influence of serum digoxin concentration in their analyses (albeit because this information is rarely collected in registries or study databases).\textsuperscript{15-19} The therapeutic reference range for digoxin reported by most laboratories remains 0.5-2.0 ng/mL,\textsuperscript{20,21} and
digoxin toxicity continues to account for almost 6% of adverse drug event hospitalizations in the elderly.\textsuperscript{22}

To provide more compelling evidence concerning the link between serum digoxin concentration and outcomes, we revisited the DIG trial dataset to test two novel and related hypotheses. First, we evaluated whether a classical pharmacological dose response relationship existed between serum digoxin concentration evaluated in a continuous fashion and all cause mortality. In addition, experimental and clinical findings indicate that low digoxin concentrations augment intracellular calcium to produce beneficial inotropic, chronotropic and neurohormonal effects.\textsuperscript{23-27} Although higher concentrations further increase calcium levels, they do not enhance these efficacious actions and often provoke arrhythmia.\textsuperscript{6-8} However, whether these basic results would translate into a predictable bidirectional dose response relationship between serum concentration and all cause mortality when compared to placebo is unknown. Second, we hypothesized that if there was a bidirectional dose response relationship between serum concentration and all-cause mortality, then similar analysis of cause-specific mortality endpoints would reveal two distinct patterns: reduction in death due to worsening heart failure at low concentrations and increased risk of cardiovascular death from presumed arrhythmia at high concentrations. Evaluating these hypotheses could provide critical support for a varying relationship of digoxin to mortality and for targeting specific low serum concentrations in patients treated with digoxin.

**METHODS**

**Study Design**

The DIG trial dataset was obtained from the National Heart Lung and Blood Institute (NHLBI) under the Limited Access Dataset option.\textsuperscript{9} The DIG trial design and primary results have been
published.\textsuperscript{4,28} The initial dose of study drug was determined by an algorithm that considered the patient's age, gender, weight, and renal function.\textsuperscript{28,29} The investigator could modify digoxin dose based on other factors, including co-administration of interacting drugs. Blinded serum digoxin concentrations were determined in an arbitrary subset of patients at their week 4 study visit by radioimmunoassay (lower limit of detection 0.5 ng/mL) in a core laboratory (SmithKline BioScience).

**Rationale for Study Endpoints**

The following mortality outcomes were utilized in the current study: 1) all-cause mortality, 2) death from progressive heart failure (includes patients who died from worsening heart failure, even if the final event was an arrhythmia), and 3) cardiovascular death (aggregate of death due to worsening heart failure, other cardiac death and other vascular death). The cause of death was determined by the blinded site investigator after reviewing the patient’s hospital chart or interviewing relatives. Investigators were given a detailed form concerning specific causes of death including death due to worsening heart failure and death due to presumed arrhythmia. In the main trial publication, death due to worsening heart failure was reported separately while other more specific causes were summarized as other cardiac (to include presumed arrhythmia) and other vascular. This convention was followed in the public dataset on which the current analysis is based.

The primary endpoint for analysis was all-cause mortality. Additional analyses focused on two complementary cause-specific mortality endpoints: death from progressive heart failure, and a derived endpoint, cardiovascular death not due to worsening heart failure. Based on event definitions outlined in the protocol and operational materials provided to investigators, this
derived endpoint would include mortality from arrhythmia without evidence of worsening heart failure. Previous work supports the likelihood that arrhythmia accounts for the great majority of deaths not due to worsening heart failure in the DIG trial. Secondary analyses of time to first all-cause hospitalization and first heart failure hospitalization were also explored.

**Dose Response Analysis**

The study analysis used dose response concepts to evaluate the relationship between serum digoxin concentration and key clinical outcomes. Serum concentration was considered as a continuous variable to represent ‘dose’ and time to key clinical outcomes to represent response; similar to a classical pharmacological drug concentration to effect analysis. Serum concentration was chosen, rather than the dose of digoxin administered, since this measurement was more likely to reliably reflect drug concentration at the extracellular site of action of digoxin in the myocardium. Evidence for a dose response relationship requires that serum concentration be an independent predictor of the clinical outcome of interest as determined by multivariable Cox proportional hazards analysis. From each Cox regression model, two distinct types of hazard ratios (HR) and their 95% confidence intervals (CI) were derived based on this fitted relationship. The first expressed the dose response relationship as the hazard ratio per 0.5 ng/mL increase in serum concentration among those randomized to digoxin. Secondly, to place the dose response relationship in clinical context, HRs and 95% CIs were derived to express the relative risk for each serum digoxin concentration compared to placebo.

