ASSESSMENT OF PLATELET ACTIVATION AND PROTHROMBOTIC RISK FOLLOWING ACUTE UPPER GASTROINTESTINAL BLEEDING AND BLEEDING IN THE CONTEXT OF ACUTE CORONARY SYNDROMES

by

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Abstract

Patients presenting with acute upper gastrointestinal bleeding have an increased incidence of cardiovascular events, with mortality invariably related to factors other than the bleeding itself, with the majority of deaths related to cardiovascular disease. The Blatchford score is used to identify low risk patients suitable for early discharge. Diurnal and seasonal variation in acute upper gastrointestinal bleeding has not been addressed in the UK population. Patients presenting with bleeding complicating acute coronary syndromes are at an increased risk of recurrent cardiovascular events and have higher short-term and long-term mortality when compared to patients with uncomplicated acute coronary syndromes.

This study has shown the preferential use of the Blatchford score in identifying patients suitable for early discharge and using a cut-off score of 2 could avoid up to 15% of admissions for acute upper gastrointestinal bleeding. The results show significant diurnal and seasonal variation in the presentation of acute upper gastrointestinal bleeding with fewer admissions in the winter months and a higher proportion of patients presented in the 12:01-18:00 time period.

This study has shown an increase in levels of platelet activation and prothrombotic markers (d-dimer and vWF) in the acute upper gastrointestinal bleeding population, which may help explain the risk of these patients developing future cardiovascular events. No such findings were seen in the acute coronary syndromes complicated by bleeding group which may in part be due to the treatments patients received or due to the small numbers of patients recruited. These novel findings may help explain the excess of cardiovascular mortality in patients with presenting upper gastrointestinal bleeding and give a biological rationale to restarting antiplatelet agents early in these patients. Further studies are required to confirm and further investigate these findings.

Declaration

I declare that the thesis submitted is my own work and that all of the studies were carried out at the University Department of Medicine, City Hospital, Dudley Road, Birmingham, UK.

Dedication

This piece of work is dedicated to all of my family and friends that have supported me during a difficult period and Lauren who gave me the motivation to complete this thesis.

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List of Abbreviations

AA	Arachidonic acid
ACS	Acute coronary syndrome
ACUITY	Acute Catheterization and Urgent Intervention Triage strategy
ADP	Adenosine diphosphate
AF	Atrial fibrillation
ANOVA	Analysis of variance
APC	Allophycocyanin
ATP	Adenosine triphosphate
AUGIB	Acute Upper Gastrointestinal Bleeding
BARC	Bleeding Academic Research Consortium
BMI	Body mass index
BMS	Bare metal stent
BP	Blood pressure
BPM	Beats per minute
BSG	British Society of Gastroenterology
CABG	Coronary artery bypass graft
CAD	Coronary artery disease

CI	Confidence interval
COX	Cyclooxygenase
CRUSADE	Can Rapid Risk Stratification of Unstable Angina Patients Suppress Adverse Outcomes With Early Implementation of the ACC/AHA Guidelines
CTAD	Citrate, Theophylline, Adenosine and Dipyridamole
CURE	Clopidogrel in Unstable angina to prevent Recurrent Events
CVA	Cerebrovascular accident
CVS	Cardiovascular
DD	D-dimer
DES	Drug-eluting stent
dL	Decilitre
DM	Diabetes mellitus
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked Immunosorbent assay
ESGE	European Society of Gastrointestinal Endoscopy
FACS	Fluorescence-Activated Cell Sorter
FITC	Fluoroscein Isothiocyante
fL	Femtolitre

FSC	Forward Scatter Channel
GBS	Glasgow Blatchford Score
GDF-15	Growth differentiation factor-15
GI	Gastrointestinal
GRACE	Global Registry of Acute Coronary Events
GUSTO	Global Utilisation of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries
H. pylori	*Helicobacter pylori*
HALT-IT	Haemorrhage alleviation with tranexamic acid – intestinal system
Hb	Haemoglobin
Hct	Haematocrit
HORIZONS-AMI	Harmonizing Outcomes With Revascularization and Stents in Acute Myocardial Infarction
HR	Hazards ratio
HRP	Horse radish peroxidase
HTN	Hypertension
H2RA	Histamine (H2) receptor antagonist
IABP	Intra-aortic balloon pump
Ig	Immunoglobulin

IHD	Ischaemic heart disease
IL-6	Interleukin-6
IQR	Interquartile range
ISTH	International Society on Thrombosis and Haemostasis
IV	Intravenous
kDa	Kilodalton
L	Litre
MFI	Mean fluorescent intensity
µL	Microlitre
NICE	National Institute for Health and Care Excellence
NSAIDS	Non-steroidal anti-inflammatory drugs
NSTEMI	Non ST elevation myocardial infarction
OASIS	Organization to Assess Strategies in Ischemic Syndromes
OGD	Oesophagogastroduodenoscopy
OR	Odds ratio
PARAGON	Platelet IIb/IIIa Antagonist for the Reduction of Acute coronary syndrome events in a Global Organisation Network
PBS	Phosphate buffered solution
PCI	Percutaneous Coronary Intervention

PE	Phycoerythrin
PerCP	Peridininchlorophyll Protein Complex
PPI	Proton pump inhibitor
PRISM	Platelet Receptor inhibition for Ischaemic Syndrome Management
PURSUIT	Platelet glycoprotein IIb/IIIa in Unstable angina: Receptor Suppression Using Integrelin Therapy
RBC	Red blood cell
RCT	Randomised Controlled Trial
REPLACE	Randomized Evaluation in PCI Linking Angiomax to Reduced Clinical Events
RGDS	L-arginyl-glycyl-L-aspartyl-L-serine
ROC	Receiver operating characteristic
RR	Relative risk
SCD	Stable coronary disease
SD	Standard deviation
SDH	Subdural haematoma
SIGN	Scottish Intercollegiate Guidelines Network
sP-sel	Soluble P-selectin
SSC	Side Scatter Channel

SRH	Stigmata of recent haemorrhage
STEMI	ST elevation myocardial infarction
SWBH	Sandwell and West Birmingham Hospitals
TIMI	Thrombolysis in Myocardial Infarction
UA	Unstable angina
VALIANT	Valsartan in acute myocardial infarction trial
vWF	Von Willebrand factor
WBC	White blood cell

CHAPTER 1 – INTRODUCTION AND BACKGROUND

1.1 Overview

This chapter provides an introduction to, and discusses in detail, two separate but related areas. These are the clinical nature, importance and management of two types of bleeding; acute upper gastrointestinal bleeding and bleeding in the context of acute coronary syndromes, and the role of platelets and prothrombotic markers in these conditions.

1.2 Non-variceal Acute Upper Gastrointestinal Bleeding

1.2.1 Introduction to non-variceal upper gastrointestinal bleeding

Acute upper gastrointestinal bleeding (AUGIB) is defined in table 1.1; it is generally the vomiting of blood and/or the passage of blood or melaena via the rectum (Hearnshaw *et al* 2010). Gastrointestinal bleeding can be split into upper or lower in origin, with upper gastrointestinal bleeding being caused by a lesion proximal to the ligament of Treitz. AUGIB is a common medical emergency and is associated with significant morbidity and mortality (Klein and Gralnek 2015). It can be split into variceal (associated with chronic liver disease) and non-variceal in its origin (associated with anti-platelet medication and *helicobacter pylori* infection) (Rotondano 2014).

In this chapter, only non-variceal AUGIB will be discussed for the following reasons. Patients who suffer from variceal bleeding generally have chronic liver disease and as such, represent a different subset of patients with a very different risk-factor profile, specific pharmacological treatments and disease-related mortality. Variceal bleeding is not commonly

triggered by anti-platelet therapy and does not commonly complicate acute coronary syndromes (ACS). Many studies show that chronic liver disease itself causes abnormal platelet function (Ogasawara *et al* 2005; Panasiuk *et al* 2001; Panasiuk *et al* 2005; Tacke *et al* 2003; Vardareli *et al* 2007; Witters *et al* 2008). Given that this study focuses on the role of platelets in the context of bleeding, variceal bleeding will not be discussed.

Table 1.1 Definitions relating to acute upper gastrointestinal bleeding (adapted from Hearnshaw *et al* 2010)

Acute upper gastrointestinal bleed (AUGIB)	Haematemesis, the passage of melaena and/or firm clinical or laboratory evidence of acute blood loss from the UGI tract within the previous 10 days. The origin of bleeding is proximal to the ligament of Treitz.
Haematemesis	Vomiting of blood or blood clots. Occasionally this can be caused after swallowing blood from a source in the nasopharynx. Coffee-ground vomitus refers to the vomiting of black material which is assumed to be blood.
Melaena	The passage of black tarry stools.
Haematochezia	The passage of fresh or altered blood per rectum. Usually due to colonic bleeding, occasionally caused by profuse AUGIB.
High risk stigmata on OGD	Red blood in the UGI tract, spurting or oozing, visible vessel, adherent clot for all diagnoses; red spot, wheal, marking or nipple sign on varix/varices.
Rebleeding	Further haematemesis, passage of fresh melaena, continuing or recurring hypotension and tachycardia ± fall in haemoglobin after the first endoscopy
All-cause mortality	Death occurring within the hospital admission up to 30 days post index AUGIB.

1.2.2 Epidemiology of acute upper gastrointestinal bleeding

Hospital admission due to AUGIB is significant problem with important implications for health economics (Campbell *et al* 2015; Cryer *et al* 2010). In the USA, the estimated incidence of AUGIB rates is 150 cases per 100000 population which translates to approximately 400000 admissions per annum (Lewis *et al* 2002). Across Europe the incidence varies between 50-170 per 100000 population (Blatchford *et al* 1997; Button *et al* 2011; Hearnshaw *et al* 2010a; Rockall *et al* 1995). The incidence in the UK has remained stable over the past decade, however, elsewhere in Europe hospitalisations have decreased (Button *et al* 2011; Lanas *et al* 2011). The incidence of AUGIB secondary to peptic ulcer disease only is between 19-57 per 100000 population (Lau *et al* 2011).

In the UK, AUGIB accounts for over 9000 deaths per annum (Crooks *et al* 2009). Mortality in the context of AUGIB is 8.2-13.1% (Blatchford *et al* 1997; Button *et al* 2011; Crooks *et al* 2011). There is evidence showing that 28-day mortality has reduced over time (1999-2007) for non-variceal haemorrhage from 14.7% to 13.1% and for variceal haemorrhage from 24.6% to 20.9% (Crooks *et al* 2011). Long term outcomes following peptic ulcer bleeding are poor. Mortality rates following peptic ulcer bleeding have been reported as 28.7% at 1-year and 46.8% at 5-years (Imhof *et al* 2007). Other studies reported 29% mortality following gastric ulcer or peptic ulcer bleeding (Hudson *et al* 1995; Smart and Langman 1986).

There is some evidence that patients presenting at the weekend have worse mortality outcomes – 13% higher for weekend admissions and 41% higher for those admitted during a bank holiday (Button *et al* 2011). In-patients that develop AUGIB have a higher mortality, 26%, compared with 6.8% in new admissions with AUGIB (Hearnshaw *et al* 2011).

Despite advances in pharmacological and endotherapy mortality remains high. This is likely to be accounted for by an aging population with increasingly complex, and multiple, co-

morbidities (Klein and Gralnek 2015). The median age of patients presenting with AUGIB is 68 years (IQR 49-81) (Hearnshaw *et al* 2011). Sixty percent of patients with peptic ulcer disease are above the age of 60 and approximately 20-25% above the age of 80 (Hearnshaw *et al* 2011; Rockall *et al* 1995; Ohmann *et al* 2005). Those patients found to have varices or malignancy at endoscopy have the highest rates of in-hospital mortality, 15 and 17% respectively (Hearnshaw *et al* 2011).

The cause of death in patients suffering from peptic ulcer disease bleeding is not necessarily from the bleeding itself. A study from Hong Kong found that of the patients that died (6.2%), only 18.4% of those died from a bleeding-related cause. Non-bleeding related mortality was due to cardiac disease (13.5%), pulmonary disease (23.5%), multiorgan failure (23.9%) and terminal malignancy (33.7%), all of which may be complicated by a bleeding event (Sung *et al* 2010a). The association between cardiovascular disease and AUGIB will be discussed later (section 1.2.11).

Many medical conditions show diurnal and seasonal variation. Examples include acute coronary syndromes, heart failure and stroke (Marsh *et al* 1990; Muller *et al* 1985; Muller *et al* 1987; Quyyami 1990; Tofler *et al* 1987; Willich *et al* 1987; Zarich *et al* 1994). Diurnal and seasonal variations in AUGIB have been previously described but the results are conflicting. A European study found biphasic peaks in peptic ulcer bleeding with haematemesis incidence peaking at both 6:45AM and 6:45PM (Minoli *et al* 1994). However, data from China has highlighted a diurnal variation in the presentation of AUGIB with a peak incidence in the nighttime hours (Du *et al* 2010). Several authors have looked for seasonal differences in AUGIB with conflicting results. Results from China suggest an increased incidence during colder months (December to April) and similar results are also seen in Israel (Du *et al* 2010; Stermer *et al* 1995). The observation of a decrease in presentation during winter is consistent with a previous European based study (Thomopoulos *et al* 1997).

1.2.3 Aetiology and pathophysiology of non-variceal acute upper gastrointestinal bleeding

Helicobacter pylori (*H. pylori*) is the main aetiological factor in the development of peptic ulcer disease. being responsible for up to 90% of duodenal and 70% gastric ulcers (Marshall *et al* 1985). In bleeding peptic ulcers the prevalence of *H. pylori* is between 55-68% (Tang *et al* 2009). One study suggests the prevalence is as high as 92.4% in bleeding duodenal ulcers, 84% in non-steroidal anti-inflammatory drugs (NSAIDS) users and 96.7% in non-NSAID users (Gisbert *et al* 2001).

The other factor associated with the development of oesophago-, gastro-, duoden- itis and peptic ulcer disease is the use of NSAIDS. Different NSAIDS have differing risk profiles for the development of AUGIB (Langman *et al* 1994):

- *Low risk* – ibuprofen (OR 2.0, 95% CI 1.4-2.8) and diclofenac (OR 4.2 95% CI 2.6-6.8)
- *Intermediate risk* – indomethacin (OR 11.3, 95% CI 6.3-20.3), naproxen (OR 9.1, 95% CI 5.5-15.1) and piroxicam (OR 13.7, 95% CI 7.1-26.3)
- *High risk* – azapropaone (OR 31.5, 95% CI 10.3-96.9) and ketoprofen (OR 23.7, 95% CI 7.6-74.2)

H. pylori infection and NSAID use are independent risk factors for peptic ulcer disease. There is, however, synergism for the development of peptic ulcer bleeding between *H. pylori* infection and NSAID use (Huang *et al* 2002). This meta-analysis found NSAID use more common in patients with AUGIB than controls, OR 4.85 (95% CI 3.77-6.23). *H. pylori* infection was again found to be more common than in controls, OR 1.79 (95% CI 0.97-3.32.

However, patients taking NSAIDS and who were *H. pylori* positive had an additive effect for risk of AUGIB with an OR 6.13 (95% CI 3.93-9.56) (Huang *et al* 2002).

Despite these causative factors between 11-44% of peptic ulcers are not associated with *H. pylori* infection or the use of NSAIDS (Hung *et al* 2005). However, in *H. pylori* and NSAID negative ulcers, the cause is surreptitious NSAID use in up to 60% of cases. Other medications associated with peptic ulcer disease include bisphosphonates, mycophenalate and potassium chloride. Other rare causes include Zollinger-Ellison syndrome, *helicobacter heilmanii*, cytomegalovirus, systemic mastocytosis, Crohn's disease and idiopathic (Quan *et al* 2002). Other risk factors for the development of peptic ulcer disease are corticosteroid use, oral anticoagulation, previous peptic ulcer, dyspepsia within the previous year, heart failure, diabetes and current smoking (Weil *et al* 2000).

The underlying cause of AUGIB found on upper GI endoscopy is most commonly peptic ulcer disease, which accounts for one third of all bleeds (Hearnshaw *et al* 2011). Other causes include oesophagitis and gastritis. Interesting no abnormality is found in up to 17% of endoscopies performed for AUGIB (Hearnshaw *et al* 2011). Table 1.2 has further details of the abnormality found on endoscopy in patients presenting with AUGIB.

Table 1.2 Abnormality found at endoscopy in patients presenting with acute upper gastrointestinal bleeding (Hearnshaw *et al* 2011)

Cause of bleeding	**Frequency (% found at endoscopy)**
Peptic ulcer	36.0
Oesophagitis	24
Gastritis or erosions	22
Erosive duodenitis	13
Varices	11.0
Portal hypertensive gastropathy	7
Malignancy	3.7
Mallory Weiss tear	4.3
Other (vascular ectasia, haemobilia)	2.6
No abnormality seen	17

1.2.4 Management of acute upper gastrointestinal bleeding

It should be noted that acute upper GI bleeding is a medical emergency associated with significant mortality (Gralnek *et al* 2015). As such, the most important initial management of a patient presenting with upper GI bleeding involves urgent assessment and risk stratification, and prompt fluid resuscitation (Gralnek *et al* 2015). Once the patient has been adequately resuscitated an upper GI endoscopy can be considered. Endoscopic therapy can be used to treat the cause of bleeding, however, if this fails to control the bleeding then interventional radiology or surgery may be required.

1.2.4.1 Pre-endoscopic management of acute upper gastrointestinal bleeding

Resuscitation

All patients should receive adequate fluid resuscitation (Gralnek *et al* 2015). Initially the blood pressure should be maintained with infusion of crystalloid. AUGIB is a common indication for red blood cell (RBC) transfusion, and accounts for 14% of all RBC units transfused in the UK (Wallis *et al* 2006). Blood transfusions are given to 43% of patients hospitalised with AUGIB in the UK (Hearnshaw *et al* 2011).

Guidelines on the use of RBC transfusion vary. Current UK guidelines recommend RBC transfusion after 30-40% of the circulating volume has been lost, based on a reduced systolic and diastolic blood pressure and a tachycardia of greater than 120bpm (SIGN 2008).

The American Society of Gastrointestinal Endoscopy (ASGE) guidelines recommend ‘that packed red blood cells should be transfused in patients with evidence of ongoing or active blood loss who have experienced significant blood loss or cardiac ischaemia’ (ASGE 2004). The latest International Consensus suggests a blood transfusion if the haemoglobin is less

than 70 g/L, with a target of 70-90 g/L in the absence of tissue hypoperfusion, coronary artery disease or acute haemorrhage (Barkun *et al* 2010; Gralnek *et al* 2015). International guidelines recommend considering transfusion when haemoglobin levels are less than 80 g/L or when cardiovascular symptoms develop in haemodynamically stable patients with pre-existing cardiovascular disease (Carson *et al* 2012).

The benefit of RBC transfusion in a massive AUGIB can be lifesaving; however, there is evidence to suggest that early transfusion in less severe bleeding can be harmful (Fabricius *et al* 2016; Hearnshaw *et al* 2010b; Subramaniam et al 2016). Recent evidence suggests early transfusion is associated with a two-fold risk of rebleeding (OR 2.26, 95% CI 1.76-2.90) and a 28% increase in mortality (OR 1.28, 95% CI 0.94-1.74) (Hearnshaw *et al* 2010b). Indeed a Cochrane review reports an increase in rebleeding and mortality rates in the transfusion arms of several studies; however, the data is limited by small numbers (Jairath *et al* 2010). This problem has been overcome by Villanueva and colleagues who investigated liberal and restrictive transfusion strategies for AUGIB (Villanueva *et al* 2013). The restrictive strategy meant blood transfusion occurred when the haemoglobin was less than 70 g/L, whereas the threshold was 90 g/L in the liberal arm. The restrictive group had fewer adverse events such as transfusion reactions and cardiac events (40% versus 48%), rebleeding (10% versus 16%, HR 0.68, 95% CI 0.47-0.98) and most importantly reduced mortality for the 45 days following admission (5% versus 9%, HR for death 0.55, 95% CI 0.33-0.92) (Villanueva *et al* 2013). The most recent National Institute for Health and Care Excellence (NICE) guidelines for blood transfusion endorse the threshold of 70 g/L with a target haemoglobin of 70-90 g/L (NICE 2015).

In practice things are not as black or white as some of the evidence suggests (i.e. choosing to transfuse a patient if the haemoglobin is less than 70 g/L). A more pragmatic view is taken in the latest NICE guidelines on AUGIB which recommend basing decisions on blood

transfusion on the full clinical picture, recognising that over-transfusion may be as damaging as under-transfusion (NICE 2012).

Platelet transfusion should be offered to patients who are actively bleeding and have a platelet count of less than 50 x 10^9/L. The appropriate use of platelet transfusion has been associated with a reduction in the need for re-endoscopy (Fabricius *et al* 2016). Fresh frozen plasma should be given to patients with either (i) a fibrinogen level of less than 1 g/L or (ii) a prothrombin time or activated partial thromboplastin time of greater than 1.5 times normal. Prothrombin complex concentrate should be offered to patients who are taking warfarin and are actively bleeding (NICE 2012).

1.2.5 Pre-endoscopy risk assessment

Scoring systems have been developed to highlight those patients who are likely to require an intervention or are at risk of rebleeding and death. The Rockall and Blatchford scores are the most well recognised such scores (Rockall *et al* 1996; Blatchford *et al* 2000).

The Rockall scoring system was devised to identify patients at risk of death or rebleeding based upon a combination of clinical and endoscopic findings (Rockall *et al* 1996a). The score is split into pre-endoscopic Rockall score which is based upon age, presence of shock and co-morbidity, whilst the post-endoscopic score also takes into account endoscopic diagnosis and stigmata of recent haemorrhage (see table 1.3). The maximum score is 7 pre-endoscopy and 11 post-endoscopy. A score of 0 pre-endoscopy would be a patient under the age of 60 years with no signs of shock and no significant co-morbidity. In this study, 15% of patients had an initial score of 0 which signifies a low risk of death (0.2%) or rebleeding

(0.2%) and these patients should be considered for discharge and early outpatient endoscopy (Rockall *et al* 1996b).

Mortality rates increase with increasing pre-endoscopic score:

- Rockall score 0 – death 0.2%
- Rockall score 1 – death 2.4%
- Rockall score 2 – death 5.6%
- Rockall score 5 – death 39.6%
- Rockall score 7 – death 50%

Of those patients who undergo endoscopy, a Rockall score ≤ 2 is associated with a 4.3% risk of rebleeding and 0.1% risk of death (Rockall *et al* 1996b). These patients should be considered for early discharge.

The Rockall score has been validated as a good tool for stratifying patients into high- and low-risk for mortality, however, it is less useful in the prediction of rebleeding (Vreeburg *et al* 1999).

The Glasgow Blatchford Scoring (GBS) system was developed to predict those patients with AUGIB that would require intervention (i.e. blood transfusion, endoscopic therapy or surgery) or death (Blatchford *et al* 2000, Stanley *et al* 2009). The score was developed in Scotland with data from 1748 patients presenting with AUGIB (Blatchford *et al* 2000).

The advantage of the Blatchford score over the Rockall score is that it is based on simple variables from a patient's history, examination and laboratory results (see table 1.4), whereas

the Rockall score is not complete until endoscopic findings are known, often meaning patients are kept in hospital unnecessarily.

On the results of simple laboratory and clinical data a GBS score between 0 and 23 is derived. A score of 0 identifies a group of patients with a very low-risk of requiring an intervention – 0.5% (Blatchford *et al* 2000).

Up to 16% of patients admitted with an AUGIB have a Blatchford score of 0 and up to 28% have a Rockall score of 0 (Stanley *et al* 2009). There is evidence to support the Blatchford score being more useful than the Rockall score for predicting low-risk patients who do not need therapeutic endoscopy and who may be suitable for outpatient management (Stanley *et al* 2009, Pang *et al* 2010). Of the patients in one study with a Blatchford score of 0, no interventions and no deaths were recorded. However, 17% of those with an initial Rockall score of 0 required an intervention and there was 1 death (Stanley *et al* 2009).

A study of low-risk AUGIB individuals, defined as GBS ≤ 2 and age < 70, identified 34.2% of all patients presenting with AUGIB of whom 10.5% were managed safely as outpatients (Stephens *et al* 2009). Other studies have also looked at a GBS of ≤ 2 to define low-risk (Masaoka *et al* 2006; Srirajaskanthan *et al* 2010). To assess the validity of the GBS at separating low and high-risk groups, receiver-operator characteristic (ROC) curves were plotted. The GBS had an area under ROC curve of 0.96 (95% CI 0.95–1.00). When a cut-off value of ≥ 3 was used, sensitivity and specificity of GBS for identifying high risk bleeds was 100% and 68%. Thus at a cut-off value of ≤ 2 the GBS is useful for distinguishing those patients with a low risk UGI bleed (Srirajaskanthan *et al* 2010). Despite this evidence current international guidelines define high-risk patients as those with a GBS ≥ 1 (Sung *et al* 2011).

The latest NICE guidelines suggest formal risk scoring in all patients presenting with AUGIB; the Blatchford score being used at first assessment and the Rockall score once an endoscopy has been performed (NICE 2012).

Table 1.3 Rockall Scoring system

Variable	0	1	2	3
Age	< 60 years	60-79 years	≥ 80 years	
Shock	No shock SBP ≥ 100mm Hg, pulse < 100 bpm	Tachycardia SBP ≥ 100mm Hg, pulse > 100 bpm	Hypotension SBP < 100mm Hg	
Co-morbidity	None		Cardiac failure, IHD	Renal failure, liver failure, metastatic malignancy
Diagnosis	Mallory-Weiss tear, no lesion and no SRH	All other diagnoses	Malignancy of upper GI tract	
Major SRH	None or dark spot only		Blood in upper GI tract, adherent clot, visible or spurting vessel	

SBP – systolic blood pressure; IHD – ischaemic heart disease; SRH – stigmata of recent haemorrhage

Table 1.4 Glasgow Blatchford Score

Clinical Variable	Score Value
Blood urea (mmol/L)	
6.5-7.9	2
8.0-9.9	3
10.0-25.0	4
>25.0	6
Haemoglobin for men (g/L)	
120-129	1
110-119	3
<100	6
Haemoglobin for women (g/L)	
100-119	1
<100	6
Systolic BP (mm Hg)	
100-109	1
90-99	2
<90	3
Other markers	
Pulse ≥ 100/min	1
Melaena	1
Syncope	2
Hepatic disease*	2
Cardiac failure†	2

*Known history, or clinical and laboratory evidence, of chronic or acute liver disease. †Known history, or clinical and echocardiographic evidence of, cardiac failure.

1.2.6 Pharmacological therapy

There is evidence suggesting that the use of pharmacological therapy to increase gastric pH by inhibiting gastric acid secretion promotes clot formation, stabilises the clot and perhaps hastens the healing of lesions (Barkun *et al* 2006). This is based upon historic *in vitro* data that suggest a pH above 6 is needed to inactivate pepsin (Green *et al* 1978). A more recent study found that by increasing the intragastric pH to ≥ 6.4, by proton pump inhibitor (PPI) therapy, gastric mucosal bleeding time was reduced (Li *et al* 2000).

