

ASSOCIATION OF DIAGNOSTIC BLOOD LOSS FROM PHLEBOTOMY AND HOSPITAL-
ACQUIRED ANEMIA DURING ADMISSION WITH ACUTE MYOCARDIAL
INFARCTION

By

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ABSTRACT

Introduction: Acute, hospital-acquired anemia (HAA) during admission with AMI is associated with higher mortality and worse health status, and often occurs in the absence of recognized bleeding. Diagnostic blood loss from phlebotomy is readily modifiable, but the relationship between diagnostic blood loss and HAA is unclear.

Methods: We studied 17,676 AMI patients in the Health Facts database who were not anemic at admission and did not undergo coronary bypass surgery, focusing on the development of moderate-severe HAA (Hgb decline to < 11 g/dl during hospitalization), since this degree of HAA has been shown to be prognostically important. Health Facts included the lab tests, as well as date and time of every blood draw. Patients' total diagnostic blood loss was calculated by multiplying the number and types of blood tubes drawn by the standard blood volume for each tube type. Hierarchical modified Poisson regression was used to test the association between phlebotomy volume and the development of moderate-severe HAA, accounting for clustering by hospital site and adjusting for demographics, comorbidities, disease severity and treatment variables. Sensitivity analyses were conducted after excluding patients with documented bleeding and after stratifying by length of stay (LOS).

Results: Moderate-severe HAA developed in 3,549 patients (20%). Total diagnostic blood loss ranged from 12ml to 1864ml, and mean phlebotomy volume was significantly higher in patients with (182±149 ml) vs. without HAA (86.2±55.9 ml, $p<0.001$). The risk of developing HAA was greater with increasing phlebotomy volume (per 50 ml: RR 1.17 (95% CI 1.13-1.21)). After multivariable adjustment, each 50 ml of blood drawn for laboratory tests was associated with a

14% increase in risk of HAA (RR 1.14 (95% CI 1.11-1.17)). Results were similar in patients without documented bleeding (RR 1.14 (95% CI 1.11-1.16) per 50 ml) and stratified by LOS (LOS \leq 4 days: RR 1.30 (95% CI 1.15-1.46) per 50 ml; LOS \geq 4 days RR 1.11 (95% CI 1.09-1.14) per 50 ml).

Conclusion:

Blood loss from more frequent phlebotomy is independently associated with the development of moderate-severe HAA. These findings suggest that HAA may be preventable by implementing strategies to limit both the number of blood draws and the volume of blood removed for diagnostic testing.

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INTRODUCTION

Both chronic anemia, which is present at the time of admission to the hospital, and hospital-acquired anemia (HAA), which is a new-onset anemia that develops during admission with AMI, are associated with greater mortality and poorer health status in patients with AMI.¹⁻⁵

Unfortunately, chronic anemia often results from chronic inflammation and underlying comorbidities such as renal failure and congestive heart failure. Administration of anemia treatments such as erythropoietin analogues effectively increase hemoglobin (Hgb) levels, however, they have also been shown to increase risk of stroke and thromboembolism in other patient populations.⁶⁻⁸ Similarly, blood transfusion offers at best a short-term benefit but has also been shown to increase mortality when administered to AMI patients.⁹⁻¹¹ Accordingly, while chronic anemia is a marker for patients at risk of poor outcomes, in many cases there are limited treatment options with potential to favorably alter risk for adverse events.

In contrast to chronic anemia, HAA is a new-onset anemia during hospitalization that may be preventable by implementing hospital-based strategies to reduce blood loss in high risk patients. Periprocedural bleeding is an important target for such HAA prevention initiatives. For instance, interventions that decrease bleeding risk at the time of percutaneous coronary intervention (such as closure devices or the use of alternative anticoagulants such as bivalirudin) are likely to reduce the incidence of HAA by reducing bleeding complications.¹² However, HAA commonly occurs in the absence of bleeding in both AMI patients and in patients admitted for percutaneous coronary intervention, suggesting that factors other than documented bleeding are important in the etiology of HAA.^{4,13} Although HAA may reflect unrecognized bleeding events in some patients, it is likely that development of HAA is multifactorial and related to other clinical factors.

Several potential risk factors may explain development of HAA in patients who do not experience bleeding events. Acute inflammation related to myocardial infarction and comorbidities such as chronic renal disease or congestive heart failure may result in blunted hematopoietic response.^{14,15} As with chronic anemia, however, most of these are not modifiable. In contrast, diagnostic blood loss occurs to a varying degree in all patients hospitalized with AMI and may be another important driver of HAA.

Prior studies in other patient populations have linked diagnostic blood loss with development of anemia. Smoller and colleagues studied 100 patients admitted for both medical and surgical care settings. They reported that phlebotomy volumes were particularly high in ICU settings and high volumes of diagnostic blood loss were more common in patients who received blood transfusion. In this ICU cohort, they reported a mean volume of blood drawn daily of 41 ml and a mean of 762 ml per hospitalization.¹⁶ Similarly, in a retrospective analysis of patients with long intensive care unit stays, Chant and colleagues found that phlebotomy volume was an independent correlate of transfusion requirement.¹⁷ More recently, Thavendiranathan reported a retrospective, single center study of the relationship between diagnostic blood loss and development of anemia including all patients admitted to a general medicine service in an academic hospital setting.¹⁸ Diagnostic blood loss averaged 75 ml but varied significantly (standard deviation 52 ml). Even after adjusting for relevant confounders, higher phlebotomy volume was strongly associated with hemoglobin declines during hospitalization.

Patients admitted with AMI may be particularly vulnerable to large blood losses from diagnostic phlebotomy. In addition to baseline laboratory investigations necessary to evaluate metabolic status, renal function and hemoglobin, AMI patients often have additional lab investigations beyond those commonly drawn for patients admitted to general medical wards.

For example, serial cardiac biomarker assessments are obtained to assess for ongoing ischemia and document the severity of the patients' myocardial infarction, pre- and post-procedural labs are required to assess for blood loss or renal insult in patients undergoing coronary angiography and serial electrolyte assessments are often ordered to ensure potassium and magnesium are normalized to reduce risk of arrhythmia. Importantly, this potential risk factor is under the locus of control of care providers who can limit scheduled phlebotomy, use pediatric blood tubes in place of standard blood collection tubes and rely more heavily on stored serum specimens in high risk patients. These strategies could reduce diagnostic blood loss and also the incidence and severity of HAA.¹⁹⁻²¹ Although reducing diagnostic blood loss is a common-sense intervention that may even be cost-saving in AMI care, few studies have evaluated the relationship between blood loss from phlebotomy and development of HAA.

The only prior study we are aware of that studied the relationship between diagnostic blood loss and HAA in patients admitted for AMI was published by Thank-Johnson in 1993.²² The authors found that patients treated with thrombolytics often had larger in-hospital hemoglobin declines than controls who did not receive thrombolysis, even in the absence of observed bleeding or invasive procedures. Patients who received thrombolytics also had greater phlebotomy volumes over the first 24 hours of hospitalization. The authors concluded that higher phlebotomy volumes were a likely contributors to the larger hemoglobin declines in the thrombolysis group. However, the study was small, represents practice patterns over 20 years ago, and no statistical test of the association between phlebotomy and in-hospital hemoglobin declines was applied. It is unclear from this study whether volume of diagnostic blood loss is an independent correlate of in-hospital hemoglobin declines after accounting for relevant confounders.

Since diagnostic blood loss is a potentially modifiable risk factor for development of HAA, we undertook the following study with the goal of describing the relationship between diagnostic blood loss and HAA. Our goals were to quantify diagnostic blood loss in a nationally representative cohort of AMI patients and see if the volume of diagnostic blood loss from phlebotomy is associated with HAA. Since this relationship is likely confounded by other variables, we also studied whether any crude relationships persisted after multivariable adjustment. The specific aims of the study were to:

1. Compare patients who developed HAA to those who do not develop HAA and determine the mean/median volume of diagnostic blood loss over the course of the hospitalization, average diagnostic blood loss per 24 hours of hospitalization, and on each individual hospital day (1-10, respectively).
2. Describe the variability in diagnostic blood loss volumes across Health Facts hospitals.
3. Understand the relationship between diagnostic blood loss and development of HAA. To understand whether this is an independent relationship, we built a multivariable regression model to understand whether diagnostic blood loss is associated with the development of HAA accounting for relevant confounders.
4. Understand the relationship between the development of HAA and length of stay. Specifically, do patients who develop HAA tend to develop it early in the hospital or later during the admission? If we hypothesize that the cumulative burden of ongoing diagnostic blood loss may lead to HAA, then we must see that some patients develop HAA late in the hospitalization. Further, we hoped to understand whether the relationship between diagnostic blood loss and HAA persisted after stratifying by length of hospital stay.

5. Perform sensitivity analyses to understand how different assumptions of the volume required to fill blood tubes influences the estimated diagnostic blood loss (since the available literature reports several different standard blood volumes) and determine how implementation of potential strategies to reduce diagnostic blood loss (specifically, using pediatric tubes in place of standard phlebotomy tubes) could influence the relationship between phlebotomy and HAA. Finally, since another important risk factor for HAA is bleeding, we will repeat our analyses after excluding patients who had known bleeding to understand whether our results are consistent in these patients.