For descriptive purposes, HRs and 95% CIs were also derived from this model for the midpoints of ranges of serum concentration of interest, including 0.5 to 0.7 ng/mL, representing
the lower end, and 1.6 to 2.0 ng/mL representing the upper end of the traditional therapeutic range.

**Details of Modeling Analysis**

Unadjusted and adjusted multivariable Cox proportional-hazards modeling assessed the relationship between serum digoxin concentration at the 4 week visit and time to each study outcome of interest during follow-up (mean 37 months) (see also Supplementary Appendix). The candidate and final predictive variables from the mortality model have been published; these were used as covariates in adjusted modeling for all study endpoints. Serum digoxin concentrations (≥0.5 ng/mL) were treated as a linear continuous variable along with an indicator for treatment assignment (digoxin or placebo) simultaneously included in the model. No categories of serum concentration based on arbitrary cut points were used to obtain parameter estimates for the models. An indicator for patients randomized to digoxin who had undetectable serum concentrations (<0.5 ng/mL) was also included in the model, treating them as an additional group to maintain an intention to treat approach. As an additional adjustment for potential selection bias, two sets of propensity scores for each patient were estimated via logistic regression for the probability of being in a low serum concentration group (0.5 to 0.7 ng/mL or 0.5 to 0.9 ng/mL). Polynomial and piecewise linear terms assessed the potential nonlinearity of the relationship between serum concentration and all-cause mortality.

**RESULTS**

**Study Patients**
A total of 6738 of the 6800 patients enrolled in the DIG trial survived for at least 4 weeks of follow-up. Data were analyzed on 5209 patients within this group, including all 3366 patients randomized to placebo and 1843 of the 3372 patients randomized to digoxin with serum concentration obtained at week 4 (eFigure 1). Baseline characteristics of patients with and without serum concentrations obtained were similar (eTable 1). Serum concentration was measured between 6 and 30 hours after the last dose of study drug in 1578 of these 1843 patients. Serum digoxin concentration was $\geq 0.5$ ng/mL in 1451 of these 1578 patients, and they had a mean digoxin dose at week 4 of $0.24 \pm 0.07$ mg/day and a mean serum concentration of $0.98 \pm 0.42$ ng/mL. The baseline characteristics of these 1578 patients, corresponding to categories of serum concentration, were compared to the 3366 placebo patients (Table 1). Patients with higher serum concentrations had worse renal function and more advanced heart failure than patients assigned to placebo, while patients with low serum concentrations (0.5 to 0.7 ng/mL) had better renal function and less severe heart failure than placebo. The frequencies of events for key study endpoints across serum concentration ranges of interest are shown in eTable 2.

**Evaluation of Dose Response in Digoxin Treated Patients**

Unadjusted and adjusted modeling analysis revealed a significant relationship between serum digoxin concentration and the risk of all-cause mortality (both $P<0.001$, Table 2). The observed relationship was linear and characterized by a positive slope with the hazard of mortality increasing as serum concentration increased. In the adjusted analysis, a 25% increase in the risk of death was observed for each 0.5 ng/mL increase in serum digoxin concentration. A nonsignificant association was found between serum digoxin concentration and death from worsening heart failure that was characterized by declining benefit as serum concentration
increased. In contrast, there was a significant linear association between serum digoxin concentration and the risk of cardiovascular death not due to worsening heart failure (Table 2).

**Evaluation of Dose Response Relative to Placebo**

*All-Cause Mortality.* The lowest serum digoxin concentration studied (0.5 ng/mL) was associated with the greatest reduction in mortality compared to placebo (Table 3 and Figure 1). There was an association of digoxin use with reduced all-cause mortality until the serum concentration exceeded 0.9 ng/mL (adjusted HR for 0.9 ng/mL = 0.88, 95% CI 0.79-0.99, \(P=0.03\)). Beginning at a serum concentration of 1.5 ng/mL, an association with increased mortality was observed (adjusted HR 1.16, 95% CI 1.02 to 1.32, \(P=0.03\)). Unadjusted modeling results were similar (eTable 3). Additional modeling strategies further supported digoxin concentration as an independent predictor of all-cause mortality (eTable 4). The lowest concentrations reported in the DIG trial (0.5 - 0.7 ng/mL) were associated with the lowest risk of all-cause mortality compared to placebo (adjusted HR 0.77, 95% CI 0.67-0.89, \(P<0.001\)) (Figure 1 and Table 4). The mortality risk of the 127 patients with undetectable serum concentrations did not differ from placebo (adjusted HR 1.01, 95% CI 0.74 -1.37, \(P=0.95\)). The linear fit of serum concentration was used, as curve fitting with higher order polynomial models (second, third, and fourth order) as well as piecewise linear models were all nonsignificant (all \(P>0.11\) for polynomial; \(P>0.76\) for piecewise linear).

