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Investigating Haemoglobin Thresholds for Red Blood Cell Transfusion in Patients with Acute Upper GI Bleeding

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Abstract

Red blood cell transfusion is frequently used to treat the harmful effects of anemia in patients with acute upper gastrointestinal bleeding. However, there is no clear consensus on when red blood cell transfusion is warranted. No studies thus far have defined the optimal threshold for transfusion, and none have looked at whether volume of blood transfused is associated with clinical outcome. This thesis attempts to addresses these gaps by analyzing hemoglobin and volume of blood transfused as predictors of patient outcomes using multivariable logistic regression models. Main results showed a statistically significant interaction between initial hemoglobin and whether a patient received a transfusion, suggesting no apparent benefit to receiving a transfusion above hemoglobin concentrations of approximately 10.5g/dL. Risk of adverse outcomes also increased with increasing volume of blood transfused. These results could contribute to improving outcomes for patients with acute upper gastrointestinal, and identify opportunities for conservation of blood resources.

Keywords

Acute upper gastrointestinal bleeding, red blood cell transfusion, hemoglobin thresholds, transfusion volumes, restrictive protocols

Summary for Lay Audience

Acute upper gastrointestinal bleeding (AUGIB) is a common gastrointestinal emergency treated in-hospital, and can be life-threatening if not properly managed. Red blood cell (RBC) transfusion is often used to combat the harmful effects of anemia in AUGIB patients. However, in cases of less severe, non-exsanguinating hemorrhage, it is not always clear when a RBC transfusion is warranted. A commonly used indication for RBC transfusion is blood hemoglobin (Hb) concentration; if a patient's Hb falls too low, RBCs are transfused to restore hemodynamic stability. Previous clinical guidelines have endorsed an Hb threshold of 10g/dL, but recent trials have suggested this threshold can be safely lowered without adversely affecting patient outcomes. However, these trials have only compared arbitrary Hb thresholds, and have not focused on the optimal threshold for transfusion that best balances the risks of anemia against the risks of transfusion. Further, no trials thus far have investigated the association between volume of RBCs transfused and clinical outcomes. The aim of this study was to address these gaps in the literature by analyzing the association between Hb and clinical outcomes for patients who received a transfusion and those that did not (i.e., transfusion status), as well as the association between number of RBC units received and clinical outcomes. This was done by re-analyzing clinical data from the 2015 TRIGGER trial of 936 patients with AUGIB in the UK. Results showed a significant interaction between Hb at presentation and transfusion status, suggesting that receiving an RBC transfusion above Hb values of approximately 10.5g/dL significantly increased the patient's probability of a negative health event such as death, re-bleeding, or other serious adverse events, within 28 days of hospital presentation. Further, patients who received more RBC units were at an increased risk of death and re-bleeding by study day 28, even after adjustment for baseline health of the patient. These results suggest the optimal threshold for RBC transfusion may not be as low as previously suggested in recent transfusion trials, and more focus should be paid to the appropriate volume of RBCs for transfusion, though further analysis of specific patient sub-groups is necessary.

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Chapter 1

1 Introduction

Acute upper gastrointestinal bleeding (AUGIB) is a common gastrointestinal emergency treated in-hospital, with an annual incidence of 100 – 150 cases per 100,000 in Western countries, and an overall case fatality of around 10% (Hearnshaw et al., 2011; Hreinsson, Kalaitzakis, Gudmundsson, & Björnsson, 2013; van Leerdam et al., 2003; Wuerth & Rockey, 2017). Although the hospitalization rate of AUGIB has been on the decline over the last few decades, the burden on healthcare systems remains high.

Older patients are more likely to experience AUGIB, with the risk of mortality and adverse outcomes increasing with age. Importantly, as the population living beyond 80 years increases, and the prognosis of patients with AUGIB worsens, the burden and risks associated with AUGIB are likely to rise at both the population and individual level. Risk factors include the use of NSAIDs, anticoagulants, and antiplatelet agents, as well as socioeconomic status, and helicobacter pylori infection, and may present targets for policy and intervention (Crooks, West, & Card, 2012; Hearnshaw et al., 2011; Jackson et al., 2009; Lanas et al., 2015; Sostres, Gargallo, & Lanas, 2013). AUGIB occurring as a result of liver cirrhosis carries a particularly high risk of complications and adverse outcomes, and the increasing incidence of liver cirrhosis worldwide due to obesity and alcohol abuse may influence incidence and mortality associated with AUGIB.

Effective management of patients with AUGIB includes intensive resuscitation, as well as early endoscopy and risk assessment to determine risk of re-bleeding, mortality, and other adverse events. Re-bleeding is a particularly significant outcome, as it in itself is a significant predictor of mortality (Hearnshaw et al., 2010; Jairath & Barkun, 2012)

Without intervention, AUGIB can lead to dangerous drops in circulatory volume and associated symptoms and risks of anemia. Initial resuscitation prioritizes restoration of hemodynamic stability through intravenous fluid replacement. Red blood cell (RBC) transfusion is often prescribed to manage further bleeding and maintain oxygen uptake and delivery; as many as 50% of patients with AUGIB receive a RBC transfusion

(Hearnshaw et al., 2011). However, RBC transfusion is also associated with risks, and specific to AUGIB may be associated with an increased risk of re-bleeding (Restellini, Kherad, Jairath, Martel, & Barkun, 2013). While previous guidelines have endorsed hemoglobin concentration (Hb) thresholds as high as 10g/dL to guide RBC transfusion practice, the results from recent clinical trials have shown this threshold can be safely lowered to values as low as 7g/dL, without adversely affecting patient outcomes (Jairath et al., 2015; Villanueva et al., 2013). Consequently, the field has seen a shift in practice concerning RBC transfusion in patients with AUGIB. Should these findings continue to be supported by further studies, the potential implications for informing clinical practice could be highly significant, and result in a global reduction in use of blood resources; it is estimated that £12.6 million are spent in the UK each year on RBCs for the treatment of AUGIB (Campbell et al., 2015).

Though the pooled results of multiple RBC transfusion strategy trials and meta-analyses provide evidence for the superiority of more restrictive RBC transfusion protocols (Holst, Petersen, Haase, Perner, & Wetterslev, 2015), all clinical trials to date have examined the Hb threshold for RBC transfusion as a dichotomized value, comparing the effects of a protocol defined by a higher Hb threshold (i.e., the liberal policy) with those of a protocol defined by a lower Hb threshold (i.e., the restrictive policy). Selection of these Hb values has been somewhat arbitrary, and varies between studies and medical specialties, and as a result it is difficult to make any conclusive recommendations on the optimal Hb threshold for RBC transfusion. Importantly, it is likely that there is not one threshold value that is best suited for all patient groups. For example, older patients may be less tolerant to lower blood Hb concentrations than younger patients, as may those with concomitant comorbidity such as ischemic heart disease.

In addition to absolute thresholds for RBC transfusion, a separate but related issue pertains to how much blood a patient should receive once a RBC transfusion is initiated. Thus far, no study has specifically explored how patient outcomes may be predicted by the amount of blood they receive via RBC transfusions. Given the frequent use of RBC transfusion to manage AUGIB, further investigation into the optimal amount of blood could potentially influence patient outcome as well as ensure efficient use of valuable

blood stores. The purpose of this thesis is to address these two areas of uncertainty around the use of RBC transfusion and Hb thresholds in the management of AUGIB. Specifically, the goals of this thesis are to investigate a potential optimal Hb threshold and optimal volume of blood for guiding RBC transfusion in patients with AUGIB.

1.1 Structure of the Thesis

This thesis follows Western University's School of Graduate and Postdoctoral Studies monograph format. Chapter two provides a comprehensive literature review of the topic, as well as the specific aims and research objectives of the thesis. Chapter three describes the methodology and statistical analyses used to address these objectives, and Chapter four describes the results of these analyses. Chapter five discusses these results in the context of previous clinical trials and existing literature, and concludes with a discussion of the implications and limitations of this study.

Chapter 2

2 Literature Review

2.1 Acute Upper Gastrointestinal Bleeding

2.1.1 Definition

Acute upper gastrointestinal bleeding (AUGIB) is defined as bleeding proximal to the ligament of Treitz, namely in the esophagus, stomach, or duodenum. It is an acute medical emergency and common cause of hospitalization worldwide. It typically presents with hematemesis (vomiting of blood) and/or melena (blood in the stool), and may also be accompanied by symptoms of hemodynamic instability, such as dizziness, anemia, and shock (Kurien & Lobo, 2015; Rockall, Logan, Devlin, & Northfield, 1995). The following chapter intends to give a comprehensive overview of AUGIB, its incidence and associated outcomes and costs, as well as risk factors, management, and treatment.

2.1.2 Incidence

Incidence rates of AUGIB vary across different studies and patient populations. In the UK, the incidence of AUGIB is 50 to 172/100,000 people per year (Blatchford, Davidson, Murray, Blatchford, & Pell, 1997; Crooks, West, & Card, 2012; Evidence & Practice, 2012; Rockall et al., 1995), and these estimates have been virtually consistent for the past 20 years (Crooks, West, & Card, 2013). Incidence in the UK may be marginally higher than AUGIB incidence in other European countries such as Iceland, that reports a per annum incidence of 87 / 100,000 population (Hreinsson et al., 2013), and the Netherlands, which reports a crude incidence rate of 48 / 100,000 people per year (van Leerdam et al., 2003). In North America, the hospitalization rate associated with AUGIB in the USA is approximately 67 cases/100,000 people per year. This estimate represents a 21% decrease in the hospitalization rate of AUGIB since 2002 (Wuerth & Rockey, 2017). This is consistent with time trend patterns in Europe—for example, in the Netherlands, age-adjusted estimates show a 23% decrease in the overall incidence rate between 1993/94 and 2000 (van Leerdam et al., 2003).

2.1.3 Mortality

Despite improvements in treatment modalities and care for AUGIB, the reported case fatality rates from population-based studies range from 2 to 10%. Mortality in the UK remains high at 10% (Crooks, Card, & West, 2011; Hearnshaw et al., 2011), while mortality rates in the USA are reported to be lower. In the USA, in-hospital mortality dropped to 2.1% in 2009, and 1.9% in 2012 (Abougergi, Travis, & Saltzman, 2015; Wuerth & Rockey, 2017). However, crude estimates can be misleading, as case fatality is influenced predominantly by patient related factors such as age and co-morbidity, etiology of bleeding, as well as other factors such as admission status at the time of bleeding. For example, in the UK, the case fatality for patients who develop bleeding whilst already hospitalized for another condition is 26%, compared to 6.8% for patients presenting to hospital with AUGIB as the primary problem (Hearnshaw et al., 2011; Jairath et al., 2014). Methods for case ascertainment will also influence these rates.

Regardless, mortality associated with AUGIB has been declining for the last two decades (Abougergi et al., 2015; Crooks et al., 2011; Hearnshaw et al., 2011; Rockall et al., 1995). This downtrend has outpaced that of all patients admitted to a hospital for any reason (Abougergi et al., 2015). This is especially meaningful in light of the rising age of the population, and thus the number of patients presenting with multiple comorbidities. Case fatality tends to increase independently with age and number of comorbidities; indeed, death from AUGIB is rare in younger patients without significant comorbidity (Hearnshaw et al., 2011). This may imply that the reduction in AUGIB mortality among an aging population may be, in part, associated with improvements in treatment and care specific to AUGIB (Abougergi et al., 2015; Crooks et al., 2011; Hearnshaw et al., 2011).

2.1.4 Healthcare costs

Despite the general trend of decreasing hospitalizations and length of stay for AUGIB, the economic burden on healthcare systems is high. In the UK for example, the average total in-hospital costs associated with AUGIB have been estimated at £2,458 (roughly $3,000 USD) per patient (Campbell et al., 2015). When combined with population figures and annual incidence of AUGIB, the total annual cost for initial hospital treatment in the

UK is estimated at roughly £155 million (roughly $190 million), accounting for approximately 0.13% of total spending by the National Health Service (NHS) in the UK (Campbell et al., 2015). Estimates in the USA are proportionally as high; AUGIB accounts for over $1 billion in direct medical costs (Wuerth & Rockey, 2017), and data from the last two decades indicate this cost is rising. At the individual patient level, the median cost associated with AUGIB hospitalization increased from $9,249 in 1989 to $20,370 in 2009. At the national level, the direct in-hospital cost of AUGIB also increased between 1989 and 2009, from $3.3 billion/year to $7.6 billion/year (Abougergi et al., 2015). Over this same time period, average length of hospital stay has fallen for patients with AUGIB, from approximately 4.5 days to 2.9 days, as well as overall hospitalization rate, from 81 to 67 cases / 100,000 per year (Abougergi et al., 2015; Wuerth & Rockey, 2017). This indicates that the increased economic burden is not simply due to an absolute increase in AUGIB hospitalizations, or length of hospital stay. As suggested above, evidence for the improvement in patient outcomes, despite the increasing age and associated poorer health of the average AUGIB patient, may be indicative of improvements in AUGIB-specific treatment modalities. The increased health care spending associated with AUGIB hospitalization may reflect these improvements.

