ANGLIA RUSKIN UNIVERSITY
FACULTY OF MEDICAL SCIENCE

"BLOOD AND BLOOD COMPONENT TRANSFUSION ON 30-DAY MORTALITY AND MORBIDITY OF INFRA-RENAL RUPTURED ABDOMINAL AORTIC ANEURYSM"

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A thesis in partial fulfillment of the requirements of Anglia Ruskin University for the degree of MD by Research.

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"The spirit of research and conquest is the permanent soul of evolution"

-Teilhard de Chardin (1881-1955) -

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ABSTRACT

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This thesis for the first time in the literature, through a single cohort (n=82), systematic review and meta-aggregation of the data, has identified that the majority (>85%) of ruptured abdominal aortic aneurysm (rAAA) do not present with coagulopathy. In addition, the thesis for the first time, through a retro and prospective cohort study, has demonstrated that the hemostatic resuscitation protocols derived from military and civilian trauma for the correction of coagulopathy with a blood product ratio of one unit of packed red blood cell to one unit of fresh frozen plasma to one pool of platelet (1:1:1), contributes to increased postoperative (30-day) morbidity and mortality, especially thrombotic complications. This was attributed to different baseline demographics, pathophysiology and coagulation status. Through a comparative study, the thesis then confirms that such transfusion practice not only contributes to adverse outcomes, but also has no impact on final coagulation status of rAAAs. In addition, through a retro and prospective cohort study, a novel hematological marker (neutrophil to lymphocyte ratio) (NLR) was identified as an independent predictor of morbidity in rAAAs. This thesis was set on the background of significant research into all factors that could contribute to pathogenesis, inhibition and progression of rAAAs.

This thesis concludes that the use of additional blood products (fresh frozen plasma and platelet) in hemostatic resuscitation of ruptured abdominal aortic aneurysms alongside packed red blood cell is not evidence based and a single protocol derived from one cohort (military and civilian trauma) of patients does not apply to another. The use of additional products in ruptured abdominal aortic aneurysms should be tailored to the individual hematological and clinical requirements and not as a part of a set transfusion ratio (1:1:1). This thesis has resulted in a change of practice and has created a platform for further search of the optimal transfusion protocol in this cohort of patients.

Keywords: Ruptured Abdominal Aortic Aneurysm (rAAA); Blood Transfusion; Coagulation status; Coagulopathy; Mortality; Morbidity.

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ABBREVIATIOBNS

AAA	Abdominal Aortic Aneurysm
AP	Atmospheric Pressure
aPTT	Activated Partial Thromboplastin Time
ASA	American Society of Anesthesiology
CI	Confidence Intervals
CINHAL	Cumulative Index of Nursing and Allied Health Literature
COPD	Chronic Obstructive Pulmonary Disease
CT	Computed Tomography
CTA	Computed tomography angiogram
DIC	Disseminated Intravascular Coagulopathy
DM	Diabetes Mellitus
ED	Emergency Department
eGFR	Estimated Glomerular Filtration Rate
EVAR	Endovascular Aneurysm Repair
FDP	Fibrinogen degradation product and/or D-Dimer
FFP	Fresh frozen plasma
Hb	Hemoglobin
HDU	High Dependency Unit
HTN	Hypertension
IL6	Interleukin-6
INR	International Normalization Ratio
IQR	Inter Quartile Range
ITU	Intensive Care Unit
LDL	Low Density Lipoprotein
LOS	Length of stay
MeSH	Medical Subject Headings
MMP	Matrix Metalloproteinase
NLR	Neutrophil lymphocyte Ratio
PCO_2	Partial Pressure of Carbon Dioxide
PF	Pro-thrombin fragment
Plt/PLT	Platelet count
Po_2	Partial Pressure of Oxygen
PRBC	Packed Red Blood Cell
PT	Prothrombin Time
rAAA	Ruptured Abdominal Aortic Aneurysm
RI	Renal insufficiency
SD	Standard Deviation
TAT	Thrombin-anti-thrombin complex
TF	Tissue Factor
t-PA	Tissue Plasminogen Activator
UK	United Kingdom
USA	United States of America
USS	Ultrasonography
vWF	von-Wilebrand Factor
WSS	Wall Shear Stress

Book Chapter, Publication & Presentations

Book Chapter

1. Ali Kordzadeh: **Abdominal Aortic Aneurysm and its Rupture: An Overview. Vascular Surgery,** *01/2016: chapter 1; SME groups. ISBN: 978-1-944685-58-4*

Journal Publications

1. Ali Kordzadeh, A.D. Parsa, A. Askari, B. Maddison, Y.P. Panayiotopoulos: **Presenting Baseline Coagulation of Infra Renal Ruptured Abdominal Aortic Aneurysm: A Systematic Review and Pooled Analysis.** *European Journal of Vascular and Endovascular Surgery 03/2016; 51(5). DOI:10.1016/j.ejvs.2016.02.009*
2. Ali Kordzadeh, Alan Askari, Ali D Parsa, Tom Browne, Yiannis P. Panayiotopoulos: **The Clinical Implication of Blood Product Transfusion on Morbidity and Mortality of Ruptured Abdominal Aortic Aneurysm**. *Clinical and Applied Thrombosis/Hemostasis DOI: 10.1177/1076029615624548*
3. Ali Kordzadeh: **Factors associated with progression of the abdominal aortic aneurysm and novel inhibition therapies. Reviews in Vascular Medicine** *09/2015;DOI:10.1016/j.rvm.2015.09.003*
4. Ali Kordzadeh, George Malietzis, Tom Browne, Ioannis Prionidis, Yiannis P. Panayiotopoulos: **Neutrophil to lymphocyte ratio (NLR) of five predicts 30-day morbidity in ruptured abdominal aortic aneurysms (rAAA): A retrospective cohort study.** *International Journal of Surgery 01/2015; DOI:10.1016/j.ijsu.2015.01.013*

Presentations (Oral & Poster)

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3. Ali Kordzadeh: **Variables associated with progression of the abdominal aortic aneurysm and novel inhibition therapies.** *Charring Cross International Vascular Symposium 27 to 29 April 2016 (Poster).*

4. Ali Kordzadeh. **The Clinical Implication of Blood Product Transfusion on Morbidity and Mortality of Ruptured Abdominal Aortic Aneurysm**. *Charring Cross International Vascular Symposium 27 to 29 April 2016 (Poster).*

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CHAPTER 1
Introduction to Abdominal Aortic Aneurysm &Their Rupture.

1.1 Brief History

The word aneurysm originates from the Greek word “Aneurysma” describing permanent cardiac or vessel dilatation. The most common site of arterial aneurysm is the infra-renal abdominal aorta (Johnston, 1991; Cervantes, 2003). The first description of the aortic aneurysm or so-called “tumor of the artery” was found in one of the oldest medical documents called “Eber Papyrus” around 1500 BC. Since then, various unsuccessful suggestions and attempts have been made by different physicians to repair the infra-renal abdominal aorta aneurysms (AAA) (Cohen, 1990). Amongst them, the most famous attempt was the wrapping of Albert Einstein’s aneurysm with “cellophane” that never prevailed. It was not until 1951, when two surgeons (Freeman and Dobust, 1952) within the time frame of one month in different continents managed to successfully repair the infra-renal abdominal aortic aneurysm by a synthetic tube graft. This technique (open repair) remained the only method of repair until the introduction of endovascular aneurysm repair (EVAR) by Volodos (1986) and its later modification by an Argentinian surgeon Parodi in 1999. Currently, both techniques (EVAR and open repair) remain the only method of treatment for AAAs. Despite advances in the field of EVAR and it is associated technology; open technique remains the commonest surgical approach in the repair of ruptured abdominal aortic aneurysms.

1.2 Epidemiology

The current reported incidence of AAA occurrence in the population is around 4.9-9.9%. Its elective repair has a mortality of 1-5% in tertiary vascular centers and up to 10 % in district general hospitals. Once the AAA ruptures, the mortality can rise to 50-80%. This is amongst the patients that make it to the hospital.

Currently, in United States (US), ruptured AAA (rAAA) claims 15,000 lives every year, and it is the 13th leading cause of death in US. In the United Kingdom (UK), rAAA accounts for 8,000 deaths annually (Melton, 1984; Holt, 2007). As mentioned earlier, there appears to be a clear familial (genetic) link for AAA. Estimated risk of AAA in first-degree relatives is 11.6 times higher than the rest of the non-affected population. A screening process of siblings of aneurysm patients has shown 6 % of sisters and 29% of brothers to have aneurysm too (Bird, 1995). AAA possesses a 2:1 male to female ratio at any given age. In addition, there appears to be a higher incidence of AAA in the Caucasian population in comparison to Afro-Caribbean ethnic backgrounds (Salem, 2009).

1.3 Terminologies & Anatomy

The native infra-renal aortic diameter in the healthy male population at its largest measures approximately 24 mm and this value is 22 mm in the female cohort (Wanhainen, 2008). Overall, the female population exhibits a smaller aortic diameter but the relative aortic diameter based on the predicted size in relation to overall body surface area, exhibits a much larger native aorta than their male counterparts (Länne, 1998). Once the

infra-renal aorta reaches 1.5 times of its mentioned size, the term aneurysm is applied, if the enlargement remains below this threshold, the term “Ectasia” is used. If this affects the entire circumference of the artery the term “Fusiform” and if partially, the term “Saccular” is applied. Each artery including the abdominal aorta is comprised of three major layers; The Tunica intima (inner most layer), Tunica media and Tunica adventitia. The aneurysmal degeneration of any artery involves all the three layers. Tunica intima is composed of multi-functional single layer endothelial cells whereas tunica media contains smooth muscle cells, elastin (12%) and collagen fibers. Tunica adventitia contains connective tissue and vasa vasorum (Rutherford, 2010).

The current cut off size for the repair of non-ruptured AAA is based on two major prospective randomized trials. Both trials suggest that AAA measuring 5-5.5 cm should be repaired as the rupture ratio at this level remains at 1% per annum. If the AAA reaches 5.5-5.9 cm in size, the incidence of rupture increases to about 9.4%. This trend continues as the AAA continues to grow in size (Kingdom & Lederle, 2002).

1.4 Pathophysiology

The most common etiology (90%) behind infra-renal abdominal aortic aneurysm formation is atherosclerotic degeneration. The second most common etiology is an inflammatory process (5%) whereas infectious (mycotic) and autoimmune disease plays a less attributing role. The

details of all accepted and suggested etiologies are tabulated in Table 1.1 of this manuscript (Kordzadeh, 2013).

Aetiology	Percentage
• *Degenerative (atherosclerosis)*	90%
• *Inflammatory (Vasculitis).*	5%
• *Infective (Mycotic).* Fungal. Bacterial (*Staphylococcus Species*, *Streptococcus Species,* Non-typhoidal Salmonellae Species, Sexual transmitted disease). Viral. Poly-microbial.	2%
• *Autoimmune/ Genetic Disorders*. Marfan Syndrome. Loeys-Dietz syndrome. Ehlers-Danlos syndrome. Turner syndrome. Genetic Mutation.	1%
• *Traumatic*	1%

Table 1.1 Aetiology of Abdominal Aortic Aneurysm (AAA).

The current evidence suggests that the path to arterial wall remodeling (aneurysm formation) is a sequence of multiple pathological factors that result in endothelial dysfunction, proteolytic degradation, destruction of the elastic media, depletion of vascular smooth muscle cells and inflammation of the aortic wall components at both the macro and micro level. The initiating trigger is believed to be the consequence of alterations in wall shear stress (WSS) due to flow changes (high versus low) (Tanweer, 2014). This process creates a unique hemodynamic and mechanical insult to the aorta and ignites the aneurysmal pathogenesis.

The cascade of events occurs mostly in the tunica media and intima. In this process the endothelial cell damage along with smooth muscle cell, elastic and collagen destruction is accelerated by enhanced metalloproteinase (MMP) and other proteinase expressions. In such circumstances, elastin reduces from 12% in a healthy aorta to 1% only. The commonest MMPs associated with AAA are MMP 2 (Gelatinase A) and MMP 9 (Gelatinase B) that exhibit elastolytic and proteolytic features (Dilme, 2014). Despite the clear understanding of the cellular pathogenesis, the exact mechanism of aneurysm formation remains undetermined to this date (Table 1.2).

1.Atherosclerosis/ Inflammation/ Infection/Trauma
2.Activation of inflammatory pathway. Damage to vessel wall (intima /media /intima). Recruitment of inflammatory cells.
3.Smooth muscle cell, Elastic and collagen destruction. Enhanced metalloproteinase (MMP-9 & MMP-2) Activation of proteinases expressions.
4.Elastin reduction to 1%. Elastolytic and proteolytic enhancement. Endothelial dysfunction. Depletion of vascular smooth muscle cells. Overall wall weakness.
5.Hemodynamic (pressure & flow) exceed wall strength. Focal dilatation of the Aorta.
6.Impact of genetic and risk factors on further growth.
7.Rupture. (Once the pressure exceeds wall strength) (Beyond 5-5.5 cm).

Table 1.2 Process of Rupture in AAAs.

Precipitating Risk Factors

Advancement in vascular biology has led to a better understanding of the ongoing degeneration of AAA at a molecular, cellular and systemic level. This, in conjunction with the recent development in pharmacological therapies aimed at associated risk factors, could serve as an important strategic tool for control or limitation of AAA expansion. Hence, in this review, each associated risk factor will be discussed with their respective management strategy (Table 1.3). In addition, the role of novel therapies in inhibition of AAA growth will be discussed in further detail (Table 1.4).

1.1 Literature Search

An electronic and comprehensive search of literature in Medline from 1966 to December 2015 using the following keywords and/or Medical Subject Headings (MeSH) terms were used: Abdominal aortic aneurysm growth and/or risk factors and/ or genetic disposition, abdominal aortic aneurysm and/or sac expansion and/or risk factors. This search was limited to adult subjects and English language only. The references were also crosschecked for any additional literature not identified in the primary search. This search produced a total of 134 articles. All abstracts were retrieved and reviewed.

1.2 Publication Selection

The following restrictions (exclusion criteria) were applied:

1). Studies with less than 50 cases.

2). Abstracts and/or conference proceedings.

3). Narrative and/or invited review articles.

4). Studies with no endpoints.

5). Non abdominal aortic aneurysm studies.

6). Post aneurysm repair sac expansion.

1.3 Results

After the application of the inclusion criterion a total of 21 articles were found eligible for this review. Outcomes of each article were presented with their respective headings and meta-aggregation in a tabulated format.

1.4 Diabetes Mellitus

Diabetes mellitus (DM) is a well-established risk factor for atherosclerotic arterial disease but interestingly DM plays a protective role in AAAs. This has been attributed to increase synthesis and decrease degradation of the matrix metalloproteinase (MMP) resulting in thicker aortic wall. Hence reducing growth and rupture incidence. This is believed to be the direct result of hyperglycemia in this cohort of patients (Shantikumar, 2010).

1.5 Smoking

Smoking remains as one of the most relevant risk factors in AAAs growth and subsequent rupture. There exists a 2.5-fold increase in smokers versus non-smokers (Lederle, 2003). It appears that Tobacco and one of its contents Nicotine, can induced MMP-2 through the activation of protein kinase alpha-2 resulting in enhanced arterial degeneration and

remodeling (Murphy, 1998). Therefore, an aggressive approach in smoking cessation plays a crucial role in early inhibition of aneurysm growth and its subsequent rupture.

1.6 Chronic Obstructive Pulmonary Disease (COPD)

Although the majority of COPD in the AAA cohort is smoking related, evidence suggests that COPD remains an important risk factor well beyond the smoking point (Meijer, 2012). This might be related to the impact of significant reduction in forced expiratory volume 1 to that of forced expiratory volume (FEV1/FEV) in AAA patients compared to non COPD group. This reduction has shown to enhance MMP 2 and neutrophil elastase (proteolytic enzymes) expression thus enhancing aneurysmal degeneration (Meijer, 2012).

1.7 Renal Failure

In recent years there has been an accumulating level of evidence to support the independent role of renal insufficiency (RI) and raised estimated Glomerular Filtration Rate (eGFR) in the prediction of vascular events in peripheral vascular disease (Go, 2004). Despite known impacts of RI and raised eGFR on small to medium arteries, such impact on AAA (large artery) remains contentious. In addition, no direct impact between MMPs and renal failure has yet been established.

1.8 Hypertension

Hypertension is a well-established and an independent risk factor for rupture of AAAs (Vardulaki, 200). Each cardiac cycle produces a discrete quanta of energy, which result in oscillation, loading and unloading of elastin and collagen fibers permitting a considerable expandability of the normal aorta within normal blood pressure (Wixon, 2009). However, due to a significant reduction of such fibers (12% to 1%) in a deformed and aneurysmal aorta, exceeding blood pressure (≥ 100mmHg) results in stiffening of the aorta and further recruitment of non-distensible fibers precipitating rupture (Kordzadeh, 2013). Therefore, a well-controlled blood pressure in conjunction with life style modifications to achieve this, is of vital importance.

1.9 Atmospheric Pressure

Current literature suggests that periods of low atmospheric pressure (AP) (meteorological parameter) irrespective of the day, season and month have a direct correlation to higher incidence of sudden rupture. This has been attributed to alteration in partial pressure of oxygen (Po_2) and carbon dioxide (Pco_2) along with changes in main arterial blood pressure. The combination of hypoxia, hypercarbia and reduction in blood pressure, activates the sympathetic stimulation, enhancing the intra and extra luminal arterial pressure and subsequent rupture (Koner, 1989). Despite various postulated hypothesis the exact mechanism of rupture in correlation to AP remains unknown (Koner, 1989).

1.10 Vitamin D (25-hydroxyvitamin D)

Another recently identified risk factor has been low levels of circulating plasma Vitamin D (25-hydroxyvitamin D). It appears that in a population-based study of three hundred and eleven older man, low levels of Vitamin D remained an independent risk factor in the enlargement of AAAs. In addition, this study also demonstrated a dose-response relationship between AAA diameter and vitamin D supplementation. The only set back of this study was the lack of calcium and parathyroid circulatory levels that are known to have a direct relationship to Vitamin D levels (Wong, 2013).

1.11 Selenium

Another recently recognized serum marker associated with AAA degeneration and enlargement has been low levels of selenium (Se). In this study seventy-three individuals (n=73), based on their aneurysm size, were categorized to three groups and their respective serum Se were obtained. The higher AAA size was associated with lower levels of circulatory Se. In Addition, an inverse relationship was observed between serum Se and AAA diameter. Hence, suggesting Se might have an independent impact on molecular degeneration of AAAs (Witkowska, 2006).

1.12 Cholesterol

High levels of cholesterol remain an independent risk factor for the atherosclerotic change of abdominal aorta and aneurysmal degeneration. Literature suggests low-density lipoprotein (LDL) cholesterol acts via

inflammatory mediated pathway in matrix degeneration of the abdominal aorta and contribute further to their expansion. Therefore, initiation of statins (HMG-CoA reductase) upon diagnosis of AAA remains essential to inhibit their further growth (Hobbs, 2003; Kerati, 2004).

Risk Factor	*Impact*	*Targeted Therapy*
Smoking	• 2.5 fold increase in expansion & rupture.	• Smoking cessation (Varenicline)
COPD	• Enhances degenerative changes & expansion.	• Control of COPD
Hypertension	• Increase aortic wall stiffness, reduction in elastin fibers.	• Regular monitoring and medication (ACE Inhibitors & Ang II receptor blockers).
Low Vitamin D	• Enhances expansion	• Multivitamins & Sun exposure
Low Selenium	• Enhances expansion	• Multivitamins
Cholesterol &LDL	• Enhances expansion through matrix degeneration	• Statins (HMG-CoA reductase)
Renal Failure	• No impact on large vessels.	• None
Diabetes Mellitus	• Inhibitor of Growth	• Normo-glycemic control
Female Gender and old Age	• Worse outcome	• Informed consent.
Low atmospheric pressure	• Higher rupture incidence	• Prioritization of AAA in such season.

Table 1.3 Risk Factors Associated with AAA Expansion & Targeted Therapies.

1.13 Gender & Age

The male population tends to present at a younger age and demonstrate a better outcome (Mortality & Morbidity) in comparison to female cohort. Overall, there is insidious difference in diagnosis, intervention and outcome of rAAA in female compared to male individuals. The most

widespread accepted explanation to date has been the protective impact of endogenous estrogen on cardiovascular disease in female patients. It has been demonstrated that estrogen inhibits the aneurysmal degeneration and negative remodeling of the aorta. This effect eventually wears off at the menopausal stage and hormone replacement therapy has failed to achieve same result in vitro studies (Makrygiannis, 1964).

1.14 Neutrophil to Lymphocyte (NLR)

A recently identified marker of morbidity and mortality in cardiovascular surgery is the hematological marker of neutrophil to lymphocyte ratio (NLR) (Duffy, 2006). The Neutrophil to Lymphocyte ratio (NLR) was first reported in 1967, is a widely used marker of systemic inflammatory response in recent literature (Bobb, 1967). The NLR has been linked to adverse outcomes in patients with colorectal, gastric, pancreatic, ovarian and hepatocellular carcinoma (Gomez, 2008; An, 2010; Malietzis, 2013).

In relation to vascular surgery, there is a single study that demonstrates NLR is a predictor of 2-year survival in patients undergoing a range of vascular interventions (Bhutta, 2011). In addition, a recent report suggests that in the elective group of AAAs, a preoperative NLR> 5 could be a predictor of 30-day mortality (Appleton, 2014). Neutrophilia has been associated with collagen remodeling, direct endothelial cell injury, pro-thrombotic state and hypercoagulability which is known to occur in rAAAs (Siminiak, 1995; Madjid, 2004; Coller, 2005). Lymphocytopenia appears

to be the result of redistribution and margination of lymphocytes within the lymphatic system and a marker of accelerated apoptosis. Low lymphocyte count indicates a state of immunosuppression and physiological stress that have adverse relation to overall patient outcome (Evans, 1995). However, this has never been assessed for ruptured AAAs to date.

Novel Therapies Inhibiting AAA Growth

1.1 Antibiotic therapy

In the last decade there has been suggestions that atherosclerotic plaque in the wall of abdominal aortic aneurysm often contains bacterial pathogens and early initiation of antibiotics may aid in the regression or inhibition of the aneurysm growth. Two randomized clinical trials, demonstrated doxycycline (150 mg daily) and roxithromycin (300mg daily) to inhibit aneurysm expansion but this was only effective in the first year of treatment and beyond that time frame no further effect was noted (Mosorin and Vammens, 2001). Rapamycin an immunosuppressive agent in a recent trial has shown 40% reduction in aneurysm expansion through reduction of Matrix metalloproteinase (MMP-9) expression (Lawrence, 2004) (Table 1.4).

1.2 Anti-inflammatory agents

Increased production of prostaglandins in the process of inflammation induces Matrix metalloproteinase production, which plays a significant role in expansion of the aneurysm. A recent study by Walton et al.

demonstrated that Celecoxib, a COX-2 inhibitor (non-steroidal anti-inflammatory, cyclooxygenase type 2) significantly decreases the incidence and severity of AAA formation. This effect was not similar in COX-1 inhibitors (Walton,1999; King, 2006) (Table 1.4).

Therapy	*Impact*	*Medication Types*
Antibiotic therapy	• Atherosclerotic Plaque. • 40% reduction (MMP-9).	• Doxycycline 150mgs. • Roxithromycin 300mgs. • Rapamycin
Anti-inflammatory Therapy	• Prostaglandins. • Induces MMP-9	• Celecoxib (COX-2 inhibitor).
Mast cell Inhibitor	• Reduces MM expression. • Reduction of AAA expansion by 40%.	• Cromolyn Sodium
Kinase Inhibitor (c-Jun N-terminal kinase) (JNK)	• Molecular protein in apoptosis. • Cell differentiation & Proliferation.	• JNK inhibitors.
Genome Therapy	• Genome regulation & expression.	• Inhibition of genome transactivation.

Table 1.4 Novel therapies in inhibition of AAA growth.

1.3 Mast Cell inhibitor

Mast cell stabilizers or inhibitors traditionally used for control of allergic disorders and bronchial asthma have shown promising outcome in reduction of aortic expansion by 40%. Amongst suggested medication is Cromolyn Sodium that inhibits histamine and macrophages in the process of the inflammation that in turn reduces MMP expression (Sun, 2007) (Table 1.4).

1.4 Kinase inhibitors

Kinase or so called c-Jun N-terminal kinases (JNK), a molecular protein that play a crucial part in apoptosis, cell differentiation and proliferation reacts to inflammatory changes as a result of stress stimuli, shock and cytokines. Presence of AAA has shown elevated levels of JNK that contribute to pro-inflammatory and MMP-9 activation. The JNK-Inhibitors not only reduce the inflammatory changes in AAA patients but also aid in restoration of aortic tissue (Yoshimura, 2005) (Table 1.4).

1.5 Genome therapy

In recent years there has been a significant emphasis on gene regulation and expression therapies. The transcription of a defined gene is up regulated by specific proteins known as transcription factor that are found in families with AAAs. Targeted therapies aiming to avoid transactivation of such factors are still in the early stages but their inhibition could contribute to complete resolution of the inflammatory and matrix degradation associated with AAA expansion (Epstein, 1996) (Table 1.4).

Mechanism & Hemodynamics of Rupture

In general, it is accepted that the natural history of AAA is further growth and eventual rupture. This might have direct correlation to the aneurysm diameter. The expansion rate of an AAA is around 0.2-0.4 cm per annum (Brady, 2004; Mofidi, 2007). This remains subjective to the individual risk factors and genetic predisposition explained in detail in this section of the introduction. In theory, the “Laplace law” has been traditionally used to

demonstrate why an AAA deforms and ruptures (Hademenos, 1994). According to this law there is a direct correlation between tension, pressure and the radius of each cylindrical structure. This aids in calculating the circumferential tension and the possibility of the rupture (Hademenos, 1994). However, this law might not be applicable to infra-renal AAAs of all shapes (Saccular versus Fusiform) as they might present more of a spherical structure than cylindrical in practice. In such cases “vessel wall stress” defined as the restoring force per unit area in circumferential and longitudinal direction is more useful in defining the rupture (Table 1.2). Glagov (1983) demonstrated a linear relationship between the thickness of the aorta (tunica media) and that of the aortic radius at a mean given blood pressure. So a larger aorta that has a thicker wall is more resistant to rupture than a smaller aorta. Therefore, if two aortas of unequal size develop an aneurysm of a same size, the aneurysm formed from a smaller aorta is at higher risk of rupture. This theory was reinforced by the work of Stringfellow (1987). In this study, they exhibited that the gradient wall stress in a six (6) cm aneurysm arising from three (3) cm and of one (1) cm aortas exhibit significant differences in longitudinal and circumferential directions. Therefore, a 6cm aneurysm arising from a 1cm native aorta has much higher chance of rupture than that of a 3 cm native aorta due to significant gradient wall stress differences. Overall, the evidence suggests that the path of rupture is multifactorial and its exact nature remains elusive to this date.

1.1 Presentation of Rupture

According to the words of Sir William Osler "Aneurysm of the abdominal aorta is very often diagnosed when not present, and when present the symptoms may be so obscure that the nature of the trouble is overlooked" (Osler, 1905). This perhaps remains the best explanation of AAA and its clinical manifestation despite significant advancements in the field of diagnostic medicine (Table 1.5).

