STUDIES OF THE CLINICAL EPIDEMIOLOGY AND PATHOPHYSIOLOGY OF ANAEMIA AND BLOOD TRANSFUSION IN CRITICALLY ILL AND ELECTIVE ORTHOPAEDIC PATIENTS

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DECLARATION

I declare that this thesis was my own composition. The majority of the work was carried out between 2001 and 2004 whilst in the post of Postgraduate research worker in the Department of Anaesthetics, Critical Care, and Pain Medicine in The University of Edinburgh.

Ezz el din Saleh Mohamed Ibrahim

March 2004

For my mother Aida, my father Saleh, my sister Ghada, my nephew Marwan, and my dear friend Tim and his family

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ABSTRACT

Anaemia commonly complicates surgery and critical illness. This thesis comprised four studies of this important clinical problem. The ***first study*** was a retrospective study based in a major teaching hospital Intensive Care Unit (ICU). Three existing datasets were merged to provide a study dataset that described the demographics and severity of illness for a cohort of critically ill patients (n = 489). The epidemiology of anaemia during ICU admission to death or discharge home from hospital was described. 81.5% of patients were discharged anaemic to the community after surviving critical illness. 35.5% had a haemoglobin concentration <100g/L. 82% of patients had normocytic normochromic blood indices at hospital discharge. The ***second study*** was a prospective observational study that further investigated blood transfusion practice in a cohort of patients (n = 185) after ICU discharge. 72.2 % of these patients were discharged home anaemic; 83% had normocytic normochromic blood indices at hospital discharge. These data confirmed the retrospective analysis. Hospital physicians used restrictive transfusion practice after ICU discharge; the median pre-transfusion Hb was 74 g/L (interquartile range: 68-76 g/L). The ***third study*** measured red blood cell 2,3-diphosphoglycerate (2,3-DPG) levels and P50, which both reflect the ability of red cells to deliver oxygen to tissues, in a prospective cohort of 111 patients admitted to the ICU. Factors that were associated with 2,3-DPG and P50 were investigated. The data showed that critically ill patients have lower levels of 2,3-DPG compared with normal healthy controls. Red cell 2,3-DPG during critical illness had a strong association with patients' acid base status. Acidemia was strongly associated with low DPG concentrations, which was counterintuitive. 2,3-DPG concentration had the strongest association with P50 raising the possibility that oxygen unloading could be impaired in critically ill patients. The ***fourth study*** merged several large existing databases to document the incidence, patterns, and likely aetiology of anaemia and the incidence of red cell transfusion in patients presenting for elective major orthopaedic surgery. The dataset comprised approximately 3500 patients presenting over one year to a single orthopaedic hospital. The relation between pre-operative haemoglobin status, other

factors, and peri-operative transfusion was investigated. 17 % of patients with known pre-admission Hb levels were anaemic. Most of these patients (64%) had normocytic normochromic anaemia. 303 patients received a total of 838 red cell units. Low per-admission Hb levels were common among those who were transfused during hospital admission.

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CHAPTER ONE

NORMAL RED BLOOD CELL PHYSIOLOGY

INTRODUCTION

Red blood cells are essential for life as they carry oxygen to the entire body's tissues (Hoffbrand 1999). Anaemia due to blood loss, red cell destruction, or ineffective erythropoiesis is a potentially serious health problem. Blood transfusion is considered one of the standard treatments of anaemia. However, despite major advances in blood safety concerns remain about the risks of blood transfusion (Mollison 1993). Two clinical areas in which anaemia and blood transfusion are common are the intensive care unit, and elective orthopaedic surgery. These two groups of patients use a considerable amount of blood. The decrease in blood availability world wide due to decrease in the number of blood donors and continuing increases in demands is an alarming problem (2004a, 2004b). Treating anaemia without allogeneic blood and using transfusion-sparing interventions such as low haemoglobin transfusion triggers could reduce the need of red blood cells. Investigating causes and types of anaemia in these settings could help in identifying alternative methods of treatment.

It has been shown that low pre-operative haemoglobin level is associated with peri-operative red cell transfusion (2004b, SIGN guideline, page 363). In this thesis, we investigated the incidence and type of pre-operative anaemia in patients undergoing elective orthopaedic patients and the associated red cell transfusion. Our intention was to obtain data that could help design strategies that decrease blood use.

A low haemoglobin transfusion trigger is used widely in the intensive care unit. However, it is unclear how patients' own red cells function during the course of the illness particularly when many patients become anaemic. In addition, it is not known how long anaemia lasts after an episode of critical illness.

In the present work red cell function in critically ill patients was investigated by measuring red cell 2,3-Diphosphoglycerate. The relationship between haemoglobin level and red cell 2,3-Diphosphoglycerate during critical illness was investigated. The relationships between red cell 2,3-Diphosphoglycerate and many other physiological variables were also examined. We also studied the prevalence of anaemia and its characteristics at the time of hospital discharge for this group of patients who were managed with low transfusion trigger strategy in the ICU.

A literature review was done for each study separately. The literature search was done through: a) The National Centre for Biotechnology Information (PubMed, a service of the National Library of Medicine), b) E-library (NHS, Education for Scotland), c) Cochrane library, and c) Edinburgh University electronic library. A priority was given to meta-analysis, Cochrane reviews, and clinical studies that carry in their title the key words that related to our studies. Other clinical studies and reviews that investigated the key words were also included. This general introduction reviews red blood cell physiology and the pathophysiology of anaemia. Chapters 2,3, and 4 review the literature relevant to each study.

ERYTHROCYTE STRUCTURE

The red cell is essentially a semi-permeable cellular membrane enclosing a viscous 30% protein solution. The protein is almost entirely haemoglobin, which comprises 95% of the dry weight of the cell. Erythrocytes are characterized by a large surface area-to-volume ratio due to their unique biconcave discoidal shape. This configuration minimizes diffusion distances between cell surface and haemoglobin molecules.

The mean red cell diameter is about 8 μm with marginal and central thickness of about 2 and 1μm.

The erythrocyte's biconcave form is apparently related to certain membrane proteins, such as spectrin, which may have contractile properties.

The outer surface of the erythrocyte membrane is composed principally of glycoprotein, which carries several major blood group antigens (A, B, M, N) (2004a, Brobeck 1979).

HAEMOGLOBIN (Hb)

Haemoglobin (mol.wt 64 500) contains four haem groups linked to four globin chains, and can bind four molecules of oxygen (Hoffbrand, Lewis, Tuddenham, editors 1999).

Haemoglobin serves as the oxygen carrier in blood, but it also plays a vital role in the transport of carbon dioxide and hydrogen ions. The capacity of haemoglobin to bind oxygen depends on the presence of the haeme group (monopolypeptide unit), which gives haemoglobin its distinctive colour. The haem consists of elemental iron and an organic component. The organic part, protoporphyrin, is made of four-pyrrole group

(tetrapyrrol). Four methyl, two vinyl, and two propionate side chains are attached to a tetrapyrrole ring to form protoporphyrine IX.

The iron atom in haem binds to the four nitrogens in the centre of the protoporphyrin ring. The iron can form two additional bonds, one on either side of the haeme plane. Haemoglobin contains 0.34% iron by weight. Thus 1ml of packed erythrocytes contains approximately 1 mg of iron (Beutler, Lichtman, Coller, Kipps, editors 1995).

ERYTHROPOIESIS

BLOOD CELL PRODUCTION (MARROW)

The marrow is one of the largest organs in the human body and is the principle site for blood cell formation. In normal adults its daily production amounts of about 2.5 billion red cells, 2.5 billion platelets, and 1.0 billion granulocytes per kilogram of body weight. The rate of production is adjusted to actual needs and can be varied from nearly zero to many times normal (Beutler, Lichtman, Coller, Kipps, editors 1995).

Mature blood cell production takes place from progenitor cells, which can undergo rapid proliferation and development in response to several cytokines

A schematic representation of the structure of the haemopoietic system is shown in figure (1.1) (Hoffbrand, Lewis, Tuddenham, editors 1999).

Erythrocytes are the most common circulating haemopoetic cells. The precursor forms within the marrow are nucleated but, when released into the peripheral blood, they no longer contain a nucleus. Red cells have a finite lifespan of around 120 days, at which time they are destroyed primarily in the reticuloendothelial system. Red

cells recently released from the bone marrow contain trace amounts of RNA that can be stained with supravital stains and recognized as reticulocytes. The reticulocyte count can give a vital insight into the synthetic function of the marrow (Webb, Shapiro, Singer, Suter, editor 1999).

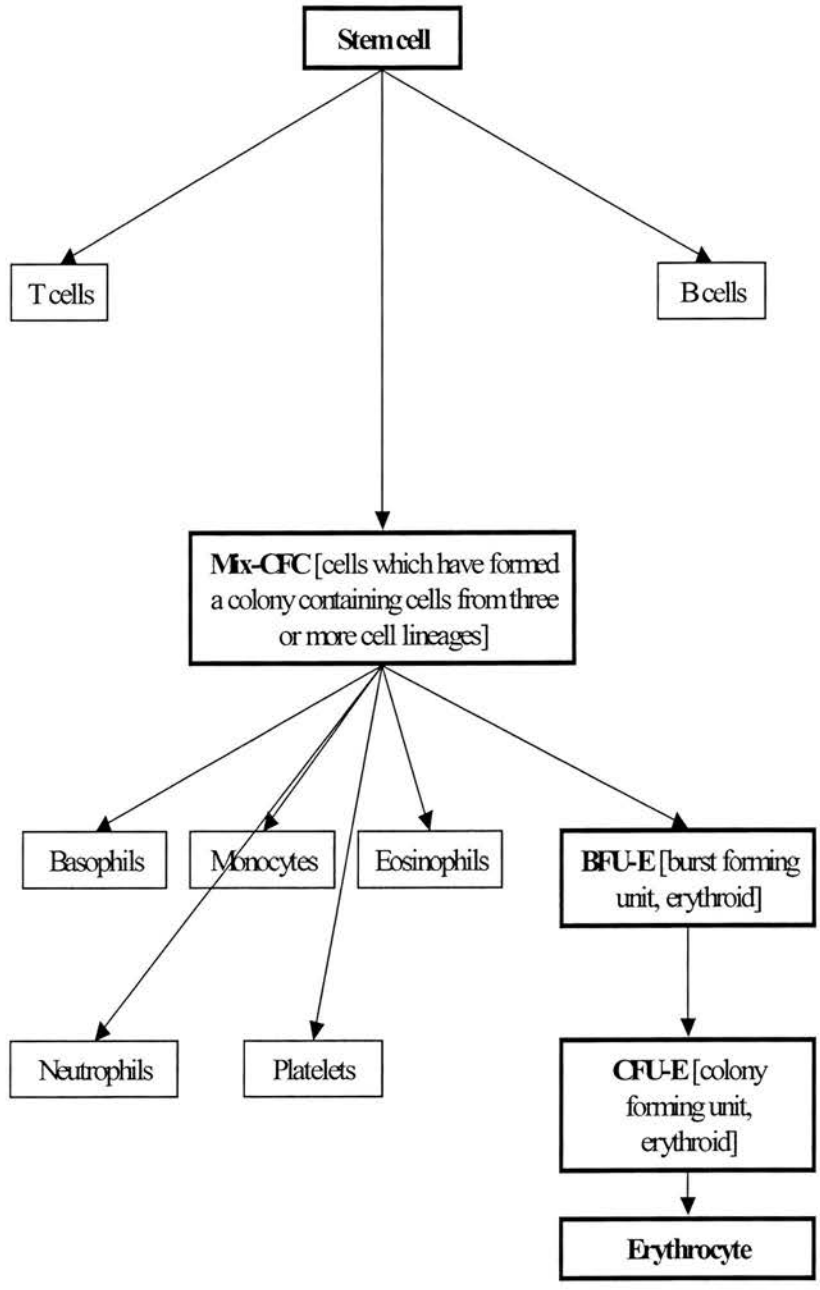


Figure (1.1): Flow chart illustrating the haemopoietic system.

FACTORS CONTROLLING ERYTHROPOIESIS

Erythropoietin

Erythropoietin, a hormone produced mainly by the kidney, stimulates bone marrow differentiation and production of red blood cells. Decreased renal oxygen delivery secondary to hypoxia, anaemia, or ischaemia augments erythropoietin synthesis. Additional stimulants of erythropoietin production include vasoconstrictors such as norepinephrine, anti-diuretic hormone (ADH), and angiotensin. Suppressants of erythropoietin synthesis include hyperoxia, increased red cell blood volume, and reduced renal mass. Furthermore, the adenosine antagonist theophylline inhibits erythropoietin production (Webb, Shapiro, Singer, Suter, editor 1999).

Iron metabolisim and transport

Iron absorption

Iron absorption depends not only on the amount of iron in the diet, but also, and more importantly, on the bioavailability of that iron, as well as the body's needs for iron. A normal western diet provides approximately 15 mg iron daily. Of that iron, digestion within the gut lumen solubilizes about one-half, from which about 3 mg may be taken up by mucosal cells and about 1 mg transferred to the portal blood in a healthy adult male. Iron absorption can be influenced at several different stages (Hoffbrand, Lewis, Tuddenham, editors 1999). The minimal daily iron requirements are shown in table (1.1). Factors affecting iron absorption are shown in table (1.2).

Table (1.1): The minimal daily iron requirements (Beutler, Lichtman, Coller, Kipps, editors 1995)

	Amount that must be absorbed for haemoglobin synthesis (mg)	Minimal amount that should be ingested daily (mg)
Infants	1	10
Children	0.5	5
Young non pregnant women	2	20
Pregnant women	3	30
Men & postmenopausal women	1	10

Table (1.2): Factors affecting iron absorption (Hoffbrand, Lewis, Tuddenham, editors 1999)

Favoured by	Reduced by
Dietary factors	
Increased haem iron	Decreased haem iron
Increased animal foods	Decreased animal food
Ferrous iron salts	Ferric iron salts
Luminal factors	
Acid pH (e.g.gastric HCl)	Alkalis (e.g. pancreatic secretions)
Low molecular weight soluble chelates (e.g.vitamin C, amino acids)	Insoluble iron compexes (e.g. phytates, tannates in tea)
Ligand in meat (unidentified)	
Systemic factors	
Iron deficiency	Iron overload
Increased erythropoiesis (e.g. after haemorrhage)	Decreased erythropoiesis
Ineffective erythropoiesis	Inflammatory disorders
Pregnancy	
Hypoxia	

Iron transport

In plasma iron is bound to transferrin, which is a glycoprotein. Normally, approximately one-third of the transferrin iron-binding sites are occupied by iron. About 200 mg of transferring carry about 100μg/dL of iron. Once an atom of iron enters the body, it is virtually in a closed system [internal iron cycle] (figure 1.2) (Beutler, Lichtman, Coller, Kipps, editors 1995). Iron compartments in normal subject are shown in table (1.3).