*Cause-specific Mortality.* Decreased risk of death due to worsening heart failure compared to placebo was observed from serum concentrations of 0.5 ng/mL (adjusted HR 0.69, 95% CI 0.53-0.89, \(P=0.005\)) to 1.1 ng/mL (adjusted HR 0.82, 95% CI 0.68-0.98, \(P=0.03\)) (Table 3 and Figure 2). Above 1.1 ng/mL, no significant association was observed between serum
concentration and death due to worsening heart failure. In contrast, serum concentrations ≥1.1 ng/mL were associated with an increased risk of cardiovascular death not related to heart failure compared to placebo (adjusted HR for 1.1 ng/mL = 1.21, 95% CI 1.04 to 1.41, P=0.01); risk was substantially increased at high serum concentrations (Table 3 and Figure 2). A sensitivity analysis demonstrated a similar pattern using an alternative cause-specific mortality endpoint, defined as death not due to worsening heart failure of worsening risk with increasing serum concentration (eTable 5, eFigure 2).

**Risk of Hospitalization.** No significant association was observed between serum digoxin concentration and risk of heart failure hospitalization (adjusted HR 1.10; 95% CI 0.98 to 1.23; P=0.11 per 0.5 ng/mL increase in serum concentration). However, there was evidence for an association between serum concentration and the risk of all-cause hospitalization (HR 1.08; 95% CI 1.00 to 1.16; P=0.05 per 0.5 ng/mL increase in serum concentration).

**DISCUSSION**

This investigation reveals novel findings concerning the relationship of serum digoxin concentration to outcomes in patients with heart failure and reduced ejection fraction. In patients treated with digoxin, models demonstrated a linear dose response relationship linking serum concentration to mortality. The slope of this dose response curve was positive so that increasing serum concentration was associated with increasing mortality. Based on this relationship, a bidirectional association was found between digoxin therapy and all-cause mortality when patients treated with digoxin were compared to placebo. There was a reduction in mortality at low serum concentrations and increased mortality at high concentrations (Figure 1), a finding that is consistent with experimental data.
Modeling serum concentration and cause-specific mortality in digoxin treated patients also demonstrated positive dose response curves with cause-specific mortality increasing as serum concentration increased (Table 2). However comparison of these dose response curves to placebo revealed disparate patterns that are consistent with experimental mechanistic underpinning for the changing association observed between digoxin and all-cause mortality from low to high serum concentrations (Figures 1 and 2). The curve for death from worsening heart failure differed from a priori expectation since there was no further reduction in risk of this endpoint as serum concentration increased. There was evidence of benefit on this endpoint until serum concentrations exceeded 1.1 ng/mL, and after this threshold, mortality due to worsening heart failure was not significantly increased even at high serum concentrations. This finding is supported by retrospective analysis of short-term trials indicating that the lowest category of digoxin concentrations (0.5 to 0.9 ng/mL) were as effective as higher concentrations in reducing the risk of worsening heart failure and maintaining exercise tolerance and left ventricular function.7,8 In contrast, digoxin had a neutral effect on the risk of cardiovascular death not due to heart failure at low serum concentrations (≤1.0 ng/mL), but higher concentrations were steeply associated with increased mortality due to presumed arrhythmia. These results are analogous to dose-dependent increases in mortality, attributed to pro-arrhythmic death, observed with other positive inotropic drugs associated with calcium loading.36-38 Consistent with this finding, digoxin toxicity has been observed with serum concentrations from 1.5 to 2.0 ng/mL.33

The current findings concerning cause specific mortality corroborate and clarify the hypothesis-generating results seen from analysis of secondary mortality endpoints in the original DIG Trial (eTable 6). Findings in this trial associated randomization to digoxin with both
reduced death due to progressive heart failure and increased risk of other cardiovascular death likely due to presumed arrhythmia versus placebo.4

The current analysis does not include serum concentrations below 0.5 ng/mL that show no clinical efficacy, so the minimal serum digoxin concentration with benefit cannot be defined. Although the linear fit does not suggest any loss of efficacy at the lowest serum concentrations studied, results are less certain at the extremes. Alternatively, whether even lower concentrations of digoxin would be clinically effective is a critical issue to resolve in future studies.

