

**Differential Mortality of Digoxin Users By Gender Over Time: An Observational Study in
the THIN Database**

**James Flory, MD/MSCE candidate (primary author)
Sean Hennessy, PhD (mentor)**

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I. Specific Aims

Primary Aim 1: test the hypothesis that in patients with heart failure (HF), digoxin use is associated with higher mortality in women but not in men (i.e., that gender is an effect modifier for the association of digoxin use with mortality).

Primary Aim 2: test the hypothesis that effect modification between digoxin use and gender on mortality hazard is attenuated at lower dosage levels (i.e., that there is a three-way effect modification between gender, digoxin, and dose).

Secondary Aim 1: test the hypothesis that digoxin serum levels mediate the gender-digoxin interaction affecting mortality (in the subset of patients for whom these data are available)

II. Background

Several interventions in cardiovascular medicine may have different safety/efficacy profiles depending on gender. For example, digoxin may increase mortality by approximately 20% in women but not in men (1).

The initial signal of this differential effect by gender was identified as part of a post-hoc analysis of the Digitalis Investigation Group (DIG) trial (1). In absolute terms, women assigned to digoxin had a 4.2% higher risk of mortality compared to placebo while men had assigned to digoxin had a 1.6% lower risk of mortality compared to placebo. After multivariable adjustment, the relative risk of mortality in women was statistically significantly increased by digoxin use and the test for an interaction between sex and digoxin affecting mortality was significant at $P = 0.014$, an effect most likely mediated by higher serum levels in women (2).

The study was accompanied by an editorial which raised three complications in interpreting these results(3). The first was that the finding was post-hoc and could be a spurious false positive. The second letter was that there were significant differences at baseline between men and women (women had higher rates of cardiomegaly, severe heart failure, diabetes, and idiopathic heart failure; men had higher rates of heart failure related to myocardial ischemia and infarction) which could lead to different mortality rates by gender. The third was that, since one of the primary results of DIG had been that digoxin might be associated with increased mortality when serum concentration was greater than 1.0 ng/ml and with reduced mortality when serum concentration was lower than 1.0 ng/ml, it was reasonable to hypothesize that higher serum concentrations in women might be driving the differential effect on mortality by gender. Subsequent letters to the editor of the New England Journal of Medicine elaborated on these points (4, 5, 6). One letter looking at hospital registry data on Sweden found that in clinical practice digoxin serum levels did tend to be higher in female patients and were more often above the therapeutic range, lending credibility to the hypothesis that, if real, the differential mortality might be due to differences in serum level (7).

A different group of investigators subsequently carried out another post hoc analysis of the DIG trial, which examined mortality in women in the control arm versus women in the digoxin arm and stratified by serum digoxin concentration (2). This study found that the hazard ratio for death favored digoxin users (0.8, 95% CI 0.62-1.13) in women with a serum digoxin concentration up to 0.9 ng/ml, while women with a serum concentration of 1.2 ng/ml or greater

had a hazard ratio of 1.33 (95% CI 1.001-1.76) for death. This study further supported the hypothesis that serum digoxin concentration differences between the genders might be driving the sex-digoxin interaction. However, it was not powered to exclude the possibility that gender interaction might be present even after digoxin serum concentration was accounted for.

Finally, an observational study was conducted on patients enrolled in the Studies of Left Ventricular Dysfunction (SOLVD) trial to test for a sex-digoxin interaction affecting mortality, and found no evidence for such an interaction (7). The reason for this disagreement with the DIG post-hoc analysis (1) was not clear, although there were several possible explanations including the fact that the DIG study randomized digoxin use while the SOLVD study did not. Variables including digoxin dose and digoxin serum levels, which might have shed light on the discrepancy, were not included in the analysis. The SOLVD paper ended with the recommendation that further observational studies in other populations be conducted (7).

Further investigation of the potential sex-digoxin (or sex-digoxin-serum-level) interaction is clinically important because digoxin is still widely used by both women and men (8). It remains unclear whether digoxin should be used differently in the different genders (7) and recent publications warn against the use of digoxin in women (9)—an attitude which may unnecessarily curb use of a drug which has been shown to reduce hospitalizations in heart failure. It is plausible that careful maintenance of low digoxin serum concentration would prevent excess mortality in either gender (3), but it is not known whether current clinical practice avoids causing excess mortality in women.