METHODS

Data Source

We studied hospital encounters in the Cerner Health Facts database, a large database of all AMI admissions to participating hospitals using the Cerner electronic medical record. Full details of the Cerner Health Facts Database have been described previously.^{23,24} The database captures the deidentified data of consecutive AMI encounters. Included patients had a primary discharge diagnosis of AMI as determined by *International Classification of Diseases, Ninth Revision, Clinical Modification* diagnostic codes 410.xx. Detailed data from admissions between January 1, 2000 and December 31, 2008 were collected including hospital characteristics, patients' demographic characteristics (limited to age, gender and race and abstracted from the electronic medical records medical records and registration data), medical history and comorbidities (using *International Classification of Diseases, Ninth Revision, Clinical Modification* diagnostic codes), laboratory studies (including venous and arterial blood draws

and time of phlebotomy events), in hospital medications and procedures, in-hospital complications and inpatient mortality.

Study Population

The total population included in this database represents 61,149 encounters from a total of 53,659 unique patients. To avoid including patients readmitted after recent AMI who may have residual HAA from the previous admission, we limited our cohort to the first AMI encounter for each patient. Among these 53,659 patients, we further confirmed the patient was admitted for AMI by excluding patients without at least 1 abnormal cardiac biomarker (troponin or CK-MB) during the admission (n=13,900 patients) and those who were discharged within the first 24 hours after admission (n= 1,337 patients). We also excluded 81 patients transferred from other acute care facilities or from hospice, since full data on processes of care were not available on patients transferred from another acute care facility and goals of care for patients on hospice may differ from the overall population. Additionally, we excluded 57 patients from hospitals with small enrollments (<20 patients) and 392 patients with lengths of stay greater than 31 days, since these patients' data are generalizable to very few patients admitted with AMI.

Exclusion criteria included coronary artery bypass grafting during the index admission (n=3,990), since post-bypass anemia is attributable to the surgery itself and is associated with distinct long term outcomes.⁴ Patients who did not have hemoglobin assessed within the first 24 hours of the admission (n=2,225), those without at least 2 hemoglobin assessments during their hospitalization (n=3,577), and those who were anemic at admission (below diagnostic thresholds for anemia on initial Hgb, n=10,424) were also excluded yielding a final analytic cohort of 17,676 patients with AMI and without anemia at admission from 57 hospitals.

Anemia and Bleeding Definitions

Patients were classified as having hospital-acquired anemia if they had a normal admission hemoglobin but nadir hemoglobin (defined as each patients' lowest hemoglobin value over the course of their hospitalization) declined below diagnostic thresholds for anemia. Anemia was defined using age, gender, and race specific criteria was described by Beutler and Waalen as a hemoglobin value less than 13.7 g/dl for white men aged 20 to 59, 13.2 g/dl for white men ≥ 60 years, 12.9 g/dl for black men aged 20-59, 12.7 g/dl for black men ≥ 60 years, 12.2 g/dl for white women and 11.5 g/dl for black women. This classification is based upon analyses of large, modern cohorts and more accurately identifies anemia than the World Health Organization definition (WHO).²⁵ There were few patients who reported a race other than white or black. For these patients, we applied the Beutler and Waalen criteria for blacks since there a fewer data to guide how the diagnostic threshold for anemia varies in other races. Anemia was classified as mild (diagnostic threshold $> \text{Hgb} > 11.0$ g/dl) or moderate-severe ($\text{Hgb} \leq 11.0$ g/dl) consistent with prior work.⁴ In contrast to moderate-severe hospital acquired anemia, mild HAA has not been shown to be independently associated with increased mortality.⁴ Because it has not been shown to be prognostically important, whereas mild HAA has not been shown to be associated with poor outcomes, moderate-severe HAA was selected as the outcome for the present study. Since the outcome of interest was moderate-severe HAA, the bleeding definition did not alter categorization of patients as having moderate-severe HAA since differences between the Beutler and Waalen and the WHO anemia definitions only influence the proportion of patients with mild HAA vs. no HAA. Bleeding events were identified by reviewing all ICD-9 codes and including any that reflected an in-hospital bleeding episode in creation of an "in-

hospital bleeding” variable. We subdivided the type of bleeding (intracranial, retroperitoneal, gastrointestinal and miscellaneous).

Ascertainment of Diagnostic Blood Loss

The date and time of every blood draw and the laboratory test that were collected with each phlebotomy event were recorded in the Cerner electronic medical record and are available in the Health Facts database. Using these data, we identified the number and types of blood tubes that would be needed to complete the laboratory tests reported in the medical record. All laboratory tests obtained at the time of each blood draw that could have been run off of a particular type of blood tube will be assumed to have been run off of a single tube. Each type of tube was assigned a conservative estimate of typical blood draw volume from the literature. Hematology tubes were assigned a volume of 5 ml, coagulation laboratory tubes 4.5 ml, chemistry/miscellaneous laboratory tubes 5 ml, arterial blood gas tubes 2 ml and blood cultures 10 ml based on estimates from prior literature.¹⁷ For each patient, we multiplied these blood volumes by the number of type of blood tubes collected during each patient’s hospitalization to arrive at total volume of blood drawn for diagnostic tests during the hospitalization. We also calculated the average phlebotomy volumes per 24 hour period during each patient’s hospitalization and mean phlebotomy volumes on the each of the first through 10th days of hospital admission. For the latter analyses, only patients who remained hospitalized on each respective day were included in the denominator.

Statistical Analyses

Baseline patient characteristics, laboratory values, in-hospital treatments and in-hospital complications of patients who developed moderate-severe HAA were compared to those who did

not develop anemia or had mild HAA. For descriptive purposes, we presented categorical data as frequencies and differences between groups were compared using chi-square tests.

Continuous variables were reported as the mean \pm standard deviation and differences were compared using independent t-tests. The Wilcoxon rank-sum was used to compare variables that have a skewed distribution, and results were reported as the median and interquartile range.

When comparing diagnostic blood loss volumes across Health Facts hospitals, we generated shrinkage estimates to account for smaller enrollments into Health Facts from small hospitals. This approach pulls estimates from smaller hospitals toward the overall mean. Estimates were generated from a linear model which included site as a random effect and no other independent variables.

We used hierarchical multivariable regression, including hospital site as a random effect to account for clustering within hospitals. The outcome of interest is prevalent based on prior literature; accordingly, we used a relative risk regression strategy using Poisson regression with robust error variance.²⁶ To identify the independent association between phlebotomy volume and development of moderate-severe HAA, we adjusted for key demographic, clinical and treatment variables that we identified *a priori* based on clinical experience. These variables represent likely confounders that could obscure the independent association between diagnostic blood loss and HAA. For instance, women, older patients, and those with chronic kidney disease or diabetes are more likely to develop HAA, and could undergo more frequent blood draws as clinicians attempt to monitor their laboratories more closely. The variables included were key demographics (age, gender and race [Caucasian vs. other]), clinical characteristics (history of chronic kidney disease, congestive heart failure, hypertension, diabetes and prior myocardial infarction) and MI type and treatments (ST-elevation MI vs. non-ST-segment elevation MI,

thrombolytics, in-hospital cardiac catheterization or PCI, use of aspirin, thienopyridines, glycoprotein IIb/IIIa inhibitors, thrombolytics, heparin, warfarin, ACE inhibitors/ARBs, beta blockers, statins and bivalirudin). We also adjusted for in-hospital complications that could confound the apparent association between diagnostic blood loss from phlebotomy and development of moderate-severe HAA. For instance, patients with acute renal failure, cardiogenic shock and mechanical ventilation would be more likely to receive frequent blood draws. Accordingly, we adjusted for each of these in the model. We also tested for statistically and clinically important interactions between model covariates and the primary exposure variable of diagnostic blood loss from phlebotomy. *A priori* two-way interactions between diagnostic blood loss volume and race, mechanical ventilation, chronic kidney disease, gender and cardiac catheterization or PCI were also tested. The calibration of the model was assessed by plotting the mean predicted risk of developing moderate-severe HAA within each decile of risk of HAA vs. the observed moderate-severe HAA rates.

Missing Data

Missing data were minimal and the data that were missing were assumed to be missing at random. This included 267 missing observations for initial creatinine and no more than 1 missing value for the other covariates in our model. Accordingly, we used multiple imputation with IVEWARE software to impute these values to allow inclusion of the entire analytic cohort in the modeling.

Sensitivity analyses

Several sensitivity analyses were conducted to confirm the robustness of our findings. Varying estimates of the volume of blood needed to fill each standard tube have been reported in

the literature, and hospitals are likely to use different standard volumes depending on equipment and the facility's awareness and support of blood conservation efforts. Therefore, we repeated our calculation of diagnostic blood loss after assuming alternative estimated volumes from the literature.^{16,27-29} We also used estimates of blood draw volumes using pediatric tubes²⁰ to estimate how this potential intervention would influence the volume of phlebotomy if pediatric tubes were used in place of standard-volume blood tubes. Estimates for tube volume derived from this paper were 1.2 ml for CBC (EDTA tube), 1.4 ml for citrate tubes (coagulation studies), and 1.1ml for other tube types. Moreover, since phlebotomy volume and length of stay are likely to be strongly associated, we conducted another sensitivity analyses stratifying patients by length of stay (less than or equal to 4 days, representing the duration of many uncomplicated AMI admissions, versus greater than 4 days). To to assess the relationship between diagnostic blood loss and development of HAA in the absence of documented bleeding, we excluded all patients with known bleeding and repeated our primary analyses. Additionally, since phlebotomy volumes reported in the literature are variable, we conducted sensitivity analyses around the estimated volume of blood needed to fill each type of tube. Specifically, analyses were repeated using various published estimates of blood volume required for each tube type.^{16,27-29}

RESULTS

Baseline Characteristics, Frequency of HAA and In-Hospital Hemoglobin Declines

One out of every 5 patients admitted with AMI developed moderate-severe HAA (3,551 patients (20.1%)). Patient characteristics of those who developed moderate-severe HAA are compared to those who did not Table 1.