- **Proton pump Inhibitors (PPI) therapy**. It appears to be commonplace for patients to receive a PPI upon presentation with AUGIB, with up to 89% of patients receiving intravenous PPI prior to endoscopy (Enns *et al*, 2004; Hearnshaw *et al* 2010a). However, the evidence for such an approach is lacking. One randomised controlled trial (RCT) assigned patients to receive either omeprazole (80mg IV bolus followed by 8mg per hour) or placebo (Lau *et al* 2007). Those in the omeprazole group had a reduced need for endoscopic therapy (19.1% versus 28.4%, P=0.007), fewer actively bleeding ulcers and more ulcers with a clean base. However, there were no differences in transfusion need, rebleeding or death (Lau *et al* 2007). Similar findings were observed in a Canadian study (Andrews *et al* 2005). Indeed, a recent Cochrane meta-analysis of 6 RCTs reached a similar conclusion (Sreedharan *et al* 2010). PPI treatment reduced the need for endoscopic therapy at initial endoscopy (OR 0.68, 95% CI 0.50-0.93). However, no significant differences were seen in mortality (OR 1.12, 95% CI 0.72-1.73), rebleeding (OR 0.81, 95% CI 0.61-1.09) or need for surgery (OR 0.96, 95% CI 0.68-1.35) (Sreedharan *et al* 2010). Interestingly, the use of pre-endoscopy PPI is cost-effective as it reduces the need for endoscopic therapy. However, this evidence is based on Asian population studies, which usually have a different case mix to western centres (less alcohol and different parietal cell mass) (Tsoi *et al* 2008). Current UK guidelines do not support the use of PPIs prior to endoscopy (SIGN 2008; NICE 2012).

- **Prokinetic agents**. The prokinetic erythromycin (a motilin receptor agonist) can be given prior to endoscopy in the case of AUGIB to stimulate gastrointestinal motility (Peeters *et al* 1989). Erythromycin given intravenously prior to endoscopy does improve endoscopic examination with improved rates of mucosal visualisation and reduces the numbers of clots, but does not impact important clinical outcomes – mortality, ability to identify source of bleed, length of hospital stay and need for blood transfusion or surgery (Carbonell *et al* 2006; Coffin *et al* 2002; Frossard *et al* 2002; Pateron *et al* 2011). This approach, however, has been shown to be cost-effective using computer modelling (Winstead *et al* 2010).

- **Tranexamic acid**. The use of tranexamic acid in AUGIB dates back almost 40 years when several small studies were carried out with conflicting results (Barer *et al* 1983; Biggs *et al* 1979; Engqvist *et al* 1979). Recent Cochrane reviews concluded that tranexamic acid may reduce mortality associated with AUGIB although due to the high drop out in previous trials further study is required and routine use cannot be currently recommended (Bennett *et al* 2014; Gluud *et al* 2012). It is not currently recommended for routine use in any guidelines. However, this may change when the results of the ongoing HALT-IT trial, a randomised controlled trial of the effect of tranexamic acid in AUGIB, become available (Roberts *et al* 2014).

1.2.7 Endoscopy for acute upper gastrointestinal bleeding

Endoscopy is the primary diagnostic and therapeutic tool for patients with AUGIB. Endoscopy should take place after adequate resuscitation or in those patients with ongoing bleeding (Gralnek *et al* 2015). Endoscopic therapy in AUGIB has reduced transfusion requirements, rebleeding rates, the need for surgery, mortality and length of hospital stay (Cappell *et al* 2008; Kovacs *et al* 2008).

Endoscopy within 24 hours of presentation (early endoscopy) allows for early risk stratification, improves patients outcomes for those with high-risk lesions found on endoscopy and allows for the early (and safe) discharge of patients with low risk lesions (Barkun *et al* 2010). UK and international guidelines advocate endoscopy within 24 hours of admission (Barkun *et al* 2010; NICE 2012; SIGN 2008). However, there are conflicting results when it comes to the timing of endoscopy. In one study, very early endoscopy (i.e. within 12 hours) did not improve the clinical outcomes of mortality, need for surgery or reduce transfusion rates (Tsoi *et al* 2009) in all comers with AUGIB. However, another study found that delay to endoscopy was associated with an increase in mortality (Wysocki *et al* 2012). Furthermore, in high-risk patients, as defined by a GBS ≥ 12, endoscopy within 13 hours of presentation has been associated with a reduction in mortality (Lim *et al* 2011). Although a more recent study has demonstrated that very early endoscopy (within 12 hours) in patients with a GBS <12 is associated with an increase in a composite outcome of death, rebleeding and the need for surgical or radiological intervention (Kumar *et al* 2016). In the UK, unfortunately only 50% of patients undergo endoscopy within 24 hours of presentation with an AUGIB (Hearnshaw *et al* 2010a). Endoscopy may be delayed if the patient is too unwell or due to co-morbid conditions such as recent ACS. The timing of endoscopy in this situation is discussed elsewhere (see section 1.3.8).

Generally, endoscopy should take place after adequate fluid resuscitation as this is associated with a reduction in cardiopulmonary complications (Barkun *et al* 2003; Kumar *et al* 2016). In certain situations where a patient is unstable with ongoing bleeding and signs of shock, early endoscopy should take place whilst the patient is being resuscitated. Early endoscopic therapy in this situation is associated with better patient outcomes (Barkun *et al* 2010; Kumar *et al* 2016; Lim *et al* 2011). Endotracheal intubation to prevent aspiration is recommended for patients with ongoing haematemesis or altered mental status (Ghassemi *et al* 2009).

Endoscopy should take place in a controlled environment with a specialist nursing team who are familiar with endoscopic techniques.

Peptic ulcers are a common cause found on endoscopy in patients with AUGIB although not all ulcers will warrant endoscopic therapy. The need for endoscopic therapy can be categorised by stigmata of recent haemorrhage (SRH). These are split into high and low-risk SRH, based on risk of rebleeding, and the endoscopic management of each lesion varies. Table 1.5 shows SRH and the risk of recurrent bleeding (Enestvedt *et al* 2008; Kovacs *et al* 2007; Laine and Peterson 1994).

Table 1.5 Stigmata of recent haemorrhage and risk of bleeding, surgery and mortality in peptic ulcer disease

Stigmata of recent haemorrhage	Forrest classification	Prevalence	Further bleeding	Surgery for bleeding	Mortality
Active spurting bleeding	IA	12% (spurting and oozing)	55% (spurting and oozing)	35% (spurting and oozing)	11% (spurting and oozing)
Active oozing bleeding	IB				
Non-bleeding visible vessel	IIA	8%	43%	34%	11%
Adherent clot	IIB	8%	22%	10%	7%
Flat pigmented spot	IIC	16%	10%	6%	3%
Clean base	III	55%	5%	0.5%	2%

1.2.8 Endoscopic therapy

The endoscopist has a myriad of options for therapeutic endoscopy and the modality used is user dependent based on familiarity with the various techniques. The goal is to prevent continued bleeding and reduce the risk of rebleeding. Therapeutic endoscopy in patients with a peptic ulcer is not indicated for low-risk stigmata and should be reserved for those with high-risk stigmata (Barkun *et al* 2010).

- **Peptic ulcer disease**. As already mentioned the management of AUGIB due to a peptic ulcer does not necessarily require endoscopic therapy. This should be reserved for patients with high risk SRH; those with active spurting or oozing or a non-bleeding visible vessel (Laine and Jensen 2012). The therapeutic options available to an endoscopist are adrenaline injection, mechanical clips, thermocoagulation, Hemospray and sclerosant injection. Adrenaline halts bleeding by 3 mechanisms: local tamponade by the injection of fluid, by vasoconstriction and by thrombosis (Kubba *et al* 1996).

Current national and international guidelines recommend the use of adrenaline plus another modality (i.e. clips or thermocoagulation) for endoscopic haemostatic therapy for patients with high-risk stigmata – active bleeding, non-bleeding visible vessel and an adherent clot (Barkun *et al* 2010; SIGN 2008, NICE 2012). Adrenaline should not be used alone, as it is inferior to both combination therapy and thermal or clips alone (Barkun *et al* 2009; Barkun *et al* 2010). One meta-analysis compared injection plus clips to injection therapy alone. Dual therapy with injection plus clips was superior in haemostasis to injection alone (88.5% versus 78.1%; RR 1.13, 95% CI 1.03-1.23) leading to a reduction in the need for surgery, however, no difference in mortality was observed (Sung *et al* 2007). A second meta-analysis found the addition of a second endoscopic modality, in addition to adrenaline injection, reduced rebleeding (18.4% to 10.6%), surgery (11.3% to 7.6%) and more importantly, mortality was

reduced from 5.1% to 2.1% (OR 0.51; 95% CI 0.31-0.84) (Calvet *et al* 2004). A subsequent Cochrane review found that adding a second modality to adrenaline injection reduced rebleeding (OR 0.51, 95% CI 0.39-0.66), need for surgery (OR 0.63, 95% CI 0.45-0.89) and mortality (OR 0.50, 95% CI 0.30-0.82) (Vergara *et al* 2007).

One area of controversy with regards to peptic ulcers is what to do with an adherent clot. Many endoscopists adopt a more cautious approach, leaving the clot *in situ* and continuing pharmacologic therapy, whereas 'the brave' remove the clot and apply treatment to the ulcer. Rebleeding rates for ulcers with an adherent clot vary between 10-40% and after clot removal is only 8% (Laine *et al* 1994, Laine *et al* 1996). The findings after clot removal are a clean ulcer base in 57%, flat spot 11%, non-bleeding visible vessel 13%, oozing 13%, and spurting 2% (Laine *et al* 1996). One randomised study compared a combination of endoscopic therapy and omeprazole infusion (80 mg bolus followed by 8 mg/hour) to omeprazole alone (Sung *et al* 2003). In this study, patients with adherent clot had this actively removed using irrigation and a mini-snare, and the underlying vessels were treated with thermocoagulation. Recurrent bleeding within 30 days was 11.6% in those treated with omeprazole alone and 1.1% rebleeding in the dual therapy group (P=0.009). This has been confirmed by further studies (Bini *et al* 2003; Bleau *et al* 2002; Jensen *et al* 2002). In these studies patients treated with endoscopic removal of the clot and endoscopic therapy had lower rates of rebleeding, decreased length of hospital stay and lower numbers of RBC transfusion. A subsequent meta-analysis drew the same conclusions (RR rebleeding of 0.35, 95% CI 0.14-0.83) (Kahi *et al* 2005). International guidelines recommend that when an adherent clot is present that endoscopic removal of the clot should be considered so that the underlying vessel can be treated (Gralnek *et al* 2015; Hwang *et al* 2012; Laine and Jensen 2012).

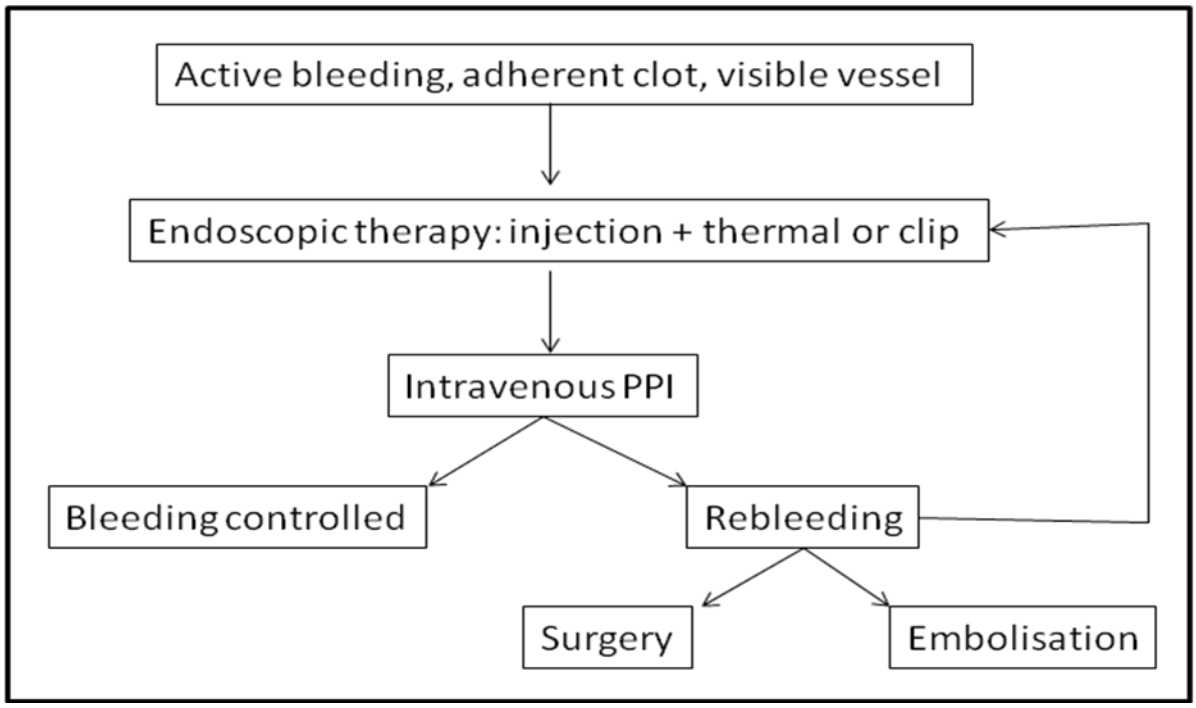


Figure 1.1 Management algorithm for the acute phase management of acute upper gastrointestinal bleeding due to peptic ulcer. This shows the algorithm of endoscopic therapy for high-risk endoscopic features, the need for subsequent intravenous PPI therapy and options should rebleeding occur.

1.2.9 Post-endoscopy management of acute upper gastrointestinal bleeding

- **Proton pump inhibitors.** The basis of using PPIs is their effect on intragastric pH. Gastric acid plays an important role in impairing haemostasis and causing clot lysis (Laine *et al* 2008). Raising the pH above 6 helps stabilise the clot by facilitating platelet aggregation (Green *et al* 1978). High-dose omeprazole can neutralise intragastric pH. An initial bolus followed by a constant intravenous infusion of omeprazole increases the intragastric pH above 6 rapidly and maintains this for the majority of a 24 hour period (Labenz *et al* 1997). The elimination half-life of oral PPIs is approximately 1 hour meaning that new proton pumps can produce acid and therefore an intravenous infusion may be preferential to maintain a high gastric pH (Laine *et al* 2008). A newer study correlated the findings of Laine *et al* and confirmed that the PPIs omeprazole, pantoprazole and rabeprazole given either orally or intravenously raise the intragastric pH above 6, significantly higher than placebo where a pH of 2.04 was seen (Javid *et al* 2009).

There is no doubt about the benefit of high dose intravenous PPI therapy post-endoscopy in patients with high-risk lesions found at endoscopy. Previous studies have shown that rebleeding is most common within the first 72 hours after endoscopy (Laine *et al* 1994). This gives the rationale to maintain intragastric pH above 6 for this period. A randomised double-blind study comparing high-dose intravenous omeprazole with placebo following endoscopic therapy for peptic ulcer bleeding found significantly reduced rates of rebleeding in the PPI group (Lau *et al* 2000). Of the 240 patients enrolled (120 in each group), rebleeding within 30 days occurred in 6.7% and 22.5% in the PPI and placebo groups respectively (HR 3.9, 95% CI 1.7-9.0). The PPI cohort had lower transfusion requirements and shorter duration of stay. Mortality was lower in the PPI group, but not significantly (P=0.14). This study was carried out in a predominately Asian population and cannot necessarily be applicable to a Caucasian population (Asians have a smaller parietal cell mass and tend to be slow metabolisers). A

newer study with a cohort of 85% Caucasians, compared intravenous esomeprazole or placebo following endoscopic therapy for patients with peptic ulcer bleeding and high-risk SRH (Sung *et al* 2009). The rate of rebleeding was significantly lower in the group randomised to esomeprazole (P=0.026), although it found that surgery and mortality rates were unchanged.

The benefit of a PPI infusion in this setting has been confirmed by various systematic reviews and meta-analyses, with this strategy also found to be cost effective (Barkun *et al* 2004; Barkun *et al* 2010; Leontiadis *et al* 2005; Leontiadis *et al* 2006; Leontiadis *et al* 2007; Leontiadis *et al* 2010). Newer evidence, from a meta-analysis studying intermittent versus continuous PPI infusion, found similar results in terms of rebleeding, mortality and length of stay between the two approaches (Sachar *et al* 2014). Future guidelines may take into account these findings which would be both cost and resource saving.

An oral PPI can be commenced once the 72 hour infusion has been finished. If *H. Pylori* is confirmed, and eradicated, an additional 3 week course of PPI is needed to ensure ulcer healing (Liu *et al* 2003). A maintenance dose is not needed unless ulcerogenic medications are to be continued.

- ***Helicobacter pylori* eradication**. The detection, and subsequent eradication, of *H. pylori* is important in the management of AUGIB. *H. pylori* is the main cause of peptic ulcers. Successful eradication is associated with reduced ulcer recurrence: 6% vs. 67% for patients with duodenal ulcers; 4% vs. 59% for patients with gastric ulcers (Hopkins *et al* 1996). *H. pylori* eradication within 6 months of a diagnosis of peptic ulcer was associated with reduced hospitalisation with major ulcer events (HR 0.57, 95% CI 0.54-0.59) (Hsaio *et al* 2011). Eradication of *H. pylori* is more effective than anti-secretory medication (H2RA or PPI) non-eradication therapy in preventing rebleeding from peptic ulcers (4.5% versus

23.7%; OR 0.18, 95% CI 0.09-0.37) (Gisbert *et al* 2004; Gisbert *et al* 2004). One study from Finland found that *H. Pylori* eradication therapy alone may be sufficient in the treatment of *H. Pylori* positive ulcers without the need for PPI therapy (Arkkila *et al* 2005). Current guidelines suggest a 14 day eradication regimen (Fallone *et al* 2016; Chey *et al* 2017).

It should be noted, however, that the detection of *H. Pylori* in the setting of a bleeding ulcer can be problematic. Studies have shown the rapid urease tests sensitivity to detect *H. Pylori* is reduced with increasing numbers of false-negatives in the context of bleeding peptic ulcers (Romero Gómez *et al* 1998; Tang *et al* 2009). A meta-analysis found biopsy-based methods of *H. Pylori* detection such as rapid urease test, histology and culture have a low sensitivity but high specificity in patients with AUGIB (Gisbert *et al* 2006). Current guidelines suggest caution of negative results in the acute setting and the need for repeated testing (Barkun *et al* 2010).

- **NSAIDS.** In patients suffering from arthritis or cardiovascular disease there may be a strong indication for continuing ulcerogenic treatment. In these cases the benefits and risks of therapy must be assessed in each individual patient.

With respect to NSAIDS and cyclooxygenase-2 (COX-2) inhibitors current evidence suggests a strong link with recurrent bleeding. One study compared celecoxib to diclofenac plus PPI in patients presenting with peptic ulcer bleeding. After 6 months recurrent bleeding was 4.9% (95%CI 3.1-6.7) in the celecoxib group and 6.4% (95%CI 4.3-8.4) in the diclofenac plus PPI group (Chan *et al* 2002). NSAID plus PPI therapy offers some gastroprotection, however, a COX-2 inhibitor plus PPI offers better protection. One study compared celecoxib against celecoxib plus esomeprazole with the primary endpoint being recurrent ulcer bleeding (Chan *et al* 2007). Recurrent bleeding occurred in 8.9% in the celecoxib group and 0% in the combined group (P=0.0004). Unfortunately, there are no head-to-head trials comparing COX-

2 plus PPI against NSAID plus PPI. If NSAIDS or COX-2 inhibitors are to be continued then a concomitant PPI should be given for the duration of treatment. In addition, *H. Pylori* should be tested for and treated to further reduce the risk of bleeding.

- **Anti-platelet agents.** Antiplatelet therapy can cause AUGIB. Antiplatelet agents should be restarted as soon as the cardiovascular benefits outweigh the risk of further bleeding. The common dilemma is should antiplatelet therapy be discontinued, and for how long? Aspirin is often stopped, in up to 63% of patients in one study, in the context of AUGIB but is often not restarted prior to discharge (Cheung *et al* 2009). The cessation of anti-platelet therapy is associated with recurrent thrombotic events (Biondi-Zoccai *et al* 2006).

One prospective RCT examined patients taking aspirin who suffered AUGIB. Patients were assigned to aspirin or placebo immediately following endoscopic therapy plus high-dose intravenous PPI. Patients who received aspirin had lower all-cause mortality, 1.3% versus 12.9% and lower mortality as a result of cardiovascular, cerebrovascular and gastrointestinal complications, 1.3% versus 10.3% (Sung *et al* 2010b). Recurrent ulcer bleeding was higher in the aspirin group, 10.3% versus 5.4% in the placebo group. As a result of this guidelines recommend the early re-introduction of antiplatelet therapy (Barkun *et al* 2010, NICE 2012). The recent European Society of Gastrointestinal Endoscopy (ESGE) suggest restarting aspirin immediately after endoscopy if low-risk stigmata is seen; in the context of high-risk stigmata the re-introduction of aspirin should be considered by day 3 provided that adequate haemostasis has been achieved (Gralnek *et al* 2015). However, this may seem counter-intuitive when a patient has been admitted with bleeding, and so clinicians have been slow to change and adopt this guidance.

Aspirin plus a PPI is superior to clopidogrel alone in terms of the prevention of ulcer rebleeding (Chan *et al* 2005; Lai *et al* 2006). Rates of rebleeding in one study was 8.6% for clopidogrel compared with 0.7% for aspirin plus esomeprazole (Chan *et al* 2005). A second study found 0% ulcer complications in the aspirin plus esomeprazole group compared with 13.6% in the clopidogrel alone group (Lai *et al* 2006). Pooled results of these two studies show a significant reduction in rebleeding with aspirin and a PPI versus clopidogrel (OR 0.06, 95% CI 0.01-0.32), but no difference on mortality (OR 0.63, 95% CI 0.24-1.64) (Barkun *et al* 2010). With debate still raging of a significant interaction between clopidogrel and PPIs, it is felt that aspirin and a PPI is the best way forward in this situation (Disney *et al* 2011). In patients receiving dual antiplatelet therapy concomitant PPI use is associated with lower rates of AUGIB (HR 0.13, 95% CI 0.03-0.56, P=0.001) (Bhatt *et al* 2010).

Currently there is no evidence for the optimum management of patients on dual antiplatelet therapy (e.g. aspirin plus a thienopyridine) who develop AUGIB. Withholding antiplatelet therapy should be individualised based upon cardiovascular and gastrointestinal risk; for example, the indication for dual antiplatelet therapy, timing of stent placement, drug-eluting versus bare metal stent. However, it should be noted that PPI use in the context of dual antiplatelet therapy does reduce the incidence of AUGIB (Bhatt *et al* 2010; Kwok *et al* 2011; Lin *et al* 2011). PPI therapy confers better protection than H2RA (Lin *et al* 2011).

In those patients remaining on aspirin it is important to recognise and eradicate *H. Pylori*. Among patients with *H. pylori* infection and a history of upper gastrointestinal bleeding who are taking low-dose aspirin, the eradication of *H. pylori* is equivalent to treatment with omeprazole in preventing recurrent bleeding (Chan *et al* 2001).

The acute management of AUGIB in the context of ACS is dealt with elsewhere (section 1.3.8).

1.2.10 Rebleeding

Peptic ulcer bleeding

Rebleeding had been thought to occur in approximately 20% (range 9-42%) of patients despite medical and endoscopic intervention (Schoenberg *et al* 2001). However, a recent systematic review found lower rates of rebleeding of 13.9% (95% CI 8.4-19.4) (Lau *et al* 2011). The most recent figures from the UK suggest a rebleeding rate of 13% (95% CI 12-14) (Hearnshaw *et al* 2011).

In the event of rebleeding there are 4 options available:

1. Medical management. The least effective option. Further fluid and RBC infusion in addition to intravenous PPI, but no further endoscopy. Only those patients deemed unfit for further intervention should be in this group.

2. Re-endoscopy. A randomised trial comparing a second endoscopy with surgery reported favourable outcomes (Lau *et al* 1999). There were more complications in the patients assigned to surgery (OR 3.45, 95% CI 1.2-9.1). Endoscopic therapy reduced the need for surgery without an increase in mortality (Lau *et al* 1999). This is the preferred strategy in current international guidelines (Barkun *et al* 2010).

3. Interventional radiology. This is fast becoming a more attractive alternative to surgery for patients deemed high-risk when endoscopic therapy has failed (Loffroy *et al* 2010). Selective angiography and embolisation of a culprit vessel can be performed under light sedation in those patients in whom surgery is not a viable option due to co-morbidity. The need for surgery varies between 2-37% and 30-day mortality rates between 4-46% (Loffroy *et al* 2010). When compared with surgery for those patients who failed endoscopic therapy several retrospective studies found transarterial embolisation reduced

the need for surgery without increasing mortality (Ripoll *et al* 2004; Wong *et al* 2011). This is despite the embolisation group being older and with more cardiac co-morbidities. More complications were seen in the surgical group (Wong *et al* 2011). Figures from the UK national gastrointestinal bleed audit reveal that 1.2% of patients underwent an interventional radiological procedure (Hearnshaw *et al* 2011).

4. Surgery. The need for surgery has been greatly reduced by the widespread use, and increasing complexities, and efficacy of therapeutic endoscopic and interventional radiology. Surgery should be reserved for those patients who have failed endoscopic and interventional radiology (Schoenberg *et al* 2001). The results from the latest UK gastrointestinal bleed audit reveal that 1.9% of patients underwent surgical intervention (Hearnshaw *et al* 2011).

1.2.11 Acute upper gastrointestinal bleeding and cardiovascular disease

Death following AUGIB is often due to co-morbid disease, rather than bleeding itself, in up to 80% of cases (Sung *et al* 2010a). Several studies show that death following AUGIB is often due to cardiovascular disease.

Up to 32% of patients in a study of peptic ulcer bleeding died from either cerebrovascular disease or myocardial infarction (Smart and Langman 1986). One study of survival of patients suffering from peptic ulcer disease followed patients for a median of 36 months. Of the 121 patients who were followed up 30 (25%) died during the study period. Of these, 66% (n=20) died from cardiovascular disease; 23% (n=7) from myocardial infarction and 20% (n=6) from a stroke (Kubba *et al* 1997). Similar associations between peptic ulcer disease and subsequent mortality from cardiovascular disease have also been reported in the UK

population (Hudson *et al* 1995). Analysis of the General Practice Research Database found that of 978 patients presenting with peptic ulcer bleeding cardiovascular disease was the leading cause of death; 30.3%, RR 1.7 (95% CI 1.2-2.5) with cerebrovascular disease accounting for a further 6.5% of deaths (Ruigómez *et al* 2000).