Common presentation of ruptured AAA (25-50%) **(Classic Triad)**
• *Sudden onset sever back pain (± abdominal pain)* • *Hypotension (± syncope)* • *Pulsatile abdominal mass*
Non-common presentation of ruptured AAA (30%)
• *Right flank pain.* • *Groin pain.* • *Testicular pain or pressure.* • *Compression effect causing transient lower limb paralysis.* • *Testicular ecchymosis (blue scrotum sign of Bryant).* • *Hernia.* • Urolithiasis.
Rare presentation of rupture AAA (5%).
• *Aortocaval fistula (high output cardiac failure, dyspnea, tachycardia, wide pulse pressure, cyanosis, and lower limb edema)* • *Aortoenteric fistula (Sever Melena and death).* • *Aorto-left renal vein fistula (abdominal pain, haematuria, silent left kidney syndrome).*

Table 1.5 Common & uncommon presentation of ruptured AAA.

Almost all patients with infra-renal AAA are asymptomatic and their diagnosis is mainly incidental. This is mainly the result of an ongoing investigation for other abdominal presentation where an ultrasound (USS) or computed tomography scan (CT) and detail clinical examination reveals a pulsatile mass. AAA becomes symptomatic when they rupture or cause complications (Assar, 2009). The most common presentation of ruptured infra-renal AAA is the classical triad of: sudden and severe back pain with or without abdominal pain, hypotension and/or syncope and pulsatile abdominal mass. However, such presentation is only seen in 25-50 % of the population and some may present in an entirely different way (Table 1.5), misleading clinicians towards other pathophysiological conditions (Fielding, 1981). It appears that the anatomical site of AAA rupture dictates its clinician presentation and this factor perhaps explain why there is 30% misdiagnosis in practice (Marston, 1992).

1.2 Rupture Site & Their Clinical Manifestation

Given the anatomical location of the abdominal aorta rupture, can occur anteriorly (peritoneal), posteriorly (retroperitoneal) or in to the adjacent anatomical structure such as bowel (Duodenum, Aorto-enteric fistula), Inferior vena cava (aorto-caval fistula), left renal vein (Aorto –left renal vein fistula) and in very rare cases contained chronic rupture. The retroperitoneal rupture (posterior) is the most common type of rupture (80%) (Rutherford, 1989). This is the result of a tear in the abdominal aortic wall that permits blood leak into the posterior compartment of abdominal cavity. This manifest itself clinically with the classical triad of

presentation as described earlier. Such patients, due to temporary sealing of the rupture site due to tamponade effect, may be stable for the few hours of presentation and normally have better prognosis due to their early diagnosis (Rutherford, 1989).

In 15% to 20% of the cases, the AAA ruptures anteriorly and into the peritoneum. In such circumstances, given the amount of free embryological space in the anterior compartment of abdominal cavity, the extent of bleeding is so severe that patient goes into sudden syncope and loses consciousness. Due to the lack of tamponade effect and loss of entire circulatory volume, patients do not make it to the hospital (Brewster, 2003). In very rare scenarios (3-4%), the presence of AAA and constant chronic inflammation can cause erosion into the neighboring anatomical structures.

Amongst them, erosion to the inferior vena cava (aorto-caval) remains the most common of all. Given the containment of the circulatory volume (blood) in such circumstance, the size of the fistula (communication) between the aorta and inferior vena cava dictates the presenting symptoms. Abdominal bruit on auscultation remains the most common clinical finding but in extreme clinical situations, high output cardiac failure (dyspnea, tachycardia, wide pulse pressure, cyanosis) and lower limb edema or pulsation has also been reported. The Majority of aortocaval fistulas are detected during elective surgery (50-90%) and they rarely present in extrermis (Davis, 1998; Iriz, 2006).

Aorto-left renal fistula remains a rare identity and following its first description by Lord et al. and its repair by DeBakey (1958) only 26 reported cases have been identified in the literature. In the majority of cases, the AAA erodes to the left renal vein, which runs anteriorly (90%) and in rare anatomical variation posteriorly (1-4%). Almost all patients, present with abdominal pain, hematuria and silent left kidney syndrome and in rare scenarios, high output cardiac failure (Debakey, 1958).

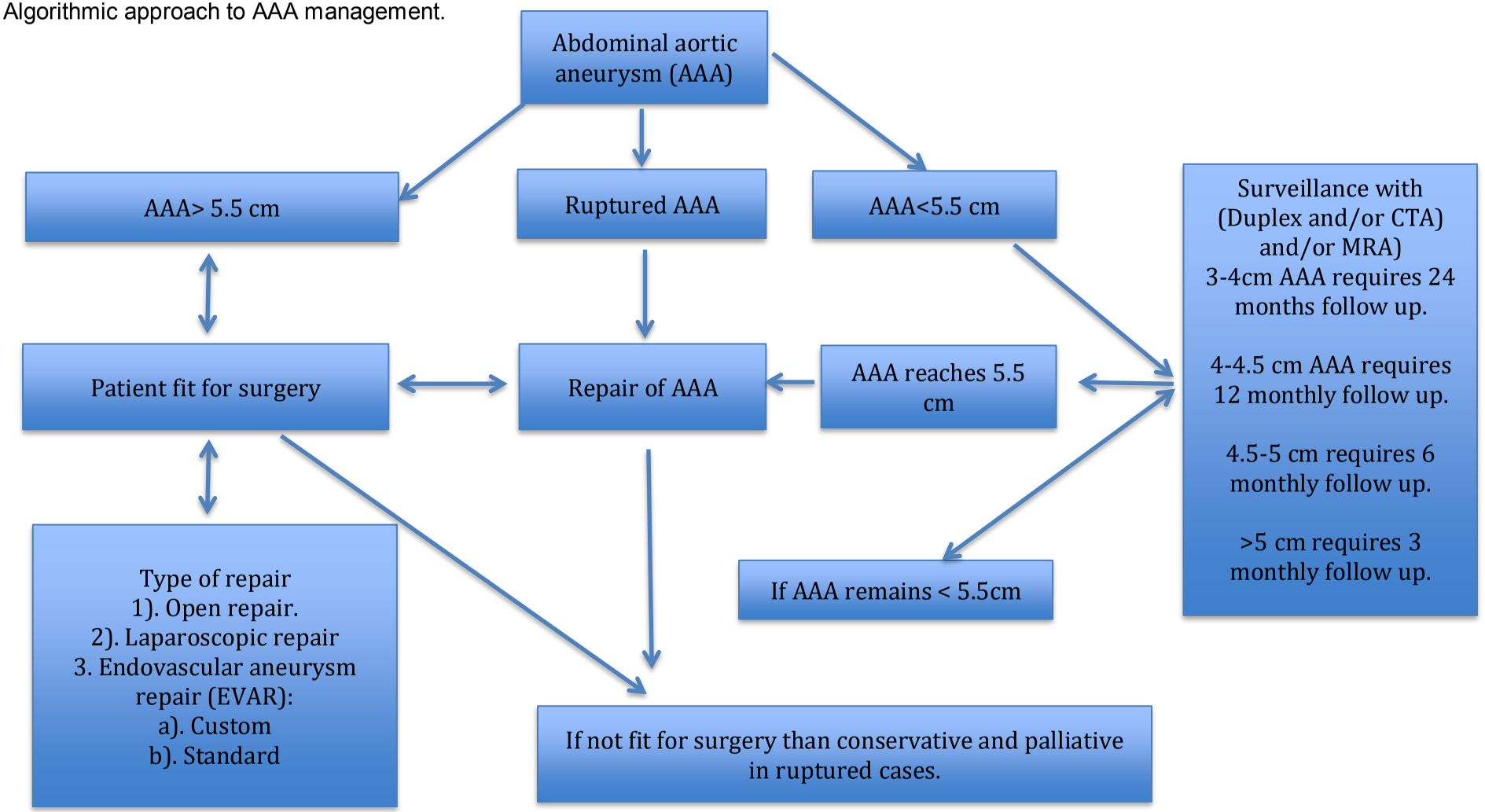
Due to the close proximity of third and fourth parts of duodenum to AAA, in extremely rare circumstances (0.5–2.3%) an aortoeneteric fistula is formed. This condition is fatal (95%) and very few patients make it to the hospital. The reported incidence of aortoeneteric fistula in general population as a result of AAA is 0.04–0.07% at autopsy (Assar, 2009).

In very rare clinical (4%) situations following posterior rupture of AAA, a large hematoma and its binding resistance due to peri-aortic tissue (tunica adventitia) can result in contained ruptured or sealed ruptured aneurysm. Some patients might escape detection for days and months due to their limiting clinical manifestation. This was first reported by Szilagyi (1961) and the majority of such patients presented with chronic pain and signs of compression (transient neuropathy). Such patients may rupture at any time and upon their detection, they should be prioritised like ruptured AAA cohort (Sazilagyi, 1961).

1.3 Surveillance & Screening

The role of a regular screening programme for AAAs has gained significant attention in the last decade. The rational is early detection of AAA in order is to avoid high mortality and morbidity from rupture. According to Cochrane review the odds ratio in favor of screening for male population in reduction of mortality was 0.60 (95%CI 0.47-0.78) (Cosford, 2007). This review recommends men aged between 65-79 will benefit significantly from routine screening for the detection of AAA. The method of screening is through ultrasonography and calculation of the aorta and/or AAA through Anterior-posterior measurement rather than transverse. The use of ultrasound is cheap and none invasive with no radiation and a minimal learning curve (Cosford, 2007). The Cochrane review did not report any reduction in mortality and morbidity associated with the use of routine screening for the detection of AAA in female population (OR 1.99; 95% CI 0.36 to 10.88) (Cosford, 2007). Once an abdominal aorta of >3 cm in size (termed as abdominal aortic aneurysm) is detected, the patient is than followed up by routine surveillance in specialized vascular and/or cardiovascular units. For AAA measuring 3-4 cm in size 12-24 monthly, for 4-4.5 cm AAA 12 monthly and above 4.6 cm 6 monthly surveillance is recommended (MASS, 2007) (Table 1.6).

Figure 1.1 Algorithmic approach to AAA management.



Preoperative Investigations

1.1 Imaging modality

Upon suspicion of ruptured AAA, the only treatment is the repair of abdominal aortic aneurysm. However, prior to surgery, if the patient is stable, the gold standard investigative modality of the choice is computed tomography angiography (CTA) (Fitzgerald, 1996). This modality demonstrates, the size, shape, site, extent and possible other variations or complications of the ruptured AAA with 95 % specificity and sensitivity. In addition, CTA can also assist the operating surgeon in minimizing the amount of dissection and define an appropriate surgical approach. In unstable individuals an ultrasound abdomen (Kuhn, 2000) can be performed to confirm diagnosis although the information obtained from this modality is not as precise and informative as CTA. This is mainly due to the patient's body habitus, bowel shadow and its intolerance by patients. In such circumstances, where time is of essence given the significant hemodynamic instability, no investigative modality should delay surgery (Table 1.6).

1.2 Other investigations

Once decision for surgery is made, all patients required detailed blood evaluation although these investigations should not delay surgery and must be parallel to final preparation for surgery. Full blood count (FBC), coagulation profile, renal function test, group and save and cross match

for blood and its component transfusion are of vital importance (Table 1.6).

Type of investigation	Information obtained
• Computed tomography angiogram (CTA).	**Size, Site, Shape, Anatomy, rupture site, defining surgical approach.**
• Ultrasonography (USS).	**Site, Size, Shape, Anatomy, No detail on site of rupture.**
• Full blood count (FBC).	**Degree of blood loss (Hemoglobin). Platelet count, Inflammatory markers.**
• Renal Function Test (RFTs).	**Electrolytes (Sodium & Potassium), Kidney function status (Urea, Cr).**
• Coagulation Profile.	**Activated Partial Thromboplastin Time (APTT), Prothrombin Time (PT) & international normalized ratio (INR).**
• Group, Save and cross match	**Type and rhesus status of patient blood for blood and blood component transfusion.**

Table 1.6 Investigative Modality & Their Relevance for rAAA.

Resuscitation Strategies

The management of a patient with rAAA (hemorrhagic shock) in the preoperative period plays a vital role in the final outcome of the patient. The ongoing loss in the circulatory volume (blood) causes compensatory and reduced perfusion to the vital organs. This explains why such patient present with syncope or loss of consciousness. In order to achieve adequate perfusion, the aim of resuscitation is to obtain a systolic blood

pressure of 100 mmHg. This is referred to as “permissive arterial hypotension” (Hamilton, 2014) and remains the main strategy in the primary resuscitation of all types of ruptured aneurysms in the body. In permissive hypotension, the objective is to gain adequate perfusion to all vital organs but not to increase the systolic pressure to a level that can result in further tearing of a rAAA and loss of present tamponade effect (Hamilton, 2014).

1.1 Traditional & Current Transfusion Strategies

The optimal transfusion strategy for rAAA has been subject to various debates in the last two decades. This debate has resulted in a significant shift in the transfusion protocols deployed in the resuscitation of hemorrhagic shock patients. The traditional resuscitation strategy was set to optimize the cardiac cycle and oxygen delivery system with active fluid resuscitation (non-blood components). In this process patients were massively transfused with colloid and crystalloid fluids intravenously. However, such protocols did not reduce the significant and ongoing mortality, therefore a new investigation into the best resuscitation protocol was initiated. The outcome of these investigations, demonstrated that patients with hemorrhagic shock do not die of inadequate cardiac cycle and oxygen delivery. The mortality was mainly due to ongoing hemorrhage secondary to coagulopathy disorders despite surgical management of the bleeding point (Duchesne, 2008).

The outcome of these investigations revealed that coagulopathy starts immediately at the time of the trauma and not what was originally believed to be several hours later. It has also been established that non-blood component (crystalloids/colloid) transfusion worsens the coagulopathy furthermore significantly increasing the mortality. In addition, recent research suggests that hemorrhagic shock activates anticoagulant protein C as a result of inadequate tissue perfusion and not from consumption of coagulation factors (Tieu, 2007). Hemorrhagic shock also results in hypothermia and subsequent acidosis. Therefore, the new resuscitation strategy is aimed at correction of the trauma triad of hypothermia, acidosis and coagulopathy with active blood and blood component transfusion (Brohi, 2003). However, these studies are not free of methodological limitations (e.g. survival bias, heterogeneity) and their application in the resuscitation of all types of haemorrhage, including those with ruptured abdominal aortic aneurysm, is not currently supported by the literature (Hunt, 2014).

Furthermore, individuals with ruptured abdominal aortic aneurysm (rAAA) have a number of demographical differences. Patients are much older, suffer with more comorbidities and are exposed to a different mechanism and type of volume depletion. In addition, aneurysmal degeneration of the aorta is a time dependent process (chronic) and indulges the coagulation cascade at various molecular and endothelial levels, which is different to the sudden impact of trauma and its implications (Kordzadeh, 2015).

Operative Methods

1.1 Open Ruptured Abdominal Aortic Aneurysm Repair

Upon diagnosis of ruptured abdominal aortic aneurysm, the only treatment is surgery. Open surgery remains the most common technique of repair worldwide, although this technique has been replaced by endovascular approach in many specialised units across Europe and USA. In open surgery the aorta after a midline laparotomy incision is exposed and an infra renal and/or a supra renal aortic clamp is applied. This is followed by exposure of the common iliac arteries and application of vascular clamps. The aneurysm is longitudinally exposed and opened through an arteriotomy incision. Depending on the size of the aorta and following the ligation of feeding lumbar arteries, a knitted Dacron graft is placed and sutured proximally and distally to the native aorta and common iliac vessels. The graft is than wrapped with the aneurysm sac and the abdomen is closed in layers (Moll, 2011).

1.2 Endovascular Ruptured Abdominal Aortic Aneurysm Repair

In this technique, patient does not require an open abdomen and the aorta and common iliac arteries are approached through bilateral common femoral arteriotomies. Upon successful cannulation of the common femoral arteries, an aortic occlusive balloon is introduced and the arterial flow is proximally inhibited by its inflation. Finally, a main body stent is introduced through the groin and the contralateral limb is deployed in the main endovascular stent from the ipsilateral common femoral artery. This

is followed by an on table angiography and closure of the vessels (Moll, 2007).

CHAPTER 2

Methodology of Thesis

2.1 Objectives

As highlighted in the resuscitation section of chapter one, the current massive transfusion protocol deployed for hemostatic resuscitation of hemorrhagic shock (derived from military and trauma cohort of patients) (Brohi, 2007; Henrikson, 2012) assumes that all patients with any type of hemorrhage are in coagulopathy. However, the search of literature does not demonstrate any evidence to support this protocol in ruptured abdominal aortic aneurysms. Therefore, the aim of this thesis is to ascertain for the first time:

1). What is the true coagulation status of infra-renal ruptured abdominal aortic aneurysms presenting to our unit?

2). What is the true coagulation status of infra-renal ruptured abdominal aortic aneurysms across the literature?

3). What is the clinical impact/s (mortality and morbidity) of current massive transfusion protocol in hemostatic resuscitation of infra-renal ruptured abdominal aortic aneurysms?

4). What is the best transfusion practice in such group of patients?

5). Are there any predictors (blood and blood components) of mortality and morbidity?

6). Does such practice have any effect on the overall correction of coagulopathy if any?

7). What is the best practice and probable future recommendations?

2.2 Ethical Approval

For the comprehensive, systematic review, Meta-analyses and/or Meta-aggregation of the literature, no ethical approval was required. In all original studies requiring patient data, ethical approval was obtained from Mid Essex Hospital services NHS Trust, Broomfield hospital (Audit and research committee) prior to the start of the thesis with clinical audit (CA) number: CA 13-226 on 08/01/2014 (Appendix 1).

All the studies were performed in accordance with the declaration of Helsinki without disclosing any patient identification. In all studies no individual was subjected to any new and/or alternative treatment/intervention and meticulous attention was paid to keep the data anonymous and in accordance with the data protection act.

2.3 Methods & Materials

Data on patients with ruptured abdominal aortic aneurysm presenting to Broomfield hospital, Mid Essex Hospital Services NHS Trust was obtained from four different local sources, to assure data precision by myself.

These were as following:

1). Patients records (notes).

2). Operative theatre records and data log.

3). Anesthetic data log

4). Blood transfusion services.

The data was retrospectively collated from the time of ethical approval (08/01/2014) to November 2007 (to the point of availability of the data) and prospectively to the end of June 2015.

This produced a total of ninety individuals (n=90). Complete data was available only for eighty-two (n=82) individuals. The lack of complete data for the remaining eight patients (n=8) was mainly due to inadequate data records from 2007 to 2008 and lack of centralization of anesthetic log and patient's records.

2.4 Data Collection

The following data (variables) were recorded into the excel sheet with pre-defined coding system for later statistical analysis:

1). Patient demographics (age, gender).

2). Patients comorbidities (chronic obstructive pulmonary disease, Smoking, Hypertension, history of previous coagulation disorder).

3). American society of anesthesiology score (ASA).

4). Initial emergency services presenting hematological markers (Hemoglobin, Neutrophil, Lymphocyte, Neutrophil lymphocyte ration (NLR).

5). Initial emergency department presenting coagulation status (Platelet count, international normalized ratio (INR), Prothrombin time (PT) and activated partial thromboplastin time (aPTT).

6). Operative data (type of operation, method of operation, cross clamp time, aneurysm size, type of conduit (prosthetic graft), suture material and size, blood loss)

7). Total Blood component transfusion on each individual (Platelet pool, Packed red blood cell, fresh frozen plasma).

8). Post transfusion coagulation status (Platelet count, international normalized ratio (INR), Prothrombin time (PT) and activated partial thromboplastin time (aPTT).

9). Duration of intensive care unit and hospital stay (in days), mortality and morbidity.

2.5. Data Inclusion

In order to increase the internal, external validity and robustness of each individual study the following inclusion were applied:

1). Data was extracted only on ruptured infra-renal abdominal aortic aneurysm cohort.

2). No mixed etiology was included (other ruptured aneurysms and/or in conjunction with).

3). Complete availability of all data (variables) on each individual as highlighted in section 2.4 of this manuscript.

4). All patients had confirmed ruptured abdominal aortic aneurysm on computed tomography angiography (CTA) and no tender aneurysm (non-ruptured but painful) was included.

2.6 Definitions

In order to avoid heterogeneity, create a uniform and replicable approach, all terminologies in this thesis were defined according to their respective and acceptable international definitions as follow:

1). Ruptured abdominal aortic aneurysm (rAAA) was defined as any infra renal abdominal aortic aneurysm that has lost its wall integrity (breech of the vessel) resulting in extravasation of the contrast and/or blood outside the aortic wall based on CTA and clinical findings.

2). All comorbidities were defined in accordance to definitions provided by world health organization (WHO).

3). Coagulopathy was defined Coagulopathy was defined as a condition in which clot formation was impaired. Therefore, patients with aPTT >40 seconds and/or international normalized ratio of >1.2 and/or platelet count of <150 $10/L^9$ were deemed coagulopathic in the broader terminology. In addition, the following definitions were also applied (Greuters, 2011; Hunt, 2014):

- Mild coagulopathy was defined as APTT >40 seconds and/or international normalized ratio between 1.2 and 1.5 and/or platelet count of 100 $\times 10/l^9$-150 $\times 10/l^9$.
- b. Significant coagulopathy was defined as APTT >40 seconds and/or international normalized ratio of more than 1.5 and/or platelet of < 100 $\times 10/l^9$.

4). Disseminated intravascular coagulation is an acquired syndrome defined by the intravascular activation of the coagulation cascade with

loss of localization arising from different causes.

5). No robust definition of contained versus free rupture of abdominal aortic aneurysm could be identified in the literature.

6). The study end point was set at 30-days for all outcomes of mortality and morbidity.

7). Morbidity was defined according Clavien-Dindo classification ≥ 3 (Dindo, 2014) (considered a major complication that requires medical and/or surgical intervention).

8).Morbidity was also recorded in thrombotic and non-thrombotic complications (hyper and/or hypo-coagulate state resulting in arterial thrombosis).

9). Mortality was defined as death from any cause within the duration of hospital stay or at 30 days.

10). All hematological values were recorded in accordance to their international and national accepted ranges and values.

11). All blood and blood component transfusions were recorded in units.

12). Time duration was calculated in minutes and days for all variables.

2.7 Coding & Statistical Analysis

After completion of the data collection by myself, all data was fed into an excel sheet (Microsoft office) and coded by myself in accordance with the

requirement of statistical package for the social sciences (SPSS) IBM version 20.0 (Chicago, IL). A different type of statistical analysis for each study was conducted and details of each are highlighted in each chapter of this thesis, however certain standards were set for all study design.

Each study design was set on the background of null hypothesis and this was put into detailed assessment. All scale variables were categorized according to their median values. All variables that had previously defined normal ranges (hemoglobin, platelet count, International normalized ratio (INR) Prothrombin time (PT) and activated partial thromboplastin time (aPTT), Neutrophil, Lymphocyte) (section 2.6 definitions) were categorized to three different groups of normal, low (hypo) and high (hyper) Levels. The best cut of value at highest sensitivity and specificity at 95 % confidence interval for values with no prior range and international value was acquired through area under curve (AUC). Time was calculated in minutes and hours and used in a uniform way throughout the entire thesis.

Test of probability was considered significant at *p* value of <0.05 at 95% confidence intervals. Logistic regression analysis (binary) was used to determine the association and/or impact of each variable to that of predefined outcomes (e.g. mortality and morbidity). Those variables that demonstrated a significance with *p* value <0.1 at univariate logistic regression were then entered to the multivariate logistic regression to assess and evaluate their independence. Other test of statistics deployed in this thesis, were Kruskal- Wallis, Chi-square and Student t-test for

assessments of differences across the outcome groups and/or variables. The agreement between two independent assessments of ruptured abdominal aortic aneurysm measurements (surgeon and radiologist) was carried out using Cohen's k (kappa) coefficient. The detail of each statistical analysis is highlighted in detail in each individual chapter.

2.8 Systematic review & Meta-Analysis

Systematic reviews and meta-analyses were performed using methodology as described by the Cochrane Collaborative (http://www.york.ac.uk/crd/guidance/). Reporting of systematic reviews and meta-analyses were carried out using the validated PRISMA (Preferred Reporting Items for Systematic Reviews & Meta-Analyses) (Appendix 2).

2.9 Literature Search

An electronic search of literature was carried out using appropriate keywords and/or medical subject headings (MeSH) headings through Medline, CINHAL, Scopus, Embase and Cochrane library database. All abstracts were retrieved and reviewed. Studies that appeared to fulfil the eligibility criteria but had insufficient information in the abstracts were also retrieved and examined in full. References within retrieved articles were manually searched for further potential studies.

2.10 Quality Assessment

All articles were assessed for their validity, bias, applicability, and

inference using a critical appraisal tool provided by the Oxford Critical Appraisal Skills Programme (CASP) (Appendix 3). The strength of evidence and their recommendation for future practice was also assessed through the National Institute for Health and Care Excellence (NICE) checklist (Appendix 4).

2.11 Statistical Analysis in Systematic Review

The pooled analysis for systematic review was conducted by calculation of the mean value in addition to pooled prevalence of coagulopathies, and 95% CI were estimated with a variance weighted random effects model. This was done using MedCalc statistical Software version 16.1.

2.12 Methodological & statistical support

The entire statistical analysis throughout this thesis was performed by myself and reassessed by my clinical supervisor Mr. Yiannis Panayiotopoulos whom has published extensively within the field of vascular surgery and has been a supervisor of various PhDs in his respective field with authorship of a few surgical books. Each individual methodology was evaluated and examined in detail for their robustness by both respected supervisors prior to their publication and peer review in respected medical journals.

CHAPTER 3

Baseline Coagulation of Ruptured Abdominal Aortic Aneurysms and Their Impact on 30-Day Mortality and Morbidity: A Single Center Cohort Study.

3.1 Introduction

For decades, the high mortality of rAAAs has been attributed to hemorrhage secondary to coagulopathy. In recent years, there has been a decline in the mortality incidence of rAAAs. In the early resuscitation phase and context, this decline has been attributed to the better understanding and correction of the trauma triad (hypothermia, acidosis and coagulopathy) and the advent of new transfusion protocols (Brohi, 2007; Henriksson, 2012). Despite such suggestions, emerging evidence speculates that unlike traumatic hemorrhagic and subsequent coagulopathy, patient with rAAA possess a normal or an activated coagulation (Fransson, 2012). Furthermore, active and liberal hemostatic resuscitation of rAAA patients with fresh frozen plasma and platelet pool (other than packed red blood cells) to correct the initial suspected and assumed coagulopathy can contribute to mortality and/or thrombotic complications (Fransson, 2012). Hence, it is vital to establish whether such patients do present with coagulopathy in the first instance and if so, do they require coagulation correction or a minimal transfusion strategy suffice. The aim of this study is to establish the baseline coagulation and hematological markers of individuals presenting with rAAA to the ED and with a secondary aim to assess their impact on the 30-day mortality and morbidity.

3.2 Material & Methods

A retrospective study of n=101 consecutive patients with suspected infra renal ruptured abdominal aortic aneurysm (rAAA) from November 2007 to June 2015 that presented to the emergency department was conducted. Five patients (n=5) were deemed to be for palliation and another five (n=5) did not have any preoperative blood assessment. Eight patients (n=8) had a mixed picture of abdominal and/or iliac and/or supra renal and/or tender aneurysm and were excluded from the study. The remaining individuals (n=82) had confirmed clinical and radiological infra-renal AAA rupture. The Mid Essex Hospital services NHS Trust, has estimated population coverage of 380,000 people. Data on patient demographics (age and gender), comorbidities (Hypertension (HTN), Chronic obstructive pulmonary disease (COPD), Active smoking,) coagulation profile (Prothrombin time (PT), Activated partial thromboplastin time (aPTT), International normalized ratio (INR), Haemoglobin (Hb), hematocrit (Ht) and platelet count (Plt) were collected. Due to a lack of any new or alternative interventions ethical approval was granted from the local hospital with audit number CA13-226.