Table (1.3):Iron compartments in normal subjects (Beutler, Lichtman, Coller, Kipps, editors 1995)

Compartment	Iron content, mg	Total body iron, %
Haemoglobin iron	2,000	67
Storage iron (ferrintin, haemosiderin)	1,000	27
Myoglobin iron	130	3.5
Labile pool	80	2.2
Other tissue iron	8	0.2
Transport iron	3	0.08

The values represent estimates for an "average" person, that is, 70 kg, 177 cm (70 in) in height.

Iron storage compartment

Iron storage exists in two distinct forms: ferritin and haemosiderin. In adult men the storage iron amounts to 800 to 1,000 mg; in adult women it is a few hundred milligrams less (Beutler, Lichtman, Coller, Kipps, editors 1995; Hoffbrand, Lewis, Tuddenham, editors 1999).

Ferritin is found in virtually all cells of the body and also in tissue fluids. Hepatocytes, and other cells, have ferritin receptors on their cell membranes that are involved in the binding and internalisation of ferritin from plasma or interstitial fluid.

Serum ferritin concentration correlates with total-body iron stores, which makes this measurement important in diagnosis of many iron disorders.

Haemosiderin is found predominantly in cells of the monocyte-macrophage system in the marrow, Kupffer cells of the liver, and in the spleen. Haemosiderin contains 25 to 30% iron by weight (Beutler, Lichtman, Coller, Kipps, editors 1995).

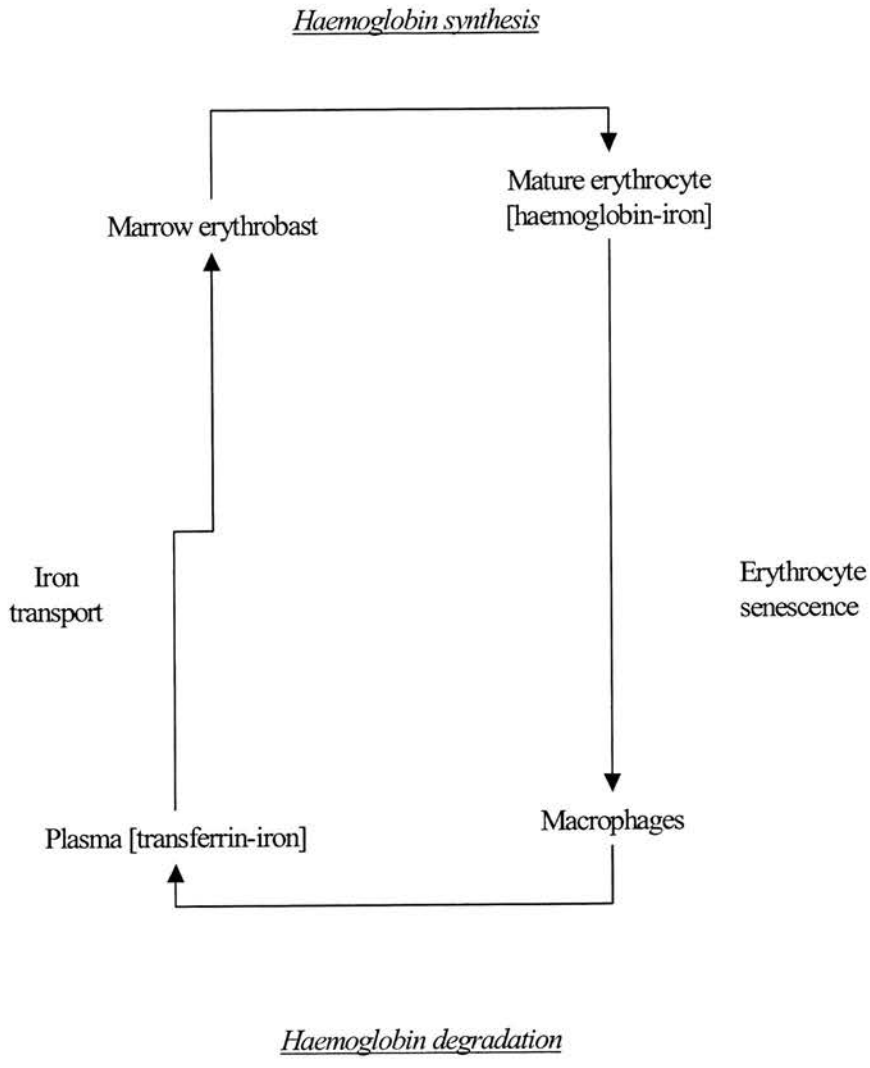


Figure (1.2): Internal iron cycle

Factors that modify erythropoiesis

Inflammation

Inter-leukins, colony stimulating factors and cytokines

Interleukins, colony-stimulating factors, and other cytokines are responsible for the regulation of blood cells production; they are molecules with bioactivity in the lympohaemopoietic pathways. Some of these molecules have stimulatory effect on red cell production such as erythropoietin, interleukin-1 (IL-1), IL-3, IL-9, KIT ligand, platelet-derived growth factor, hepatocye growth factor, insulin-like growth factor II, and membrane bound burst-promoting activity.

Others have inhibitory effect on red cell production, such as H-subunit ferritin, transforming growth facor-β, prostaglandin E1 and E2, inhibin, lactoferrin, and seraspenide (Beutler, Lichtman, Coller, Kipps, editors 1995; Hoffbrand, Lewis, Tuddenham, editors 1999).

Iron and haematenics

Deficiencies of iron, vitamin B_{12}, or folic acid on the one hand and/or the presence of primary bone marrow disease on the other may result in a decreased production of red blood cells. Underlying chronic diseases (i.e. malignancies, systemic diseases, metabolic disturbances) or (chronic) infections may also result in a decreased red cell production. Anaemia in the latter group of disorders is characterized by an underproduction of red blood cells owing to a decreased availability of iron. Depletion of the body iron stores during chronic infection probably has an immunological aetiology and is modulated by the production of cytokines (Webb, Shapiro, Singer, Suter, editor 1999).

Vitamin B_{12} and folate

Deficiency is caused by: poor intake, drugs, alcoholism (folate), and malabsorption. Vitamin B_{12} deficiency occurs with ileal and gastric pathology. Effects include megaloblastic anaemia, glossitis, and stomatitis. Neuropathy or neuromyelopathy (subacute combined degeneration) occur with B_{12} deficiency. The normal requirements are 3 mg/kg/day folate and 2 μg/day vitamin B_{12} (Webb, Shapiro, Singer, Suter, editor 1999).

STRUCTURAL HAEMOGLOBIN VARIANTS

Many structural haemoglobin variants have been described, most of which result from single amino acid substitutions. Single amino acid substitutions may cause clinical disorders as they may alter the stability and the functional properties of the haemoglobin molecule and causes abnormal oxygen binding (Hoffbrand, Lewis, Tuddenham, editors 1999).

HAEMOGLOBIN VARIANTS THAT CAUSE ABNORMAL OXYGEN BINDING

The high oxygen affinity haemoglobin variants

These result from single amino acid substitutions at critical parts of the haemoglobin molecule that are involved in the configurational changes which underlie haem-heam interaction and the production of a sigmoid oxygen dissociation curve. Many occur at the junctions between the α and β subunits. Others involve the amino acids that are involved with the binding of 2,3-DPG to haemoglobin.

Fetal haemoglobin has a high oxygen affinity (left-shifted the haemoglobin oxygen dissociation curve) because it cannot interact with 2,3-DPG; mutations of the DPG binding sites have similar effect (Hoffbrand, Lewis, Tuddenham, editors 1999).

All the high oxygen affinity variants have a left-shifted oxygen dissociation curve with a reduced P50. Thus, the variant haemoglobin holds on to oxygen more avidly than normal haemoglobin; this potentially lead to tissue hypoxia. This in turn causes an increased output of erythropoietin and an elevated red cell mass. Most patients with this type of Hb are healthy and are only identified when a routine haematological examination shows an unusual high Hb level or packed cell volume.

Low oxygen affinity variants

At least six haemoglobin variants with reduced oxygen affinity have been reported such as Kansas haemoglobin. The amino acid substitution is at the interface between the α and β globin chains. This condition should be thought of in any patient with an unexplained congenital cyanosis (Hoffbrand, Lewis, Tuddenham, editors 1999).

Sickling disorder

Sickling disorder occurs due to the presence of an abnormal Hb variant, namely haemoglobin S (HbS). HbS has increased oxygen affinity. Due to the number of relevant genes, the propotion of α-chain variant is half that of a β-chain variant. HbS undergoes liquid crystal formation as it becomes deoxygenated due to unusual solubility characteristics. Fully oxygenated sickle cells are more rigid than normal and tend to adhere to vascular endothelium. Factors such as hypoxia and infection can trigger sickle cell crises that characterised by cyanosis, haemolysis, and thrombo-embolic manifestations.

Acidosis profoundly affects the sickling of red cells, even when oxygen saturation is normal. Alkalosis, on the other hand, by shifting the equilibrium toward the oxy confrontation, tends to retard sickilng but impairs oxygen release to tissue. In standard conditions, HbS shows low P50 levels [12-15 mmHg] with markedly shift

of the oxygen haemoglobin dissociation curve to the left (Beutler, Lichtman, Coller, Kipps, editors 1995; Hoffbrand, Lewis, Tuddenham, editors 1999).

OXYGEN TRANSPORT

OXYGEN CONTENT

Each 100 ml of blood contains about 20 ml of oxygen, almost all of which is carried on the haemoglobin. Oxygen is poorly soluble in blood.

The oxygen content of blood (Co2) is: (Aitkenhead, Smith, editors 1996)

CO_2 (ml.100 ml^{-1} blood) = 0.023 x PO_2 (kPa) + (1.34 x Hb ($g.dl^{-1}$) x SO_2 (%)/100.

From the above-mentioned equation, blood oxygen content can be affected by: Anaemia, carbon mono-oxide (CO) poisoning, pulmonary diseases, and abnormal haemoglobin structure (Brobeck 1979).

OXYGEN DELIVERY

Oxygen delivery is a function of cardiac output and arterial O2 content. Arterial O2 content is dependent on haemoglobin concentration and percentage of O2 carried by the haemoglobin molecule, or saturation. Oxygen dissolved in plasma contributes a negligible amount to overall delivery (Thamer, Richard, Klinkmann, Ivanovich, Lang, Cotter 2000).

Oxygen transport variables

a. DO2 = CO X CaO2

DO2= O2 delivery

CO= cardiac out-put

CaO2= arterial oxygen content

b. CaO2= % saturation of haemoglobin X 1.34 (mL/g) X [Hg] (g/L)

c. CO= HRX SV

HR= heart rate

SV= stroke volume (Webb, Shapiro, Singer, Suter, editor 1999; Thamer, Richard, Klinkmann, Ivanovich, Lang, Cotter 2000).

An absolute reduction in DO_2 could occur as a result of a reduction in haemoglobin, PaO_2, and hence SaO_2 (% of Hb oxygen saturation), or from a reduction in CO which could be the result of low stroke volume or heart rate (Webb, Shapiro, Singer, Suter, editor 1999).

CARBON DIOXIDE TRANSPORT

Carbon dioxide is carried in the blood in three forms; dissolved, as bicarbonate, and in combination with proteins as carbamino compounds.

Haemoglobin is an important element in the carriage of carbon dioxide. Oxygenation of the haemoglobin molecule causes the release of a proton, which in turn, reacts with bicarbonate to liberate CO2 according to the following reaction

$$HHB \text{ (deoxy Hb)} + O2 \rightleftarrows HbO2^{-} + H^{+}$$

$$H^{+} + HCO3^{-} \rightleftarrows H2CO3 \rightleftarrows H2O + CO2$$

The reactions take place in the lungs in the direction indicated; in peripheral tissues the reactions are reversed and allow more CO2 to be carried in venous blood.

Within the red cell, a zinc-containing enzyme, carbonic anhydrase (c.a.), greatly facilitates the transport of CO2 from the tissue to the lungs

$$H2O + CO2 \rightleftarrows H2CO3 \text{ (in the presence of c.a.)}$$

Haemoglobin also forms carbamino complexes to aid in the transport of carbon dioxide.

$$RNH_2 + CO_2 \rightleftarrows RNHCOOH$$

Deoxyhaemoglobin binds more CO_2 as a carbamino compound than does the oxyhaemoglobin, and the haemoglobin shuttle between oxyhaemoglobin and reduced haemoglobin provides an additional mechanism for CO_2 transport (Brobeck 1979).

ANAEMIA

DEFINITIONS

Anaemia in an individual is defined as a haemoglobin concentration in blood that is below the expected value, when age, gender, pregnancy and certain environmental factors, such as altitude, are taken into account (Emmanuel, McClelland, Page, editors 1997).

It is also defined as a reduction in red cell mass, resulting in a lowered haemoglobin and haematocrit level, and leading to a decrease in the oxygen-carrying capacity of the blood (Webb, Shapiro, Singer, Suter, editor 1999).

World Health Organization Criteria to define anaemia based on normal haemoglobin range at the see level are shown in table (1.4) (Emmanuel, McClelland, Page, editors 1997).

Table (1.4): Normal haemoglobin levels (Emmanuel, McClelland, Page, editors 1997)

Gender	Normal Hb g/L	Anaemic if Hb less than: g/L
Adult males	130-170	130 (Hct 39%)
Adult females: non-pregnant	120-150	120 (Hct 36%)
Adult females: pregnant		
First trimester: 0-12 weeks	110-140	110 (Hct 33%)
Second trimester 13-18 weeks	105-140	105 (Hct 31%)
Third trimester 29 weeks-term	110-140	110 (Hct 33%)

These values simply define anaemia. They are often used as thresholds for investigation and treatment, but not indications for transfusion.

The National Cancer Institute (NCI) considers normal haemoglobin levels as 120 to 160 g/L for women and 140 to 180 g/L for men. The World Health Organization (WHO) and the National Cancer Institute have provided scales for characterizing haemoglobin levels, shown in table (1.5) (Yount, Lai, Cella 2002).

Table (1.5): NCI and WHO scales of anaemia (Yount, Lai, Cella 2002)

Grade (severity)	NCI scale	WHO scale
0 (none)	Normal limits**	>110
1 (mild)	100 to normal	95 to 109
2 (moderate)	80 to 100	80 to 94
3 (severe)	65 to 79	65 to 79
4 (life-threatening)	<65	<65

Hb values (g/L).

** 140 to 180 g/L for mean and 120 to 160 g/L for females.

CLASSIFICATION OF ANAEMIA

Anaemia can be classified according to its pathological causes in several ways. Two common approaches are:

1. According to red blood indices namely mean red cell volume, mean red cell corpuscular haemoglobin and mean corpuscular haemoglobin concentration [MCV, MCH and MCHC respectively] (table 1.6)
2. According to pathophysiology (table 1.7)

Table (1.6): Classification of anaemia according to red blood cell indices (Emmanuel, McClelland, Page, editors 1997).