The largest study (> 10000 patients) of outcomes from peptic ulcer bleeding found that cardiovascular disease was responsible for 19% of all deaths; ACS and heart failure 13.5% and cerebrovascular disease 5.4% (Sung *et al* 2010a). A study by the same group found that the early resumption of low-dose aspirin (used for secondary prevention), rather than withholding for 8 weeks, following peptic ulcer bleeding resulted in lower cardiovascular events (Sung *et al* 2010b). Another study found a strong association between the discontinuation of aspirin following a peptic ulcer bleed and the risk of death and acute cardiovascular events during the first six months following admission with bleeding (Derogar *et al* 2013). In this study aspirin was discontinued in 40% of patients with peptic ulcer bleeding. Those patients with cardiovascular co-morbidities who discontinued aspirin had a 7-fold increase (HR 6.9, 95% CI 1.4-34.8) in the risk of death or acute cardiovascular events within the first 6 months.

To conclude there appears to be sufficient evidence to show an association between non-variceal AUGIB and mortality related to cardiovascular disease.

1.3 Bleeding in acute coronary syndromes

1.3.1 Introduction to bleeding in acute coronary syndromes

Cardiovascular disease is the leading cause of death in industrialised countries, with coronary artery disease (CAD) the most prevalent manifestation (McAloon *et al* 2016). The presentation of CAD varies from silent ischaemia through to stable angina, unstable angina, myocardial infarction, heart failure and sudden death (Hamm *et al* 2011).

The acute coronary syndromes encompass a spectrum of unstable coronary artery disease including unstable angina (UA), non-ST segment elevation myocardial infarction (NSTEMI) and ST segment elevation myocardial infarction (STEMI). It is diagnosed based upon the specific characteristics of each element of the triad of: clinical presentation (including a history of coronary artery disease), electrocardiographic changes (such as ST segment elevation) and biochemical cardiac markers (such as an elevated troponin level) (SIGN 2013).

The initial management of ACS involves patient stabilisation and pain relief. The continuing management depends upon the presentation. A patient with a STEMI invariably undergoes coronary angiography and primary percutaneous coronary intervention with subsequent antiplatelet and antithrombotic medications. However, patients with UA and NSTEMI are typically initially treated with antiplatelet and antithrombotic medications with subsequent coronary angiography with, or without, primary percutaneous coronary intervention (Hamm *et al* 2011; Steg *et al* 2012).

There are numerous pharmacological interventions used in the management of ACS such as anti-platelet agents (e.g. aspirin, thienopyridines/P2Y12 inhibitors, glycoprotein IIb/IIIa

inhibitors) and anti-thrombotic agents (e.g. heparin, direct thrombin inhibitors). These medications are regularly used in combination.

The advent of potent anti-platelet and anti-thrombotic agents over the past decade has resulted in significant improvement in reducing ischaemic events in ACS, which has resulted in a reduction in the risk of death (Pham *et al* 2011). Bleeding was once regarded as merely a reversible side effect of treatment to Cardiologists; themselves favouring the use of treatments aimed at reducing recurrent ischaemia and the risk of death (Armstrong *et al* 1998). However, times have changed and bleeding has now been established as not just a side effect easily rectified by stopping treatment, but as an independent risk for poor short- and long-term outcomes in the context of ACS. Bleeding is the most frequent non-ischaemic complication observed in the management of ACS. In fact bleeding is an area of much interest and is now covered in detail in international guidelines for the treatment of ACS (Hamm *et al* 2011).

1.3.2 Epidemiology and aetiology of bleeding in acute coronary syndromes

Historically, trials examining the treatment of ACS with antithrombotic agents have reported major bleeding rates between 0.4% - 10.6% (GUSTO IIb 1996, PRISM 1998, PURSUIT 1998, PARAGON-B 2002). The incidence of bleeding is likely to be higher outside randomised controlled trials in the 'real-life' population as high-risk patients are often excluded in therapeutic trials. The incidence of major bleeding from registry data is as high as 11.5% with overall bleeding rates between 5.2 - 36% (Sobieraj-Teague *et al* 2008). Interestingly, despite the use of more aggressive antithrombotic treatment regimens, data from the Global Registry of Acute Coronary Events (GRACE) registry from 2000-2007 suggests that the incidence of major bleeding has decreased over time from 2.6% to 1.8%

(Fox *et al* 2010). This may indicate that clinicians are now more aware of bleeding complications and as a result have adapted management strategies accordingly (Hamm *et al* 2011).

The first large observational study to identify risk factors for bleeding in ACS was published in 2003 (Moscucci *et al* 2003). This study analysed data from 24045 patients from the GRACE registry; the incidence of bleeding was 3.9%. Older age, female gender, histories of bleeding and chronic kidney disease were all independently associated with major bleeding. Interventions associated with bleeding were percutaneous coronary intervention (PCI), GP IIb/IIIa receptor blockers and pulmonary artery catheters (Moscucci *et al* 2003).

The first authors to associate bleeding with poor outcomes in the context of ACS found that mild bleeding occurred in 16.6%, moderate in 9.8% and severe in 1.2% of patients (Rao *et al* 2005). This study examined 26452 patients with ACS from 4 clinical trials (GUSTO IIb, PURSUIT and PARAGON A and B). The Global Utilisation of Streptokinase and Tissue Plasminogen Activator for Occluded Coronary Arteries (GUSTO) definition of bleeding was used. Risk factors for bleeding were old age, reduced body weight, female gender and the presence of cardiac risk factors such as diabetes, hypertension.

Numerous studies have evaluated risk factors for major bleeding (Eikelboom *et al* 2006; Khan *et al* 2015; Kinnaird *et al* 2003; Manoukian *et al* 2007; Mehran *et al* 2009a; Moscucci *et al* 2003; Rao *et al* 2005; Spencer *et al* 2007). Risk factors for bleeding can be classified into patient characteristics and therapeutic.

More recently there has been interest in biomarkers associated with bleeding events and one recent study found that higher levels of growth differentiation factor-15 (GDF-15) is associated with risk of bleeding (Hagström *et al* 2016). Patients with higher levels of the renal function biomarkers, beta-trace protein and cystatin C, are at increased risk of major

bleeding (López-Cuenca *et al* 2013). Point of care platelet function testing has also been studied; those patients with low on-treatment platelet reactivity had a higher risk of bleeding (Cuisset *et al* 2013; Holm *et al* 2014; Huczek *et al* 2013; Patti *et al* 2011). Although of academic interest, the use of platelet function testing has yet to become routine clinical practice (Aradi *et al* 2015).

A summary of risk factors for bleeding in ACS can be seen in table 1.6 and figure 1.2.

Table 1.6 Risk factors for bleeding in acute coronary syndromes

Patient characteristics	Treatment related risk factors
Older age	GP IIb/IIIa inhibitors
Female gender	Unfractionated heparin
Lower body mass index	Thienopyridines
Comorbidity: chronic kidney disease, hypertension, diabetes, previous stroke	Diuretics
Lower blood pressure and higher pulse on admission	Inotropes
Smoking	Invasive therapy e.g. PCI, coronary artery bypass graft (CABG), intra-aortic balloon pump (IABP)
Prior history of bleeding	
ST-segment deviation	
Baseline anaemia	

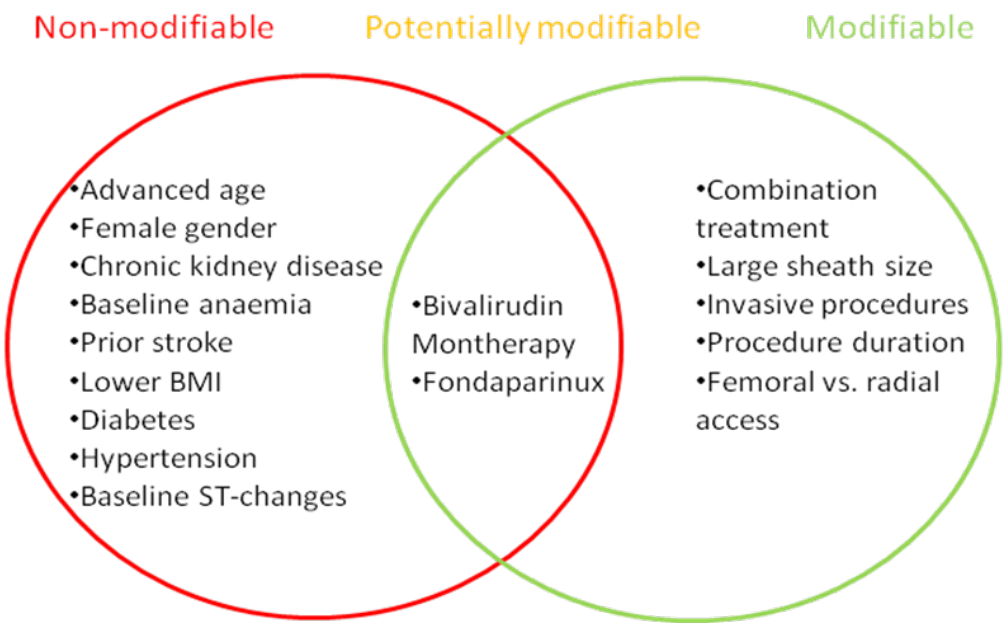


Figure 1.2 Risk factors for bleeding in ACS. An outline of the risk factors that are non-modifiable, such as patient characteristics, with those that can be modified such as treatments chosen and procedure characteristics (adapted from Pham *et al* 2011).

1.3.3 Site of bleeding

The most common sites for bleeding complications are, in descending order (Moscucci *et al* 2003, Spencer *et al* 2007):

- Gastrointestinal bleeding 31.5%
- Vascular access site 23.8 – 29%
- Retroperitoneal 6.0%
- Genitourinary 4.8%
- Intracerebral 6%

In patients undergoing PCI the most common site of bleeding is the vascular access site. The site of access for PCI is important. Femoral arterial site complications (e.g. haematoma and pseudo-aneurysm) are responsible for a significant portion of the bleeding complications occurring during treatment for ACS (Doyle *et al* 2008; Moscucci *et al* 2003; Segev *et al* 2005). Several studies show that a trans-radial approach (i.e. an arterial puncture at the wrist as opposed to the groin which is used with the femoral approach) results in a lower incidence of bleeding events, with major bleeding events reduced by 73% in one study when the trans-radial approach was used (Agostini *et al* 2004; Jolly *et al* 2009). In addition a trans-radial approach, as compared to femoral, has been shown to reduce mortality and major adverse cardiovascular events (Andò and Capodanno 2015; Karrowni *et al* 2013).

1.3.4 Definition of bleeding in acute coronary syndromes

The wide range of definitions used when assessing various anti-platelet or anti-thrombotic agents causes confusion with interpretation of the rate of bleeding and in the association of bleeding with poor outcomes. Lack of standardisation makes it difficult to optimally organise key clinical trial processes such as adjudication, and even more difficult to interpret relative safety comparisons of different antithrombotic agents across studies, or even within a given trial, because results may vary according to the definition(s) used for bleeding (Mehran *et al* 2011a).

The Thrombolysis in Myocardial Infarction (TIMI) and Global Use of Strategies to Open Occluded coronary arteries (GUSTO) definitions of major bleeding were first developed in the thrombolytic era to assess the short-term bleeding associated with thrombolytic agents in the treatment of patients with a STEMI. As a result of these classifications being historic and orientated towards thrombolytic treatment they may underestimate episodes of bleeding in current clinical trials (Pham *et al* 2011). In addition, the TIMI definition for bleeding has evolved over time, the current definition being shown in table 1.7 (Mehran *et al* 2011a). Notably, the GUSTO criteria for bleeding is unique from other definitions in that it does not require changes in haemoglobin, nor does it quantify the amount of blood transfused (Mehran *et al* 2011a). More recent definitions of bleeding (e.g. CURE, OASIS, ACUITY) have tried to overcome the shortcomings of TIMI and GUSTO by combining both laboratory and clinical parameters.

The definition used for bleeding has an effect on the clinical impact of bleeding found in clinical trials. For example, one observation from pooled analysis of the GUSTO trial found that there was a stepwise increase in the adjusted hazard of 30-day death or MI with worsening GUSTO bleeding (HR [95% CI], GUSTO mild 1.20 [1.05 to 1.37]; moderate 3.28

[2.88 to 3.73]; severe 5.57 [4.33 to 7.17]), whereas increased risk but no stepwise progression was observed with all three levels of TIMI bleeding (TIMI minimal 1.84 [1.63 to 2.08]; TIMI minor 1.64 [1.31 to 2.04]; major 1.45 [1.23 to 1.70]). When both bleeding scales were included in the same model, the risk with GUSTO bleeding persisted; however, the association between TIMI bleeding and outcome was no longer significant (Rao *et al* 2006). This suggested that clinically significant bleeding added prognostic value to laboratory markers. In another study of patients undergoing PCI, the GUSTO, ACUITY and TIMI classifications identified bleeding rates of 2.3%, 1.9% and 2.1% respectively; the GUSTO criteria identified all bleeding events but there were 22 (18.6%) and 8 (6.8%) that did not meet ACUITY and TIMI criteria respectively (Fleming *et al* 2011).

The various definitions used in therapeutic trails can be seen in table 1.7.

Due to the problems with definitions of bleeding, the International Society on Thrombosis and Haemostasis (ISTH) criteria defined universal criteria for bleeding (Schulman *et al* 2005). That is:

- Fatal bleeding, and/or
- Symptomatic bleeding in a critical area or organ, such as intracranial, intraspinal, intraocular, retroperitoneal, intraarticular or pericardial, or intramuscular with compartment syndrome, and/or
- Bleeding causing a fall in haemoglobin level of 2 g/dL or more, or leading to transfusion of two or more units of whole blood or red cells.

The ISTH criteria were therefore the criteria adopted for the purpose of the study. At the time of study conception the standardised Bleeding Academic Research Consortium (BARC)

definition of bleeding for cardiovascular clinic trials had not yet been published and as a result were not considered in the design of this study (Mehran *et al* 2011a).

Table 1.7 Definitions of major bleeding used in cardiovascular trials

Studies	**TIMI 1987**	**GUSTO 1993**	**GRACE 2003**	**ACUITY 2006**	**REPLACE-2 2007**	**HORIZONS-AMI 2008**	**OASIS-5 2009**
Clinical	Intracranial bleed	Intracranial bleed Haemodynamic compromise requiring intervention	Death Haemorrhagic SDH Stroke	Intracranial, or intraocular bleed Access site bleeding requiring intervention Haemotoma > 5cm	Intracranial, intraocular or retroperitoneal bleed	Intracranial, intraocular bleed Access site haematoma > 5cm or requiring intervention Reoperation for bleeding	Clinically overt fatal bleed Symptomatic intracranial, retroperitoneal or intraocular bleeding leading to significant visual loss

			Reoperation for bleeding			
Transfusion requirement		≥ 2 units RBC		≥ 2 units RBC	Blood product transfusion	≥ 2 units RBC
Laboratory parameters	↓ Hb > 5g/dL or ↓ Hct > 15%	Absolute ↓ Hct ≥ 10%	↓ Hb ≥ 4g/dL without overt bleeding source or ↓ Hb ≥ 3g/dL with source	Any ↓ Hb ≥ 4g/dL ↓ Hb ≥ 3g/dL with overt bleeding	↓ Hb ≥ 4g/dL without overt bleeding source or ↓ Hb ≥ 3g/dL with source	↓ Hb ≥ 3g/dL

1.3.5 Prognosis and implications following a bleeding event

Once thought of as an inevitable side effect of the treatment for ACS, bleeding is now widely recognised as an independent risk factor for further morbidity and mortality. Major bleeding was first recognised to be associated with an increased risk of hospital death in 2003 with an adjusted OR 1.64 (95% CI 1.18-2.28) (Moscucci *et al* 2003). Bleeding is also associated with an increased risk of further ischaemic events (Rao *et al* 2005).

Rao and colleagues were amongst the first to associate major bleeding with both short- and long-term adverse outcomes (Rao *et al* 2005). In this analysis of 26452 patients (using the GUSTO definition of bleeding) 27.6% of patients had ≥ 1 bleeding event (mild, moderate or severe). Severity of bleeding was associated with a step-wise increase in adverse outcomes (see table 1.8). Those with severe bleeding had an in-hospital mortality of 25.7% compared with 2.9% in those without bleeding. Patients were also noted to have had an increase in recurrent ischaemic events.

In the short term, analysis from the GRACE registry of 40000 patients with ACS found that those patients, who suffered major bleeding, were 4 times more likely to die during the admission than those without bleeding (20.9% versus 5.6%; P<0.001) (Spencer *et al* 2007). Similarly, those who survived major bleeding in hospital were more likely to die during post-discharge follow up than those who did not (7.9% versus 5.2%, P=0.002).

Similar results were found by Eikelboom who analysed data from OASIS-1, OASIS-2 and CURE (34146 patients with ACS). Major bleeding was associated with higher rates of 30-day mortality (12.8% versus 2.5%, P<0.0001) (Eikelboom *et al* 2006).

Bleeding is not only a short-term problem (i.e. related to in hospital death) but also translates into a poorer prognosis in the longer term. The ACUITY investigators found that bleeding was not only an independent predictor of 30-day mortality but that this phenomenon was still present at 1 year (Manoukian *et al* 2007; Mehran *et al* 2009). This 1-year association of bleeding with increased risk of mortality is further demonstrated with the pooled analysis of the REPLACE-2, ACUITY and HORIZONS-AMI trials; major bleeding was associated with a HR 4.89 (95% CI 3.61-6.63) for mortality at 1 year (Mehran *et al* 2011b). Analysis from the CRUSADE registry took things one step further. The authors found that major bleeding continued to be significantly associated with higher mortality for 3 years; 31 days to 1 year (HR 1.19, 95% CI 1.10-1.29), 1 year to 3 years (HR 1.09, 95% CI 1.01-1.18) (Lopes *et al* 2012).

Further studies examining the association between major bleeding and adverse events are summarised in table 1.9.

Table 1.8 Severity of bleeding and adverse events (Adapted from Rao *et al* 2005)

Outcome	**Degree of bleeding**			
	None	**Mild**	**Moderate**	**Severe**
Unadjusted rates (%)				
30-d end points				
Death	549/19110 (2.9%)	155/4387 (3.5%)	154/2499 (5.9%)	79/307 (25.7%)
MI	1412/19110 (7.4%)	501/4373 (11.5%)	605/2591 (23.4%)	100/306 (32.7%)
Death or MI	1758/19110 (9.3%)	572/4372 (13.1%)	675/2591 (26.1%)	151/306 (49.4%)
6-m end points				
Death	983/18886 (5.2%)	273/4358 (6.3%)	253/2566 (9.9%)	107/305 (35.1%)
Adjusted HR (95%CI)				
30-d end points				
Death	1	1.6 (1.3-1.9)	2.7 (2.3-3.4)	10.6 (8.3-13.6)
Death or MI	1	1.3 (1.2-1.5)	3.3 (2.9-3.7)	5.6 (4.6-6.8)
6-m end point				
Death	1	1.4 (1.2-1.6)	2.1 (1.8-2.4)	7.5 (6.1-9.3)

30-d = 30 day, 6-m = 6 months

Table 1.9 Studies examining bleeding and adverse outcomes (adapted from Pham *et al* 2011)

Trials/study design	Bleeding definition	Outcomes	Reference
Meta-analysis (GUSTO IIb, PURSUIT, PARAGON A & B)	GUSTO criteria	30-d hazard of death by bleeding severity Mild: HR 1.6 (1.3-1.9) Moderate: HR 2.7 (2.3-3.4) Severe: HR 10.6 (8.3-13.6) 6-m mortality Mild: HR 1.4 (1.2-1.6) Moderate: HR 201 (1.8-2.4) Severe: HR 7.5 (6.1-9.3)	Rao *et al* 2005
OASIS-1, OASIS-2, CURE	Transfusion ≥2 units RBC, or life threatening	30-d hazard of death HR 5.37; P<0.0001 Between 30-d - 6-m	Eikelboom *et al* 2006

		HR 1.54; P=0.047	
OASIS-5	*	30-d & 180-d composite endpoints ↑4-fold in those with bleeding	Budaj *et al* 2009
GRACE registry	GRACE criteria	In-hospital death higher with bleeding (HR 1.9, 95% CI 1.6-2.2)	Spencer *et al* 2007
Phase III ACUITY	ACUITY criteria	30-d mortality, composite ischaemia, and stent thrombosis higher with bleeding (P<0.0001)	Manoukian *et al* 2007
ACUITY	ACUITY criteria	1-year mortality: ↑3.5 times in those with major bleeding	Mehran *et al* 2009
REPLACE-2	*	30-d mortality ↑ in patients with bleeding (5.1% vs.	Feit *et al* 2007

		0.2%; P<0.001) 6-m mortality ↑ in patients with bleeding (6.7% vs. 1.0%; P<0.001) 1-year mortality ↑ in patients with bleeding (8.7% vs. 1.9%; P<0.001)	
Retrospective analysis of >10000 patients undergoing PCI	TIMI criteria	Major bleeding independent risk factor of in-hospital mortality (OR 3.5; P=0.0001)	Kinnaird *et al* 2003
Meta-analysis (ISAR-REACT, -SWEET, -SMART-2, REACT-2)	TIMI criteria	Bleeding independent risk factor for mortality (HR 2.96; P<0.001)	Ndrepepa *et al* 2008

*= Major bleeding: clinically overt fatal bleeding, symptomatic intracranial, retroperitoneal, intraocular bleed, transfusion ≥2 units RBC, ↓Hb ≥3g/dL

1.3.6 Management of bleeding complications

The prevention of bleeding is as important as the treatment of the bleeding complication itself. As AUGIB accounts for a large proportion of bleeding complications the use of PPIs have been recommended to protect against bleeding (Bhatt *et al* 2008). PPIs have been found to reduce the frequency of overt AUGIB when prescribed with clopidogrel – HR 0.13 (95% CI 0.03-0.56) (Ng *et al* 2008, Bhatt *et al* 2010). Other preventative strategies include the use of radial over femoral access (Jolly *et al* 2009), and the correct choice and dosage of medications depending on the patients bleeding risk (Alexander *et al* 2010).

The treatment of bleeding largely depends upon the site and severity of bleeding. In general terms, treatment is supportive. Minor bleeding can be managed conservatively without the interruption of antiplatelet and antithrombotic therapy, however, in major bleeding, cessation and reversal of antiplatelet and antithrombotic therapy may be required. The risk of interrupting antiplatelet therapy should be weighed against the risk of a recurrent thrombotic event especially stent thrombosis following PCI. Treatment should be restarted once haemorrhage has been controlled for at least 24 hours (Hamm *et al* 2011).

Blood products may be required to correct abnormalities in haemostasis, haemoglobin and platelet count (e.g. fresh frozen plasma, platelet transfusion, red blood cell transfusion). These abnormalities may be the due to either the bleeding itself or as a side effect of drug treatment (e.g. heparin, GP IIb/IIIa inhibitors). It should be noted however, that blood transfusion itself is associated with poor outcomes in the context of ACS (Alexander *et al* 2008; Jolicoeur *et al* 2009; Rao *et al* 2004; Shishebor *et al* 2009). Transfusion has also been shown to have adverse effects on mortality in

critically ill patients; in a study of restrictive versus liberal blood transfusion strategies those in the restrictive strategy group had a lower mortality (Hébert *et al* 1999; Villanueva *et al* 2013).

1.3.7 Explanation for poor outcomes

The mechanism by which an episode of bleeding leads to poor outcomes (recurrent ischaemic events and death) is at present poorly understood although several mechanisms have been suggested.

One explanation is the need to discontinue antiplatelet and antithrombotic medications when bleeding occurs. This, however, leads to an increased risk of recurrent thrombotic events, particularly stent thrombosis after PCI (Hamm *et al* 2011).

Other potential mechanisms by which bleeding leads to poor outcomes include (Disney *et al* 2011; Eikelboom *et al* 2006; Fitchett 2007; Manoukian *et al* 2007; Mehran *et al* 2009a; Spencer *et al* 2007):

- Adverse effects of hypotension on end organs
- Platelet and coagulation activation associated with anaemia
- Adverse effects of blood transfusion
- Interventions required to control bleeding (e.g. surgery)
- Bleeding may unmask a covert diagnosis with a poor prognosis (e.g. malignancy)

1.3.8 Gastrointestinal bleeding in the context of acute coronary syndromes

Gastrointestinal bleeding is the most common site of bleeding in patients treated with ACS. In patients who present with AUGIB in the setting of ACS, the bleed may have precipitated the ACS or occurred as a consequence of anti-platelet and other anticoagulants given during ACS (Lin *et al* 2006).

AUGIB complicates approximately 0.7-3% of patients following ACS (Abbas *et al* 2005; Chin *et al* 2007; Lin *et al* 2006; Moukarbel *et al* 2009; Nikolsky *et al* 2009). Bleeding occurs at a mean time of 2.8 days in one study (Chin *et al* 2007).

The risk of AUGIB in this patient group is commonly due to the use of anti-platelet therapy. Anti-platelet agents are important for 2 reasons; firstly, they reduce recurrent ischaemic events, and secondly, they prevent the risk of stent thrombosis. Late stent thrombosis, a phenomenon related to drug eluting stents, means prolonged use of dual anti-platelet therapy is needed. Currently, patients with a stent in situ require a minimum of 4 weeks (bare metal stents) and 12 months (drug eluting stents) of anti-platelet therapy (Grines *et al* 2007). Following this period, patients continue treatment with aspirin monotherapy.

Non-compliance or withdrawal of aspirin increases the risk of recurrent cardiovascular events. Aspirin non-adherence or withdrawal is associated with three-fold higher risk of major adverse cardiac events (OR=3.14 [95% CI 1.75-5.61], P=0.0001). This risk was magnified in patients with intracoronary stents, as discontinuation of antiplatelet treatment was associated with an even higher risk of adverse events (OR=89.78 [29.90-269.60]) (Biondi-Zoccai *et al* 2006).

- **Aspirin**. The risk of AUGIB with aspirin is dose related (Weil *et al* 1995). The 75 mg dose, that is commonly used for the secondary prevention of

cardiovascular events, carries an OR of 2.3 (95% CI 1.2-4.4) for AUGIB. A more recent study found that aspirin alone had an OR 1.8 (95% CI 1.5-2.1) (Hallas *et al* 2006).

- **Clopidogrel**. The OR for AUGIB for patients taking clopidogrel alone is 1.9 (95% CI 0.6-2.1) (Hallas *et al* 2006).

- **Dual antiplatelet therapy**. Patients taking both aspirin and clopidogrel have an OR of 7.4 (95% CI 3.5-15) and a HR 3.18 (95% CI 1.91-5.29) for the development of AUGIB (Hallas *et al* 2006; Moukarbel *et al* 2009).

- **Warfarin**. Patients on warfarin have an 8 fold increased risk of AUGIB compared with controls (OR 7.8, 95% CI 2.8-21.5) (Weil *et al* 2000).

The suggested treatment of patients with AUGIB in the context of recent coronary stent insertion is shown in figure 1.3.