Parameters:

The computed tomography angiography (CTA) of the entire cohort were assessed by two separate investigators (interventional vascular radiologist & vascular surgeon) and the aneurysm size was calculated and presented in millimetres. The maximum diameter was taken as the true size of the

AAA. The agreement level in size measurement was assessed using Cohen's Kappa Coefficient (κ=0.82). No patient demonstrated loss of consciousness prior to surgery and the Glasgow Coma Scale (GCS) of all patients were 15/15. No patient exhibited hypothermia upon arrival to the emergency department. None of the patients were on any local (aneurysm) screening programme or had any known coagulopathy and/or haematological disorders. Eighty individuals (n=80) were already on Aspirin 75 mgs upon arrival to the emergency department and none were on any other antiplatelet or anticoagulants. All patients had an open repair of the ruptured abdominal aortic aneurysm with straight knitted Dacron graft, as endovascular option is not routinely available. All patients received 5000 IU of heparin upon application of the aortic clamp (infra-renal).

Definitions

1. The study endpoint was set at 30 days for all outcomes of mortality. The mortality was defined as death from any cause.

2. Coagulopathy was defined as international normalisation ratio (INR)> 1.2 (normal value: 0.9 to 1.2) and platelet count of less than 150×10^9/l. (Normal value: 150×10^9/l to 400150×10^9/l) (Hunt, 2104).

3. Significant coagulopathy was defined as Platelet count of < 100×10^9/l and INR > 1.5 (Hunt, 2014).

4. Thrombosis was defined as complications arising from thromboembolic phenomenon resulting in blockage of the corresponding vessel (Hunt, 2014)

5. Morbidity was defined according to the Clavien-Dindo (2013) classification ≥3 (considered a major complication).

Statistical Analysis

Statistical analyses were undertaken using IBM Statistical Package for the Social Sciences (SPSS) version 20.0. Scale variables such as age, haemoglobin (Hb), platelet count (Plt), haematocrit (Ht), prothrombin time (PT), Activated partial thromboplastin time (aPTT) and international normalisation (INR) were categorised according to their respective median values. Differences between the two groups (normal coagulation versus coagulopathy) were determined using Pearson Chi-Squared tests of a difference. A p value of 0.05 or less was deemed to be statistically significant.

3.3 Results

The median age of the cohort was 75 years (range, 51-92) with male predominance (male n=66, 85% vs. female n=16, 19.5%, Table 1). The median size of rAAA was 75.52 mm (range, 40-100). Pre-existing hypertension was found in 60.9%(n=50), chronic obstructive pulmonary disease (COPD) in 28 %(n=23), active smokers in 23% (n=19) and acute renal failure was noted in three patients (3.9%). The median presenting value for Hb was 11.3 mg/dl (Interquartile Range (IQR), 9.45-13.3,) whilst the median Hematocrit was 34.0% (IQR, 28.25-39.0%) and the platelet count was 176×10^9/l (IQR, 122×10^9/l -210×10^9/l). The median value for PT

was 13.1 (IQR, 10.1-35, mean= 14.79), for APTT was 29.55 (IQR 20.4-240, mean=34.7) and for INR was 1.12 (IQR, 0.9-2.91, mean= 1.25) (Table 3.1) (Table 3.2).

Coagulation & Mortality

According to the defined parameters only 17.1% (n=14/82 vs n=68/82) ($p<0.001$) of the entire cohort was found to have coagulopathy (INR>1.2 and PLT< 150×10^9/l). Only one patient was found to have significant coagulopathy (INR>1.5 and PLT <100×10^9/l). Overall, no patient expired from hemorrhage

		Non Coagulopathic		Mild Coagulopathy		*p*=
		n=	%	n=	%	
Gender	Male	54	79.4%	12	85.7%	0.588
	Female	14	20.6%	2	14.3%	
Age	18-75 years	37	54.4%	5	35.7%	0.202
	>75 years	31	45.6%	9	64.3%	
ASA Grade	2	15	22.1%	2	14.3%	0.038
	3	25	36.8%	2	14.3%	
	4	24	35.3%	6	42.9%	
	5	4	5.9%	4	28.6%	
Hypertension	No Hypertension	25	36.8%	7	50.0%	0.355
	Hypertensive	43	63.2%	7	50.0%	
COPD	No COPD	46	67.6%	13	92.9%	0.056
	COPD	22	32.4%	1	7.1%	
Smoking Status	Non-Smoker	51	75.0%	12	85.7%	0.387
	Smoker	17	25.0%	2	14.3%	
Hemoglobin Pre-Op	Up to 11.5g/l	35	51.5%	9	64.3%	0.381
	>11.5g/l	33	48.5%	5	35.7%	

Table 3.1 Patient demographics in the normal & coagulopathy group.

secondary to coagulopathy and the overall mortality incidence was 14.6% (n=12/82), with significant male predominance (n=10/12). The Chi-Square test of difference amongst the two groups (normal versus coagulopathy) showed no statistically significant difference in demographics, comorbidities and the presenting hemoglobin. There was also no statistically significant difference (*p=0.10*) in mortality outcomes associated with both groups at 30-days (Table 3.2).

		Number	Percentage
Variables	**Parameters**		
Age	18-75 years	42	51.2%
	>75 years	40	48.8%
Gender	Male	66	80.5%
	Female	16	19.5%
Hb	Up to 11.5g/l	44	53.7%
	>11.5g/l	38	46.3%
Haematocrit	Up to 0.3500	45	54.9%
	0.35	37	45.1%
APTT	Up to 30.00	47	57.3%
	>30.00	35	42.7%
PT	Up to 13.000	37	45.1%
	>13.000	45	54.9%

Table 3.2 Hematological markers.

Coagulation & Morbidity

A total of 22 patients (26.8% of the entire population) experienced morbidity within 30-days post rAAA repair. Postoperative thrombotic events (thromboembolic phenomenon) occurred in 21.9% (n=18/82) of the entire

cohort. Those that lead to mortality were 8.3% (n=7/82) contributing to 58.5% of the overall mortality (n=7/12). They were due to bowel (n=4) and limb ischemia leading to irreversible acidosis (n=3). Other thrombotic events were ischemic stroke, Ischemic myocardial infarction, and renal artery thrombosis. The overall 30-day thrombotic events in both groups (Chi-Square) (normal versus coagulopathy) showed no statistically significant difference (p=0.51). Furthermore, thrombosis led mortality could not achieve a statistically significant difference in both groups (p=0.67) (Table 3.3 & 3.4).

		Non Coagulopathic		**Mild Coagulopathy**		**p=**
		n=	%	n=	%	
Thrombosis (30-Day)	No Thrombosis	54	79.4%	10	71.4%	0.511
	Thrombotic event	14	20.6%	4	28.6%	
Mortality (30-day)	Alive	60	88.2%	10	71.4%	0.105
	Deceased	8	11.8%	4	28.6%	

Table 3.3 30-Day Thrombosis & Mortality Events Post rAAA Repair.

3.4 Discussion

The outcome of this study demonstrates that individuals with rAAAs do not commonly present with coagulopathy to the ED. It appears that a very limited number of individuals (n=14, 17.1%) (INR>1.2 and PLT< 150×10^9/l)

show signs of coagulopathy and this may not necessarily require any active correction. My finding seems to complement the work of Reed and colleague, where no coagulopathy in rAAAs on their initial presentation to the ED was detected (Reed, 2013).

In recent years, there has been a significant emphasis on early haemostatic resuscitation and coagulation correction of haemorrhagic shock. These suggestions and /or guidelines are mainly from research, derived from military and civilian trauma (Graham, 2012). In fact, no evidence in the literature could support the active and liberal use of fresh frozen plasma and platelet pool in the early resuscitation phase of rAAAs. Furthermore, emerging evidence suggests that patients with rAAA may be in a pro-coagulant state and might not have the same pathophysiological alterations that are witnessed in haemorrhagic shock following trauma (Mantovani, 1997).

		Non Coagulopathic		**Mild Coagulopathy**		
		n=	%	n=	%	p=
Thrombosis (30-Day)	No Thrombosis	3	37.5%	2	50.0%	0.679
	Thrombotic event	5	62.5%	2	50.0%	

Table 3.4 Rates of Thrombotic Events in Patients Who Died Within 30-Days Of rAAA Repair.

The first major difference (rAAAs vs Trauma) is related to the time dependent and chronic process of aneurysm formation. During this stage,

injury to the endothelium acts as a nidus for the adhesion, migration of platelets and the generation of fibrin. The release of von Willbrand Factor (vWF) and other hematological factors over months and/or years promote the activation of tissue factor (TF) that generates thrombin formation through factors X and VII (Edington, 1991). In addition, the size of mural thrombus exhibits a direct (positive) correlation to the aneurysm size and its anatomical tortuosity (Yamazumi, 1998; Adam, 1999), thus, indicating an ongoing active coagulation cascade. Furthermore, in a study by Adam et al. even non-ruptured AAAs exhibited significant coagulation differences to that of rAAAs. The authors demonstrated that AAA individuals are more pro-coagulant once they rupture and such a pro-coagulant state persists throughout the duration of the operation and resolves in the postoperative period (≥ 24 hours) (Roberts, 2006).

Secondly, the initial resuscitation strategy commonly adopted in rAAA patients is “permissive hypotension” (Illig, 1997). Such hypotension may well result in hypo-perfusion and early coagulopathy, but unlike trauma patients, evidence in the rAAA cohort demonstrates no significant relationship between hypotension and/or hypo-perfusion, the degree of coagulopathy and fibrinolytic parameters. In addition, there are suggestions that the elevation of fibrinolytic factors is related to the application of supra-celiac cross clamp during surgery and not on the initial presentation of the patients to the ED (Packman, 1994; Gertler, 1996).

In this study, thrombotic complications contributed to more than half of the 30-day mortalities (n=7/12, 58%). Platelets have a significant and potent role in thrombosis. Their adhesion to sub-endothelium of the vasculature

forms thromboxane A2 and results in the secretion of platelet granules accelerating platelet aggregation and thrombin generation (Sakgius, 2007). The study by Skagius et al. (2008) demonstrated that rAAAs possess a higher thrombin-antithrombin, prothrombin fragment, von Wilbrand factor antigen and D-dimers in comparison to non-ruptured aneurysm, indicating an active coagulation cascade (Tamyraja, 2008). Therefore, liberal and proactive haemostatic resuscitation (platelet pool and Fresh frozen plasma) could increase thrombotic complications even further.

The outcome of this study demonstrates that the application of the new haemostatic resuscitation (strategies) derived from military and civilian trauma such as the active transfusion of platelet pool and fresh frozen plasma in addition to the required red blood cells in the ED might not be necessarily applicable to the rAAAs. This is based on the evidence that rAAAs do not present with coagulopathy and/or hypothermia noticed in the trauma individuals. In addition, hypotension in rAAAs does not necessarily result in hypo perfusion. Therefore, refinement of transfusion protocols in light of emerging evidence for the active resuscitation of rAAAs is highly advocated. This study does not challenge the transfusion of red blood cells in individuals with haemorrhagic shock secondary to rAAAs but does call into question the active transfusion of other products such as platelet and fresh frozen plasma in the early resuscitation phase.

This study inherits the weakness of being retrospective in nature and a higher number of individuals in a randomized controlled trial and/or comparative study would have been optimal. However, given the nature of

rAAAs, their associated high morbidity and mortality, such methodology might not be feasible and current practice may have to rely on observational studies. Furthermore, the assessment of other variables or the deployment of various scoring systems such as Glasgow Aneurysm Score (GAS), Hardman Index (HI), Edinburgh ruptured Aneurysm Score (ERAS) and Physiological and Operative Severity Score for the enUmeration of Mortality and Morbidity (POSSUM) have failed to predict survival in rAAAs and cannot be used as reliable markers (Gatt, 2009).

3.5 Conclusion

Patients with ruptured abdominal aortic aneurysms do not present to the ED with coagulopathy and if they do, the coagulopathy is often mild and does not require correction. The presence or the lack of coagulopathy does not have any impact on thrombotic induced morbidity or peri-operative (30-day) mortality. Ruptured abdominal aortic aneurysms exhibit different baseline coagulation to those of trauma and further research in this area is advocated. In next chapter, the finding from our unit will be assessed and evaluated in contrast to what is known in the literature with regards to the coagulation status of ruptured abdominal aortic aneurysms.

CHAPTER 4

Presenting Baseline Coagulation of Infra Renal Ruptured Abdominal Aortic Aneurysm: A Systematic Review and Pooled Analysis.

4.1 Introduction

The lack of high quality evidence regarding the coagulation status of patients with rAAAs has led to speculations, reliance on anecdotal experience and suggestions about their appropriate haemostatic resuscitation. Furthermore, individuals with ruptured abdominal aortic aneurysm (rAAA) have a number of demographical differences. Patients are much older, suffer with more comorbidities and are exposed to a different mechanism and type of volume depletion. In addition, aneurysmal degeneration of the aorta is a time dependent process (chronic) and indulges the coagulation cascade at various molecular and endothelial levels, which is variant to the sudden impact of trauma and its implications (Kordzadeh, 2015).

In order to optimise patient care, diminish disparities and objectify the current protocols, the first systematic review of the literature was performed to establish the baseline coagulation status of infra renal ruptured abdominal aortic aneurysms. In this review I did not settle with the term “coagulopathy” alone and investigated their subtypes in detail (mild versus significant) against the defined standards and definitions provided by the literature.

4.2 Material & Methods:

Search Strategy

A systematic search of the literature from 1966 to July 2015 was conducted in Medline, CINHAL, Scopus Embase and Cochrane library. The following keywords and/or medical subject headings (MeSH) were used: “ruptured abdominal aortic aneurysm” and/or “ruptured infra renal abdominal aortic aneurysm” and/or “coagulation” and/or “coagulopathy”. The search was performed in accordance to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher, 2009). Bibliographies of the retrieved articles were also manually checked for any additional studies not identified in the primary search. All abstract was retrieved and reviewed. Studies that appeared to fulfil the eligibility criteria but had insufficient information in the abstract were also retrieved and examined in full.

Results

The electronic search returned a total of 71 studies (Figure 4.1) (Moher, 2009). A further manual search revealed 2 more articles. Of the total 73 studies, 12 articles were found to be duplicates leading to 61 articles in total. The abstracts of these articles were screened and 30 studies were further (language restriction n=7 and non-relevant n=23) excluded. The full texts of 31 studies were assessed for eligibility and of these 24 were excluded against the inclusion criterion. A total of seven studies (Table 4.1) were included in this systematic review.

Exclusion Criteria

The following articles were excluded:

1). Non-human and non-adult studies.

2). Abstracts and conference proceedings.

3). Case reports, narrative reviews, commentary and opinions.

4). Other studies that did not report the outcomes of coagulation/coagulopathy or investigated coagulopathy only post rAAA repair.

5). Articles that were not in English language.

Quality Assessment & statistical analysis

In order to achieve an informed conclusion and an evidence base approach the included articles were assessed for their validity, bias, applicability and inference through critical appraisal tool provided by Oxford critical appraisal skills Programme (CASP, 2015). Due to lack of consistency and uniformity of the provided data, as well as heterogeneity in the population across the studies, a meaningful statistical analysis (meta-analysis) was not deemed plausible. However, the data was presented in a tabulated format and a pooled analysis was performed. Data extraction included the type of the studies, number of recruited individuals, coagulation factors, outcome, and conclusion. The strength of evidence and their recommendation for future practice was also assessed through National Institute for health and Care Excellence (NICE) checklist (Weightman, 2015). In addition, recommendations for future research were formulated based on the current systematic review outcome.

Standards & Definitions

In order to avoid heterogeneity and create a uniform approach, all terms were defined according to their respective and acceptable definitions (Greuters, 2011; Hunt, 2014).

1. Coagulopathy was defined as a condition in which clot formation was impaired. Therefore, patients with aPTT > 40 seconds and/or international normalized ratio of more than 1.2 and/or platelet count of < 150 ×10/l^{9} were deemed coagluopathic in the broader terminology. In addition, the following definitions were also applied:

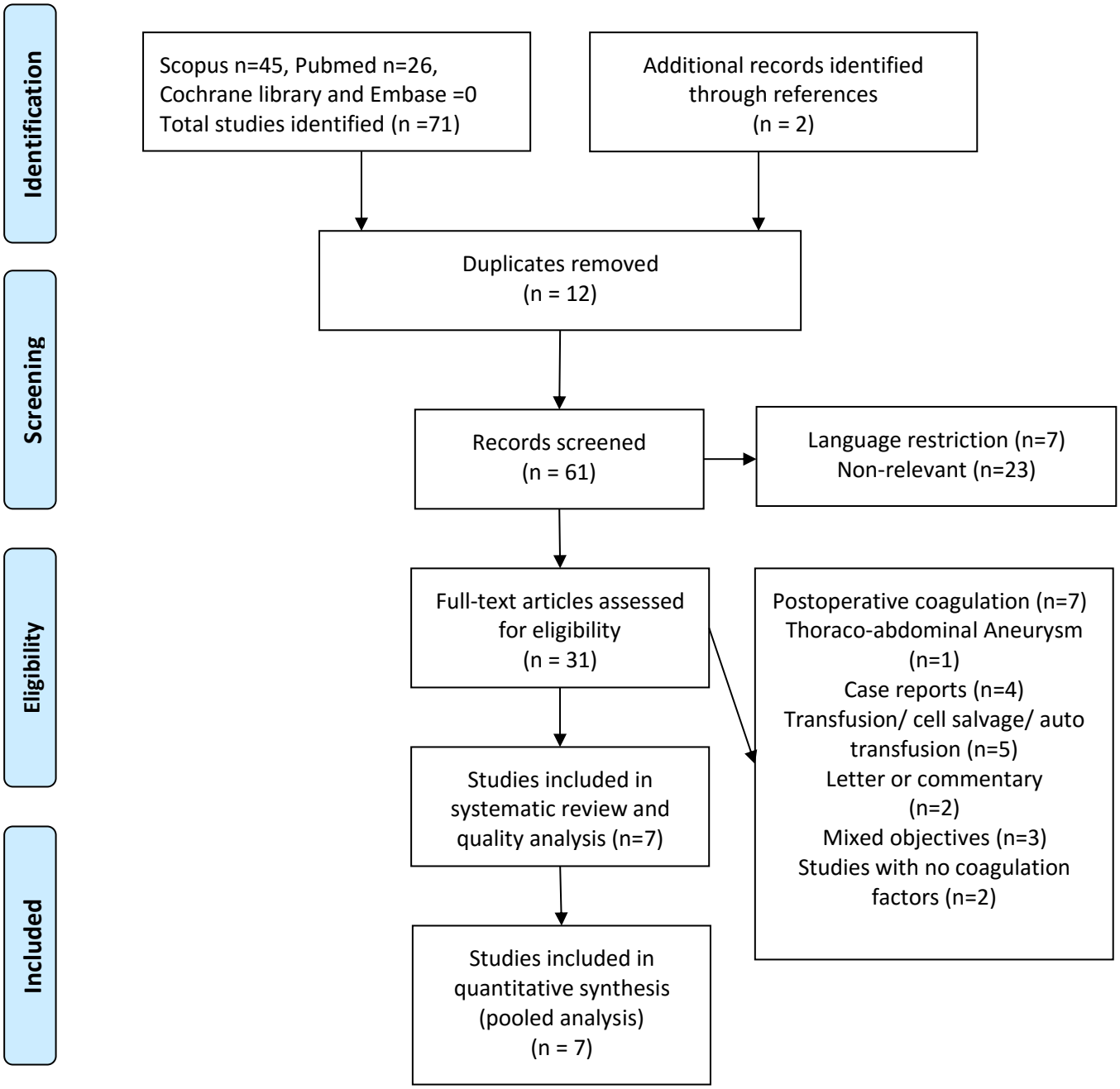
    a. Mild coagulopathy was defined as APTT > 40 seconds and/or international normalized ratio between 1.2-1.5 and/or platelet count of 100×10/l ^{9}-150 ×10/l ^{9}.
    b. Significant coagulopathy was defined as APTT > 40 seconds and/or international normalized ratio of more than 1.5 and/or platelet count of < 100 ×10/l ^{9}.

2. Disseminated intravascular coagulation (DIC) was defined as an acquired syndrome defined by the intravascular activation of the coagulation cascade with loss of localization arising from different causes.

	Study/ Population	Assessed Factors	Outcome	Conclusion
Montan et al. 2015.	Retrospective Cohort. N= 91	1.Hemoglobin. 2.Platelet count. 3.International normalization ratio. 4.Activated partial thromboplastin time. 5.Fibrinogen. 6.Prothrombin ratio.	• Platelet & Fibrinogen show linear relationship to blood pressure (70mmHg). • Hypotension results in low Fibrinogen Level (<1.5) (p=0.001). • Low Fibrinogen Level is associated with 10 fold increase in intraoperative bleed > 2 liters (p=0.009).	No significant coagulopathy. Shock results in coagulopathy.
Reed et al. 2013.	Retrospective cohort. N= 119	1.Hemoglobin. 2.Platelet count. 3.International normalization ratio. 4.Activated partial thromboplastin time. 5.prothrombin time.	• 14.3% had INR ≥1.5. • 9.2 % had platelet < 100 $\times 10^9$/l. • APTT (p=0.353) was not significant. • PT (p =0.25) was not significant.	No significant coagulopathy.
Fransson et al. 2012.	Retrospective cohort. N=55	1.Platelet count. 2.International normalization ratio. 3.Activated partial thromboplastin time. 4.Acidosis ≥ 6mmol.	• 12.7 % had INR ≥1.5. • N=2 had DIC. • High incidences of thrombotic mortality.	No significant coagulopathy/ activated coagulation.
Fujita et al. 2012.	Retrospective cohort. N=64	1.Hemoglobin. 2.Platelet count. 3.International normalization ratio. 4.Activated partial thromboplastin time. 5.Fibrinogen. 6.D-dimmers.	• Difference in platelet, aPPT & Fibrinogen in survivors versus non- survivors as a consequence of shock. • Coagulopathy noted in shocked individuals only. • D-Dimmer is high in rAAA.	Limited coagulopathy. Shock results in coagulopathy.
Skagius et al. 2008.	Comparative Study N=55	1.Hematocrit. 2.Platelet count. 3. International normalized ratio. 4.Activated partial thromboplastin time. 5. D-dimers	• Higher platelet count and lower aPTT in ruptured group. • D-dimmer is high in ruptures.	Activated coagulation in ruptures.
Davies et al. 1993.	Prospective Cohort. N=50	1.Platelet count 2. International normalized ratio.	• 40 % showed coagulopathy • Platelet count << 100 $\times 10^9$/l and/or INR> 1.5 is associated with mortality.	Degree of coagulopathy.
Getaz et al. 1977.	Retrospective Cohort. N=27	1.Platelet count. 2. International normalized ratio. 3. Fibrinogen.	• Low platelet <100$\times 10^9$/l (n=5). • Coagulopathy noted in shocked patients.	Shock associated coagulopathy/ 56% coagulopathic.

Table 4.1 Information obtained from the recruited studies (n=7) referring to type, size, coagulation factors and outcome.

Figure 4.1 PRISMA-Flow Chart



The total population across these seven studies was 461 patients. These patients all presented with rAAA and were subjected to coagulation assessment of: 1). Platelet count. 2). International normalized ratio (INR). 3). Activated partial thromboplastin time (aPTT). 4). Fibrinogen and 5). D-dimer or fibrin degradation product (FDP) prior to surgery to establish their coagulation baseline (coagulopathy versus none). This included six (n=6) retrospective cohort and one comparative study (n=1). No randomized clinical trial (RCT), systematic review and/or meta-analysis were identified.

Study Quality

Of the seven included studies, three studies scored maximum marks (8/8) according to the CASP checklist (Skagius, 2008; Fransson, 2012; Montan, 2015). Two studies scored 7/8 (Davies, 1993; Fujita, 2012) and the remaining two studies (Getaz, 1997; Reed, 2013) scored 6/8 (Table 4.2). The strength and grade of each study were also measured against (NICE) checklist.

Normal Coagulation vs. Coagulopathy

All of the seven studies (n=7) with 461 patients had assessed the range of platelet count in detail. Pooled analyses of these studies demonstrated 86.3% (n=398) of the population to have a platelet count of >150×10/l ^{9}. Only 12.1%(n=56) had a platelet count of <150×10/l ^{9}. Data on the platelet counts of seven patients were unavailable (n=7/461). Data on coagulation status using international normalized ratio (INR) was available on 363 patients.

Within this group, 84.8% (n=308/363) had an INR <1.2 whilst 15.1% (n=55/363) had INR>1.2. The activated partial thromboplastin time (aPTT) was available and measured in 396 patients. The aPTT was less than 40 second in 86.2% (n=340/396) of the cohort and more than 40 seconds 13.7% (n=54) (Table 3) (Figure 4.2).

Investigator/Year	Clear aim	Recruitment Bias	Exposure Bias	Outcome Measurement	Confounding Factors	Follow-up	Results	Applicability	Total Score	Level Of Evidence
Montan et al. 2015.[12]	Yes	No	No	Clear	Considered	Yes	Clear	Yes	8/8	2
Reed et al. 2013.[32]	Yes	No	Yes	Clear	Maybe	Yes	Clear	Yes	6/8	3
Fransson et al.[33] **2012.**	Yes	No	No	Clear	Considered	Yes	Clear	Yes	8/8	2
Fujita et al. 2012.[34]	Yes	No	No	Clear	Maybe	Yes	Clear	Yes	7/8	2/3
Skagius et al. 2008.[35]	Yes	No	No	Clear	Considered	Yes	Clear	Yes	8/8	2
Davies et al. 1993.[36]	Yes	No	No	Clear	Considered	Yes	Clear	Yes	7/8	2/3
Getaz et al. 1977.[37]	Yes	No	Yes	Clear	Maybe	Yes	Clear	Yes	6/8	3

Table 4.2 Quality assessment of (n=7) by critical appraisal tool provided by Oxford Critical Appraisal Skills Programme (CASP).