Costs and burden associated with AUGIB extend beyond use of in-hospital treatments and interventions. In the UK, post-discharge health and social care costs account for an additional £391 per patient. Lost working days averaged 11.6 days across all patients by day 28 post-discharge, corresponding to a cost of £275/patient in productivity losses (Campbell et al., 2015). Further, patients with AUGIB report health-related Quality of Life (HRQoL) scores lower than that of the general population in the UK (EQ-5D scores of 0.735 versus 0.86, respectively); this is likely to confer additional burden and use of resources, at both the individual and national level, though this is difficult to quantify (Campbell et al., 2015).

2.2 Risk Factors for AUGIB

2.2.1 Age

Patients with AUGIB are likely to be older. In the UK, the median age of AUGIB patients is 68 years, although this estimate is higher for in-patient bleeds (77 years) compared to new admissions (65 years) (Hearnshaw et al., 2011). This is consistent with trends in North America; the average age of patients with AUGIB in Canada is 66 to 67 years, and more than half the patients hospitalized for AUGIB in the US are over 65 years (Barkun et al., 2004; Wuerth & Rockey, 2017) There is an increase in incidence with age (Hearnshaw et al., 2011; Hreinsson et al., 2013), and increasing age is a significant predictor of mortality both for patients with variceal and non-variceal bleeding (Crooks et al., 2011). Thus, it is not surprising that in-patients, who are considerably older than new admissions, show significantly higher rates of mortality and re-bleeding (Crooks et al., 2011; Hearnshaw et al., 2011).

Age as a risk factor becomes increasingly relevant as the proportion of elderly patients in the population rises. In the 1993/1994 nationwide UK audit of AUGIB, only 32% of in-patients were over 80 years old. In the 2007 audit, which was conducted with almost identical methodology, this figure had risen to 42% (Hearnshaw et al., 2011). In a study of the Canadian Registry of patients with Upper Gastrointestinal Bleeding and Endoscopy (RUGBE), the odds of mortality in AUGIB patients with high-risk stigmata increased by 50% for each 10 year increase in age (Barkun et al., 2004). Given that comorbid illness is also a risk factor for AUGIB and associated adverse outcomes, and there is a logical correlation between age and comorbid illness, it is possible that the trend between AUGIB incidence and mortality, and increasing age, is partially explained by increasing number of comorbidities in the elderly. Nonetheless, multivariable analyses still report a non-linear trend for increasing age and all-cause mortality in patients with AUGIB, even after adjustment for other factors such as number of comorbidities (Crooks et al., 2011; Rockall, Logan, Devlin, & Northfield, 1996)

2.2.2 Co-Morbid illness

Approximately half of emergency admissions for AUGIB present with at least one comorbidity, and there is a strong association between severity and/or number of comorbidities and mortality (Hearnshaw et al., 2011). In the Canadian RUGBE study, patients with greater than two comorbidities had almost three times the odds of mortality relative to patients with two or fewer comorbidities (Barkun et al., 2004). Renal failure, liver failure, and malignancy carry a particularly high risk of mortality and re-bleeding, with cardiac failure, ischemic heart disease, and any other major comorbidity lending a slightly lower but still significant risk (Blatchford et al., 1997; Hearnshaw et al., 2011; Rockall et al., 1995).

As mentioned previously, there is a clear association between increasing age and number of comorbidities, and comparisons between the 1993/1994 and 2007 national UK audits show an increase in the average number of comorbidities for patients with AUGIB (Hearnshaw et al., 2011). Accordingly, consideration of baseline health status and comorbid illness, and how this may influence appropriate management and treatment, has become increasingly relevant for attending physicians.

2.2.3 Medications

Given the substantial co-morbidity amongst patients with AUGIB, use of multiple medications is not uncommon, and their potential contribution to the development of GI bleeds and complications is relevant. Aspirin and other non-steroidal anti-inflammatories (NSAIDs) are some of the more commonly reported medications, as well as anticoagulant and antiplatelet agents. In the 2007 UK audit of AUGIB, one in three new admissions was taking aspirin, clopidogrel (an antiplatelet), or warfarin (an anticoagulant) at presentation (Hearnshaw et al., 2011).

2.2.3.1 Aspirin and other non-selective non-steroidal anti-inflammatories (NSAIDs)

Aspirin is a type of NSAID, and is frequently used for its analgesic, anti-inflammatory, and antiplatelet properties. Aspirin and other NSAIDs are the most commonly prescribed medications among patients with AUGIB, with up to 28% of patients having used, or

recently used, aspirin at presentation for GI bleeding (Hearnshaw et al., 2011; Lanas et al., 2006). The highest use is reported in patients presenting with peptic ulcer bleeding—a nonvariceal source of upper GI bleeding due to complication of sores present in the upper GI tract. In the Netherlands, up to 50% of patients with peptic ulcer disease were currently using aspirin or other NSAIDs (Hearnshaw et al., 2011; van Leerdam et al., 2003).

There is a well established association between NSAID use and GI bleeding— users of aspirin and other NSAIDs are 4.7 to 5.3 times more likely to develop AUGIB than non-users (Rodríguez & Jick, 1994; Hreinsson et al., 2013; Lanas et al., 2006; Sostres, Gargallo, & Lanas, 2013). Although the relative risk for aspirin users is approximately the same as the overall relative risk for all NSAIDs, these estimates diverge when comparing individual non-aspirin NSAIDs. For example, ibuprofen, diclofenac, aceclofenac and naproxen have been shown to be some of the least toxic NSAIDs with respect to GI ulcers and perforation, with relative risk estimates of approximately 4.2 or less (Baigent et al., 2013). Conversely, the risk associated with use of NSAIDs such as piroxicam, azapropazone, or ketorolac can be substantially higher than the risk associated with NSAIDs overall, with reported relative risks as high as 14.4 (Rodríguez & Jick, 1994; Lanas et al., 2006). Importantly, the risk associated with NSAID use is dose-dependent, with higher doses and longer half-lives increasing the risk of upper GI ulcers, bleeds, or perforation (Lanas et al., 2006).

2.2.3.2 COX-2 Inhibitors

Coxibs, or cyclooxygenase-2 (COX-2) inhibitors, are a class of NSAID that selectively target the COX-2 enzyme, and are meant to reduce inflammation and swelling by acting on COX-2, without interfering with the GI-protective effects of the COX-1 enzyme (Brooks, Rijswijk, Warner, & Zeidler, 1999). Accordingly, current users of non-selective NSAIDs have a reduced risk of AUGIB relative to current users of coxibs, although non-users still have less than half the risk of coxib users (Baigent et al., 2013; Lanas et al., 2006). In the Canadian RUGBE cohort, 3.3% of patients were taking COX-2 inhibitors at presentation.

However, concomitant use of coxibs with aspirin or other antiplatelet agents negates the safety benefits of independent coxib use, suggesting that the antiplatelet effect of aspirin, even at low doses, is a principal mechanism leading to GI lesions (Lanas et al., 2006).

2.2.3.3 Concomitant use of NSAIDs with proton pump inhibitors

Proton pump inhibitors (PPIs) are a class of strong acid suppressants, and are frequently prescribed in the treatment of gastritis, duodenitis, and most ulcers (van Leerdam et al., 2003). Use of oral PPIs is associated with a significantly lower risk of AUGIB relative to non-users; when used concomitantly with NSAIDs, PPIs minimize the NSAID's harmful effect on the stomach lining, and combats the increased risk of GI hemorrhage that is characteristic of NSAID use (Lanas et al., 2006).

2.2.3.4 Anticoagulants

11% of patients in the Canadian RUGBE cohort were taking heparin at hospital presentation (Barkun et al., 2004), and approximately 7% of patients with AUGIB in the UK are taking warfarin around the time of presentation to hospital (Hearnshaw et al., 2011). Warfarin is one of the more commonly prescribed dicumarinics— a class of oral anticoagulants. Use of dicumarinics increases the risk of AUGIB in a dose-dependent fashion (Lanas et al., 2006).

Novel oral anticoagulants (NOACs) such as dabigatran and rivaroxaban are now frequently prescribed as alternatives to warfarin. This practice is increasingly popular for patients with AUGIB. Previous studies have reported some concern that NOACs increase the rate of GI bleeds in excess of that seen for warfarin use (Holster, Valkhoff, Kuipers, & Tjwa, 2013). A recent population-based cohort study comparing NOACs to warfarin found that similar rates of bleeding were reported among NOAC users and warfarin users overall, but that rate of bleeding may increase with age at a greater rate for users of NOACs compared to warfarin (Abraham et al., 2015).

Overall, use of anticoagulants is associated with roughly a four-fold increase in risk of AUGIB relative to non-users, surpassing that of other commonly reported medications such as antiplatelet agents, aspirin, and some individual NSAIDs (Lanas et al., 2015). In

light of the global aging population, this finding is particularly relevant, as the proportion of patients with AUGIB taking an anticoagulant is likely to increase.

2.2.3.5 Antiplatelets

Use of clopidogrel is reported in 3.9 – 5.3% of patients with AUGIB (Hearnshaw et al., 2011). Clopidogrel and ticlopidine belong to a class of antiplatelet agents known as thienopyridines, and are associated with as much as a 2.8 times greater risk of AUGIB relative to non-users (Lanas et al., 2006; Lanas et al., 2015) .

When used concomitantly with aspirin or other NSAIDs, a synergistic interaction produces an excess risk of AUGIB beyond what would be expected from a simple additive effect of the two drugs. That is, estimates of relative risk for NSAID users and antiplatelet users are 5.3 and 2.9, respectively; when used in combination, the relative risk of AUGIB jumps from 15.2 to 16.4 (Lanas et al., 2006). A similar effect is seen for concomitant use of aspirin and thienopyridines.

2.2.4 Helicobacter Pylori infection

Helicobacter pylori (H. pylori) infection is one of the most important risk factors for the development of peptic ulcer disease. The lifetime risk of peptic ulcer among those infected ranges from 3 to 25% (Suerbaum & Michetti, 2002). H. pylori is a common bacterial infection; roughly 26% of the population in the UK has a positive serology for H. pylori, with infection more common among men, smokers, and older populations (Jackson et al., 2009). Prevalence is also strongly associated with socioeconomic status, estimated between 20% and 50% in industrialized countries, but as high as 80% in some developing countries (Jackson et al., 2009; Suerbaum & Michetti, 2002).

Without intervention, H. pylori can cause chronic gastric inflammation, leading to the development of peptic ulcer, ulcer-related bleeding or perforation, and gastric cancer (Yip & Teoh, 2018). Thus, timely eradication of H. pylori via antibiotic therapy is essential in AUGIB patients with positive H. pylori serology. When eradication treatment is delayed from a month to a year or longer, the risk of recurring ulcers and ulcer-related hemorrhage increases by a factor of 3.55 and 6.14, respectively (Yip & Teoh, 2018).

The independent contribution of H. pylori infection to pathogenesis of peptic-ulcer disease (PUD) can be difficult to tease apart from other risk factors—namely, use of NSAIDs. NSAID use and H. pylori infection are widely regarded as the two most prevalent risk factors in PUD, and subsequent complication and bleeding (Lanas & Chan, 2017). Previous studies have estimated a three- to four-fold increase in the risk of peptic ulcer disease associated with H. pylori infection and use of NSAIDs, without a clear distinction between the individual contributions of each factor, and their relevance to ulcer bleeding. More recently, evidence indicates that H. pylori may only marginally increase the risk of ulcer bleeding relative to NSAID use, as those infected with H. pylori were 1.79 times more likely to experience ulcer bleeding, irrespective of NSAID use. However, the odds of NSAID use among patients with bleeding ulcer were 4.85 times higher than that of controls. When considered together, there appears to be an additive interaction between H. pylori infection and NSAID use (Huang, Sridhar, & Hunt, 2002).

2.2.5 Socioeconomic deprivation

Socioeconomic disadvantage is associated with higher incidence of AUGIB and higher risk of mortality. A study conducted in Scotland over twenty years ago showed the incidence of AUGIB was 2.2 times greater in the most socioeconomically deprived quarter than in the least deprived quarter of the study region, as defined by the Carstairs deprivation score (Blatchford et al., 1997). Mortality in the least deprived quarter was found to be double that of the most affluent quarter (Blatchford et al., 1997). More recent studies of healthy inequality across all of England show that some of these trends still hold: those who live in the most disadvantaged areas show a two to three times higher rate of hospitalization for AUGIB than those who live in the most affluent areas, though there does not seem to be any significant trend in 28-day mortality rates by socioeconomic status. Overall, socioeconomic deprivation seems to be indirectly responsible for as many as 10,000 excess hospitalizations and 1,000 excess deaths due to AUGIB (Crooks, West, & Card, 2012).

It is likely that this association between socioeconomic status and incidence of AUGIB is mediated by known and modifiable risk factors. For example, given the strong association between H. pylori prevalence and socioeconomic deprivation, it is possible

that the association between socioeconomic status and AUGIB is at least partly explained by peptic ulcer disease resulting from H. pylori infection (Suerbaum & Michetti, 2002).