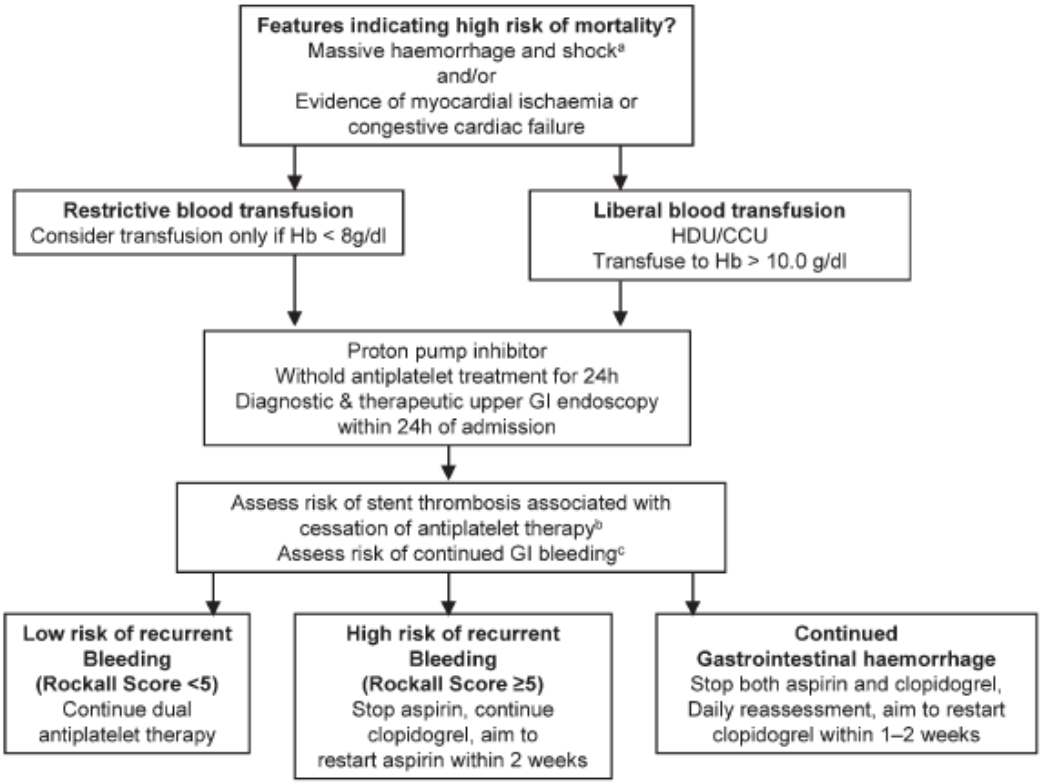


Figure 1.3 Suggested management algorithm for the management of acute upper gastrointestinal bleeding after recent stent implantation (Tan *et al* 2009).

[a]Systolic blood pressure > 100mmHg, pulse > 100 beats per minute.

[b]Factors associated with a high risk of stent thrombosis: impaired LV systolic function, diabetes mellitus, renal failure, long segment of stent (>20mm) and recent coronary intervention (within 3 months of BMS, within 12 months of DES).

[c]Factors associated with increased risk of continued bleeding: endoscopic diagnosis (visible vessel and stigmata of recent haemorrhage).

1.3.8.1 Outcomes following acute upper gastrointestinal bleeding in acute coronary syndromes

Short term

Haematological indices are affected in those patients with GI bleeding. In particular, patients with GI bleeding have a higher incidence of acquired thrombocytopenia (21.6% vs. 10.5%, $p < 0.001$) and haemoglobin drop of $\geq$ 3g/dL (30.4% vs. 1.3%) (Nikolsky *et al* 2009). High-risk lesions on endoscopy were identified in 33% of patients and rebleeding occurred in 4.5% of patients (Chin *et al* 2007).

Bleeding has a profound effect on outcomes. Length of hospital stay is increased from a median of 3 to 6 days in patients with gastrointestinal bleeding (Abbas *et al* 2005; Nikolsky *et al* 2009). In-hospital mortality is higher in patients suffering from an AUGIB; 10% versus 2.8% in patients not suffering from bleeding (Abbas *et al* 2005). In one case-control study 30-day mortality was 11.9% in patients with GI bleeding, compared to 0.5% for controls (Chin *et al* 2007). Further evidence suggests GI bleeding is an independent predictor of all-cause mortality (HR 4.87, IQR 2.61-9.08), cardiac mortality (HR 5.35, IQR 2.71-10.59) and composite ischaemia (HR 1.94, IQR 1.14-3.30) (Nikolsky *et al* 2009) – see figure 1.4.

Long term

In addition to an increase in short term mortality, evidence supports GI bleeding as a predictor of mortality at 1 year (Nikolsky *et al* 2009). It should be noted that the majority of patients with AUGIB did not meet the criteria for major bleeding.

In the valsartan in acute myocardial infarction trial (VALIANT) analysis 35.7% (n=35) died during follow up (median follow-up was 24.7 months). GI bleeding was associated with an increased risk of all-cause death, with a HR of 3.57 (95% CI 2.56-5.00), even after adjustment for important confounding factors. It should be noted that the majority of deaths in this study were from cardiovascular causes (60%) rather than the bleeding itself (11.4%) (Moukarbel *et al* 2009).

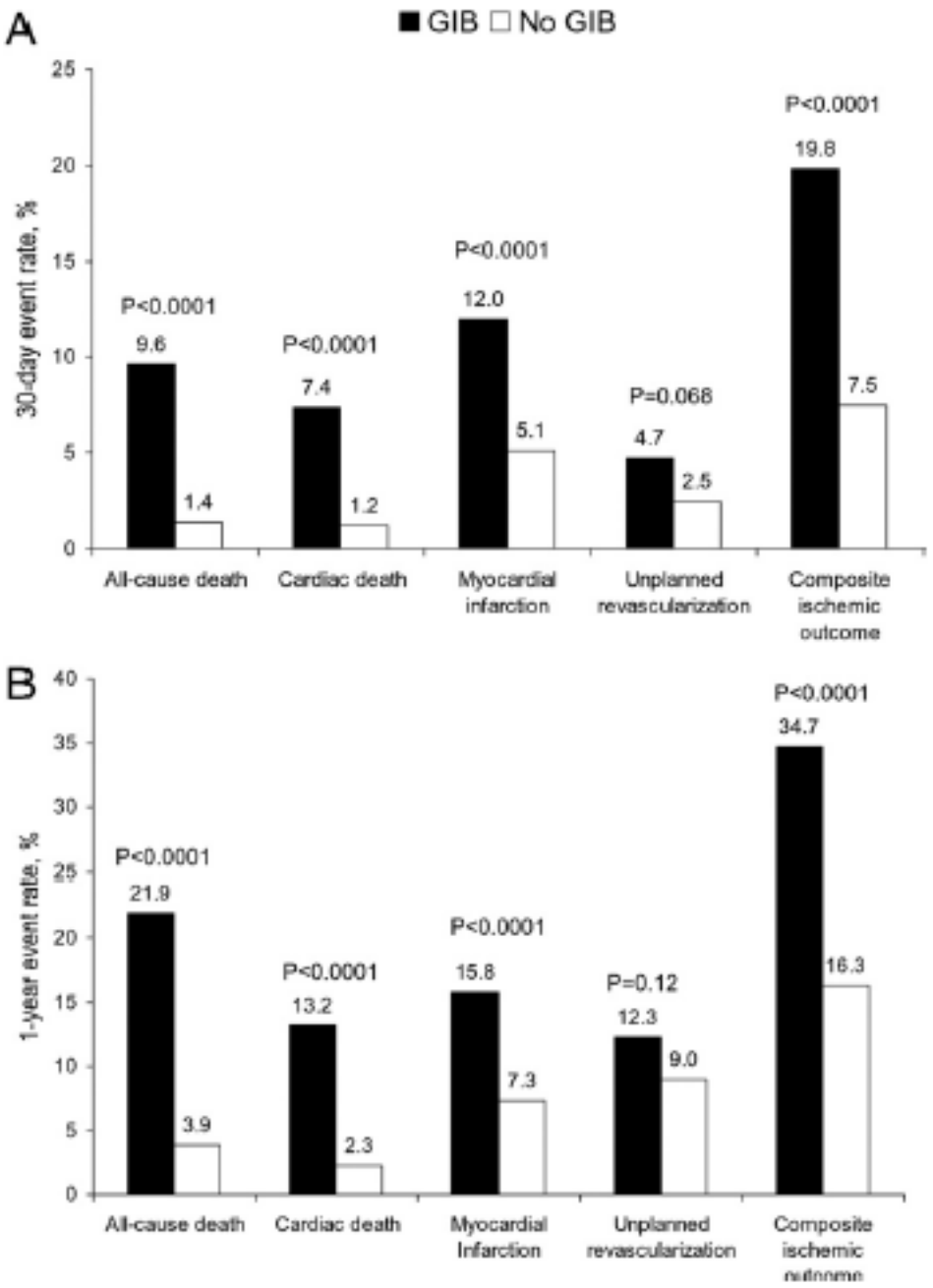


Figure 1.4 Ischaemic events and mortality at 30 days and 1 year in patients with ACS, with and without gastrointestinal bleeding. A = 30 day, B = 1 year, GIB = gastrointestinal bleeding. This figure demonstrates that patients who suffer from AUGIB in the context of ACS have a higher incidence of recurrent cardiac events and mortality; both in the short term and long term (Nikolsky *et al* 2009).

1.3.9 Use of proton pump inhibitors for the prevention of acute upper gastrointestinal bleeding in acute coronary syndromes

Proton pump inhibitor use reduces the risk of GI bleeding associated with aspirin use (Chan *et al* 2005, Bhatt *et al* 2010).

The use of proton pump inhibitors after PCI is associated with a reduction in the rate of GI bleeding (Chin *et al* 2007). Logistical regression analysis found that PPI therapy was associated with reduced rates of upper GI bleeding (OR 0.08, 95% CI 0.02-0.40 (Chin *et al* 2007). Other studies have found PPIs to reduce the risk of AUGIB; HR 0.13 (95% CI 0.03-0.56) (Bhatt *et al* 2010), RR 0.17 (95% CI 0.04-0.76) (Lin *et al* 2011), RR 0.04 (95% CI 0.002-0.21) (Ng *et al* 2008).

1.3.9.1 The interaction between clopidogrel and proton pump inhibitors

There has been much controversy over the use of PPIs as gastroprotection in patients on dual antiplatelet therapy, particularly clopidogrel.

Concerns were initially raised of an interaction between clopidogrel and PPIs in 2008 (Gilard *et al* 2008). A study from 2006 found clopidogrels antiplatelet activity was diminished in patients receiving PPI treatment (Gilard *et al* 2006). Several retrospective studies further suggested a link between co-prescription of a PPI and clopidogrel, and an increased risk of adverse clinical outcomes (Dunn *et al* 2008; Ho *et al* 2009; Juurlink *et al* 2009; Pezalla *et al* 2008). However, as with any retrospective analysis the influence of compounding factors is problematic and may lead to inaccurate results and conclusions.

Several studies have found no clinically significant interaction including the only prospectively designed randomised controlled trial (Bhatt *et al* 2010; O'Donoghue *et al* 2009; Rassen *et al* 2009).

Current thinking is that PPIs offer clear protection against AUGIB in patients on dual antiplatelet therapy and the concern over clinically relevant interactions with clopidogrel, although biologically plausible, is not yet proven (Disney *et al* 2011).

In summary, the evidence suggests that a bleeding event in ACS increases risk of subsequent cardiovascular events and mortality in both the short and long term; a phenomenon that has yet to fully explained.

1.4 Platelets and Platelet activation

1.4.1 Platelet structure and function

Platelets are small fragments of megakaryocyte cytoplasm with an average volume of 7-8 fL. When studied with electron microscopes non-activated platelets are shaped like biconvex discs, have a convoluted surface and contain mitochondria, granules, two systems of cytoplasmic membranes, microfilaments, microtubules and many glycogen molecules. When platelets change their shape during activation, the microtubules break their connections with the cell membrane and contract inwards (Wickramasinghe *et al* 2011).

There are 4 types of platelets granules:

1. Dense bodies (δ granules) – these contain ADP, ATP, calcium, adrenaline and serotonin – involved in platelet aggregation.

2. α granules – contain platelet factor 4, β-thromboglobulin, fibrinogen, fibronectin.

3. Lysosomal (λ) granules – contain acid phosphatase, cathepsin, β-glucuronidase, and β-galactosidase.

4. Peroxisomes – contains catalase.

The normal range for platelet count in peripheral blood is between 150-450 x 10^9/L. The life span of normal platelets is 8-10 days (Wickramasinghe *et al* 2011).

Platelets release an array of agonists, such as ADP; adhesive molecules, such as P-selectin, thrombospondin, fibrinogen and vWF; coagulation factors; and growth factors (Jennings 2009).

The main function of platelets is their role in haemostasis with the formation of a haemostatic plug/thrombus in response to endothelial damage (Thachil 2015). Under physiological conditions platelets do not interact with the vessel wall (Gawaz *et al* 1999). However, when endothelial cells of vessel walls are damaged and shed, platelets adhere to subendothelial connective tissue via vWF attached to a specific receptor on the platelet membrane, glycoprotein Ib-IX. This leads to activation of the platelet and the release of the contents from platelet granules; initially from the dense granules followed by α granules. The ADP released from the dense bodies causes an interaction between platelets, leading to aggregation. Aggregation is preceded by an alteration of the cell membrane, leading to a calcium-dependent binding of fibrinogen to platelet receptors on the membrane glycoprotein IIb-IIIa. The process of aggregation continues until a platelet plug occludes the damaged vessel (Wickramasinghe *et al* 2011).

Platelet activation by agonists results in rapid changes in platelet morphology, platelet aggregation, granule secretion and involvement of the cell surface in coagulation reactions (Jennings 2009). Activated platelets release contents from storage granules and generate thromboxane A_2 which is a potent vasoconstrictor and platelet agonist leading to further platelet activation (Reilly and Fitzgerald 1993).

The process of platelet activation is a receptor-mediated response of resting platelets to a variety of specific stimuli originating either from activated proteins of the coagulation cascade (e.g. thrombin), subendothelial matrix proteins (e.g. collagen), or specific mediators such as ADP and adrenaline. The receptor mediated signals leads to ion fluxes, phosphoinositol breakdown and protein phosphorylation which regulate the reorganisation of the cytoskeleton, shape change, glycoprotein redistribution, expression of procoagulant surface and the release reaction. Furthermore, inside-out

signalling transform GPIIb/IIIa complex to a conformational state that is competent for binding of ligands such as fibrinogen or vWF. This process is the prerequisite for platelet aggregation and for thrombus formation on subendothelial matrices (Schmitz *et al* 1998). The changes that occur upon platelet activation are summarised in figure 1.5.

In addition to their haemostatic role, platelets are protagonists of inflammation and are activated in inflammation (Gawaz *et al* 2005; Ripoche *et al* 2011). Platelet adhesion to monocytes is mediated by the platelet surface expression of P-selectin. As platelets are involved in both inflammation and thrombosis the formation of monocyte-platelet aggregates may represent targeting of both cell types to specific inflammatory or thrombotic sites (Furman *et al* 2001).

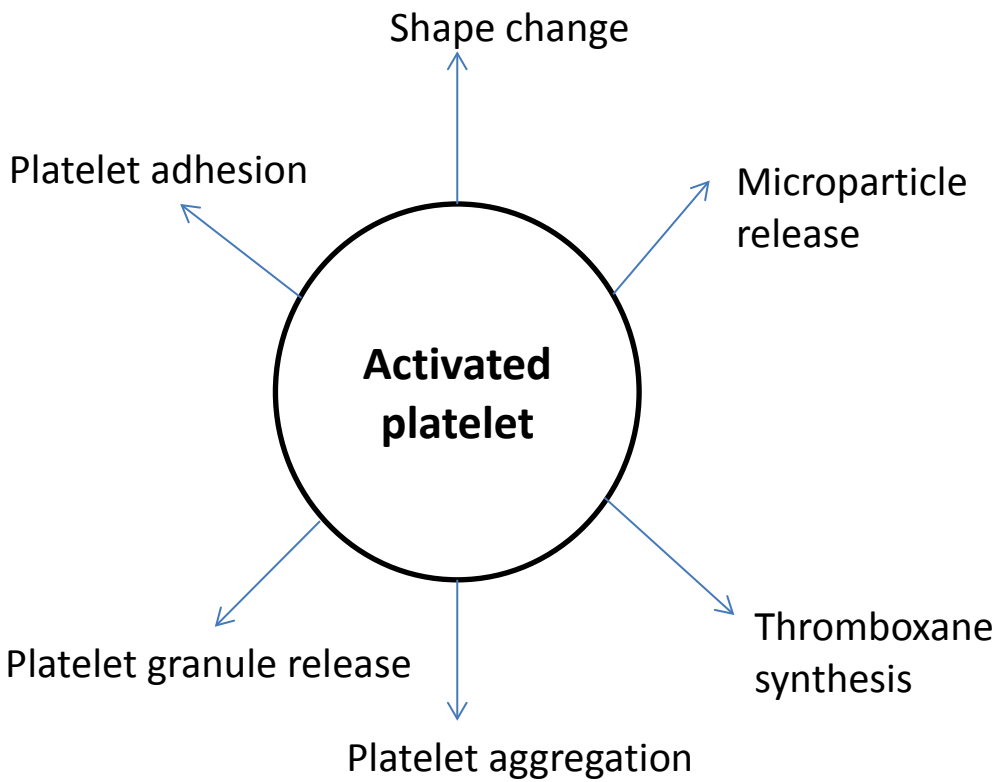


Figure 1.5 The changes that occur during platelet activation.

These are also the targets for direct and indirect methods to measure platelet activation (Tan and Lip 2005).

1.4.2 Platelets in upper gastrointestinal bleeding

Very little is known about the role of platelets with respect to AUGIB. In particular there is no study that looks at platelet activation or function in the context of AUGIB, however, it is well known that anti-platelet agents are associated with AUGIB and as a result, the role of platelets in AUGIB is a vitally important, yet unexplored area (Hallas *et al* 2006).

The role of platelets in AUGIB is of particular interest given the results from a recent study (Sung *et al* 2010a). This study group investigated 156 patients presenting with AUGIB with a history of aspirin use as secondary prophylaxis for cardiovascular diseases. Following endoscopic therapy for peptic ulcer bleeding, patients were randomised to either restart low-dose aspirin immediately or placebo for 8 weeks. During follow up recurrent ulcer bleeding occurred in 10.5% in the aspirin group and 5.4% in the placebo group (difference 4.9%, 95% CI -3.6-13.4). Patients receiving aspirin had lower all-cause mortality (1.3% versus 19.5%; difference 11.6%, 95% CI 3.7-19.5) and lower mortality due to cardiovascular, cerebrovascular and gastrointestinal complications when compared to placebo (1.3% versus 10.3%; difference 9%, 95% CI 1.7-16.3) (Sung *et al* 2010b). One could hypothesise from the results that bleeding may increase the activity of platelets and as a result lead to higher rates of thrombotic events.

A subsequent study from Norway investigated platelet function in gastrointestinal bleeding (Kringen *et al* 2011). They found that patients with gastrointestinal bleeding had lower arachidonic acid (AA) induced platelet aggregation and lower AA- and collagen-induced p-selectin expression (assessed by flow cytometry) when compared with healthy controls. However, the patients included in this study had either upper or

lower gastrointestinal bleeding and were not age-matched. Patients with bleeding related to alcohol abuse were also included, a group of patients known to have problems with platelet number and function (Miceli *et al* 2003; Ogasawara *et al* 2005; Panasiuk *et al* 2005; Witters *et al* 2008; Zhang *et al* 2000). In terms of absolute platelet numbers, this study found that patients suffering from gastrointestinal bleeding have lower numbers when compared to healthy controls (Kringen *et al* 2011).

1.4.3 Platelets in cardiovascular disease

Platelet activation has been implicated in a number of cardiovascular disorders, e.g. acute coronary syndromes, recurrent ischaemic events following angiography, stroke, hypertension and peripheral vascular disease (Braunwald *et al* 2001; Chung and Lip 2006; McCabe *et al* 2005; Neumann *et al* 1997; Ott *et al* 1996). Even patients with stable coronary artery disease have activated circulating platelets (Furman *et al* 1998). Platelets, with fibrin, are predominant components of the thombi that occludes arteries, but may also participate in the development and progression of the atherosclerotic plaques. Thus, platelets are central to the process of atherothrombosis (Gawaz *et al* 2005; Jennings 2009; Ruggeri 2002).

Platelets play a role in the initiation of the atherosclerotic lesion formation, but contribute to plaque progression as well (Braunwald *et al* 2008). Platelets do this by participating in the inflammatory and matrix-degrading processes of coronary atherosclerosis (Massberg *et al* 2003).

Persistent platelet activation poses a serious problem among patients with ACS and those who have undergone PCI, placing them at risk for ischaemic events and subacute stent thrombosis (Braunwald *et al* 2008).

As a result of this knowledge, patients with ACS receive treatment targeting platelets and their activation. Numerous antiplatelet agents are now available against differing therapeutic targets within the platelet and with different risk/benefit profiles. They are widely used in the primary and secondary prevention of cardiovascular disease.

1. Aspirin – the 'reference' antiplatelet agent. The mechanism of action of aspirin lies in its ability to irreversibly block cyclooxygenase (COX)-1, which inhibits the production of thromboxane A_2 (Coccheri 2010). Aspirin resistance is associated with an increase in the risk of death, myocardial infarction and thromboembolic stroke (Grinstein and Cannon 2012; Kasotakis *et al* 2009).
2. Thienopyridines e.g. clopidogrel, prasugrel, ticlopidine. This class of drugs act by irreversibly inhibiting ADP binding to P2Y12 receptors on the platelet surface. Clopidogrel is more effective than aspirin in reducing cardiovascular events and death (CAPRIE 1996). Again resistance to these medications is associated with an increased risk of cardiovascular events (Nguyen *et al* 2005; Sharma *et al* 2009).
3. Ticagrelor. This is a non-thienopyridine reversible ADP receptor blocker (Nawarskas and Clark 2011). Ticagrelor has been found to be superior to clopidogrel for patients with ACS (Cannon *et al* 2010).
4. GPIIb/IIIa inhibitors e.g. tirofiban. This class of drug prevent platelet aggregation and activation by inhibiting the GPIIb/IIIa receptor on the

platelet surface and are therefore used as adjunctive therapy for high-risk patients undergoing angioplasty (Coller *et al* 1996).

1.4.4 Markers of platelet activation and the prothrombotic state

1.4.4.1 Platelet activation

Platelet activation is important in the pathogenesis of cardiovascular disease and is an important marker in this study. There are several targets (see figure 1.5) and methods for measuring platelet activation. These are described as direct and indirect; direct methods are useful in monitoring rapid changes in platelet activation status, whereas indirect methods refer to the measurement of modified metabolites of activated platelets in either plasma or urine (Tan and Lip 2005).

The direct and indirect methods for studying platelet activation are discussed below (Mylotte *et al* 2011; Tan and Lip 2005).

Direct methods:

1. Electron microscopy – this can be used to study the changes in platelet shape and vesiculation. However, it is a costly and time-consuming method.

2. Flow cytometry – this is the most widely used technique to study platelet activation as it is simple, quick to perform and very sensitive to picking up as little as 1% of activated platelets (Shattil *et al* 1987). In essence, fluorochrome-conjugated monoclonal or polyclonal antibodies directed against platelet glycoproteins or their receptor-ligands are used in combinations that allow simultaneous identification of platelets in whole blood with sensitive analysis of platelet antigen expression (Schmitz *et al*

1998). This suspension is then passed through a laser beam in the flow cytometer which detects those platelets staining positive for the specific antibodies and expresses this as a percentage and as mean fluorescent intensity (MFI). In addition, the size of the platelet can be judged by the forward scatter (FSC) and the granularity by side scatter (SSC). Examples of fluorochrome-conjugated antibodies include CD62P, recognising platelet surface P-selectin, and PAC-1, which is an antibody that recognises an epitope on the glycoprotein GPIIb/IIIa complex of activated platelets (Lu and Malinauskas 2011).

Indirect methods measure metabolites of platelet activation. This can be achieved by techniques such as enzyme-linked Immunosorbent assay (ELISA). These assays are not as sensitive to dynamic changes in platelet activation as the direct methods. The metabolites commonly measured are β-thromboglobulin, platelet factor 4, P-selectin and thromboxane A_2 (Mylotte *et al* 2011; Tan and Lip 2005).

1.4.4.2 Prothrombotic markers

Biomarkers of specific interest (proinflammatory and prothrombotic) with regards to this work will be discussed here.

P-selectin

P-selectin (also known as CD62P) is worth particular attention. It is the largest of the selectins, with a mass of 140 kDa and is a component of the membrane of the alpha and dense granules of platelets (Blann *et al* 2003). P-selectin becomes present on the surface of platelets and endothelial cells following activation and is a cell adhesion

molecule. P-selectin binds to P-selectin glycoprotein ligand-1 (PSGL-1) which is expressed on almost all leukocytes, which leads to adhesion between these molecules. P-selectin is also important for inter-platelet aggregation (Blann *et al* 2003). Raised levels of P-selectin are associated with adverse cardiovascular risk (Blann *et al* 1997; Hillis *et al* 2002; Ridker *et al* 2001).

Interleukin-6

Interleukin-6 (IL-6) is a multifunctional pro-inflammatory cytokine (Abeywardena *et al* 2009; Simpson *et al* 1997). IL-6 plays a central role in diverse host defence mechanisms such as the immune response, haematopoiesis and acute-phase reactions (Simpson *et al* 1997). It is commonly produced at local tissue sites and is released in almost all situations of homeostatic perturbation, such as endotoxaemia, trauma and acute infection (Streetz *et al* 2000). Increased levels have been associated with a variety of pathologies such as cancer and cardiovascular disease (atherosclerosis and myocardial infarction) (Abeywardena *et al* 2009; Waldner *et al* 2012). IL-6 is the key cytokine responsible for the stimulus of synthesis and secretion of CRP. IL-6 activates cell surface signalling via the assembly of IL-6, the IL-6 receptor (IL-6R) and the signalling receptor glycoprotein 130 (Abeywardena *et al* 2009). IL-6 activates the membrane-bound receptor in hepatocytes and leucocytes, thereby initiating downstream proinflammatory cascades that increase hepatic production of C-reactive protein, fibrinogen and other acute-phase reactants (Sarwar *et al* 2012). These pathways have a causal association with coronary artery disease and could be a novel therapeutic approach to preventing coronary artery disease (Hingorani and Casas 2012; Sarwar *et al* 2012).