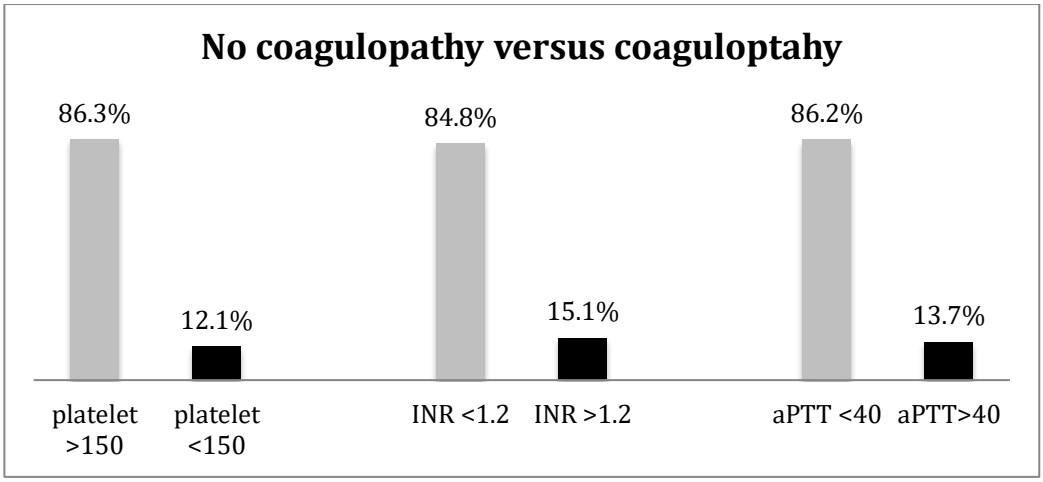


Figure 4.2 Chart demonstrating the comparison between two groups of patients with no coagulopathy versus coagulopathy

Mild Versus Significant Coagulopathy

Sub-group analysis was performed to establish the severity of the coagulopathy. Further examination of 56 patients (12.1% of the overall population) with the coagulopathy, demonstrated that more than half of the individuals (n=31/56, 6.69%) had a platelet count range between 100-150×10/l^{9}. The remaining 25 patients (n=25/56, 5.4%) had a platelet count of less than 100×10/l^{9}**.** Amongst 55 individuals (15.1%) that had INR >1.2, 6 % (n=22/55) had an INR range between1.2 to 1.5. The remaining 9% (n=33/55) had an INR> 1.5. The activated partial thromboplastin time was more than 40 seconds in 13.7% (n=54) of the entire cohort (n= 396) (Table 4.4) (Figure 4.3).

Fibrinogen, D-Dimer & DIC

Only three studies (n=3) with population of 165 patients, reported the serum levels of Fibrinogen (normal range, 150-400 mg/dL). Four individuals (2.4%) reportedly had a high levels of fibrinogen > 400md/dL and one individual had (0.6%) fibrinogen of less than 150 md/dL. Overall 97% of patients had normal

Fibrinogen levels. The D-dimer or fibrin degradation product (FDP) was reported in two articles with population of 119 and it was significantly elevated in 46.2% (n=55) of the individuals. Only 4 (2.4%) individual in the entire cohort demonstrated DIC.

	Population	Platelet >150 ×10/l ^{9}	Platelet <150 ×10/l ^{9}	INR<1.2	INR>1.2	aPTT< 40 seconds	aPTT> 40seconds
Montan et al. 2015.[12]	n=91	n=68	n=23	n=91	n=0	n=68	n=33
Reed et al. 2013.[32]	n=119	n=111	n=8	n=91	n=28	n=119	n=0
Franson et al. 2012.[33]	n=55	n=55	n=0	n=48	n=7	n=55	n=0
Fujita et al. 2012.[34]	n=64	n=64	n=0	_	_	n=43	n=21
Skaguis et al. 2008.[35]	n=55	n=55	n=0	n=55	n=0	n=55	n=0
Davies et al. 1993.[36]	n=50/55*	n=23	n=20	n=23	n=20	_	_
Getaz et al. 1977.[37]	n=27	n=22	n=5	_	_	_	_
Total	**n=461**	**n= 398 (86.3%)**	**n=56 (12.1%)**	**n=308/363 (84.8%)**	**n=55 (15.1%)**	**n=340 (86.2%)**	**n=54 (13.7%)**

*No data available on n=7 patients.
- no data provided in the original manuscript.
% was produced from the total provided data on each category.

Table 4.3 Data extraction on the studies that provided the Platelet count, international normalized ratio (INR), Activated Partial thromboplastin Time (aPTT) (Normal coagulation vs. Coagulopathy).

	Platelet 100-150 ×10/l [9]	Platelet <100 ×10/l [9]	INR= 1.2-1.5	INR>1.5	aPTT> 40seconds
Montan et al. 2015.[12]	n=23	n=0	n=0	n=0	n=33
Reed et al. 2013.[32]	n=8	n=0	n=15	n=13	n=0
Franson et al. 2012.[33]	n=0	n=0	n=7	n=0	n=0
Fujita et al. 2012.[34]	n=0	n=0	_	_	n=21
Skaguis et al. 2008.[35]	n=0	n=0	n=0	n=0	n=0
Davies et al. 1993.[36]	n=0	n=20	n=0	n=20	_
Getaz et al. 1977.[37]	n=0	n=5	_	_	_
Total	**n=31/461 (6.69%)**	**n=25/461 (5.4%)**	**n=22/363 (6%)**	**n=33/363 (9%)**	**n=54/394 (13.7%)**

*No data available on n=7 patients.
- No data provided in the original manuscript.
% Was produced from the total provided data on each category.

Table 4.4 Pooled analysis on subtype of coagulopathy (mild vs. significant) based on 12.1% deranged platelet count, 15.1% deranged INR, Activated Partial thromboplastin Time (aPTT).

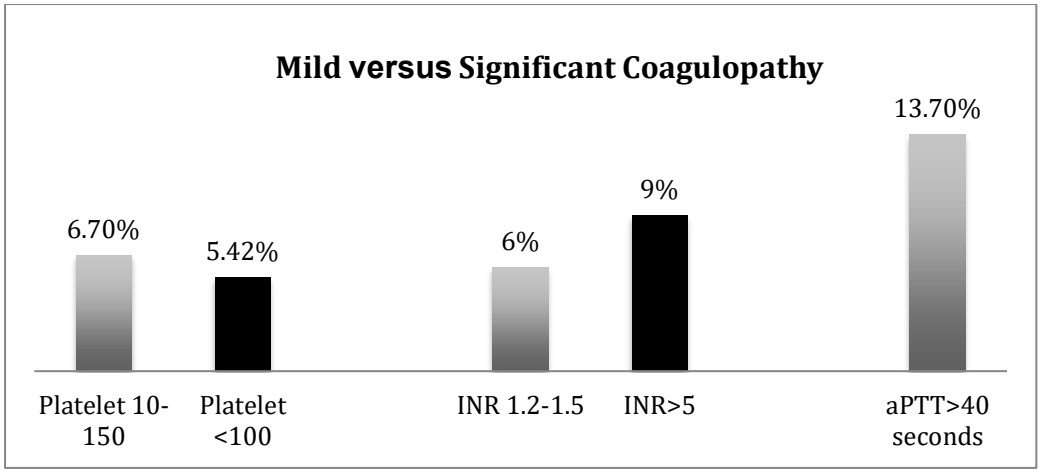


Figure 4.3 Chart demonstrating the comparison between the sub-types of coagulopathy (mild versus significant).

4.3 Discussion

Pooled analysis of the included studies in this review suggests that the majority (85%) of ruptured abdominal aortic aneurysms (rAAA) do not present with coagulopathy to the emergency services. Amongst the individuals that do, significant coagulopathy is limited to only 6% and disseminated intravascular coagulation (DIC) to 2.4% of the entire population. Furthermore, fibrinogen serum concentration was normal in 97% (n=165) and the D-dimer or fibrin degradation product (FDP) level was elevated in 46.2% (n=55/119) of the cohort. These findings appear to be in conflict with earlier assumptions about the baseline coagulation (and assumed coagulopathy) of rAAAs. This review questions the current and the ongoing comparison of rAAAs to that of traumatic (civilian and military) hemorrhage induced coagulopathy in the literature (Mell, 2010; Kauvar, 2012). This conflict could be explained by the presence and/or lack of certain variables and/or pathophysiological processes and postulations.

Firstly, in the study by Montan et al. (2015) a sub-group analysis, found a linear relationship between the preoperative presenting blood pressure, platelet count and the fibrinogen concentration levels. A cut-off blood pressure value of ≤ 70 mmHg was found to have a positive linear relationship to low serum levels of fibrinogen < 150md/dL (p=0.001) (normal range, 150-400 mg/dL) and reduction of circulatory platelet count (<150×10/l ^{9}) (p=0.05). Therefore, it appears prior evidence relating coagulopathy to that of hypotension is attested but there appears to be a further cut-off in the blood pressure measurements (≤ 70 mmHg) beyond which significant coagulopathy starts to occur in rAAAs. Given fibrinogen concentration is first to fall to a critically low level in major hemorrhage as the part of coagulation cascade (Levy, 2014), the presence of normal fibrinogen in the majority of (97%) individuals suggest hemodynamic instability may not be as profound as that of traumatic hemorrhage. This outcome is also complemented by Al-Barjas et al. (2006) study where nine (n=9) rAAAs with no hemodynamic instability demonstrated normal fibrinogen levels on their presentation to the hospital. Furthermore, randomized placebo-clinical trial (RCT) of Rahe-Meyer et al. (2013) demonstrated total avoidance of blood and blood product transfusion when fibrinogen concentration versus placebo was within normal limits.

Secondly, there is a clear and distinctive anatomical and mechanical cause for hemodynamic instability and circulatory volume loss in these two cohorts of patients (rAAAs and trauma). Anatomically, the majority of trauma victims (civilian and trauma) exhibit an ongoing and unopposed blood loss that can explain the significant hypotension and consequential hypo perfusion and

coagulopathy. However, 80% of patients with ruptured AAAs present with retroperitoneal rupture (posterior-lateral) and the aortic (infra renal) tear is often sealed and contained (Brewster, 2003; Rutherford, 1989). This permits a short phase of physiological and hemodynamic volume adjustment and accounts for the lack of profound circulatory shock in rAAAs. This tamponade effect could complement the presence of cut off blood pressure measurements noted in Montan et al. (2015) study. However, further research is required to ascertain the complex interplay of such variables (blood pressure, coagulation cascade and site of rupture) in this cohort of patients.

Thirdly, aneurysm formation is a time depend pathophysiological process as opposed to sudden impact of trauma and its implications and its morphology alters and indulges the coagulation cascade constantly and chronically during its degeneration. The assault to the endothelium in aneurysm formation acts as a nidus for the adhesion and migration of platelets and generation of fibrin. The release of von Willebrand Factor (vWF) and the presence of various other hematological variables promote the activation of tissue factor (TF) (Edington,1991). Tissue factor that is expressed in the adventitia and the medial smooth muscle, generates thrombin formation through factors X and VII. In addition, the presence of mural thrombus during this process that exhibits a direct (positive) correlation to the aneurysm size and its anatomical tortuosity, activates the coagulation cascade furthermore (Mantovani, 1997; Yamazumi, 1998). Upon rupture, the loss of aortic wall integrity exposes the collagen and sub-endothelial matrix to more blood that results in significant and additional thrombin formation (Furie, 2008). This process can account for

the elevated serum levels of D-dimer or fibrin degradation product (FDP) in 46.2% of the individuals in this review.

The emerging literature that has evaluated the complex factors of coagulation in abdominal aortic aneurysms have highlighted that even the baseline coagulation of ruptured AAAs, are vastly different to those of non-ruptured cases. In two comparative studies, the markers of thrombin generation: Thrombin-anti-thrombin complex (TAT) and Pro-thrombin fragment (PF) 1+2 concentration were statistically higher in rAAAs in comparison to the non-ruptured cases (135.5 µg/L Vs. 61 µg/L, $p \leq 0.02$). This outcome was also noted in the markers of fibrinolysis (tissue- plasminogen activator antigen) (ruptured AAA 15.7 ng/mL vs. Non ruptured AAA 6.6 ng /mL, $p \leq 0.05$) (Skagius, 2007; Adam, 1999). Thus, suggests an activated coagulation cascade widely different to those noted in the trauma cohort.

The initial resuscitation strategy most commonly adopted in rAAA patients across vascular units is “permissive hypotension” (Roberts, 2006). Such hypotension may well result in hypo-perfusion and early coagulopathy, although unlike trauma patients, evidence in the rAAA cohort demonstrates no significant relationship between hypotension and hypo perfusion with the degree of coagulopathy and fibrinolytic parameters. In addition, an earlier suggestion about elevation of fibrinolytic factors in rAAAs could be related to the application of supra-celiac cross clamp that in turn, reduces the hepatic blood flow and results in elevation of fibrinolytic factors (Gertler, 1996; Illig, 1997). Furthermore, studies in the literature have suggested improved

mortality, morbidity with minimal pre and perioperative blood loss as the result of systolic blood pressure titration between 70-100 mmHg as the part of the initial resuscitation strategy adopted in permissive hypotension of rAAAs (Riley, 2000; Dutton, 2002).

Finally, another major variable that has been associated with significant coagulopathy is the presence of hypothermia. A high number of victims in the military and civilian trauma are exposed to a hostile and exposed environment with delayed presentation to the emergency services and hospital resuscitation facilities (Kheirbek, 2009; Wade, 2011). Therefore, hypothermia might be more profound in this population as compared to rAAA patients that mainly present to emergency services direct from a controlled environment.

Strengths & Limitations

This is the first systematic review to investigate the baseline coagulation of ruptured abdominal aortic aneurysms in the literature. Despite the lack of statistical analysis (meta-analysis), this review can serve as a platform for future research. The careful application of recognized definitions provides future investigators with a more focused approach towards the topic in hand. This study is relatively low in power for an objective inference and a larger cohort would have been optimal. Furthermore, the level and grade of evidence according to NICE guidelines (Table 4.2) were suboptimal in two studies.

The included studies in this review did not report the serological markers of coagulation in a collective and a unified statistical manner. In addition, there appears to be a lack of consensus on the definition and interpretation of theoretical and clinical coagulopathy. Overall, the coagulation cascade is a complex interplay of various parameters and focus on a single factor in few included studies could not be a representative of the broader status alone. These factors should be assessed in context of other various markers of coagulation. This review emphasizes, the need for robust methodology in the future research.

4.4 Conclusion

In conclusion, the majority (≥ 85%) of ruptured abdominal aortic aneurysms do not present with coagulopathy. Amongst the individuals that do, significant coagulopathy is limited to only 6% with only 2.4% of the population demonstrating disseminated intravascular coagulation (DIC). Almost half of the population (46.2%) showed elevated levels of the D-dimer or fibrin degradation product (FDP) and the majority (97%) have normal concentration of fibrinogen.

CHAPTER 5

The Clinical Implication of Blood Product Transfusion on 30-day Morbidity and Mortality of Ruptured Abdominal Aortic Aneurysm.

5.1 Introduction

Previous reports in the literature have suggested that mortality following the repair of ruptured abdominal aortic aneurysm (rAAA) has been predominantly due to hemorrhage secondary to coagulopathy. However, the current literature suggests that fatal hemorrhage secondary to coagulopathy is now rare and there has been a significant shift towards post procedure mortality and morbidity as a consequence of thrombotic complications (Davies, 1993; Henriksson, 2012). This has been attributed to the new transfusion and resuscitation techniques where crystalloids and colloids have been replaced by blood and blood component transfusion (Duchesne, 2008).

The new transfusion (blood & blood component) protocols have evolved as a result of research surrounding military and civilian trauma patients with catastrophic hemorrhage, where a transfusion ratio of 2:1:1 and/or 1:1:1 of packed red blood cell (PRBC) to fresh frozen plasma (FFP) to platelets (PLT) conferred a mortality benefit (Duchesne, 2008). These protocols have since been used in the resuscitation of all patients presenting with hemorrhagic shock including those with rAAAs. Evidence suggests that the transfusion of packed red blood cells (PRBC) in rAAAs resuscitation has significantly reduced mortality however, (Fujita, 2012) the impact of the transfusion of FFP and PLT with the aforementioned ratio has yet to be determined. The aim of this study is to determine the effect of transfusion of blood and blood components such

as FFP and Platelets on the 30-day morbidity and mortality of patients presenting with rAAAs.

5.2 Material & Methods

A retrospective consecutive study of all patients presenting with ruptured infra-renal AAA at Mid Essex Hospitals NHS Trust from November 2007 to June 2015 was carried out. The hospital, surgical and anaesthetic database for all ruptured cases was searched and a total of ninety patients (n=90) were identified. However, only eighty-two (n=82) patients had a complete record. None of the patients were on any local screening programme and all patients had an open repair of the infra-renal ruptured abdominal aortic aneurysm. All individuals had a similar method of repair. A straight knitted Dacron graft according to the aorta size (size 16-20mm) was anastomosed proximally and distally with Polyprolene Monofilament suture size 3 and 4 respectively (Surgipro^{TM} Covidien). Furthermore, none of the patients had any known coagulopathy and/or haematological disorder. Eighty individuals (n=80) were on Aspirin 75 mgs preoperatively and none were on any other antiplatelet and/or anticoagulant medication. The transfusion policy was based on clinical expertise of the anaesthetic department and (not on any protocol) this was applicable to all individuals.

The patient demographics on age, gender, co-morbidities, American society of Anesthesiology score (ASA), blood loss and blood component transfusions of units of Pack Red Blood Cell (PRBC), units of Fresh Frozen Plasma (FFP), and pools of Platelet (PLT) were retrieved. None of the individuals received any other additional clotting factors or were subjected to auto transfusion. All

patients received 5000 IU of heparin intravenously after the application of the aortic clamp. Postoperatively all patients received Aspirin 75mgs along with Enoxaparin Sodium 40mgs, subcutaneously once daily. Individual preoperative Computed tomography angiograms (CTA), of the entire cohort was assessed by two separate investigators (interventional vascular radiologist & consultant vascular surgeon) and the aneurysm size was calculated and presented in millimeters. The maximum diameter was taken as the true size of the AAA. The agreement level in size measurement was assessed using Cohen's Kappa Coefficient (κ=0.82). The study endpoint was set at 30 days for all outcomes of mortality, morbidity (thrombotic and non-thrombotic complications). Morbidity was defined according Clavien-Dindo classification ≥3 (considered a major complication) (Dindo, 2004) Mortality was defined as death from any cause. Due to lack of any new or alternative intervention and availability of the data for clinical purpose ethical approval was granted with audit number CA13-226.

Statistical Analysis

For the purposes of the statistical analyses, scale variables were categorized according to their median values. Differences across the outcome groups were examined using Kruskal-Wallis and Chi Squared analyses. Logistic regression analyses were undertaken to determine factors associated with each outcome (30-day mortality, 30-day morbidity and 30-day thrombotic complications). Each variable was tested individually for association with the outcome. Those variables that demonstrated

		No Morbidity or Mortality		30-Day Morbidity		30-Day Mortality	
		n=	%	n=	%	n=	%
Gender	Male	39	81.2%	17	77.3%	10	83.3%
	Female	9	18.8%	5	22.7%	2	16.7%
Age	18-75 years	26	54.2%	12	54.5%	4	33.3%
	>75 years	22	45.8%	10	45.5%	8	66.7%
ASA	2	13	27.1%	4	18.2%	0	0.0%
	3	20	41.7%	4	18.2%	3	25.0%
	4	12	25.0%	11	50.0%	7	58.3%
	5	3	6.2%	3	13.6%	2	16.7%
PRBC (Unit)	No Transfusion	9	19.1%	4	18.2%	2	16.7%
	1-3 Units Transfused	23	48.9%	3	13.6%	1	8.3%
	>3 Units Transfused	15	31.9%	15	68.2%	9	75.0%
FFP (Unit)	No Transfusion	23	48.9%	8	36.4%	1	8.3%
	1-3 Units Transfused	12	25.5%	4	18.2%	3	25.0%
	>3 Units Transfused	12	25.5%	10	45.5%	8	66.7%
PLT (Pool)	No Transfusion	32	66.7%	10	45.5%	3	25.0%
	1 or more pools	16	33.3%	12	54.5%	9	75.0%
ITU Stay (hours)	0-72 hours	25	71.4%	6	31.6%	3	27.3%
	>72 hours	10	28.6%	13	68.4%	8	72.7%
Length of Stay (Days)	0-10 days	31	66.0%	8	38.1%	4	33.3%
	>10 days	16	34.0%	13	61.9%	8	66.7%

Table 5.1 Demographic detail of patients in each category.

a significance of p ≤0.10 were entered into a multivariable logistic regression model. At multivariable analyses, variables with a p value of p≤0.05 were deemed to be statistically significant and independently associated with the outcome. All statistical analyses were carried out using Statistical Package for the Social Sciences (SPSS), IBM version 22.0.

5.3 Results:

A total of 82 patients had complete data for analyses. The median age at time of rAAA was 75 years (range, 51-92). The majority of the population was male (n=66, 80.5%). The median size of rAAA was 75.52 mm (range, 40-100mm). The American Society of Anesthesiology score (ASA) in the order of prevalence was ASA=4 (n=36, 36.6%), ASA=3 (n=27, 32.9%), ASA=2 (n=17, 20.7%) and ASA=5 (n=8, 9.8%). Hypertension was found in 60.9%(n=50), chronic obstructive pulmonary disease (COPD) in 28.0% (n=23/82) and active smokers in 23.2% (n=19/82) of the cohort. The median intra-operative blood loss was 2,100 ml (range, 500-5,000). The median length of intensive care unit stay was 72 hours (range, 13-574) and the overall median length of hospital stay was 10 days (range, 5-29) (Table 5.1).

Mortality

Overall 14.6% (n=12) died within the first 30-days of surgery. Of these, the vast majority (n=10/12) were males. Univariate analyses demonstrated increasing ASA score (p=0.028), transfusion of fresh frozen plasma of more 3 units (p=0.034) were significantly associate with mortality for the overall 14.6%

(n=12, male=10 vs. female= 2) mortality. At multivariable logistic regression only fresh frozen plasma (> 3 units) remained an independent predictor of mortality (OR, 11.27, CI (1.13-96.72), p=0.027) (Table 5.2).

Morbidity

A total of 22 patients (26.8% of the entire population) experienced morbidity within 30-days post rAAA surgery. Logistic regression analyses were undertaken to ascertain determinants of morbidity. Univariate analyses demonstrated transfusions of PRBC >3 units (p=0.008), FFP >3 units (p=0.006) and platelet >1 pool were significantly associated with 30-day morbidity (Table 5.3). At multivariable level, no variable was found to be independently associated with 30-day morbidity. However, further sub-group analyses of 30-day morbidity (thrombotic vs. non thrombotic complications) at multivariable regression analysis demonstrated transfusion of platelet pool of more than 1 is an independent predictor of thrombotic events (OR, 4.3, 95% CI: 1.37-13.6) p=0.012) accounting for 50% (n=11/22) of all morbidities and mortality (n=6/12) (Table 5.4). Postoperative thrombotic events (thromboembolic phenomenon) that lead to mortality were bowel ischemia (n=4) and limb ischemia (n=2). The others included, ischemic stroke (n=1), Ischemic myocardial infarction (n=1) and three (n=3) cases of renal artery thrombosis. The median length of stay for the deceased was 14 days (IQR8-23) as opposed to median of 8 days (IQR 6-14) for patients that made it through the surgery. Their details are highlighted in table 5 of this manuscript.

		Univariate	Multivariate		
			OR	CI	p=
Gender	Male	0.788	1 (Ref)		
	Female				
Age	18-75 years	0.188	1 (Ref)		
	>75 years				
ASA Grade	2	0.028	1 (Ref)		
	3		not estimable		0.998
	4		not estimable		0.998
	5		not estimable		0.998
COPD	No COPD	0.660	1 (Ref)		
	COPD				
Smoking Status	Non-Smoker	0.871	1 (Ref)		
	Smoker				
Blood Loss (mls)	Up to 2L	0.534	1 (Ref)		
	>2L				
Packed Red Blood Cells Transfused (Units)	No Transfusion	0.168	1 (Ref)		
	1-3 Units Transfused				
	>3 Units Transfused				
Fresh Frozen Plasma Transfused (Units)	No Transfusion	0.016	1 (Ref)		
	1-3 Units Transfused		5.81	0.56-60.48	0.141
	>3 Units Transfused		11.27	1.31-96.72	0.027
Platelets Transfused (Pools)	No Transfusion	0.034	1 (Ref)		
	1 or more pools		1.44	0.23-9.04	0.696

Table 5.2 Regression analysis (Univariate & multivariate) on factors associated with 30-day mortality.

Logistic regression analysis: factors associated with 30-day morbidity					
		Univariate	Multivariate		
			OR	CI	p=
Gender	Male	0.750			
	Female				
Age	18-75 years	0.392			
	>75 years				
Hypertension	No Hypertension	0.272			
	Hypertensive				
COPD	No COPD	0.898			
	COPD				
Smoking Status	Non-Smoker	0.382			
	Smoker				
Packed Red Blood Cells Transfused (Units)	No Transfusion	0.008	1 (Ref)		
	1-3 Units Transfused		0.27	0.06-1.32	0.106
	>3 Units Transfused		2.03	0.51-8.0	0.313
Fresh Frozen Plasma Transfused (Units)	No Transfusion	0.006	1 (Ref)		
	1-3 Units Transfused		1.26	0.25-6.35	0.774
	>3 Units Transfused		0.79	0.12-5.29	0.806
Platelets Transfused (Pools)	No Transfusion	0.007	1 (Ref)		
	1 or more pools		2.49	0.85-7.31	0.098

Table 5.3. Regression analysis (Univariate & Multivariate) factors on 30-day Morbidity.

5.4 Discussion:

The outcome of this study suggests FFP transfusion coincides with increased risk of 30-day comorbidity, namely thrombo-embolic events. The traditional crystalloid/colloid infusion has now been replaced with PRBC, FFP and PLT which is based on massive transfusion protocols (Stansbury, 2009; Holcomb, 2010). The initial strategy was aimed at the optimization of cardiac output and oxygen delivery system and later, at the correction of coagulopathy believed to develop several hours after the onset of hemorrhagic shock (Cosgriff, 1997; Ledgerwood, 2003). However, recent research suggests that traumatic coagulopathy in hemorrhagic shock is from the activation of anticoagulant protein C pathway due to tissue hypo-perfusion and not from the consumption of coagulation factors. Thus, the resuscitation protocols should now aim at limiting hypo perfusion and its duration, which can be assessed by the degree of acidosis (Brohi and Tieu, 2007). This means the new transfusion strategy aims at correcting the trauma triad of hypothermia, acidosis, and coagulopathy (Mikhail, 1999).

The current transfusion strategy has reduced mortality from catastrophic hemorrhage in rAAA patients. However, with increased survival there has been an emergence of a new pattern of mortality and morbidity, which is related to thrombotic events. There is a degree of evidence to support the use of PRBCs in this cohort of patients. However, the difference between a traumatically injured and rAAA patient needs to be considered before the administration of other blood components like FFP and PLT (Henriksson, 2012). In contrast to the trauma population, the average age in vascular patient is much higher (75

vs. 45 years), they exhibit far more comorbidities and they do not commonly present with hypothermia (Mell, 2010). Aneurysm formation is a time depend process and its morphology alters the coagulation cascade in comparison to the sudden impact of trauma on overall physiology and its immediate effect on the coagulation cascade.

Furthermore, during aneurysm degeneration, the assault to the endothelium acts as a nidus for the adhesion of platelets, migration of platelets and generation of fibrin. The release of von Willebrandt Factor (vWF) and the presence of various other hematological factors promote the activation of tissue factor (TF) that in turn generates thrombin formation through factors X and VII. In addition, the size of mural thrombus exhibits a direct (positive) correlation to the aneurysm size and its anatomical tortuosity that are much higher in rAAA (Edington, 1991; Mantovani, 1997; Yamazumi, 1998). This indicates rAAAs present with a pro-coagulant rather than coagulopathic state and therefore do not require the current suggested ratio of FFP and PLT as a rule.