Table 1: Patient Characteristics of Patients with and Without Moderate-Severe Hospital Acquired Anemia

	Moderate-severe HAA		P-Value
	Yes (n = 3,551)	No (n = 14,125)	
Age (mean±SD)	71.9 ± 13.0	64.5 ± 14.5	< 0.001
Race (% Caucasian)	2933 (82.6%)	12314 (87.2%)	< 0.001
Gender (% female)	2481 (69.9%)	4651 (32.9%)	< 0.001
Length of stay (Median (IQR))	6.5 (4.1, 10.1)	3.5 (2.5, 5.0)	< 0.001
Dyslipidemia	1054 (29.7%)	7056 (50.0%)	< 0.001
Heart failure	1377 (38.8%)	2995 (21.2%)	< 0.001
Hypertension	1793 (50.5%)	7833 (55.5%)	< 0.001
History of percutaneous coronary intervention	109 (3.1%)	923 (6.5%)	< 0.001
Chronic kidney disease	454 (12.8%)	648 (4.6%)	< 0.001
Peripheral arterial disease	101 (2.8%)	293 (2.1%)	0.005
IV Heparin	2671 (75.2%)	10286 (72.8%)	0.004
Diabetes	1134 (31.9%)	3566 (25.2%)	< 0.001
History of coronary artery bypass surgery	97 (2.7%)	642 (4.5%)	< 0.001

	Moderate-severe HAA		P-Value
	Yes (n = 3,551)	No (n = 14,125)	
Prior myocardial infarction	131 (3.7%)	909 (6.4%)	< 0.001
Current smoking	483 (13.6%)	4635 (32.8%)	< 0.001
History of Stroke/transient ischemic attack	167 (4.7%)	320 (2.3%)	< 0.001
Acute renal failure	513 (14.4%)	510 (3.6%)	< 0.001
Bleeding event from ICD9 diagnosis codes	499 (14.1%)	436 (3.1%)	< 0.001
Cardiogenic shock	336 (9.5%)	305 (2.2%)	< 0.001
In hospital mechanical ventilation	390 (11.0%)	422 (3.0%)	< 0.001
Bleeding event type from ICD9 diagnosis codes			< 0.001
Miscellaneous site	290 (58.1%)	301 (69.0%)	
GI bleed	196 (39.3%)	100 (22.9%)	
Intracranial	13 (2.6%)	35 (8.0%)	
ST-Elevation MI	1547 (43.6%)	6127 (43.4%)	0.840
Non ST-Elevation MI	1874 (52.8%)	7543 (53.4%)	0.503
ACE inhibitor or ARB	2431 (68.5%)	9242 (65.4%)	< 0.001

	Moderate-severe HAA		P-Value
	Yes (n = 3,551)	No (n = 14,125)	
Thienopyridine	2379 (67.0%)	9924 (70.3%)	< 0.001
Aspirin	3091 (87.1%)	12304 (87.1%)	0.952
Beta blockers	3049 (85.9%)	11957 (84.7%)	0.066
Diuretics	1935 (54.5%)	4376 (31.0%)	< 0.001
Glycoprotein IIa/IIIb inhibitor	1702 (47.9%)	7213 (51.1%)	< 0.001
Bivalirudin	129 (3.6%)	656 (4.6%)	0.009
Statin	2301 (64.8%)	9833 (69.6%)	< 0.001
Thrombolytics	184 (5.2%)	575 (4.1%)	0.003
Warfarin	507 (14.3%)	1295 (9.2%)	< 0.001
In hospital coronary angiogram	2253 (63.4%)	10230 (72.4%)	< 0.001
In hospital percutaneous coronary intervention	1719 (48.4%)	7970 (56.4%)	< 0.001
Initial hemoglobin (g/dl, mean±SD)	13.64 ± 1.15	14.71 ± 1.26	< 0.001
Minimum hemoglobin (g/dl, mean±SD)	9.78 ± 1.14	13.09 ± 1.22	< 0.001
Final hemoglobin (g/dl, mean±SD)	10.67 ± 1.05	13.41 ± 1.30	< 0.001

	Moderate-severe HAA		P-Value
	Yes (n = 3,551)	No (n = 14,125)	
Time from admission to moderate-severe HAA (days)	2.7 ± 2.6	-	-
Admission creatinine	1.4 ± 1.2	1.2 ± 0.7	< 0.001
Continuous variables compared using Student's T-test.			
Categorical variables compared using chi-square or Fisher's exact test.			

Patients who developed moderate-severe HAA were older and greater proportions were female and non-white race. Several comorbidities were more common in those who developed moderate-severe HAA including heart failure, chronic renal failure and diabetes. In contrast, dyslipidemia, hypertension and prior PCI or CABG were less likely in those with moderate-severe HAA. Several important differences in in-hospital treatments were noted. Fewer moderate-severe HAA patients received thrombolytics, glycoprotein IIb/IIIa inhibitors and bivalirudin and a greater proportion received heparin and warfarin. Moderate-severe HAA patients less frequently had coronary angiography or percutaneous coronary intervention. They were also more likely to develop acute complications of cardiogenic shock and acute renal failure. Consistent with more complicated admissions in the moderate-severe HAA group, the median length of stay was 6.5 days (IQR 4.1, 10.1) among patients with moderate-severe HAA vs. 3.5 days (IQR 2.5, 5.0) for patients with mild or no HAA (p<0.001). The mean hemoglobin declined during hospitalization in both groups, with greater declines in those with moderate-

severe HAA (3.86 g/dl \pm 1.64 vs. 1.62 \pm 1.09, $p < 0.001$). In-hospital bleeding was significantly more common in patients with moderate-severe HAA (14.1% vs. 3.1%, $p < 0.001$).

Diagnostic Blood Loss

Mean, median and range of diagnostic blood loss during the course of hospitalization and per 24 hours are presented in Table 2, as well as the breakdown of phlebotomy volume per type of blood tube drawn. Mean blood loss from phlebotomy was nearly 100 ml higher over the course of the hospitalization among patients who developed moderate-severe HAA (182.1 \pm 148.5 ml vs. 86.2 \pm 55.9 ml, $p < 0.001$), while mean blood loss per 24 hours hospitalized was slightly greater in those with moderate-severe HAA (25.7 \pm 43.2 vs. 23.5 \pm 25.6, $p < 0.001$). Accordingly, mean differences in total diagnostic blood loss volumes largely reflect differences in length of stay and ongoing daily blood draws during prolonged hospitalizations.

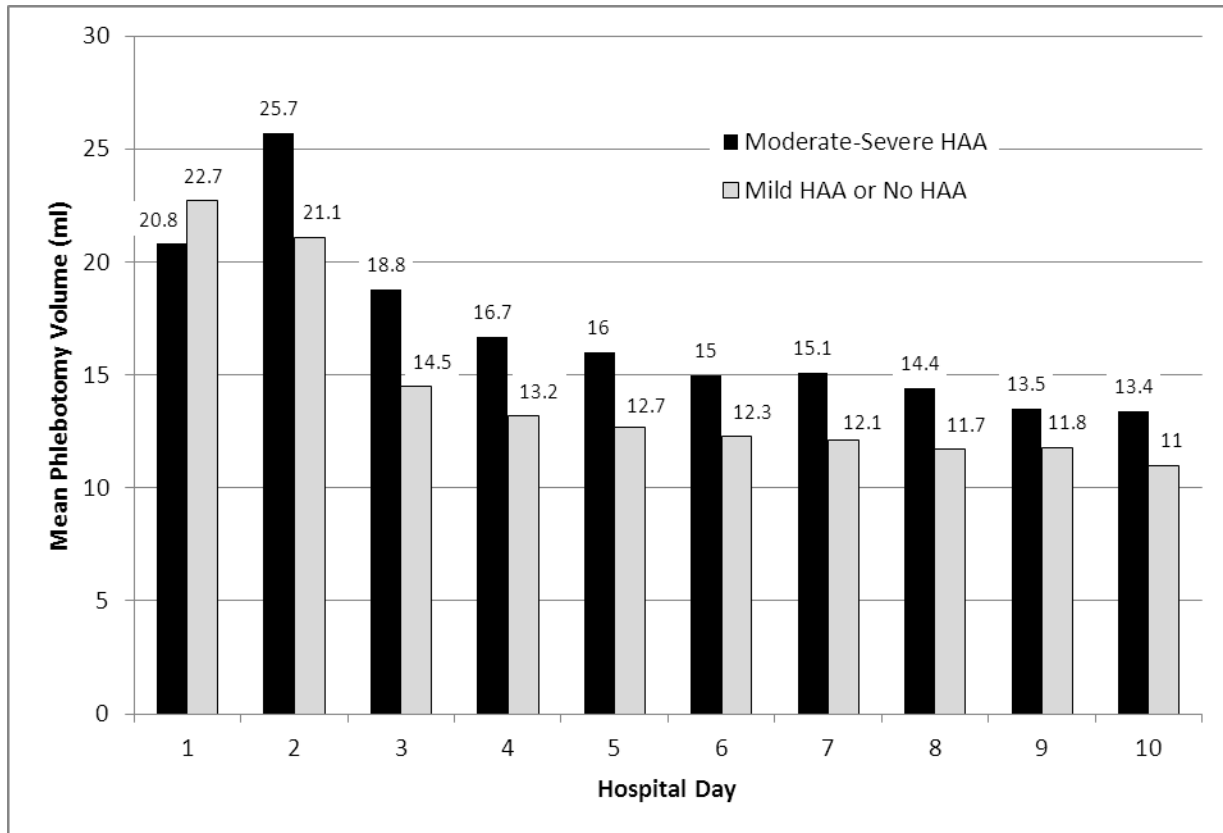
Table 2: Diagnostic Blood Loss Estimates During Hospitalization with Acute Myocardial Infarction