While our findings on serum concentration and risk of all-cause mortality suggest a wider margin of safety than previous post hoc analyses, the margin of safety for digoxin is not consistent across all clinically relevant endpoints. Risk of cardiovascular death not related to heart failure was increased at serum concentrations well within the traditionally accepted therapeutic range (i.e. 1.0 to 1.1 ng/mL, Table 3). The relationship between serum concentration and risk of all-cause hospitalization provides further evidence of attenuated benefit with increasing serum concentration. These observations leave no clinical rationale for targeting higher serum concentrations, since maximum efficacy and safety for all study endpoints was achieved at low serum digoxin concentrations. They provide additional support for the recent ACCF/AHA heart failure guidelines suggesting digoxin should be dosed to achieve serum concentrations of 0.5 to 0.9 ng/mL.13 However, current results suggest targeting an even lower serum concentration range, 0.5 to 0.7 ng/mL, may have both greater efficacy and safety. Low serum digoxin concentrations are generally readily achievable by prescribing smaller doses, e.g. ≤0.125 mg/day or every other day, and monitoring serum concentration during initial administration.1 For many patients, annual monitoring should be sufficient, with supplemental levels as dictated by changes in renal function or concomitant therapies.
This study was designed to extend the important findings of previous post-hoc analyses of serum concentration and outcomes in the DIG Trial. The present investigation is hypothesis-driven and draws upon biologically plausible dose response relationships to account for study findings. Prior work helps understand digoxin’s effect within wide concentration ranges. The current analysis increases precision and is more informative concerning specific serum concentrations where the likelihood of benefit versus harm may change. Hypothesis testing was conducted in all available patients (men and women) with serum concentration measured across a comprehensive set of key outcomes including a novel analysis of cause-specific mortality.

Study findings should be interpreted in the context of certain limitations. Patients were not randomly allocated to specific digoxin concentration ranges. Therefore, causality cannot be determined and these findings may be subject to unknown bias and residual confounding. However, several unique aspects of our analysis mitigate concerns about the nonrandomized design. The robust evidence for dose response adds critical support for an association between serum concentration and outcome, since dose response is widely considered to reduce the likelihood that bias accounts for the findings of a nonrandomized study. Using serum concentration rather than digoxin dose per se coupled with the extracellular location of binding for digoxin on the Na⁺/K⁺-ATPase molecule gives credence to our dose response findings. A foundation of biological plausibility enhances the likelihood that results from nonrandomized studies are valid. In experimental studies, digoxin has a predictable dose response relationship with intracellular calcium characterized by concentration dependent increases in calcium levels attributed to progressive inhibition of Na⁺/K⁺-ATPase and subsequent changes in activity of the Na⁺/Ca++ exchanger. Although calcium influx promotes a positive inotropic response and
other potentially beneficial actions, calcium overload with high serum concentrations has been linked to arrhythmias, cell death, and adverse metabolic effects from increased myocardial oxygen consumption.6

Several considerations reduce the chance that study findings are due to confounding. Patients were analyzed as randomized to digoxin or placebo, and the investigators were blinded to serum concentrations. Blinding significantly reduces the potential for confounding since knowledge of digoxin levels could not influence dosing. The large event rate in the DIG trial allowed construction of a robust multivariable model for all-cause mortality (eTable 7). Adjustment based on this model plus a propensity score did not alter study findings. Worsening renal function and to some extent increasing severity of clinical heart failure were associated with both higher serum concentration and reduced survival (eTable 8). However, a significant association between serum concentration and all-cause mortality remained despite adjustment for these particular confounders. The distinct patterns observed in the association between serum concentration and cause-specific mortality are difficult to explain by uncontrolled confounding. These findings are inconsistent with severity of heart failure as the explanation for the observed association between higher serum digoxin concentration and increased mortality.