2.3 Etiology of AUGIB

There are a number of different underlying etiologies for AUGIB. The primary cause of bleeding is often broadly categorized as variceal or non-variceal in origin because of their distinct prognoses and treatment strategies. For non-variceal AUGIB, peptic ulcer disease is the most common etiology, accounting for 36% of cases in the UK and almost 50% of cases in the US (Barkun et al., 2004; Hearnshaw et al., 2011; Wuerth & Rockey, 2017). Other causes of non-variceal bleeding, such as oesphagitis, gastritis, and duodenitis account for approximately 9%, 7%, and 2% of cases, respectively.

Bleeding from varices is a consequence of liver disease and cirrhosis of the liver, and formation of gastroesophageal varices occurs in at least 50% of cases, with an overall case fatality rate of around 15% (Jairath et al., 2014). Variceal bleeding and GI malignancy (accounting for just under 4% of cases), are associated with the highest case fatality rates among patients with AUGIB. These causative lesions are not necessarily mutually exclusive; about 30% of patients have more than one endoscopic diagnosis (Hearnshaw et al., 2011).

It is worth noting that figures arising from the 2007 UK audit may not represent prevalence in other patient populations. For example, PUD is consistently reported as the most common cause of AUGIB, yet it appears to be significantly more common in some countries (e.g., the Netherlands, with PUD accounting for 50% of AUGIB cases), and less common in others (e.g., the US, with PUD accounting for 30% of cases) (van Leerdam et al., 2003; Wuerth & Rockey, 2017). However, it is difficult to make conclusive claims on differing distributions between populations, as differing case definitions and methodologies may explain many of these differences.

2.3.1 Peptic ulcer disease

Due to its presence in the vast majority of non-variceal AUGIB, international guidelines often equate non-variceal GI hemorrhage treatment to peptic ulcer disease treatment (A.

N. Barkun et al., 2010). Although PUD remains the most common diagnosis for AUGIB, accounting for 40 – 60% of cases (Lanas et al., 2011) the incidence has declined significantly over the past two decades. From 2002 to 2012, a nationwide analysis in the USA found that the hospitalization rate of PUD decreased from 41 to 30 cases / 100,000 population, a 30% decline (Wuerth & Rockey, 2017). Similar trends of declining prevalence have been found in the UK. Between 1994 and 1998, the rate of PUD incidence dropped by more than 50% among patients aged less than 65 years old. Declining incidence among patients over 65 years old was not as steep, but still significant—33% and 29% for females and males, respectively (Kang, Tinto, Higham, & Majeed, 2002). In the UK, PUD is almost twice as prevalent in the most deprived areas than in the least deprived areas (Kang et al., 2002).

The decline in PUD hospitalization and complication is often attributed to the discovery and eradication of H. pylori infection, as well as the introduction of acid suppressants such as proton pump inhibitors (Pérez-Aisa, Del Pino, Siles, & Lanas, 2005; Wuerth & Rockey, 2017).

2.3.2 Variceal bleeding

Variceal hemorrhage typically arises because of portal hypertension (a pathological increase in the pressure gradient within the portal venous system) in patients with cirrhosis of the liver. Among industrialized countries, approximately 90% of cases of portal hypertension are a result of cirrhosis of the liver (Bosch, Abraldes, Berzigotti, & Garcia-Pagan, 2008; Stanley & Hayes, 1997). Variceal hemorrhage is one of the most severe complications of portal hypertension, and AUGIB patients with advanced liver disease and cirrhosis are at a particularly high risk of further complication and mortality (Bosch et al., 2008; Hearnshaw et al., 2011; Jairath et al., 2014). The risk of variceal bleeding increases with the severity of liver disease, with the majority of bleeds occurring from esophageal varices (Bosch et al., 2008).

In 2007, variceal bleeding accounted for approximately 11% of cases of AUGIB in the UK. This figure is more than twice as large as that reported in the 1995 AUGIB audit (Hearnshaw et al., 2011). In the US however, figures from a 2002 - 2012 nationwide

analysis indicated that hospitalization for variceal hemorrhage has remained relatively consistent (Wuerth & Rockey, 2017), although direct comparisons are difficult due to differing methodologies and source populations. In the UK, the increasing frequency of variceal bleeding is presumed to be linked to the rising prevalence of alcohol abuse (Hearnshaw et al., 2011).

Regardless, variceal bleeding is consistently associated with a higher risk of mortality and re-bleeding than most non-variceal bleeds, with case fatality reaching upwards of 20% in the UK, in part due to the high probability of comorbid liver disease (Crooks et al., 2011; Hearnshaw et al., 2011; Jairath et al., 2014). Risk of death rises substantially for cirrhotic patients who re-bleed (Jairath et al., 2014). As a result, immediate intervention and proper management is particularly important for patients with comorbid liver disease and suspected variceal hemorrhage.

2.4 Processes of care for AUGIB

Patients presenting with AUGIB in hospital are promptly assessed to determine their immediate risk of adverse outcomes, such as mortality and re-bleeding. Re-bleeding, an adverse event in itself, is also a strong predictor of mortality, and thus preventing its re-occurrence is a principal priority (Palmer, 2011; Rockall et al., 1996).

2.4.1 Risk assessment

Patients admitted emergently with suspected AUGIB should be promptly assessed to determine their risk of further adverse events and outcomes. A validated risk assessment tool is preferred, such as the complete Rockall score or Glasgow-Blatchford score. The complete Rockall score (RS) is usually calculated following endoscopy, and considers factors such as age, presentation of shock, number and severity of comorbidities, and primary diagnosis. Higher RS indicate a higher risk of adverse outcome, and necessitate more immediate and intensive intervention (Rockall et al., 1996). The RS is limited by its reliance on endoscopic findings, and thus a pre-endoscopy Rockall score (pRS), which does not require endoscopic information, is often utilized in acute settings.

The Glasgow-Blatchford bleeding score (GBS) functions similarly to the Rockall score, but is unique in its intention to identify patients in need of clinical intervention, rather than identifying those at risk of death. In addition to factors such as presence of cardiac failure and/or liver disease, the GBS also considers laboratory data such as blood urea, Hb concentration, pulse, and systolic blood pressure, as well as clinical symptoms such as melena and syncope, to identify patients requiring clinical intervention (Blatchford, Murray, & Blatchford, 2000). Because the GBS and RS work to identify different patient groups (i.e., those at risk of death versus those in need of treatment), current guidelines recommend calculating both. However, comparisons of the performance of these two scores suggest the GBS outperforms the pRS at predicting mortality and composite poor outcomes, as well as the need for RBC transfusion (Oakland et al., 2019; Stanley et al., 2011).

Development of a new international scoring system (the CANUKA score) may be particularly valuable in reliably identifying patients at low risk of mortality or harm. The ability to identify low-risk patients is important, as it allows quick discernment of patients that do not need to be admitted, and can be treated on an outpatient basis, avoiding the unnecessary costs and resources associated with hospitalization (Oakland et al., 2019).

2.4.2 Endoscopy

Early endoscopy is a well-established clinical practice for patients with suspected AUGIB, and is performed in over three-quarters of patients. It is highly useful as a means of diagnosing the primary cause of bleeding, informing prognosis, and allowing for endoscopic intervention (Hearnshaw et al., 2011; Kurien & Lobo, 2015; van Leerdam et al., 2003). Use of endoscopy is increasing, with endoscopy being performed for more patients, and earlier in hospitalization (Abougergi et al., 2015; Hearnshaw et al., 2011; van Leerdam et al., 2003). It has been purported that early endoscopy is associated with improved patient outcomes (Hearnshaw et al., 2011; van Leerdam et al., 2003), though this is contradicted by a nationwide study showing no difference in mortality or re-bleeding between patients who underwent endoscopy within versus beyond 12 hours of presentation (Jairath et al., 2012). Regardless, intensive resuscitation should take priority

in an emergency setting, and endoscopy should not be performed until unstable patients are resuscitated and managed (Kurien & Lobo, 2015).

2.4.3 Initial resuscitation

Initial resuscitation following the principles of airway, breathing, and circulation (ABC) is the most primary concern when a patient presents to an emergency room with suspected AUGIB; patients who receive earlier intervention for correction of hemodynamics, hematocrit, and coagulopathy have a reduced risk of mortality relative to those who do not receive, or receive delayed intensive initial resuscitation (Baradarian et al., 2004). Restoring hemodynamic stability typically necessitates intravenous fluid replacement with colloids or crystalloids. In some circumstances, early RBC transfusion is necessary to manage further bleeding and maintain oxygen uptake and delivery (Kurien & Lobo, 2015; Palmer, 2011). RBC transfusion specific to patients with AUGIB will be discussed further in a subsequent section.

2.5 Red blood cell transfusion

Early RBC transfusion is a life-saving intervention for patients with massive exsanguinating haemorrhage. However, among patients with less severe bleeding, indications for RBC transfusion are not as clear. In these cases, the decision to transfuse is often dictated by the patient's blood Hb concentration; if a patient's Hb falls too low, a RBC transfusion may be used to treat anemia, and maintian global oxygen delivery. This decision is largely influenced by severity of bleeding, patient characteristics, clinical guidelines and clinician discretion. Historically, clinical guidelines have endorsed a RBC transfusion threshold, of 10g/dL—that is, transfusion is recommended when Hb concentration falls below 10g/dL, and the patient may be liberally transfused until Hb concentration is restored to 10 to 12 g/dL (Jairath, 2013).

While the effects of anemia can be severe, RBC transfusion comes with its own risks of harm and adverse events. A large Canadian observational study found that patients with nonvariceal AUGIB who received a RBC transfusion showed a significantly higher risk of re-bleeding, and a trend towards increased risk of mortality, though this effect was not significant (Restellini et al., 2013).

Supporting these findings is a growing body of evidence across a diverse set of patient populations showing that restrictive RBC transfusion strategies (i.e., guidelines with lower Hb thresholds) may be just as safe as, and potentially favourable to liberal RBC transfusion strategies, corroborating the notion that RBC transfusion could confer more harm than it alleviates (Holst et al., 2015).

In a large, multi-centre randomized controlled trial (RCT) of critically ill, non-bleeding patients, a restrictive RBC transfusion threshold of 7 g/dL, with a post-transfusion target of 7 to 9 g/dL, was found to be at least as safe as a liberal RBC transfusion threshold of 10g/dL (Hebert et al., 1999). Importantly, rates of mortality were significantly lower in the restrictive group versus the liberal group in a sub-group analysis of those who were younger and less acutely ill (i.e., with an acute Physiology and Chronic Health Evaluation II score of ≤ 20, and age less than 55 years), suggesting that lower Hb concentrations may be especially tolerable among healthier patients (Hebert et al., 1999). Similar evidence for the safety of restrictive RBC transfusion thresholds has also been shown among critically ill children—paediatric intensive care unit (ICU) patients assigned to a restrictive RBC transfusion policy (i.e., Hb concentration < 7 g/dL) showed no statistically significant differences in multiple-organ dysfunction syndrome, or any other adverse events, relative to patients assigned to a liberal RBC transfusion policy (i.e., Hb concentration < 9.5 g/dL) (Lacroix et al., 2007).

This pattern holds even amongst patient groups that one might expect to be particularly vulnerable to symptoms of anemia. In the Transfusion Trigger Trial for Functional Outcomes in Cardiovascular Patients Undergoing Surgical Hip Fracture Repair (FOCUS), there were no significant differences between patients assigned to a restrictive RBC transfusion strategy (i.e., threshold of 8g/dL) versus a liberal RBC transfusion strategy (i.e., threshold of 10 g/dL) with respect to clinical outcomes such as mortality, ability to walk 60 days post-surgery, myocardial infarction, and length of hospital stay (Carson et al., 2011). The lack of benefit to liberal RBC transfusion even among such a high-risk group of patients, with an average age of over 80 years and a history of cardiovascular disease, is highly suggestive of the safety of lower Hb thresholds, especially among younger and healthier patients.

However, there are still inconsistencies and contradictions concerning restrictive RBC transfusion strategies within the research literature. An RCT of high-risk oncological surgery patients found a liberal RBC transfusion strategy (i.e., a Hb threshold of 9g/dL) to be significantly safer than a restrictive transfusion strategy (i.e., a Hb threshold of 7g/dL) with respect to outcomes such as mortality and severe clinical complications (de Almeida et al., 2015). Similar findings evidencing the potential benefit of RBC transfusion in septic patients have also been seen in observational studies and propensity-matched analyses (Park et al., 2012; Sakr et al., 2010). There are several possible explanations for the variable results across the literature. First, different study populations may be differentially tolerant of anemia and RBC transfusions, resulting in inconsistent outcomes between treatment assignments. Second, type of blood used for transfusion and the storage time of the blood is rarely uniform, and could partly explain some of the fluctuation in the effects of RBC transfusion across trials (de Almeida et al., 2015). The third, and perhaps most conspicuous explanation, is the inconsistency in operational definitions of “restrictive” versus “liberal” RBC transfusion strategies. In a systematic review and meta-analysis of 31 randomized trials, the threshold values for the restrictive group varied from Hb concentration 7.0 g/dL to 9.7 g/dL, hematocrit 24% to 30%, or symptoms of anemia. The thresholds for the liberal RBC transfusion group varied from Hb concentration 9 g/dL to 13 g/dL, and hematocrit 30% to 40% (Holst et al., 2015). Therefore, it is possible that the uncertainty around the safety of restrictive RBC transfusion strategies can be at least partly owed to the uncertainty around the definition of a restrictive RBC transfusion threshold. Furthermore, the quality of evidence of many transfusion trials is jeopardized by small sample sizes, large confidence intervals, and lack of power. Despite these limitations, a trial sequential analysis of all restrictive RBC transfusion trials indicated that it is unlikely that restrictive transfusion strategies are associated with overall harm (Holst et al., 2015).