Von Willebrand Factor

Von Willebrand Factor (vWF) is a multimeric glycoprotein encoded on chromosome 12 and is produced almost exclusively by endothelial cells (Speil *et al* 2008). It plays a vital role in mediating platelet adhesion to arterial walls suggesting a possible contributory prothrombotic role (Constans et al 2006). It is an essential factor in primary haemostasis, and a hereditary deficiency of vWF is consequently associated with an increased likelihood of bleeding (Jansson *et al* 1991). vWF has a role in both platelet adhesion and aggregation, and has been found to be elevated in various conditions of endothelial damage. It has therefore been proposed as a useful marker of endothelial dysfunction with multiple studies showing that vWF is a predictor of cardiovascular events (Jansson *et al* 1991; Spiel *et al* 2008; Thompson *et al* 1995). IL-6 and vWF have been used as part of a biomarker panel to help predict adverse cardiovascular prognosis (Omicron Hartaigh *et al* 2013).

D-dimer

D-dimer is derived from the breakdown of fibrinogen and is termed a fibrin degradation product. It is a biomarker of fibrin turnover and hence, thrombosis (Danesh *et al* 2001; Wang *et al* 2006). It is formed from the activation of the following enzymes in sequence; thrombin, factor XIIIa and plasmin (Adam *et al* 2009). D-dimer is only present in circulating blood in response to activation of the coagulation cascade, and is a specific marker of fibrin clot formation and fibrinolysis (Adam *et al* 2009).

Elevated d-dimers levels have been shown to predict future myocardial infarction in the elderly and overall cardiovascular mortality (Cushman *et al* 1999; Danesh *et al* 2001; Folsom *et al* 2009; Lowe *et al* 1998; Lowe *et al* 2004).

Elevated d-dimer levels are also associated with a number of conditions such as venous thromboembolism, functional decline in patients with peripheral vascular disease, stroke and cancer mortality (Christersson *et al* 2014; Folsom *et al* 2009; Folsom *et al* 2016; Halaby *et al* 2015; McDermott *et al* 2003; Wiseman *et al* 2014). In apparently healthy individuals a raised d-dimer is independently associated with all-cause mortality (Di Castelnuovo *et al* 2013).

1.5 Study hypothesis

- The use of risk scores for acute upper gastrointestinal bleeding in real-life practice is poor. The use of the Blatchford score is better able to identify patient's suitable for outpatient management than the Rockall score. To demonstrate the presence of diurnal and seasonal variation in the presentation of acute upper gastrointestinal bleeding.

- Clinically significant bleeding (acute upper gastrointestinal bleeding, bleeding in the context of an ACS) leads to long-term, as well as short-term increased platelet activation and other prothrombotic markers, which may contribute to the poor clinical outcomes, including thrombotic events observed in these conditions

1.6 Study aims

1. To assess the management of patients with acute upper gastrointestinal bleeding including the use of risk stratification to assess patients for early discharge
2. To assess for a diurnal and seasonal variation in the presentation of acute upper gastrointestinal bleeding
3. To characterise temporal changes in platelet activation and other prothrombotic markers in the following patients:

    a) Acute upper gastrointestinal bleeding

    b) Bleeding in the context of acute coronary syndrome

CHAPTER 2 – MATERIALS AND METHODS

2.1 Materials

2.1.1 General Reagents

Reagents were obtained from the following sources:

- **Abcam, Cambridge, UK**

  D-dimer ELISA kit (Mouse monoclonal anti-D-dimer antibody)

- **Abnova, Taipei, Taiwan**

  Fibrinogen (Human) ELISA kit

- **Alpha Laboratories, Hampshire, UK**

  200 µL yellow pipette tips, 1250 µL clear pipette tips

- **Becton Dickinson, Oxford, UK**

  Polystyrene round-bottom tubes (5 mL), FACS Flow, FACS Clean, Lysing solution, venous blood collection tubes (3.8% citrate, CTAD, EDTA, serum)

- **Bio/Data Corporation, Pennsylvania, USA**

  Arachidonic acid 5 mg/mL

- **Dako, Ely, Cambridgeshire, UK**

  Cytocount (Count control beads), Polyclonal rabbit anti-human vWF; Polyclonal rabbit anti-human vWF/HRP (Horse Radish Peroxidase)

- **Invitrogen, Paisley, Renfrewshire, UK**

  Phosphate buffered saline (PBS)

- **Labsystems, Basingstoke, Hampshire, UK**

  0.5-10 µL, 40-200 µL, 200-1000 µL single channel pipettes, 40-200 µL eight channel pipette

- **R & D Systems, Abindgon, UK**

  Hydrogen peroxide; IL-6 Immunoassay ELISA kit; Human P-Selectin Immunoassay ELISA Kit; sulphuric acid; tetramethylbenzidine

- **Sigma-Aldrich, Dorset, UK**

  L-arginyl-glycyl-L-aspartyl-L-serine (RGDS); Adenosine diphosphate (ADP); Bovine serum albumin; Citric acid; Washing manifold; phosphate buffered saline tablets; Triton X-100; fibrinogen; Hydrogen peroxide (30% w/w); Hydrochloric acid; Sodium carbonate; Sodium hydrogen carbonate; Sodium hydrogen phosphate; Ortho-phenylene diamine

- **Thermo Electron Corporation, Basingstoke, Hampshire, UK**

  Flat-bottomed 96 well microtitre plates

2.1.2 Antibodies

The antibodies utilised for flow cytometry are shown in table 2.1 below along with their source.

Table 2.1 Antibodies and isotype controls used for flow cytometry

TARGET ANTIGEN	FLUROCHROME	CATALOGUE NUMBER & CLONE	TYPE	SUPPLIER
CD42a (Anti-gpIX)	PerCP	340537 Beb1	Mouse IgG_1	Becton Dickinson, Oxford, UK
CD62P	APC	550888 AK-4	Mouse IgG_1	Becton Dickinson, Oxford, UK
PAC-1	FITC	340507 PAC-1	Mouse IgM	Becton Dickinson, Oxford, UK
FITC Mouse IgM, Isotype Control	FITC	551448 G155-228	Mouse IgM	Becton Dickinson, Oxford, UK
APC Mouse IgG_1 Isotype Control	APC	IC0002A	Mouse IgG_1	R&D Systems, Abingdon, Oxford, UK

CD144	PE	FAB9381P 123413	Mouse IgG_{2B}	R&D Systems, Abingdon, Oxford, UK
CD42b	APC	551061 HIP1	Mouse IgG_1 κ	Becton Dickinson, Oxford, UK
CD14	PerCP	FAB3832C 134620	Mouse IgG_1	R&D Systems, Abingdon, Oxford, UK

APC = allophycocyanin; PerCP = Peridininchlorophyll Protein Complex; FITC = fluoroscein isothiocyanate; PE = phycoerythrin

2.2 Venepuncture

2.2.1 Venepuncture technique

A standardised venepuncture protocol was used for all patients in accordance to local guidelines.

All subjects were required to rest for 10 minutes prior to venepuncture to avoid artefactual platelet activation that can be influenced by adrenergic hormones and physical exercise (Hjemdahl *et al* 1991). Blood samples were drawn from an antecubital fossa vein into vacutainers using a 20-gauge needle with minimal stasis (Janes and Goodhall 1994). The initial aliquots were used to obtain EDTA and serum samples to minimise artificial platelet activation as a result of venepuncture. Subsequent aliquots were collected in citrate and CTAD (citrate, theophylline, adenosine and dipyridamole) vacutainers to assess platelet activation via flow cytometry (citrate) and ELISA (CTAD). Citrated blood was used in accordance to manufacturer's guidelines (BD Biosciences). CTAD vacutainers were used as although EDTA vacutainers have been used previously, PAC-1 will not bind to EDTA-treated blood (Shattil *et al* 1986).

2.3 Flow Cytometry for the determination of platelet activation

An assay was developed to accurately measure platelet activation. Flow cytometry is a sensitive and reliable method to directly measure expression of activation-dependent surface markers on platelets in whole blood with minimal handling therefore greatly reducing the chances of *in vitro* platelet activation.

Flow cytometry was performed in accordance with the consensus document for the flow cytometric characterisation of platelet function (Schmitz *et al* 1998).

2.3.1 Technique

Venous blood was taken as described in 2.2.1. Citrated samples were used for flow cytometric analysis of platelet activation. EDTA vacutainers were avoided as PAC-1 binding to the fibrinogen receptor is both pH and calcium sensitive (Shattil *et al* 1986). As a result PAC-1 does not bind with EDTA-treated blood.

CaliBRITE three-colour beads and FACSComp software were used to set photomultiplier tube voltages, fluorescence compensation, spectral overlap and sensitivity.

After incubation data acquisition and analysis were performed with CELLQuest software version 3.1 in the flow cytometer (FACSCalibur, BD Biosciences, Oxford, UK). Ten thousand activation-independent platelet events were collected for each sample.

The FACSCalibur was calibrated using FACSComp software with CaliBRITE beads on a daily basis.

2.3.2 Identification of platelets

Platelets were identified on the basis of their forward (FSC) and side scatter characteristics (SSC) and the presence of CD42a, a pan-platelet marker. FSC and SSC were set on logarithmic gains.

2.3.3 Titration of antibodies

Platelet activation was assessed by the presence of the monoclonal antibodies PAC-1 and CD62P. The concentration of all antibodies used for activation analysis has to be titrated towards a saturating titre. Antibodies at varying concentrations were titrated against a blood sample stimulated maximally with the addition of ADP (2×10^{-4} M) – see 2.3.4 – for use in all future experiments.

The following volumes of antibodies were optimal for all future flow cytometry experiments:

- 3 µL CD42a-PerCP
- 5 µL CD62P-APC
- 5 µL PAC-1-FITC

2.3.4 Platelet stimulation with adenosine diphosphate

Various strengths of ADP were used to stimulate whole blood, ranging between (0 – 2 x 10-4 M), using the following method:

- Within 10 minutes of venepuncture, 50 µL of ADP was pipetted into a test tube.
- 0.45 mL of whole blood was added and gently swirled to mix.
- This was incubated at room temperature for 2 minutes.
- The sample was analysed immediately.

For the purpose of the study, 2 x 10^{-4} M ADP was used throughout as this was adequate for maximal platelet activation in all future experiments. Stimulating the platelets will serve as a positive control in the experiments and measure the reactivity of platelets to an agonist in various clinical conditions.

2.3.5 Platelet stimulation with arachidonic acid

Various strengths of arachidonic acid (AA) were used to stimulate whole blood using the following method:

- Within 10 minutes of venepuncture, 2.6 µL of AA was pipetted into a test tube.
- 98 µL of whole blood was added and gently swirled to mix.
- This was incubated at room temperature for 5 minutes.

- The sample was analysed immediately.

Given the availability of ADP and the large intra-assay variability found with AA, ADP was used in all future experiments.

2.3.6 Intra-assay Variability

Intra-assay reproducibility is 3.7% for CD62P and 16.9% for PAC-1 positive platelets at rest and 0.9% for CD62P and 2.8% for PAC-1 when stimulated with ADP.

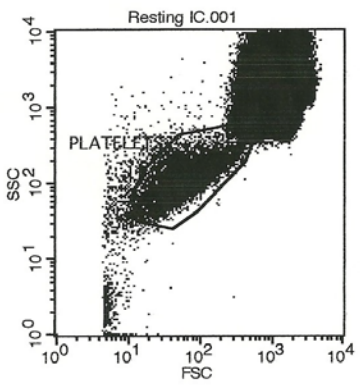
2.3.7 Summary of technique to determine platelet activation with flow cytometry

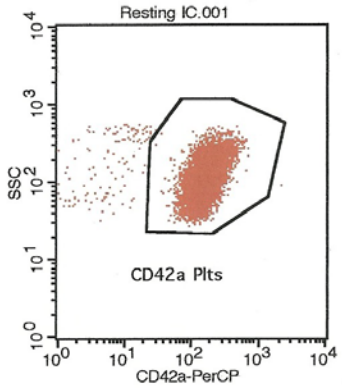
Within 10 minutes of collection 5µL of 3.8% citrated whole blood was incubated at room temperature for 15 minutes with monoclonal mouse-antihuman antibodies against constitutively expressed platelet marker CD42a-PerCP (Clone Beb1, BD), and markers of platelet activation PAC-1-FITC (Clone PAC-1, BD), and CD62P-APC (Clone AK-4, BD). In parallel negative control samples including L-arginyl-glycyl-L-aspartyl-L-serine (RGDS, Sigma-Aldrich, Dorset, UK) and IgG_1-APC isotype control (R&D Systems, Abingdon, UK) were utilised. Incubation was terminated by 1:20 dilution in pH 7.2 phosphate buffered saline (PBS, Invitrogen, Paisley, UK).

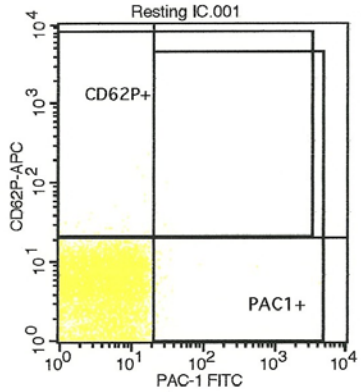
Response to an agonist, in this case adenosine diphosphate (ADP), was assessed by stimulating 3.8% citrated blood with $2x10^{-4}$M ADP (Sigma-Aldrich) for 2 minutes prior to incubation with antibodies as described above.

After incubation, samples were analysed using a FACSCalibur flow cytometer (BD). Platelets were identified on the basis of their forward and side scatter properties and the presence of the CD42a platelet-specific marker. CD62p and PAC-1 expression were measured by mean fluorescence intensity (MFI) and the percentage of platelets expressing these markers was recorded.

Example flow cytometry figures of isotype controls, resting platelets and platelets stimulated with ADP are shown in figures 2.1, 2.2 and 2.3.







Acquisition Date: 01-Jul-10
Gate: G7
Gated Events: 8368
Total Events: 195775

Quad	Events	% Gated	X Geo Mean	Y Geo Mean
UL	42	0.50	4.91	30.13
UR	2	0.02	30.64	54.74
LL	8270	98.83	3.56	4.04
LR	54	0.65	32.14	4.42

Gate: No Gate
Gated Events: 195775
Total Events: 195775

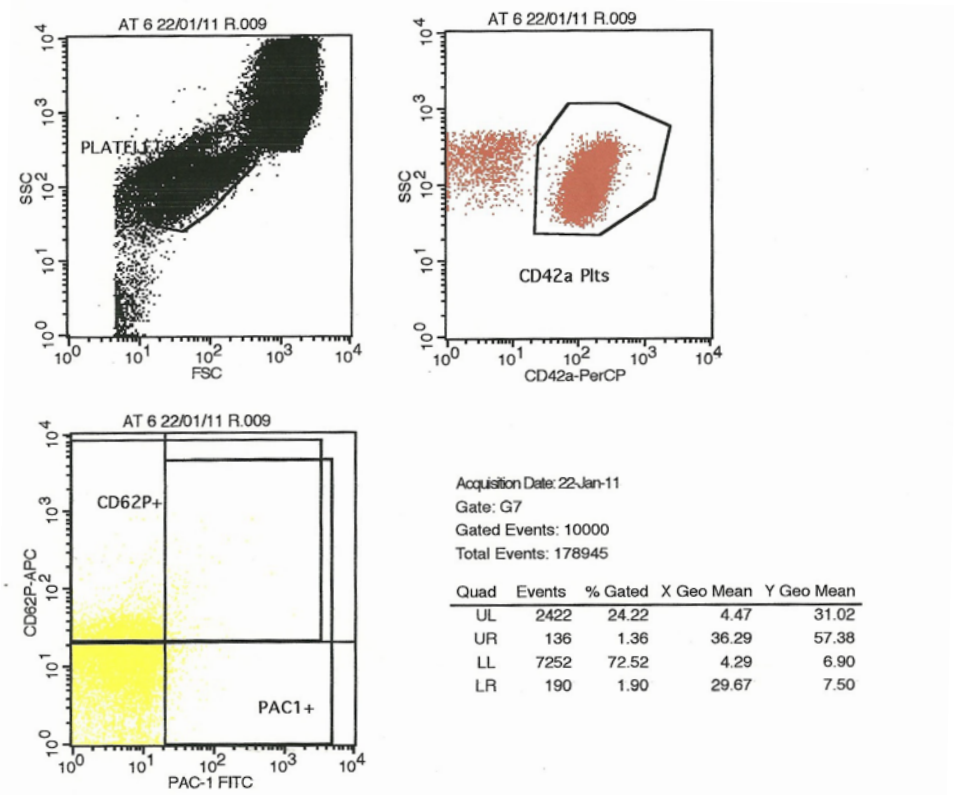
Region	Events	% Gated	% Total
PLATELETS	8499	4.34	4.34
CD42a Plts	10000	5.11	5.11
CD62P+	836	0.43	0.43
PAC1+	9954	5.08	5.08

Gate: G1
Gated Events: 8499
Total Events: 195775

Region	Events	% Gated	% Total
PLATELETS	8499	100.00	4.34
CD42a Plts	8368	98.46	4.27
CD62P+	50	0.59	0.03
PAC1+	72	0.85	0.04

Region	X Geo Mean	Y Geo Mean
PLATELETS	3.62	4.08
CD42a Plts	3.62	4.08
CD62P+	5.27	33.34
PAC1+	31.16	4.83

Figure 2.1 Assessment of platelet activation using flow cytometry – isotype control. This figure shows an example of a typical flow cytometry plot with isotype control staining. The top right box shows platelets staining for CD42a, a pan-platelet marker. The bottom left corner box shows platelets staining postive for either CD62P, PAC-1, or both. As expected with an isotype control those staining positive is minimal.



Acquisition Date: 22-Jan-11
Gate: G7
Gated Events: 10000
Total Events: 178945

Quad	Events	% Gated	X Geo Mean	Y Geo Mean
UL	2422	24.22	4.47	31.02
UR	136	1.36	36.29	57.38
LL	7252	72.52	4.29	6.90
LR	190	1.90	29.67	7.50

Gate: No Gate
Gated Events: 178945
Total Events: 178945

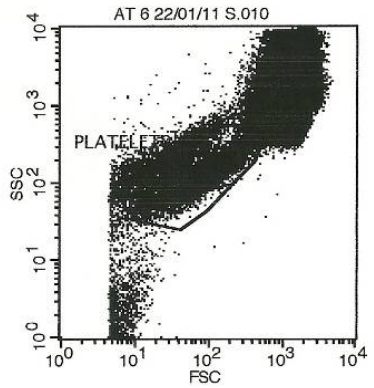
Region	Events	% Gated	% Total
PLATELETS	11364	6.35	6.35
CD42a Plts	11982	6.70	6.70
CD62P+	44573	24.91	24.91
PAC1+	10461	5.85	5.85

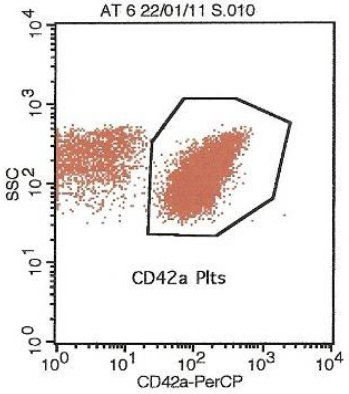
Gate: G1
Gated Events: 11364
Total Events: 178945

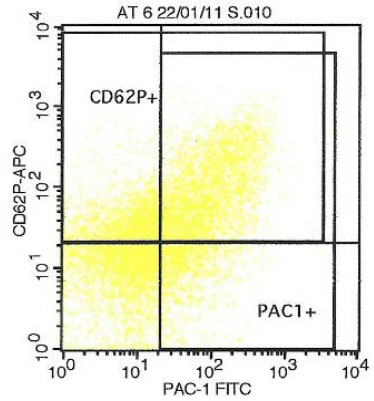
Region	Events	% Gated	% Total
PLATELETS	11364	100.00	6.35
CD42a Plts	10000	88.00	5.59
CD62P+	2617	23.03	1.46
PAC1+	404	3.56	0.23

Region	X Geo Mean	Y Geo Mean
PLATELETS	4.63	10.23
CD42a Plts	4.63	10.23
CD62P+	5.11	33.51
PAC1+	32.01	17.34

Figure 2.2 Assessment of platelet activation using flow cytometry – resting platelets. As per figure 2.1, however, in this figure no isotype control was used. This bottom left box shows typical figures for staining of CD62P and PAC-1 whilst platelets are in a resting state (i.e. taking whole blood and running the analysis without any additional platelet stimulation).







Acquisition Date: 22-Jan-11
Gate: G7
Gated Events: 10000
Total Events: 274517

Quad	Events	% Gated	X Geo Mean	Y Geo Mean
UL	2145	21.45	7.04	38.45
UR	3223	32.23	91.58	92.00
LL	3214	32.14	6.21	7.64
LR	1418	14.18	47.51	7.78

Gate: No Gate
Gated Events: 274517
Total Events: 274517

Region	Events	% Gated	% Total
PLATELETS	12115	4.41	4.41
CD42a Plts	12169	4.43	4.43
CD62P+	71465	26.03	26.03
PAC1+	21811	7.95	7.95

Gate: G1
Gated Events: 12115
Total Events: 274517

Region	Events	% Gated	% Total
PLATELETS	12115	100.00	4.41
CD42a Plts	10000	82.54	3.64
CD62P+	5636	46.52	2.05
PAC1+	4773	39.40	1.74

Region	X Geo Mean	Y Geo Mean
PLATELETS	20.26	24.16
CD42a Plts	20.26	24.16
CD62P+	33.90	67.31
PAC1+	74.57	43.06

Figure 2.3 Assessment of platelet activation using flow cytometry – stimulated platelets. As per figures 2.1 and 2.2. In this example platelets have been stimulated with 2 x 10^{-4} M ADP for 2 minutes. The bottom left box shows a right-sided shift in the staining of platelets indicating increased staining for both CD62P and PAC-1 as would be expected for stimulated platelets which have undergone activation.

2.4 Enzyme-linked immunosorbent assay

A brief outline of ELISA techniques will be described here, for full standard operating procedures refer to appendix 2. Levels of vWF, soluble P-selectin, IL-6 and fibrinogen were measured using commercially available ELISA kits. Experiments were carried out in accordance to manufacturer's instructions.

2.4.1 Determination of von Willebrand Factor

In brief, a microtitre plate was coated with 100 µL of a dilution of the primary antiserum at room temperature for 60 minutes. After washing, a 1:40 dilution of plasma, and standards, was added in duplicate and incubated for 60 minutes. After washing, 100 µL of the secondary antiserum was added and incubated for 45 minutes. After washing, 100 µL of substrate was added and the reaction terminated with 50 µL hydrochloric acid. Optical densities were read at 492 nm on a microplate reader.

2.4.2 Determination of soluble P-selectin

Microtitre plates were coated with the capture antibody and incubated overnight at room temperature. Unbound antibody was washed, and plates blocked and incubated for 1 hour and washed again. Samples, and standards, were then added to the plates in duplicate and incubated for 2 hours at room temperature. After washing, the detection antibody was added and after incubating for another 2 hours streptavidin-horseradish peroxide was added to bind the detection antibody. After washing, tetramethylbenzidine substrate solution was added to the wells and a blue colour

developed. This reaction was stopped with sulphuric acid and optical densities were read using a microplate reader set to 450 nm.

2.4.3 Determination of interleukin-6

The principle for IL-6 is the same as soluble P-selectin determination (see section 2.4.2).

2.4.4 Determination of d-dimer

In brief, 50 µL of plasma and standards was added in duplicate to pre-prepared microtitre plates followed by 50 µL of the antibody cocktail and incubated for 60 minutes on a plate shaker. After washing 100 µL of substrate was added to each well and incubated for 10 minutes in the dark on a plate shaker. The reaction was terminated with 100 µL of stop solution and placed on a plate shaker for 1 minute. Optical densities were read at 450 nm on a microplate reader.

2.5 Audit of acute upper gastrointestinal bleeding

A retrospective observational study was undertaken on all patients admitted to Sandwell and West Birmingham Hospitals NHS Trust in 2009 suffering from AUGIB. This study was approved by SWBH NHS Trust audit department.

Patients were identified by interrogation of the ADAM® medical documentation system (Fujinon Europe GmbH, Willich, Germany) database using the search: 'haematemesis,' 'melaena' and 'suspected upper gastrointestinal haemorrhage' and Endoscopy department logbooks. Using a custom designed proforma (see appendix 3) data was collected from 'Unisoft' and hospital records including: patient characteristics, co-morbidities, clinical findings in order to calculate Rockall and Blatchford scores, use and timing of endoscopy, treatment including endoscopic and blood transfusion. Rebleeding and mortality rates (30 day) were also recorded.

Audit standards were based on recent British Society of Gastroenterology (BSG) / Scottish Intercollegiate Guidelines Network (SIGN) Guidelines (SIGN 2008).

2.6 Study of platelet activation and prothrombotic markers

2.6.1 Cross-sectional study

Patients admitted to Sandwell and West Birmingham Hospitals NHS Trust with an ACS and a bleeding episode, and with AUGIB, were compared to control groups. All patients had baseline demographic details recorded including age, gender, race, BMI, pulse, blood pressure, smoking status and any co-morbidity. Documentation of any medications stopped during a period of bleeding were taken.

Bleeding was defined according to the International Society on Thrombosis and Haemostasis criteria (e.g. Symptomatic bleeding in a critical area such as intracranial, gastrointestinal, retroperitoneal, clinically observed haemorrhage or access site haematoma) or covert (drop in haemoglobin [Hb] of >2g or drop in Hb needing transfusion of 2 or more units of blood).

Control groups were:

a: ACS patients with no bleeding episode,

b: Patients having endoscopy but no bleeding or cardiac disease,

c: Stable patients with controlled angina who have undergone PCI over 6 months previously.

Control groups were age and sex matched.

2.6.2 Longitudinal study

Patients admitted with a bleeding episode had blood samples taken on admission, 4 and 12 weeks later with patients followed up clinically for a period of 6 months. Episodes of further cardiovascular and bleeding episodes were recorded in addition to any other hospital admissions for other reasons.

Markers of platelet activation were compared between the admission and blood tests taken at 3 months, to see if any changes seen was prolonged, and whether it was different from the control groups.

Inclusion Criteria:

- Participant is willing and able to give informed consent for participation in the study
- Male or Female, aged 18 years or above

Exclusion Criteria:

The participant may not enter the study if any of the following apply:

- Patient on warfarin or equivalent oral anticoagulation
- Previous gastrointestinal haemorrhage in past 3 months
- Previous ACS and PCI in last 3 months
- Major trauma in last 3 months
- Surgery in last 3 months
- Current malignancy

- Current sepsis
- Active systemic inflammatory disease (e.g. rheumatoid arthritis)
- Pregnancy
- Liver disease

2.7 Data analysis and study statistics

Statistical advice was obtained from Dr Andrew Blann, Senior Lecturer and Fellow of the Royal Statistical Society.

The sample size calculation was based on plasma levels of d-dimers (DDs), which would therefore be the test statistic. The local method (immunturbidimetry, Stago) takes as cut off value of 0.5 fibrinogen equivalent units/mg/mL (FEUs/mg/mL) as being raised and of clinical significance. A reference range of median 0.25 (interquartile range 0.18-0.26) FEUs/mg/mL and a pathological levels of 0.6 (0.58-0.66) FEUs/mg/mL to be expected from patients with cardiovascular disease was modelled.