We propose a longitudinal observational study in the THIN database to help answer this question, with prespecified outcomes looking both at gender-digoxin interaction on mortality and at gender-digoxin-dose interaction on mortality. The key advantages which THIN can offer over previously published research include a much larger sample size, use of data from routine clinical practice rather than the artificial environment of a clinical trial, the ability to adjust for digoxin dose within that large sample, and the ability to observe trends over a very long period of use (from the early 1990s up until 2007). Availability of digoxin serum levels on a minority of patients will add further value to the study. If there is a differential effect on mortality by gender, exploration of dose-dependency and serum concentration dependency will give insight into whether there is a way to avoid such effects. Indeed, as the average dose of digoxin has declined over time (8), a longitudinal database may allow a natural experiment to assess whether there was a differential effect by gender in the past which no longer exists under modern dosing practices. Since concerns about digoxin use in women continue to appear in the literature (9), either a positive or a negative finding would help guide clinical decision making, as long as confounding and other forms of bias are acceptably controlled.

III. Research and Methods

A. Overview

Using a retrospective cohort design, we will assess whether the mortality risk for digoxin users interacts with gender. We will also assess whether any interaction depends on the digoxin dose or has changed over the years. The database to be used (THIN, see below) provides

laboratory values on some patients, so it should also be possible to do a subgroup analysis adjusting for serum concentration of digoxin.

The clinical benefit of this study is that it will identify whether digoxin, as it is really being used, is harming women. Exploration of dose-dependency and serum concentration dependency will also give insight into whether there is a way to avoid such effects. Since concerns about digoxin use in women continue to appear in the literature, either a positive or a negative finding will help guide clinical decision making.

B. Rationale for Study Design

The proposed study design is a retrospective cohort study to estimate the hazard-ratio of all-cause deaths in patients chronically taking digoxin versus patients not taking digoxin. A retrospective observational cohort study was chosen for two reasons. First, while a randomized trial provided the initial evidence that digoxin may pose different mortality risks by gender, no randomized set of patients is available with sufficient numbers of outcomes to address all the aims of this protocol. Second, we wanted to study the effects of digoxin on mortality as it has actually been used in ordinary patients over time. This made data collected from routine clinical practice more relevant than data from randomized controlled trial data, provided problems with confounding and bias can be adequately addressed.

Confounding will be an issue in the design of this study, but the problem of confounding will be attenuated in two ways. The most problematic comparison would be to assess the direct effect of digoxin on mortality, since it is quite likely that patients who receive digoxin differ systematically from those who do not. However, the first primary outcome of this study is an assessment of drug-gender interaction. For this comparison to be confounded, a baseline difference between digoxin users and non-users would have to also differ by gender, a less likely scenario. Furthermore, the second primary outcome of this study is an assessment of drug-gender-dose interaction. Changes in dosing practices for digoxin are unlikely to be influenced strongly by gender; indeed, there is a strong secular trend towards lower doses of digoxin (8), which suggests that much variation in dosing practices is driven by factors other than patient characteristics.

C. Source Population/Database Description

The research design for this study is a retrospective cohort study. The source of the cohort will be a database from the United Kingdom called THIN (The Health Improvement Network). This database contains over 5 million individuals and has been validated for a variety of outcomes (10). The analysis will be restricted to individuals with HF, with digoxin as the exposure of interest, and assess hazard ratios for several relevant outcomes (all cause mortality as the primary outcome, with sudden cardiac death, digoxin toxicity, and hospitalization as secondary outcomes). THIN provides lab values on a subset of patients, so it should also be possible to execute a subgroup analysis adjusting for actual serum concentration of digoxin.

At the Center for Clinical Epidemiology and Biostatistics (CCEB), THIN can be used at minimal cost. A random 10% sample of the whole database is available for pilot work and can be used immediately for refinement of power calculations and development of analytic code for this

project. The primary author is familiar with the software packages (particularly MySQL and SAS) needed for this and will write the analytic code. The SAS package in particular includes procedures for the COX proportional hazard regression that will be used in the primary analysis (see next section).

D. Subjects

The analysis will be restricted to individuals with heart failure (HF). As detailed below, subjects must be treated with medication for this chronic disease.

1. Exposed Population

Digoxin exposure will be defined as consecutive, recurrent prescriptions (2 or more) of digoxin. Individuals will contribute exposed person-time from 90 days after their first prescription of digoxin until 30 days after their last prescription.

Individuals will contribute exposed person-time from the date of their first prescription of digoxin until 30 days after their last prescription. Exposure for this study is meant to capture current use of digoxin.

A last prescription is defined as a prescription not followed by another within 90 days. To clarify, if somebody takes digoxin for many years but during that time has a single gap of greater than 90 days, they will contribute two blocks of exposed time.