	Moderate-severe HAA		P-Value
	Yes (n = 3,551)	No (n = 14125)	
Total Diagnostic Blood Loss (ml)			< 0.001
Mean \pm SD	182.1 \pm 148.5	86.2 \pm 55.9	
Median (IQR)	137.0 (91.0, 221.0)	70.5 (51.5, 102.5)	

	Moderate-severe HAA		P-Value
	Yes (n = 3,551)	No (n = 14125)	
Diagnostic Blood Loss per 24 hours (ml)			< 0.001
Mean±SD	25.7 ± 43.2	23.5 ± 25.6	
Median (IQR)	21.9 (16.3, 28.8)	20.8 (15.7, 27.3)	
Range of Diagnostic Blood Loss (ml)			< 0.001
Min	20.0	12.0	
Max	1864.0	732.0	
Total Number of Blood tubes (mean±SD)	40.5 ± 33.2	19.3 ± 12.2	< 0.001
Blood tubes per 24 hours (mean±SD)	5.7 ± 7.8	5.2 ± 4.9	< 0.001
Range of Number of Blood tubes			< 0.001
Min	4.0	3.0	
Max	443.0	172.0	
Blood Loss from Arterial Blood Gas (mean±SD)	18.3 ± 19.7	7.7 ± 6.9	< 0.001
Blood Loss from Chemistries (mean±SD)	69.6 ± 63.3	35.8 ± 23.5	< 0.001
Blood Loss from Coagulation labs (mean±SD)	26.6 ± 28.5	15.3 ± 16.8	< 0.001
Blood Loss from CBCs (mean±SD)	47.4 ± 38.7	21.3 ± 12.5	< 0.001
Blood Loss from Blood Cultures (mean±SD)	20.3 ± 36.4	6.1 ± 18.0	< 0.001

When diagnostic blood loss was estimated for each hospital day, the mean diagnostic blood loss was highest on the first two hospital days and declined over subsequent days (Figure 1).

Figure 1: Mean Volume of Diagnostic Blood Loss On Each Day From Hospital Day 1 Through 10



Mean blood loss from phlebotomy was nearly 100 ml higher over the course of the hospitalization among patients who developed moderate-severe HAA (182.1 ± 148.5 ml vs. 86.2 ± 55.9 ml, $p < 0.001$), while mean blood loss per 24 hours hospitalized was slightly greater in those with moderate-severe HAA (25.7 ± 43.2 vs. 23.5 ± 25.6 , $p < 0.001$). Accordingly, mean differences in total diagnostic blood loss volumes largely reflect differences in length of stay and ongoing daily blood draws during prolonged hospitalizations. When diagnostic blood loss was estimated for each hospital day, the mean diagnostic blood loss was highest on the first two hospital days and declined over subsequent days (Figure 1).

Variability in Diagnostic Blood Loss Across Hospitals

There was important variability in the estimated diagnostic blood loss from phlebotomy across Health Facts hospitals. Regardless of HAA status, the mean volume of diagnostic blood loss from phlebotomy varied between 53.8 ml and 145.2 ml. After adjusting for the size of each hospital's patient population, this range was 69.6 ml (95% CI 61.1-78.1 ml) to 144 ml (95% CI 138.0-150.0 ml). There was even greater variability among those with moderate-severe HAA. The unadjusted total diagnostic blood loss ranged from 66.5ml to 400.1 ml while shrinkage adjusted values ranged from 129.0 ml (95% CI 100.3 ml-157.7 ml) to 244.2 ml (95% CI 183.4 ml- 305.0 ml) across hospitals.

Multivariable Model for Development of Moderate-Severe HAA

The multivariable modified Poisson model for development of moderate-severe HAA is presented in Table 3.

Table 3: Multivariable Model Predicting Moderate-Severe HAA

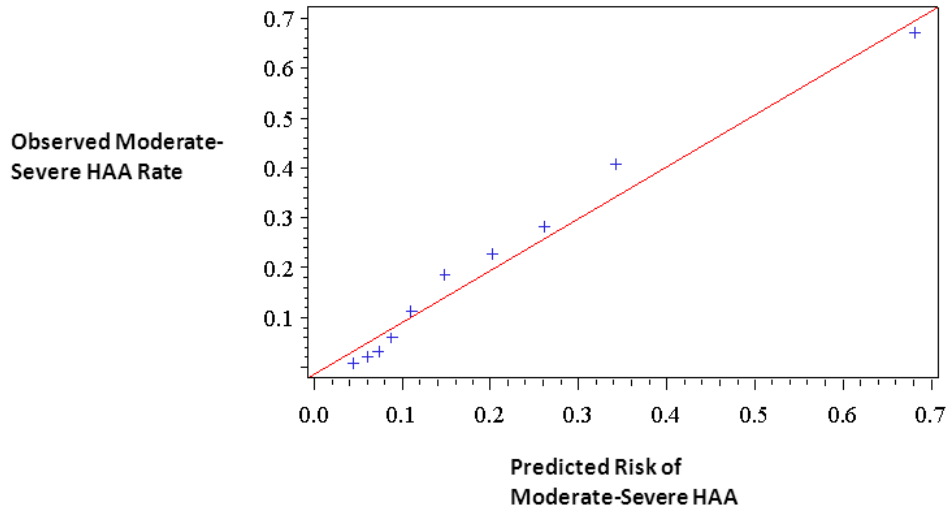
Variable	Relative	Lower CI	Upper CI	p-value
	Risk			
Diagnostic blood loss (per 50 ml increase)	1.14	1.13	1.15	<.0001
Age (10 year increase)	1.24	1.21	1.28	<.0001
Female	2.97	2.75	3.20	<.0001

Variable	Relative Risk	Lower CI	Upper CI	p-value
Non-Caucasian	1.30	1.19	1.43	<.0001
ST elevation MI	1.16	1.07	1.25	.0001
Acute Renal Failure	1.22	1.09	1.36	0.0003
Heparin	1.13	1.04	1.23	0.002
Cardiogenic Shock	1.14	0.99	1.30	0.0625
In hospital Cath or PCI	1.15	1.04	1.26	0.005
Glycoprotein IIb/IIIa inhibitor	1.22	1.13	1.33	<0.001
Bivalirudin	0.86	0.72	1.03	0.106
Antiplatelet	1.04	0.95	1.14	0.351
Beta-Blocker	1.04	0.94	1.16	0.445
Statin	0.98	0.90	1.06	0.615
ACE/ARB	1.15	1.06	1.24	0.0007
Aspirin	0.91	0.81	1.03	0.126
Thrombolytics	1.07	0.92	1.26	0.378
Mechanical ventilation	1.41	1.26	1.58	<.0001
CKD	1.51	1.36	1.69	<.0001
Prior MI	0.80	0.67	0.95	0.013

Variable	Relative	Lower CI	Upper CI	p-value
	Risk			
Diabetes	1.10	1.02	1.18	0.0005
Heart failure	1.06	0.98	1.14	0.0965
Hypertension	0.87	0.81	0.93	<.0001

No interactions were included in the final model. Interactions of diagnostic blood loss volume by race, mechanical ventilation and cardiac catheterization or PCI were not statistically significant. Interactions of chronic kidney disease and gender with volume of diagnostic blood loss were statistically significant but did not result in clinically important differences in the relative risk of developing moderate-severe HAA. The model had excellent capacity to discriminate moderate-severe HAA with a c-statistic of 0.831, and model calibration was acceptable (Figure 2).