It was hypothesised that levels of DDs will remain high at 3 months compared to 6 weeks (i.e. statistically unchanged), in those patients who have had PCI and who have subsequently suffered a haemorrhagic endpoint. This group was compared to the group of PCI patients whose high DDs at 6 weeks (i.e. in the region of 0.60 (0.58-0.65) FEUs/mg/mL) will resolve at 3 months in falling by 50% towards the reference range at, i.e. to 0.30 (0.30-0.50) FEUs/mg/mL. This drop is significant at $p<0.01$ and 1-beta = 0.8 if good paired data from n=25 are analysed. To achieve a difference of $p<0.05$ and a power of 0.80, 25 subjects are required in each group. However,

recognising the possibility of drop out and increased variability in a clinical setting, the aim was to collect samples from 30 patients per group.

Following application of the Shapiro-Wilk W test, to determine distribution, non-categorical data distributed normally was analysed by *t* test, and data distributed non-normally by the Mann-Whitney U test. Correlations were sought with Spearman's rank method. Categorical data (e.g. sex and smoking) was analysed by the chi-squared test. Power calculations were performed on Minitab 16 software, statistical analysis of the raw data was performed with SPSS 19.0 software.

A probability of 0.05 was considered as statistically significant.

2.8 Ethical considerations

The study was conducted in accordance with the declaration of Helsinki. Ethical approval has been obtained from the Warwickshire Research Ethics Committee: reference number 09/H1211/67.

Written informed consent was obtained from all patients.

CHAPTER 3 – AUDIT OF ACUTE UPPER GASTROINTESTINAL BLEEDING

3.1 Introduction

Acute upper gastrointestinal bleeding (AUGIB) poses a significant health problem associated with significant costs to the NHS (Klein and Gralnek 2015). Across Europe the incidence varies between 50-170 per 100000 population (Blatchford *et al* 1997; Button *et al* 2011; Hearnshaw *et al* 2010a; Rockall *et al* 1995).

Overall in the UK, AUGIB accounts for over 9000 deaths per annum (Crooks *et al* 2009). In the UK, mortality due to AUGIB is between 8.2-13.1% (Blatchford *et al* 1997; Button *et al* 2011; Crooks *et al* 2011). Mortality figures appear to be decreasing for variceal haemorrhage but only minimally changing for non-variceal haemorrhage. From 1999-2007, 28-day mortality for non-variceal haemorrhage was reduced from 14.7% to 13.1%, and for variceal reduced from 24.6% to 20.9% (Crooks *et al* 2011).

The aim of this retrospective study was to assess the management of AUGIB in SWBH NHS Trust, including the use of risk scoring systems (i.e. Rockall and Glasgow Blatchford scores) and the need for intervention (i.e. endoscopic therapy or transfusion). A further objective was to study the diurnal and seasonal variation of the presentation of AUGIB given that many diseases related to platelet activation or function show diurnal variability (e.g. ACS, sudden cardiac death, stroke), with a peak incidence in the morning hours (Marsh *et al* 1990; Muller *et al* 1985; Muller *et al* 1987; Quyyami 1990; Tofler *et al* 1987; Willich *et al* 1987; Zarich *et al* 1994). A European study found biphasic peaks in peptic ulcer bleeding with haematemesis incidence peaking at both 6:45AM and 6:45PM (Minoli *et al* 1994). However, data

from China has highlighted a diurnal variation in the presentation of AUGIB with a peak incidence in the nighttime hours (Du *et al* 2010). There has been recent interest into the possibility of poor outcomes associated with blood transfusion in patients with AUGIB, therefore, this area will also be addressed (Hearnshaw *et al* 2010b).

3.2 Methods

This was a retrospective study of all patients presenting with AUGIB requiring an endoscopy to SWBH NHS Trust, between 1st January and 31st December 2009. SWBH NHS Trust serves an urban population over approximately 500000 over two hospital sites. Patients were identified using the ADAM® medical documentation system (Fujinon Europe GmbH, Willich, Germany) and Endoscopy department logbooks.

A 3 page custom-designed questionnaire (see appendix 3) was used to collect the following information on patients; baseline demography, co-morbidity, endoscopic diagnosis and therapy, transfusion requirements, length of stay and 30 day mortality. Data was collected to allow the calculation of the Glasgow Blatchford and Rockall scores. Patients were followed up for 30 days to assess mortality at this time point. Audit standards for the management of AUGIB were taken from the current guidelines approved by the British Society of Gastroenterology (SIGN 2008).

To investigate for seasonal variation of the presentation of AUGIB the year was split into the four seasons; spring (March, April and May), summer (June, July and August), autumn (September, October and November) and winter (December, January and February). To investigate for diurnal variation of the presentation of AUGIB, the

day was split into equal 6-hour periods (00:01-06:00; 06:01-12:00; 12:01-18:00; 18:01-00:00). For new admissions, the time was recorded as the time of presentation to the Emergency Department; for established inpatients the time was recorded as the time of onset of symptoms.

Statistical analysis

Data was analysed using SPSS (Statistical Package for Social Sciences) software 19 (SPSS Inc., Chicago, Illinois), unless otherwise stated. Diurnal and seasonal differences in presentation were analysed using the χ^2 (chi-squared) test; differences in Rockall and Glasgow Blatchford scores were analysed using the Kruskal-Wallis test, followed by the Mann Whitney U test, with Bonferroni correction, to assess differences between individual groups. Trend changes in scores over the 24 hour period were determined by Altman's linear ordered groups method. A p-value of <0.05 was considered as statistically significant.

3.3 Results

3.3.1 Baseline patient characteristics

The initial search drew results of 474 patients who were admitted suffering from an AUGIB. Four sets of notes could not be retrieved and as a result they were excluded from all subsequent analysis. Data relating to 470 patients were included in the analysis.

The baseline patient characteristics are shown in Table 3.1. Of all cases of AUGIB 67.2% (n=316) were male and 32.8% (n=154) were female. The median age of the patients was 68 years (IQR 40-96) with an age range of 18-99 years. With regards to ethnicity, 70.6% (n=332) were Caucasian, 17.7% (n=83) Asian, 10.4% (n=49) Afro-Caribbean, with the ethnicity unknown in 1.3% (n=6) of cases.

The age distribution of cases was as follows:

- <60 years 36.2% (n=170)
- 60-79 years 40.8% (n=192)
- ≥80 years 23.0% (n=108)

Current inpatients accounted for 23.4% (n=110) of cases. Patients were admitted during a weekday in 78.5% (n=369), with 21.5% (n=101) admitted at the weekend.

Delayed presentation to hospital was defined as greater than 24 hours from symptom onset to hospital attendance. This occurred in 34.7% (n=163) of cases.

Haematemesis was the presenting symptom in 70.6% (n=332) of cases, melaena in 58.3% (n=274) and syncope in 11.3% (n=53). A digital rectal examination was

performed in 74.5% (n=350) of cases. Alcohol intake was documented 76.6% (n=360) of the time.

Pre-admission medications are shown in table 3.1. However, 14.5% (n=68) of patients were on prophylactic enoxaparin at the time of AUGIB. It should be noted that 54.5% (n=256) of patients received PPI therapy pre-endoscopy. This was administered intravenously in 69.5% (n=178) and orally in 30.5% (n=78) of cases. In the case of a potential variceal haemorrhage (70 cases) terlipressin was given 58.6% (n=41) of the time.

3.3.2 Blood transfusion

A transfusion of packed red cells was given to 51.7% (n=243) of patients. These patients were older than those who did not require transfusion (mean age 67.2 ± 16.0 years compared to 60.5 ± 20.8 years; P<0.001). As would be expected those who received a transfusion had a lower haemoglobin level than those who did not (mean Hb 7.6 ± 2.0 g/dL compared to 12.6 ± 2.5 g/dL; P<0.001).

Mortality and blood transfusion

Of those patients who received a blood transfusion (n=243), 30 day mortality was 21.0% (n=51). Of those who did not receive a blood transfusion (n=227), 30 day mortality was 10.1% (n=23). There was a significantly increased 30 day mortality for those receiving transfusion; 21% versus 10.1%; P=0.001.

Table 3.1 Baseline patient characteristics of patients with acute upper gastrointestinal bleeding

Characteristic	Percentage (n=470)
Gender	
Male	67.2 (n=316)
Female	32.8 (n=154)
Age	
Overall (mean ± standard deviation)	64.0 (± 18.8)
Male	62.5 (± 18.5)
Female	67.1 (± 19.3)
Admission status	
New admission	76.6 (n=360)
Co-morbidity	
IHD	14.5 (n = 68)
Cardiac failure	8.1 (n = 38)
Cancer	11.7 (n=55)
Hypertension	36.4 (n = 171)
Stroke	8.7 (n = 41)
Diabetes	19.4 (n = 91)
AF	9.6 (n = 45)
Pre-admission medication	
Aspirin	32.6 (n=153)
Clopidogrel	7.9 (n=37)
NSAIDS	11.3 (n=53)
Warfarin	6.2 (n=29)
PPI	30.9 (n=145)

3.3.3 Pre-endoscopy risk assessment

Only 20% (n=94) had a pre-endoscopy risk score documented.

Rockall scores for patients are shown in table 3.2. A Rockall score of 0 accounted for 13.8% (n=65) of cases.

Glasgow Blatchford scores are shown in table 3.3 and figure 3.1. A Glasgow Blatchford score of 0 accounted for 6.0% (n=28) of cases.

Table 3.2 Pre-endoscopy Rockall scores

Pre-endoscopy Rockall score	Percentage
0 – 1	34.7 (n=163)
2 – 3	33.0 (n=155)
4 – 5	28.3 (n=133)
6 – 7	3.6 (n=17)
Unknown	0.4 (n=2)

This table demonstrates the pre-endoscopy Rockall scores of patients admitted with AUGIB. Approximately one third of patients admitted had a low risk pre-endoscopy Rockall score of 0-1.

Table 3.3 Glasgow Blatchford scores

Blatchford score	Percentage
0 – 3	19.2 (n=90)
4 – 7	20.6 (n=97)
8 – 11	24.9 (n=117)
12 - 15	29.6 (n=139)
16 - 19	5.3 (n=25)
Unknown	0.4 (n=2)

This table demonstrates the Glasgow Blatchford scores of patients admitted with AUGIB. The spread of Glasgow Blatchford scores are evenly spread with a lower proportion presenting with a very high Blatchford score.

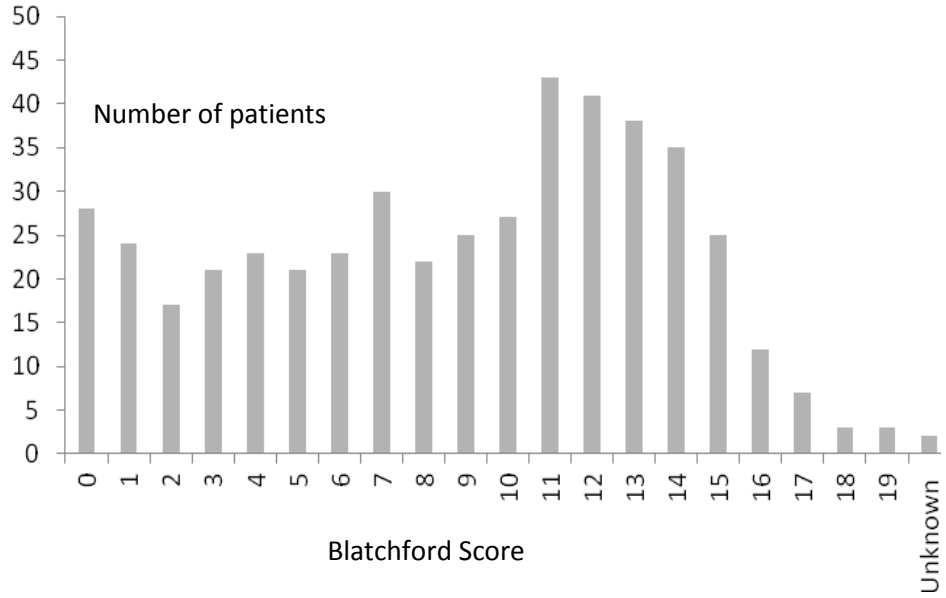


Figure 3.1 Glasgow Blatchford scores

A significant proportion of patients presenting with AUGIB have a low risk GBS of zero and a large proportion have high risk scores (i.e. GBS greater than 12). Very few patients are admitted with very high risk bleeds.

3.3.4 Pre-endoscopy risk and need for intervention

Pre-endoscopy risk assessment can be used to assign a patients risk of mortality or need for intervention. The need for intervention for the patients in this cohort, dependent on their Rockall or Glasgow Blatchford score, was assessed.

The need for intervention (i.e. transfusion, endoscopic therapy or surgery) and mortality for a given GBS is shown in table 3.4 and figure 3.2. Of note, patients with a GBS ≤ 2 had no need for intervention and had no mortality (figure 3.2). This group of patients accounts for 14.7% (n=69) of all patients admitted with AUGIB. The need for intervention increased with a higher GBS with patients potentially requiring more than 1 intervention e.g. undergo endoscopic therapy and need a blood transfusion. Those patients with a Blatchford score ≤ 2 were younger than those with a score ≥ 2, with a mean age of 44.1 ± 17.5 years versus 67.4 ± 18.8 years ($P<0.001$).

The need for intervention, for a given pre-endoscopy Rockall score is shown in table 3.5 and figure 3.3. It should be noted, that those patients with a Rockall score of 0 (i.e. low risk) had a need for intervention in a number of cases; 13.8% (n=9) required a blood transfusion and 6.2% (n=4) received endoscopic therapy. There were however, no deaths in this group. A post-endoscopy risk score of 0-1, shown in table 3.6, was associated with fewer interventions (16.4% had a transfusion and 4.1% received endoscopic therapy) but of note, there were no deaths at 30 days.

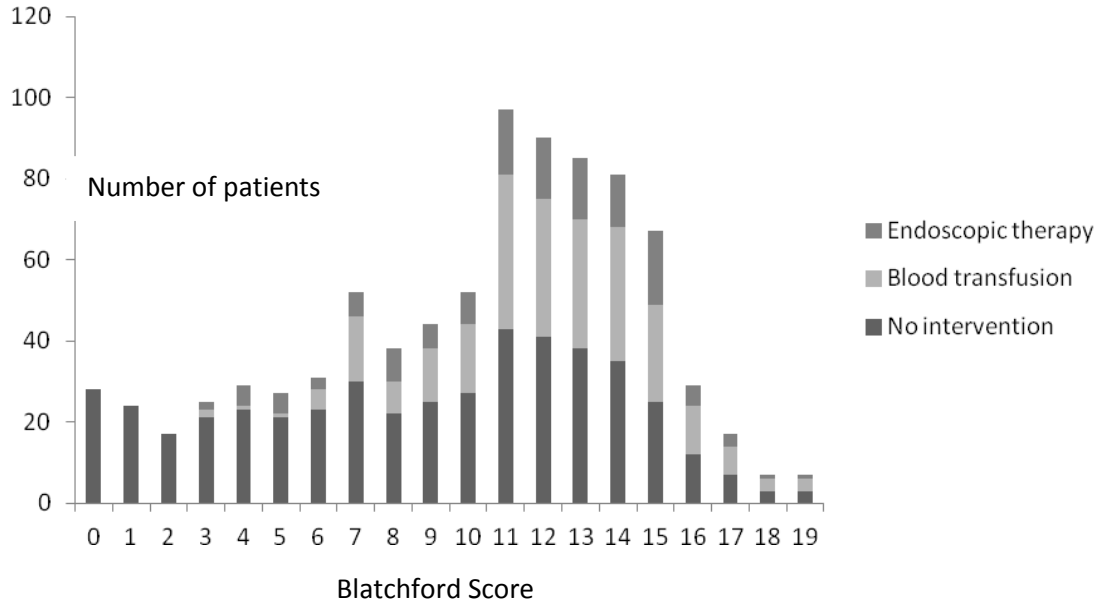


Figure 3.2 Glasgow Blatchford score and subsequent need for intervention

Patients with a GBS of 2 or less did not require an intervention, such as blood transfusion or endoscopic therapy.

Table 3.4 Glasgow Blatchford score and need for intervention

Score	No. of pts	Age (mean ±SD)	Blood transfusion (%)	Endoscopic therapy (%)	30-day mortality (%)
0	28	42.4±20.3	0	0	0
1	24	42.2±17.0	0	0	0
2	17	49.6±20.6	0	0	0
3	21	57.7±19.4	10	10	14 (5 from GIB)
4	23	63.4±19.6	4	22	13 (4 from GIB)
5	21	68.5±17.2	5	24	5
6	23	62.7±21.4	22	13	9
7	30	61.0±18.1	53	20	10
8	22	69.8±18.4	36	36	18
9	25	68.0±13.1	52	24	8
10	27	68.6±13.2	63	30	4
11	43	70.7±14.5	88	37	14 (5 from GIB)
12	41	70.9±16.0	83	37	29 (5 from GIB)
13	38	68.1±15.5	84	39	24 (8 from GIB)
14	35	66.1±15.1	94	37	17 (6 from GIB)
15	25	71.3±13.8	96	72	36 (16 from GIB)
16	12	71.7±18.1	100	42	50 (17 from GIB)
17	7	70.7±11.0	100	43	0
18	3	71.7±10.4	100	33	0
19	3	65.7±16.4	100	33	33
Unknown	2	83.5±7.8	50	0	50

Table 3.5 Pre-endoscopy Rockall scores and need for intervention

Score	No. of pts	Age (mean ±SD)	Blood transfusion (%)	Endoscopic therapy (%)	30-day mortality (%)
0	65	37.4±12.1	14	6	0
1	98	50.2±16.0	35	17	2 (1 from GIB)
2	82	74.2±15.8	61	27	16 (4 from GIB)
3	73	69.9±12.0	71	37	11 (3 from GIB)
4	77	69.7±16.8	62	44	22 (4 from GIB)
5	56	67.7±13.5	77	38	39 (14 from GIB)
6	16	81.4±7.7	75	25	31
7	1	89.0±0.0	100	100	100
Unknown	2	83.5±7.8	50	0	50

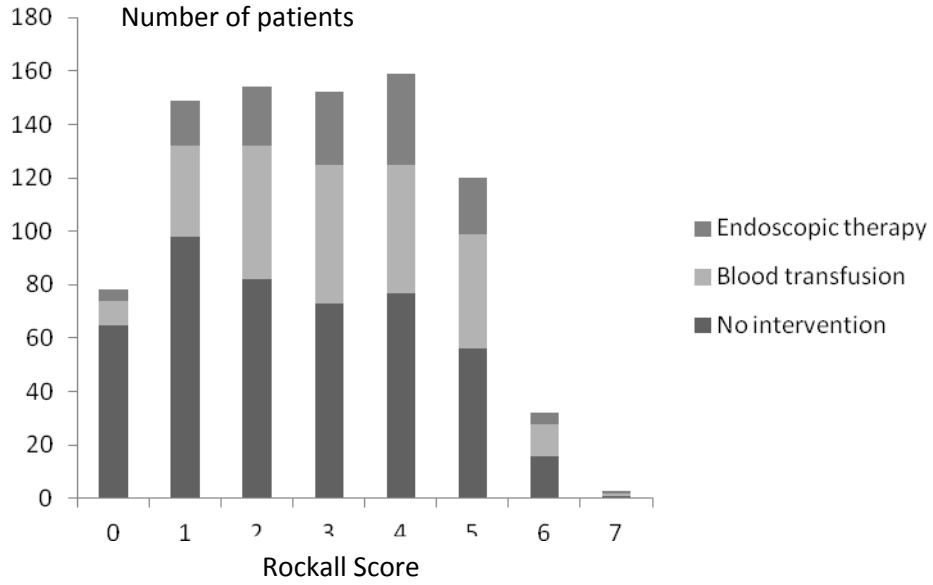


Figure 3.3 Need for intervention based on the pre-endoscopy Rockall score

In patients with a low risk pre-endoscopy Rockall score (i.e. 1) a significant proportion of patients required either blood transfusion or endoscopic intervention. This is in contrast to the GBS where patients with a score of 0 did not require any intervention.

Table 3.6 Post-endoscopy Rockall score and need for intervention

Score	No. of pts	Age (mean ±SD)	Blood transfusion (%)	Endoscopic therapy (%)	30-day mortality (%)
0	23	64.0±19.0	17	0	0
1	50	63.7±19.1	16	6	0
2	74	63.4±18.9	31	5	1
3	59	64.0±18.9	59	10	10 (2 from GIB)
4	57	66.7±18.9	54	32	14 (5 from GIB)
5	75	68.9±18.9	63	40	19 (3 from GIB)
6	57	69.4±18.8	79	47	23 (5 from GIB)
7	37	73.0±18.8	73	62	38 (5 from GIB)
8	22	64.8±18.8	86	64	41 (23 from GIB)
9	6	82.5±18.6	100	67	0
10	1	89.0±0	100	100	100
Unknown	9	67.6±18.8	33	0	33 (11 from GIB)

3.3.5 Timing of Endoscopy

The timing of endoscopy is important. Patients should have an endoscopy within 24 hours of admission. Overall, 61.3% (n=288) of patients had an endoscopy within 24 hours. Disappointingly, a significant proportion of patients, 23.0% (n=108), waited greater than 48 hours for endoscopic examination. The timing of endoscopy is shown in table 3.7.

Out of hours endoscopy occurred in 10.6% (n=50) of cases. The majority of cases took place in the endoscopy unit, 90.4% (n=425). Endoscopy was performed in the intensive care unit in 4.3% (n=20) and in theatres in 2.8% (n=13) of cases.

Table 3.7 Timing of endoscopy

Time to endoscopy	Percentage
<12 hours	30.2% (n=142)
12 – 24 hours	31.1% (n=146)
24 – 48 hours	15.7% (n=74)
48 – 72 hours	6.8% (n=32)
> 72 hours	16.2% (n=76)

3.3.6 Endoscopic diagnosis

Data on endoscopic diagnosis are shown in table 3.8. Peptic ulcer disease was the most common diagnosis found on endoscopy. Of note, a normal endoscopy was found in 9.1% (n=43) of cases.

Table 3.8 Diagnosis found on endoscopic examination

Diagnosis	Percentage (n=470)
Peptic ulcer	34.5 (n=162)
Varices	11.3 (n=53)
Malignancy	1.9 (n=9)
Oesophagitis	31.7 (n=149)
Gastritis	23.0 (n=108)
Duodenitis	14.7 (n=69)
Mallory Weiss Tear	4.3 (n=20)
Bleeding post-sphincterotomy	1.1 (n=5)
Other*	3.0 (n=14)
Normal	9.1 (n=43)

*angiodysplasia, Dieulafoy lesion, aorto-enteric fistula

3.3.7 Endoscopic therapy

Of all patients with AUGIB only 27.7% (n=130) required an endoscopic intervention, meaning that in the vast majority 72.3%, (n=340) of cases no endoscopic intervention was required.

Of those patients requiring endoscopic therapy for oesophageal varices, endoscopic band ligation was used in 54.7% of cases (n=29). One patient (2%) had endoscopic band ligation with sclerotherapy.

One hundred patients with non-variceal AUGIB required endoscopic therapy. Of these patients, mono-therapy was used 53% (n=53) of the time; adrenaline alone 47% (n=47), endoscopic clip 3% (n=3), thermal therapy 3% (n=3). Dual therapy was used 47% (n=47) of the time.

3.3.8 Patient outcomes

Rebleeding occurred in 8.9% of patients (n=42) and 3.2% required surgery (n=15).

A total of 14.7% of patients died during the index admission (n=69). These patients were older than those who survived admission (70.4±15.1 years versus 62.9±19.2 years; P=0.002).

3.3.9 Diurnal and seasonal variation of acute upper gastrointestinal bleeding

To investigate for diurnal and seasonal variation of the presentation of AUGIB the day was split into equal 6 hour periods and 4 seasons as outlined in section 3.2. Monthly admission rates for AUGIB are shown in figure 3.4. This highlights a peak incidence during the month of October, after which admission rates decline in November and December. Lowest admission rates were found in February. No difference was observed between the monthly admission rate (χ^2=17.05, P=0.106). However, when examining the seasonal admission rates fewer admissions were observed during the three winter months (χ^2=9.10, P=0.028) (see figure 3.5).

Admission rates throughout the 24-hour period are shown in table 3.12. There is significant variation throughout the day with a higher proportion of patients admitted during the 1201-1800 time period (χ^2=18.83, P<0.001).

Rockall and Glasgow Blatchford scores of patients, for the 4 time periods of the day, were analysed to determine whether or not 'sicker' patients presented at any specific time point. Differences were observed between time of admission and Rockall score (p=0.048). Patients have a significantly higher median Rockall score during the 06:01-1200 (median Rockall score=3) time period when compared to 12:01-18:00 (median Rockall score=2) period (P=0.006). No diurnal difference was observed for the Glasgow Blatchford score (P=0.39). However, there is a clear linear trend starting from the 06:01-12:00 (median Glasgow Blatchford score=10) time period through to the 00:01-06:00 (median Glasgow Blatchford score=8) time period (P<0.01). There was no diurnal difference in in-hospital mortality (χ^2=0.62, P=0.89) or 30 day mortality (χ^2=3.06, P=0.38). These results are shown in table 3.12.

There was no difference in Rockall score (P=0.88) or Glasgow Blatchford score (P=0.39) over the four seasons. No difference was observed in the in-patient mortality between the four seasons (χ^2=3.32, P=0.35) or 30-day mortality (χ^2=3.03, P=0.39) – see table 3.13.

Non-variceal AUGIB

For non-variceal AUGIB, significant variation was seen in both seasonal and diurnal presentation. Presentation was more common in autumn (n=129) and lowest in winter (n=82) with these differences found to be significant (χ^2=10.811, P=0.013). Presentation was more common in the 12:01-18:00 period (n=139) and lowest during 00:01-06:00 (n=80) (χ^2=16.925, P=0.001).

No significant differences were seen in the seasonal Rockall (P=0.83) or Glasgow Blatchford scores (P=0.52). The Rockall score does show diurnal variation (P=0.003). As with the variceal and non-variceal groups combined this difference is observed between the 06:01-1200 (median Rockall score = 3) time period when compared to 12:01-18:00 time period (P=0.003). However, the Glasgow Blatchford score does not show any variation (P=0.18).

Variceal AUGIB

No seasonal variation was observed in the presentation of variceal haemorrhage (χ^2=2.522, P=0.471). A large proportion of variceal bleeds presented in the 12:01-18:00 (n=16) time period. However, there is no significant diurnal variation (χ^2=2.522, P=0.471). No significant differences were seen in the seasonal Rockall (P=0.31) or Glasgow Blatchford scores (P=0.39), nor in diurnal variation (Rockall: P=0.80; Glasgow Blatchford Score: P=1.0).