Of note, individuals in the control population are required to be on other medications associated with HF in order to contribute followup time. A parallel requirement is not in place for the cases (who contribute person-time even if digoxin is their only medication) in the primary analysis. There is a possibility that this would create bias by allowing more individuals with less severe or symptomatic HF into the case arm. However, in a secondary analysis, cases will be restricted to those with another medication in addition to digoxin.

At least one diagnostic code for heart failure will be required for any case.

2. Control Population

The control population will consist of individuals with HF who are being treated with medication for that condition. Only person time during such medical treatment (or for 30 days after the last prescription) will be included in the study. The reason for this rule is that a diagnostic code for HF unaccompanied by any medical treatment is likely to reflect either a mistaken code; an extremely mild case, or a patient so ill that they cannot adhere to chronic outpatient medication. Chronic medication use also implies frequent followup with a physician such that morbidities and mortality will be appropriately recorded. Medications commonly used chronically for heart failure include beta-blockers, diuretics (including aldosterone antagonists for severe cases), and ACE inhibitors.

Because all of these medications have other indications in addition to heart failure, at least one diagnostic code for heart failure will be required for any control. The diagnostic code must appear within 6 months of initiation of medication, to minimize ambiguous situations in which use of a medication like a beta-blocker may precede the onset of heart failure.

3. Exclusion Criteria

The only exclusion criterion for this study is that patients must have one year or more of follow-up time in the database prior to their incident diagnosis.

E. Covariates

Covariates in the analysis will include calendar year, age, sex, BMI and smoking status, (39% missing data, so BMI and smoking will only be included in a sensitivity analysis), history of hypertension, history of MI, history of stroke, history of hospitalization, and history of diabetes. Baseline statin use will also be used as a covariate. The most useful covariates (ie, consistently available and associated with the outcome) are likely to be medication use. The cornerstones of HF pharmacotherapy are beta-blockers, ACE inhibitors, and diuretics. These drugs will be entered as covariates and, numbers permitting, cases and controls will be matched by medication regimen. Diagnostic codes defining ejection fraction, severity of HF, or type of HF (systolic versus diastolic) are unlikely to be available on enough patients to be used in any but secondary analyses. Please see code appendix for additional details.

Baseline covariates will be assessed in the year prior to the index date for the study. For both cases and controls, the index date is the date of initiation of medical treatment for HF. Medication exposure at baseline consist of the constellation of medications initiated before or within 90 days of the beginning of treatment for HF. Medication exposure will also be analysed as a time-varying covariate as a sensitivity analysis; if this sensitivity analysis alters the study conclusions then more sophisticated methods for analyzing time varying covariates, such as a marginal structural model, will be employed.

F. Outcome

The primary outcome for this study is death, which is recorded in the THIN database as a death date. Secondary outcomes, such as death from cardiac arrhythmia and digoxin toxicity, will be defined using medical diagnosis codes (see code appendix).

G. Codes

Medical and drug codes were identified through text searches of the THIN medical codes databases, hand screening, and consultation with other researchers at the University of Pennsylvania who have done research on digoxin and on HF. The codes are included as an appendix.

H. Analysis

The initial analysis will be descriptive, comparing exposed and unexposed individuals. Differences between groups will be tested for significance using chi-square and t-tests depending on whether the variable of interest is dichotomous or continuous. Incidence rates in each group will be calculated, then hazard ratios will be calculated using Cox proportional hazards models. Both unadjusted and adjusted models will be run. Adjusted models will include all variables outlined in the Covariate section above.

The final analysis to address the first primary aim will most likely be presented as two multivariable Cox models, one for males and one for females. The formal significance test for a digoxin-gender interaction, however, will most easily be done via a single Cox model which includes sex, digoxin exposure, and a sex-digoxin exposure interaction term in the model.

To address the second primary aim, average daily dose of digoxin will be calculated and the analysis will be stratified by quartiles of average daily dose. Because of the strong secular downward trend in digoxin dose with time, it will be particularly important to control for calendar year in this analysis. In an exploratory component of this aim, we will investigate whether calendar year, possibly in combination with physician prescribing preference, can be used as an instrumental variable and whether this adjustment changes the results of the analysis. In theory, an instrumental variable can control for confounding that is not necessarily captured by conventional multivariable adjustment (11).

To address the third (and secondary) aim, we will identify patients for whom digoxin serum levels are available in the database. For that subset of patients, the analyses described for aim 1 can be repeated but including serum level of digoxin in the model. It is possible that there will be insufficient data of this kind for us to complete this aim.

In all aims, sensitivity analysis for the potential effect of unmeasured confounding will also be conducted (12).