Our study has other potential limitations. There was lack of independent adjudication and specific detail for the cause-specific mortality endpoints used in the study analysis. Translation of current findings into contemporary practice must consider the caveat that digoxin has never been tested for efficacy as an add-on to current evidence-based therapy for heart failure with reduced ejection fraction. Whether lower serum concentration would result in better outcomes in patients optimally treated with ACE-inhibitors, beta-blockers, mineralocorticoid receptor antagonists, and device therapies is unknown. In everyday clinical practice, an
important minority of patients may not be candidates for these drugs. Further, contemporary therapy may mitigate the risk of presumed arrhythmic death in patients treated with digoxin. Many patients will have an implantable cardioverter defibrillator in place or be candidates for this device. Mineralocorticoid receptor antagonists reduce the risk of hypokalemia, which may lessen pro-arrhythmia with digoxin. Finally, beta-blocker use in heart failure patients is associated with a significantly reduced risk for sudden cardiac death.45

In conclusion, this study defines dose response relationships between serum digoxin concentration and key clinical outcomes in patients with heart failure and reduced ejection fraction. The lowest serum concentrations studied (0.5 to 0.7 ng/mL) were associated with the most favorable outcomes for all endpoints, including all-cause mortality. Our findings favor targeting this lower range when choosing which doses of digoxin to administer to patients with heart failure and reduced ejection fraction.
Acknowledgements

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Covis Pharmaceuticals, Inc. provided an unrestricted research grant that was used to support this statistical analysis of the DIG dataset. Covis had no role in the design or conduct of the analysis; collection, management, analysis, or interpretation of the data; or preparation, review, or approval of the manuscript. Covis did not review the manuscript prior to submission.

Conflict of Interest Disclosures

Kirkwood F. Adams, Jr: Covis Pharmaceuticals, Inc. (Consultant/Advisory Board, Research Grants); Novartis (Consultant/Advisory Board, Research Grants); Amgen (Consultant, Research Grants); Duke Clinical Research Institute (Research Grants); NIMH (Research Grants); Otsuka (Research Grants); Cardiorentis (Research Grants); Sorbent (Research Grants)

Javed Butler: Amgen (Consultant); Bayer (Consultant); Cardiocell (Consultant); Celladon (Consultant); Covis Pharmaceuticals, Inc. (Consultant); Medtronic (Consultant); Novartis (Consultant); Trevena (Consultant); NIH (Research Grants); European Union (Research Grants); FDA (Research Grants)

J. Herbert Patterson: Covis Pharmaceuticals, Inc. (Advisory Board); Novartis (Advisory Board)
Wendy Gattis Stough: Covis Pharmaceuticals, Inc. (Advisory Board/Consultant); Medtronic (Consultant); Relypsa (Consultant), Stealth Peptides (Consultant)

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FIGURE LEGENDS

Figure 1. Relationship shown for serum digoxin concentration and all-cause mortality. Results are derived from proportional hazards regression and expressed as adjusted hazard ratio and 95% confidence interval for serum digoxin concentrations from 0.5 to 2.0 ng/mL compared to placebo (adjusted analysis).

Figure 2. Relationships shown for serum digoxin concentration with cardiovascular death not due to worsening heart failure and death due to worsening heart failure. Results are derived from proportional hazards regression and expressed as adjusted hazard ratio and 95% confidence interval for serum digoxin concentrations from 0.5 to 2.0 ng/mL compared to placebo (adjusted analysis).
Table 1. Baseline Characteristics of Study Patients According to Digoxin Concentration Ranges (ng/mL)†