		Univariate	Multivariate		
			OR	CI	p=
Gender	Male	0.319			
	Female				
Age	18-75 years	0.345			
	>75 years				
ASA Grade	2	0.182			
	3				
	4				
	5				
Hypertension	No Hypertension	0.249			
	Hypertensive				
COPD	No COPD	0.977			
	COPD				
Smoking Status	Non-Smoker	0.601			
	Smoker				
Blood Loss (ml)	Up to 2L	0.039	1 (Ref)		
	>2L		1.91	0.52-7.07	0.334
Packed Red Blood Cells Transfused (Units)	No Transfusion	0.559			
	1-3 Units Transfused				
	>3 Units Transfused				
Fresh Frozen Plasma Transfused (Units)	No Transfusion	0.179			
	1-3 Units Transfused				
	>3 Units Transfused				
Platelets Transfused (Pools)	No Transfusion	0.012	1 (Ref)		
	1 or more pools		4.33	1.37-13.67	0.012

Table 5.4 Regression analysis (Univariate & Multivariate) on factors associated with thrombotic complications.

The initial resuscitation strategy most commonly adopted in rAAA patients across vascular units is “permissive hypotension” (Roberts, 2006). Such hypotension may well result in hypo-perfusion and early coagulopathy, although unlike trauma patients, evidence in the rAAA cohort demonstrates no significant relationship between hypotension and/or hypo perfusion, the degree of coagulopathy and fibrinolytic parameters. In addition, an earlier suggestion about elevation of fibrinolytic factors in rAAAs could be related to the application of supra-celiac cross clamp that in turn, reduces the hepatic blood flow and results in elevation of fibrinolytic factors (Gertler, 1996; Illig, 1997; Adam, 1999).

In the study by Adam et al. (1999) even non-ruptured AAAs exhibited significant coagulation status difference to that of rAAAs. The markers of thrombin generation: Thrombin-anti-thrombin complex (TAT) and Pro-thrombin fragment (PF) 1+2 concentration were statistically higher in rAAAs in comparison to the non-ruptured cases (135.5 µg/L Vs. 61 µg/L, $p \leq 0.02$). This was also significant in the markers of fibrinolysis (tissue- plasminogen activator antigen) (15.7 ng/mL vs. 6.6 ng /mL, $p \leq 0.05$) (Adam, 1999). The above data in addition to the earlier discussion, demonstrates that unlike the trauma group of patients, AAA individuals are pro-coagulant and more so if they are in ruptured circumstances. It appears that such a pro-coagulant state persists throughout the operation and resolves in the postoperative period (≥ 24 hours) (Adam, 1999). Therefore, liberal or extensive use of fresh frozen plasma (FFP) and platelets may well contribute to postoperative thrombotic events and mortality.

Data suggests that FFP transfusion of more than three units is an independent predictor of 30-day mortality in rAAAs. Other authors have reported similar results. In a study comparing the number and types of transfusions of survivor and non-survivor groups in rAAA, the authors demonstrated the unit of FFP transfused in the survivor group to be significantly lower than the non-survivor group. The postoperative complications leading to mortality in the study by Fransson et al. (2012), was related to micro and macro cardiovascular thrombotic complications. In the same direction, Kauvar et al. (2012) study could not establish a reduction in mortality with increase FFP transfusion in rAAA patients ($p \geq 0.56$). Furthermore, the study by Holcomb et al. (2008) highlighted that the new transfusion protocols could not address other contributing independent impacts of FFP on the endothelial structure, fibrinolysis, plasma proteins and increase platelet count on their effect on the overall mortality. The use of FFP transfusion as a volume replacement in addition to PRBC also seems to have no rationale in the literature.

Another important issue surrounding the repair of rAAA is the use of un-fractionated heparin. It appears that other than tissue plasminogen activator (t-PA) no other fibrinolytic component is affected by heparin. Considering the status of rAAA and the potential loss of significant volume, surgeons are reluctant to use heparin in such settings. The judicial use of heparin after the application of the aortic clamp may reverse and/or decrease the pro-coagulant state and the associated mortality induced by higher FFP transfusion. In our cohort all individuals received Heparin and this may justify the lower mortality incidences compared to the other reported series. Graham et al. review also

recommends the use of heparin in the repair of rAAA for the reversal of the ongoing pro coagulant state and post procedural thrombotic events (Marsh, 1990; Thompson, 1996).

Platelet transfusion of more than one pool was an independent marker of thrombotic complications in our cohort of patients. In a recent analysis of preoperative bloods (Platelet count, Prothrombin time (PT), activated partial thromboplastin time (APTT) performed on individuals with rAAAs, no coagulopathic state was identified. In addition, no thrombocytopenia and/or platelet count of less than 100 10/l was noted in the majority of the individuals (Reed, 2013). This outcome clearly indicates that patient with rAAA, do not necessarily require active platelet transfusion and if so it has to be based on ongoing laboratory result rather than one for all transfusion protocol. In addition, active transfusion of platelet in a patient with adequate platelet count for a major surgery could explain the ongoing trend and shift towards the occurring thrombotic complications in our cohort.

The current study has several strengths and weaknesses. This is the first study in the literature that has evaluated the role of PRBC, FFP and PLT (massive transfusion protocol and its ratio) in the resuscitation of rAAAs. Data appears to be in agreement with earlier studies indicating that rAAAs unlike trauma patients are pro-coagulant and liberal use of FFP and PLT can contribute to mortality, morbidity and thrombotic complications. In addition, this study highlights that the mortality incidence of rAAA has improved and these patients are no longer succumbing to hemorrhagic shock, but instead are expiring as a

result of thrombotic complications later in the recovery phase. This study inherits the weakness of being retrospective in nature and a higher number of individuals in a randomized controlled trial would have been optimal. However, given the nature of the surgery, its associated high morbidity and mortality, such methodology might not feasible.

		n=	%
Gender	Male	10	83.3%
	Female	2	16.7%
Age	18-75 years	4	33.3%
	>75 years	8	66.7%
ASA Grade	2	0	0.0%
	3	3	25.0%
	4	7	58.3%
	5	2	16.7%
Hypertension	No Hypertension	8	66.7%
	Hypertensive	4	33.3%
COPD	No COPD	8	66.7%
	COPD	4	33.3%
Smoking Status	Non-Smoker	9	75.0%
	Smoker	3	25.0%
Blood Loss	Up to 2L	5	41.7%
	>2L	7	58.3%
PRBC	No Transfusion	2	16.7%
	1-3 Units Transfused	1	8.3%
	>3 Units Transfused	9	75.0%
FFP	No Transfusion	1	8.3%
	1-3 Units Transfused	3	25.0%
	>3 Units Transfused	8	66.7%
PLT	No Transfusion	3	25.0%
	1 or More Pools	9	75.0%
HB	Up to 115 g/l	8	66.7%
	>115 g/l	4	33.3%

Table 5.5 Information on the deceased patients (n=12).

Age	Sex	HB	HT	Platelet	PTT	APTT	INR
87	Female	8.8	0.300	168	13.1	37.3	1.10
88	Male	9.4	0.271	65	14.9	50.9	1.25
82	Male	9.6	0.244	140	13.3	28.2	1.12
76	Male	10.5	0.313	99	16.7	47.9	1.40
74	Male	12.0	0.376	149	12.8	25.0	1.07
72	Male	14.7	0.264	136	11.3	27.5	1.01
87	Male	7.0	0.210	35	32.6	240.0	1.20
75	Female	11.6	0.380	69	16.6	36.0	1.45
80	Male	10.7	0.340	177	24.9	113.3	2.34
85	Male	10.4	0.230	190	16.3	46.6	1.42
64	Male	14.8	0.430	146	14.5	24.1	1.23

Table 5.6. Laboratory results of deceased Patients prior to transfusion.

5.5 Conclusion

The findings of this study are that certain blood products such as FFP and platelets may be associated with increased postoperative morbidity post rAAA repair. The traditional transfusion protocol and its suggested ratios of blood products to PRBC need to be questioned. Despite reduction in mortality associated with transfusion of PRBC, the use of other blood components such as FFP and PLT should be considered in the context of the patient's requirement based on clinical and laboratory data. The liberal use of FFP and PLT in rAAA patients might have a different outcome to that of trauma patients. Thus, further research assessing such factors is required to determine what the ideal transfusion protocol would be.

CHAPTER 6

Blood markers on 30-day mortality & morbidity of ruptured abdominal aortic aneurysm: A retro & prospective cohort study.

6.1 Introduction

To deliver a more personalized form of vascular care, there is a need to identify novel, readily available and consistent predictive factors for risk stratification in rAAA surgery. In recent years there has been an accumulating level of evidence that elevated levels of systemic inflammatory response (SIR) are associated with increased cardiovascular incidents (Duffy, 2006). The Neutrophil to Lymphocyte ratio (NLR) first reported in 1967, is a widely used marker of SIR in recent literature (Bobb, 1967). The NLR has been linked to adverse outcomes in patients with colorectal, gastric, pancreatic, ovarian and hepatocellular carcinoma (Gomez, 2008; An, 2010; Malietzis, 2013).

In relation to vascular surgery, there is a single study that demonstrates NLR is a predictor of 2-year survival in patients undergoing a range of vascular interventions (Bhutta, 2011). In addition, a recent report suggests that in the elective group of AAAs, a preoperative NLR> 5 could be a predictor of 30-day mortality (Appleton, 2014). Although there is emerging evidence to support the prognostic importance of NLR in the outcomes of a patient having a vascular procedure, no study has examined the role of NLR on patients with ruptured infra-renal abdominal aortic aneurysms (rAAA).

The aim of this study is therefore to assess the predictive value of NLR in relation to 30-day morbidity and mortality of patients undergone repair of ruptured infra-renal abdominal aortic aneurysms (rAAA).

6.2 Material & Methods:

A retrospective consecutive cohort study of all ruptured infra-renal AAA from November 2007 to June 2015 that presented for repair at Mid Essex Hospital NHS Trust with estimated population coverage of 360,000 was designed. The hospital, surgical and anesthetic dataset for all ruptured cases was searched and a total of eighty patients (n=80) were identified. None of the patients were on any local screening programme and all patients had an open repair of the ruptured aneurysm. None of the patients exhibited any loss of consciousness prior to the operation. The data on age, sex, AAA size, blood loss, length of stay (LOS) and comorbidities (hypertension (HTN), active smoking (S), renal failure (RF), chronic obstructive pulmonary disease (COPD)) were recruited. A full blood count (FBC) was obtained preoperatively on all individuals and the NLR was calculated by dividing the Neutrophil by Lymphocyte count to obtain a ratio. Individual preoperative computed tomography angiogram (CTA) of the entire cohort was assessed by two separate investigators (interventional vascular radiologist & consultant vascular surgeon) and the aneurysm size was calculated and presented in millimeters. The maximum diameter was taken as the true size of the AAA. The agreement level in size measurement was assessed using Cohen's Kappa Coefficient (κ=0.78). The primary study endpoint was set at 30-day morbidity and mortality, measured from the time of surgery. Morbidity was defined according Clavien-Dindo (2004) classification

≥3 (considered a major complication). Due to lack of any new or alternative intervention and availability of the data for clinical purpose ethical approval was granted with audit number CA13-226.

Statistical Analysis

The primary study end point was 30d morbidity and mortality. I determined the optimal discriminator value for NLR using receiver operating characteristic (ROC) curve analysis. At each value, the sensitivity and specificity for each outcome under study were plotted. The ratio closest to the point with maximum sensitivity and specificity was selected as the cutoff value. The relationship between NLR and other clinical parameters was assessed using nonparametric statistics. Stepwise multivariate logistic regression analysis was performed. The level of significance permitting multivariate analysis inclusion and the statistical significance for all other tests used was set at $P < 0.1$. All analyses were performed using the statistical software, Statistical Package for the Social Sciences, version 20.0 (SPSS, Inc, Chicago, IL).

6.3 Results

The median patient age was 75 (range, 51 to 92) with 82.5% of the population being male (n=66/80). Of the total cohort forty-three patients (64.2%) were hypertensive (HTN). Out of the nineteen active smokers (25%), seventeen patients (n=17, 21.8%) had the confirmed diagnosis of COPD.

Preoperative acute renal failure was noted in three patients (3.9%). The median AAA size in millimeters was 75.50 mms (range, 45 to 120 mm). The

median preoperative hemoglobin was 11.3 mg/dl (range, 4.5 to 16 mg/dl). Total intraoperative blood loss was 2500ml (range 500ml to 5620ml).
Table 1. The median intensive care unit (ITU) stay was 72 hours and the median duration in hospital stay was 13 days. The overall 30-day morbidity was 51.2% (n=41/80) and the overall 30-day mortality was 13.8% (n=11/80) (Table 6.1).

An ROC curve analysis was performed in relation to the presence of 30d mortality and morbidity. For all 80 rAAAs, an NLR of 5.0 had the highest sensitivity and specificity for both outcomes. Thereafter, patients were stratified into 2 groups: low NLR (≤5) and high NLR (>5). Table 6.2 shows the difference in clinic-pathological features of the two NLR groups. Overall, 25 patients (31.2%) had a low NLR and 55 patients (68.8%) had a high NLR. A high NLR was significantly associated with low Hemoglobin (Hb) levels and it was not associated with sex, age, AAA size, history of HTN, COPD, smoking and Renal failure. Patients with HNLR had higher 30d morbidity compare with the LNLR group (35 Vs. 6, p=0.001) but no difference in the other short term outcomes studies such as intraoperative blood loss, Intensive care unit stay, primary length of stay and 30-day mortality.

To investigate whether the NLR was associated with clinical outcome, univariate and multivariate logistic regression analysis for 30-day morbidity were performed. Analysis for 30d mortality was not performed due to the small number of events i.e. 30-day deaths. Univariate analysis identified the presence of HTN history (OR=0.39, 95%CI (0.14-1.14), p=0.08) as a negative and high

NLR (OR=5.54, 95%(1.9-10.15), p=0.02) as poor prognostic indicators of 30d morbidity.

To determine the independent prognostic significance of NLR on 30-day morbidity, multivariate analysis using a binary logistic regression model was performed. Multivariate analysis included HTN history and NLR. Only NLR variable was an independent prognostic factors for 30d morbidity (OR=4.28, 95% (1.27-14.42), p=0.02) as outlined in Table 6.3.

6.4 Discussion:

To the best of my knowledge, this is the first study that has evaluated the predictive role of NLR in rAAA. The outcome suggests that NLR > 5 is an independent predictor of post-operative morbidity. This appears to compliment the work of Appleton et al. (2014) where a NLR > 5 was associated with significantly higher mortality and a lower 2-year survival as a consequence of morbidity in the elective repair of infra-renal abdominal aortic aneurysms (AAA). Ruptured abdominal aortic aneurysms are a major cause of mortality in the western hemisphere and currently account for 2% of all deaths in the UK (Office of population Census and Surveys. Mortality Statistics: cause, England and Wales). Despite advances in the field of vascular surgery and allied health the cumulative perioperative mortality still persists at 40-50% and in some series up-to 80% (Norman, 2004; Sakalihasan, 2005). Assessment of such patients is rapid and remains subjective. Risk stratification of rAAAs has led to the development of various score designs. Three such models are the Edinburgh Ruptured Aneurysm Score (ERAS), Hardman Index (HI) and Glasgow Aneurysm Score (GAS) (Samy, 1994; Hardman, 1996; Tambyraja, 2008).

However, the study by Gatt et al. (2009) demonstrated that HI and GAS are poor predictors of outcome after rAAA repair and no thorough validation of ERAS in another cohort has been performed. None of these scoring systems assess the inflammatory response in their analysis.

		Median	25 %	75%	Count	Percentage
Sex	**Female**				14	17.5%
	Male				66	82.5%
HTN	**No**				24	35.8%
	Yes				43	64.2%
	No				61	78.2%
	Yes				17	21.8%
Smoking	**No**				57	75.0%
	Yes				19	25.0%
Renal Failure	**No**				73	96.1%
	Yes				3	3.9%
AAA size in mm		75.50	65.00	87.50		
Hb		11.30	9.50	13.30		
NLR		9.40	4.14	13.69		
Blood Loss (ml)		2500	1500	4750		
ITU stay (hours)		72	24	176		
LOS (days)		13	8	23		
30-day Morbidity	**None**				39	48.8%
	Yes				41	51.2%
30-day Mortality	**No**				69	86.2%
	Yes				11	13.8%

Table 6.1 Patients Demographics.

		LNLR					HNLR					p-value
		Median	Percentile 25	Percentile 75	N=	%	Median	Percentile 25	Percentile 75	N=	%	
Age		76.00	72.00	87.00			75.00	69.00	81.00			0.301
Sex	**Female**				5	20.0%				9	16.4%	0.755
	Male				20	80.0%				46	83.6%	
HTN	**No**				4	23.5%				20	40.0%	
	Yes				13	76.5%				30	60.0%	0.221
COPD	**No**				19	76.0%				42	79.2%	
	Yes				6	24.0%				11	20.8%	0.746
Smoking	**No**				17	70.8%				40	76.9%	
	Yes				7	29.2%				12	23.1%	0.569
Renal	**No**				23	95.8%				50	96.2%	
Failure	**Yes**				1	4.2%				2	3.8%	0.686
AAA size (millimeters)		80.00	60.00	88.00			75.00	65.00	85.00			0.942
Hb		13.25	11.35	14.05			11.00	9.10	12.20			**0.001**
Blood Loss (mL)		2500	1500	3500			3000	1500	5000			0.606
ITU stay (Hours)		57	24	96			94	32	192			0.205
LOS (Days)		11	9	20			13	8	23			0.867
30-day	**None**				19	76.0%				20	36.4%	
Morbidity	**Yes**				6	24.0%				35	63.6%	**0.001**
30-day	**No**				23	92.0%				46	83.6%	
Mortality	**Yes**				2	8.0%				9	16.4%	0.488

Table 6.2 Clinical features & outcomes of patients with high & low NLRs included.

In recent years the role of NLR as an independent predictor of mortality and morbidity has gained significant attention in colorectal, upper GI and cancer surgery. The use of this single and inexpensive prognostic factor has been on the rise in clinical and academic settings. In relation to cardiovascular surgery, recent evidence suggests that a raised NLR (NLR > 5) is associated with poorer survival following coronary artery bypass grafting (CABG) (Janion, 2007). In vascular surgery, Bhutta et al. (2011) assessment of 1021 individuals undergoing vascular interventions (non-emergency) revealed a lower independent 2-year survival in patients with NLR > 5. This has been attributed to the direct relationship between leucocytes and adverse cardiovascular outcomes such as myocardial infarction, coronary artery ectasia, arrhythmia, p-wave depressions and its sequelae (Janion, 2007; Papa, 2008; Isik, 2013). This appears to be in line with complications noted in our data irrespective of the age, gender, AAA size, comorbidities (COPD, HTN, RF, AS), blood loss, length of hospital and ITU stay of the entire cohort.

Aneurysmal degeneration of the abdominal aorta is a time dependent process and NLR represents a chronic and a low-grade systemic inflammatory response. Neutrophilia has been associated with collagen remodeling, direct endothelial cell injury, pro-thrombotic state and hypercoagulability which is known to occur in rAAAs (Siminiak, 1995; Madjid, 2004; Coller, 2005). Neutrophilia is the product of neutrophil de-

margination, delayed apoptosis and stimulation of stem cells by interleukin 6 (IL-6) and granulocyte colony stimulating

		Univariate Logistic Regression		Multivariate Logistic Regression	
		OR 95% CI	**P-value**	**OR 95% CI**	**P-value**
Age		1.02 (0.97-1.07) b=0.02	0.424		
Sex	**Female**	1			
	Male	0.52 (0.16-1.73)	0.29		
HTN	**No**	1		1	
	Yes	0.39 (0.14-1.14)	0.08	0.47 (0.15-1.36)	0.15
COPD	**No**	1			
	Yes	0.63 (0.21-1.88)	0.41		
Smoking	**No**	1			
	Yes	0.49 (0.17-1.42)	0.19		
Renal Failure	**No**	1			
	Yes	0.49 (0.04-5.60)	0.56		
NLR	**Low**	1		1	
	High	5.54 (1.9-10.15)	0.02	4.28 (1.27-14.42)	**0.02**
AAA size		0.98 (0.95-1.01) b=-0.17	0.27		
Hb		0.94 (0.79-1.13)	0.53		

Table 6.3 Univariate & Multivariate analyses of clinical & Bio-chemical parameters for the prediction of 30d morbidity in rAAAs

factors (G-CSF). Sudden neutrophilia could be a representative of acute on chronic inflammatory response as neutrophils encourage plaque rupture by releasing arachidonic acid derivatives and proteolytic enzymes. In addition, Haumer et al. study demonstrated increased neutrophil activation as a consequence of endothelial cell injury in formation of micro and macro vascular occlusive plugs (Haumer, 2005).

Chung et al. (2010) review of 252 thoracic endovascular aneurysm repairs (TEVAR) demonstrated preoperative leukocytosis to be an independent marker of overall mortality. Recent literature suggests low lymphocyte to be associated with poor outcome in patients with cardiac pathology (Ischemic heart disease) (Evans,1995). Lymphocytopenia appears to be the result of redistribution and margination of lymphocytes within the lymphatic system and a marker of accelerated apoptosis. Low lymphocyte count indicates a state of immunosuppression and physiological stress that have adverse relation to overall patient outcome. Analysis of the components contributing to a raised NLR in this series suggests that neutrophilia is much more significant than lymphocytopenia (compared to their base line) and low Hb might be a surrogate marker of the severity of the rupture despite its statistical insignificance (Onmen, 1997).

The examination of a single, readily available, inexpensive index of NLR during the rapid assessment of rAAAs might be more feasible than calculation of a multifactorial scoring system in clinical practice. Despite accumulating evidence in support of high NLR; as a marker of worse outcome in elective and rAAAs,

whether preoperative NLR can be altered before intervention and consequently influence outcome remains to be establish. Further research, assessing this on a cellular and systemic level might be advocated. The authors believe there are some limitations to the current research. The low number of individuals (n=80) the retrospective methodology and the small percentage of missing data might subject this study to statistical bias. Therefore, future research should aim at recruiting a higher number of subjects and perhaps in a randomized controlled trial setting. However, given the nature of rAAAs and its associated high mortality and morbidity such methodology might not be feasible.

In summary, no single prognostic marker or scoring system could be a complete replacement for the surgeon to patient clinical assessment and decision-making process. However, in an era where service provision, utilization of resources and informed consent is based on statistical outcomes, NLR might prove to be of clinical importance.

6.5 Conclusion:

A preoperative NLR> 5, irrespective of the age, gender, AAA size, blood loos, length of stay and comorbidities is an independent marker of 30-day morbidity and not mortality in ruptured abdominal aortic aneurysms.

CHAPTER 7

Pre & Post Coagulation Profile Following Blood Component Transfusion in Ruptured Abdominal Aortic Aneurysm Irrespective of the Outcomes of 30-Day Mortality & Morbidity.

7.1 Introduction

Prior studies evaluating the clinical impact of blood product transfusion indicated that both blood product components (fresh frozen plasma and platelet) as part of hemostatic resuscitation could independently contribute to mortality and morbidity (Kordzadeh, 2016). The overall aim of such protocols is to correct the suspected coagulopathy of rAAAs. Irrespective of earlier findings with regards to minimal coagulopathy, mortality and morbidity associated with such protocols, the aim of this current study is to assess whether such products actually do have any impact on the correction of identified coagulopathic individuals.

7.2 Material and Methods

A retrospective study of n=101 consecutive patients with suspected infra renal ruptured abdominal aortic aneurysm (rAAA) from November 2007 to June 2015 that presented to the emergency department was conducted. Five patients (n=5) were deemed to be for palliation and another five (n=5) did not have any preoperative blood assessment. Eight patients (n=8) had mixed picture of abdominal and/or iliac and/or supra renal and/or tender aneurysm and were excluded from the study. The remaining individuals (n=82) had confirmed clinical and radiological infra-renal AAA rupture. The Mid Essex Hospital services NHS Trust, has estimated population coverage of 380,000 people.

Data on patient demographics (age and gender), comorbidities (Hypertension (HTN), Chronic obstructive pulmonary disease (COPD), Active smoking, coagulation profile (Prothrombin time (PT), Activated partial thromboplastin time (APTT), International normalized ratio (INR), Haemoglobin (Hb), platelet count (Plt) were recorded. Following the identification of patients with coagulopathy the immediate postoperative data on coagulation profile (Prothrombin time (PT), Activated partial thromboplastin time (APTT), International normalized ratio (INR), Haemoglobin (Hb), platelet count (Plt), blood and blood component transfusion were also recorded.

Statistical Analysis

Statistical analyses were undertaken using IBM Statistical Package for the Social Sciences (SPSS) version 21.0. Scale variables such as age, haemoglobin (Hb), platelet count (Plt), haematocrit (Ht), prothrombin time (PT), Activated partial thromboplastin time (aPTT) and international normalisation (INR) were categorised according to their respective median values and presented with inter quartile range (IQR). Upon recognition of the coagulopathy group, a paired t test was performed to evaluate the impact of blood and blood component transfusion on respective values of platelet count (Plt), haematocrit (Ht), prothrombin time (PT), Activated partial thromboplastin time (aPTT) and international normalisation (INR) (before and after). A p value of 0.05 or less was deemed to be statistically significant at 95% confidence interval.

7.3 Results

The median age of the cohort was 75 years (IQR, 51-92) with male predominance (male n=66, 85% vs. female n=16, 19.5%, Table 1). The median size of rAAA was 75.52 mm (IQR, 40-100). Pre-existing hypertension was found in 60.9%, n=50, chronic obstructive pulmonary disease (COPD) in 28 %, n=23, active smokers in 23%, n=19 and acute renal failure was noted in three patients (3.9%). The median presenting value for Hb prior to operation was 11.3 mg/dl (IQR, 9.45-13.3,) whilst the median platelet count was 176×10^9/l (IQR, 122×10^9/l -210×10^9/l). The median value for PT was 13.1 (IQR, 10.1-35, mean= 14.79), for APTT was 29.55 (IQR 20.4- 240, mean=34.7) and for INR was 1.12 (IQR, 0.9-2.91, mean= 1.25).

		n=	%
Gender	Male	12	85.7%
	Female	2	14.3%
Age	18-75 years	5	35.7%
	>75 years	9	64.3%
ASA Grade	2	2	14.3%
	3	2	14.3%
	4	6	42.9%
	5	4	28.6%
Hypertension	No Hypertension	7	50.0%
	Hypertensive	7	50.0%
COPD	No COPD	13	92.9%
	COPD	1	7.1%
Smoking Status	Non-Smoker	12	85.7%
	Smoker	2	14.3%
Hemoglobin Pre-Op	Up to 11.5g/l	9	64.3%
	>11.5g/l	5	35.7%

Table 7.1 Demographic of patients with coagulopathy(n=14/82)

Pre Transfusion Coagulation Profile

According to the defined parameters only 17.1% (n=14/82) of the entire cohort was found to have coagulopathy (INR>1.2 and PLT< 150×10^9/l). Only one patient was found to have significant coagulopathy (INR>1.5 and PLT <100×10^9/l) (Table 7.1) (Table 7.2). The median value for Hemoglobin (Hb) was 9.95mg/dl (IQR, 6.9 to14.8), for PT was 15.3 (IQR, 14.5 to 35), for aPTT 34.55 (IQR, 24.1 to 69.4), Plt 113.5×10^9/l (IQR,62-146×10^9/l) and for INR was 1.35 (IQR, 1.22 to 2.91).