Blood film	Red cell indices	Cause
Microcytic, hypochromic with abnormal red cells	Low mean cell volume (MCV) Low mean cell haemoglobin (MCH) Low mean cell haemoglobin concentration (MCHC)	**Acquired** • Iron deficiency • Sidroblastic anaemia • Anaemia of chronic disorder **Congemital** • Thalassaemia • Sideroblastic anaemia.
Macrocytic, normochromic	Increased MCV	**With megaloblastic marrow** • Deficiency of vitamin B12 or folic acid **With normoblastic marrow** • Alcohol excess • Myelodysplasia
Macrocytic polychromasia	Increased MCV	Haemolytic anaemia

Blood film	Red cell indices	Cause
Normocytic, normochromic	Normal MCV, MCH, MCHC	• Chronic disorder • Infection • Malignancy • Autoimmune disorders • Renal failure • Hypothyrodism • Hypopituitarism • Aplastic anaemia • Red cell aplasia • Marrow infection
Leucoerthroblastic	Indices may be abnormal due to early and numerous forms of red and white cells	• Myelodyspalsia • Leukaemia • Metastatic cancer • Myelofibrosis • Severe infections

Table (1.7): Classification of anaemia according to its pathophysiology (Beutler, Lichtman, Coller, Kipps, editors 1995)

Anaemia predominantly caused by decreased red cell production	Anaemia caused by increased erythrocyte destruction or loss
a. Disturbance of proliferation and differentiation of haemopoietic stem cells (1) Aplastic anaemia (2) Anaemia of leukaemia and myelodysplastic syndrome b. Disturbance of proliferation and differentiation of erythroid progenior or precursor cells (1) Pure cell aplasia (2) Anaemia of chronic renal failure (3) Anaemia of endocrine disorders (4) Congenital dyserythropoietic anaemia c. Disturbance of DNA synthesis (megaloblastic anaemia) (1) Vitamin B12 deficiency (2) Folic acid deficiency (3) Acquired and congenital defects in purine and primidine metabolism d. Disturbance of haemoglobin synthesis (hypochromic anaemia) (1) Iron deficiency anaemia (2) Congenital transferrinaemia and idiopathic pulmonary haemosidrosis (3) Thalassemia e. Disturbance of erythropoietic regulation (1) Low oxygen affinity haemoglobinopathies	a. Intrinsic abnormality (1) Membrane defect (a) Hereditary spherocytosis, ellipocytosis, and pyropoikilocytosis (b) Hereditary acanthocytosis and stomatocytosis (2) Enzyme deficiency (a) Glucose-6-phosphate dehydrogenase deficiency (b) Pyruvate kinase deficiency (c) Prophyria (3) Globin abnormality (a) Sickle cell anaemia (b) Unstable haemoglobin (4) Paroxysmal nocturnal haemoglobinuria b. Extrinsic abnormality (1) Mechanical (a) March haemoglobinuria and sport anaemia (b) Traumatic cardiac haemolytic anaemia (c) Microangiopathic haemolytic anaemia (2) Chemical or physical (a) Haemolytic anaemia due to chemical or physical agents

Anaemia predominantly caused by decreased red cell production	Anaemia caused by increased erythrocyte destruction or loss
f. Unknown multiple mechanisms (1) Anaemia of chronic disorders (2) Anaemia associated with marrow infiltration (3) Anaemia associated with nutritional deficiencies Sideroblastic anaemia	(3) Infection (a) Haemolytic anaemia due to infection with microorganisms (4) Anti-body mediator (a) Acquired haemolytic anaemia due to warm-reacting autoantibodies (b) Cryopathic haemolytic syndrome (c) Drug reaction involving antibodies reacting with reythrocytes (d) Alloimmune haemolytic disease of the newborn (5) Hyperactivity of the monocyte-macrophage system (a) hypersplenism (6) Blood loss (a) Acute blood loss anaemia

CLINICAL IMPORTANCE OF ANAEMIA

Anaemia is a condition of low haemoglobin level, which is the main oxygen carrier to the tissues. A lower Hb level can potentially affect oxygen delivery to all of the body's organs. This can lead to serious health problems. Areas that are often highlighted in literature are as follow:

Impaired quality of life and fatigue

Symptoms of anaemia generally affect quality of life. It has been shown that 31% of patients with severe anaemia due to renal causes have severe impairment of quality of life (Valderrabano 2000). Anaemia due to malignancy contributes to fatigue and low quality of life scores (Sabbatini 2000). Some studies have shown that increasing Hb level in anaemic patients with different diseases such as chronic renal failure, malignancy, inflammatory bowel diseases, and rheumatoid is associated with better quality of life (Gasche, Dejaco, Waldhoer et al. 1997; McMahon, Mason, Skinner, Burge, Grigg, Becker 2000; Quirt, Robeson, Lau et al. 2001).

Increased cardiovascular morbidity

Compensatory haemodynamic changes during anaemia, which lead to increased cardiac output and systemic blood flow are associated with cardiac morbidity due to increased cardiac work at a time of decreased oxygen supply to the heart muscles (Eckardt 1999). For example, it has been shown that renal patients with Hct $\leq$ 33% have higher cardiac death rates than patients with Hct >33% (Collins, Li, St Peter et al. 2001). The presence of coronary disease is widely acknowledged as a factor that may decrease tolerance to anaemia.

Hinder cognitive function

Low oxygen delivery to the brain due to low Hb level is associated with impairment in cognitive function (Kusunoki, Kimura, Nakamura, Isaka, Yoneda, Abe 1981). It has been shown that elevating Hct level in patients with cerebrovascular disorders was associated with significant improvement in cognitive function. In anaemic patients with renal disease, correction of Hb levels to its normal level was shown to improve neurophysiological parameters indicative of cognitive function and memory (Marsh, Brown, Wolcott et al. 1991; Pickett, Theberge, Brown, Schweitzer, Nissenson 1999).

CHAPTER TWO

PREVALENCE OF ANAEMIA AMONG SURVIVORS OF CRITICAL ILLNESS MANAGED WITH CONSERVATIVE TRANSFUION TRIGGERS

Anaemia is common in the critically ill. The pathogenesis of anaemia in this group of patients is very complex. Regardless of the heterogeneity of underlying diseases and complications necessitating ICU treatment, anaemia is extremely common in this patient population. Many patients are already anaemic when admitted to the ICU. This holds true not only for post-operative admissions, but also for patients with medical diseases (Rogiers, Zhang, Leeman et al. 1997; Hebert 2000; Vincent, Baron, Reinhart et al. 2002; Eckardt 2002).

REVALENCE OF ANAEMIA IN CRITICALLY ILL PATIENTS

AT ADMISSION TO ICU

A recent history of anaemia before ICU admission is associated with the incidence and the degree of anaemia at the time of admission. Vincent et al found that at the time of ICU admission, 13% of patients had a recent history of anaemia; of those patients, 36.6% had an admission haemoglobin less than 10 g/dL. Of all patients with an admission haemoglobin level less than 10 g/dL, 48.9% had neither a history of anaemia nor of acute bleeding before admission (Vincent et al. 2002).

Some studies have investigated the prevalence of anaemia at the time of ICU admission. Eckardt et al showed that nearly half of ICU admissions had reduced haemoglobin concentration. In this study patients with an Hb < 120 g/L had a significantly worse prognosis than those admitted with higher Hb values (Eckardt 2002).

Table (2.1) shows the reported published ICU admission haemoglobin in some studies. None of these studies have mentioned the normal reference ranges for Hb or Hct.

Table (2.1): Published ICU admission Haemoglobin levels.

Author	Type of patients	ICU admission Hb (g/L) or haematocrit (%)
Von Ahsen et al.(von Ahsen, Muller, Serke, Frei, Eckardt 2001)	Non operative admission	113, 92-121 g/L (median, IQ)
	Cardiac surgery	108, 94-116 g/L (median, IQ)
	Other surgery	99, 109-118 g/L (median, IQ)
Vincent et al (Vincent et al. 2002)	Medical	119 g/L (mean)
	Elective surgery	110 g/L (mean)
	Emergency surgery	108 g/L (mean)
	Trauma	115 g/L (mean)
	Other	120 g/L (mean) (SD not mentioned)
Hebert et al(Hebert, Wells, Tweeddale et al. 1997)	Medicosurgical ICUs	
	ICU survivors (n = 3469)	39.6% had mean Hb<95 g/L
	Non ICU survivors (n=1001)	59% had mean Hb <95 g/L

Author	Type of patients	ICU admission Hb (g/L) or haematocrit (%)	Percentage of patients (%)
Tan et al (Tan, Lim 2001)	Not mentioned in the paper	Mean Hct <36% (SD not mentioned)	75 (% Of all ICU admissions)
Spiess et al.(Spiess, Ley, Body et al. 1998)	Cardiac surgery	≤24% (haematocit value) 25%-33% (haematocrit value) > 34% (haematocrit level)	11 70 19 (% of ICU admissions with recorded Hct results n= 2202)
Vinh Nguyen Ba et al.(Nguyen, Bota, Melot, Vincent 2003)	Multidisciplinary (n = 91) (medico-surgical)	122 ± 20g/L (overall mean ± SD Hb at ICU admission)	
Petra Hobisch-Hagen et al.(Hobisch-Hagen, Wiedermann, Mayr et al. 2001)	Trauma patients (n = 23)	100, 68-129 g/L (overall mean, range Hb at ICU admission)	

DURING ICU STAY

Progression of anaemia during the period of ICU treatment is reflected by further reduction in mean Hb levels and frequent blood transfusion. Some studies have specifically investigated Hb levels and their change during ICU stays.

In a prospective observational cohort study, which took place in a large Scottish ICU unit, Chohan et al found that 55% of ICU patients who spent > 24 hours in ICU had Hb ≤ 90g/L at least once during their ICU stay. 52% of these patients had Hb ≤ 90g/L on the first day of ICU admission, and 77% had Hb ≤ 90g/L within 2 days of admission (Chohan, McArdle, McClelland, Mackenzie, Walsh 2003). In another study, von Ahsen et al. also showed that 77% of all ICU admissions had Hb level of ≤ 90g/L by day two of ICU stay (von Ahsen, Muller, Serke, Frei, Eckardt 2001).

Some authors found that by intensive care unit day 3, almost all patients (95%) admitted became anaemic (Eckardt 2002) (Goldhill, Boralessa, Boralessa 2002) (Hobisch-Hagen et al. 2001).

In a multi-centre Canadian randomised control trial, Hebert et al studied patients where Hb was ≤ 90 g/L within 72 hours of ICU admission. The average ICU Hb concentration in patients randomised to a Hb transfusion trigger < 70 g/L and transfused with one red cell unit on each occasion was 85 g/L (Hebert, Wells, Marshall et al. 1995).

Nguyen and colleagues studied the time course of haemoglobin concentrations in non-bleeding intensive care unit patients. They found that for the entire ICU stay, the fall in haemoglobin concentrations averaged 0.52 g/dL/day. In those patients who stayed in the ICU for >3 days, haemoglobin concentrations decreased by

0.66 g/dL/day for the first 3 days and by 0.12 g/dL/day thereafter. For patients who stayed at least 1 week, haemoglobin concentration decreased by 0.58 g/dL/day during the first 3 days and by 0.21 g/dL/day thereafter (Nguyen, Bota, Melot, Vincent 2003).

CAUSES OF ANAEMIA DURING CRITICAL ILNESS

In general there are three potential causes of anaemia in critically ill patients: 1) blood loss, 2) reduced red cell life span and 3) disturbed erythropoiesis.

BLOOD LOSS

Von Ahsen et al. estimated total blood loss of patients during their intensive care unit stay and found that total blood loss, as estimated from the difference between the initial and last measured Hb concentration and the amount of Hb transfused during the ICU stay, was a median of 128 mL per patient per day (von Ahsen, Muller, Serke, Frei, Eckardt 2001). The authors suggested that blood loss in ICU patients is mainly due to frequent diagnostic blood sampling (diagnostic), hidden and major bleeding (non-diagnostic).

Blood sampling

ICU patients may face the dilemma of an iatrogenic transfusion requirement because of multiple blood sampling. Andrews et al quantified the mean number of blood gas samples taken per patient and estimated the mean blood loss resulting from this, in the critically ill. They found that patients who were ventilated for ≥24 hours had a statistically significant greater blood loss (mean 50.16 ml per day) when compared to those who were ventilated for less than a day (mean 34.33 ml per day). A subgroup of patients who underwent renal

replacement therapy had the greatest blood loss (mean 55.18 ml per day). This increased loss was significantly greater when compared to patients not in acute renal failure (mean 42.18 ml per day). When patients underwent multiple therapies such as total parental nutrition, potassium replacement and insulin therapy that are frequently thought to be associated with increased sampling, there was no statistically significant difference in blood loss (mean 48.28 ml per day) (Andrews, Waterman, Hillier 1999).

Vincent et al. determined the frequency of blood drawing and the associated volume of blood lost. They found that the number and volume of blood samples drawn in the 24-hour period varied widely across patients. The mean number of draws per patient was 4.6, the mean volume per drawn was 10.3 ml, and the average total volume was 41.1 ml for the 24-hour period. They also studied the relation between number of blood samples draw and organ dysfunction, and they found that there was a significant positive correlation between organ dysfunction and number of blood draws and total volume drawn (Vincent et al. 2002).

Von Ahsen et al. found that the median volume of diagnostic blood loss declined from 41 mL on day 1 to less than 20 mL after 3 weeks of ICU admission and contributed 17% (median) to estimate total blood loss. Acute renal failure, fatal outcome, and high-simplified acute physiology score on admission were associated with increase in total blood loss (von Ahsen, Muller, Serke, Frei, Eckardt 2001).

142 ICU patients were studied by Corwin et al. to determine the amount of diagnostic blood loss. These authors found that these patients were phlebotomized a total of 176 L of blood for diagnostic tests during their ICU

stay. The mean daily blood sample volume withdrawn from non-transfused patients was 45 ml/day, for patients who were transfused with 1 to 5 units was 61 ml/day and for patients who were transfused more than 10 units was 70 ml/day. They commented in their study that phlebotomy for diagnostic tests can lead to reduced haemoglobin levels and increase the likelihood of red cell transfusion, and that patients who had a daily phlebotomy were more likely to be transfused compared with those who did not (Corwin, Parsonnet, Gettinger 1995).