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<th>Characteristic</th>
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<td>White race - %*</td>
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<td>80</td>
<td>87</td>
<td>84</td>
<td>91</td>
<td>88</td>
<td>87</td>
<td>88</td>
</tr>
<tr>
<td>Previous MI - %</td>
<td>65</td>
<td>65</td>
<td>66</td>
<td>64</td>
<td>67</td>
<td>69</td>
<td>72</td>
<td>60</td>
</tr>
<tr>
<td>Current angina - %</td>
<td>26</td>
<td>28</td>
<td>27</td>
<td>26</td>
<td>26</td>
<td>29</td>
<td>25</td>
<td>30</td>
</tr>
<tr>
<td>Hypertension - %</td>
<td>46</td>
<td>42</td>
<td>43</td>
<td>41$^{\dagger\dagger}$</td>
<td>42</td>
<td>42</td>
<td>50</td>
<td>45</td>
</tr>
<tr>
<td>Ischemic etiology - %</td>
<td>71</td>
<td>70</td>
<td>72</td>
<td>70</td>
<td>72</td>
<td>75</td>
<td>75</td>
<td>71</td>
</tr>
<tr>
<td>Diabetes - %</td>
<td>29</td>
<td>22</td>
<td>28</td>
<td>26</td>
<td>29</td>
<td>26</td>
<td>32</td>
<td>30</td>
</tr>
<tr>
<td>Age – years***</td>
<td>$64 \pm 11$</td>
<td>$60 \pm 11$</td>
<td>$63 \pm 10$</td>
<td>$62 \pm 11^{\dagger\dagger}$</td>
<td>$64 \pm 10$</td>
<td>$65 \pm 10$</td>
<td>$65 \pm 10$</td>
<td>$65 \pm 8.9$</td>
</tr>
<tr>
<td>BMI - kg/m$^2$***</td>
<td>$27 \pm 5.2$</td>
<td>$28 \pm 5.0$</td>
<td>$27 \pm 5.1$</td>
<td>$27 \pm 5.0$</td>
<td>$27 \pm 4.9$</td>
<td>$26 \pm 5.2$</td>
<td>$27 \pm 5.8$</td>
<td>$26 \pm 4.8$</td>
</tr>
<tr>
<td>LVEF - %</td>
<td>$29 \pm 8.8$</td>
<td>$29 \pm 9.1$</td>
<td>$29 \pm 8.8$</td>
<td>$29 \pm 8.6$</td>
<td>$29 \pm 8.6$</td>
<td>$28 \pm 9.1$</td>
<td>$28 \pm 9.3$</td>
<td>$27 \pm 8.6$</td>
</tr>
<tr>
<td></td>
<td>Duration of CHF – months**</td>
<td>SBP – mmHg</td>
<td>DBP – mmHg***</td>
<td>Heart rate – bpm</td>
<td>CT Ratio</td>
<td>CHF Score***</td>
<td>NYHA Class***</td>
<td>eGFR - ml/min/1.73m²***</td>
</tr>
<tr>
<td>------------------------</td>
<td>-----------------------------</td>
<td>------------</td>
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<td>------------------</td>
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<td>--------------------------</td>
</tr>
<tr>
<td></td>
<td>30 ± 37</td>
<td>126 ± 20</td>
<td>75 ± 11</td>
<td>79 ± 13</td>
<td>0.53 ±</td>
<td>11 ± 5.3</td>
<td>2.2 ± 0.7</td>
<td>64 ± 25</td>
</tr>
<tr>
<td></td>
<td>28 ± 33</td>
<td>128 ± 18</td>
<td>79 ± 9.9</td>
<td>79 ± 13</td>
<td>0.53 ±</td>
<td>10 ± 5.3</td>
<td>2.1 ± 0.7</td>
<td>72 ± 21</td>
</tr>
<tr>
<td></td>
<td>34 ± 39</td>
<td>125 ± 20</td>
<td>74 ± 11</td>
<td>78 ± 13</td>
<td>0.53 ±</td>
<td>12 ± 5.6</td>
<td>2.2 ± 0.7</td>
<td>63 ± 19</td>
</tr>
<tr>
<td></td>
<td>32 ± 36</td>
<td>126 ± 18</td>
<td>76 ± 10††</td>
<td>77 ± 13††</td>
<td>0.53 ±</td>
<td>11 ± 5.4</td>
<td>2.1 ±</td>
<td>69 ± 18††</td>
</tr>
<tr>
<td></td>
<td>34 ± 42</td>
<td>124 ± 20</td>
<td>74 ± 11</td>
<td>78 ± 13</td>
<td>0.53 ±</td>
<td>12 ± 5.5</td>
<td>2.2 ± 0.7</td>
<td>64 ± 18</td>
</tr>
<tr>
<td></td>
<td>35 ± 38</td>
<td>124 ± 20</td>
<td>73 ± 11</td>
<td>77 ± 13</td>
<td>0.54 ±</td>
<td>12 ± 5.8</td>
<td>2.2 ± 0.7</td>
<td>60 ± 17</td>
</tr>
<tr>
<td></td>
<td>40 ± 48</td>
<td>125 ± 19</td>
<td>75 ± 11</td>
<td>79 ± 13</td>
<td>0.54 ±</td>
<td>14 ± 6.0††</td>
<td>2.3 ± 0.7</td>
<td>59 ± 21</td>
</tr>
<tr>
<td></td>
<td>34 ± 33</td>
<td>125 ± 21</td>
<td>73 ± 11</td>
<td>79 ± 11</td>
<td>0.54 ±</td>
<td>14 ± 6.0††</td>
<td>2.4 ±</td>
<td>55 ± 18††</td>
</tr>
</tbody>
</table>