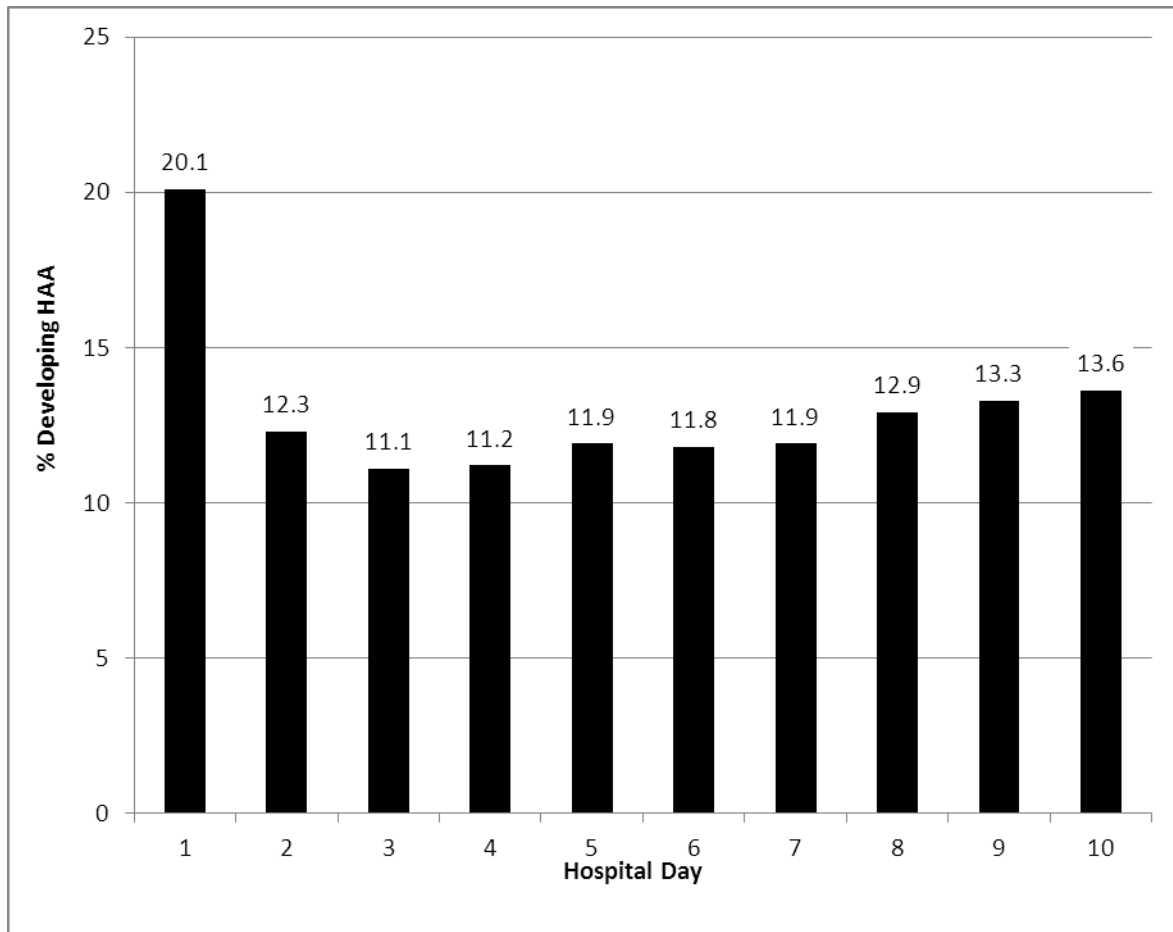
Figure 2: Plot of Mean Predicted Risk of Moderate-Severe HAA within Deciles of Predicted Risk vs. Observed Rates of Moderate-Severe HAA



Relationship Between Diagnostic Blood Loss and Development of Moderate-Severe HAA

The risk of developing moderate-severe HAA was highest on the first hospital day, when 1 in 5 patients developed moderate-severe HAA (Figure 3).

Figure 3: Proportion of Patients Developing Moderate-Severe HAA on Hospital Days 1 Through 10



On hospital days 2 through 10, the proportion of patients who developed new moderate-severe HAA ranged between 11.1% and 13.6%. There was a significant unadjusted relationship between volume of diagnostic blood loss and development of moderate-severe HAA. Each 50 ml of blood drawn was associated with a 17% increase in risk of HAA (RR 1.17, 95% CI 1.14-1.21, $p < 0.001$). After adjusting for demographic and clinical confounders, diagnostic blood loss remained an independent predictor of moderate-severe HAA (per 50 ml: RR 1.14, 95% CI 1.11-1.17, $p < 0.001$).

Sensitivity Analyses

Our first sensitivity analysis focused on the influence of length of stay on the association between diagnostic blood loss and moderate-severe HAA. There was a significant interaction between length of stay and diagnostic blood loss ($p < 0.001$). Accordingly, we stratified these analyses by stratified by of length of stay. Among patients with a length of stay of 4 days or less ($N=9,461$), the risk of HAA was greater with increasing diagnostic blood loss (per 50 ml: RR 1.30, 95% CI 1.15-1.46, $p < 0.001$) than in patients with lengths of stay greater than 4 days ($N=8215$; per 50 ml: RR 1.11, 95% CI 1.09-1.14, $p < 0.001$).

Results of several other sensitivity analyses were consistent with our primary findings. After excluding patients who suffered documented bleeding episodes ($N=16741$), the risk of HAA remained unchanged (RR 1.14, 95% CI 1.11-1.16, $p < 0.001$). Estimates of total diagnostic blood loss generated by varying the volume of blood required to fill each tube based upon alternative reports from the prior literature are presented in Table 4.

Table 4: Estimated Diagnostic Blood Loss Across Range of Potential Tube Volumes

Study Reporting Blood Tube Volumes	Diagnostic blood loss: Moderate-Severe HAA (mean±SD)	Diagnostic Blood loss: Mild HAA or no HAA (mean±SD)	Risk of Moderate-Severe HAA (per 50 ml)
Smoller, et. al, 1986 ⁹	233.0±187.0	114.5±69.6	1.11 (95% CI 1.08-1.14)
Wisser, et. al., 2003 ²⁰	144.5±122.4	67.8±46.5	1.17 (95% CI 1.13-1.21)
Shaffer, 2007 ²¹	140.1±115.7	67.8±46.5	1.19 (95% CI 1.15-1.23)

Pabla et. al., 2009 ^{22*}	155.8±128.6	73.0±48.8	1.17 (95% CI 1.13- 1.20)
---------------------------------------	-------------	-----------	-----------------------------

Among patients who developed moderate-severe HAA, the estimated total phlebotomy volume ranged from 140.1±115.7 ml using the most conservative estimates of blood volume required to fill standard tubes to 233.0±187.0 ml using the largest blood volume estimates from prior literature. The relative risk of developing moderate-severe HAA remained similar when using these different estimates of phlebotomy volumes. If pediatric blood tubes were used in place of standard tubes for all blood draws in Health Facts, the mean estimated diagnostic blood loss volume in the overall cohort would decline to 35.5 ± 39.0 (65.3 ± 62.2 ml (range 4ml to 689 ml) in those with moderate-severe HAA and 28.0 ± 25.5 ml (range 3.4 to 433 ml) in those with mild or no HAA).

Discussion

In this large, cohort of unselected AMI patients from hospitals across the United States, diagnostic blood loss from phlebotomy was substantial and greater use of phlebotomy was independently associated with a higher risk of developing moderate-severe HAA. Diagnostic blood loss during hospitalization with AMI varied dramatically within this cohort and also varied across hospitals, suggesting that process of care differences may influence the volume of blood taken for diagnostic tests. Importantly, we found that the mean daily volume of diagnostic blood loss remained consistent even during prolonged hospitalizations, suggesting that the common clinical practice of daily, scheduled phlebotomy can lead to large cumulative blood loss for patients who experience long hospitalizations. Accordingly, a large fraction of patients' total

diagnostic blood loss volumes reflect differences in length of stay and ongoing daily blood draws during prolonged hospitalizations, which may represent a preventable cause of HAA. Moreover, we found that on each hospital day over 10% of patients who began the day with a normal Hgb developed HAA, confirming an ongoing risk of HAA throughout the course of hospitalization with AMI. Finally, the association between amount blood collected for diagnostic tests and the development of moderate-severe HAA was significant, and remained robust after multivariable adjustment and in several sensitivity analyses.

Prior studies have shown that HAA and in-hospital Hgb declines are common and are associated with poor post-AMI outcomes, including higher mortality and worse health status.^{4,13,30} In our study, HAA developed in over half of patients admitted for AMI who did not have baseline anemia or undergo coronary bypass surgery, 1 in 5 patients developed moderate-severe HAA. Several mechanisms play a role in development of HAA. Although some of these are not modifiable (age, gender, chronic kidney disease, inflammation in the setting of AMI),^{4,31} two are clearly under the locus of control of healthcare providers – prevention of periprocedural bleeding and limitation of diagnostic blood loss from phlebotomy. Alternative anticoagulants such as bivalirudin, closure devices, and radial access at the time of coronary angiography all reduce the incidence of periprocedural bleeding.^{12,32-34} However, no clear bleeding event is identified in many patients who develop HAA.^{4,13} Although phlebotomy has been suggested as a cause of in-hospital Hgb declines in AMI patients,²² no studies have established this relationship. Our study is the first to directly assess the association between diagnostic blood loss and HAA, and leverages a large, contemporary cohort reflecting real world patient care, and included detailed data on the number and timing of blood draws. Our finding that diagnostic blood loss was often substantial was an independent correlate of higher risk of

moderate-severe HAA sheds important light on a potentially modifiable risk factor for moderate-severe HAA. It is important that future HAA prevention efforts focus on multi-modal interventions, and limiting diagnostic blood loss may be an important component of these efforts.