There are many causes for blood sampling in ICU patients. Vinh Nguyen Ba et al, described briefly the number, volume and different causes for withdrawing blood from patients in ICU and they reported that the mean volume of a standard blood sample was 3 to 4 mL. The mean volume of a blood gas sample was 1.5 mL, with an additional 2 mL of discarded blood. Blood culture sampling involved a specimen of 10 mL, usually taken three times. The average number of blood samples per day was 11.7, and the total volume of blood taken per day was 40.3 mL. The number of blood samples each day was 14.0 for septic patients and 10.9 for non-septic patients. The mean blood volume drawn daily was 49.0 mL in the septic patients and 36.7 mL in the non-septic patients (Nguyen, Bota, Melot, Vincent 2003).

Non-sampling blood loss

Non-acute blood loss in critically ill patients is considered by some to play a major role in ICU anaemia. It is possible that much of it is caused by occult bleeding from the gastrointestinal tract due to impairment in mucosal integrity, which can be found in many critically ill patients. However, such losses are difficult to quantify and can take different forms such as haematemesis, grossly

bloody nasogastric aspirates, and nasogastric aspirates containing coffee grounds material, and melaena (Cook, Fuller, Guyatt et al. 1994; Eckardt 2001).

Stress ulceration can cause overt and clinically important bleeding in critically ill patients. Although the pathophysiology is not completely understood, factors that may play an aetiological role include decreased gastric pH, increased gastric mucosal permeability, and ischaemia. In a prospective study by Schuser et al. of 174 patients in a medical/respiratory ICU to evaluate the occurrence of upper gastrointestinal bleeding, they found that 14% of ICU patients had overt or occult gastrointestinal bleeding after ICU admission. These patients had higher mortality and increased length of ICU stay than non-bleeding patients (Schuster, Rowley, Feinstein, McGue, Zuckerman 1984; Cook, Heyland, Griffith, Cook, Marshall, Pagliarello 1999).

Von Ahsen et al. recorded the amount of diagnostic blood loss and estimated the total blood loss from the changes in Hb levels and the amount of red cells transfused. They found in many patients treated for long periods in the ICU that the contribution of diagnostic blood loss to the total blood loss amounted to 17% (range, 10%-28%), they referred the other 83% of total blood loss to various other reasons, e.g., occult gastrointestinal bleeding and renal replacement therapy. However, occult blood losses, by definition, were not directly measured in this study (von Ahsen, Muller, Serke, Frei, Eckardt 2001).

Cook et al. described briefly in a multi-centre randomised study the blood loss during ICU stay for 1200 patients who required mechanical ventilation. They found 23.3% of all cases had gastrointestinal bleeding episodes such as haematemesis, bloody nasogastric aspirate, and melaena in the first 5 days of

ICU stay, on days 6-10 (43.3% of cumulative cases), days 11-15 (73.3% of cumulative cases), day 16-20 (80.0% of cumulative cases), and after day 21 (100.0% of cumulative cases). The overall incidence of clinically important bleeding was 1.93 per 1000 ICU days. The authors defined clinically important bleeding as a) overt gastrointestinal bleeding with a spontaneous decrease in blood pressure ≥20 mmHg within 24 hours of bleeding, b) an increase in pulse rate of 20 beats/min with decrease in systolic blood pressure of 10 mmHg, c) decrease in Hb of ≥ 20 g/L within 24 hours and transfusion of 2 units of red blood cell, and d) failure of the Hb to increase by at least 20 g/L after transfusion with 4 units of red cells (Cook, Heyland, Griffith, Cook, Marshall, Pagliarello 1999).

In another multi-centre study, Cook et al. investigated the risk factors for clinically important gastrointestinal bleeding in critically ill patients. Clinically important bleeding occurred in 1.5% of all patients after a mean of 14 days from admission. The risk factors were respiratory failure, coagulopathy, hypotension, sepsis, hepatic failure, renal failure, enteral feeding, glucocorticoids administration, organ transplantation, and anti-coagulant therapy. They found that respiratory failure and coagulopathy were the only independent risk factors. 31% of patients with high risk factors and 0.1% of patients at low risk had clinically significant bleeding (Cook et al. 1994).

In another multi-centre study by Cook et al. to estimate the effect of clinically important gastrointestinal bleeding on mortality and ICU length of stay, the authors studied 1666 critically ill patients, of whom 59 patients developed clinically important bleeding. The authors found when they used different

analytic approaches to explore the risk of death in relation to clinically important gastrointestinal bleeding that this risk was increased in bleeders (relative risk = 2.9, 95% confidence interval (CI)= 1.6-5.5). They found also that bleeding was associated with a significant increase in the length of ICU stay (median length of stay attributable to clinically important bleeding = 3.8, 95% CI = -0.01 to 7.6 days) (Cook, Griffith, Walter et al. 2001).

Cook et al. did a multi-centre randomised trial to compare the use of sucralfate with H2- receptor antagonist for the prevention of upper gastrointestinal bleeding. They found that critically ill patients who received H2 receptor antagonist had a significantly lower rate of clinically important bleeding (Cook, Guyatt, Marshall et al. 1998).

Some studies related clinically important gastrointestinal bleeding to different risk factors. Schuster et al evaluated prospectively the risk of upper gastrointestinal bleeding after admission to a medical intensive care unit. They found that there were no statistically significant relationships with patient's age, chronic obstructive pulmonary disease, hypotension and/or shock. There were statistically significant relationships with number of days in ICU, coagulopathy, acute respiratory illness, malignancy, sepsis, requirement for mechanical ventilation, and days of mechanical ventilation (Schuster, Rowley, Feinstein, McGue, Zuckerman 1984).

REDUCED RED CELL SURVIVAL

Little is known about red cell life span during critical illness. However considering the systemic inflammatory response and complement activation, a premature destruction of red cells is possible. In particular the survival time of transfused red cells may be reduced in this group of patients. Under conditions during which the intra-vascular lifetime or red cells are reduced, either due to external blood loss or due to premature destruction, the ability to increase red cell production in the marrow becomes particularly relevant (Eckardt 2001).

Disturbed erythropoiesis

Disturbed eryrthropoiesis is increasingly recognised to be a potentially major contributor to anaemia in association with critical illness. Measuring reticulocyte count or percent can assess erythropoietic activity of the bone marrow (Beutler, Lichtman, Coller, Kipps, editors 1995; Seidenfeld, Piper, Aronson 2002). Several studies have shown that anaemic critically ill patients have low or normal reticulocyte count and/or reticulocyte percent. Table (2.2) summarises mean Hb or Hct values and the corresponding reticulocyte count or percent in studies related to the critical care setting.

Table (2.2): Summary of mean values of Hb or Hct and reticulocyte % measured in the critically ill patients.

Author	Hct (%) or Hb (g/L)	Reticulocyte percent (%) or count ($x10^9$/L)
Corwin et al. (Corwin 2001c)	30.4 ± 3.7 %	1.7 ± 1.1%
Rodriguez et al. (Rodriguez, Corwin, Gettinger, Corwin, Gubler, Pearl 2001)	103 ± 12 g/L	1.66 ± 1.09%
Van Iperen(van Iperen, Gaillard, Kraaijenhagen, Braam, Marx, van de 2000)	98 ± 10 g/L	56 ± 33 $x10^9$/L
Corwin et al. (Corwin 2001a)	99.7 g/L	1.82%

All the above-mentioned studies agreed that in anaemic critically ill patients there is disturbance in erythropoiesis that is reflected by low reticulocyte count or percent. However, they did not mention the normal reference ranges for Hb, Hct, reticulocyte count, or reticulocyte percent.

Corwin et al. showed that when the mean reticulocyte percent increased by 2.5% (from the baseline to the final value), the mean Hct also increased by 4.8%. In another group of patients when the mean reticulocyte percent increased by 0.8%, the mean Hct increased by 1.4%. This results emphasis the fact that any change in reticulocyte production is usually accompanied by change in the degree of anaemia.

There are several possible mechanisms of abnormal and/or inadequate red cell production [table (2.3)].

Table (2.3): Disturbed erythropoiesis in critically ill patients

Iron metabolism
Inappropriate erythropoietin response
Nutritional deficiencies
Effect of inflammation on bone marrow

Iron metabolism during critical illness

Anaemia in many critically ill patients as well as in patients with chronic inflammatory diseases is characterized by an imbalance in iron homeostasis. During the immune response, iron is shifted from the environment into immune cells, where it is incorporated into the storage protein ferritin. Consequently, the anaemia of chronic disease is characterized by low serum iron concentrations despite increased storage iron. The iron transport protein transferrin may be at the lower limit or below normal. Recent suggestions focus on an association between activation of the immune system and the development of anaemia in critically ill patients (Hobisch-Hagen et al. 2001).

More than 90% of ICU patients have low serum iron, low total iron binding capacity, and low serum iron/total iron binding capacity ratio but do have a normal or, more usually, an elevated serum ferritin concentration. There is usually no evidence of adequate reticulocyte response (Corwin 2001a; Corwin 2001b; Weiss 2002). von Ahsen et al. measured the parameters of erythropoiesis in critically ill patients during the course of their disease. The median reticulocyte count was 57 $x10^{15}$/L during the first 2 days of ICU admission, during the period between 6^{th} and 8^{th} day of admission was 47 $x10^{15}$/L; and nearly after 2 weeks of

admission it was 45 $x10^{15}$/L.The authors did not mention the normal reference range (von Ahsen, Muller, Serke, Frei, Eckardt 2001). However, Bellamy et al. suggested that at ICU admission, 35% of patients could have functional iron deficiency, suggested by the presence of hypochromic red cells and reduced reticulocyte Hb concentration (Bellamy, Gedney 1998). Functional iron deficiency is thought to indicate adequate iron stores but a block to incorporating iron into newly formed red cells. Bellamy et al. also found that patients with functional iron deficiency had longer stays in intensive care compared with patients without functional iron deficiency. They explained the results by suggesting that functional iron deficiency may be a marker of nutritional status or general health (Bellamy, Gedney 1998).

Research in animals and humans has suggested that adequate iron scores are important for immune function and a deficiency may, therefore, predict patients with inappropriate immune responses. Patients may also develop functional iron deficiency in response to immune activation (Bellamy, Gedney 1998).

Rodriguez RM et al. carried out a multi-centre cohort study to determine the prevalence of iron, vitamin B12, and folate deficiency in long-term intensive care unit patients as a part of a study to evaluate erythropoietin response in this cohort. Out of 5288 patients admitted to their ICUs only 184 patients were screened for iron, B12, and folate deficiency. The authors defined B12 and folate deficiency if their levels were below the hospital laboratory reference range (the actual levels were not mentioned in the paper). Iron deficiency was diagnosed when iron/total iron binding capacity ratio of <15% combined with a ferritin <100 ng/L was present. Percentage of patients with iron deficiency was 9%. The authors

described the characteristics of the 160 anaemic critically ill patients not iron, folate, or B12 deficient [table (2.4)]. The mean Hb for this group of patients was 103 g/L (Rodriguez, Corwin, Gettinger, Corwin, Gubler, Pearl 2001).

Table (2.4): Summary of the characteristics of critically ill patients not iron, folate, or B12 deficient (Rodriguez, Corwin, Gettinger, Corwin, Gubler, Pearl 2001).

Percentage	83%
Mean ± SD age	60.3 ± 19.2 years old
Mean ICU length of stay	18 ± 16.5 days
Three most common admitting diagnosis	24% pneumonia, 21% other respiratory illness, and 15%post-trauma
Mean APACHEII score	18.3 ± 5.4
Hospital mortality	28%
Mean Hb	103 ± 12 g/L
Mean reticulocyte	1.66 ± 1.09%
Mean serum iron	27.5 ± 32 µg/dl
Mean total iron binding capacity	170.2 ± 65.3 µg/dl
Mean ferritin	726.8 ± 1203.7ng/mL

The authors did not mention the normal reference ranges for the parameters.

These findings emphasise the fact that at an early point of critical illness, most patients have iron studies similar to the anemia of chronic disease (Rodriguez, Corwin, Gettinger, Corwin, Gubler, Pearl 2001).

Two other studies recorded parameters of iron metabolism during the course of the critical illness. Table (2.5) summarizes data from these studies.

Table (2.5): Summary of iron metabolism parameters during the course of critical illness.

Author	ICU days	Iron	Ferritin	Transfer-rin	Transferri-n saturation
*von Ahsen et al.(von Ahsen, Muller, Serke, Frei, Eckardt 2001)		Median	Median	Median	Median
	1-2	4.8	471	144	16
	6-8	6	767	126	15
	13-15	6.5	795	131	22
	20-25	8.1	774	142	24
	31-40	7.8	723	152	20
**van Iperen et al.(van Iperen, Gaillard, Kraaijenhagen, Braam, Marx, van de 2000)		Mean	Mean	Mean	Mean
	0	20.5	891	1.4	0.12
	10	28.4	1273	1.5	0.14
	21	43.3	776	1.7	0.18

* Normal reference ranges: iron = 7-30 µmol/L, ferritin = 10-300 ng/ml, transferrin = 200-400 mg/dL, and transferrin saturation (%) = not mentioned.

** Normal reference ranges: iron = 55-170 µg/dL, ferritin = 10-200 µg/L, transferrin = 2-3.6 g/L, and transferrin saturation = 0.20-0.45 (fraction).

Erythropoietin concentration during critical illness

Increasing knowledge in the critical care setting indicates that the anaemia commonly seen in critically ill patients represents a combination of blood loss

and an "acute" anaemia of chronic disease. EPO levels that are inappropriately low for the degree of anaemia characterize this type of anaemia (Goodnough 2001).

In contrast to patients with iron-deficiency anaemia, most critically ill patients display blunted erythropoietin response to physiologic stimuli despite comparable haemoglobin concentrations. Inflammatory mediators appear to cause the blunted EPO response by inhibiting the genomic regulation of transcriptional and/or posttranscriptional events (Hobisch-Hagen et al. 2001; Corwin 2001a).

Serum EPO concentrations are only mildly elevated, with little evidence of reticulocyte response to endogenous EPO in critically ill patients (Corwin 2001b). Rodriguez et al. aimed to evaluate erythropoietin (EPO) response to anaemia in a cohort of long-term intensive care unit patients. The mean and median day 2 EPO levels in their study were not significantly higher than the normal level and serial EPO levels in most persistently anaemic patients remained within the normal range (Rodriguez, Corwin, Gettinger, Corwin, Gubler, Pearl 2001).

Table (2.6) summarizes published studies that have measured EPO levels in critically ill patients during ICU stay. Comparing the results of these studies was difficult as the authors used different reference ranges for EPO level and some authors did not mention the normal reference range.

Table (2.6): Summary of published EPO level in critically ill patients.