2.5.1 Restrictive thresholds in patients with AUGIB

Patients with AUGIB represent a unique subpopulation in patients eligible for RBC transfusion, and transfusion requirements may differ in this group. As mentioned previously, patients with AUGIB tend to be elderly and in relatively poor health; the

burden of comorbid disease is high and often associated with concomitant use of anticoagulants and other medications with hemodynamic effects, and development of anemia and hemodynamic instability can occur rapidly (Odutayo et al., 2017).

Not surprisingly, AUGIB is one of the leading indications for RBC transfusion, and accounts for 10 – 13% of RBC units transfused in the UK (Tinegate et al., 2013; Wallis, Wells, & Chapman, 2006). At a price of £128.99/RBC unit in the UK, the movement to more restrictive RBC transfusion policies for patients with AUGIB could result in savings of £2.48 million or more, and have monumental implications on finances and resources for health-care organizations (Campbell et al., 2015; Jairath et al., 2015; National Health Service, 2018).

Encouragingly, the results of a number of trials suggest that restrictive RBC transfusion strategies can be safely applied in patients with non-exsanguinating AUGIB. The first large-scale study—a single-centre RCT in Spain—of transfusion strategies in patients with AUGIB found that patients assigned to a restrictive RBC transfusion threshold of 7 g/dL had a reduced rate of re-bleeding, mortality, and length of hospital stay relative to those assigned to a liberal RBC transfusion threshold of 9 g/dL (Villanueva et al., 2013). More recently, a large pragmatic multi-centre study in the UK (the TRIGGER trial) showed similar results—a restrictive RBC transfusion threshold of 8 g/dL was found to be at least as safe as a liberal threshold of 10 g/dL, with respect to patient outcomes such as mortality, re-bleeding, length of hospital stay, and other serious adverse events. Although trends in the outcome data favoured the restrictive policy, there was no statistically significant difference in outcomes between the patients assigned to the restrictive versus liberal RBC transfusion policy. (Jairath et al., 2015).

There are a number of possible explanations for why more liberal RBC transfusion may be harmful among patients with AUGIB. First, RBC transfusion may interfere with normal coagulation in patients with an acute GI bleed. Results from a small clinical trial found that patients with GI hemorrhage may show an acute hypercoagulable response to bleeding, as evidence by shortened clotting times [e.g., the Biobridge Impedance Clotting Time (ICT) and Koalin Cephalin Clotting Times (KCCT)]. RBC transfusion, however,

appears to negate this effect—patients that received an early RBC transfusion (i.e., within 24 hours of admission) showed significantly longer clotting times than patients who had not received a RBC transfusion (Blair, Janvrin, McCollum, & Greenhalgh, 1986). Importantly, this practice of early RBC transfusion and subseqeuent reversal in the hypercoagulation response was associated with increased rates of re-bleeding (Blair et al., 1986). Second, possible circulatory overload associated with RBC transfusion could be particularly dangerous among patients with ischemic heart disease and patients with liver cirrhosis (through the worsening of portal hypertension), two significant subpopulations within AUGIB patients (Restellini et al., 2013). Third, varying methods of blood storage could result in adverse changes to RBC units, and reduce their capacity to deliver oxygen (Restellini et al., 2013). Lastly, the pro-inflammatory and immunomodulatory consequences arising from RBC transfusion could significantly inflate a patient's risk of acquiring an in-hospital infection, and subsequently impede an uncomplicated recovery (Odutayo et al., 2017).

Aside from the Villanueva et al. (2013) and TRIGGER trials, the remainder of evidence informing restrictive RBC transfusion strategies in AUGIB comes from observational studies and a few small, underpowered RCTs. Although a pooled analysis of these studies indicate there is no clear benefit to employing a liberal RBC transfusion strategy in the management of AUGIB, like the broader field as a whole, uncertainty still exists as to the overall treatment effects of restrictive RBC transfusion strategies (Odutayo et al., 2017). Studies of RBC transfusion strategies have been primarily limited to comparisons of restrictive versus liberal strategies without consistent Hb thresholds. Consequently, the only conclusions that can be drawn from such comparisons is that one threshold is better than, or noninferior to the other. As mentioned previously, the definition of a restrictive RBC transfusion threshold is vague; in the broader field of restrictive transfusion strategies overall, the restrictive threshold values for some studies are actually larger than the liberal threshold values in others. As a result, conclusions on the safety of restrictive transfusion strategies are somewhat arbitrary, and do not address the more relevant question of the *optimal* Hb threshold for RBC transfusion. Furthermore, studies of RBC transfusion strategies have paid little attention to the association between the amount of blood transfused and clinical outcomes, resulting in further uncertainty on best practices

once RBC transfusion has been indicated.

2.6 Aims and outline of thesis

The aim of this thesis is to address the two important gaps in the literature on restrictive RBC transfusion strategies in patients with AUGIB; that is, the lack of attention to a potential critical Hb threshold, and optimal volume of blood for RBC transfusion. This aim was achieved by answering two specific research objectives:

(1) Investigate if there is an *optimal* Hb threshold to guiding RBC transfusion decisions among patients with non-exsanguinating AUGIB;

(2) Investigate *how much blood* should be administered to non-exsanguinating AUGIB patients receiving an RBC transfusion.

This project will be unique in its treatment of Hb concentration as a continuous variable, rather than a dichotomous measure, in establishing specific Hb thresholds and targets that optimize clinical outcomes.

Chapter 3

3 Methods

To address the research questions outlined in Chapter 2, data from the 2015 Transfusion in Gastrointestinal Bleeding Trial (TRIGGER) (Jairath et al., 2015) was acquired and reanalyzed. The TRIGGER trial, published in the *Lancet* in 2015, was a multi-center, cluster randomized feasibility trial of six university hospitals in the UK, comparing restrictive versus liberal RBC transfusion protocols in patients with AUGIB. Presented below is a brief description of the original TRIGGER trial, and methods used for the current data analysis to address the research questions of this thesis.

3.1 TRIGGER trial

The objective of the TRIGGER trial was to investigate the safety of restrictive RBC transfusion protocols for patients with non-exsanguinating AUGIB, and to assess whether implementing these protocols in routine clinical practice is pragmatic and feasible. The TRIGGER trial collected data on clinical characteristics and outcomes, as well as feasibility outcomes such as adherence to the study protocol and recruitment rates. Given the clinical nature of the current research objectives, feasibility outcomes were not directly relevant and were not included in my secondary analysis of the dataset.

3.1.1 Eligibility and recruitment

Eligibility criteria at the cluster level required that the center have:

- Greater than 20 admissions with AUGIB per month
- Greater than 400 hospital beds
- 24-hour access to endoscopy, on-site intensive care, and surgical support

Within each center, patients were recruited from emergency departments and acute admissions units over the span of 6 months, and patients were eligible for inclusion in the trial if they were aged 18 years or older, and presented with AUGIB (defined by symptoms of hematemesis or melena). Patients were excluded from eligibility if they met any of the following criteria:

- Deemed to be in immediate need of RBC transfusion regardless of initial Hb

- Had a known myocardial infarction or cerebrovascular event within 30 days prior to admission
- Pregnant
- Unable or refused to receive blood components
- Existing hospital in-patient

Written informed consent was sought from all participating patients, or the representative responsible for making decisions on their behalf. At the end of the recruitment period, 936 patients had been enrolled, 905 of which were followed up on further bleeding up until day 28 of the study. All patients, physicians and staff were unmasked to the treatment assignment of the hospital and its respective participating patients.

3.1.2 Randomization and intervention protocols

To reduce the possibility of contamination between interventions, and simplify intervention delivery, randomization occurred at the cluster level; each center was assigned to either the restrictive or liberal RBC transfusion protocol, and all enrolled patients were treated according to the protocol assigned at cluster randomization. Presenting Hb values were recorded for all patients following enrolment. In keeping with a pragmatic trial design, clinicians were able to deviate from this protocol at their own discretion whenever deemed necessary, and simply document their reasons for protocol deviations.

3.1.2.1 Restrictive protocol

Patients assigned to the restrictive RBC transfusion group were transfused with red blood cells if their presenting Hb was ≤ 8.0g/dL, with the aim of restoring Hb to within 8.0 – 10.0g/dL. The number of RBC units transfused, and the timing of subsequent Hb measurements was decided by the responsible physician. Further transfusion of red blood cells was warranted by the trial protocol only when Hb fell to a value of 8.0g/dL or less. This practice was continued for the remainder of the patient's hospital stay, up until death, hospital discharge, or day 28 after enrolment (i.e., whichever came first).

3.1.2.2 Liberal protocol

Patients assigned to the liberal RBC transfusion group were transfused with red blood cells if their presenting Hb was ≤ 10 g/dL, with the aim of restoring Hb to within 10.1 – 12.0 g/dL. The number of RBC units transfused, and the timing of subsequent Hb measurements was decided by the responsible physician. Further transfusions of red blood cells were warranted by the trial protocol only when Hb fell to a value of 10.0 g/dL or less. This practice was continued for the remainder of the patient's hospital stay, up until death, hospital discharge, or day 28 after enrolment (i.e., whichever came first).

3.1.3 Baseline clinical measures

Baseline clinical characteristics were gathered for all patients admitted with AUGIB and enrolled in the trial. These data included, but were not limited to, age, biological sex, blood pressure, heart rate, Rockall score, Blatchford score, current medications, and various biochemical laboratory measures.

3.1.4 Data collected during intervention period

Following recording of baseline variables and characteristics, routine medical records were also utilized to provide information on all laboratory Hb values measured, all RBC transfusion episodes, total volume of intravenous fluid prescribed, platelet and/or fresh frozen plasma transfusions, date and time of index endoscopy, endoscopic diagnosis, stigmata of hemorrhage, therapeutic procedures, infusion of PPIs, antibiotics, or vasopressin or analogue.

3.1.5 Clinical outcome measures

Where possible, data was collected on the following clinical outcomes up until day 28 from hospital presentation:

- Further bleeding
- Death
- Need for therapeutic intervention at index endoscopy
- Need for surgery or radiological intervention
- Thromboembolic and/or ischaemic events (e.g., myocardial infarction, stroke, pulmonary embolus, deep vein thrombosis)
- Transfusion reactions

- Infections
- Length of hospital stay
- Health-related quality of life (using EuroQol EQ-5D)

Clinical outcome measures were recorded up until hospital discharge or death. After hospital discharge, participants were contacted at study day 28 to establish if there were any further episodes of re-bleeding, mortality, infections, thromboembolic, or ischemic events since discharge. Health-related quality of life was also measured at this time using the EuroQoL-5D questionnaire.

3.2 Statistical Analyses

The original dataset from the TRIGGER trial was acquired from the Joint Chief Investigator, and included baseline, intervention, and outcome measures for the 936 patients enrolled in the study. All subsequent secondary analyses of the dataset were performed in STATA 13 (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP).

Since the research questions addressed in this thesis were different from that of the original trial, the variable on which the patients were randomly assigned (i.e., RBC transfusion protocol) was no longer the predictor of interest; as such, the dataset was treated as observational in nature by disregarding clustering and treatment assignments. Accordingly, protocol deviations and measures of adherence were not considered, as these pertained to the original trial design and protocol, and potential confounding variables had to be carefully considered and controlled. Two separate models were built and analyzed to address the two specific objectives of this project: first, to determine if there is an optimal Hb range to indicate RBC transfusion, and second, to determine if there is an optimal amount of RBC units, for patients receiving a RBC transfusion. The specific statistical approaches utilized to address these two objectives are described in sections 3.2.3 and 3.2.4, respectively.

3.2.1 Clinical outcomes

As mentioned previously, the TRIGGER trial recorded a number of clinical outcomes pertaining to adverse health events for each patient. All of the outcomes analyzed in this

study were assessed at study day 28. I chose to focus on a composite outcome of further bleeding or mortality by day 28 as my primary outcome of interest. The decision to use this composite outcome was motivated by two factors.

First, the event rate for both death by day 28 and further bleeding by day 28 were less than 10% in the dataset. This created some concern that with a sample size of less than 1000 patients, and a relatively rare outcome event, traditional logistic regression methods may produce biased estimates and results. To combat this potential bias, I combined these two outcomes into a composite binary outcome of death *or* re-bleeding by day 28, where an event "success" was coded with a value of one, and indicated the patient had re-bled or died by day 28 of the study period.