Our findings have important clinical implications. We found that, on average, diagnostic blood loss remains relatively constant throughout the course of patients' hospital stays after the first two days of hospitalization. The majority of diagnostic evaluation and therapeutic interventions often occur relatively early during hospitalization with AMI, and it is likely that much of the blood drawn later in long hospitalizations represents routine, scheduled lab draws that could lead to substantial blood loss. Although much of this phlebotomy may be unnecessary, few data have linked this practice to adverse outcomes, but our findings indicate greater use of phlebotomy is an important risk factor for HAA. Measures to reduce unneeded blood draws could limit the development and severity of HAA with no additional cost to the hospital (using stored serum samples) and potential cost savings (eliminating unnecessary, scheduled blood draws), while also reducing patient discomfort and potentially risk for HAA. Greater use of pediatric tubes in high risk individuals who require diagnostic testing may also reduce diagnostic blood loss. We found that estimates of blood loss from phlebotomy were dramatically lower when estimated using blood volumes required to fill pediatric tubes, highlighting a potentially promising intervention to limit diagnostic blood loss.

The finding that high diagnostic blood loss volumes are common over the course of AMI hospitalizations also warrants further discussion. Among patients who developed moderate-severe HAA, the mean estimated phlebotomy volume was 182 ml. This equivalent to nearly half a unit of whole blood, and while significant, is likely one contributor among several others in the development of HAA. Moreover, many patients had modest diagnostic blood losses and smaller

in-hospital hemoglobin declines, implementing strategies that reduce unneeded, scheduled phlebotomy could have other benefits including cost savings and reduced patient discomfort. Our data underscore the importance of studying comprehensive interventions, that go beyond focusing only on reducing bleeding, to prevent in hospital hemoglobin declines.

Although our study focused on the association between diagnostic blood loss and moderate-severe HAA, we found in prior work that HAA often developed in the absence of documented bleeding despite careful, prospective collection of bleeding data.⁴ The question remains - is this a reflection of undocumented bleeding episodes or other factors. While it may be common that minor bleeding episodes are underreported, these episodes have not been shown to be prognostically important and are unlikely to result in the large Hgb declines required to reach diagnostic thresholds for moderate-severe HAA. Bleeding prevention should clearly be an important focus of HAA prevention efforts, but our findings suggest that phlebotomy is also an important factor in the development of moderate-severe HAA that may be a cause of HAA both in the presence and absence of bleeding.

Limitations

Several potential limitations of our study should be considered in the interpretation of these data. Our calculation of phlebotomy volume relied on estimates of the amount of blood required for each blood tube. These estimates are consistent with prior literature, and we included several conservative assumptions in our calculations (we did not account for wasted blood during blood draws, assumed only 1 tube was drawn when many labs drawn at the same time could have potentially been run off of only 1 tube but may have required several tubes of blood). Moreover, we conducted sensitivity analyses in which we used alternative volumes for

the volume of blood required for each tube, and still found that diagnostic blood loss was substantial. Similarly, different hospitals may have different standard required blood volumes to fill each tube, and some may use pediatric tubes in select adult patients. Future studies with prospective collection blood draw volume are needed to further confirm our findings. We were also unable to assess the impact of hemodilution from administration of intravenous fluids or from fluid retention in the setting of acute heart failure on Hgb concentrations. However, since large Hgb declines are usually required to develop moderate-severe HAA, hemodilution likely had little effect on development of this grade of HAA. Our assessments of hemoglobin reflected clinical practice and therefore were not drawn at protocol driven intervals for each patient. Although there was variability in the number and timing of Hgb assessments, these data are generalizable to real-world clinical practice where these labs are obtained at the discretion of the treating physician. The Health Facts database lacks long-term patient follow-up, and accordingly we were unable to confirm the impact of diagnostic blood loss on long-term outcomes. Although the literature supports a strong association between HAA and long-term outcomes, further study of the impact of diagnostic blood loss on long-term mortality and health status are needed. Finally, given the retrospective nature of these analyses residual confounding cannot be excluded and no causal inference can be drawn from these observational data.

Conclusions

In conclusion, blood loss from more frequent phlebotomy is often significant in patients admitted with AMI and is independently associated with the development of moderate-severe HAA. Over ten percent of patients who had not previously developed moderate-severe HAA developed new moderate-severe HAA on each hospital day, even late in the course of hospitalization. This reinforces the potential importance of ongoing blood loss and the

cumulative effect of large volumes of diagnostic blood loss over the course of prolonged hospitalizations. This is further underscored by finding that diagnostic blood loss from phlebotomy remains independently associated with development of moderate-severe HAA in patients with long lengths of stay after stratifying by length of hospitalization. These findings suggest that HAA may be preventable by implementing blood conservation strategies that limit both the number of blood draws and the volume of blood removed for diagnostic testing. Further studies are needed to determine whether these measures reduce the incidence of HAA and improve clinical outcomes.

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Appendix

Example SAS code:

Note: Base code presented below. Multiple sensitivity analyses conducted with the same base data/models/code. Most variables were already coded for a prior project – code not presented.

```
libname hf 'c:\work\temp s\health facts data';
libname phleb 'C:\Documents and Settings\p08806\Desktop\KU MS-CR
Classes\Thesis Materials';
options fmtsearch=(hf phleb source);
options nofmterr;

proc format lib=phleb;
  value yesno 1='(1) Yes' 0='(0) No';
  value bleedtp
    1 = '(1) Misc site'
    2 = '(2) GI bleed'
    3 = '(3) Retroperitoneal'
    4 = '(4) Intracranial'
    5 = '(5) Intraocular';
  value sevdichot
    0 = '(0) Mild/None'
    1 = '(1) Moderate/Severe';
run;

options fmtsearch=(hf phleb) nofmterr;

*frequency of mod-severe HAA;

proc freq data=phleb.bw_091610;
tables modsevhaa ;
run;

*histogram of phlebotomy ml;

PROC UNIVARIATE DATA=phleb.bw_091610 NOPRINT;

  VAR totalml;

  HISTOGRAM /NORMAL (COLOR=RED W=5) NROWS=3;

RUN;

*descriptives for Table 1. We used the standard MAHI macro to generate this
to spare the tedium
given the large number of variables. These wont print out on my laptop so
I've provided the RTF
files separately;
```

```

ods rtf file="C:\Documents and Settings\p08806\Desktop\KU MS-CR
Classes\Thesis Materials\table1 &rundate..rtf";

title1 'descriptives overall';
%wordfmt;
%report (format=rtf);
  %set(data=phleb.BW_analysis_091610,showmiss=t);
  %table;
  %freq(admitted_yr); %freq(discharge_year);

%stat(min_hgb,stat=mediqr,ndec=2);
%stat(min_hgb,ndec=2);
%stat(mean_hgb,stat=mediqr,ndec=2);
%stat(mean_hgb,ndec=2);
%stat(adm_hgb,stat=mediqr,ndec=2);
%stat(adm_hgb,ndec=2);
%stat(final_hgb,stat=mediqr,ndec=2);
%stat(final_hgb,ndec=2);
%stat(adm_min_change,ndec=2);%stat(adm_min_change,stat=mediqr,ndec=2);
%stat(num_hemoglobin_labs); %stat(num_hemoglobin_labs,stat=mediqr);

%freq(HAA);%freq(modsevhaa);
%stat(timetohaa_modsev); %stat(timetohaa_modsev,stat=mediqr);
%freq(severity); %freq(severity_cat);
%stat(totalml);%stat(totalml,stat=mediqr);%stat(mlperday);
%stat(mlperday,stat=mediqr);

%stat(num_phleb_labs); %stat(mean24hr);

%stat(totalml,stat=min max);%stat(mlperday,stat=min max);
%stat(num_phleb_labs,stat=min max);

%stat(numtube_A); %stat(numtube_C); %stat(numtube_CL); %stat(numtube_H);
%stat(numtube_BC);
%stat(totalml_A); %stat(totalml_C); %stat(totalml_CL); %stat(totalml_H);
%stat(totalml_BC);

%freq(bleeding_event);%freq(bleeding_event_type);

%stat(AGE); %freq(RACE);%freq(caucasian);
%freq(raceblack);%freq(MARITAL_STATUS); %freq(GENDER); %freq(femalesex);
%stat(LOS);%stat(LOS,stat=mediqr);%freq(losgt31); %freq(Acute_resp_failure);
%freq(Acute_Renal_Failure);

%freq(pvd); %freq(anticoag_hep); %freq(dm); %freq(dialysis);
%freq(hx_arrest); %freq(hx_cabg); %freq(Hx_CAD); %freq(Cardio_shock);
%freq(CBVD); %freq(CKD);
%freq(LungDisease); %freq(Dementia); %freq(Dialysis_diag); %freq(Dm_Pr_Sec);
%freq(Dm_Pr);
%freq(End_Stage_Renal_Disease); %freq(Ty2_dm); %freq(Ty1_dm); %freq(Dyslip);
%freq(HF); %freq(syst_hf);
%freq(dias_hf); %freq(comb_hf); %freq(oth_hf); %freq(HTN); %freq(hx_pci);
%freq(Kidney_Transplant);
%freq(liver_dis); %freq(PriorMI); %freq(PVD1); %freq(Any_shock);
%freq(Other_Shock); %freq(Sep_Shock);
%freq(Smoking); %freq(Stroke_TIA); %freq(hx_stroke_Tia); %freq(Stemi);
%freq(Nstemi); %freq(unknown_ami);