Author	Normal reference range	EPO level	Hb level (g/L)	Time from ICU admission	Number of patients
Rodriguez et al.(Rodriguez, Corwin, Gettinger, Corwin, Gubler, Pearl 2001)	4.2 - 27.8 mIU/ml	Mean ±SD mIU/ml	Not included in the paper		
		35.2 ± 35.6		Day 2	113
		28.3 ± 31.5		Week 1	41
		33 ± 54.4		Week 2	29
		21.5 ± 16.7		Week 3	12
		29.8 ± 23.6		Week 4	9
Hobisch-Hagen P et al.(Hobisch-Hagen et al. 2001)	Not included in the paper	Mean, range U/L	Mean, range	Day 1	23
		49.8, 3.6-157.7	100, 68-129		
(Rogiers et al. 1997) P et al.	36 IU/L	Mean ± SD IU/L	Mean ± SD Hct	After 1 week	10
		199 ± 93	29.8 ± 1 %		

Author	Normal reference range	EPO level	Hb level (g/L)	Time from ICU admission	Number of patients
von Ahsen et al (von Ahsen, Muller, Serke, Frei, Eckardt 2001)	11-13 U/L	Median (IQ) U/L	Median (IQ)	Days	
		25 (21-25)	113 (102-132)	1-2	71
		25 (21-35)	109 (97-117)	6-8	51
		28 (22-33)	104 (98-118)	13-15	25
		24 (19-34)	104 (98-115)	20-25	17
		24 (19-40)	112 (109-114)	31-41	8
Van Iperen et al. (van Iperen, Gaillard, Kraaijenhagen, Braam, Marx, van de 2000)	5-20 U/L	Mean ± SD U/L	Mean ± SD		
		21 ± 8	98 ± 10	0	12
		19 ± 8	100 ± 9	10	10
		28 ± 19	108 ± 8	21	8
Krafte-Jacobs et al.(Krafte-Jacobs et al.1994)	< 19 mU/ml (mean 6.5)	Mean ±SD mU/mL	Mean ± SD	< 1 day	10
		13.5 ± 10.5	116 ± 5		
Corwin et al (Corwin 2001c)	nL 25 mU/mL	Mean ±SD mU/mL30 ± 29	Mean ± SD Hct 30.4 ± 3.7%	<3 days	80

Nutritional deficiencies during critical illness

Malnutrition is a common problem in critically ill patients that can be present upon admission to the ICU or can develop during the course of critical illness. Deficiencies of folate and/or vitamin B_{12} could occur as a part of the malnutrition process that occurs during critical illness (Cerra, Benitez, Blackburn et al. 1997). Few studies have investigated the prevalence of different types of anaemia during ICU admission. Rodriguez et al. aimed to determine the prevalence of vitamin B12, and folate deficiency. They found that 2% were B12 deficient, and 2% were folate deficient on the second or third day of ICU admission, however, the authors did not mentioned serum levels of these parameters (Rodriguez, Corwin, Gettinger, Corwin, Gubler, Pearl 2001).

In another prospective clinical study in a medical intensive care unit, von Ahsen and colleagues measured parameters of erythropoiesis and red blood cell metabolism that included vitamin B12, and folic acid levels during ICU stay. The normal reference range used for B12 was 240-1100 ng/L and for folate was >3.6 µg/L.Table (2.7) summarises the median values of Hb, B12, and folate for the study during the follow up period (von Ahsen, Muller, Serke, Frei, Eckardt 2001).

Rogiers el al. measured vitamin B12 and folic acid levels in critically ill patients in one of their studies. They did not show the data, however, they mentioned that their levels were within normal limits (Rogiers et al. 1997).

Table (2.7): Summary of the median levels of Hb, B12, and folate in critically ill patients (von Ahsen, Muller, Serke, Frei, Eckardt 2001).

Day	Hb g/L	B12 ng/L	Folate µg/L
1-2	113 (n = 71)	565 (n = 30)	4.1 (n = 31)
6-8	109 (n = 51)	612 (n = 32)	4 (n = 31)
13-15	104 (n = 25)	784 (n = 15)	4.6 (n = 15)
20-25	104 (n = 17)	723 (n = 14)	3.9 (n = 14)
31-40	112 (n = 8)	983 (n = 8)	5 (n = 8)

n = number of patients

Effect of inflammation on the marrow during critical illness

Released pro-inflammatory cytokines, e.g., tumour necrosis factor-[alpha] (TNF-[alpha]), interleukin-1 (IL-1), and interleukin-6 (IL-6) inhibit erythroid precursor cells formation from the bone marrow and reduce the formation of erythropoietin (EPO). The mechanism of this type of anaemia is still unclear. There are some different explanations for mechanisms by which cytokines might affect erythropoiesis such as; a) direct inhibitory effect on red cell production, b) inflammation may impair iron availability for erythropoiesis, c) critically ill patients have inappropriately low erythropoietin concentrations for the degree of anaemia (Gabrilove 2000; McLellan, McClelland, Walsh 2003).

Another explanation for the effect of inflammatory cytokines on erythropoiesis was given in a review by Ebert et al. where they clarified that the production of erythropoietin is under the influence of a gene (EPO gene). Inflammatory cytokines affect EPO gene expression and thus affect EPO production and erythropoiesis (Ebert, Bunn 1999).

Rogiers P. et al. did a study to investigate the relationship between endogenous EPO and haematocrit levels in different groups of critically ill patients. They included ambulatory patients with iron-deficiency anaemia, without acute illness, or acute renal failure as a control group. The mean EPO and Hct levels of the study are shown in table (2.8)

Table (2.8): Summary of the mean EPO level and Hct in critically ill patients (Rogiers et al. 1997).

Group	EPO level (IU/L)	Haematocrit(%)
Control (iron deficiency anaemia)	845	30.3
Sepsis without acute renal failure	124	28.9
Sepsis with acute renal failure	136	28.2
Non-sepsis without acute renal failure	199	29.8
Non-sepsis with acute renal failure	103	32.4

The authors concluded that EPO response to anaemia is blunted in critically ill patients, in acute renal failure, and in the presence of sepsis. They suggested this might result from the production of pro-inflammatory mediators (Rogiers et al. 1997).

Hobisch-Hagen et al assessed the relation between anaemia, serum erythropoietin, iron status, and inflammatory mediators in multipally traumatized critically ill patients. They measured serum EPO, some inflammatory markers, some serum cytokines, and iron status. They found that haemoglobin concentration was low at admission and did not increase during the observation time. Serum EPO

concentration was low and did not show significant increases thereafter. No correlation was found between EPO and haemoglobin concentrations. Serum iron was significantly decreased on day 2 post-trauma and remained low during the study. Serum ferritin increased steadily from day 2, reaching its maximum on day 9. In contrast, concentrations of transferrin were low from admission onward. TNF-α remained within the normal range. sTNF-rI was high at admission and increased further. IL1-ra was above the normal range. IL-6 was very high at admission and did not decrease thereafter. The initial neopterin concentration was normal, but increased until day 9. Significant correlations over time were found for EPO/IL1-ra, EPO/ferritin, and reticulocytes/ferritin. Haemoglobin was not correlated with any of the measured parameters (Hobisch-Hagen et al. 2001).

In summary, studies that investigated the relation between inflammatory mediators and anaemia of critical illness have shown that there is an association between some erythroid and inflammatory indices. However, these studies did not prove that it was a causative relationship.

CRITICAL HAEMOGLOBIN CONCENTRATION IN CRITICAL ILNESS

The minimal haemoglobin level tolerated in the absence of haemorrhage without organ dysfunction is often referred to as "critical haemoglobin." Below this level, compromised oxygen delivery can occur, resulting in a decrease in oxygen consumption or an increase in lactate. Decrease in oxygen delivery, and in turn oxygen consumption is called the "critical oxygen delivery". It is important to realize that the critical delivery is not a fixed value, but varies between organs and is dependent on the metabolic activity of the tissue (Spahn, Casutt 2000; McLellan, McClelland, Walsh 2003).

The critical haemoglobin in the critical care setting is uncertain because of lack of data from controlled trials, and practice guidelines are based primarily on expert consensus and, therefore, vary in their recommendations. The decision to accept a certain level of Hb does not depend only on Hb levels, but it is usually based on factors such as volume status, acuteness of the anaemia, severity of the symptoms, age and the presence of co-morbid conditions, particularly cardiovascular and pulmonary diseases (Sherk, Granton, Kapral 2000).

Few studies have investigated the critical haemoglobin values in humans, as it is difficult to do such studies. Valuable data are available from: a) healthy volunteers, b) surgical setting, c) case reports and data collected from Jehovah's Witness patients, and d) animal studies. Table (2.9) summarises some of these studies.

Table (2.9): Summary of published studies that investigated critical Hb levels

Author	Type of study	Aim of the study	Settings	Results and conclusions
Haisjackl et al.(Haisjackl, Luz, Sparr et al. 1997)	Animal study in pigs.	To study the effects of progressive isovolaemic haemodilution on the intestinal functions in pigs.	12 pigs used for the study, 5 used as control group and 7 were haemodiluted gradually to haematocrits of 20%, 15%, 10%, and 6%.	Arterial pH decreased significantly at hematocrit <10%. Intestinal oxygen supply was well maintained to systemic hematocrit values of approximately 10%.
Spahn et al.(Spahn, Seifert, Pasch, Schmid 1998)	Surgical patients underwent first time mitral valve replacement surgery.	To investigate the effect of low Hb levels on haemodynamic parameters and oxygen consumption after isovolaemic haemodilution.	20 patients with significant mitral valve disease, after phlebotomy, mean haemoglobin value was decreased from 130 g/L to 100 g/L with maintaining central venous pressure to the base line value.	During haemodilution, PaO_2 tended to increase whereas $PaCO_2$ decreased slightly and arterial pH was unchanged. Arterial base excess and bicarbonate decreased slightly and mixed venous oxygen saturation as well as body temperature decreased. The authors did not reach a defined value for the critical haemoglobin in this group of patients, however, they commented that Hb level of 103 g/L is well tolerated.

Author	Type of study	Aim of the study	Settings	Results and conclusions
Spahn DR. et al.(Spahn, Zollinger, Schlumpf et al. 1996)	Elderly patients without known cardiac disease undergoing major abdominal surgery.	To study minimal haemoglobin values that can be tolerated using isovolaemic haemodilution technique.	20 patients with a mean age of 76 years, mean Hb was decreased from 116 to 88 g/L, and central venous pressure values were kept to baseline values.	Cardiac index was increased due to increase in stroke volume, no patients developed signs of cardiac ischaemia, PaO_2 increased whereas $PaCO_2$ was unaltered. Base excess, pH, and bicarbonate decreased slightly during haemodilution. Mixed venous haemoglobin saturation decreased during haemodilution. The authors concluded that Hb level of 88g/L is well tolerated in elderly patients without known cardiac disease.
Spahn et al.(Spahn, Smith, Veronee et al. 1993)	Patients with coronary artery disease receiving beta-adrenergic blockers chronically.	To investigate haemodilution tolerance during cardio-pulmonary bypass surgery.	Randomised control trial. 60 patients underwent isovolaemic haemodilution, mean Hb level was decreased from 126 to 99 g/L, and 30 controls.	In the study group, cardiac index and O_2 extraction increased resulting in stable O_2 consumption. Haemoglobin value of 99 g/L was tolerated in chronically beta-blocked patients with coronary artery disease

Author	Type of study	Aim of the study	Settings	Results and conclusions
Weiskopf et al.(Weiskopf, Viele, Feiner et al. 1998)	Human healthy volunteers.	To investigate the effect of acute isovolemic reduction of blood Hb concentration to 50 g/L in healthy resting humans on cardiovascular system oxygen transport.	2 groups, 11 conscious healthy patients prior to anaesthesia and surgery and 21 volunteers not undergoing surgery. Mean Hb was reduced by venipuncture from 131 to 50g/L and isovolaemia was mentained by intravenous fluids. Cardiovascular parameters, arterial and mixed venous oxygen content, oxyhemoglobin saturation, and arterial blood lactate were measured before and after haemodilution. Electrocardiogram using Holter monitor were recorded continuously.	Systemic vascular resistance was decreased. Heart rate, stroke volume, and cardiac index were increased. No evidence of inadequate oxygenation was found and plasma lactate concentration did not change. No pathological ECG changes were recorded during the study. Hb level of 50g/L can be tolerated by healthy people without affecting either systemic oxygen delivery nor cardiac function.

Author	Type of study	Aim of the study	Settings	Results and conclusions
Toy et al.(Toy, Feiner, Viele, Watson, Yeap, Weiskopf 2000)	Healthy adult volunteers.	To investigate the effect of acute reduction of Hb to 50 g/L on the cardiovascular system and whether it would result in fatigue.	8 subjects, Hb level was decreased to 50 g/L by venesection and isovolaemia was maintained. Energy levels at various times during blood collection were measured using visual analog scale. Heart rate and blood pressure were recorded.	Energy level was lower at Hb of 70, 60, and 50 g/L compared to the baseline. Heart rate increased without change in supine blood pressure. The authors concluded that these findings are not necessarily indications for blood transfusion and can be tolerated by healthy adults.
Viele et al.(Viele, Weiskopf 1994)	Jehovah's Witnesses patients	To assess the lowest tolerable Hb concentration.	61case reports review for Jehovah's Witnesses patients with Hb $\leq$ 80 g/L or Hct $\leq$ 24%	50 patients died, 23 associated with Hb $\leq$ 50g/L, however 3 of them died after cardiac surgery. 25 patients survived with Hb $\leq$ 50 g/L. The data suggested that low Hb can be tolerated and survival is possible.

Clinical experiences in the non-critical care setting suggest that a Hb < 50 g/L is associated with a higher mortality. In young healthy volunteers undergoing isovolaemic haemodilution, Hb concentrations can fall to < 50 g/L without any serious side effects. The risks are greater in those with compromised coronary blood flow; they appear to need a higher Hb value (Goldhill, Boralessa, Boralessa 2002) (Weiskopf et al. 1998).

COMPLICATIONS OF ANAEMIA DURING CRITICAL ILNESS

CARDIAC ISCHAEMIA

Anaemic critically ill patients with ischaemic heart disease are of particular concern. This population may be at particular risk from anaemia due to underlying coronary artery disease. However, they may also be more predisposed to complications of red blood cell transfusion such as volume overload (Hebert, Szick 2000).

Some clinical trials have examined the effect of anaemia on myocardial ischaemia. Nelson et al. studied the effect of postoperative anaemia on myocardial ischaemia and morbid cardiac events in high-risk vascular patients in the intensive care unit. 27 patients who underwent major vascular surgery were attached to continuous ambulatory electrocardiographic monitoring from the evening before surgery up to 80 hours during the postoperative period. All patients had a mean baseline haematocrit value of >30%. The haematocrit value of each patient during the study period was the average of all haematocrit value obtained on post-operative day one. Patients were divided into 2 groups according to their post-operative haematocrit levels; a) patients with post-

operative haematocrit <28%, 77% developed cardiac ischaemia and 46% had a morbid cardiac event, and b) patients with post-operative haematocrit level ≥28, 14% developed cardiac ischaemia and 0% morbid cardiac event (cardiac ischaemia was defined as ischaemic ECG changes and morbid cardiac event as cardiac death, myocardial infarction, and ischaemic pulmonary oedema) (Nelson, Fleisher, Rosenbaum 1993).