Age	Sex	AAAsize	Platelet	HB	PT	APTT	INR
88	M	90	65	9.4	14.9	50.9	1.25
88	M	70	116	6.9	35	30	2.91
87	M	60	116	6.9	35	30	2.91
87	M	53	145	13.3	15.4	26.9	1.31
80	M	90	113	13.7	16	33.1	1.34
78	F	60	114	4.5	18.5	50.9	1.64
76	M	84	95	11.3	15.3	30.1	1.28
76	M	80	99	10.5	16.7	47.9	1.4
76	M	70	135	9.1	19.6	51.9	
75	F	100	69	11.6	16.6	36	1.45
71	M	90	62	7.6	24.3	69.4	2.03
68	M	95	72	12.5	14.6	31.5	1.22
64	M	80	146	14.8	14.5	24.1	1.23
63	M	65	122	7.8	15.7	39	1.35

*AAA size is in mms.

Table 7.2 Age, gender & coagulation profile of patients
(Prior to transfusion of blood & blood component transfusion).

Post Transfusion Coagulation Profile

Following the transfusion of blood and blood component products (Table 7.4) in 14 individuals that were found to be coagluopathic according to the defined

parameters, nine individuals (n=9/14, 64%) were still found to be in coagulopathy following the repair of rAAAs. The median value for Hb was 10.3 mg/dl (IQR, 7.5 to 12.9), for PT was 16.1 (IQR, 13.1 to 22.9), for aPTT 32.6 (IQR, 25.7 to 123), Plt 95×10^9/l (IQR,46-275×10^9/l) and for INR was 1.31 (IQR, 1.1 to 2.5).

Age	Sex	Platelet	Hb	PT	APTT	INR
88	M	89	12.9	13.1	34.3	1.1
88	M	126	9.5	16.5	25.7	1.38
87	M	116	10.5	17.5	27.7	1.46
87	M					
80	M	74	12.4	14.9	29.7	1.25
78	F	82	7.5	16.9	44.7	1.49
76	M	95	11.3	15.3	30.1	1.28
76	M	115	10.1	16.1	32.6	1.35
76	M	160	11	21.8	46.5	?
75	F	275	10.3	22.9	26.4	2.15
71	M	58	9.6	13.8	33.4	1.16
68	M	84	11.4	13.2	29.4	1.11
64	M	109	8.4	14.3	39.1	1.21
63	M	46	7.8	19	123.5	1.7

Table 7.3 Age, gender & coagulation profile of patients (Post transfusion of blood & blood component transfusion).

Age	Sex	PRBC	FFP	PLT
88	M	11	14	2
88	M	5	4	1
87	M	5	4	1
87	M	2	0	0
80	M	14	4	2
78	F			
76	M	10	6	0
76	M	0	7	1
76	M	16	4	3
75	F	10	4	0
71	M	14	6	2
68	M	12	4	2
64	M	12	2	2
63	M			

***** The empty space indicates the lack of data

Table 7.4 Details of blood & blood component transfusion in all coagulopathic patients.

Impact of Transfusion

Statistical analysis demonstrated no significant change of coagulation profile (coagulopathy) following transfusion of blood and blood component transfusion in any values of PT (p=0.53), aPPT (p=0.98), INR (p=0.17) and Plt (p=0.66) at 95% confidence interval (Table 7.5).

Markers /Mean	Std. D	Std. Error Mean	95% Confidence Interval		P value
			Lower	Upper	
Pre & post PT/1.6	9.49493	2.53763	-3.85007	7.11435	.531
Pre & post aPPT/ 0.13077	27.94368	7.75018	-16.75542	17.01696	.987
Pre & Post INR/ 0.28083	.66977	.19335	-.14472	.70638	.174
Pre & Post Plt/ -8.07692	66.45482	18.43125	-48.23517	32.08132	.669

Table 7.5 Paired t-test analysis of pre & post transfusion of blood & blood component transfusion.

7.4 Discussion

The findings of this study demonstrates that transfusion of blood products of fresh frozen plasma and platelet has no impact on correction of coagulopathy in limited number of individuals (n=14) that do present with such status. Fresh frozen plasma (FFP) normally contains two important components, namely factor V and factor VIII (Mumford et al., 2014). The use of FFP remains vital in known coagulation disorders of factor V, Antithrombin III deficiency and reversal of anticoagulants such as warfarin (Keeling et al., 2011). Despite these findings, their empiric use as a part of massive transfusion protocol has

been on the rise in last one decade (Duchesne et al.,2008). The depletion of coagulation factors of V and VIII in patients with hemorrhagic shock are not common and the majority of coagulation disorders are secondary to the loss of circulatory volume and its subsequent effect on platelet count. The universal transfusion of FFP to reverse hemostatic disorders should be limited to individuals with known factor deficiencies (factor V and VIII) and/or replacement of presumed depleted factors (Keeling et al., 2011). The finding of this study appears to complement earlier research that prophylactic transfusion of FFP as a part of set transfusion ratio in not indicated and may result in adverse outcomes in rAAAs (Kordzadeh, 2015: Calballero, 2014). Furthermore, the transfusion of FFP irrespective of the cause of the hemorrhage could contribute to transfusion related acute lung injury (TRALI), increase surgical wound infections and cardiac failure (Rudman, 2005). In exceedingly dire circumstances where no information on patient past medical history is available (factor V deficiency, Warfarin use), the use of FFP in combination to packed red blood cells and/ or instead may be justifiable. However, in cases of ruptured abdominal aortic aneurysm and availability of various hematological tests during the perioperative period this might not be clinically recommended. Another argument might be, once a patient had massive transfusion of PRBCs, some derangements of factors might eventually occur and transfusions of FFP might be advisable. However, in cases of ruptured abdominal aortic aneurysm, the major factor is limitation of further circulatory volume depletion and if not perioperative blood test could aid in prompt identification of hematological deficiencies (Assar and Zarins, 2009). Overall, it appears that the use of FFP in resuscitation of rAAAs is not evidence

based and their empirical use should be limited to known coagulation disorders.

According to the definition of coagulopathy (Greuters, 2011; Hunt, 2014)., only one patient was identified to suffer from severe coagulopathy (INR>1.5 and PLT <100×10^9/l) on arrival to the emergency department whereas the rest of the cohort had mild coagulopathy (INR>1.2 and PLT< 150×10^9/l). In the mild coagulopathy group, the transfusion of platelet pool not only contributed to thrombotic complications at 30-days but had no significant impact (*p*=0.66) on the platelet count of individuals that presented with lower levels (113.5×10^9/ vs 95×10^9/l). The transfusion of platelet pool was only effective in the case of severe coagulopathy (n=1). This outcome complements earlier indications for the independent transfusion of platelet in any surgical patients with values less than 50×10^9/l in practice, known cases of platelet disorders and severe coagulopathy (Van Veen et al., 2010). The other relevant use of platelet, is in cases of platelet dysfunction. This commonly occurs in individuals whom regularly take medications such as aspirin (Acetylsalicylic Acid) and/ or Clopidogrel (thienopyridine anti-platelet). However, evidence suggests that the risk of bleeding in such individuals is directly proportional to the volume of the medication and may not have the same profound effect as previously thought (Schrör, 1997). In addition, if such status is suspected (platelet dysfunction), transfusion is not necessarily the only possible option and instead platelet function and count during the perioperative period can be measured and accordingly adjusted based on clinical and laboratory data. This omits the possible adverse outcomes associated with platelet transfusion (fever,

transfusion reaction, immune-mediated platelet destruction and thrombosis) and helps in evidence driven approach in adjustments of such abnormalities (Reddy et al., 2015). Furthermore, recent evidence suggests the impact of such medication may not necessarily cause dysfunctional or inhibitory impact after all (Bartels et al., 2015).

7.5 Conclusion

Overall, it appears that empiric and/or protocol driven transfusion of fresh frozen plasma does not have any impact on correction of any type coagulopathy (mild versus sever) noted in ruptured abdominal aortic aneurysms. It should be reserved only for known cases of factor V and VIII deficiency. The transfusion of platelet pool does not have any bearing on the correction of mild coagulopathy and should be used in severe cases that are very rare.

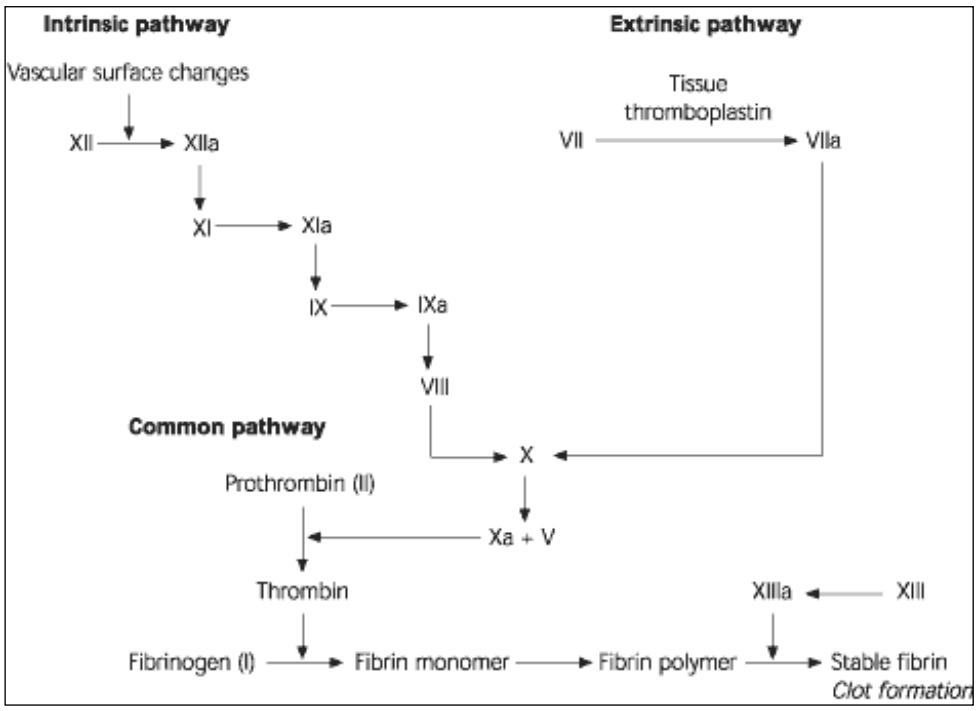
CHAPTER 8

Discussion of Thesis

The lack of literature in support of hemostatic resuscitation of ruptured abdominal aortic aneurysms (rAAA) and adoption of a protocol derived from civilian and military trauma initiated the clinical question and the platform for this thesis. A meticulous evaluation of the literature identified two major issues. Firstly, the foundation of hemostatic resuscitation in the trauma/military cohort is based entirely on the presence of coagulopathy in the vast majority of their cohort. This premise was applied to rAAAs without any conclusive and robust evidence (Kordzadeh et al., 2016). Secondly, the impact of such transfusion on ruptured abdominal aortic aneurysms was never studied nor investigated. Simply an assumption was made that hemorrhage in any type of setting mimics same presentation and pathophysiology to that of aforementioned groups of individuals and should be treated equally.

In order to identify what is the true coagulation status of ruptured abdominal aortic aneurysms, a search for a robust definition of the term “coagulopathy” was performed. Despite numerous academic definitions, only one applicable definition and its sub group terminology in daily practice was identified (Greuters, 2011, Hunt, 2014). This search highlighted that coagulopathy could be of two major sub types of mild and severe. The mild sub-type only refers to a non-significant derangement of coagulation markers that do not necessitate and/or require any correction whereas the second subtype (severe coagulopathy) demands an immediate and a responsive correction (Greuters, 2011, Hunt, 2014).

The coagulation cascade and its normal physiological process take place through two independent and well-defined pathways. These are the extrinsic and intrinsic pathways as highlighted in figure 8.1 of this chapter. In order to assess both pathways in the entire cascade every hospital and/or unit in the world refers and evaluates certain independent markers that are universal. Amongst them Prothrombin time (PT) and international normalized ratio (INR) remain the markers of extrinsic pathway and activated partial thromboplastin time (aPTT) the marker of intrinsic pathway. Another additional factor that is evaluated alongside coagulation profile (PT, aPTT and INR) is platelet count (Plt) (Hoffbrand,2006; Pallister, 2010). The platelet is an important constitute of blood that plays a vital role in clot formation and/or the coagulation process (Berridge, 2006). The normal and universally accepted range for all these markers is highlighted in Appendix (Appendix 5) of this thesis.



*All numbers refer to various factors.

Figure 8.1 Simplified coagulation cascade in formation of clot through extrinsic & intrinsic facto pathway.

With these parameters and definitions in hand I embarked on performing the first consecutive cohort study assessing the coagulation status of the ruptured abdominal aneurysm on their presentation to emergency services of Mid Essex Hospital services NHS trust. The outcome of this study demonstrated that only 17.1% of patients (n=14/82) with rAAAs present with coagulopathy. Amongst them only 7.1% (n=1/14) exhibited significant and/or severe coagulopathy that required actual correction. The remaining population had mild coagulopathy that did not demand any correction but received correction irrespective. In this study I also evaluated the impact of presenting coagulation on both outcomes of 30-day morbidity (p=0.51) and mortality (p=0.10) that demonstrated no statistical significance. Due to the outcome of this study being in conflict with prior reports and invited commentaries (Davies, 1993; Fransson, 2012) the decision was made to perform the first systematic review and meta-analysis of

the literature. The total population identified from 1966 to 2015 was 461 patients. The pooled analysis by calculation of the mean value and variance-weighted prevalence of coagulation exhibited that the majority (≥ 85%) of patients with rAAA do not present with coagulopathy. Only 6% of the entire population showed significant and/or severe coagulopathy. This finding complemented my primary study outcome of 7.1% (Kordzadeh et al., 2016) concluding that rAAAs do not present with coagulopathy and prior assumptions in practice held no merit.

Another notable outcome of this systematic review was the elevated levels of D dimer or fibrin degradation product (FDP) in 46.2% of the entire population. This product (D dimer or FDP) is the component of clot degeneration by fibrinolysis and is deployed as a blood marker (test) to determine thrombosis. This in turn suggests that 46.2% of patients with rAAA could have an activated coagulation cascade rather than coagulopathy that was not noted before. This finding in combination to the emerging evidence (Englberger et al., 2006) suggests, thrombosis might be a contributing factor to that of morbidity and mortality rather than coagulopathy secondary to hemorrhage. Given (in last decade) the method of rAAA repair has not altered and has even improved, the aforementioned finding (thrombosis) does not take its root from the surgical techniques and must have another potentiating source. This in combination with outcomes noted in my daily practice initiated the clinical question as to whether the new resuscitation strategies (blood and blood component transfusion) are contributing to these adverse outcomes (thrombosis resulting in mortality and morbidity) that might have not been noted before.

Now that it has been established that rAAAs do not present with coagulopathy and if they do it only includes a very small proportion (6-7%) and perhaps they might have an activated status (hyper coagulate). It remained crucial to understand the foundation of massive transfusion protocol in practice and to identify why rAAAs might behave different to that of military and trauma cohort.

The current evidence recommends the use of packed red blood cell (PRBC) instead of fluids as the first line therapy for the depleted volume of circulation as a consequence of hemorrhagic shock in the resuscitation period. This practice results in rapid volume expansion, optimizes oxygen transportation and/or cardiac cycle with improved survival. However, in the later phase of these studies, it was noted that the addition of other blood products such as fresh frozen plasma and platelet might also improve survival of trauma patients by inhibition of coagulopathy and/or their correction during the resuscitation phase. This resulted in recommendation of a 2:1:1 and/or 1:1:1 ratio of blood and blood component (fresh frozen plasma and platelet) transfusion for all hemorrhagic shock patients including rAAAs (Borgman et al., 2007). However, in none of these studies any patient with rAAA was included nor investigated. So this raised the clinical question that if rAAAs are not in coagulopathy and might be in hyper coagulate status does later addition of such products (fresh frozen plasma and platelet pool) contribute to thrombosis or even needed?

Therefore, thesis embarked on investigating the clinical implication of such protocol on 30-day morbidity and mortality of rAAAs. The evaluation of blood product transfusion on 30-day mortality and morbidity of rAAAs highlighted that

the transfusion of > 3 units of fresh frozen plasma (FFP) regardless of blood loss and transfusion of packed red blood cell (PRBC) is associated with increased mortality (p=0.02) in the rAAA cohort. In addition, transfusion of > 1 pool of platelet (Plt) independent of blood loss and transfusion of packed red blood cells (PRBC), was associated with morbidly as a result of thrombotic complications. The result of this (consecutive cohort) study coupled with activated coagulation status of rAAA exhibited that earlier blood product transfusion ratio might have far different results in rAAAs than that of hemorrhagic shock from trauma. It simply highlights that the suggested ratio of transfusion (2:1:1) (1:1:1) is not applicable to all patients and such transfusion strategies should be based on clinical and laboratory results instead of one protocol for all.

Further examination of blood markers other than those associated with the coagulation cascade in this cohort of patients (rAAA) identified another unique and previously unknown marker of morbidity. This was the neutrophil to lymphocyte ratio (NLR). The neutrophil is an immune cell that circulates in the blood and is the first cell to respond to an inflammation and/or infection within the circulation. Neutrophilia (increase in neutrophil count) has been associated with collagen remodeling, direct endothelial cell injury, pro-thrombotic state and hypercoagulability in rAAAs (Siminiak, 1995; Madjid, 2004; Coller, 2005). Lymphocytes, a subtype of white cell blood count is representative of the immune system. Lymphocytopenia (decrease in lymphocyte count) appears to be the result of redistribution and margination of lymphocytes within the lymphatic system and a marker of accelerated apoptosis (designed cell death).

Low lymphocyte count indicates a state of immunosuppression and physiological stress (Evans, 1995). The NLR > 5 were associated with increased morbidity in rAAAs (p=0.001). Given 50% of morbidity (n=11/22) was from thrombotic events, this blood marker demonstrated the active state of coagulation, physiological stress and the possible clinical impact of blood product transfusion (FFP and Plt) on this group of patients. This marker (NLR) remains the cheapest predictor of morbidity in rAAAs to date. It is a very useful tool for stratification of this group of patients during their postoperative recovery phase.

This thesis did not suffice with the impact of blood transfusion, identification of blood markers and base line coagulation of rAAAs. I also investigated the impact of such protocols on postoperative markers of coagulation (corrected vs non corrected) (Chapter 6). This is a crucial step as to whether (despite all outcomes of mortality and morbidity) the primary aim of blood product transfusion (FFP and PLT) has been effective in achieving the correction of the assumed coagulopathy.

This final study demonstrated that transfusion of blood products (FFP and Plt) does not have any (statistical) significant impact on the overall correction of coagulation. Out of 14 individuals nine remained coagulopathy post transfusion. The only correction was in one case of reported sever coagulopathy by platelet transfusion only. It appeared that the transfusion of fresh frozen plasma does not have any impact on correction of any type

coagulopathy (mild versus sever) in ruptured abdominal aortic aneurysms at all and it should be reserved for known cases of factor V and VIII deficiency only.

In conclusion, majority of ruptured abdominal aortic aneurysms do not present with coagulopathy and if they do it is not severe. Effort should be made to minimize the blood loss at first instance and if transfusion is recommended it should be limited to packed red blood cells and in sever advocated case platelet pool of limited amount. The fresh frozen plasma has no rule in correction of coagulopathy in rAAAs and should be reserved for known depleted cases of factor V and VII deficiency.

CHAPTER 9

Recommendations for Practice

This thesis has produced certain results that were now known before in the clinical practice and the following conclusion for practice was made:

- No assumption should be made that all ruptured abdominal aortic aneurysms presenting to the emergency services are in coagulopathy.
- All ruptured abdominal aortic aneurysm should have a coagulation profile and/or screening on arrival to the emergency services.
- Only 6-7 % of all ruptured abdominal aortic aneurysm present to emergency services with severe coagulopathy.
- Ruptured abdominal aortic aneurysms present with elevated levels of D dimer and/or fibrin degradation product (FDP) in 46.2% of cases.
- Ruptured abdominal aortic aneurysms might possess a hyper coagulate status rather than coagulopathy.
- If hemostatic resuscitation is indicated, packed red blood cell (PRBC) can be transfused and it is evidence based.
- No additional blood product (Fresh frozen plasma and platelet pool) should be transfused as a part of any protocol unless clearly indicated through available laboratory results.
- Platelet pool of more than 1 contributes to thrombotic complications independent of any other blood and/or blood component transfusion, therefore their use should be limited and subjected to clinical and laboratory findings.

- The fresh frozen plasma has no rule in correction of coagulopathy in rAAAs and should be reserved for known depleted cases of factor V and VII deficiency.
- Fresh frozen plasma (FFP) transfusion of more than 3 units, independent of any other blood and/or blood component transfusion can contribute to mortality and their use beyond this point should be clinically justified.
- Neutrophil to lymphocyte ratio (NLR) > 5 can be used as a predictive marker of morbidity.
- Neutrophil to lymphocyte ratio (NLR) can be used as risk stratification tool to justify longer and closer post-operative monitoring though intensive care and/or high dependency unit.
- Coagulopathy in rAAAs is a complex event and no specific blood product transfusion can single handily rectify this cascade and attention to other possible products may be required.

Recommendations for Future Research.

- Protocols derived from different cohorts of patients cannot, in modern day practice, be applied to another field of medicine without prior investigation.
- Ruptured abdominal aortic aneurysms (rAAA) possess a different cellular, physiological and coagulation status to that of trauma cohort and this requires substantive research.
- A larger number of patients (rAAA) might be needed to complement and/or refute the current findings.
- The possible hyper coagulate status of rAAAs requires further in-depth (cellular, molecular and physiological) investigation.
- Optimal transfusion ratio in rAAAs should await further investigations.
- All future investigations should attempt to report coagulation in a universally accepted manner for optimal collation of the data.

REFERENCES

Adam, D.J., Ludlam, C.A., Ruckley, C.V. and Bradbury, A.W.,1999. Coagulation and fibrinolysis in patients undergoing operation for ruptured and nonruptured infrarenal abdominal aortic aneurysms. Journal of Vascular Surgery, 30(4), pp.641–650.

Al-Barjas, H.S., Ariens, R., Grant, P. and Scott, J.A., 2006. Raised plasma Fibrinogen concentration in patients with abdominal Aortic Aneurysm. Angiology, 57(5), pp.607–614.

Angel Caballero, A.C., 2014. Prophylactic use of fresh frozen plasma in patients undergoing liver Resection: Does it make any sense? Journal of Anesthesia & Clinical Research, 05(11).

Appleton, N.D., Bailey, D.M., Morris-Stiff, G. and Lewis, M.H., 2014. Neutrophil to Lymphocyte ratio predicts Perioperative mortality following open elective repair of abdominal Aortic Aneurysms. Vascular and Endovascular Surgery, 48(4), pp.311–316.

Assar, A.N. and Zarins, C.K., 2009. Ruptured abdominal aortic aneurysm: A surgical emergency with many clinical presentations. Postgraduate Medical Journal, 85(1003), pp.268–273.

Association of neutrophil to lymphocyte ratio with presence of isolated coronary artery ectasia, 2013. Turk Kardiyoloji Dernegi Arsivi-Archives of the Turkish Society of Cardiology, 41(2), pp.123–130.

Baird, P.A., Sadovnick, A.D., Cole, C.W. and Cole, L., 1995. Sibling risks of abdominal aortic aneurysm. The Lancet, 346(8975), pp.601–604.

Bartels, A., Sarpong, Y., Coberly, J., Hughes, N., Litt, J., Quick, J., Kessel, J., Nelson, C., Coughenour, J., Barnes, S.L., Litofsky, N.S., Hammer, R.D. and Ahmad, S., 2015. Failure of the Platelet function assay (PFA)-100 to detect antiplatelet agents. Surgery, 158(4), pp.1012–1019.

Berridge, M.J., 2014. Module 11: Cell stress, inflammatory responses and cell death. Cell Signalling Biology, 6, p.csb 0001011.

Bhutta, H., Agha, R., Wong, J., Tang, T.Y., Wilson, Y.G. and Walsh, S.R., 2011. Neutrophil-Lymphocyte ratio predicts medium-term survival following elective major vascular surgery: A cross-sectional study. Vascular and Endovascular Surgery, 45(3), pp.227–231.

Bobb, G.A. and Fairchild, E.J., 1967. Neutrophil-to-lymphocyte ratio as indicator of ozone exposure. Toxicology and Applied Pharmacology, 11(3), pp.558–564.

Borgman, M.A., Spinella, P.C., Perkins, J.G., Grathwohl, K.W., Repine, T., Beekley, A.C., Sebesta, J., Jenkins, D., Wade, C.E. and Holcomb, J.B., 2007. The ratio of blood products transfused affects mortality in patients receiving massive transfusions at a combat support hospital. The Journal of Trauma: Injury, Infection, and Critical Care, 63(4), pp.805–813.

Brady, A.R., 2004. Abdominal Aortic Aneurysm expansion: Risk factors and time intervals for surveillance. Circulation, 110(1), pp.16–21.

Brewstera, D.C., Cronenwett, J.L., Hallett, J.W., Johnston, K.W., Krupski, W.C. and Matsumura, J.S., 2003. Guidelines for the treatment of abdominal aortic aneurysms: Report of a subcommittee of the joint council of the American association for vascular surgery and society for vascular surgery. Journal of Vascular Surgery, 37(5), pp.1106–1117.

Brohi, K., Cohen, M.J., Ganter, M.T., Matthay, M.A., Mackersie, R.C. and Pittet, J.-F., 2007. Acute traumatic Coagulopathy: Initiated by Hypoperfusion. Annals of Surgery, 245(5), pp.812–818.

Brohi, K., Singh, J., Heron, M. and Coats, T., 2003. Acute traumatic Coagulopathy. The Journal of Trauma: Injury, Infection, and Critical Care, 54(6), pp.1127–1130.

Cervantes, J., 2003. Reflections on the 50th anniversary of the first abdominal aortic aneurysm resection. World journal of surgery., 27(2), pp.246–8.

Chung, J., Corriere, M.A., Veeraswamy, R.K., Kasirajan, K., Milner, R., Dodson, T.F., Salam, A.A. and Chaikof, E.L., 2010. Risk factors for late mortality after endovascular repair of the thoracic aorta. Journal of Vascular Surgery, 52(3), pp.549–555.

Cohen, J. and Graver, L., 1990. The ruptured abdominal aortic aneurysm of Albert Einstein. Surgery, gynecology & obstetrics., 170(5), pp.455–8.

Coller, B.S., 2005. Leukocytosis and Ischemic vascular disease morbidity and mortality: Is it time to intervene? Arteriosclerosis, Thrombosis, and Vascular Biology, 25(4), pp.658–670.

Cosford, P. and Leng, G., 2007. Screening for abdominal aortic aneurysm. The Cochrane database of systematic reviews.

Cosgriff, N., Moore, E.E., Sauaia, A., Kenny-Moynihan, M., Burch, J.M. and Galloway, B., 1997. Predicting life-threatening Coagulopathy in the massively transfused trauma patient. The Journal of Trauma: Injury, Infection, and Critical Care, 42(5), pp.857–862.

Critical Appraisal Skills Programme (CASP). Available at: <http://www.casp-uk.net/#!checklists/cb36> [Accessed 25 October 2015]> [Accessed 14 January 2017a].