††Abbreviations denote the following: BMI = body mass index, bpm = beats per minute, CHF = congestive heart failure, CT = Cardiothoracic, DBP = diastolic blood pressure, eGFR = estimated glomerular filtration rate, LVEF = left ventricular
ejection fraction, MI = myocardial infarction, NYHA = New York Heart Association, SBP = systolic blood pressure, SDC = serum digoxin concentration. †<0.5 = the group of patients who were randomized to digoxin but for whom the core lab reported serum digoxin concentration <0.5 ng/mL. §All SDC includes the subset of patients with detectable week 4 serum digoxin concentrations drawn between 6 and 30 hours after the last digoxin dose.  P values represent significance with *P<0.05; **P<0.01; ***P<0.001 for any difference among all SDC ranges and placebo and ††P<0.05 and †††P<0.01 for pairwise comparison of the SDC ranges 0.5 to 0.7 ng/mL and 1.6 to 2.0 ng/mL versus Placebo. Results shown as Mean ± SD or %, as appropriate.
Table 2. Relative Risk of Mortality with Increasing Serum Digoxin Concentrations in Digoxin Treated Patients

<table>
<thead>
<tr>
<th>Outcome Endpoint</th>
<th>Relative Risk per 0.5 ng/mL Increase in Serum Digoxin Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unadjusted</td>
</tr>
<tr>
<td>All-Cause Mortality</td>
<td>1.37 (1.26 - 1.49), P&lt;0.001</td>
</tr>
<tr>
<td>*Death due to WHF</td>
<td>1.37 (1.18 - 1.58), P&lt;0.001</td>
</tr>
<tr>
<td>†CV Death not due to WHF</td>
<td>1.33 (1.18 - 1.50), P&lt;0.001</td>
</tr>
</tbody>
</table>

*WHF = worsening heart failure; †CV = cardiovascular
Table 3. Analysis of Key Mortality Outcomes Versus Placebo Based on Individual Serum Digoxin Levels – Adjusted*

<table>
<thead>
<tr>
<th>SDC</th>
<th>All-Cause Mortality</th>
<th>Death due to Worsening HF</th>
<th>CV Death not due to Worsening HF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
<td>P Value</td>
</tr>
<tr>
<td>0.5</td>
<td>0.74</td>
<td>0.64 – 0.86</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>0.6</td>
<td>0.77</td>
<td>0.67 – 0.89</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>0.7</td>
<td>0.81</td>
<td>0.71 – 0.92</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>0.8</td>
<td>0.85</td>
<td>0.75 – 0.95</td>
<td>0.005</td>
</tr>
<tr>
<td>0.9</td>
<td>0.88</td>
<td>0.79 – 0.99</td>
<td>&lt;0.027</td>
</tr>
<tr>
<td>1.0</td>
<td>0.92</td>
<td>0.83 – 1.03</td>
<td>0.14</td>
</tr>
<tr>
<td>1.1</td>
<td>0.97</td>
<td>0.87 – 1.07</td>
<td>0.53</td>
</tr>
<tr>
<td>1.2</td>
<td>1.01</td>
<td>0.91 – 1.13</td>
<td>0.83</td>
</tr>
<tr>
<td>1.3</td>
<td>1.06</td>
<td>0.95 – 1.18</td>
<td>0.32</td>
</tr>
<tr>
<td>1.4</td>
<td>1.11</td>
<td>0.98 – 1.25</td>
<td>0.10</td>
</tr>
<tr>
<td>1.5</td>
<td>1.16</td>
<td>1.02 – 1.32</td>
<td>0.03</td>
</tr>
<tr>
<td>1.6</td>
<td>1.21</td>
<td>1.05 – 1.40</td>
<td>0.009</td>
</tr>
<tr>
<td>1.7</td>
<td>1.27</td>
<td>1.08 – 1.48</td>
<td>0.003</td>
</tr>
<tr>
<td>1.8</td>
<td>1.33</td>
<td>1.12 – 1.58</td>
<td>0.001</td>
</tr>
<tr>
<td>1.9</td>
<td>1.39</td>
<td>1.15 – 1.67</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2.0</td>
<td>1.45</td>
<td>1.18 – 1.78</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
*Abbreviations denote the following: CI = confidence interval; CV = cardiovascular; HF = heart failure; HR = hazard ratio;

SDC = serum digoxin concentration
Table 4. Adjusted Risk* for Mortality Outcomes for Digoxin Versus Placebo At the Midpoint of Specific Serum Concentration Ranges (ng/mL)