Other studies support an association between anaemia and cardiac morbidity in patients with coronary heart disease. Carson et al did a retrospective cohort study in 1958 patients who underwent surgery and declined blood transfusion for religious reasons. Overall 30-days mortality was 3.2%; the mortality was 1.3% in patients with preoperative haemoglobin 12 g/dL or greater and 33.3% in patients with preoperative haemoglobin less than 6 g/dL. The increase in risk of death associated with low preoperative haemoglobin was more pronounced in patients with cardiovascular disease than in patients without. The effect of blood loss on mortality was larger in patients with low preoperative haemoglobin than in those with higher preoperative haemoglobin. The results were similar in analyses of postoperative haemoglobin and 30-day mortality or in-hospital morbidity (Carson, Duff, Poses et al. 1996). Another Canadian analysis by Hebert at al. of a large cohort of critically ill patients found that in patients with cardiac disease, there was a trend toward an increased mortality when haemoglobin values were < 95 g/L (55% versus 42%) as compared with anaemic patients with other diagnoses (Hebert et al. 1997).

Wu et al. conducted a retrospective study in a large cohort of patients (78,974 elderly subjects≥ 65 years old) who were hospitalized with acute myocardial

infarction to evaluate whether there was an association between improving anaemia with blood transfusion and 30-day mortality. Patients were categorized according to the haematocrit on admission. The authors found that anaemic patients with low haematocrit values on admission had higher 30-day mortality rates. In this study the outcome of anaemic elderly patients with acute myocardial infarction was related to the degree of anaemia. Improving anaemia by blood transfusion was associated with a lower short-term mortality rate among patients if they were anaemic with a haematocrit on admission $\leq 30\%$ (Wu, Rathore, Wang, Radford, Krumholz 2001).

Not all studies show associations between low haematocrit and adverse outcomes in patients with ischaemic heart disease. Spies et al investigated the haematocrit value and its effect on myocardial oxygen supply after coronary artery bypass grafting. They found that high haematocrit $\geq 34\%$ was associated with increased rate of myocardial infarction, severe left ventricular dysfunction and mortality rate. By multivariate analysis they found that ICU admission haematocrit was the most significant predictor of adverse outcomes (Spiess et al. 1998).

Walsh TS et al suggested that patients with stable or mild coronary artery disease could probably be managed with transfusion triggers of 70 to 80 g/L unless they have evidence of worsening ischaemia or infarction. Patients with severe, symptomatic disease should probably be close to 100 g/L if there is evidence of ischaemia, recent infarction, or acute cardiac dysfunction (Walsh, McClelland 2003).

RESPIRATORY STATUS/WEANING

Patients without pulmonary disease may tolerate anaemia for two reasons. First, adaptive mechanisms occur (including an increase in cardiac output, and an increase in the 2,3-diphosphoglycerate content of the red blood cells). A second factor, however, is that these patients have a normal PaO_2; minor reductions in oxygen delivery to the tissues may thus not be critical. However, the case may be different in patients with acute or chronic respiratory disease (Schonhofer, Wenzel, Geibel, Kohler 1998).

Most ICU patients in the UK are ventilated at sometime during their ICU stay. Weaning from the ventilator is therefore an important part of critical care during which oxygen delivery and therefore Hb may be important. Marini and colleagues documented that anaemic patients with chronic obstructive pulmonary disease (COPD) might be weaned successfully from mechanical ventilation after haemoglobin is boosted to levels above the currently accepted guidelines for blood administration. The authors related their findings to; a) reduced physiological dead space, b) alteration in mixed venous gas, c) improving oxygen carrying capacity to vital organs such as heart, brain, and neuromuscular system by red cell transfusion (Marini 1998; Toy, Feiner, Viele, Watson, Yeap, Weiskopf 2000).

Schonhofer et al hypothesised that in patients with severe chronic obstructive pulmonary disease (COPD) anaemia might induce an increase in ventilation requirements. If so, transfusion of red blood cells would lead to a reduction in minute ventilation (VE), which should result in reduced work of breathing (WOB). They tested this hypothesis in patients with severe COPD. Anaemic

patients without pulmonary disease served as controls. The authors explored whether blood transfusion reduces minute ventilation and work of breathing (WOB) in patients with COPD. They prospectively evaluated VE and WOB in 20 anaemic adults (haemoglobin of <11 g/dL). Ten patients had severe COPD and ten patients were without lung disease (Schonhofer, Wenzel, Geibel, Kohler 1998).

In both groups, the haemoglobin and haematocrit increased significantly after transfusion. When compared with the baseline values, there was a significant post-transfusion reduction of VE; WOB decreased in patients with COPD. In patients without lung disease VE did not change after transfusion (Schonhofer, Wenzel, Geibel, Kohler 1998).

Schonhofer, in another publication, reported a case series of five anaemic patients (Hb 87 g/L) with chronic obstructive lung disease in whom trials of weaning from the ventilator were unsuccessful. After transfer to a weaning centre, blood was transfused to increase the haemoglobin value to 120 g/L or higher. Subsequently, all patients were weaned successfully. They concluded that in anaemic patients with chronic obstructive lung disease there should not be a fixed transfusion threshold. In anaemic patients in whom difficulty in weaning from the ventilator is experienced, blood transfusion should be tailored to the individual patient's needs. Transfusion in those with chronic obstructive airways disease may lead to successful weaning (Schonhofer, Bohrer, Kohler 1998).

In contrast, Corwin H.L. et al included 1302 critically ill patients in an EPO trial (detail of the study discussed later). Hb increased significantly in the treatment group compared to the placebo group. In a sub-group of patients who were

mechanically ventilated, the increase in Hb levels was not associated with shorter ventilation times (Corwin, Gettinger, Pearl et al. 2002).

Hebert et al. studied a sub-group of 713 patients from the TRICC study (the study discussed in detail later). This group of patients were mechanically ventilated, 357 patients in the restrictive group and 356 patients in the liberal group. Table (2.10) summarises ventilatory characteristics for both groups.

Table (2.10): Summary of mean values of the ventilatory characteristics of a sub-group of patients in the TRICC study (Hebert, Blajchman, Cook et al. 2001).

	Restrictive group	Liberal group
Durations of mechanical ventilation (days)	8.3	8.3
Ventilator-free days (days)	17.5	16.1
Patients successfully weaned (%)	82	78
Relative risk of extubation success	1.07	1.1

From these results, the authors concluded that there was no evidence that maintaining a higher Hb level by red cell transfusion shortened mechanical ventilation time in critically ill patients (Hebert et al. 2001).

NEUROLOGICAL STATUS

Acute reduction of haemoglobin concentration to 7g/dl does not affect healthy human cognitive function. However, further reduction to 6 and 5g/dl produces subtle, reversible increases in reaction time and impaired immediate and delayed memory, and these effects can be reversed by blood transfusion (Toy, Feiner, Viele, Watson, Yeap, Weiskopf 2000).

Weiskopf et al. studied nine healthy volunteers with a mean age of 29 years. The authors tested their memory abilities before the study using computerized neuropsychologic tests. Acute isovolaemic reduction of their Hb was carried out to 70, 60, and 50 g/L. Neuropsychologic tests were carried out at each Hb level. The authors found no change from baseline at Hb level of 70g/L. Reaction time to external stimuli increased at Hb 60g/L, and immediate and delayed memory was affected at Hb 50g/L. When Hb increased to 70g/L with blood transfusion, the neuropsychologic tests returned to the baseline again. The authors suggested that the reversibility of the cognitive function was due to the improvement in the brain oxygenation caused by the transfused red blood cells (Weiskopf, Kramer, Viele et al. 2000).

Granberg et al. performed a prospective qualitative investigation of the ICU syndrome/delirium. The aim of their study was to explore the relationship between the ICU syndrome/delirium and age, gender, length of ventilator treatment, length of stay and severity of disease, as well as factors related to arterial oxygenation and the amount of drugs used for sedation/analgesia. They observed nineteen mechanically ventilated patients who had stayed in the ICU for more than 36 hours, and interviewed then in depth twice after discharge. Demographic, administrative and medical data were collected as a part of the observation study (Granberg Axell 2002).

The authors found that patients with severe delirium had significantly lower haemoglobin concentrations than those with moderate or no delirium. Patients suffering from severe delirium spent significantly longer time on the ventilator and in the ICU, and were treated with significantly higher daily doses of both

fentanyl and midazolam in comparison with those reporting only moderate or no symptoms of delirium. There were no significant differences in the Therapeutic Intervention Scoring System scores, reflecting the degree of illness, between patients with and without delirium.

They concluded that ICU syndrome/delirium was associated with decreased haemoglobin concentrations and extended times on the ventilator. The authors suggested that prolonged ICU stays and treatment with higher doses of sedatives and opioids in patients with delirium could be a secondary phenomena rather than a cause (Granberg Axell 2002). However, the observational nature of the study means a causative link between anaemia and delirium is not proven.

ERYTHROPOIETIN THERAPY DURING CRITICAL ILLNESS

In recent years a number of case reports, small trials and two larger trials have been published on the use of erythropoietien therapy in patients treated in ICU. Following the administration of high doses of erythropoietin, the majority of these reports demonstrated an increase in reticulocyte counts within a few days and subsequent rise in Hb levels (Eckardt 2002). The blunted production of endogenous erythropoietin in response to anaemia suggests that pharmacological doses of EPO may be useful in critically ill patients to reduce the number of red cell transfusions and/or improve anaemia (Corwin 2001a).

There are many issues related to the use of EPO therapy during critical illness. Inflammatory mediators that blunt the action of endogenous EPO and reduce bone marrow activity may have similar effects on exogenous EPO. It is unclear from the present clinical trials the optimum time to start EPO therapy in this

group of patients. Furthermore, it is unclear if this treatment can be effective in preventing and treating anaemia of critical illness, and decreasing the need of allogeneic blood transfusion. The optimum EPO dose requires further evaluation and more studies are needed to show which sub-group of critically ill patients receive greatest benefit.

The studies investigating the role of EPO therapy in the critical care setting are summarised in table (2.11).

Table (2.11): Summary of clinical trials that investigated the use of EPO in the critical care setting.

Author	Aim of the study	Intervention	Results
Van Iperen et al.(van Iperen, Gaillard, Kraaijenhagen, Braam, Marx, van de 2000)	Randomised clinical trial to investigate endogenous EPO production and the response to exogenous EPO in critically ill patients.	36 critically ill patients with haemoglobin levels of <11.2g/dl, or in case of cardiac disease, < 12.1g/dl were included in the study. Study subjects were randomly selected to receive intra-venous (iv) folic acid (control group), iv folic acid and iv iron saccharate (iron group), or iv folic acid, iv iron saccharate and EPO (EPO group). APACHE II score, total blood count, reticulocyte count, EPO concentration, serum transferrin receptors, serum iron, transferrin and serum ferritin were measured. Zinc, vitamin B12, folic acid, creatinine and c reactive protein were measured as well.	Serum EPO concentrations were inappropriately low for the degree of anaemia at baseline, with no difference between patients with and without renal failure. Exogenous EPO administration increased EPO levels to a maximum on day 10. Reticulocyte count increased exclusively in EPO group. Serum transferrin receptors rose only in the EPO group. Haemoglobin concentration and platelet count remained identical in the three-study groups. There were low serum iron, low transferrin and transerrin saturation, and very high serum ferritin. CRP was high in all groups of patients at all time points. Vitamin B12 and folic acid concentrations were normal in all the subjects.

Author	Aim of the study	Intervention	Results
Corwin (Corwin 2001c)	Prospective, randomised, double blind, placebo-controled, multicenter trial to determine whether the administration of EPO to critically ill patients would reduce the number of red blood cell transfusions.	160 Patients were included in the study on ICU day 3. Subcutaneous EPO or placebo was administered and continued daily for 5 days. The subsequent dosing schedule was every other day, minimum for 2 weeks or until ICU discharge. All patients received oral iron on study day one. All patients were followed up for 6 weeks. Blood transfusion requirements were compared. There was no specific transfusion protocol.	The cumulative number of units of red blood cell transfused was significantly less in the EPO group than in the placebo group. The final haematocrit concentration of the EPO group was significantly greater than in placebo group. There were no significant differences between the two groups either in mortality or in the frequency of adverse events.
Corwin et al. (Corwin et al. 2002)	A prospective, randomised, double blind, placebo-controlled, multi-centre trial to evaluate the efficacy of a weekly dose of EPO to decrease red cell transfusion in critically ill patients.	1302 critically ill patients who stayed ≥2 days in ICU were included in the study. 650 patients were randomised to receive a weekly dose of EPO for 3 weeks and if any patient stayed in the ICU >3 weeks he/she received a 4^{th} dose.652 patients were included in a placebo group. Iron therapy was given daily from the first day of the study in a form (oral or iv), which was well tolerated by each individual. Treatment was withheld if the haematocrit was 38%. Patients were followed up to 28 days. Transfusion threshold was similar in both groups, although there was no transfusion protocol. The mean pre-transfusion Hb was 85.7g/L in the placebo group and 85.3g/L in the EPO group.	During the study period, blood transfusion was significantly lower in EPO group. 50.5% of patients in EPO group received red cell transfusion compared to 60.4% in placebo and there was 19% reduction in total red cell units transfused in EPO group. Hb concentration increased more significantly in EPO group. There was no significant difference in 28-day mortality, median length of hospital & ICU stays, and mechanical ventilator-free days.

BLOOD TRANSFUSION IN CRITICALLY ILL PATIENTS

There are arguments both for and against the use of red blood cell transfusion to maintain a high haemoglobin concentration in the critical care setting (Jean-Francois 2001). Table (2.12) summarize the potential benefits and adverse effects of red cell transfusion in this group of patients.

Table (2.12): Benefits and adverse effects of red cell transfusion in the critical care setting (Jean-Francois 2001).

Benefits	Adverse effects
▪ Restoration of intra-vascular volume status.	▪ Immunomodulation.
▪ Improving tissue oxygenation.	▪ Potential for increasing organ failure and mortality.
▪ Improve haemostasis.	▪ Effects due to red blood cell storage lesion.
▪ Increase the safety margin for bleeding.	
▪ Improve cardiac function.	

PREVALENCE OF BLOOD TRANSFUSION IN THE CRITICAL CARE SETTING

The decision to transfuse a critically ill patient can be considered under two headings: a) patient factors and b) physician factors (Hebert, Wells, Martin et al.

1998; Van Der 2001). Table (2.13) shows factors that may influence blood transfusion decisions in ICU.

Table (2.13): Factors influence prevalence of blood transfusion in ICU (Hebert et al. 1998).