```

```

%freq(Unstable_Angina); %freq(Stable_Angina); %freq(ace_inhibit);
%freq(anticoagulant); %freq(anti_plate);
%freq(arb); %freq(aspirin); %freq(aspirin_dipyridamole); %freq(bblk);
%freq(bronchodilators); %freq(ccb);
%freq(dipyridamole); %freq(diuretics); %freq(gp2a3b); %freq(heparin);
%freq(insulin); %freq(long_insulin);
%freq(short_insulin); %freq(lipid_lowering); %freq(nsaid); %freq(nitrate);
%freq(oral); %freq(plat_inhibit);
%freq(statin); %freq(thrombolytics); %freq(warfarin);
%freq(Coronary_Angiography); %freq(AVR_MVR); %freq(Cath);
%freq(PCI); %freq(cathpci); %freq(CABG); %freq(dialysis_proc);
%freq(mech_vent); %freq(PVD2);

%freq(bival);

%stat(adm_cr, stat=mediqr, ndec=2); %stat(adm_cr);
%stat(mean_cr, stat=mediqr, ndec=2);
%stat(adm_glucose, stat=mediqr, ndec=2);
%stat(mean_glucose, stat=mediqr, ndec=2);
%stat(num_glucose); %stat(num_glucose, stat=mediqr);
%freq(max_cr, stat=mediqr, ndec=2); %freq(num_cr, stat=mediqr, ndec=2);
%freq(iv_insulin2, stat=mediqr, ndec=2);

%stat(NCR40);

%stat(first_hct, stat=mediqr, ndec=2); %stat(first_hgb, stat=mediqr, ndec=2);
%stat(first_trop, stat=mediqr, ndec=2); %stat(first_wbc, stat=mediqr, ndec=2);
%stat(first_ckmb, stat=mediqr, ndec=2);

%stat(GFR_MDRD4_Init_BC, stat=mediqr, ndec=2);
%stat(adm_cr, stat=mediqr, ndec=2); %stat(max_cr, stat=mediqr, ndec=2);

%freq(hospdeath); %freq(death24hr);

%freq(AKI); %freq(acearb);

%endtable;
%endreport;
ods rtf close;

ods rtf file="C:\Documents and Settings\p08806\Desktop\KU MS-CR
Classes\Thesis Materials\table1 &rundate..rtf";

*missing data for model covariates??? ;

proc contents data=phleb.bw_091610 varnum ; run;

ods rtf file="C:\Documents and Settings\p08806\Desktop\KU MS-CR
Classes\Thesis Materials\missingness of predictors &rundate..rtf";

title 'missingness of predictors';
proc means data=phleb.bw_091610 n nmiss min max ndec=0;
var age femalesex caucasian

```

```

stemi acute_renal_failure num_phleb_labs heparin cardio_shock cathpci gp2a3b
bival
    anti_plate aspirin thrombolytics ckd adm_cr priormi dm hf htn totalml
mild_anemia mod_anemia sev_anemia smoking adm_hgb thrombolytics mech_vent
lungdisease cath pci
gp2a3b bival heparin aspirin anti_plate bblk statin acearb totalml hospdeath
haa modsevhaa bleeding_event;
run;

ods rtf close;

*got a help with the following as I do not have multiple imputation software
or know how to do it;
/*
* impute missing creatinine values and a few other missing ;

data modelvars;
set phleb.bw_091610;
keep patient_nbr hospital_id
haa mild_anemia mod_anemia sev_anemia modsev_vs_mildnone hospdeath
age femalesex caucasian
stemi acute_renal_failure num_phleb_labs heparin cardio_shock cathpci gp2a3b
bival
    anti_plate aspirin thrombolytics ckd adm_cr priormi dm hf htn totalml
mild_anemia mod_anemia sev_anemia smoking adm_hgb thrombolytics mech_vent
lungdisease cath pci
gp2a3b bival heparin aspirin anti_plate bblk statin acearb totalml hospdeath
haa modsevhaa bleeding_event ;
run;

options set = SRCLIB 'C:\Work\SAS\addons\iveware\ive_sas_windows'
sasautos=('!srclib' sasautos);

data _null_;
file "impute.set";
put "title imputation;";
put "datain modelvars;";
put "dataout phleb.bw_imputed_091610 all;";
put "default categorical;";
put "continuous age num_phleb_labs totalml adm_hgb adm_cr;";
put "transfer patient_nbr hospital_id;";
put "iterations 2;";
put "multiples 1;";
put "seed 20071205;";
put "run;";
run;

* use IVEWARE to impute ;
%impute(name=impute, dir=.);

data phleb.bw_imputed_091610 ;
set phleb.bw_imputed_091610 ;
if adm_cr<0 then adm_cr=0;
run;
*/

```

```

title 'missingness of predictors after imputation';
proc means data=phleb.bw_imputed_091610 n nmiss min max ndec=0;
var age femalesex caucasian
stemi acute_renal_failure num_phleb_labs heparin cardio_shock cathpci gp2a3b
bival
anti_plate aspirin thrombolytics ckd adm_cr priormi dm hf htn totalml
mild_anemia mod_anemia sev_anemia smoking adm_hgb thrombolytics mech_vent
lungdisease cath pci
gp2a3b bival heparin aspirin anti_plate bblk statin acearb totalml hospdeath
haa modsevhaa hospital_id;
run;

*
HAA models
;

*model without covariates to get unadjusted RRs accounting only for site;

title 'predictors of moderate/severe HAA';
title2 'hierarchicial modified poisson model';
title3 'variables are site centered, random site effect ';
title4 '***** unadjusted RR for phlebotomy and mod/sev HAA
*****';
proc glimmix data=phleb.bw_imputed_091610 empirical ;
class hospital_id ;
model modsev_vs_mildnone = totalml_ / dist=poisson link=log ;
estimate 'Total phlebotomy ml (50 ml increase)' totalml_w 50 /exp cl;
random int / subject=hospital_id ;
nloptions tech=trureg /*(or nrridg) */ maxiter=1000;
run;

title 'model results predicting moderate/severe HAA ';
title2 'modified poisson model, relative risks';
proc print data=haaest2;
var label expeestimate explower expupper probt;
run;

title 'model results predicting HAA ';
title2 'modified poisson model, relative risks';
proc print data=haaest;
var label expeestimate explower expupper probt;
run;

*multivariable model;

title 'predictors of moderate/severe HAA';
title2 'hierarchicial modified poisson model';
title3 'variables are site centered, random site effect ';

ods graphics on;
ods rtf file="C:\Documents and Settings\p08806\Desktop\KU MS-CR
Classes\Thesis Materials\plots.rtf";

```

```

proc glimmix data=phleb.bw_imputed_091610;
  class hospital_id;
  model modsev_vs_mildnone = age femalesex caucasian stemi
acute_renal_failure totalml heparin cardio_shock cathpci gp2a3b bival
  anti_plate bblk statin acearb aspirin thrombolytics mech_vent
ckd priormi dm hf htn
  / dist=poisson link=log ;
  estimate 'Age (10 year increase)' age 10 /exp cl;
  estimate 'Female' femalesex 1 /exp cl;
  estimate 'Non-Caucasian' caucasian -1 /exp cl;
  estimate 'ST elevation MI' stemi 1 /exp cl;
  estimate 'Acute Renal Failure' acute_renal_failure 1 /exp cl;
  estimate 'Heparin' heparin 1 /exp cl;
  estimate 'Cardiogenic Shock' cardio_shock 1 /exp cl;
  estimate 'In hospital Cath or PCI' cathpci 1 /exp cl;
  estimate 'Gp2a3b' gp2a3b 1 /exp cl;
  estimate 'Bivalirudin' bival 1 /exp cl;
  estimate 'Antiplatelet' anti_plate 1 /exp cl;
  estimate 'Beta-Blocker' bblk 1 /exp cl;
  estimate 'Statin' statin 1 /exp cl;
  estimate 'ACE/ARB' acearb 1 /exp cl;
  estimate 'Aspirin' aspirin 1 /exp cl;
  estimate 'Thrombolytics' thrombolytics 1 /exp cl;
  estimate 'Mechanical ventilation' mech_vent 1 /exp cl;
  estimate 'CKD' ckd 1 /exp cl;
  estimate 'Prior MI' priormi 1 /exp cl;
  estimate 'Diabetes' dm 1 /exp cl;
  estimate 'Heart failure' hf 1 /exp cl;
  estimate 'Hypertension' htn 1 /exp cl;
  estimate 'Total phlebotomy ml (50 ml increase)' totalml 50 /exp cl;
  random int / subject=hospital_id ;
  nloptions tech=trureg /*(or nrridg) */ maxiter=1000;
ods output estimates=haaest2;
output out=pred2 pred(ilink blup)=pred;

run;
ods rtf close;
ods graphics off;

*assessing calibration of model - mean within each decile of predicted vs.
observed;

%cut(data=pred2,var=pred,ngroups=10,cutvar=decile);

proc sort data=pred2;

by decile;

run;

proc means data=pred2;

var modsev_vs_mildnone pred;

by decile;