Second, outcomes of death or re-bleeding are of primary concern for physicians treating patients with AUGIB, and patients who re-bleed are at a 4-5 fold greater risk of death than patients who do not experience further bleeding (Jairath & Barkun, 2012). As such, re-bleeding is a recommended end-point in clinical trials for AUGIB based on expert consensus, and its close association with mortality supports analyzing these two endpoints as one composite outcome (Laine et al., 2010).

Death and re-bleeding are not the only adverse health events experienced by patients with AUGIB. Additional analyses were conducted on a secondary composite outcome of death, re-bleeding, thromboembolic or ischemic events, or any other serious adverse events by study day 28. This was analyzed as a binary indicator for "any adverse event", where patients were coded as positive for the outcome if they experienced any of these events by study day 28.

3.2.2 New variables derived from original dataset

To address the research objectives of the current thesis, it was necessary to derive additional variables that were unavailable in the original trial dataset. These additional variables are described below.

- *Hb_final* is the last Hb measurement taken for a patient before either death or discharge from hospital.

- *Hb_indication* is the Hb measurement that would have been used to inform a RBC transfusion decision for a patient. For transfused patients, this value is the Hb measurement taken most recently before the first RBC transfusion was given. For patients that did not receive any RBC transfusions during the study period, this value is their presenting Hb value. This value may also be referred to as "*initial Hb"*.

- *Fbleed_died_d28* is a binary, composite outcome measuring whether the patient died or re-bled by study day 28, coded "1" in the event of death or re-bleeding, and "0" if the patient neither died nor re-bled by day 28.

- *Aae_d28* (i.e., "any adverse event") is a binary, composite outcome measuring whether the patient died, re-bled, experienced a thromboembolic or ischemic event, or other serious adverse event by study day 28. This variable was coded "1" if the patient experienced any of these events, and "0" if the patient experienced none of these events by day 28.

- *Bleed_temp_sat* is a binary indicator of whether or not temporality requirements for causation were satisfied for timing of RBC transfusion relative to the patient's re-bleeding event. This variable was coded "1" if the patient's re-bleeding event occurred after their first RBC transfusion, or "0" if the re-bleed was before the patient's first RBC transfusion, or if there was uncertainty that the RBC transfusion preceded the re-bleeding event.

    - Additional similar variables pertaining to whether temporality requirements for causation were satisfied were also created for thromboembolic and ischemic events, and serious adverse events.

3.2.3 Establishing temporality

An important consideration in all statistical analyses concerned the temporal sequence of a patient's RBC transfusion relative to occurrence of an outcome event. In order to investigate RBC transfusion as a potential predictor of patient outcomes, the transfusion must have preceded the outcome of interest. This was addressed by comparing time-to-

event data for the outcome of interest against the day of the first transfusion for all patients that received a transfusion and experienced the outcome. This variable was then coded as a binary indicator of whether the temporality requirements of causation were satisfied (as described in section 3.2.2); patients that did not satisfy this requirement were excluded from the analysis, as it was not possible to determine that a transfusion occurring after the outcome did not still adversely affect the patient's prognosis.

3.2.4 Objective 1 analysis

The overarching approach for Objective 1 was to model the association between the patient's Hb at baseline and their clinical outcome, modified by whether or not the patient received at least one RBC transfusion over the course of the study period (i.e., transfusion status). This approach was motivated by the following logic: if there is a certain point on a scale of Hb values at which RBC transfusion is warranted, this will be indicated by an intersection in the predicted probability of adverse outcomes for those that received a RBC transfusion, and those that did not. Above this point (i.e., for higher Hb values), it is expected that risk would be higher for those that received a RBC transfusion than those that did not. Below that intersection (i.e., for lower Hb values), it is expected that risk would be lower for those that received a RBC transfusion relative to those that did not.

Therefore, the relationship was modeled by regressing the log-odds of death or re-bleeding by day 28 on initial Hb, with an interaction term for transfusion status (i.e., at least one RBC transfusion during study period). Because an interaction term was included for initial Hb and transfusion status, the model required some variability for each of these predictors. In the dataset of 936 patients, all patients with an initial Hb of less than 7 g/dL were transfused at some point during the study period; thus, there was no ability to look for an interaction between initial Hb and RBC transfusion in this group, and these 117 patients were excluded from the analyses for the first objective.

The following five steps were used to build the final model:

Step 1: Simple logistic regression was used to model the probability of death or re-bleeding by day 28, as predicted by initial Hb, transfusion status, and the interaction between these two main predictors.

Step 2: Assumptions of linearity and fit of this simple model were assessed. The Hosmer-Lemeshow goodness-of-fit test was used to check for lack of fit, and inclusion of cubic splines and higher order terms were tested to check for inadequacy of the linear model.

Step 3: Potential confounders were considered and adjusted for in the model. First, all baseline covariates controlled for in the original TRIGGER trial were evaluated in bivariate analyses with the outcome, and initial Hb, to check for plausibility as a potential confounder. Covariates that showed no association with Hb or death or re-bleeding were excluded. The remaining variables were sequentially added to the model, and their contribution to the model was assessed using Likelihood Ratio (LR) tests. Covariates that significantly improved the predictive ability of the model (as indicated by an LR test with $p < .05$) were included in the final model.

Step 4: The final model was used to produce predicted probabilities for 802 patients; 17 patients were excluded from the analysis because their transfusion was administered after the event of re-bleeding by day 28. Predicted probabilities were used to plot predictive margins for Hb by transfusion status.

Step 5: Results from the above regression analysis were used to produce equations for the regression lines for both the transfused group and non-transfused group. To derive the point of intersection between these two lines, these two regression line equations were set as equal (i.e., with the same predicted probability), and solved for the Hb value at which these two lines intersected.

3.2.5 Objective 2 analysis

The overarching approach for Objective 2 was to model the association between the number of RBC units a patient received over the study period and clinical outcome. This approach was motivated by the following logic: if there is an optimal amount of blood to give a patient, or an optimal post-transfusion Hb target, this would likely be reflected in

the association between amount of RBC units received and probability of re-bleeding or death. If there is an optimal range to aim for when transfusing a patient, it would be expected to observe beneficial effects of receiving more blood for those with a lower initial Hb, and beneficial effects of receiving relatively less blood for those with a higher initial Hb. Importantly, this question is only relevant to those who actually received a RBC transfusion; thus, this analysis only looked at the patients who received at least one RBC transfusion during the study period, and whose RBC transfusion preceded at least one of the study outcome events.

Analyses to address this research question are highly prone to confounding by indication—critically unwell patients are more likely to be given more blood, and may have already had a greater risk of death or re-bleeding at baseline. Therefore, inclusion of covariates to adjust for baseline health of the patient was a key factor in modeling this association. The final model was built in four steps.

Step 1: Simple logistic regression was used to model the probability of death or re-bleeding by day 28, as predicted by the number of RBC units a patient received over the course of the study period.

Step 2: Assumptions of linearity and fit of this simple model were assessed. The Hosmer-Lemeshow goodness-of-fit test was used to check for lack of fit, and inclusion of cubic splines and higher order terms were tested to check for inadequacy of the linear model.

Step 3: Potential confounders were considered and adjusted for in the model. The baseline covariates measured in the TRIGGER trial were evaluated to determine which variables would best indicate the baseline health of the patient, and be of most relevance in transfusion-related decisions (i.e., when to transfuse, and how much blood to transfuse). These variables were sequentially added to the model, and their contribution to the model was assessed using Likelihood Ratio (LR) tests. Covariates that significantly improved the predictive ability of the model (as indicated by an LR test of $p < .05$) were included in the final model.

Step 4: The final model was used to produce predicted probabilities of death or re-bleeding for 358 patients; 22 of the transfused patients were excluded from the analysis because their RBC transfusion was administered after the event of death or re-bleeding by day 28. These predicted probabilities and the fitted regression line were plotted as a function of number of RBC units transfused.

Chapter 4

4 Results

This chapter summarizes the results from my secondary analysis of the TRIGGER dataset, as described in Chapter 3. These results are presented separately for each of the two main objectives, and interpreted further in Chapter 5. Descriptive statistics are included for both analyses.

4.1 Objective 1: Investigating the optimal Hb threshold for RBC transfusion

4.1.1 Descriptive statistics

Descriptive statistics on baseline characteristics, signs and symptoms, and comorbidities are presented in Table 1 for those that received at least one RBC transfusion during the study period, and those that did not. Predictably, there are clear differences between the two groups. Patients that received a RBC transfusion were older on average, and more critically unwell, as evidenced by higher Rockall scores and Blatchford scores. The following section summarizes the results of my first regression analysis, investigating Hb at presentation or RBC transfusion as a predictor of re-bleeding or death by day 28. Adjustment for a number of covariates indicating baseline health of the patient is supported by the differences seen in table 1 for those transfused and not transfused.

Table 4.1: Baseline characteristics, signs and symptoms, comorbidities, and clinical outcomes by transfusion status

	Transfused (n = 264)	Not Transfused (n = 555)
Baseline characteristics		
Male	153 (57.95%)	342 (61.62%)
Age (years)	65.96 (18.33)	54.62 (20.12)

Rockall score	3 (2 to 4)* *(1)*	1 (0 to 3)
Blatchford score	9 (7 to 12)* *(1)*	3 (1 to 5)* *(4)*
Signs and symptoms		
Melena	181 (68.56%)	194 (34.95%)* *(2)*
Hematemesis	130 (49.24%)	342 (61.62%)
Heart rate (beats per min)	98.03 (22.18)* *(1)*	93.52 (20.29)* *(1)*
Respiratory rate (breaths per min)	18.34 (3.75)* *(7)*	17.61 (3.26)* *(13)*
Systolic blood pressure (mmHg)	120.70 (22.65)	131.0 (21.96)* *(1)*
Pre-existing comorbidities		
Ischemic heart disease	46 (17.42%)	74 (13.33%)
Cardiac failure	15 (5.68%)* *(1)*	12 (2.16%)
Hypertension	85 (32.20%)* *(1)*	108 (19.46%)
Respiratory disease	28 (10.61%)* *(1)*	111 (20.00%)
Renal disease	23 (8.71%)	20 (3.60%)
Liver disease	48 (18.18%)	65 (11.71%)
Cancer	39 (14.77%)* *(1)*	47 (8.47%)
Stroke	25 (9.47%)* *(1)*	24 (4.32%)
First recorded laboratory data		
Hemoglobin (g/dL)	9.63 (1.83)	13.68 (2.15)

Urea (mmol/L)	13.75 (7.96)* *(1)*	7.44 (5.08)* *(8)*
Albumin (g/L)	33.23 (6.51)* *(24)*	39.20 (6.80)* *(54)*
Clinical outcomes		
Death by day 28	17 (6.44%)	14 (2.52%)
Re-bleeding by day 28	24 (9.09%)* *(8)*	9 (1.62%)* *(14)*
Thromboembolic or ischemic event by day 28	13 (4.92%)* *(24)*	14 (2.52%)* *(17)*
Serious adverse event by day 28	58 (21.97%)	57 (10.27%)
Infection by day 28	81 (30.68%)* *(62)*	94 (16.94%)* *(184)*
Acute transfusion reaction (in hosp.)	5 (1.89%)* *(1)*	N/A
Surgical or radiological intervention (in hosp.)	16 (6.06%)	2 (0.35%)
Therapeutic intervention (in hosp.)	130 (49.24%)	57 (10.27%)

Data are n (%), mean (SD), or median (IQR)

***Data missing for *(n)* patients**

4.1.2 Univariate analysis

Data from 802 patients (17 subjects excluded because their transfusion did not precede the outcome) were analyzed in a simple regression of probability of death or re-bleeding by day 28 as predicted by Hb indication and transfusion status. There was a significant interaction between Hb indication and transfusion status (p = .01); patients who did not receive a RBC transfusion had a 29% reduction in odds of death or re-bleeding by day 28 for each one-unit increase in Hb at presentation (OR = .71, p = .001), whereas patients

who did receive at least one RBC transfusion experienced a 62% increase in odds of death or re-bleeding by day 28 for each one-unit increase in Hb at first RBC transfusion (OR = 1.62, p = .01).

4.1.3 Adjusted regression

Table 4.1 shows that patients receiving RBC transfusion were, on average, older and in poorer health than those that did not receive a RBC transfusion. To control for this difference in baseline health in the analysis of Hb as a predictor of re-bleeding or death, a number of baseline covariates were considered and tested for inclusion in the regression model.

Specifically, the covariates assessed were age, systolic blood pressure (SBP), heart rate, respiratory rate, coagulopathy, syncope, hematemesis, cancer, ischemic heart disease, respiratory disease, renal disease, and liver disease as potential predictors of the outcome and confounders in the association between Hb and the outcome. Of these variables, only four contributed significantly to the model: age, SBP, hematemesis, and cancer.

After adjustment for these covariates, there was still a significant interaction between Hb and transfusion status in predicting death or re-bleeding (p = .008). Similar to the results seen in the unadjusted analysis, patients who did not receive a RBC transfusion experienced a 24% decrease in odds of death or re-bleeding by day 28 for every one-unit increase in Hb at presentation (OR = .76, p = .02), whereas patients who received a RBC transfusion experienced a 68% increase in odds of death and re-bleeding by day 28 for a one-unit increase in Hb at transfusion (OR = 1.68, p = .008).