Patients' factors	Physician's factors
▪ Age. ▪ Severity of the disease. ▪ Presence of pre-admission bleeding or anaemia. ▪ Co-morbidity. ▪ Cardiac status e.g. ischaemic heart and congestive heart failure. ▪ Pulmonary condition e.g. COPD ▪ Special disorders e.g. haemostatic and blood disorders.	▪ Experience. ▪ Speciality. ▪ Type of ICU e.g. medical and/or surgical. ▪ Country. ▪ Transfusion threshold policy.

Some studies reported the rate of blood transfusion among critically ill patients. Vincent et al. studied 3534 patients and showed that 37% of this cohort were transfused during their ICU stay. von Ahsen studied 96 patients and showed that 39% of them received red cell transfusion. These results were very similar to a study carried out by Walsh et al, which found that 39.5% of ICU admissions

were transfused (Hebert, Szick 2000; von Ahsen, Muller, Serke, Frei, Eckardt 2001; Vincent et al. 2002; Walsh, Lee, Maciver, Garrioch, McClelland 2003a). Table (2.14) shows some of published studies that have been carried out in different ICUs to explore transfusion practice.

Table (2.14): Summary of published data about blood transfusion in critical care setting.

Author.	Type of patients.	Percentage of patients transfused.	Transfusion threshold.	Reason for transfusion.
Chohan S.S et al.(Chohan, McArdle, McClelland, Mackenzie, Walsh 2003)	Medico-surgical.	52 % of patients who stayed >24 hours in ICU (n = 176).	Median Hb 78g/L	18% Haemorrhage, 70% reduced physiological reserves, 11% the reason was not recorded, and 1%due to other causes.
Maciver C.R. et al.(Maciver, Walsh, Lee, MacKirdy, Garrioch, McClelland 2002)	Medico-surgical.	39%	Median Hb 78g/L	They did not mention causes in detail but reported that 60% of transfusion episodes were not related to bleeding.

Author.	Type of patients.	Percentage of patients transfused.	Transfusion threshold.	Reason for transfusion.
Corwin et al.(Corwin, Parsonnet, Gettinger 1995)	Multi-disciplinary ICU.	85% (121/142) of patients who stayed > one week.	Mean Hct 27% 27% 24% 28% 26% 27% 27% SD not mentioned	 No indication reported (29%). Surgical bleeding (24%). Low haematocrit (19%). Low cardiac output (14%). Myocardial ischaemia (2%). Oxygen transport defect (8%). Other (5%).
Babineau et al.(Babineau, Dzik, Borlase, Baxter, Bistrian, Benotti 1992)	Surgical patients. Authors studied all patients managed with consecutive transfusions strategy.	Number of patients = 33 patients.	Mean ± SD Hb 94 ± 2 g/L	Not given in the paper.

Author.	Type of patients.	Percentage of patients transfused.	Transfusion threshold.	Causes of transfusion.
French et al.(French, Bellomo, Finfer, Lipman, Chapman, Boyce 2002)	Multi-centre study in 18 Australian and New Zealand ICUs.	19.8%	Median, range Hb 82, 44-187 g/L 79, 54-119 g/L 82, 44-118 g/L 81, 78-94 g/L 90, 53-129 g/L	Acute blood loss (60.1%). ↓ physiological reserves (28.8%). Altered tissue perfusion (5.3%). Coronary artery disease (0.4%). Indication not defined (5.4%).
Vincent JL et al.(Vincent et al. 2002)	Surgical and medical.	37%	Mean ± SD Hb 84 ± 14 g/L 84 ± 10 g/L 84 ± 12 g/L 87 ± 9g/L 84 ± 12 g/L	Acute bleeding (55.5%). ↓ physiological reserves (28%). Altered tissue perfusion (16.8%). Coronary artery disease (8.2%). Others (11.2%).

Rao et al. studied red blood cell transfusion practice in critically ill patients in London hospitals and found that the average pretransfusion haemoglobin concentration was < 90 g/L in 75% of transfusion episodes. The common indications for transfusion were low haemoglobin (72%) and haemorrhage (25%) (Rao, Boralessa, Morgan et al. 2002).

Vincent et al. showed that transfusion rates differed significantly by hospital type, with community hospitals transfusing at a rate of 36.4%, regional hospitals at a rate of 39.8%, and academic hospitals at the highest rate of 44.2%. This was attributed to differences in illness severity for patient populations (Vincent et al. 2002). In a review by Corwin et al., the authors reported that in the USA, approximately 25% to 30% of transfusions occur in critically ill patients (Corwin 2001a).

A Canadian study by Hebert et al. found that transfusion practice in ICU varied between practitioners. The authors found an association between having an academic affiliation and transfusing patients at lower Hb concentrations. In this study, the number of years spent working in ICU; year of graduation, and primary speciality did not have significant effect on transfusion practice (Hebert et al. 1998).

HOW MANY RBC TRANSFUSIONS ARE GIVEN TO THE CRITICALLY ILL?

The amount of blood transfused to critically ill patients is influenced by factors such as the patient case mix, and the transfusion practice of the clinicians.

In a prospective study by von Ahsen and colleagues77% of the patients received blood transfusions. The authors showed that, for the transfused patients, the number of red blood cell transfusions in individual patients varied between one and 32. Of these patients, 40% received >5 units (von Ahsen, Muller, Serke, Frei, Eckardt 2001).

Corwin et al. studied all patients who stayed in a tertiary care center ICU for more than a week over a one-year period. 141(23%) patients stayed more than one week. Of these patients, 121 (85%) patients received blood transfusion at a mean rate of 9.5 units per patient. 40% of RBCs were transfused during the first week of admission; thereafter transfusion requirement was 2 to 3 units per patient per week (Corwin, Parsonnet, Gettinger 1995).

In a Canadian cohort study, Hebert et al. compared two cohorts of ICU patients from 23 centres, before and after the introduction of routine leukodepletion. They included all patients admitted to these hospitals during the study period (14 786 patients) who received blood transfusion. The authors recorded the number of red cell units transfused and reported the subgroup of patients who received the transfusion. They found that the mean number of units transfused (leukoreduced vs control periods) was the same (3.5 units) for cardiac/surgical patients; 5.4 vs 5.6 units for critically ill/multiple trauma patients; and 2.6 vs 2.5 units for patients with hip fracture (Hebert, Fergusson, Blajchman et al. 2003). These data related entire hospital admission, rather than ICU stay alone.

In the UK, Chohan et al. prospectively collected transfusion data in a large medico/surgical UK teaching ICU over a 6-months period. They found that total red cell use was 3.1 units per admission, comprising 1.6 units and 1.2 units per

admission for reduced physiological reserve and haemorrhage respectively, (0.47 units per patient day). The median number of units per transfusion episode was 2 units (Chohan, McArdle, McClelland, Mackenzie, Walsh 2003). Walsh et al. showed in a multi-centre study of 10 Scottish ICUs that the overall transfusion rate was 1.9 units per ICU admission (0.34 red cell units/24 hours). The authors showed that 1023 critically ill patients in these 10 ICUs utilized a total of 1890 red cell units in 100 days (Garrioch, Walsh, Maciver, McClellan 2002; Maciver, Walsh, Lee, MacKirdy, Garrioch, McClelland 2002).

In another multi-centre Australian and New Zealand study French et al. studied 1808 critically ill patients, 19.8% admissions (357 patients) received blood transfusion. The rate of RBCs transfusion in ICU was 2.5-units/ICU transfusion days (French, Bellomo, Finfer, Lipman, Chapman, Boyce 2002).

Massive red cell transfusion

Vaslef, Steven N et al did a five years, retrospective chart review of 7734 trauma patients admitted to a trauma centre. They found that 0.6% of the patients who had been admitted to the centre's intensive care unit received >50 units of red cell units on the first day of admission. Of these admissions, 36.4% patients received >75 RBCs units and 63.5% received > 25 RBCs units. The greatest number of transfused blood products over 24 hours in a survivor was 116 units (Vaslef, Knudsen, Neligan, Sebastian 2002).

POSSIBLE COMPLICATIONS OF TRANSFUSION THAT ARE OF CONCERN IN CRITICALLY ILL PATIENTS

Immunomodulation

An immunosuppressive effect of blood transfusion may be clinically beneficial in selected patients (e.g., renal transplant recipients). However, in other settings it could have adverse effects such as increased cancer recurrence and increased rates of post-operative infection. Because considerable morbidity and mortality is related to infection in the ICU population, transfusion related immunosuppression is an issue of concern. The incidence of infection appears to increase with the number of transfused red blood cell units, and in some studies red cell transfusion remains associated with infection even after adjusting for confounding factors (Corwin 2001a).

The immunomodulatory effect of red cell transfusion has been investigated in cohort studies, randomised controlled trials, and meta-analysis. Some of these studies compared this effect in transfused and non-transfused patients; other studies compared transfusing leukodepleted and non-leukodepleted red blood cells.

a) Cohort studies

Taylor et al. investigated retrospectively whether critically ill patients who receive allogeneic packed red blood cell transfusions are at increased risk of developing nosocomial infections during hospitalisation. Nosocomial infection rates were compared among three groups: 1) the entire cohort, 2) the transfusion group, and 3) the non-transfusion group. The nosocomial infection rate for the entire cohort was 5.94%, and for the transfusion group and the non-transfusion

group were 15.38% and 2.92%, respectively. There was an association between an increased number of units of RBCs transfused and the chance of infection. The transfusion group was six times more likely to develop nosocomial infection compared with the non-transfusion group. The mean number of RBC units transfused for patients with nosocomial infection was almost four times greater. Mortality rate for the entire cohort was 13.6%. In the transfusion group, mortality was 24.3%, while mortality in the nontransfusion group was 10.2%. The length of ICU stay and hospital stay were significantly longer in the transfused group compared with the nontransfused group (Taylor, Manganaro, O'Brien, Trottier, Parkar, Veremakis 2002). As it was a retrospective cohort, the significance of these associations is unclear.

Leal-Noval et al showed similar results in their cohort study when they investigated the influence of blood derivatives on the occurrence of severe postoperative infection in patients undergoing heart surgery. 738 patients were included in the study from a postoperative ICU of a tertiary-level university hospital. The authors studied the influence of 36 variables on the development of severe post-operative infection in general and individually for pneumonia, mediastinitis, and/or septicemia. The influence of red blood cells and blood derivatives (plasma and platelets) on infections was assessed. 9.4% had severe post-operative infections. Using multivariate analysis, the variables associated with severe post-operative infection were reintubation, sternal dehiscence, mechanical ventilation, reintervention, neurologic dysfunction, transfusion of ≥ 4 units of red blood cell, and systemic arterial hypotension. The mortality rate and

the length of ICU stay were greater in transfused patients (Leal-Noval, Rincon-Ferrari, Garcia-Curiel et al. 2001).

b) Randomised controlled trials

Hebert et al did a multi-centre study to evaluate a national pre-storage leukoreduction program. They studied different types of patients such as cardiac surgery, repair of hip fracture, intensive care patients and multiple traumas. In the study they classified the patients into three groups; patients transfused with leukodepleted blood, a second group received non-leukodepleted and non-transfused groups (control group). The mean number of units transfused was the same. In the leukodepleted group, the proportion of patients with fever episodes was lower, and the use of antibiotics also decreased. However, serious nosocomial infections did not decrease (Hebert et al. 2003).

In a randomised trial by Tartter et al. the incidence of postoperative infections, hospital stays, and hospital charges of gastrointestinal surgery patients transfused with packed red cells (non- leukocyte-depleted) or leukocyte-depleted cells were compared. 221 patients were included in the study. Of these 59 (27%) patients received blood transfusion. The authors found that operative site and nosocomial infections, postoperative stays, and hospital charges were significantly more frequent in patients transfused with packed red cells compared with patients transfused with leukocyte-depleted red cells (Tartter, Mohandas, Azar, Endres, Kaplan, Spivack 1998).

Another retrospective study by Baron and colleagues was done to evaluate the effect of universal leukodepletion of packed red blood cells on postoperative infections in high-risk patients undergoing abdominal aortic surgery. They

studied 2 groups of patients; leukodepleted group (195 patients) and non-leukodepleted group (192 patients). The mean number of units received in both groups was nearly the same. There was no significant difference in morbidity or mortality between the two groups and cardiovascular and respiratory outcomes were not significantly different between the control and leukodepleted groups. The incidence of postoperative infections, severe infectious complications, and multiple organ failure was not significantly different (Baron, Gourdin, Bertrand et al. 2002).

c) Meta-analysis

Several meta-analyses of randomised controlled trials that compared the risk of post-operative infection in patients receiving allogeneic blood transfusion have been done.

Hill GE et al. reviewed 20 articles published over 4 years with a total of 13 152 patients included in the analysis (5,215 in the blood transfused group and 7,937 in the non-transfused group). The common odds ratio for all studies included in this analysis evaluated the association of allogeneic blood transfusion with post-operative bacterial infection. The authors found that allogeneic blood transfusion was a significantly associated risk factor for the development of post-operative bacterial infection in surgical patients. Blood transfusion was a greater risk factor in trauma patients compared to elective surgical patients and this may be due to larger number of units received by this group of patients (Hill, Frawley, Griffith, Forestner, Minei 2003).

Vamavakas EC did a meta-analysis to investigate the association between allogeneic blood transfusion and mortality when transfusing leukocyte-depleted

and non- leukocyte-depleted blood. The author reviewed 14 studies, which recorded mortality as primary or second outcome. Summary odds ratios of mortality in the group that received non-leukocyte-depleted red cells versus the leukocyte-depleted group were calculated from the studies. The author found that there was no association between allogeneic blood transfusion and either long term or short-term mortality. However, in a sub-group (open heart surgery patients) the author found that the association between non-leukocyte-depleted blood and short term-mortality might exist which could be improved by filtering the allogeneic blood from the white blood cells before transfusion (Vamvakas 2003).

Another meta-analysis by Vamavakas EC was done to compare the risk of postoperative infection between recipients of allogeneic and autologous blood transfusion. In this meta-analysis the author calculated the odds ratios of post-operative infection in patients who received allogeneic blood transfusion versus autologous blood. He found that there was no difference in reducing the risk of post-operative infection (Vamvakas 2002).

The evidence is controversial, but there is a strong suggestion from many studies and meta-analysis that leucocytes in the transfused blood have effects on infection and possibly mortality. It is not known if leucodepleted blood has significant immunomodulation effects.

Storage lesion

Transfusing packed red blood cells to maintain a normal haemoglobin concentration has been proposed to represent the most effective means of increasing systemic oxygen delivery in critically ill patients with sepsis. If sepsis

is characterized by inadequate tissue oxygenation secondary to inadequate delivery, increasing arterial oxygen content with RBC transfusion should be accompanied by an increase in systemic oxygen uptake (Marik, Sibbald 1993). It has been suggested that in some circumstances, transfused blood may actually have adverse effects in critically ill patients. Temporarily decreased concentrations of 2,3-DPG and ATP, caused by storage of blood, impair red blood cell deformability and interfere with the ability of the red blood cell to unload oxygen (Corwin 2001a).