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Adjusted</th>
<th></th>
<th></th>
<th>Adjusted + PS†</th>
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<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
<td>P Value</td>
<td>HR</td>
<td>95% CI</td>
<td>P Value</td>
</tr>
<tr>
<td>All Cause Mortality</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDC 0.5 - 0.7</td>
<td>0.77</td>
<td>0.67-0.89</td>
<td>&lt;0.001</td>
<td>0.77</td>
<td>0.67-0.89</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SDC 0.8 - 0.9</td>
<td>0.86</td>
<td>0.77-0.97</td>
<td>0.01</td>
<td>0.87</td>
<td>0.77-0.97</td>
<td>0.01</td>
</tr>
<tr>
<td>SDC 1.0 - 1.2</td>
<td>0.97</td>
<td>0.87-1.07</td>
<td>0.53</td>
<td>0.97</td>
<td>0.87-1.08</td>
<td>0.55</td>
</tr>
<tr>
<td>SDC 1.3 - 1.5</td>
<td>1.11</td>
<td>0.98-1.25</td>
<td>0.10</td>
<td>1.11</td>
<td>0.98-1.25</td>
<td>0.10</td>
</tr>
<tr>
<td>SDC 1.6 - 2.0</td>
<td>1.33</td>
<td>1.12-1.58</td>
<td>0.001</td>
<td>1.33</td>
<td>1.12-1.58</td>
<td>0.001</td>
</tr>
<tr>
<td>Death due to WHF‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDC 0.5 - 0.7</td>
<td>0.71</td>
<td>0.56-0.90</td>
<td>0.004</td>
<td>0.70</td>
<td>0.55-0.89</td>
<td>0.004</td>
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<tr>
<td>SDC 0.8 - 0.9</td>
<td>0.76</td>
<td>0.63-0.92</td>
<td>0.006</td>
<td>0.76</td>
<td>0.62-0.92</td>
<td>0.005</td>
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<tr>
<td>SDC 1.0 - 1.2</td>
<td>0.82</td>
<td>0.68-0.98</td>
<td>0.03</td>
<td>0.82</td>
<td>0.68-0.98</td>
<td>0.03</td>
</tr>
<tr>
<td>SDC 1.3 - 1.5</td>
<td>0.90</td>
<td>0.73-1.11</td>
<td>0.30</td>
<td>0.89</td>
<td>0.72-1.10</td>
<td>0.29</td>
</tr>
<tr>
<td>SDC 1.6 - 2.0</td>
<td>1.01</td>
<td>0.74-1.37</td>
<td>0.96</td>
<td>1.01</td>
<td>0.74-1.36</td>
<td>0.97</td>
</tr>
<tr>
<td>CV§ Death not due to WHF</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SDC 0.5 - 0.7</td>
<td>0.97</td>
<td>0.80-1.18</td>
<td>0.77</td>
<td>0.97</td>
<td>0.80-1.18</td>
<td>0.77</td>
</tr>
<tr>
<td>SDC 0.8 - 0.9</td>
<td>1.08</td>
<td>0.92-1.28</td>
<td>0.33</td>
<td>1.08</td>
<td>0.92-1.28</td>
<td>0.33</td>
</tr>
<tr>
<td>SDC 1.0 - 1.2</td>
<td>1.21</td>
<td>1.04-1.41</td>
<td>0.01</td>
<td>1.21</td>
<td>1.04-1.41</td>
<td>0.02</td>
</tr>
<tr>
<td>SDC 1.3 - 1.5</td>
<td>1.38</td>
<td>1.15-1.65</td>
<td>&lt;0.001</td>
<td>1.38</td>
<td>1.15-1.65</td>
<td>&lt;0.001</td>
</tr>
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<td>----------</td>
</tr>
<tr>
<td>SDC 1.6 - 2.0</td>
<td>1.65</td>
<td>1.28-2.12</td>
<td>&lt;0.001</td>
<td>1.65</td>
<td>1.28-2.12</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Results shown as HR and 95% CI derived based on the dose response analysis with digoxin compared to placebo as described in methods section. †PS = adjusted for propensity score derived based on probability of being in 0.5 to 0.7 ng/mL serum concentration group, ‡WHF = worsening heart failure; §CV = cardiovascular
Figure 2

Hazard Ratio

Cardiovascular Death Not Due To Worsening HF

Death D/T Worsening HF

Serum Digoxin Concentration (ng/mL)
Click here to access/download
Supplementary Material
SDC Outcomes Supp Append.doc
Dose Response Characterization of the Association of Serum Digoxin Concentration with Mortality Outcomes in the Digitalis Investigation Group Trial

Corresponding Author: Kirkwood F. Adams, Jr., MD

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