```

```

output out=calibplot;

run;

proc freq dataq=pred2;

tables decile;

run;

data calibplot; set calibplot;

if _stat_ = 'MEAN';

run;

title 'Calibration plot';

data anno;

function='move'; xsys='1'; ysys='1'; x=0; y=0; output;

function='draw'; xsys='1'; ysys='1'; color='red'; x=100; y=100; output;

run;

proc gplot data=calibplot;

plot modsev_vs_mildnone*pred/anno=anno haxis=axis1 vaxis=axis2;;

plot y=x;

run;quit;

run;

* we generated the figures with R - this macro will not work on my laptop,
only at SLH;

%r(data=haaest2,cmd=source('C:\\work\\R\\functions.r');
library(biostat);
win.metafile("C:\\work\\salisbury\\phlebotomy in health facts\\2010-9-
16\\forest plot model results predicting modsev HAA
&rundate....wmf",width=9,height=10,pointsize=15);

forestplot(expeestimate,explower,expupper,label,col.ref='red',frame=F,log=T,xl
im=c(.5,3.5),ann='right',interp=c('<<<< Lower Risk','Higher Risk
>>>>'),cex.interp=.9,digits.ann=2,xlab='',lwd.ref=2,lwd.est=2);
dev.off();
);

```



```
ods rtf file="C:\Documents and Settings\p08806\Desktop\KU MS-CR
Classes\Thesis Materials\model results predicting HAA &rundate..rtf";
```

```
*used our groups C statistic macro;
```

```
title 'C-statistic for model results predicting HAA';
title2 'modified poisson model, relative risks';
/* GET THE C STATISTIC HAA model */
data predout;
set pred; run;
/* THEN RUN THE FOLLOWING CODE.
C-STATISTIC WILL PRINT OUT IN THE OUTPUT WINDOW */
proc iml;
*reset print;
use predout;
/* CHANGE VARIABLE NAMES IN PARENTHESES TO ACTUAL AND PREDICTED */
read all var {haa} into y; *vector of binary outcomes*;
read all var {Pred} into x; *vector of predictor variable*;
x1 = x[loc(y = 0 & x ^= .),]; **predvar values corresponding to y = 0**;
x2 = x[loc(y = 1 & x ^= .),]; **predvar values corresponding to y = 1**;
n1 = nrow(x1);
n2 = nrow(x2);
concordant = 0; ties = 0; discordant = 0; **initialize**;
do i = 1 to n1;
do j = 1 to n2;
concordant = concordant + (x1[i] < x2[j]);
discordant = discordant + (x1[i] > x2[j]);
ties = ties + (x1[i] = x2[j]);
end;
end;
cstat = (concordant + .5*ties)/(n1*n2);
print cstat concordant discordant ties;
quit;
```

```
title 'C-statistic for model predicting moderate/severe HAA (BW)';
title2 'modified poisson model, relative risks';
/* GET THE C STATISTIC HAA model */
data predout2;
set pred2; run;
/* THEN RUN THE FOLLOWING CODE.
C-STATISTIC WILL PRINT OUT IN THE OUTPUT WINDOW */
proc iml;
*reset print;
use predout2;
/* CHANGE VARIABLE NAMES IN PARENTHESES TO ACTUAL AND PREDICTED */
read all var {modsev_vs_mildnone} into y; *vector of binary outcomes*;
read all var {Pred} into x; *vector of predictor variable*;
x1 = x[loc(y = 0 & x ^= .),]; **predvar values corresponding to y = 0**;
x2 = x[loc(y = 1 & x ^= .),]; **predvar values corresponding to y = 1**;
n1 = nrow(x1);
n2 = nrow(x2);
```

```

concordant = 0; ties = 0; discordant = 0; **initialize**;
do i = 1 to n1;
  do j = 1 to n2;
    concordant = concordant + (x1[i] < x2[j]);
    discordant = discordant + (x1[i] > x2[j]);
    ties = ties + (x1[i] = x2[j]);
  end;
end;
cstat = (concordant + .5*ties)/(n1*n2);
print cstat concordant discordant ties;
quit;

ods rtf close;

*test for interaction between gender and phlebotomy volume;

title 'predictors of moderate/severe HAA';
title2 'hierarchical modified poisson model';
title3 'variables are site centered, random site effect ';
proc glimmix data=phleb.bw_imputed_091610 empirical ;
  class hospital_id ;
  model modsev_vs_mildnone = age_w femalesex_w caucasian_w
    stemi_w acute_renal_failure_w totalml_w heparin_w cardio_shock_w
cathpci_w gp2a3b_w bival_w
    anti_plate_w bblk_w statin_w acearb_w aspirin_w thrombolytics_w
mech_vent_w ckd_w priormi_w dm_w hf_w htn_w
    totalml_w* femalesex_w
  / dist=poisson link=log ;
random int / subject=hospital_id ;
  nloptions tech=trureg /*(or nrridg) */ maxiter=1000;
run;

proc glimmix data=phleb.bw_imputed_091610 empirical ;
  class hospital_id ;
  model modsev_vs_mildnone = age_w caucasian_w
    stemi_w acute_renal_failure_w totalml_w heparin_w cardio_shock_w
cathpci_w gp2a3b_w bival_w
    anti_plate_w bblk_w statin_w acearb_w aspirin_w thrombolytics_w
mech_vent_w ckd_w priormi_w dm_w hf_w htn_w
    bleeding_event
  / dist=poisson link=log ;
estimate 'Total phlebotomy ml (50 ml increase)' totalml_w 50 /exp cl;
random int / subject=hospital_id ;
  nloptions tech=trureg /*(or nrridg) */ maxiter=1000;
  where femalesex=1;
run;

*so RR for 50 ml of diagnostic blood loss among females is 1.12;

proc glimmix data=phleb.bw_imputed_091610 empirical ;
  class hospital_id ;
  model modsev_vs_mildnone = age_w caucasian_w
    stemi_w acute_renal_failure_w totalml_w heparin_w cardio_shock_w
cathpci_w gp2a3b_w bival_w

```

```

        anti_plate_w  bblk_w  statin_w  acearb_w  aspirin_w  thrombolytics_w
mech_vent_w  ckd_w  priormi_w  dm_w  hf_w  htn_w
        bleeding_event
        / dist=poisson link=log ;
estimate 'Total phlebotomy ml (50 ml increase)' totalml_w 50 /exp cl;
random int / subject=hospital_id ;
        nloptions tech=trureg /*(or nrridg) */ maxiter=1000;
        where femalesex=0;
run;

*and RR for 50 ml of diagnostic blood loss among males is 1.15;
*this is not a large difference even if the risk was applied across 100-150
ml increments, accordingly, this
interaction seems statistically significant but clinically insignificant. I
left it out of the final model;

*next step is understanding the proportion that develop HAA on each hospital
day out of the
total at risk - ie, on hospital day #1, how many develop HAA? then throw out
the patients with
HAA and determine the proportion of those w/o HAA at the start of day 2
develop HAA on day 2, and so
on;
*thanks to Kimberly for help with the data step/proc sql on this;

proc sort data=phleb.bw_091610; by patient_nbr; run;

/* transpose data to vertical days for los for each patient */
data modsevHAA;
set phleb.BW_091610;
timetohaa_modsev = floor(timetohaa_modsev);
if timetohaa_modsev=0 then timetohaa_modsev=1;
format timetohaa_modsev 2.0;
keep patient_nbr modsevhaa timetohaa_modsev bleeding_event;
run;

data los;
set phleb.BW_091610;
los = floor(los);
if los=0 then los=1;
format los 2.0;
if los^=.;
keep patient_nbr los ;
run;

data verticallos;
set los;
do i= 1 to los;
        day=i ;
        output ;
end;
drop i;
run;

proc sql;

```

```

create table both as select *
from verticalallos as a
left join modsevHAA as b
on a.patient_nbr = b.patient_nbr ;
quit;

proc sort data=both; by patient_nbr day; run;

* only keep records up until development of mod sev HAA ;
data time;
set both;
if (modsevhaa=1 and day<= timetohaa_modsev) or modsevhaa=0 ;
run;

ods rtf file="C:\Work\Salisbury\phlebotomy in health facts\2010-9-16\Time to
development of HAA &rundate..rtf";

title 'Time to development of moderate/severe HAA by day in hospital';
proc freq data=time ;
tables    day*modsevhaa ;
run;

title 'Time to development of moderate/severe HAA by day in hospital';
title2 ' among those without a bleeding event';
proc freq data=time ;
tables    day*modsevhaa ;
where bleeding_event=0;
run;

ods rtf close;

*ml of phlebotomy on each day of hospital stay both median and mean;

title 'median and IQR of blood drawn per day (up through 10th day)';
proc means data= phleb.BW_analysis_091610 n q1 median q3  ndec=1;
var  TotalmlDay1 TotalmlDay2 TotalmlDay3 TotalmlDay4 TotalmlDay5 TotalmlDay6
TotalmlDay7 TotalmlDay8
TotalmlDay9 TotalmlDay10 ;
run;

title 'mean and stddev of blood drawn per day (up through 10th day)';
title2 'among those without bleeding events';
proc means data= phleb.BW_analysis_091610 n mean stddev  ndec=1;
var  TotalmlDay1 TotalmlDay2 TotalmlDay3 TotalmlDay4 TotalmlDay5 TotalmlDay6
TotalmlDay7 TotalmlDay8
TotalmlDay9 TotalmlDay10;where bleeding_event=0;
run;

```