Davies, M.J., Murphy, W.G., Murie, J.A., Elton, R.A., Bell, K., Gillon, J.G., McL. Jenkins, A. and Ruckley, C.V., 1993. Preoperative coagulopathy in ruptured abdominal aortic aneurysm predicts poor outcome. British Journal of Surgery, 80(8), pp.974–976.

Davis, P.M., Gloviczki, P., Cherry, K.J., Toomey, B.J., Stanson, A.W., Bower, T.C. and Hallett, J.W., 1998. Aorto-caval and ilio-iliac arteriovenous fistulae. The American Journal of Surgery, 176(2), pp.115–118.

Debakey, M.E., Cooley, D.A., Morris, G.C. and Collins, H., 1958. Arteriovenous Fistula involving the abdominal aorta. Annals of Surgery, 147(5), pp.646–658.

Dilmé, J.., Bellmunt, S., Camacho, M., Solà-Villà, D., Romero, J.., Escudero, J.. and Vila, L., 2014. Influence of cardiovascular risk factors on levels of matrix Metalloproteinases 2 and 9 in human abdominal Aortic Aneurysms. European Journal of Vascular and Endovascular Surgery, 48(4), pp.374–381.

Dindo, D., Demartines, N. and Clavien, P.-A., 2004. Classification of surgical complications. Annals of Surgery, 240(2), pp.205–213.

Dubost, C., 1952. Resection of an aneurysm of the abdominal aorta. A.m.a. archives of surgery, 64(3), p.405.

Duchesne, J.C., Hunt, J.P., Wahl, G., Marr, A.B., Wang, Y.-Z., Weintraub, S.E., Wright, M.J.O. and McSwain, N.E., 2008. Review of current blood transfusions strategies in a mature level I trauma center: Were we wrong for the last 60 years? The Journal of Trauma: Injury, Infection, and Critical Care, 65(2), pp.272–278.

Duffy, B.K., Gurm, H.S., Rajagopal, V., Gupta, R., Ellis, S.G. and Bhatt, D.L., 2006. Usefulness of an elevated Neutrophil to Lymphocyte ratio in predicting long-term mortality after Percutaneous coronary intervention. The American Journal of Cardiology, 97(7), pp.993–996.

Dutton, R.P., Mackenzie, C.F. and Scalea, T.M., 2002. Hypotensive resuscitation during active hemorrhage: Impact on in-hospital mortality. The Journal of Trauma: Injury, Infection, and Critical Care, 52(6), pp.1141–1146.

Edgington, T., Mackman, N., Brand, K. and Ruf, W., 1991. The structural biology of expression and function of tissue factor. Thrombosis and haemostasis., 66(1), pp.67–79.

Englberger, L., Savolainen, H., Jandus, P., Widmer, M., Do, D.D., Haeberli, A., Baumgartner, I., Carrel, T.P. and Schmidli, J., 2006. Activated coagulation during open and endovascular abdominal aortic aneurysm repair. Journal of Vascular Surgery, 43(6), pp.1124–1129.

Epstein, F.H. and Latchman, D.S., 1996. Transcription-factor mutations and disease. New England Journal of Medicine, 334(1), pp.28–33.

Evans, M.A., Hodge, D.O., Smars, P.A., Thomson, S.P. and Raymond, J.G., n.d. The prognostic value of the leukocyte differential in the evaluation of patients presenting with chest pain. Circulation, 8 (Supplement)(92), p.I664.

Ferrante, F., 1999. Recent advances in anaesthesia and analgesia A.P. Adams, M.B., B.S., and J.N. Cashman, B.Sc., M.B., Eds. Churchill-Livingstone, London. Regional Anesthesia and Pain Medicine, 24(5), p.489.

Fielding, J.W., Black, J., Ashton, F., Slaney, G. and Campbell, D.J., 1981. Diagnosis and management of 528 abdominal aortic aneurysms. BMJ, 283(6287), pp.355–359.

Fitzgerald, E.J. and Spence, L.D., 1996. Pre-operative computed tomography in abdominal aortic aneurysms. Postgraduate Medical Journal, 72(850), pp.484–486.

Fransson, M., Rydningen, H. and Henriksson, A.E., 2011. Early Coagulopathy in patients with ruptured abdominal Aortic Aneurysm. Clinical and Applied Thrombosis/Hemostasis, 18(1), pp.96–99.

Fujita, H., Nishimura, S., Hazama, Y., Moriyama, A., Daibo, K., Ohtake, C., Shinozuka, K., Fujimoto, S. and Kamesaki, M., 2012. Coagulopathy and transfusion product usage in relation to ruptured abdominal aortic aneurysm scoring systems in a tertiary care centre in Japan10(3).

Furie, B., 2008. Mechanisms of Thrombus formation. New England Journal of Medicine, 359(9), pp.938–949.

Gatt, M., Goldsmith, P., Martinez, M., Barandiaran, J., Grover, K., El-Barghouti, N. and Perry, E.P., 2009a. Do scoring systems help in predicting survival following ruptured abdominal Aortic Aneurysm surgery? The Annals of The Royal College of Surgeons of England, 91(2), pp.123–127.

Gatt, M., Goldsmith, P., Martinez, M., Barandiaran, J., Grover, K., El-Barghouti, N. and Perry, E.P., 2009b. Do scoring systems help in predicting survival following ruptured abdominal Aortic Aneurysm surgery? The Annals of The Royal College of Surgeons of England, 91(2), pp.123–127.

Gertler, J.P., Cambria, R.P., Brewster, D.C., Davison, J.K., Purcell, P., Zannetti, S., Johnson, S., L'Italien, G., Koustas, G., LaMuraglia, G.M., Laposata, M. and Abbott, W.M., 1996. Coagulation changes during thoracoabdominal aneurysm repair. Journal of Vascular Surgery, 24(6), pp.936–945.

Getaz, E.P. and Louw, J.H., 1977. The coagulopathy associated with aortic aneurysms. Postgraduate Medical Journal, 53(625), pp.668–671.

Gibson, P.H., Croal, B.L., Cuthbertson, B.H., Small, G.R., Ifezulike, A.I., Gibson, G., Jeffrey, R.R., Buchan, K.G., El-Shafei, H. and Hillis, G.S., 2007. Preoperative neutrophil-lymphocyte ratio and outcome from coronary artery bypass grafting. American Heart Journal, 154(5), pp.995–1002.

Go, A., Chertow, G. and Fan, D., 2005. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. Journal of Vascular Surgery, 41(1), p.177.

Gomez, D., Morris-Stiff, G., Toogood, G.J., Lodge, J.P.A. and Prasad, K.R., 2008. Impact of systemic inflammation on outcome following resection for intrahepatic cholangiocarcinoma. Journal of Surgical Oncology, 97(6), pp.513–518.

Graham, A.P., Fitzgerald O'Connor, E., Hinchliffe, R.J., Loftus, I.M., Thompson, M.M. and Black, S.A., 2012. The use of heparin in patients with ruptured abdominal aortic aneurysms. Vascular, 20(2), pp.61–64.

Greuters, S., van den Berg, A., Franschman, G., Viersen, V.A., Beishuizen, A., Peerdeman, S.M., Boer, C. and ALARM-BLEEDING investigators, T., 2011. Acute and delayed mild coagulopathy are related to outcome in patients with isolated traumatic brain injury. Critical Care, 15(1), p.R2.

Hademenos, G.J., Massoud, T., Valentino, D.J., Duckwiler, G. and Vinuela, F., 1994. A nonlinear mathematical model for the development and rupture of intracranial saccular aneurysms. Neurological Research, 16(5), pp.376–384.

Hallett, J.W., Mills, J.L. and Earnshaw, J., 2014. Comprehensive vascular and Endovascular surgery: Expert consult - online and print. 2nd ed. Philadelphia, PA: Mosby/Elsevier.

Hamilton, H., Constantinou, J. and Ivancev, K., 2014. The role of permissive hypotension in the management of ruptured abdominal aortic aneurysms. The Journal of cardiovascular surgery., 55(2), pp.151–9.

Hardman, D.T.A., Fisher, C.M., Patel, M.I., Neale, M., Chambers, J., Lane, R. and Appleberg, M., 1996. Ruptured abdominal aortic aneurysms: Who should be offered surgery? Journal of Vascular Surgery, 23(1), pp.123–129.

Haumer, M., Amighi, J., Exner, M., Mlekusch, W., Sabeti, S., Schlager, O., Schwarzinger, I., Wagner, O., Minar, E. and Schillinger, M., 2005. Association of neutrophils and future cardiovascular events in patients with peripheral artery disease. Journal of Vascular Surgery, 41(4), pp.610–617.

Henriksson, A.E., 2012. The impact of blood component transfusion practices on patient survival after abdominal Aortic Aneurysm surgery. Vascular and Endovascular Surgery, 47(1), pp.38–41.

Hobbs, S.D., Claridge, M.W.C., Quick, C.R.G., Day, N.E., Bradbury, A.W. and Wilmink, A.B.M., 2003. LDL cholesterol is associated with small abdominal Aortic Aneurysms. European Journal of Vascular and Endovascular Surgery, 26(6), pp.618–622.

Hoffbrand, A.V., Pettit, J.E. and Moss, P., n.d. Essential haematology. 4th ed. Malden, MA: Blackwell Publishing.

Holcomb, J.B., 2010. Traditional transfusion practices are changing. Critical Care, 14(3), p.162.

Holcomb, J.B., Wade, C.E., Michalek, J.E., Chisholm, G.B., Zarzabal, L.A., Schreiber, M.A., Gonzalez, E.A., Pomper, G.J., Perkins, J.G., Spinella, P.C., Williams, K.L. and Park, M.S., 2008. Increased plasma and Platelet to red blood cell ratios improves outcome in 466 massively transfused civilian trauma patients. Transactions of the ... Meeting of the American Surgical Association, 126, pp.97–108.

Holt, P.J.E., Poloniecki, J.D., Loftus, I.M., Michaels, J.A. and Thompson, M.M., 2007. Epidemiological study of the relationship between volume and outcome after abdominal aortic aneurysm surgery in the UK from 2000 to 2005. British Journal of Surgery, 94(4), pp.441–448.

Hunt, B.J., 2014. Bleeding and Coagulopathies in critical care. New England Journal of Medicine, 370(9), pp.847–859.

Illig, K.A., Green, R.M., Ouriel, K., Riggs, P.N., Bartos, S., Whorf, R., DeWeese, J.A., Chhibber, A., Marder, V.J. and Francis, C.W., 1997. Primary fibrinolysis during supraceliac aortic clamping. Journal of Vascular Surgery, 25(2), pp.244–254.

Iriz, E., Ozdogan, M.E., Erer, D., Uslu, M., Koksal, P. and Kurtipek, O., 2006. A giant aortocaval fistula due to abdominal aortic aneurysm. International Journal of Cardiology, 112(3), pp.e78–e80.

Janion, M. and Kurzawski, J., 2008. Atrial fibrillation in patients with atrial septal aneurysm. Cardiology journal., 14(6), pp.580–4.

Johnston, K.W., Rutherford, R.B., Tilson, M.D., Shah, D.M., Hollier, L. and Stanley, J.C., 1991. Suggested standards for reporting on arterial aneurysms. Journal of Vascular Surgery, 13(3), pp.452–458.

Kaufman, J.L., 2002. Regarding 'the 50th anniversary of abdominal aortic reconstruction'. Journal of Vascular Surgery, 35(3), pp.623–624.

Kauvar, D.S., Sarfati, M.R. and Kraiss, L.W., 2012. Intraoperative blood product resuscitation and mortality in ruptured abdominal aortic aneurysm. Journal of Vascular Surgery, 55(3), pp.688–692.

Keeling, D., Baglin, T., Tait, C., Watson, H., Perry, D., Baglin, C., Kitchen, S. and Makris, M., 2011. Guidelines on oral anticoagulation with warfarin - fourth edition. British Journal of Haematology, 154(3), pp.311–324.

Kertai, M.D., Boersma, E., Westerhout, C.M., Domburg, R. van, Klein, J., Bax, J.J., Urk, H. van and Poldermans, D., 2004. Association between long-term statin use and mortality after successful abdominal aortic aneurysm surgery. The American Journal of Medicine, 116(2), pp.96–103.

Kheirbek, T., Kochanek, A.R. and Alam, H.B., 2009. Hypothermia in bleeding trauma: A friend or a foe? Scandinavian Journal of Trauma, Resuscitation and Emergency Medicine, 17(1), p.65.

King, V.L., 2006. Selective Cyclooxygenase-2 inhibition with Celecoxib decreases angiotensin II-Induced abdominal Aortic Aneurysm formation in mice. Arteriosclerosis, Thrombosis, and Vascular Biology, 26(5), pp.1137–1143.

Kingdom, U., 2002. Long-term outcomes of immediate repair compared with surveillance of small abdominal Aortic Aneurysms. New England Journal of Medicine, 346(19), pp.1445–1452.

Kordzadeh, A., 2015. Factors associated with progression of the abdominal aortic aneurysm and novel inhibition therapies. Reviews in Vascular Medicine, 3(3), pp.22–26.

Kordzadeh, A., Askari, A. and Panayiotopoulos, Y., 2013. Atmospheric pressure and infra-renal abdominal aortic aneurysm rupture: A single observational study and a comprehensive review of literature. International Journal of Surgery, 11(6), pp.458–462.

Kordzadeh, A., Askari, A., Parsa, A.D., Browne, T. and Panayiotopoulos, Y.P., 2015. The clinical implication of blood product transfusion on morbidity and mortality of ruptured abdominal Aortic Aneurysm. Clinical and Applied Thrombosis/Hemostasis, DOI: 10.1177/1076029615624548

Kordzadeh, A., Rhodes, K.M., Hanif, M.A., Scott, H. and Panayiotopoulos, Y., 2013. Ruptured Cryptogenic Mycotic Abdominal Aortic Aneurysm by salmonella enteritidis. Annals of Vascular Surgery, 27(7), p.973.e9–973.e17.

Kordzadeh,A., Parsa, A.D., Askari, A., Maddison, B. and Panayiotopoulos, Y.,2016. Presenting Baseline Coagulation of Infra Renal Ruptured Abdominal Aortic Aneurysm: A Systematic Review and Pooled Analysis. European Journal of Vascular and Endovascular Surgery, 51(5).

Kordzadeh,A., Malietzis, G., Browne,T., Prionidis,I., Panayiotopoulos, Y.,2015 Neutrophil to lymphocyte ratio (NLR) of five predicts 30-day morbidity in ruptured abdominal aortic aneurysms (rAAA): A retrospective cohort study. International Journal of Surgery DOI:10.1016/j.ijsu.2015.01.013

Korner, P.I., 1989. Baroreceptor resetting and other determinants of baroreflex properties in hypertension. Clinical and Experimental Pharmacology and Physiology, 16(s15), pp.45–64.

Kuhn, M., Bonnin, R.L.L., Davey, M.J., Rowland, J.L. and Langlois, S.L.P., 2000. Emergency department ultrasound scanning for abdominal Aortic Aneurysm: Accessible, accurate, and advantageous. Annals of Emergency Medicine, 36(3), pp.219–223.

Lawrence, D.M., Singh, R.S., Franklin, D.P., Carey, D.J. and Elmore, J.R., 2004. Rapamycin suppresses experimental aortic aneurysm growth. Journal of Vascular Surgery, 40(2), pp.334–338.

Lederle, F.A., Nelson, D.B. and Joseph, A.M., 2003. Smokers' relative risk for aortic aneurysm compared with other smoking-related diseases: A systematic review. Journal of Vascular Surgery, 38(2), pp.329–334.

Lederle, F.A., Wilson, S.E., Johnson, G.R., Reinke, D.B., Littooy, F.N., Acher, C.W., Ballard, D.J., Messina, L.M., Gordon, I.L., Chute, E.P., Krupski, W.C., Busuttil, S.J., Barone, G.W., Sparks, S., Graham, L.M., Rapp, J.H., Makaroun, M.S., Moneta, G.L., Cambria, R.A., Makhoul, R.G., Eton, D., Ansel, H.J., Freischlag, J.A. and Bandyk, D., 2002. Immediate repair compared with surveillance of small abdominal Aortic Aneurysms. New England Journal of Medicine, 346(19), pp.1437–1444.

Ledgerwood, A. and Lucas, C., 2003. A review of studies on the effects of hemorrhagic shock and resuscitation on the coagulation profile. The Journal of trauma., 54.

Levy, J.H., Welsby, I. and Goodnough, L.T., 2013. Fibrinogen as a therapeutic target for bleeding: A review of critical levels and replacement therapy. Transfusion, 54(5), pp.1389–1405.

Länne, T., Sandgren, T. and Sonesson, B., 1998. A dynamic view on the diameter of abdominal aortic aneurysms. European Journal of Vascular and Endovascular Surgery, 15(4), pp.308–312.

Madjid, M., Awan, I., Willerson, J.T. and Casscells, S.W., 2004. Leukocyte count and coronary heart disease. Journal of the American College of Cardiology, 44(10), pp.1945–1956.

Makrygiannis, G., Courtois, A., Drion, P., Defraigne, J.-O., Kuivaniemi, H. and Sakalihasan, N., 2014. Sex differences in abdominal Aortic Aneurysm: The role of sex hormones. Annals of Vascular Surgery, 28(8), pp.1946–1958.

Malietzis, G., Giacometti, M., Askari, A., Nachiappan, S., Kennedy, R.H., Faiz, O.D., Aziz, O. and Jenkins, J.T., 2014. A Preoperative Neutrophil to Lymphocyte ratio of 3 predicts disease-free survival after curative elective Colorectal cancer surgery. Annals of Surgery, 260(2), pp.287–292.

Mantovani, A. and Garlanda, C., 1999. Cytokine activation of endothelial cells: New molecules in an old paradigm. Atherosclerosis, 144, p.86.

Mantovani, a., Sozzani, s. and Introna, m., 1997. Endothelial activation by cytokines. Annals of the new york academy of sciences, 832(1 Phagocytes), pp.93–116.

Markov, N.P., DuBose, J.J., Scott, D., Propper, B.W., Clouse, W.D., Thompson, B., Blackbourne, L.H. and Rasmussen, T.E., 2012. Anatomic distribution and mortality of arterial injury in the wars in Afghanistan and Iraq

with comparison to a civilian benchmark. Journal of Vascular Surgery, 56(3), pp.728–736.

Marsh, N., 1990. Annotation DOES HEPARIN STIMULATE FIBRINOLYSIS? British Journal of Haematology, 76(2), pp.163–167.

Marston, W.A., Ahlquist, R., Johnson, G. and Meyer, A.A., 1992. Misdiagnosis of ruptured abdominal aortic aneurysms. Journal of Vascular Surgery, 16(1), pp.17–22.

Meijer, C.A., Kokje, V.B.C., van Tongeren, R.B.M., Hamming, J.F., van Bockel, J.H., Möller, G.M. and Lindeman, J.H.N., 2012. An association between chronic obstructive pulmonary disease and abdominal Aortic Aneurysm beyond smoking. European Journal of Vascular and Endovascular Surgery, 44(2), pp.153–157.

Mell, M.W., O'Neil, A.S., Callcut, R.A., Acher, C.W., Hoch, J.R., Tefera, G. and Turnipseed, W.D., 2010. Effect of early plasma transfusion on mortality in patients with ruptured abdominal aortic aneurysm. Surgery, 148(5), pp.955–962.

Melton, L., Bickerstaff, L., Hollier, L., Peenen, V., Lie, J., Pairolero, P., Cherry, K. and O'Fallon, W., 1984. Changing incidence of abdominal aortic aneurysms: A population-based study. American journal of epidemiology., 120(3), pp.379–86.

Mikhail, J., 1999. The trauma triad of death: Hypothermia, Acidosis, and Coagulopathy. AACN Clinical Issues: Advanced Practice in Acute and Critical Care, 10(1), pp.85–94.

Mofidi, R., Goldie, V.J., Kelman, J. and Dawson, A.R.W., 2007. Influence of sex on expansion rate of abdominal aortic aneurysms. Journal of Vascular Surgery, 45(6), p.1286.

Moher, D., 2009. Preferred reporting items for systematic reviews and Meta-Analyses: The PRISMA statement. Annals of Internal Medicine, 151(4), p.264.

Moll, F.L., Powell, J.T., Fraedrich, G., Verzini, F., Haulon, S., Waltham, M., van Herwaarden, J.A., Holt, P.J.E., van Keulen, J.W., Rantner, B., Schlösser, F.J.V., Setacci, F. and Ricco, J.., 2011. Management of abdominal Aortic Aneurysms clinical practice guidelines of the European society for vascular surgery. European Journal of Vascular and Endovascular Surgery, 41, pp.S1–S58.

Montán, C., Johansson, F., Hedin, U. and Wahlgren, C.M., 2015. Preoperative hypofibrinogenemia is associated with increased intraoperative bleeding in ruptured abdominal aortic aneurysms. Thrombosis Research, 135(3), pp.443–448.

Mosorin, M., Juvonen, J., Biancari, F., Satta, J., Surcel, H.-M., Leinonen, M., Saikku, P. and Juvonen, T., 2001. Use of doxycycline to decrease the growth

rate of abdominal aortic aneurysms: A randomized, double-blind, placebo-controlled pilot study. Journal of Vascular Surgery, 34(4), pp.606–610.

Mumford, A.D., Ackroyd, S., Alikhan, R., Bowles, L., Chowdary, P., Grainger, J., Mainwaring, J., Mathias, M. and O'Connell, N., 2014. Guideline for the diagnosis and management of the rare coagulation disorders. British Journal of Haematology, 167(3), pp.304–326.

Murphy, E.A., Danna-Lopes, D., Sarfati, I., Rao, S.K. and Cohen, J.R., 1998. Nicotine-stimulated Elastase activity release by neutrophils in patients with abdominal Aortic Aneurysms. Annals of Vascular Surgery, 12(1), pp.41–45.

Norman, P.E., 2004. Population based randomised controlled trial on impact of screening on mortality from abdominal aortic aneurysm. BMJ, 329(7477), pp.1259–0.

Office of population Census and Surveys. Mortality Statistics: cause, England and Wales, n.d. London: HMSO. Available at: <www.ons.gov.uk/.../mortality-statistics--cause--england-and-wales.../mort> [Accessed 1 January 2016]> [Accessed 14 January 2017b].

Ommen, S.R., Gibbons, R.J., Hodge, D.O. and Thomson, S.P., 1997. Usefulness of the Lymphocyte concentration as a Prognostic marker in coronary artery disease. The American Journal of Cardiology, 79(6), pp.812–814.

Osler, W., 1905. Aneurysm of the abdominal aorta. The Lancet, 166(4285), pp.1089–1096.

Packham, M.A., 1994. Role of platelets in thrombosis and hemostasis. Canadian Journal of Physiology and Pharmacology, 72(3), pp.278–284.

Pallister, C.J., Watson, M. and Garner, A., n.d. Haematology. United Kingdom: Scion Publishing.

Papa, A., Emdin, M., Passino, C., Michelassi, C., Battaglia, D. and Cocci, F., 2008. Predictive value of elevated neutrophil–lymphocyte ratio on cardiac mortality in patients with stable coronary artery disease. Clinica Chimica Acta, 395(1-2), pp.27–31.

Parodi, J.C., Palmaz, J.C. and Barone, H.D., 1991. Transfemoral Intraluminal graft implantation for abdominal Aortic Aneurysms. Annals of Vascular Surgery, 5(6), pp.491–499.

Rahe-Meyer, N., Solomon, C., Hanke, A., Schmidt, D.S., Knoerzer, D., Hochleitner, G., Sørensen, B., Hagl, C. and Pichlmaier, M., 2013. Effects of Fibrinogen concentrate as First-line therapy during Major Aortic replacement surgery. Anesthesiology, 118(1), pp.40–50.

Reddy, G.D., Gopinath, S. and Robertson, C.S., 2015. Transfusion in traumatic brain injury. Current Treatment Options in Neurology, 17(11).

Reed, M.J. and Burfield, L.C., 2013. Initial emergency department coagulation profile does not predict survival in ruptured abdominal aortic aneurysm. European Journal of Emergency Medicine, 20(6), pp.397–401.

Roberts, K., Revell, M., Youssef, H., Bradbury, A.W. and Adam, D.J., 2006. Hypotensive resuscitation in patients with ruptured abdominal Aortic Aneurysm. European Journal of Vascular and Endovascular Surgery, 31(4), pp.339–344.

Rudman, S.V., 2005. Textbook of blood banking and transfusion medicine. second ed. Philadelphia: Elsevier, Saunders.

Rutherford, R.B., Cronenwett, J.L. and Johnston, W.K., n.d. Rutherford's Vascular Surgery. 6th ed. Philadelphia: Saunders.

Rutherford, R.B. and McCroskey, B.L., 1989. Ruptured abdominal Aortic Aneurysms: Special considerations. Surgical Clinics of North America, 69(4), pp.859–868.

Sakalihasan, N., Limet, R. and Defawe, O., 2005. Abdominal aortic aneurysm. The Lancet, 365(9470), pp.1577–1589.

Salem, M.K., Rayt, H.S., Hussey, G., Rafelt, S., Nelson, C.P., Sayers, R.D., Naylor, A.R. and Nasim, A., 2009. Should Asian men be included in abdominal Aortic Aneurysm screening programmes? European Journal of Vascular and Endovascular Surgery, 38(6), pp.748–749.

Samy, A., Murray, G. and MacBain, G., 1994. Glasgow aneurysm score. Cardiovascular surgery (London, England)., 2(1), pp.41–4.

Schrör, K., 1997. Aspirin and Platelets: The Antiplatelet action of aspirin and its role in thrombosis treatment and Prophylaxis. Seminars in Thrombosis and Hemostasis, 23(04), pp.349–356.

Scott, R., 2002. The Multicentre Aneurysm screening study (MASS) into the effect of abdominal aortic aneurysm screening on mortality in men: A randomised controlled trial. The Lancet, 360(9345), pp.1531–1539.

Shantikumar, S., Ajjan, R., Porter, K.E. and Scott, D.J.A., 2010. Diabetes and the abdominal Aortic Aneurysm. European Journal of Vascular and Endovascular Surgery, 39(2), pp.200–207.

SIMINIAK, T., 1992. Neutrophil interactions with platelets and endothelium: Participation of cell-to-cell signals in the development of cardiovascular injury. Journal of Molecular and Cellular Cardiology, 24, p.91.

Skagius, E., Siegbahn, A., Bergqvist, D. and Henriksson, A., 2008. Activated Coagulation in patients with shock due to ruptured abdominal Aortic Aneurysm. European Journal of Vascular and Endovascular Surgery, 35(1), pp.37–40.

Skagius, E., Siegbahn, A., Bergqvist, D. and Henriksson, A.E., 2007. Fibrinolysis in patients with abdominal aortic aneurysm with special emphasis

on rupture and shock. Journal of Thrombosis and Haemostasis, ja(ja), p.071008055017001.

Stansbury, L.G., Dutton, R.P., Stein, D.M., Bochicchio, G.V., Scalea, T.M. and Hess, J.R., 2009. Controversy in trauma resuscitation: Do ratios of plasma to red blood cells matter? Transfusion Medicine Reviews, 23(4), pp.255–265.

Stringfellow, M.M., Lawrence, P.F. and Stringfellow, R.G., 1987. The influence of aorta-aneurysm geometry upon stress in the aneurysm wall. Journal of Surgical Research, 42(4), pp.425–433.