I. Sample Size

Pilot data indicate that approximately 800,000 patients in the THIN database have had multiple prescriptions for digoxin at some point and that 30,000 of these patients have died. While these pilot numbers do not incorporate exclusion criteria or assess how many of these deaths occurred while the patient was taking digoxin, they suggest that the number of eligible outcomes is likely to be large and will certainly support aims 1 and 2. Even if only 1% of these deaths are eligible outcomes in digoxin-exposed individuals, the study should be 90% powered at an alpha of 0.05 to detect a hazard ratio for the digoxin-gender interaction of 1.3 (analysis done using Schoenfeld's equations for time-to-event analysis).

J. Plan for reporting and following up results

This project is intended for peer-reviewed publication. The primary parameter estimates from this research will be a hazard ratio for a digoxin-gender interaction (primary aim 1, regarding the basic question of whether such an interaction is present). The results of primary aim 2 will be more complicated to present but we anticipate reporting multiple digoxin-gender interaction hazard ratios after stratification by calendar year.

K. Anticipated Limitations

- **Representativeness of Cohort:** Since exposed and unexposed are drawn from a large population sample and treated as a cohort, they should be representative of the UK population.
- **Misclassification of Exposure:** The exposure is use of a prescription drug. Prescriptions issued are automatically captured by the systems that contribute data to THIN and these data are considered highly reliable. As a proxy for use of the drug, this is not a perfectly valid measure because patients may never fill or take the prescription. However, such misclassification should be minimal and nondifferential. Dosing levels will be computed from prescription data as has been done in other studies (8); similar concerns about nondifferential misclassification apply.
- **Misclassification of Outcome:**
 - **Mortality is recorded in THIN as a mortality code.** The specificity of this measure should be good and can be confirmed by checking to make sure that medical followup ends at the time of reported death. Sensitivity may be imperfect if some deaths are not recorded and merely appear as the end of followup in THIN; however, this is unlikely to be a major problem and would most likely result in nondifferential bias to the null.
- **Confounding by Indication:** confounding by indication is likely to be a less severe problem because this study focuses on interactions rather than on the main drug effect. Adjustment for covariates will also be employed.

IV. Implications

This project has implications for basic pharmacologic science, clinical practice, epidemiological methods, and future research.

A. Basic Science

The major basic science contribution of this project would be to assess whether there is a dose-gender-digoxin three-way interaction. A dose-response relationship, which is unlikely to be due to confounding, would support the current hypothesis that differential mortality is due to the same dosing level resulting in different serum concentration in the different genders.

B. Clinical Practice

This project is focused on clinical practice: the question is whether there is a need for different usage guidelines for digoxin based on gender. In particular, primary aim 2 will address the hypothesis that modern dosing practices equalize the risk between genders.

C. Epidemiological Methods

This project will result in careful examination of two methodological questions. The first is whether interactions are typically less confounded than direct effects. The second is whether a strong secular trend in how a drug is used can lead to a convincing demonstration of how the changing practice impacts outcomes.

V. Timeline and Logistics

A. Human Subjects Protections

1. Approval from Penn IRB
2. Approval from ethics bodies governing THIN

B. Grant Support

For support for this study, I will apply to the CTSA-ACARD Internal Small Grant Program (attached). It is possible to apply for any of 5 levels of support in working with the THIN database. For this project, the most appropriate is level 4.

Level 4 support includes exporting data files from THIN to a PC environment. ACARD intends to fund small grants to export analytic data files from the full THIN (residing on a server) for use on a PC. This funding is for researchers who have pilot-tested their variable creation, cohort selection criteria, and statistical code using the 10% sample data residing on a dedicated PC (known as ‘Victoria’). These grants are for \$1000 each, all of which must be used for the cost of the Biostatistical Analysis Center (BAC) to export the data from the full THIN database for further analysis by the investigators. The BAC will implement the SQL, SAS, and/or STATA code provided by the applicant to create the study data files. The BAC will not be responsible for determining the integrity of the code. If selected for funding, the funds will be directly transferred from ACARD to BAC.

I am writing my own SQL, SAS, and STATA code to conduct this analysis with the guidance of my thesis advisor and my biostatistics advisor. PC resources available within the CCEB will be sufficient to implement this code.

C. Timeline

To do	Sep. '08	Oct.	Nov. '08	Dec.	Jan.	Feb.
<i>Develop protocol</i>	*****					
<i>Penn IRB approval (done)</i>	*****					
<i>UK Ethics Approval</i>	*****					
<i>Write, pilot, and debug SAS/SQL code</i>	*****					
<i>Data extraction and</i>	*					

<i>analysis</i>	
<i>Writeup primary results</i>	*****

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