Table 3.9 Season of admission with acute upper gastrointestinal bleeding

Season	Percentage of patients (n=470)
Spring	25.1 (n=118)
Summer	25.5 (n=120)
Autumn	29.6 (n=139)
Winter	19.8 (n=93)

Table 3.10 Time of admission and percentage of patients presenting during 6 hour periods

Time of admission	Percentage of patients (n=470)
0001 – 0600	19.1 (n=90)
0601 – 1200	24.0 (n=113)
1201 – 1800	33.0 (n=155)
1801 – 0000	23.8 (n=112)

Table 3.11 Diurnal variability for Glasgow Blatchford and Rockall Scores and mortality

	0001-0600 (n=90)	0601-1200 (n=113)	1201-1800 (n=155)	1801-0000 (n=112)
GBS Median (IQR)	8 (4-12)	10 (6-13)	9 (5-12)	9 (5-13)
Rockall (Pre-OGD) Median (IQR)	2 (1-4)	3 (1-4)*	2 (1-3)*	3 (1-4)
In-Hospital Mortality (%)	15.6 (n=14)	15.9 (n=18)	14.8 (n=23)	12.5 (n=14)
30 Day Mortality (%)	11.1 (n=10)	19.5 (n=22)	14.8 (n=23)	17.8 (n=20)

*p=0.006

Table 3.12 Seasonal variability for Glasgow Blatchford and Rockall scores and mortality

	Spring (n=118)	Summer (n=120)	Autumn (n=139)	Winter (n=93)
GBS Median (IQR)	9 (5-14)	10 (6-14)	9 (5-13)	11 (7-14)
Rockall (Pre-OGD) Median (IQR)	2 (1-4)	3 (1-4)	2 (1-4)	2 (1-3)
In-Hospital Mortality (%)	11.0 (n=13)	19.2 (n=23)	13.7 (n=19)	15.1 (n=14)
30 Day Mortality (%)	11.9 (n=14)	20.0 (n=24)	16.5 (n=23)	15.1 (n=14)

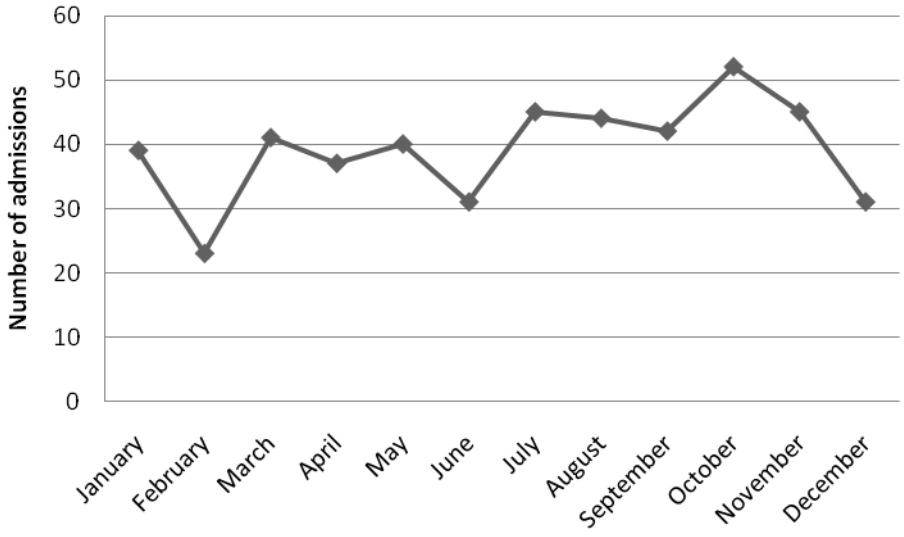


Figure 3.4 Monthly admission with acute upper gastrointestinal bleeding

Monthly admissions for AUGIB are shown. Note the lowest number of AUGIB occurred in June, December and February with the highest number in October.

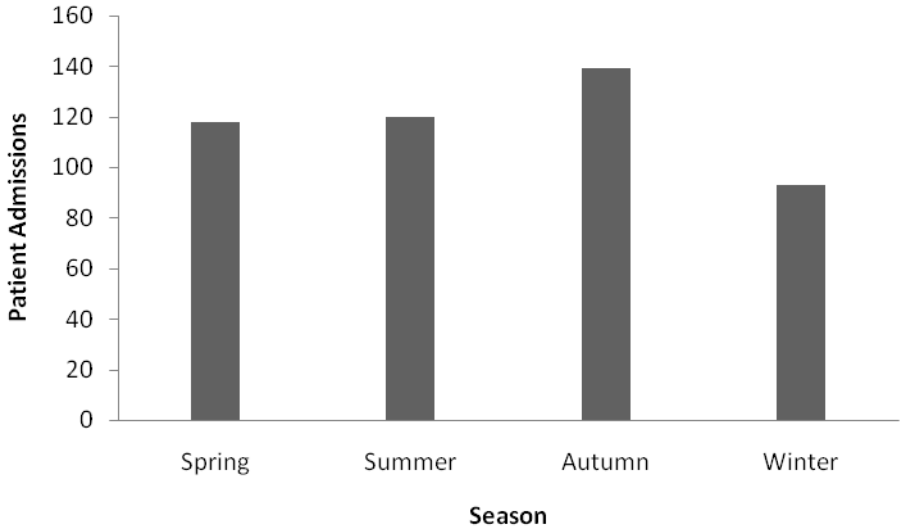


Figure 3.5 Seasonal admission with acute upper gastrointestinal bleeding

Fewer patients are admitted during the winter months (Spring n = 118, Summer n = 120, Autumn n = 139, Winter n = 93).

3.4 Summary and discussion

This chapter examines the presentation and management of AUGIB. One interesting observation regards the use of scores to risk assess AUGIB. Only 20% of patients had a risk score documented which is very low given that it guides management and is considered mandatory by National and International guidelines (SIGN 2008; NICE 2012; Gralnek et al 2015). Low risk patients can be considered for early discharge and outpatient endoscopy.

The Glasgow Blatchford score performed better than the Rockall score in identifying patients at low risk for requiring an intervention (transfusion, endoscopic therapy, surgery) or dying. A patient with a Glasgow Blatchford score of ≤ 2 did not require an intervention in this population. In contrast, 13.8% of patients with a Rockall score of 0 received a blood transfusion and 6.2% required endoscopic therapy. Given these findings the Glasgow Blatchford score should be used as the more accurate system in our local population for pre-endoscopy risk assessment. Indeed, the NICE guidelines on AUGIB endorse the preferential use of the Glasgow Blatchford score for pre-endoscopic risk assessment.

Current guidelines define low risk patients as a Glasgow Blatchford score of 0, and that these patients should be considered for early discharge and outpatient endoscopy (Sung *et al* 2011; NICE 2012). The data from this audit suggests that a Glasgow Blatchford score of ≤ 2 can be used to identify low risk patients. This accounts for 14.7% of admissions compared to 6.0% who score 0 suggesting significantly more patients could be considered for early discharge. Indeed, several recent studies have investigated what can be considered as a low risk Glasgow Blatchford score, suggesting that a score of ≤ 2 could be used to define low risk patients (Masaoka *et al* 2006; Stephens *et al* 2009; Srirajaskanthan *et al* 2010). An observational study over a

5 year period found that patients with a Glasgow Blatchford score of ≤ 2 and aged below 70 could be safely managed as an outpatient (McLaughlin *et al* 2012).

In this chapter, it has been shown for the first time that there is a significant diurnal and seasonal variation in AUGIB in the UK population. Fewer admissions are seen in the winter months and a higher proportion of patients presented in the 12:01-18:00 time period. In addition, a diurnal variation to the Rockall score was seen with a higher score being observed during the 06:01-1200 time period when compared to 12:01-18:00.

Diurnal and seasonal variations in AUGIB have been previously described but the data is conflicting. One European study found biphasic peaks in peptic ulcer bleeding (Minoli *et al* 1994). However, data from China has highlighted a diurnal variation in the presentation of AUGIB with a peak incidence in the night-time hours (Du *et al* 2010). Several authors have investigated seasonal differences in AUGIB with conflicting results; results from China suggest higher rates during winter months whereas data from Greece suggests the opposite (Du *et al* 2010; Thomopoulos *et al* 1997). Others have described higher rates in winter and autumn, or no variation at all (Benz *et al* 1993; Csendes *et al* 1995; Sezgin *et al* 2007; Stermer *et al* 1995; Zimmerman *et al* 1992).

One previous study has reported a diurnal variation in the presentation of AUGIB, with symptom onset during the morning hours (Manfredini *et al* 1994). In the majority of the patients in this study, it is time of presentation to the hospital rather than symptom onset that was recorded, which may explain the peak presentation during the hours of 1201-1800. These results conflict with recent data from China, where more cases of AUGIB presented at night (Du *et al* 2010).

Previous reports have shown a diurnal variation of variceal bleeding in patients with cirrhosis, with higher rates in the evening and early morning (Mann *et al* 1999; Merican *et al* 1993; Siringo *et al* 1996). This has been explained by changes in portal blood flow throughout the day (Alvarez *et al* 1994). This study includes just 11% with variceal haemorrhage and as a result of these small numbers no diurnal variation was seen.

A large proportion of patients in this study suffered from peptic ulcer bleeding (34.5%). These findings are consistent with previous studies that have demonstrated seasonal variation for the incidence of duodenal and gastric ulcers (Bendahan *et al* 1992; Manfredini *et al* 2010; Savarino *et al* 1996; Shih *et al* 1993; Tenías Burillo *et al* 2001; Tsai and Lin 1998). Diurnal variation has been previously described, and is thought to be related to circadian changes in gastric acid secretion, intragastric pH, pepsinogen and gastrin (Manfredini *et al* 1994; Moore & Halberg 1986; Saitoh *et al* 2001; Tarquini *et al* 1987).

Several authors have looked for seasonal differences in AUGIB with conflicting results. Results from China suggest an increased incidence during colder months (December to April) and similar results are also seen in Israel (Du *et al* 2010; Stermer *et al* 1995). The observation of a decrease in presentation during winter is consistent with a previous European based study (Thomopoulos *et al* 1997). This was thought to be due to higher use of salicylate drugs in the winter months, although later studies found opposing results (Langman 1964; Stermaer *et al* 1995; Zimmerman *et al* 1992). A study from Germany found no seasonal difference in peptic ulcer bleeding; however, other endoscopic diagnoses were not included in the analysis (Benz *et al* 1993). Seasonal fluctuation has been thought to be due to the fluctuation of the incidence of duodenal ulcer (Zimmerman *et al* 1992). The difference between Europe

and Asia may be explained by differing patient characteristics and climate. Climate has been thought to play an important role in gastric ulcer bleeding with there being an inverse relationship between the number of gastric ulcers and mean temperature and mean vapour pressure (Nomura *et al* 2001).

This is the first time that a diurnal and seasonal variation amongst AUGIB risk scores (i.e. Rockall and Glasgow Blatchford scores) and mortality has been investigated. Despite there being a statistically significant diurnal variation to Rockall scores and a linear trend of Blatchford score over a 24 hour period, it is unlikely that these findings are clinically relevant.

This work has several strengths. Firstly, the analysis included all patients with AUGIB, which is more pertinent to real life practice. Secondly, the endoscopic diagnosis for all patients was known and recorded. One weakness is one does not know the exact time of symptom onset but rather the time of presentation to the Emergency Department. Secondly, small numbers in some of the analyses may account for non-significant findings with regards to Rockall and Glasgow Blatchford scores and diurnal and seasonal variations.

These results however, do support both a diurnal and seasonal variation in the presentation of AUGIB. The reasons for such differences remain unclear. This may have implications for the provision of endoscopy services. No evidence was observed to suggest that those patients presenting out of hours are any more unstable than those presenting during the daytime. The diurnal presentation means many patients are more likely to be clerked, resuscitated and made ready for upper GI endoscopy by the end of the working day. This is in contrast to many endoscopy units where morning lists are ring fenced for AUGIB. Further studies are required to examine diurnal and seasonal variation in the UK AUGIB population.

CHAPTER 4 – PLATELET ACTIVATION AND PROTHROMBOTIC MARKERS IN NON-VARICEAL ACUTE UPPER GASTROINTESTINAL BLEEDING

4.1 Introduction

Acute upper gastrointestinal bleeding (AUGIB) is the most common gastroenterological emergency, a common reason for medical admissions and is associated with significant morbidity and mortality (Klein and Gralnek 2015). Studies have previously noted an excess of cardiovascular (CVS) events in patients who have suffered from an AUGIB (Hudson *et al* 1995). Patients who have aspirin withheld for 8 weeks following admission with AUGIB have significantly higher rates of CVS events (Sung *et al* 2010b).

It was hypothesised that significant changes in platelet activation and prothrombotic markers would be observed in patients undergoing AUGIB.

4.2 Methods

Patients presenting to hospital with AUGIB were recruited and compared with dyspeptic patients undergoing an OGD as controls. Only patients with non-variceal bleeding were recruited. Patients were excluded from the control group if upper gastrointestinal pathology was found (e.g. upper GI malignancy or other bleeding lesion).

The methods for quantifying platelet activation and prothrombotic markers have been described in Chapter 2.

Statistical analysis

Data is expressed as mean±SD for normally distributed parameters and median (interquartile range) for non-normally distributed parameters. Normality was tested using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Statistical significance, of the changes in platelet activation and prothrombotic markers, was determined by t-test, for normally distributed data, and Mann Whitney for non-normally distributed data. Analysis of related samples was determined by Wilcoxon Signed-Rank test. A value of $p<0.05$ was considered statistically significant. Statistical analysis was performed with SPSS 19.0 software.

4.3 Results

4.3.1 Baseline patient characteristics

A total of 31 patients with AUGIB and 25 controls were recruited to the study. Of these patients, 23 and 18 completed 12 week follow up respectively.

Baseline patient characteristics are documented in table 4.1. Patients suffering from an AUGIB had a mean age of 66.4±18.2 years and 67.7% were of male gender. The control group was well matched for age, gender, BMI and comorbidities. AUGIB was associated with significant changes in both systolic ($P=0.05$) and diastolic blood pressure ($P=0.013$) and heart rate ($P<0.001$) as compared to controls, indicating that these patients had significant enough bleeding to induce haemodynamic changes. Patients with AUGIB had significantly lower haemoglobin levels when compared to controls ($P<0.001$).

The diagnoses seen in the AUGIB group were mainly peptic ulceration (83.9%) with the other patients suffering from minor GI lesions. Endoscopic therapy was required in 54.9% of patients, which is higher than that seen in the general cohort of AUGIB (described in Chapter 3), in which only 27.7% required an intervention (table 4.2). This suggests that the patients recruited had significant episodes of bleeding.

Table 4.1 Baseline patient characteristics

Characteristics	AUGIB (n=31)	Controls (n=25)	P-value
Age (years)	66.4 (18.2)	62.8 (6.1)	0.311
Gender			
• Male	67.7% (n=21)	72% (n=18)	0.730
BMI (kg/m^2)	26.4 (5.0)	28.1 (4.8)	0.197
Systolic BP (mm Hg)	123 (26)	135 (18)	0.05
Diastolic BP (mm Hg)	70 (15)	80 (14)	0.013
Heart rate (beats per minute)	92 (17)	75 (15)	<0.001
Co-morbidity			
• DM	16 % (n=5)	8% (n=2)	0.361
• HTN	42% (n=13)	36% (n=9)	0.651
• IHD	13% (n=4)	16% (n=4)	0.742
Medications			
• Aspirin	48.4% (n=15)	20% (n=5)	0.028
• Clopidogrel	3.2% (n=1)	4% (n=1)	*
• Prasugrel	3.2% (n=1)	0	*
• PPI	19.4% (n=6)	64% (n=16)	<0.001
• NSAIDS	25.8% (n=8)	4% (n=1)	0.027
• Steroids	3.2% (n=1)	0	*
Current smoker	22.6% (n=7)	20% (n=5)	0.815
Laboratory			
• Hb (g/dL)	8.8 (2.4)	14.3 (1.4)	<0.001
• WBC (10^9/L)	9.7 (3.2)	7.09 (2.5)	0.002
• Platelets (10^9/L)	265 (123)	223 (46)	0.088
• eGFR	74 (16)	71 (11)	0.24

Data presented as mean (standard deviation) (analysed by t-test), or as number of subjects (analysed by chi-squared test). *analysis inappropriate.

Table 4.2 Endoscopic diagnosis and therapeutics administered in patients with acute upper gastrointestinal bleeding

	Percentage (n=31)
Diagnosis	
Duodenal ulcer	61.3% (19)
Gastric ulcer	22.6% (7)
Oesophageal ulcer	6.5% (2)
Oesophagitis	3.2% (1)
Gastric erosions	3.2% (1)
Mallory Weiss tear	3.2% (1)
Therapy	
Injection	51.6% (16)
Clip	19.4% (6)
Thermal	19.4% (6)
Number of modalities used	
None	45.1% (14)
Single	19.4% (6)
Dual	29.0% (9)
Triple	6.5% (2)

4.3.2 Platelet activation in acute upper gastrointestinal bleeding

Patients with AUGIB, had higher levels of circulating percentage of platelets staining positive for CD62P (P=0.001) and dual staining for CD62P and PAC-1 (P=0.03), when compared with controls. These results suggest that during the index admission, higher levels of platelet activation were seen in patients with AUGIB. No changes were seen in the reactivity of blood in response to ADP.

After 12 weeks, patients with an AUGIB had higher levels of circulating platelets with positive staining for CD62P (P<0.001) and dual staining for CD62P and PAC-1 (P=0.003). Significant differences are also seen in platelets staining for PAC-1 after 12 weeks (P=0.001). These results suggest prolonged changes in platelet activation following an admission with AUGIB, lasting for a minimum of 12 weeks.

No differences were seen in the longitudinal levels of platelet activation, for patients with AUGIB, between index admission and 12 weeks. No differences were seen in patients undergoing diagnostic OGD with respect to platelet activation. These results suggest that levels of platelet activation are maintained for at least 12 weeks following an AUGIB and that undergoing a diagnostic endoscopy does not induce longitudinal changes in platelet activation.

Results are shown in tables 4.3-4.6.

Table 4.3 Platelet activation at baseline in patients with acute upper gastrointestinal bleeding

	AUGIB (n=31)	Controls (n=25)	P-value
CD62P at rest			
• %	18.3 (5.8)	13.9 (3.7)	0.001
• MFI	30.2 (2.5)	28.9 (1.6)	0.023
PAC-1 at rest			
• %	5.6 (1.6-9.6)	4.0 (2.3-5.6)	0.108
• MFI	32.2 (3.1)	32.5 (4.1)	0.777
CD62P+PAC-1+ %	1.5 (0.7-2.2)	0.8 (0.5-1.2)	0.030
CD62P after ADP			
• %	67.9 (15.9)	63.1 (17.6)	0.288
• MFI	87.0 (24.8)	77.4 (27.0)	0.175
PAC-1 after ADP			
• %	73.2 (23.1)	77.4 (15.6)	0.488
• MFI	150.9 (71.4)	122.4 (46.8)	0.082
CD62P+PAC-1+ % after ADP	65.6 (45.3-85.8)	63.7 (51.7-76.7)	0.613
Soluble P-selectin (ng/mL)	26 (10)	32.6 (14.6)	0.074

Table 4.4 Platelet activation after 12 weeks in patients with acute upper gastrointestinal bleeding

	AUGIB (n=24)	Controls (n=18)	P-value
CD62P at rest			
• %	17.4 (3.8)	12.9 (2.8)	<0.001
• MFI	29.6 (3.6)	28.1 (0.8)	0.047
PAC-1 at rest			
• %	7.0 (3.7)	4.0 (1.8)	0.001
• MFI	31.0 (3.4)	32.2 (3.7)	0.274
CD62P+PAC-1+ %	1.3 (0.7-2.0)	0.7 (0.6-0.9)	0.003
CD62P after ADP			
• %	66.1 (14.7)	61.2 (9.7)	0.227
• MFI	72.5 (21.6)	59.5 (12.7)	0.020
PAC-1 after ADP			
• %	79.4 (14.6)	85.1 (9.7)	0.163
• MFI	131.2 (49.4)	115.1 (32.6)	0.238
CD62P+PAC-1+% after ADP	60.5 (17.0)	57.1 (10.2)	0.414
Soluble P-selectin (ng/mL)	33.5 (21.4)	27 (7.4)	0.414

Table 4.5 Longitudinal changes in platelet activation in patients presenting with acute upper gastrointestinal bleeding

	Baseline (n=31)	Week 12 (n=24)	P-value
CD62P at rest			
• %	18.3 (5.8)	17.4 (3.8)	0.463
• MFI	30.2 (2.5)	29.6 (3.6)	0.509
PAC-1 at rest			
• %	5.6 (1.6-9.6)	7.0 (3.7)	0.950
• MFI	32.2 (3.1)	31.0 (3.4)	0.175
CD62P+PAC-1+ %	1.5 (0.7-2.2)	1.3 (0.7-2.0)	0.639
CD62P after ADP			
• %	67.9 (15.9)	66.1 (14.7)	0.664
• MFI	87.0 (24.8)	72.5 (21.6)	0.028
PAC-1 after ADP			
• %	73.2 (23.1)	66.1 (14.7)	0.227
• MFI	150.9 (71.4)	131.2 (49.4)	0.238
CD62P+PAC-1+% after ADP	65.6 (45.3-85.8)	60.5 (17)	0.890
Soluble P-selectin (ng/mL)	26 (10)	33.5 (21.4)	0.023

Table 4.6 Longitudinal changes in platelet activation following OGD

	Baseline (n=25)	Week 12 (n=18)	P-value
CD62P			
• %	13.9 (3.7)	12.9 (2.8)	0.386
• MFI	28.9 (1.6)	28.1 (0.8)	0.034
PAC-1			
• %	4.0 (2.3-5.6)	4.0 (1.8)	0.308
• MFI	32.5 (4.1)	32.2 (3.7)	0.823
CD62P+PAC-1+%	0.8 (0.5-1.2)	0.7 (0.6-0.9)	0.098
CD62P-ADP			
• %	63.1 (17.6)	61.2 (9.7)	0.654
• MFI	77.4 (27.0)	59.5 (12.7)	0.006
PAC-1-ADP			
• %	77.4 (15.6)	85.1 (9.7)	0.054
• MFI	122.4 (46.8)	115.1 (32.6)	0.575
CD62P+PAC-1+-ADP %	63.7 (51.7-76.7)	57.1 (10.2)	0.992
Soluble P-selectin (ng/mL)	32.6 (14.6)	27 (7.4)	0.095

4.3.3 Prothrombotic markers in acute upper gastrointestinal bleeding

Patients with AUGIB had higher levels of both d-dimer (P=0.011) and vWF (P<0.001), when compared to controls, during the index admission. There is a trend seen towards an increase in IL-6 in patients with AUGIB (P=0.074). After 12 weeks, significant elevations of vWF in the AUGIB group persisted (P=0.022). These results suggest significant changes in prothrombotic markers, in patients with AUGIB, persist for a minimum of 12 weeks and that suffering from AUGIB causes a proinflammatory response.

The levels of prothrombotic markers changed over the 12 week study period in patients with AUGIB; levels decreased significantly for vWF (P<0.001) and IL-6 (P=0.029) when compared to the index admission. Longitudinal changes were not seen with d-dimer during the study period. This suggests that AUGIB may induce an inflammatory response. The increases seen in WBC in patients with AUGIB support this theory.

As expected no significant changes in d-dimer, vWF or IL-6 were seen in the control group over the 12 week study period.

Results are shown in tables 4.7-4.10.

Table 4.7 Prothrombotic markers at baseline

Marker	AUGIB (n=31)	Control (n=25)	P-value
D-dimer (µg)	1.0 (0.1-2.2)	0.8 (0.6-1.2)	0.011
vWF (IU/dL)	145 (60)	99 (34)	<0.001
IL-6 (pg/mL)	12.4 (3.4-21.3)	4.0 (2.9-10.8)	0.074

Table 4.8 Prothrombotic markers after 12 weeks

Marker	AUGIB (n=23)	Control (n=20)	P-value
D-dimer (µg)	2.1 (1.0-3.3)	0.8 (0.7-0.9)	0.073
vWF (IU/dL)	103 (24)	89 (10)	0.022
IL-6 (pg/mL)	6.1 (1.1-13.4)	5.3 (1.8-8.9)	0.295

Table 4.9 Prothrombotic markers in patients with AUGIB at 0 and 12 weeks

Marker	Baseline (n=31)	12 weeks (n=23)	P-value
D-dimer (µg)	1.0 (0.1-2.2)	2.1 (1.0-3.3)	0.796
vWF (IU/dL)	145 (60)	103 (24)	<0.001
IL-6 (pg/mL)	12.4 (3.4-21.3)	6.1 (1.1-13.4)	0.029

Table 4.10 Prothrombotic markers in controls at 0 and 12 weeks

Marker	Baseline (n=25)	12 weeks (n=2)	P-value
D-dimer (µg)	0.8 (0.6-1.2)	0.8 (0.7-0.9)	0.108
vWF (IU/dL)	99 (34)	89 (10)	0.126
IL-6 (pg/mL)	4.0 (2.9-10.8)	5.3 (1.8-8.9)	0.212

4.4 Summary and discussion

In this chapter, changes in platelet activation and prothrombotic markers, as a result of acute upper gastrointestinal bleeding, have been examined. The results are discussed below and some interesting conclusions have been drawn.

An episode of acute upper gastrointestinal bleeding is associated with a loss of circulating blood volume, which leads to haemodynamic changes (SIGN 2008). This is noted in this study; those patients with AUGIB had a lower systolic and diastolic blood pressure, in addition to an increase in heart rate, compared to controls indicating a significant episode of bleeding had occurred.

The main finding in this study was that patients with AUGIB, when compared with controls, have significantly higher circulating activated platelets, as measured by expression of CD62P and PAC-1. Patients with AUGIB also demonstrated increased fibrinolysis (increased d-dimer), increased vascular dysfunction (increased vWF) but no difference in inflammation (IL-6). In addition, platelet activation and vascular dysfunction remained significantly higher in the AUGIB group 12 weeks after the initial admission. These findings cannot be explained by differences in patient characteristics as patients were; age, gender and BMI matched. No differences were seen in conditions associated with the development of cardiovascular disease such as diabetes, hypertension, known ischaemic heart disease and current smoking.

Therefore the differences in platelet activation and vascular function are likely to be a direct consequence of the AUGIB itself. Nearly 50% of the patients with AUGIB were taking aspirin at the time of admission compared to only 20% of controls, and this may be a cause of their admission. Aspirin, being an anti-platelet agent, would have been expected to reduce the levels of platelet activation seen in the AUGIB

group. However, despite the higher levels of aspirin use, the AUGIB group had higher levels of platelet activation compared to controls, which means these results have much more importance. Unfortunately, due to the small numbers within this study this could not be adjusted for. Other possible explanations for the differences seen in platelet activation could be patient comorbidities, however there was no significant difference in major cardiovascular risk factors such as current smoking, diabetes, hypertension and prior ischaemic heart disease. *H. pylori* status was not assessed in our patient cohort and could be a potential confounding factor; previous studies have found elevated platelet activation in patients positive for *H. pylori* and a decreased platelet count, although the numbers in these studies were small (Elizalde *et al* 1997, Yeh *et al* 2010). In addition *H. pylori* has been associated with cardiovascular disease, although this claim has been refuted (Danesh *et al* 1999, Zhu *et al* 2002).