Sun, J., Sukhova, G.K., Yang, M., Wolters, P.J., MacFarlane, L.A., Libby, P., Sun, C., Zhang, Y., Liu, J., Ennis, T.L., Knispel, R., Xiong, W., Thompson, R.W., Baxter, B.T. and Shi, G.-P., 2007. Mast cells modulate the pathogenesis of elastase-induced abdominal aortic aneurysms in mice. Journal of Clinical Investigation, 117(11), pp.3359–3368.

Szilagyi, D.E., 1961. Expanding and ruptured abdominal Aortic Aneurysms. Archives of Surgery, 83(3), p.395.

Tambyraja, A.L., Lee, A.J., Murie, J.A. and Chalmers, R.T.A., 2008. Prognostic scoring in ruptured abdominal aortic aneurysm: A prospective evaluation. Journal of Vascular Surgery, 47(2), pp.282–286.

Tanweer, O., Wilson, T., Metaxa, E., Riina, H. and Meng, H., 2014. E-016 A comparative review of the Hemodynamics and pathogenesis of cerebral and abdominal Aortic Aneurysms: Lessons to learn from each other. Journal of NeuroInterventional Surgery, 6(Suppl 1), pp. A45.

Thompson, J.F., Mullee, M.A., Bell, P.R.F., Campbell, W.B., Chant, A.D.B., Darke, S.G., Jamieson, C.W., Murie, J., Parvin, S.D., Perry, M., Ruckley, C.V., Wolfe, J.N. and Clyne, C.A.C., 1996. Intraoperative heparinisation, blood loss and myocardial infarction during aortic aneurysm surgery: A joint vascular research group study. European Journal of Vascular and Endovascular Surgery, 12(1), pp.86–90.

Tieu, B.H., Holcomb, J.B. and Schreiber, M.A., 2007. Coagulopathy: Its Pathophysiology and treatment in the injured patient. World Journal of Surgery, 31(5), pp.1055–1065.

Tsolakis, J.A., Papadoulas, S., Kakkos, S.K., Skroubis, G., Siablis, D. and Androulakis, J.A., 1999. Aortocaval Fistula in ruptured Aneurysms. European Journal of Vascular and Endovascular Surgery, 17(5), pp.390–393.

Vammen, S., Lindholt, J.S., Ostergaard, L., Fasting, H. and Henneberg, E.W., 2001. Randomized double-blind controlled trial of roxithromycin for prevention of abdominal aortic aneurysm expansion. British Journal of Surgery, 88(8), pp.1066–1072.

Van Veen, J.J., Nokes, T.J. and Makris, M., 2010. The risk of spinal hematoma following neuraxial anesthesia or lumbar puncture in thrombocytopenic individuals. British Journal of Hematology, 148(1), pp.15–25.

Vardulaki, K.A., Walker, N.M., Day, N.E., Duffy, S.W., Ashton, H.A. and Scott, R.A.P., 2000. Quantifying the risks of hypertension, age, sex and smoking in patients with abdominal aortic aneurysm. British Journal of Surgery, 87(2), pp.195–200.

Volodos, N.L., Karpovich, I.P., Troyan, V.I., Kalashnikova, Y., Shekhanin, V.E., Volodos, S.N., Ternuk, N.E. and Ustinov, N.I., 1998. Endovascular Stented Grafts for Thoracic, abdominal Aortic, and iliac arterial disease: Clinical experience in the Ukraine from 1985. Seminars in Interventional Radiology, 15(01), pp.89–95.

Wade, C.E., Salinas, J., Eastridge, B.J., McManus, J.G. and Holcomb, J.B., 2011. Admission hypo- or hyperthermia and survival after trauma in civilian and military environments. International Journal of Emergency Medicine, 4(1), p.35.

Walton, L.J., Franklin, I.J., Bayston, T., Brown, L.C., Greenhalgh, R.M., Taylor, G.W. and Powell, J.T., 1999. Inhibition of prostaglandin E2 synthesis in abdominal Aortic Aneurysms: Implications for smooth muscle cell viability, inflammatory processes, and the expansion of abdominal Aortic Aneurysms. Circulation, 100(1), pp.48–54.

Wanhainen, A., Themudo, R., Ahlström, H., Lind, L. and Johansson, L., 2008. Thoracic and abdominal aortic dimension in 70-year-old men and women – A population-based whole-body magnetic resonance imaging (MRI) study. Journal of Vascular Surgery, 47(3), pp.504–512.

Witkowska, A.M., Borawska, M.H. and Gacko, M., 2006. Relationship among TNF-α, sICAM-1, and Selenium in Presurgical patients with abdominal Aortic Aneurysms. Biological Trace Element Research, 114(1-3), pp.31–40.

Wong, Y.Y.E., Flicker, L., Yeap, B.B., McCaul, K.A., Hankey, G.J. and Norman, P.E., 2013. Is Hypovitaminosis D associated with abdominal Aortic Aneurysm, and is there a Dose–response relationship? European Journal of Vascular and Endovascular Surgery, 45(6), pp.657–664.

Xu, R.-H. and Jiang, W.-Q., 2010. Elevated neutrophil to lymphocyte ratio predicts survival in advanced pancreatic cancer. Biomarkers, 15(6), pp.516–522.

Yamazumi, K., Ojiro, M., Okumura, H. and Aikou, T., 1998. An activated state of blood Coagulation and Fibrinolysis in patients with abdominal Aortic Aneurysm.The American Journal of Surgery, 175(4), pp.297–301.

Yoshimura, K., Aoki, H., Ikeda, Y., Fujii, K., Akiyama, N., Furutani, A., Hoshii, Y., Tanaka, N., Ricci, R., Ishihara, T., Esato, K., Hamano, K. and Matsuzaki, M., 2005. Regression of abdominal aortic aneurysm by inhibition of c-jun n-terminal kinase. *Nature Medicine*, 11(12), pp.1330–1338.

APPENDICES

Appendix 1. Ethical Approval

For audit department use only

- **Audit registration number:**

Mid Essex Hospital Services NHS
NHS Trust

- **Clinical Audit Project Proposal Form**

This form is for the use of all NHS staff who are about to undertake Clinical Audit activity at MEHT. If you are unsure about whether the project you wish to undertake is clinical audit, please complete the table on page 6 to distinguish between clinical audit, research and service evaluation projects **before completing the registration form.**

If you remain unsure about which category your project falls into, please contact either a member of the Clinical Audit Department on telephone extension 4572 or 6316 or the Research & Development department on telephone extension 4210 to discuss the project.

If completing the form electronically, please use the arrow or tab keys to navigate

Please ensure that your directorate audit lead signs this form or confirms their approval by email before it is submitted to the clinical audit team.

Once your project is finished, you should complete an MEHT report template and submit a copy to the Clinical Audit Department. The template is available on the Trust Intranet site.

Where significant non compliance has been identified by the audit, you must work with relevant stakeholders to develop an action plan with identified leads and timescales.

Project Title:
Intraoperative blood and blood component transfusion on ruptured abdominal aortic aneurysm .

Project Lead(s):

Name(s): Ali Kordzadeh
Directorate: General/ vascular and renal Department
Specialty: Vascular Surgery
Grade SPR Bleep: 6400055
Telephone: Email: ali.kordzadeh@mehl.nhs.uk
Rotation / leaving date (if applicable) : None

Name of Clinical Lead / Directorate Manager who has approved this audit:

Name: Mr. Panayiotopoulos
Title: Consulatnat general and vascular surgeon.

Priority level of topic:

Level	Priority	Detail
1	National Priority	National Clinical Audit, NCEPOD
2	Trust Priority	Patient safety concerns, complaints, NPSA Alerts, Risk Assurance Framework, regional audit, re-audit or audit of compliance with national guidance including NICE
3	Clinician Interest	Locally initiated audit not covered by the above

Please identify priority level of audit

Level 1 ☐ **Level 2** ☐ **Level 3** ☒

*It is fully recognised that there is a need to undertake locally initiated projects. These projects represent innovative ideas from clinicians and can provide valuable educational experience for junior staff. However there should be a transparent system to aid decision making about whether a locally conceived project should be undertaken. The quality impact assessment on **page 6** provides a weighted score to identify the level of significance of the audit topic.*

If topic is priority level 3, please calculate the Quality Impact Assessment number:
***Refer to page 6**: the scores can range between 0 and 42, with higher scores demonstrating higher priority.*

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The blood and blood component transfusion ratios is adopted by all vascular and trauma units in the country in accordance to literature suggesting decrease mortality and morbidity.

Define the standards of care

The purpose of any audit is to show that the care provided, is consistent with good practice. It is vital to clarify the type of audit to be undertaken and to research any existing guidelines or standards before proceeding.

Is the Clinical Audit you wish to undertake, standards based? Yes ☐ No ☒
i.e. measuring current practice agreed standards of care

If yes, please specify these standards eg NICE guidance, local guideline, royal college guidance

If no, is this a baseline audit? Yes ☒ No ☐
Baseline audits are carried out with the intention to set guidelines when there is currently no agreed best practice. The team should define 'good practice' to set auditable standards for a baseline audit.

If the project is not a clinical audit, is it:

Service evaluation? ☒ **Patient satisfaction survey?** ☐
Refer to the table on page 6

Define key criteria to be assessed

A criterion is a measurable element of healthcare which describes quality and can be used to assess it.

Define key criteria, consider any exceptions and the acceptable threshold of compliance

Criterion	Exceptions (cases to be excluded)	Target % threshold of compliance
Mortality and Morbidity in ruptured abdominal aortic aneureym		>50%

Public and Patient Involvement:

Please indicate how the public / patients have been, or will be, involved in this project

Identification of audit topic	☐	Carrying out audit project	☐
Dissemination of audit results	☐	Developing audit idea / project design	☐
Patient / Carer Survey	☐	Not suitable for public / patient involvement	☐

Individuals involved

Audits have a greater chance of success if all staff likely to be affected by the audit findings are involved from the start.
Please gain agreement from senior staff in the areas or organisations affected.
Please identify the individuals who will support or be involved in the audit.
Ali Kordzadeh, Mr.Panayiotopoulos

None ☐ Other, please specify

Do you hope to publish the outcome of this audit outside the Trust? Yes ☒ No ☐
If yes, please note that permission must be sought on completion of the audit in accordance with the Clinical Audit Strategy and Policy

Project Lead:

I understand that non-anonymised audit data must not be presented or taken outside the Trust and I confirm that as a data asset owner for this audit I will act in accordance with the principles of the Data Protection Act and Trust Information Security policies. In addition, I confirm that:

- I have considered the ethical issues involved in this audit
- I have informed senior staff in areas that will be affected by this audit about the project
- I will provide a report of the findings of the audit to the Directorate Audit Lead and the clinical audit team upon completion
- Where non compliances are identified by the audit, I will ensure an action plan is developed to address these and that any identified risks are added to the relevant Risk Assurance Framework
- If I wish to publish the audit findings outside the Trust I will gain appropriate permission beforehand

Signature of project lead: **Date:** 01/01/2014
If paper copy, otherwise email submission will act as verification

Name (printed if paper copy): Ali Kordzadeh

Directorate Audit Lead:

I approve this project and I confirm that this audit project is supported within my directorate and that consideration has been given regarding the priority of the topic within the directorate and Trust.

Signature of Directorate Audit Lead: **Date:** 08/01/14
If paper copy, otherwise email submission will act as verification

Name (printed if paper copy): Mr Jayanthi

Completion by Clinical Audit Department only

Date received: ___/___/_____

Date Registered: ___/___/_____

Registered as Clinical Audit ☐ Registration Number:

Registered as Baseline Assessment ☐ Registration number:

Registered as Service Evaluation ☐ Registration number:

Registers as Patient Satisfaction Survey ☐ Registration Number:

Thank you for completing this form

Please return this form to:
Clinical Audit Department, B346 Governance Corridor, Broomfield Hospital

Upon receipt of this form, the Clinical Audit Department will issue you with an audit registration number and place your project on the MEHT annual register of audit activity.

Research, Clinical Audit or Service Evaluation?

Please complete the tick chart below to help you decide whether the project you wish to undertake is Clinical Audit. If any of the criteria for **research** apply to your project, you should approach the R&D for assistance.

Research	Clinical Audit	Service Evaluation
Designed and conducted to generate new knowledge ☐	Designed and conducted to provide new knowledge to provide best care ☒	Designed and conducted to define current care ☐
Quantitative research hypothesis based – Qualitative research explores themes following established methodology ☐	Designed to answer the question: "Does this service reach a predetermined standard?" ☐	Designed to answer the question: "What standard does this service achieve?" ☒
	Measures against a standard ☐	Measures current service without reference to a standard ☒
May involve a new treatment ☐	Doesn't involve a new treatment ☐	Doesn't involve a new treatment ☒
May involve additional therapies, samples or investigations ☐	Involves no more than administration of questionnaire or record analysis ☐	Involves no more than administration of simple interview, questionnaire or record analysis ☒
May involve allocation to treatment groups NOT chosen by HCP or patient ☐	Does not involve allocation to treatment groups: the HCP and patients choose treatment ☐	Does not involve allocation to treatment groups: the HCP and patients choose treatment ☒
May involve randomization ☐	Does NOT involve randomization ☐	Does NOT involve randomization ☒
ALTHOUGH ANY OF THESE THREE MAY RAISE ETHICAL ISSUES, UNDER CURRENT GUIDANCE:-		
SEARCH **REQUIRES** REGIONAL ETHICS COMMITTEE REVIEW	UDIT **DOES NOT REQUIRE** R.E.C. REVIEW	RVICE EVALUATION **DOES NOT REQUIRE** R.E.C. REVIEW

Project Quality Impact Assessment Scoring

Issue / Score	No relevance (0)	Some relevance (1)	Almost met (2)	Fully Met (3)	Score
High frequency / volume service				3	
High cost services / procedure				3	
High risk – morbidity, disability, mortality				3	
Existence of evidence base, clear standards of good practice	0			3	
Direct involvement of patients, linked to service users priorities	0				
Known problems / concerns with care including variation in practice	0				
Potential for change / improving effectiveness of care				3	
Total score				15	

If the criterion has no relevance *score = 0*
If the criterion has some relevance *score = 1*
If the criterion is met in parts *score = 2*
If the criterion is fully met *score = 3*

Projects with the highest scores are likely to result in the greatest impact and should be prioritised.

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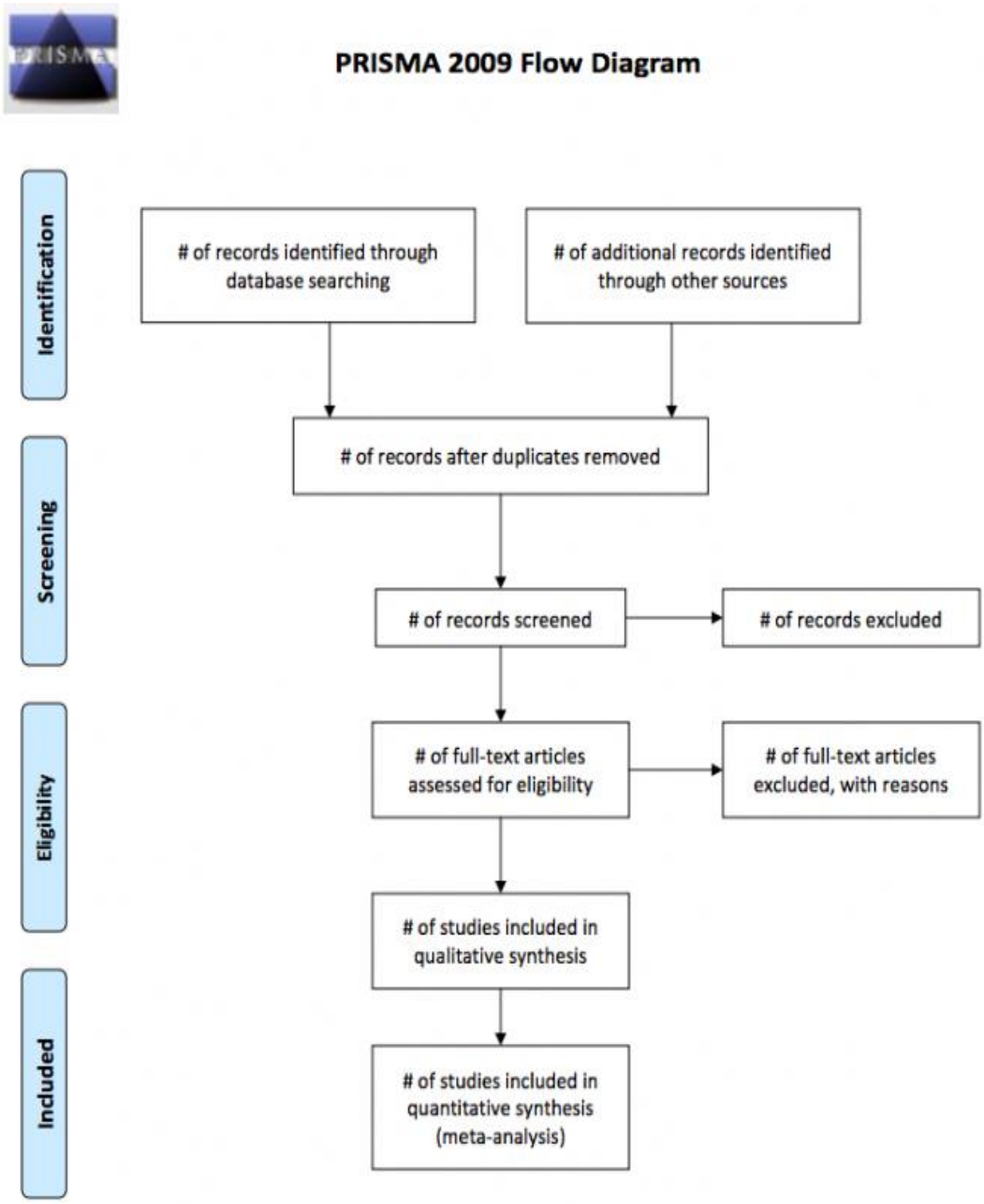
Appendix 2. PRIMSA Flow Chart.



From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement.* PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

Appendix 3. Oxford critical appraisal tool for assessing a cohort study.

12 Questions To Help You Make Sense Of Cohort Study

How to use this appraisal tool

Three broad issues need to be considered when appraising a cohort study:

Are the results of the study valid? What are the results?
Will the results help locally?

(Section A) (Section B) (Section C)

The 12 questions on the following pages are designed to help you think about these issues systematically. The first two questions are screening questions and can be answered quickly. If the answer to both is “yes”, it is worth proceeding with the remaining questions.

There is some degree of overlap between the questions, you are asked to record a “yes”, “no” or “can’t tell” to most of the questions. A number of italicised prompts are given after each question. These are designed to remind you why the question is important. Record your reasons for your answers in the spaces provided.

These checklists were designed to be used as educational pedagogic tools, as part of a workshop setting, therefore we do not suggest a scoring system. The core CASP checklists (randomised controlled trial & systematic review) were based on JAMA 'Users’ guides to the medical literature 1994 (adapted from Guyatt GH, Sackett DL, and Cook DJ), and piloted with health care practitioners.

For each new checklist a group of experts were assembled to develop and pilot the checklist and the workshop format with which it would be used. Over the years overall adjustments have been made to the format, but a recent survey of checklist users reiterated that the basic format continues to be useful and appropriate.

Referencing: we recommend using the Harvard style citation, i.e.: Critical Appraisal Skills Programme (2017). CASP (insert name of checklist i.e. Cohort Study) Checklist. [online]

Available at: *URL*. Accessed: *Date Accessed.*

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(A) Are the results of the study valid?

Screening Questions

1. Did the study address a clearly focused issue?

HINT: A question can be 'focused' In terms of

- 🕐 The population studied
- 🕐 The risk factors studied
- 🕐 The outcomes considered
- 🕐 Is it clear whether the study tried to detect a beneficial

  or harmful effect?

  2. Was the cohort recruited in an acceptable way? ❒Yes

  HINT: Look for selection bias which might compromise the generalisability of the findings:

- 🕐 Was the cohort representative of a defined population?
- 🕐 Was there something special about the cohort?
- 🕐 Was everybody included who should have been included?

  Is it worth continuing?

  Detailed questions

  3. Was the exposure accurately measured to ❒Yes

  minimise bias?

  HINT: Look for measurement or classification bias:

  - 🕐 Did they use subjective or objective measurements?
  - 🕐 Do the measurements truly reflect what you want them

    to (have they been validated)?

  - 🕐 Were all the subjects classified into exposure groups

    using the same procedure

❒Yes ❒Can't tell ❒No

❒Can't tell ❒No

4. Was the outcome accurately measured to ❒Yes ©Critical Appraisal Skills Programme (CASP) Cohort Study Checklist 13.03.17

❒Can't tell ❒No

❒Can't tell ❒No 2

minimise bias?

HINT: Look for measurement or classification bias:

- Did they use subjective or objective measurements?
- Do the measures truly reflect what you want them to

  (have they been validated)?

- Has a reliable system been established for

  detecting all the cases (for measuring disease

  occurrence)?

- Were the measurement methods similar in the

  different groups?

- Were the subjects and/or the outcome assessor

  blinded to exposure (does this matter)?

5. (a) Have the authors identified all important ❒Yes ❒Can't tell ❒No confounding factors?

List the ones you think might be important, that the author missed.

(b) Have they taken account of the

confounding factors in the design and/or analysis?

HINT: Look for restriction in design, and techniques e.g. modelling, stratified-, regression-, or sensitivity analysis to correct, control or adjust for confounding factors

❒Yes ❒Can't tell ❒No

6. (a) Was the follow up of subjects complete ❒Yes ❒Can't tell ❒No enough?

(b) Was the follow up of subjects long ❒Yes ❒Can't tell ❒No enough?

HINT: Consider
🕐 The good or bad effects should have had long enough

to reveal themselves

- 🕐 The persons that are lost to follow-up may have

  different outcomes than those available for assessment

- 🕐 In an open or dynamic cohort, was there anything special

  about the outcome of the people leaving, or the exposure of the people entering the cohort?

  (B) What are the results?

  7. What are the results of this study?

  HINT: Consider

- 🕐 What are the bottom line results?
- 🕐 Have they reported the rate or the proportion between

  the exposed/unexposed, the ratio/the rate difference?

- 🕐 How strong is the association between exposure and

  outcome (RR,)?

- 🕐 What is the absolute risk reduction (ARR)?

  8. How precise are the results?

  HINT: Look for the range of the confidence intervals, if given.

9. Do you believe the results?

HINT: Consider

- 🕐 Big effect is hard to ignore!
- 🕐 Can it be due to bias, chance or confounding?
- 🕐 Are the design and methods of this study sufficiently

  flawed to make the results unreliable?

- 🕐 Bradford Hills criteria (e.g. time sequence, dose-response

  gradient, biological plausibility, consistency)

  (C) Will the results help locally?

❒Yes ❒Can't tell ❒No

10. Can the results be applied to the local population? ❒Yes ❒Can't tell ❒No HINT: Consider whether

- 🕐 A cohort study was the appropriate method to answer this question
- 🕐 The subjects covered in this study could be sufficiently different from your population to cause concern
- 🕐 Your local setting is likely to differ much from that of the study
- 🕐 You can quantify the local benefits and harms

11. Do the results of this study fit with other available evidence?

❒Yes ❒Can't tell ❒No

12. What are the implications of this study for practice?

HINT: Consider

- 🕐 One observational study rarely provides sufficiently robust evidence to recommend changes to clinical practice or within health policy decision making
- 🕐 For certain questions observational studies provide the only evidence
- 🕐 Recommendations from observational studies are always stronger when supported by other evidence

Appendix 4.
National Institute for Health and Care excellence (NICE) Checklist (https://www.nice.org.uk/process/pmg10/chapter/appendix-g-methodology-checklist-qualitative-studies#checklist-6)

Study identification Include author, title, reference, year of publication		
Guidance topic:	**Key research question/aim:** .	
Checklist completed by:		
	Circle or highlight 1 option for each question	
Section 1: theoretical approach		
1.1 **Is a qualitative approach appropriate?** *For example:* Does the research question seek to understand processes or structures, or illuminate subjective experiences or meanings (in social care this would apply to how care and support is organised and service user or carer experience)? Or could a quantitative approach better have addressed the research question?	Appropriate Inappropriate Not sure	Comments:
1.2 **Is the study clear in what it seeks to do?** *For example:* Is the purpose of the study discussed – aims/objectives/research question(s)? Are the values/assumptions/theory underpinning the purpose of the study discussed?	Clear Unclear Mixed	Comments:
Section 2: study design		
2.1 **How defensible/rigorous is the research design/methodology?** *For example:* Are there clear accounts of the rationale/justification for the sampling, data collection and data analysis techniques used?	Defensible Not defensible Not sure	Comments:
Section 3: data collection		
3.1 **How well was the data collection carried out?** *For example:*	Appropriate Inappropriate	Comments:

Are the data collection methods clearly described? Were the data collected appropriate to address the research question?	Not sure/ inadequately reported	
Section 4: validity		
4.1 **Is the context clearly described?** *For example:* Are the characteristics of the participants and settings clearly defined? Were observations made in a variety of circumstances and from a range of respondents? Was context bias considered (that is, did the authors consider the influence of the setting where the study took place)?	Clear Unclear Not sure	Comments:
4.2 **Were the methods reliable?** *For example:* Were data collected by more than 1 method? Were other studies considered with discussion about similar/different results?	Reliable Unreliable Not sure	Comments:
Section 5: analysis		
5.1 **Are the data 'rich'?** *For example:* How well are the contexts of the data described? Has the diversity of perspective and content been explored? Has the detail of the data that were collected been demonstrated? Are responses compared and contrasted across groups/sites?	Rich Poor Not sure/not reported	Comments:
5.2 **Is the analysis reliable?** *For example:* Did more than 1 researcher theme and code transcripts/data? If so, how were differences resolved? Were negative/discrepant results addressed or ignored? Is it clear how the themes and concepts were derived from the data?	Reliable Unreliable Not sure/not reported	Comments:
5.3 **Are the findings convincing?** *For example:* Are the findings clearly presented? Are the findings internally coherent (that is, are the results credible in relation to the study	Convincing Not convincing Not sure	Comments:

question)? Are extracts from the original data included (for example, direct quotes from participants)? Are the data appropriately referenced so that the sources of the extracts can be identified? Is the reporting clear and coherent?		
5.4 **Are the conclusions adequate?** *For example:* How clear are the links between data, interpretation and conclusions? Are the conclusions plausible and coherent? Have alternative explanations been explored and discounted? Are the implications of the research clearly defined? Is there adequate discussion of any limitations encountered?	Adequate Inadequate Not sure	Comments:
Section 6: ethics		
6.1 **Was the study approved by an ethics committee?**	Yes No Not sure/not reported/not applicable	Comments:
6.2 **Is the role of the researcher clearly described?** *For example:* Has the relationship between the researcher and the participants been adequately described? Is how the research was explained and presented to the participants described?	Clear Not clear Not sure/not reported	Comments:
Section 7: Overall assessment		
As far as can be ascertained from the paper, how well was the study conducted (see guidance notes)	++ + –	Comments

Appendix 5.

Normal and universally accepted ranges for hematological markers.

Hematological Markers	The normal range
Platelet count (Plt)	150 and 450 $\times 10^9$/l
Prothrombin time (PT)	10.7 to 13.6 seconds
International Normalised ratio (INR)	0.9 to 1.2
Activated partial thromboplastin time (aPPT)	21.0 to 34.0 seconds