4.1.3.1 Separate analyses for death and re-bleeding

In separate regressions for death by day 28, and re-bleeding by day 28, a similar trend was observed to that seen for a composite outcome of the two events. Patients who did not receive a RBC transfusion had almost half the odds of re-bleeding for each one-unit increase in initial Hb (OR = 0.58, p = .004), whereas patients who did receive a RBC transfusion experienced more than double the odds of re-bleeding for the same increase in Hb at transfusion (OR = 2.57, p = .001). Similarly, non-transfused patients were less

likely to die as initial Hb increased (OR = 0.91, p = .49), and transfused patients were more likely to die with increasing Hb values (OR = 1.10, p = .73), though these results were not statistically significant.

4.1.4 Model fit

The adequacy of this model was assessed using the Hosmer-Lemeshow goodness-of-fit (GOF) test and corresponding calibration plot. The GOF test showed no gross lack of fit in the model (p = .85), and a calibration plot of predicted versus observed outcomes showed a relatively close match between these values. Further, efforts to improve the model with cubic splines or quadratic terms did not yield any significant contributions to the predictive ability of the model. Therefore logistic regression was determined to be a sufficient means of modeling the relationship between the predictors and the log odds of death or re-bleeding.

4.1.5 Deriving the threshold value

The predictive margins were plotted from the predicted probabilities of the fit model, as a function of Hb indication by transfusion status. This plot showed an intersection in probability of the outcome for those transfused and those not transfused, at an initial Hb value of just over 10g/dL. The precise value of this intersection was derived by setting the regression equations for the transfused group and non-transfused group equal to each other, and solving for Hb, holding all other variables constant:

$$\text{Logit}(Y=1 \mid \text{Transfused}) = \beta_0 + \beta_1(\text{Hb}) + \beta_2(\text{Tx\_status}) + \beta_3(\text{Hb}*\text{Tx})$$
$$\text{Logit}(Y=1 \mid \text{Transfused}) = \beta_0 + \beta_1(\text{Hb}) + \beta_2(1) + \beta_3(\text{Hb}*1)$$
$$\text{Logit}(Y=1 \mid \text{Transfused}) = \beta_0 + \beta_1(\text{Hb}) + \beta_2 + \beta_3(\text{Hb})$$

$$\text{Logit}(Y=1 \mid \text{Not Transfused}) = \beta_0 + \beta_1(\text{Hb}) + \beta_2(\text{Tx\_status}) + \beta_3(\text{Hb}*\text{Tx})$$
$$\text{Logit}(Y=1 \mid \text{Not Transfused}) = \beta_0 + \beta_1(\text{Hb}) + \beta_2(0) + \beta_3(\text{Hb}*0)$$
$$\text{Logit}(Y=1 \mid \text{Not Transfused}) = \beta_0 + \beta_1(\text{Hb})$$

$$\text{Logit}(Y=1 \mid \text{Transfused}) = \text{Logit}(Y=1 \mid \text{Not Transfused})$$
$$\beta_0 + \beta_1(\text{Hb}) + \beta_2 + \beta_3(\text{Hb}) = \beta_0 + \beta_1(\text{Hb})$$
$$\beta_1(\text{Hb}) + \beta_3(\text{Hb}) = \beta_1(\text{Hb}) - \beta_2$$
$$\beta_3(\text{Hb}) = -\beta_2$$
$$\text{Hb} = -\beta_2 / \beta_3$$

Hb = -(-5.314671) / 0.5177227
Hb = 10.26

Thus, the plot shows that below a Hb value of 10.26g/dL, those who were transfused had a lower risk of death or re-bleeding. However, those that were transfused with an initial Hb of greater than 10.26g/dL showed a higher risk of death or re-bleeding relative to those that did not receive a RBC transfusion at all.

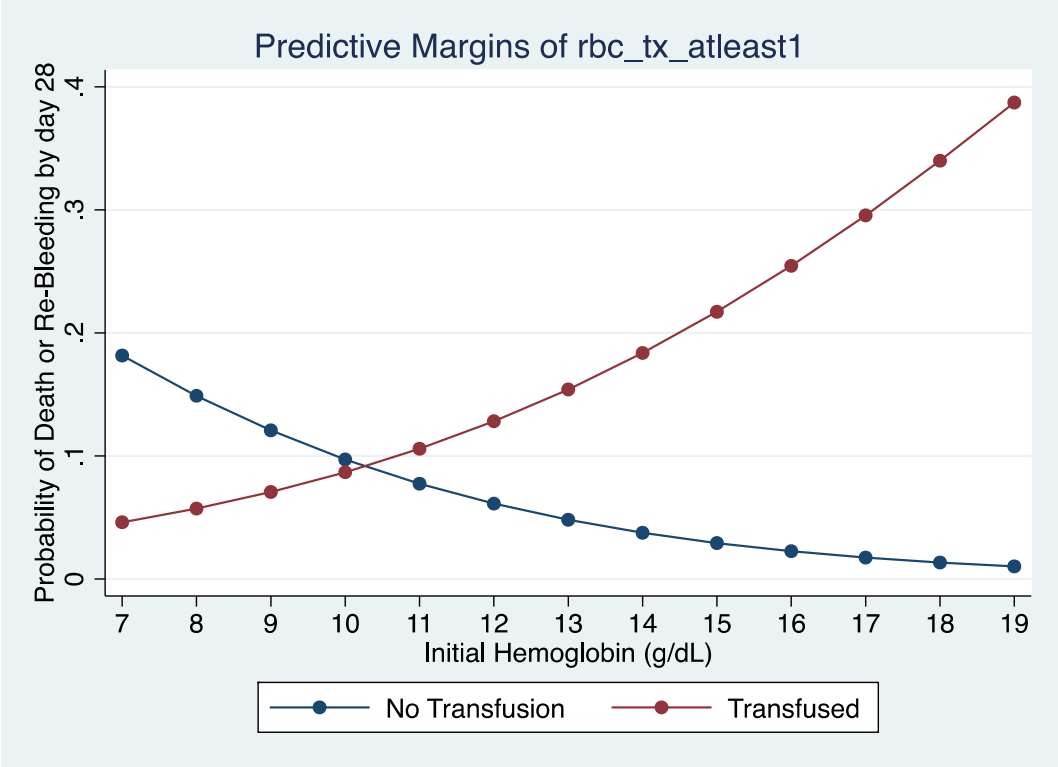

Figure 4.1: Predicted probabilities of death or re-bleeding by day 28 by transfusion status

4.1.6 Sub-group analyses

In managing and treating patients with AUGIB, patients with variceal bleeding and patients with ischemic heart disease and/or other cardiac comorbidities represent unique sub-groups in this patient population, and may be differentially tolerant of anemia or RBC transfusion. Although it is often not possible to know the etiology of the patient's bleed at presentation, variceal bleeding is often a complication of cirrhosis of the liver. Thus, patients with liver disease and patients with cardiac comorbidities (i.e., ischemic

heart disease, cardiac failure, or hypertension) may show different threshold values for RBC transfusion.

4.1.6.1 Patients with and without liver disease

When conducting a separate regression for patients with liver disease, there was no significant effect of Hb or RBC transfusion status on risk of death or re-bleeding, and no significant interaction between these two factors. However, less than 15% of patients had liver disease, leaving a sample size of only 110 patients with liver disease. Thus, it is possible that any analyses on this small group of patients may be biased, or underpowered.

An alternative approach to gauge whether patients with liver disease warrant different management is to exclude patients with liver disease from the analysis (i.e. complete case analysis), and assess any deviations from the results found in the total sample of patients. In a regression of 691 patients free of liver disease, transfused and non-transfused patients showed similar trends as those seen in analysis of the whole dataset (OR = 1.45, $p = .12$; OR = .80, $p = .09$, respectively), but these results were not statistically significant.

4.1.6.2 Patients with and without cardiac comorbidities

Similar limitations were encountered when attempting to analyze patients with ischemic heart disease, cardiac failure, or hypertension separately; approximately 30% of patients identified with one or more of these comorbidities, resulting in a group of 272. There was no significant association between Hb or transfusion status and the outcome for this group of patients.

After excluding this group of patients, the same trend emerged as that seen for the total group of patients; that is, a negative relationship between Hb and the outcome for non-transfused patients (OR = .57, $p = .007$), and a positive relationship for transfused patients (OR = 2.91, $p < .001$). However, the point of intersection for these two groups is lower than the value of 10.26 obtained from the whole group analysis. Among patients *without* ischemic heart disease, cardiac failure, or hypertension, risk of death or re-

bleeding among those transfused versus not transfused intersects at a Hb value of 9.71 (Figure 4.2). This value was calculated with the same formula as that used in section 4.1.5.

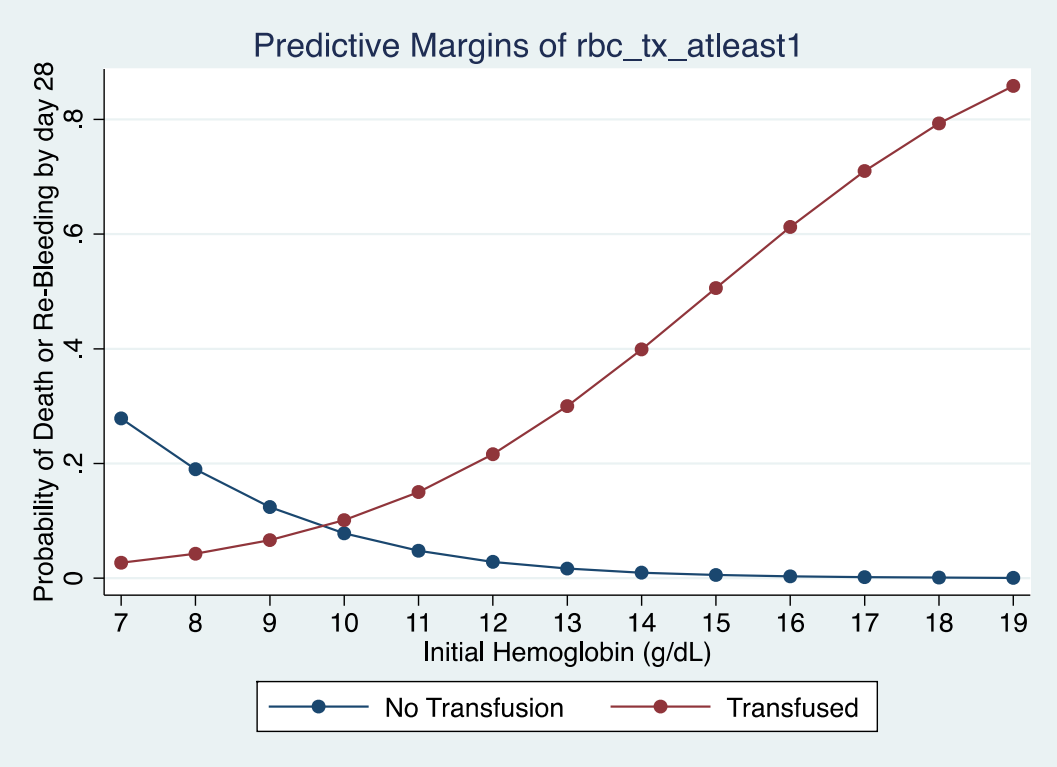

Figure 4.2: Predicted probabilities of death or re-bleeding by day 28 by transfusion status, for patients *without* ischemic heart disease, cardiac failure, and hypertension

4.1.7 Other clinical outcomes

Although endpoints for patients with AUGIB can typically be categorized as resolution of bleeding, further bleeding, or death, patients are often vulnerable to a number of other clinical outcomes and events. It is possible that decisions regarding if and when to transfuse a patient may be relevant to more than just probability of death or re-bleeding. An additional regression analysis was conducted looking at the probability of any adverse event (AAE) (i.e., death, re-bleeding, thromboembolic or ischemic events, or other serious adverse events) by study day 28 as a function of initial Hb, for both transfused and non-transfused patients.

A simple, unadjusted regression of probability of AAE by day 28 on Hb indication and transfusion status, with an interaction between the two predictors, showed similar trends to that seen for probability of death or re-bleeding. Non-transfused patients experienced a 25% reduction in odds of AAE by day 28 for a one-unit increase in initial Hb (OR = .75, $p < .001$), whereas transfused patients experienced a 60% increase in odds of AAE by day 28 for the same increase in initial Hb (OR = 1.60, $p = .001$). The point of intersection for this interaction was 10.89 g/dL (Figure 4.3), similar to that seen for death or re-bleeding.

This trend was consistent when the analysis was adjusted for age, SBP, heart rate, and symptoms of coagulopathy (OR = .75, $p < .001$ for non-transfused patients; OR = 1.70, $p = .001$ for transfused patients). A GOF test showed no gross lack of fit for this model, and inspection of a calibration plot showed a close match between the predicted and observed values. Inclusion of cubic splines or quadratic terms did not improve the fit of the model.