As noted in previous studies a significant proportion of patients who suffer with AUGIB have a history of cardiovascular diseases (IHD, cardiac failure and stroke) (Hearnshaw *et al* 2010a; Sung *et al* 2010b). These patients are often taking aspirin or other antiplatelet agents, as part of secondary prevention of further cardiovascular events, which puts them at risk of AUGIB initially. Antiplatelet agents are frequently stopped on patients presenting with AUGIB; with no real consensus on when they should be recommenced. A previous study showed that in such patients taking aspirin for secondary prevention the recommencement of aspirin for secondary prevention, once bleeding had been controlled, resulted in lower mortality rates than those who had aspirin withheld for 8 weeks (Sung *et al* 2010b). The authors speculated that AUGIB might lead to a higher mortality rate in those patients whose aspirin was withheld, because these patients are more vulnerable to atherothrombosis and therefore cannot tolerate bleeding well (Sung *et al* 2010b). A second study,

investigating the discontinuation of aspirin following peptic ulcer bleeding, found an almost 7-fold increase in risk of death or acute cardiovascular events compared to patients who continued aspirin during the first 6 months of follow up (Derogar *et al* 2013). The results presented in this thesis have particular relevance to the aforementioned studies, as the majority of patients recruited to our study had peptic ulcer disease. A recent study concluded that the use of antiplatelet or anticoagulants were not associated with adverse outcomes in patients with non-variceal AUGIB (Solakoglu *et al* 2014; Teles Sampaio *et al* 2016).

The results demonstrated in this study may, in part, explain the excess of cardiovascular mortality in these patients; that is, patients suffering from AUGIB have higher levels of platelet activation, and platelet activation is associated with cardiovascular diseases (Muller *et al* 1985; Tofler *et al* 1987). This, in combination with the cessation of antiplatelet agents is a second hit, in terms of risk of cardiovascular events.

Patients with AUGIB had higher baseline levels of d-dimer (35% rise) and vWF (31% rise) with a trend towards higher levels of soluble P-selectin and IL-6 when compared with controls. vWF levels remained higher in patients with AUGIB than controls 12 weeks following index admission. However, the level of vWF in AUGIB does significantly reduce between index admission and 12 weeks. There is a trend towards higher IL-6 levels in patients with AUGIB. In this patient group IL-6 levels decline significantly over the 12 week study period.

Elevated d-dimer has been associated with a poor prognosis in patients with both variceal and non-variceal AUGIB (Gutiérrez *et al* 2001; Primignani *et al* 2008; Violl *et al* 1996). Although these markers have not been studied exclusively in the non-

variceal AUGIB population. Elevated levels of d-dimer and vWF have previously been noted in patients with bleeding gastrointestinal angiodysplasia (Junquera *et al* 2005).

Elevated levels of d-dimer and vWF are associated with the occurrence of cardiovascular diseases such as stroke and myocardial infarction (Lowe and Rumley 1999; Pradhan *et al* 2004; Ridker *et al* 1994; Wannamethee *et al* 2012). P-selectin levels are elevated in disorders of arterial thrombosis such as acute myocardial infarction, stroke and peripheral vascular disease (Ikeda *et al* 1994; Merten and Thiagarajan 2004;). IL-6 is also associated with the development of cardiovascular events (IL6R MR *et al* 2012; Miwa *et al* 2013; Ridker *et al* 2000). The elevation of d-dimer and vWF in patients with AUGIB may explain the reason for recurrent cardiovascular events seen in this patient group.

In this study, patients with AUGIB have a lower haemoglobin and elevated white blood cell count. There was a trend towards a higher platelet count in those with bleeding, which is in contrast to a previous study investigating platelets in gastrointestinal bleeding (Kringen *et al* 2011).

The investigation of platelet activation and prothrombotic markers following AUGIB alone has not been studied in detail before. These results do however pose a dilemma for treating clinicians as it seems counterintuitive to continue a patient on what is the cause of the bleed in the first instance (i.e. the antiplatelet agent). A clinician's first intuition may be to worry about further bleeding. This study suggests a mechanism to help explain the clinical risk of thrombosis, and enable clinicians to understand the rationale for antiplatelet agents despite a recent bleeding event. However, recent guidelines regarding AUGIB do suggest, on the basis of the current evidence, that

low-dose aspirin used for the secondary prevention of vascular events should be continued once haemostasis has been achieved (Gralnek *et al* 2015; NICE 2012; SIGN 2008). Those with more complicated anti platelet regimens, such as those having had recent angioplasty, require multidisciplinary input from both Gastroenterologists and Cardiologists regarding the risks and benefits of starting, or withholding, medication.

The findings of this study are applicable to patients admitted with AUGIB to UK hospitals. The baseline demographics of the vast majority of patients being of male gender with a mean age of 66 years is similar to that found in the recent nationwide UK AUGIB audit (Hearnshaw *et al* 2010a).

Further studies will be required on the risk of cardiovascular diseases, following an episode of AUGIB and the optimum timing to recommence antiplatelet therapy in this context.

CHAPTER 5 – ACUTE CORONARY SYNDROMES COMPLICATED BY BLEEDING

5.1 Introduction

In Chapter 4, AUGIB was found to be associated with increased levels of platelet activation. As previously discussed, bleeding during the treatment of acute coronary syndromes (ACS) is associated with poor outcomes (short- and long- term) due to recurrent cardiovascular events and death (Eikelboom *et al* 2006; Manoukian *et al* 2007; Mehran *et al* 2009; Moscucci *et al* 2003; Rao *et al* 2005). Even bleeding classified as minor is associated with a poor outcome (Nikolsky *et al* 2009; Rao *et al* 2005). It was hypothesised that an episode of clinically significant bleeding in patients with acute coronary syndromes caused a prolonged prothrombotic state, as evidenced by an increase in platelet activation and prothrombotic markers, which increases long term mortality.

5.2 Methods

Patients admitted to hospital with ACS complicated by bleeding were recruited. ACS without bleeding and stable coronary artery disease patients were used as controls. Bleeding in the context of ACS was defined according to the International Society on Thrombosis and Haemostasis criteria (e.g. symptomatic bleeding in a critical area such as intracranial, gastrointestinal, retroperitoneal, clinically observed haemorrhage or access site haematoma) or covert (drop in Hb of >2g or drop in Hb needing transfusion of 2 or more units of blood) (Schulman and Kearon 2005). The methods

for quantifying platelet activation and prothrombotic markers have been described in Chapter 2.

Statistical analysis

Data is expressed as mean±SD for normally distributed parameters and median (interquartile range) for non-normally distributed parameters. Normality was tested using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Statistical significance of the changes in platelet activation was determined by repeated measures ANOVA for normally distributed data and Kruskal-Wallis for non-normally distributed data. Post-hoc analysis was performed using Tukey for normally distributed and Mann-Whitney U with Bonferroni correction for non-normally distributed data. A value of $p<0.05$ was considered statistically significant. Statistical analysis was performed with SPSS 19.0 software.

5.3 Results

5.3.1 Baseline characteristics

A total of 16 patients with ACS complicated by bleeding were recruited (index group). These were matched with 30 patients with ACS not complicated bleeding and 29 patients with stable coronary artery disease (control groups). Of the initial 16 patients in the index group, 9 completed 12 week follow up. Of the control groups 25 patients in the ACS group completed 12 week follow up. Those in the stable coronary

artery disease group had blood taken at the initial time point only, as platelet activation was felt unlikely to vary during the period of follow up.

Baseline characteristics are shown in table 5.1. Patients with ACS complicated by bleeding had a mean age of 65.9±12.5 years with 81.3% being of male gender. Significant haemodynamic changes were seen between groups. *Post hoc* analysis showed that patients with ACS complicated by bleeding had a lower systolic blood pressure, as compared with those patients with uncomplicated ACS (P=0.01) and a higher heart rate when compared to stable coronary artery disease patients (P=0.01). Patients with ACS complicated by bleeding, also had a lower platelet count when compared to both control groups (P=0.03). However, patients with stable coronary disease had lower WBC when compared to both groups (P<0.001).

The types of bleeding observed in the index group were as follows; gastrointestinal bleeding, access site haematoma, and covert (i.e. 2 g/dL drop in haemoglobin) with the most common being access site haematoma and gastrointestinal bleeding – see table 5.2.

Table 5.1 Baseline patient characteristics

Characteristics	ACS & Bleed (n=16)	ACS (n=30)	SCD (n=29)	P-value
Age (years)	65.9 (12.5)	63.8 (11.6)	61.6 (8.5)	0.42
Gender				
• Male	81.3% (n=13)	86.7% (n=26)	75.9 (n=22)	0.51
BMI (kg/m^2)	25.1 (3.7)	27.2 (4.8)	27.4 (4.3)	0.22
Systolic BP (mm Hg)	124 (29)	146 (23)	135 (18)	0.01
Diastolic BP (mm Hg)	75 (18)	83 (14)	75 (13)	0.06
Heart rate (beats per minute)	82 (24)	77 (14)	68 (12)	0.01
Laboratory				
• Hb (g/dL)	13.3 (3.0)	14.1 (1.6)	13.8 (1.6)	0.35
• WBC (10^9/L)	9.3 (2.4)	10.3 (2.8)	6.9 (1.8)	<0.001
• Platelets (10^9/L)	169 (56)	257 (69)	220 (54)	0.03
• eGFR	64 (23)	70 (15)	74 (16)	0.19

Post hoc analysis:

For systolic BP: differences between bleed/ACS and ACS

For heart rate: difference between bleed/ACS and SCD

For WBC: difference between ACS and SCD and Bleed and SCD

For Platelets: difference between Bleed and ACS

Table 5.2 Nature of bleeding episode in patients with acute coronary syndromes

Nature of bleed	Percentage of patients
Vascular access site bleed	37.5 (n=6)
Gastointestinal bleeding	37.5 (n=6)
≥ 2 g/dL drop in haemoglobin	25 (n=4)

5.3.2 Platelet activation and prothrombotic markers in acute coronary syndromes complicated by bleeding

Significant differences in platelet activation were seen across the study groups during the index admission. Patients with ACS complicated by bleeding and patients with ACS, had higher levels of circulating percentage of platelets staining positive for CD62P, when compared to patients with stable coronary disease (P=0.001). No differences were seen in PAC-1 or dual positivity for CD62P and PAC-1. Significant changes were also seen in the response to ADP. Patients with ACS complicated by bleeding and patients with ACS had a significantly lower response to ADP, when compared to those patients with stable coronary disease; these changes were for percentage of platelets staining positive for CD62P (P=0.035), PAC-1 (P=0.002) and dual positivity for CD62P and PAC-1 (P=0.001). No differences were observed between patients with ACS complicated by bleeding and patients with ACS.

With respect to prothrombotic markers, there were significant differences in the levels of vWF seen during the index admission. Higher levels of vWF were observed in patients with ACS complicated by bleeding and patients with ACS when compared to stable coronary disease controls (P<0.001). No significant differences were seen after 12 weeks.

At baseline, and after 12 weeks, no significant differences were seen with any prothrombotic markers or markers of platelet activation.

These results can be seen in tables 5.3-5.6.

Table 5.3 Baseline platelet activation

	ACS & Bleed (n=16)	ACS (n=30)	SCD (n=29)	P-value
CD62P at rest				
• %	21.9 (11.6)	18.7 (5.9)	14.1 (4.4)	0.001
• MFI	30.1 (3.0)	29.1 (1.5)	29.0 (2.1)	0.121
PAC-1 at rest				
• %	2.2 (0.8-3.7)	3.1 (1.4-4.9)	2.8 (1.6-4.0)	0.383
• MFI	31.2 (4.3)	30.6 (2.9)	31.9 (3.2)	0.393
CD62P+PAC-1+%	0.6 (0.3-1.0)	0.90 (0.3-1.6)	0.7 (0.4-0.9)	0.680
CD62P after ADP				
• %	34.4 (11.9)	35.3 (16.2)	51.8 (24.8)	0.035
• MFI	43.8 (11.3)	46.8 (20.3)	68.1 (27.1)	0.003
PAC-1 after ADP				
• %	31.4 (21.0)	37.0 (25.0)	60.2 (29.0)	0.002
• MFI	53.5 (18.6)	60.3 (33.4)	115.8 (71.5)	0.001
CD62P+PAC-1+% after ADP	11.8 (6.8-16.8)	15.7 (6.1-25.2)	41.4 (13.4-69.4)	0.001
Soluble P-selectin (ng/mL)	25.8 (8.8)	29.1 (12.2)	26.22 (6.7)	0.802

Posthoc analysis:

CD62P: difference between bleed and SCD, and ACS and SCD.

CD62P-ADP (% and MFI): differences between bleed and SCD, and ACS and SCD.

PAC-1-ADP (% and MFI): differences between bleed and SCD, and ACS and SCD.

CD62P and PAC-1-ADP: differences between bleed and SCD, and ACS and SCD.

Table 5.4 Platelet activation at 12 weeks

	ACS & Bleed (n=9)	ACS (n=25)	SCD (n=29)	P-value
CD62P				
• %	15.4 (5.4)	17.4 (5.4)	14.05 (4.38)	0.053
• MFI	28.4 (1.1)	29.2 (2.0)	29.0 (2.1)	0.640
PAC-1				
• %	3.2 (1.3)	4.2 (2.3-6.1)	2.8 (1.6-4.0)	0.130
• MFI	30.8 (2.3)	31.6 (4.1)	31.9 (3.2)	0.655
CD62P+PAC-1+%	0.7 (0.2)	1.1 (0.7-1.5)	0.7 (0.4-0.9)	0.065
CD62P-ADP				
• %	40.3 (24.4)	41.6 (19.0)	51.8 (24.8)	0.246
• MFI	51.4 (21.4)	52.0 (24.9)	68.1 (27.1)	0.051
PAC-1-ADP				
• %	49.2 (27.2)	51.5 (22.0)	60.2 (29.0)	0.317
• MFI	85.9 (47.6)	74.6 (30.1)	115.8 (71.5)	0.115
CD62P+PAC-1+ % after ADP	23.9 (0.0-49.0)	23.5 (9.0-37.9)	41.4 (13.4-69.4)	0.301
Soluble P-selectin (ng/mL)	26.8 (11.7)	26.9 (8.3)	26.22 (6.7)	0.993

Table 5.5 Prothrombotic markers at baseline

Marker	ACS & bleed (n=16)	ACS (n=30)	SCD (n=29)	P-value
D-dimer (µg)	2.6 (1.1)	2.56 (2.13-2.99)	2.73 (2.13-3.34)	0.641
vWF (IU/dL)	141.0 (45.1)	131.6 (45.7)	87.5 (17.8)	<0.001

Post hoc analysis:

vWF: No difference between ACS&bleed and ACS, P<0.001 for bleed and SCD and also for ACS and SCD

Table 5.6 Prothrombotic markers after 12 weeks

Marker	ACS & bleed (n=9)	ACS (n=25)	SCD (n=29)	P-value
D-dimer (µg)	2.5 (1.1)	2.60 (2.01-3.19)	2.73 (2.13-3.34)	0.824
vWF (IU/dL)	94.7 (16.7)	97.6 (25.7)	87.5 (17.8)	0.210

5.4 Summary and Discussion

In this chapter, changes in platelet activation and prothrombotic markers, as a result of bleeding in the context of acute coronary syndromes have been examined. This is the first study to investigate platelet activation and prothrombotic markers in this setting.

In patients presenting with ACS, and ACS complicated by bleeding, there are higher levels of platelet activation as evidenced by increased CD62P when compared to controls with stable coronary artery disease. This is an expected finding. In addition, there was a reduced platelet activity in these groups for ADP-stimulation when compared to stable coronary disease controls. These results are in contrast to those seen in the AUGIB group and could have several explanations. Firstly, PAC-1 recognises an epitope on the GP IIb/IIIa complex on activated platelets at or near the platelet fibrinogen receptor. Clearly, any medication that interferes with this receptor will therefore have an impact on results. Patients with ACS are treated with powerful antiplatelet agents including GP IIb/IIIa inhibitors (e.g. tirofiban). Other medications used to treat ACS will also impact on platelet activation and reactivity and thrombotic markers, notably aspirin, thienopyridine/P2Y12 inhibitors (clopidogrel, prasugrel, ticlopidine and ticagrelor), heparin and direct thrombin inhibitors. Patients admitted with ACS are a diverse group of patients and often undergo varying treatment strategies, depending upon individual patient characteristics. In those patients with ACS complicated by bleeding a proportion continued on these medications whereas some had the offending or contributory medications stopped for a brief period. Given the small numbers involved in this study it would be difficult to comment on whether the continuation or cessation of offending medications had any effect on the results observed.

Secondly, it could be that in contrast to patients suffering from AUGIB, excess platelet activation is not the cause of recurrent cardiovascular events. Several other reasons for poor outcomes have been suggested; discontinuation of antiplatelet agents, adverse effects of blood transfusion, adverse effects of hypotension and interventions required to control bleeding (Hamm *et al* 2011; Spencer *et al* 2007; Eikelboom *et al* 2006; Fitchett *et al* 2007; Manoukian *et al* 2007; Mehran *et al* 2009; Disney *et al* 2011).

No differences were observed in platelet activation or reactivity to ADP after 12 weeks of follow up. This may be due to the fact that changes in platelet activation in these patients do not differ. However, an alternative explanation would be that insufficient numbers of patients were recruited and followed up. Patients with ACS complicated by bleeding, were uncommon during the study period and difficult to recruit. One of the reasons for the paucity of this group of patients was due to a change in practice by many Cardiologists who began to use radial access preferentially over femoral access for coronary angiography. Previously many bleeding episodes in patients with ACS were at the access site, and the change of practice to using radial has resulted in lower rates of access site bleeding, need for transfusion and mortality (Bavishi et al 2016; Nathan and Rao 2012; Piccolo et al 2014; Rajani *et al* 2015; Ruiz-Rodriguez et al 2016; Valgimigli *et al* 2015). The data, however, is conflicting with one recent study found that major bleeding events have increased over the past 10 years, although vascular access site bleeding did not impact on mortality (Sabbag *et al* 2015). Further studies have also shown that mild-moderate vascular access site bleeding (using GUSTO definitions) does not increase risk of adverse outcomes (Kikkert et al 2014; Vavalle *et al* 2013). In addition to this, bleeding has become more widely recognised as have poorer short and long term

outcomes and therefore the treatments decision were made with the potential side effect of bleeding borne in mind.

Other factors may have played a part in poor patient numbers. Patients with ACS and bleeding have a prolonged hospital stay, often resulting in additional interventions to treat bleeding. These patients are monitored closely, which involves repeated venepuncture. Unfortunately, as a result of this a proportion of patients were unwilling to participate in this study. In addition to the problems with recruitment there were also problems with patient follow up. Of those patients with ACS complicated by bleeding that were recruited, 7 were unwilling to undergo repeat venepuncture after 12 weeks; this represents a drop out of approximately 44%. This appears to be a reflection of the general sickness and co-morbidity of this patient group, as only 17% of those patients with ACS without bleeding did not complete follow up.

The female gender has a greater risk of bleeding in the context of ACS. However, in this study, the majority of those recruited were male. This could impact on these results although one recent study showed that it is the bleeding complication rather than gender that explains excess mortality (Ng *et al* 2016).

Patients in this study with ACS and bleeding had an elevated WBC as compared to controls. This phenomenon has been noted previously and is an independent predictor of major bleeding, in addition to 1 year mortality (Palmerini *et al* 2011; Palmerini *et al* 2013).

CHAPTER 6 – CONCLUSIONS AND FUTURE WORK

The results from Chapter 3 show that the use of pre-endoscopy risk scores is disappointingly low (20%). This is despite the fact the current guidelines suggest that these scores are a mandatory part of the initial assessment of patients with AUGIB, and that patients with a GBS of zero can be discharged early with an urgent outpatient endoscopy. In this study, it was found that patients with a GBS ≤ 2 did not require an intervention and did not die. A GBS ≤ 2 accounted for approximately 15% of patients in this study population, however; further larger studies are required to demonstrate that those patients with a GBS ≤ 2 could be safely managed on an outpatient basis.

It was also found that there is both a diurnal and seasonal variation in the presentation of AUGIB, although the reasons for such differences remain unclear. These findings may have implications for the provision of endoscopy services. No evidence was observed to suggest that those patients, presenting out of hours, are any more unstable than those presenting during the daytime. The diurnal presentation means many patients are more likely to be clerked, resuscitated and made ready for upper GI endoscopy by the end of the working day. This is in contrast to many endoscopy units, where morning lists are ring fenced for AUGIB. Further studies are required to examine diurnal and seasonal variation in the UK AUGIB population and therefore allow better endoscopy provisions in the future.

The work described in Chapter 4 demonstrates that AUGIB is associated with increased levels of platelet activation and the prothrombotic markers d-dimer and vWF. Work in Chapter 5 shows that patients who suffer from a bleeding episode, during treatment for ACS, have a higher level of platelet activation than stable coronary disease controls. Although no differences were observed between the ACS

with bleeding and ACS groups, longitudinal studies are required to see if platelet activation is prolonged in the ACS with bleeding group. Further studies are also needed to address the problem of adverse outcomes in patients that suffer from bleeding during the treatment of ACS.

Overall, these studies show that there is a higher level of platelet activation in patients suffering from AUGIB. These are novel findings. In addition, those with AUGIB had higher levels of d-dimer and vWF. These findings may explain the increased risk of cardiovascular events seen in patients with AUGIB. Patients presenting with AUGIB have prolonged levels of platelet activation for at least 12 weeks following the index event. This phenomenon may be further prolonged and further studies are required to examine this. In patients with high cardiovascular risk, earlier, and bolder, re-introduction of antiplatelet therapy should be initiated. More in depth studies will be required on the risk of cardiovascular diseases following an episode of AUGIB and the optimum timing to recommence antiplatelet therapy in this context. These studies could include larger numbers, examining the length of change in platelet activation and prothrombotic markers, and whether the early reintroduction of anti-platelet agents can alter this response.

This work highlights several unresolved questions that will require future studies to address the following issues. In patients with AUGIB, it is unclear for how long the higher levels of platelet activation and prothrombotic markers is a phenomenon for, and whether the early re-introduction of anti-platelet agents will make a difference on long term outcomes; it seems apparent that this strategy improves outcomes in the short-term. In patients with AUGIB it is unclear whether the differences we have seen are clinically relevant; although it would appear from previous studies showing the cardiovascular risk that they are. Unfortunately, in patients with bleeding during an

episode of ACS this study does not resolve the reasons behind their poor outcome; high drop out rates and difficulties in patient recruitment affected the results and larger studies are required to investigate further.

To conclude, the work in this thesis supports the use of the pre-endoscopy risk scores in patients with AUGIB, preferentially the Glasgow Blatchford score, and that there is a diurnal and seasonal variation in the presentation of AUGIB. In addition, patients with AUGIB have been shown to have higher levels of platelet activation (as measured by CD62P and PAC-1), and prothrombotic markers (increased vascular dysfunction and increased fibrinolysis), for a period of at least 12 weeks, which has treatment implications. Finally, and unfortunately, no such changes were seen to explain the poor outcomes in patients with ACS complicated by bleeding, although this may be due to problems with recruitment.

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APPENDICES

APPENDIX 1:

STUDY RELATED PUBLICATIONS AND PRESENTATIONS

Publications

1. Disney BR, Watson RD, Blann AD, Lip GY, Anderson MR. Review article: proton pump inhibitors with clopidogrel - evidence for and against a clinically important interaction. Aliment Pharmacol Ther. 2011; 33(7): 758-767.

Papers in submission

1. Disney BR, Watson RD, Blann AD, Lip GY, Tselepis C, Anderson MR. Platelet activation and prothrombotic markers following acute non-variceal upper gastrointestinal bleeding: evidence of prolonged elevation.
2. Disney BR, Watson RD, Blann AD, Lip GY, Tselepis C, Anderson MR. Seasonal and diurnal variation in the presentation and severity of acute upper gastrointestinal bleeding.

Abstracts

1. Disney BR, Watson RD, Blann AD, Lip GY, Tselepis C, Anderson MR. Prolonged platelet activation in patients with acute upper gastrointestinal bleeding. Gut 2013; 62: Suppl 1 A10-A11.

2. Disney BR, Watson RD, Blann AD, Lip GY, Tselepis C, Anderson MR. Platelet activation in non-variceal acute upper gastrointestinal bleeding. Gastroenterology 2013; 144 (Suppl 1): S-166.
3. Disney BR, Watson R, Blann A, Lip G, Tselepis C, Anderson M. Use of the Blatchford score to identify low-risk upper gastrointestinal bleeds. Gut 2012; 61 (Suppl 2): A156.
4. Disney BR, Watson R, Blann A, Lip G, Tselepis C, Anderson M. Platelet activation in acute upper gastrointestinal bleeding. Gut 2012; 61 (Suppl 2): A361.
5. Disney BR, Watson R, Blann A, Lip G, Tselepis C, Anderson M. Seasonal and diurnal variation in the presentation and severity of acute upper gastrointestinal bleeding. Gut 2012; 61 (Suppl 2): A362.

International presentations

1. Platelet activation in non-variceal acute upper gastrointestinal bleeding. Digestive Disease Week, Orlando, USA. Oral presentation. May 2013.

National presentations

1. Prolonged platelet activation in acute upper gastrointestinal bleeding. British Society of Gastroenterology Conference. Oral presentation. June 2013.
2. Use of the Blatchford score to identify low-risk upper gastrointestinal bleeds. British Society of Gastroenterology Conference. Poster presentation. June 2012.

3. Platelet activation in acute upper gastrointestinal bleeding. British Society of Gastroenterology Conference. Poster presentation. June 2012.
4. Seasonal and diurnal variation in the presentation and severity of acute upper gastrointestinal bleeding. British Society of Gastroenterology Conference. Poster presentation. June 2012.
5. Upper Gastrointestinal Bleeding in the context of Acute Coronary Syndromes. West Midlands Deanery Cardiology Registrars Training Day. 2010.

Regional presentations

1. Disney BR, Watson RD, Blann AD, Lip GY, Tselepis C, Anderson MR. Platelet activation in acute upper gastrointestinal bleeding. Midlands Gastroenterology Society 2012.
2. Disney BR. Gastrointestinal bleeding in acute coronary syndromes. West Midlands Cardiology SpR training day December 2010.

Prizes

British Society of Gastroenterology National Conference 2012. Endoscopy Section Best Poster Award. Use of the Blatchford score to identify low-risk upper gastrointestinal bleeds – what is low-risk?