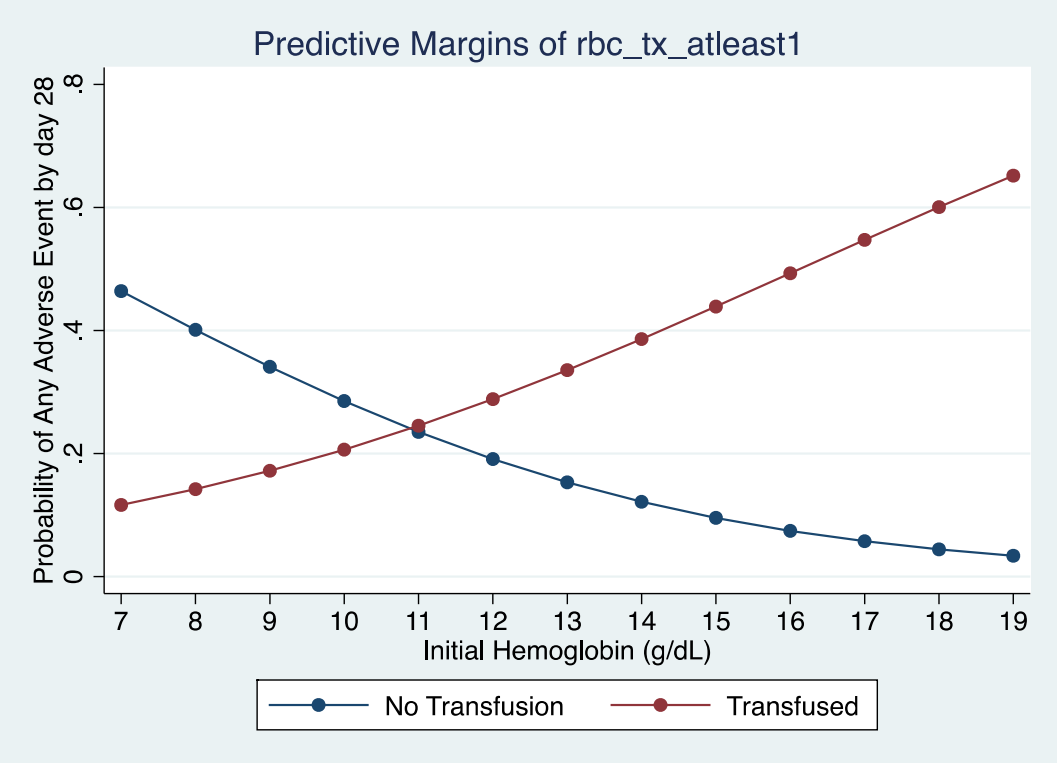

Figure 4.3: Predicted probabilities of any adverse event by day 28 by transfusion status

4.2 Objective 2: Investigating amount of blood transfused as a predictor of patient outcomes

4.2.1 Descriptive statistics

Descriptive statistics on baseline characteristics, signs and symptoms, comorbidities, and clinical outcomes are presented in Table 4.2 for those that received at least one RBC transfusion during the study period.

Table 4.2: Baseline characteristics, signs and symptoms, comorbidities, and clinical outcomes by transfusion status

	Patients who received at least one RBC transfusion (n = 380)
Baseline characteristics	

Male	224 (58.95%)
Age (years)	66.28 (18.14)
Rockall score	3 (2 to 4)* *(1)*
Blatchford score*	10 (8 to12)* *(3)*
Signs and symptoms	
Melena	280 (73.68%)
Hematemesis	169 (44.47%)
Heart rate (beats per min)	97.71 (21.31)* *(2)*
Respiratory rate	18.41 (4.27)* *(11)*
Systolic blood pressure (mmHg)	119.44 (22.10)
Pre-existing comorbidities	
Ischemic heart disease	63 (16.58%)
Cardiac failure	27 (7.11%)* *(1)*
Hypertension	124 (32.63%)* *(1)*
Respiratory disease	47 (12.37%)* *(1)*
Renal disease	34 (8.95%)
Liver disease	71 (18.68%)
Cancer	52 (13.68%)* *(1)*
Stroke	35 (9.21%)* *(1)*

RBC Transfusion characteristics	
Day of first RBC transfusion	1 (1 to 2)
Number of RBC units transfused over study period	3 (2 to 5)
Clinical outcomes	
Death by day 28	28 (7.37%)* *(1)*
Re-bleeding by day 28	35 (9.21%)* *(16)*
Thromboembolic or ischemic event by day 28	18 (4.74%)* *(35)*
Serious adverse event by day 28	88 (23.16%)* *(1)*
Infection by day 28	120 (31.58%)* *(84)*
Acute transfusion reaction (in hosp.)	11 (2.89%)* *(2)*
Surgical or radiological intervention (in hosp.)	19 (5.0%)
Therapeutic intervention (in hosp.)	177 (46.58%)

Data are n (%), mean (SD), or median (IQR)

***Data missing for *(n)* patients**

4.2.2 Univariate analysis

A total of 358 subjects (22 subjects excluded because their transfusion did not precede the outcome) were analyzed in an unadjusted regression of probability of death or re-bleeding by day 28 as predicted by number of RBC units transfused over the study period. There was a positive relationship between number of RBC units transfused and probability of death or re-bleeding by day 28; for each additional unit received, odds of death or re-bleeding increased by 30% (OR = 1.30, $p < .001$).

4.2.3 Adjusted regression

An unadjusted regression of death or re-bleeding by day 28 on number of RBC units transfused is likely to be prone to confounding by indication—patients who are more critically unwell are more likely to receive a RBC transfusion, and thus were already at higher risk of the outcome at baseline. An adjusted regression was carried out in an attempt to control for the baseline health of the patient, and determine if number of RBC units transfused was still a significant predictor of patient outcomes. Specifically, the following baseline covariates were considered as potential predictors of patient outcomes: age, SBP, Hb at presentation, heart rate, respiratory rate, coagulopathy, suspected active bleed, shock, hematemesis, cancer, ischemic heart disease, cardiac failure, respiratory disease, renal disease, and liver disease.

When all of these variables are included in the original model, the relationship between number of RBC units transfused and probability of death or re-bleeding by study day 28 became even stronger; the odds of the outcome increase by 43% for each additional RBC unit transfused (OR = 1.43, $p < .001$) (Figure 4.4). Though inclusion for so many variables may result in over-fitting, these results speak to the robustness of the relationship between number of RBC units transfused, and risk of death or re-bleeding among patients with AUGIB.

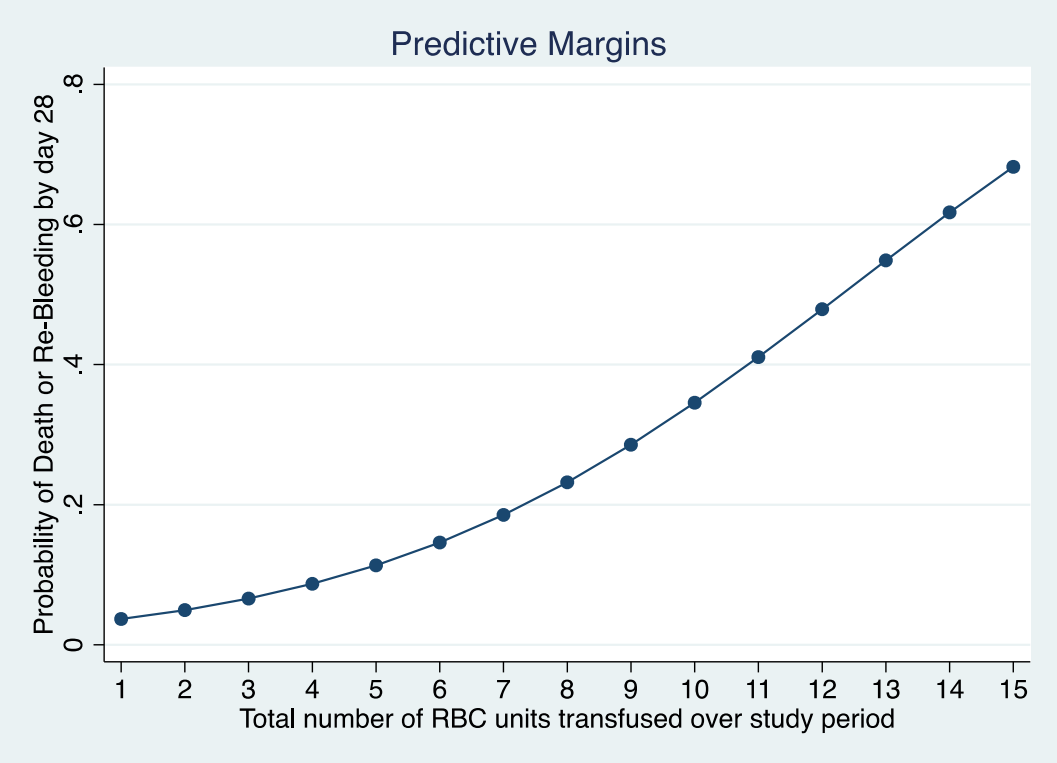

Figure 4.4: Predicted probabilities and fitted values for death or re-bleeding as a function of number of RBC units transfused

4.2.4 Model fit

The adequacy of this model was assessed using the Hosmer-Lemeshow GOF test and a calibration plot of observed and predicted values. The GOF test showed no gross lack of fit in the model (p = .82), and the calibration plot showed a close match between observed and predicted outcomes. A specification link test showed no benefit to including higher order terms, and cubic splines did not improve the fit of the model. Therefore, a linear logistic regression was determined to be a sufficient means of modeling the relationship between number of RBC units transfused and the log odds of death or re-bleeding.

4.2.5 Other clinical outcomes

It is possible that the number of RBC units a patient receives is predictive of adverse health outcomes beyond just death and re-bleeding. In an additional regression analysis,

number of RBC units was also found to be a significant predictor of AAE by study day 28; for each additional RBC unit transfused, the odds of AAE increased by 35% (OR = 1.35, $p < .001$). Furthermore, this trend was seen even when controlling for various indicators of baseline health of the patient; in fact, the strength of the association increased (OR = 1.41, $p < .001$). GOF and model specification tests showed no lack of fit, and suggested the linear logistic regression model also sufficed for modeling the outcome of AAE.

Chapter 5

5 Discussion

The results described in Chapter 4 came from a secondary analysis of clinical trial data collected from the 2015 TRIGGER trial (Jairath et al., 2015). To our knowledge, this is the first analysis to explore Hb thresholds for RBC transfusion as a predictor of patient outcomes, maintaining Hb as a continuous measure. Further, this thesis is unique in its consideration of number of RBC units transfused as a possible predictor of patient outcomes. The following sections further interpret and discuss the results of the analyses addressing the two research objectives of this study. This is followed by a discussion of the overall strengths and limitations of this study, and its implications and conclusions.

5.1 Objective 1: Is there an optimal Hb threshold for guiding RBC transfusion in patients with AUGIB?

In the logistic regression analyses used to address the first research objective, initial Hb was shown to be a significant predictor of death or re-bleeding by study day 28. However, this association differed for those who received at least one RBC transfusion, versus those who did not receive any RBC transfusions during the study period. Specifically, patients who received at least one RBC transfusion experienced a linear increase in the log odds of death for increasing Hb values at transfusion; the higher the patient's Hb at transfusion, the greater their probability of dying or re-bleeding during the 28-day follow-up period. Conversely, patients who did not receive a RBC transfusion demonstrated the opposite relationship with respect to initial Hb; the higher the patient's Hb at presentation, the smaller the probability of death or re-bleeding. This trend was seen for both crude and adjusted analyses, increasing confidence that this result is not a product of confounding by indication, or some other factor. Furthermore, similar trends were seen when analyzing the outcomes of death and re-bleeding separately, supporting the combination of these outcomes into one composite outcome.

A graphical display of these relationships (Figure 4.1) shows an intersection in predicted probabilities for transfused versus non-transfused patients at an initial Hb value of

10.26g/dL. This is an important finding; as described in Chapter 2, although RBC transfusion may be an essential intervention for managing risk of anemia in bleeding patients, previous trials have demonstrated that there may be no benefit to liberal RBC transfusion protocols (Jairath et al., 2015; Odutayo et al., 2017; Villanueva et al., 2013), and unnecessary RBC transfusions may confer more risk than they prevent. Conceptually, this suggests there may be a critical threshold where the risks associated with anemia outweigh the risks associated with RBC transfusion, and the intersection in predicted probabilities seen in Figure 4.1 may be indicative of this "tipping point". For patients whose initial Hb was lower than 10.26g/dL, receiving a RBC transfusion was beneficial even after adjustment for a number of baseline clinical covariates—transfused patients below this point showed lower risk of death or re-bleeding than patients who presented with similarly low Hb values and did not receive a RBC transfusion. However, the inverse is true when looking at patients that were transfused with initial Hb values greater than 10.26g/dL. In this case, transfused patients showed a greater risk of death or re-bleeding relative to patients that also presented with Hb values greater than 10.26g/dL, but did not receive a RBC transfusion. Confidence in these results is strengthened by the similar trend seen when the definition of the outcome was broadened to any adverse event—transfused patients experienced an increase in risk for increasing Hb levels, while non-transfused patients experienced a decrease in risk, with an intersection in these two plots at 10.89g/dL.

These results suggest that unnecessary RBC transfusions could worsen a patient's prognosis through a number of different mechanisms, and this concept of an "unnecessary" RBC transfusion can perhaps be defined by the threshold values produced above. For the average patient with a presenting Hb around 10 to 11g/dL, there does not appear to be any apparent benefit of receiving a RBC transfusion with respect to a number of major clinical outcomes. However, it is important to note that no two patients are the same, and any given patient may not be well represented by the average patient. This is exemplified by the sub-group analysis of patients free of cardiac comorbidities including ischemic heart disease, cardiac failure, and hypertension. In this group of patients, the threshold value indicating this inversion in relative risk between transfused and non-transfused patients was lower—around 9.7g/dL; this is biologically plausible

since this is likely to represent a lower risk cohort of patients. Thus it is possible that the higher threshold seen for the entire dataset could be partially driven by patients with cardiac comorbidities, for whom anemia may be less tolerable, or more likely to result in adverse events. This is just one example of how an average guideline may not apply to a specific patient, and more extensive analyses of specific patient sub-groups are warranted.

5.2 Objective 2: Is there an optimal amount of blood to give AUGIB patients receiving a RBC transfusion?

In the logistic regression analyses used to address the second research objective, number of RBC units transfused was shown to be a significant predictor of death or re-bleeding by study day 28 among patients who received at least one RBC transfusion. Importantly, this relationship did not appear to be obviously explained by another variable; despite inclusion for an extensive list of various indicators of baseline health, none of these variables significantly influenced the relationship between number of RBC units and risk of death or re-bleeding. In fact, this relationship was only strengthened when these variables were included in the model. Furthermore, a similar odds ratio and positive relationship were seen when the outcome definition included any adverse event by day 28, and this relationship was similarly unaffected by inclusion of any baseline covariates or potential confounders.

These results may suggest that RBC transfusion guidelines for AUGIB patients should not just consider if and when a patient should be transfused, but also how much blood is optimal once care providers have determined that a RBC transfusion is warranted. This matter may require slightly different considerations; however, the results of this analysis indicate that, on average, there may be no clinically significant benefit to transfusing more than one unit of RBCs at a time, even for patients with lower presenting Hb and poorer baseline health. As mentioned previously, it is important to note that variability between AUGIB patients may warrant significantly different approaches to RBC transfusion practices, and further research on differential tolerance for anemia and RBC transfusions among specific patient sub-groups will be crucial in informing routine

clinical practice. It is also important to note that the TRIGGER trial excluded patients with exsanguinating bleeding, where clearly this inference cannot be made.

5.3 Implications

Overall, the results of this thesis have a number of implications for future research and practice. Firstly, there may be a critical threshold above which there is no incremental benefit, and even harm, for receiving RBC transfusion in patients with AUGIB, which has not been addressed from clinical trials that simply compare two dichotomous Hb thresholds for RBC transfusion. Rather than directly compare two arbitrary threshold values for RBC transfusion, an interaction between initial Hb and transfusion status was examined for predicting adverse patient outcomes, and it was observed that the relationship between initial Hb and adverse events was opposite for those who received a RBC transfusion relative to those that had not. Importantly, this interaction results in an intersection between these two plots, above which there does not appear to be any benefit to receiving a RBC transfusion. This pattern of results supports the hypothesis that, for any given patient, there is an optimal threshold to guide RBC transfusion— if the patient's Hb falls too far below this point, they experience excess risk from anemia and hemodynamic instability, whereas if they are transfused above that point, they experience excess risk from the transfusion itself. Nevertheless, it is worth highlighting the discrepancy between the Hb threshold found in this thesis and the threshold values used in previous trials. Specifically, our value of roughly 10.3g/dL is appreciably larger than Hb values used to define both the restrictive and liberal RBC transfusion protocols in the TRIGGER trial, and Villanueva et al.'s (2013) trial. Given that Villanueva et al. (2013) found a threshold of 7g/dL to be superior to 9g/dL, and Jairath et al. (2015) found no significant difference between a threshold of 8g/dL and 10g/dL, at first glance the results of this thesis seem contradictory to previous findings. However, there may be a number of explanations for this discrepancy.

First, the TRIGGER trial was designed to be pragmatic in nature, thus most patients with AUGIB were eligible for inclusion in the study regardless of complications and comorbidities. This differs from the design of the 2013 trial by Villanueva et al., which excluded patients with an acute coronary syndrome, symptomatic peripheral

vasculopathy, stroke, or transient ischemic attack in the previous 90 days, a recent history of trauma or surgery, or lower GI bleeding (Villanueva et al., 2013). These stricter inclusion criteria are likely to have resulted in a sample with fewer complications and co-morbidities, and better tolerance for low Hb levels than the sample of patients in the TRIGGER trial. Additionally, one third of patients in the Villanueva trial had cirrhosis of the liver and bleeding from varices, where the pathophysiology of bleeding differs as a result of portal hypertension.

An additional consideration concerns the matter of statistical significance. Although plots of predicted probabilities for transfused and non-transfused patients showed a clear interaction between these two groups, it is highly unlikely that their point of intersection is the only point where these two groups show no statistically significant difference in probability of the outcome. Rather, it is more likely that this point of intersection (i.e., at 10.26g/dL) represents the center of an interval falling around this Hb threshold, within which there is no statistically significant difference in risk for those transfused versus those that did not receive a RBC transfusion. In that case, the results of the current study are compatible with the results of the original TRIGGER trial, which found no significant difference between those assigned to a threshold of 8g/dL versus 10g/dL. Perhaps both the original results and the current results are a reflection of some range of Hb values for which, on average, there is no benefit to transfusing within or above that range. Although these results indicate a slightly higher threshold than even the liberal value in the original trial, it should be noted that the analyses in this thesis did not use all 936 patients in the full dataset, which could partly explain why these results found a higher threshold value than either of those compared in the original trial.

Another possible explanation for this relatively high threshold value may be found in the results from the second research objective. A regression analysis of number of RBC units transfused as a predictor of adverse health events showed that as number of RBC units increased, so too did risk of death, re-bleeding, ischemic or thromboembolic events, or serious adverse events by day 28. However, neither of the previous trials discussed here controlled for amount of blood transfused; thus, it is possible that the results of these trials have been partially driven by the amount of blood patients received, rather than

simply when they were transfused. In the Villanueva trial, patients randomly assigned to the liberal policy received an average of 3.7 units of RBCs, whereas patients assigned to the restrictive policy received only 1.5 units; the difference between these groups was statistically significant ($p < .001$) (Villanueva et al., 2013). In the TRIGGER trial, patients assigned to the liberal group received more blood, on average, than those in the restrictive group, but this difference was not statistically significant (Jairath et al., 2015). It may not be a coincidence that these characteristics align with the main results of the trials; that is, there was a significant difference in outcome measures for the two groups in the Villanueva trial, but not the TRIGGER trial. Further, the pragmatic nature of the TRIGGER trial allowed for more flexible protocol delivery— number of RBC units transfused was per clinician discretion, and attending clinicians could deviate from the assigned protocol when deemed necessary. The stricter protocol of the Villanueva trial may have produced more RBC transfusions and resulted in a greater amount of RBC units used, particularly in the liberal group, as physicians may have been less likely to deviate from the protocol when they believed a RBC transfusion to be unnecessary. This disparity may also partly explain the significantly better outcomes seen in the restrictive group relative to the liberal group.

Taken together, these findings may suggest that the sole consideration is not the absolute Hb threshold for RBC transfusion, but rather more attention should also be directed to the appropriate *amount* of blood to transfuse, in order to best manage this group of patients. Rather than allowing patient Hb levels to fall as low as 7g/dL before administering RBC transfusion, it may be prudent to transfuse patients at higher Hb values, but limit this practice to single transfusions and then clinical reassessment of the need for further RBC transfusion. This of course does not apply to patients with exsanguination who were specifically excluded from this clinical trial.

It has been suggested that broad implementation of restrictive RBC transfusion strategies could drastically reduce the burden of AUGIB on blood resources and healthcare systems (Campbell et al., 2015). Although the results of this thesis suggest that simply lowering thresholds may not be beneficial to patient wellbeing, they may shed light on a unique opportunity for conservation of blood resources; that is, through a more conservative

approach to the *volume* of blood transfused for each patient. Among the 380 patients in the TRIGGER trial that received at least one RBC transfusion, the average amount of blood transfused was 3.87 units, with 10% of those patients receiving 7 or more units. If the average patient received only one unit of blood, or increments of one unit as clinically indicated, this could result in substantial blood conservation.

5.4 Limitations

This thesis had several limitations. First, the study was limited by a small sample size, as not all 936 patients in the original dataset were relevant to the aims of this thesis. For example, all patients with an initial Hb less than 7g/dL received a RBC transfusion, making it impossible to look for an interaction between Hb and RBC transfusion status in this group of 116 patients. Similarly, only 358 patients in the total dataset received at least one transfusion over the course of the study that preceded any outcome event, and thus were the only observations that could be analyzed to address the second research objective. This loss in sample size will have resulted in a loss in power and precision. Despite this, the two main models built for each of the respective thesis objectives showed significant results, and similar trends regardless of adjustment for various covariates, and broader outcome definitions.

A small sample size also limited the ability to conduct meaningful sub-group analyses for specific sub-groups of interest. For example, ischemic heart disease and cirrhosis of the liver are common co-morbidities among patients with AUGIB, and previous research suggests that the optimal Hb threshold for RBC transfusion may be higher for patients with ischemia, and lower for patients with cirrhosis or variceal hemorrhage (Yen, 2018). This dataset contained fewer than 200 patients with either liver disease or ischemic heart disease, and very few events among these patients, compromising the ability to draw inferences from these groups. Further research and study is warranted to examine how these results may differ for these patient sub-groups, and others.

Furthermore, there was a relatively low event rate in the total dataset for each of the outcomes individually, necessitating the use of composite outcomes. However, separate analyses for death and re-bleeding supported the results of the composite outcome, and alleviated some concern that combining these events into one measure was not appropriate. Additional analyses looking at other adverse events, such as thromboembolic and ischemic events, showed almost identical trends.

One final limitation concerns issues of temporality. Because the thesis aimed to look at Hb and RBC transfusion as predictors of adverse events, it is important that transfusion preceded the outcome, and was not administered in response to the outcome. This required comparing time-to-event data to the day of the first RBC transfusion, for all patients that received at least one transfusion, to determine if this temporality requirement was met. This process was necessary for all outcomes but death, and resulted in several patients being excluded from the analyses because either their RBC transfusion came after every outcome event, or there was insufficient information to make this determination. This limitation necessitated selectively excluding events only among the transfused group— the non-transfused group received no RBC transfusions, so there were no concerns regarding temporality. However, this is not simply a flaw of this specific study; in practice, it can be difficult to distinguish a re-bleed (i.e., resolution of the bleed, followed by another bleeding event) from further or continuous bleeding (i.e., a bleed that was never fully resolved), and just because a RBC transfusion came after a bleeding event does not negate the possibility that a transfusion still impedes cessation of the bleed. For this reason, patients whose event came before the RBC transfusion were excluded from the analysis, as opposed to treated as “non-events”. It is unclear how future researchers may address this issue in design and analysis. However, it is worth noting that including these patients in the analysis does not drastically change findings— the results still show a significant interaction between Hb and transfusion status, and similar plots for both the transfused and non-transfused groups. The threshold value falls only slightly, to roughly 9.5g/dL. Thus, although excluding these patients could have underestimated the risks of transfusion, it seems that the overall trend in the data, and evidence for an optimal threshold for RBC transfusion, is fairly robust.

5.5 Conclusions

The results of this thesis support the notion that there may be a critical Hb threshold for guiding RBC transfusion among patients with AUGIB. These results indicated no benefit to receiving a transfusion above Hb values of 10.26g/dL, though this value likely represents a range of Hb values for which transfusion begins to offer little to no benefit. Further, the optimal threshold for transfusion will not be consistent across all patients, and certain patients are likely to be more or less tolerant to the risks of anemia and more restrictive transfusion thresholds.

In addition, the number of RBC units a patient receives was shown to be significantly associated with clinical outcomes. Previous clinical trials on restrictive RBC transfusion have not controlled for the volume of blood transfused, and it is possible that the results seen in these trials may be partly explained by how much blood a patient receives, rather than solely their Hb at RBC transfusion.

Going forward, future researchers in this field should consider maintaining the continuous nature of Hb in their analyses, in order to better investigate a critical threshold for RBC transfusion, rather than pairwise comparisons of arbitrary thresholds. Further, more focus should be given to the number of RBC units a patient receives and how this interacts with Hb concentration at transfusion to predict clinical outcomes such as death and re-bleeding. Lastly, future trials should prioritize sub-group analyses for patients with ischemic heart disease and liver disease, respectively, who comprise a significant proportion of patients with AUGIB, and may require distinct RBC transfusion guidelines.

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