Some studies investigated the effect of the age of transfused blood in the surgical and critical care settings. Marik et al studied the effect of red blood cell transfusion on gastrointestinal and whole-body oxygen uptake in ventilated critically ill patients with sepsis within 48 hours of developing sepsis. The measured systemic oxygen up take did not increase significantly during the study. The authors found an inverse association between the change in gastric intramucosal pH and the age of the transfused blood. In those patients receiving blood that had been stored for more than 15 days, the gastric intramucosal pH consistently decreased following the red blood cell transfusion. These data suggested that patients receiving old transfused red blood cells developed evidence of splanchnic ischaemia (Marik, Sibbald 1993).

Offner et al. did a prospective cohort study to determine the consequences of massive transfusion on traumatized critically ill patients. They studied patients who survived longer than 48 hours who were transfused with 6 to 20 U of red blood cells in the first 12 hours after injury. The age of each unit of blood was determined. They found that transfusion of older blood was associated with

subsequent infection; patients who developed infections received 11.7 and 9.9 U of red blood cells older than 14 and 21 days, respectively, compared with 8.7 and 6.7 in patients who did not develop infections. Multivariate analysis suggested that age of blood was an independent risk factor for major infections (Offner, Moore, Biffl, Johnson, Silliman 2002).

Some data from other studies suggests that this is not the case with leucodepleted red cells. Walsh et al. did a prospective double blind randomised study to determine if transfusion of red cells either ≤5 days or ≥20 days from donation alters tonometric indices of gastric mucosal oxygenation or global oxygenation parameters, in euvolemic anaemic critically ill patients without ongoing haemorrhage. In this study, patients were randomised to receive 2 units of leucodepleted red cells that were either ≤5 days (10 patients) or ≥20 days (12 patients). Changes in gastric to arterial PCO_2 gap (Pg-$PaCO_2$ gap), gastric intramucosal pH (pHi), arterial pH, arterial base excess, and arterial lactate concentrations were measured during baseline (2.5 hours), during transfusion (3 hours), and for 5 hours after transfusion. Mean age of red cells stored ≤5 days was 2 days; red cells stored ≥20 days, 28 days. Haemoglobin concentration increased by 150g/Land 166g/L respectively in the fresh and stored groups. There were no significant differences between the groups either using treatment-by-time analysis or comparing the pre- and post-transfusion periods either for Pg-$PaCO_2$ gap or pHi. The mean change within each group from pre- to post-transfusion period for Pg-$PaCO_2$ gap and pHi respectively were: "fresh" red cells 0.56 kPa and –0.018 pH units, and "stored" red cells 0.52 kPa and –0.033 pH units. There was no statistically or clinically significant improvement in any

other oxygenation index over time for either group in comparison to baseline values. This study suggests that the age of blood does not significantly affect the global indices of tissue oxygenation parameters in euvolemic anaemic critically ill patients.(Walsh, McArdle, McLellan et al. 2004)

Organ failure and effect on mortality

Augmenting systemic oxygen delivery using various treatment modalities, including red cell transfusion, may decrease mortality in critically ill patients. Increasing haemoglobin concentrations using allogeneic red cell transfusion could result in this positive effect by improving arterial oxygen content. Few studies have investigated the effect of age of transfused red cells on the patients' outcome. One of these studies carried out by Purdy et al. determined, retrospectively, the age of packed red blood cells transfused to critically ill patients admitted to the ICU and correlated this variable with patients' outcome. They recorded the number and the age of red blood cell units transfused to patients, who had been admitted during a one-year period of time. The authors found a correlation between mortality and the age of the transfused red blood cell units. They concluded that the cause of this association was unclear, but could indicate an adverse effect from transfusion (Purdy, Tweeddale, Merrick 1997). This study was carried out when all red cells were non-leucodepleted.

Maetani, investigated the role of blood transfusion in organ system failure following major abdominal surgery. He included many factors that could contribute to organ system failure such as blood transfusion, age, pre-operative haematocrit, organ failure risk (respiratory failure, gastrointestinal stress bleeding, renal failure, non-obstructive, non-hepatitic jaundice, and

coagulopathy), operative time, blood loss, and post-operative highest haematocrit. He found that except for preoperative haematocrit, all the factors were statistically significant contributors and that blood transfusion was the most significant (Maetani, Nishikawa, Tobe, Hirakawa 1986). As with other retrospective cohort studies, the significance of this result is uncertain.

Vincent et al. compared mortality rates for transfused and non-transfused patients during ICU stay after using statistical techniques to match patients for other risk factors. The authors found that ICU mortality was 13.5% and 28-day overall mortality was 20.2%. Both ICU and overall mortality rates were significantly higher for transfused versus non-transfused patients (ICU: 18.5% vs 10.1%, respectively; overall: 29.0% vs 14.9%, respectively). Transfused patients had longer ICU length of stay, more severe organ failure, and higher mortality rates than non-transfused patients (Vincent et al. 2002). Another study also showed that transfused patients had a greater mortality rate (13.3% vs 8.9%, respectively) and a longer mean stay in the ICU (6.1days vs 3.7 days, respectively) than those not transfused (Leal-Noval et al. 2001).

Corwin et al. did some studies investigated the use of erythropoietin in ICU. Mean haemoglobin concentrations for patients who received EPO treatment were similar compared to patients who received blood transfusions. Mortality rate was the same in both groups (Corwin, Gettinger, Rodriguez et al. 1999; Corwin 2001a; Corwin 2001c). These studies provide indirect evidence against an adverse effect from RBC transfusion, but were not powered to detect this.

In summary, there are no large randomised control trials investigating the association between the storage age of transfused blood and patient outcome (McLellan, Walsh, McClelland 2002).

TRANSFUSION TRIGGERS IN THE CRITICAL CARE SETTING

Few large clinical studies have been done aiming to find the optimal Hb concentration and transfusion triggers in critically ill and high-risk patients. Bracey AW et al. did a study to determine if lowering the haemoglobin threshold for red cell transfusion to 80g/L after coronary artery bypass graft surgery would reduce blood use without adversely affecting patient outcome. The authors compared the study group (received blood transfusion post-operatively when Hb $\leq$ 80 g/L) to a control group (patients who were transfused when their post-operative Hb $\leq$ 90 g/L). There was no difference in clinical outcome, including morbidity and mortality rates, in the two groups. A haemoglobin transfusion trigger of 80g/L did not adversely affect patient outcome after the procedure (Bracey, Radovancevic, Riggs et al. 1999).

***TRICC study** (**T**ransfusion **R**equirements **I**n **C**ritical **C**are)* (Hebert, Wells, Blajchman et al. 1999)

The TRICC study is the key background study that investigated red cell transfusion in critically ill patients. It was a multi-centre, randomised, controlled clinical trial to compare the effect of a restrictive strategy of red-cell transfusion with a liberal strategy on critically ill patients. The authors included patients who were expected to stay in ICU $\geq$ 24 hours and had a Hb $\leq$ 90 g/L within 72 hours after admission. 838 patients were randomised to one of two groups: a) 418

patients in a restrictive strategy group, these patients were transfused one RBC unit when their Hb fell below 70 g/L. Hb levels were maintained in the range of 70 to 90 g/L. b) 420 patients in a liberal strategy group, patients were transfused when Hb was $\leq$ 100 g/L and was maintained between 100 and 120 g/L.

The mean APACHE II score was 21 for all patients with no significant difference between the two groups. Table (2.15) summarises the main findings of the study.

Table (2.15): Summary of the main findings in the ***TRICC*** study (Hebert et al. 1999)

	Restrictive strategy group N= 418	Liberal strategy group N = 420
Average daily Hb	85 g/L	107 g/L
Red cell units transfused per patient	2.6	5.6
Percentage of patients not transfused	33%	0%
30 days mortality after admission.	18.7%	23.3%
Mortality rate during hospitalisation	22.2%	28.1%
Mortality rate during the entire ICU stay.	13.9%	16.2%
Rate of cardiac events (primarily pulmonary oedema and myocardial infarction).	41%	44%
Infectious complications	3%	4%
Multi-organ failure (more than 3 organs)	37%	32%
30 days mortality rate in subgroups		
Cardiac patients (n = 151)	20.5%	22.9%
Septic patients (n = 141)	22.8%	29.7%
Trauma patients (n = 100)	10%	8.8%

The authors suggested that critically ill patients with Hb < 70g/L should be transfused to keep their Hb between 70 and 90 g/L as a restrictive strategy of red cell transfusion is at least as effective as and possibly superior to a liberal transfusion strategy (Hebert et al. 1999).

The authors published details of a sub-group of patients who were mechanically ventilated to show the effect of blood transfusion on their outcome. This study has been discussed in details in a previous section (detail see page 59) (Hebert et al. 2001). The authors published details of another sub-group of patients who had coronary artery disease (detail see page 55) (Hebert et al. 1997).

A Cochrane review by Hill et al. concluded that in patients who do not have serious coronary heart disease, transfusion can be withheld in the presence of haemoglobin concentration as low as 70g/L if there is no obvious bleeding. More large clinical trials should include critically ill patients with renal and cardiac diseases to assess the impact of lower transfusion threshold on their clinical outcome and quality of life (Hill, Carless, Henry et al. 2002).

MATERIAL AND METHODS

RETROSPECTIVE STUDY INVESTIGATING THE PREVALENCE OF ANAEMIA AMONG SURVIVORS OF CRITICAL ILLNESS MANAGED WITH CONSERVATIVE TRANSFUSION TRIGGERS

AIMS

PRIMARY AIM

To determine the prevalence of anaemia among ICU survivors who were managed with conservative transfusion triggers during ICU stay at the time of discharge home from hospital.

SECONDARY AIM

To describe the pattern of anaemia at hospital discharge.

To determine factors, during ICU stay, that are associated with anaemia at hospital discharge.

PATIENTS STUDIED

Two cohorts of general ICU patients were included in this study. Both cohorts of patients had been studied previously during their ICU stay as part of prospective blood transfusion audits

- First cohort period: between 27/1/2000 and 08/7/2000.
- Second cohort period: between 4/6/2001 and 12/9/2001.

FIRST COHORT PERIOD

Data were prospectively collected daily for the study period on haemoglobin concentration, red cell transfusion and indications for transfusion during ICU stay. Only patients who stayed in ICU more than 24 hours were included in the analysis.

For non-haemorrhage transfusion episode, the median pre-transfusion Hb concentration was 78 g/L (interquartile range: 74-84 g/L).

SECOND COHORT PERIOD

During the study period, data were prospectively collected in a similar way as for the first cohort. All patients were included in this audit during the ICU stay. The median pre-transfusion Hb was 78 (interquartile range: 73-85 g/L).

In the present study all patients were included [not only patients who stayed > 24 hours]. General characteristics of the study population and ICU demographic data were described for the combined cohorts. Transfusion thresholds were low and identical during both periods. The results indicate that ICU clinicians followed a conservative transfusion strategy during the periods of both cohorts.

STUDY DESIGN

For both cohorts a retrospective computer based study was designed by obtaining two datasets:

- ICU dataset.
- Haematology dataset.

ETHICS

The studies were carried out in the form of audit. No interventions were carried out and patient management was not altered in any way on the basis of the data collected. We obtained clear guidance from the Local Regional Ethics Committee that ethical review was not required. Guidelines from the data protection act were followed and anonymisation of data after linkage was carried out. Patients'

identifiers were deleted and ICU key number was used as a primary key in analyzing the data.

DATASETS

FORMAT

Each dataset was obtained in excel files.

ICU DATASET

For both cohorts, the following identifiers were obtained from the ICU database (Scottish Intensive Care Society (SICS) Audit Group database). Each dataset contained demographic and outcome data for all patients admitted to the unit during the study periods. Table (2.16) shows the fields which were extracted from SICS database [ICU dataset]. The two cohorts were merged in one excel file to obtain one ICU dataset.

HAEMATOLOGY DATASET

From the hospital haematology database, the haematological results were obtained for all patients who were in the Royal Infirmary, Edinburgh between 01/01/2000 & 31/10/2000 and 01/06/2001 & 30/11/2001. The following data were extracted from the hospital haematological laboratory database for each patient table (2.17).

Table (2.16): The extracted fields from SICS database [ICU dataset]

Field	Content
ICU ID (ICU key number)	Numeric value
Surname	Alpha characters <30 characters
Forename	Alpha characters <30 characters
Sex	M/F
Date of birth	dd/mm/ccyy
Age	Numeric value
Hospital ID	ISD Hospital code
Date of Discharge from ICU	dd/mm/ccyy
Date of discharge from hospital	dd/mm/ccyy
APACHEII score	Numeric value
ICU length of stay	Numeric value
ICU outcome	Alpha characters <30 characters
Hospital outcome	Alpha characters <30 characters
Destination on discharge from ICU	Alpha characters <30 characters
Outcome on discharge from hospital	Alpha characters <30 characters
Destination on discharge from hospital	Alpha characters <30 characters
Type of destination on discharge from hospital	Alpha characters <30 characters

Table (2.17): The extracted fields from the hospital haematology laboratory database [haematology dataset]

Field	Content
Hospital ID	ISD Hospital code
Surname	Alpha characters <30 characters
Forename	Alpha characters <30 characters
Sex	M/F
Date of birth	dd/mm/ccyy
Sample request date	dd/mm/ccyy
Haematological results	
Haemoglobin concentration (g/L)	Numeric value
Mean red cell corpuscular volume (MCV)	Numeric value
Mean red cell corpuscular haemoglobin concentration (MCHC)	Numeric value
White blood cell count	Numeric value

LINKAGE

This process involved the linking of each ICU patient's record, using the patient identifier (surname, forename, date of birth, hospital number, and sex) to an extract from the haematological laboratory database. This extract contained the specified patient denomination data in order to carry out the linking between the two databases. The process of linkage is described in detail in appendix (I).

STUDY DATABASE

From the linkage of ICU dataset and the haematology dataset, a study database was prepared consisting of complete records of each patient containing the data shown in table (2.18)

From the final study database, the following parameters were extracted and calculated for each patient table (2.19). The definitions of each parameter, which were used to extract the data points, are shown.

Table (2.18): Fields of the study database

Fields	Contents
ICU ID (ICU key number)	Numeric value
Surname	Alpha characters <30 characters
Forename	Alpha characters <30 characters
Sex	M/F
Date of birth	dd/mm/ccyy
Age	Numeric value
Hospital ID	ISD Hospital code
Date of Discharge from ICU	dd/mm/ccyy
Date of discharge from the Hospital	dd/mm/ccyy
APACHEII score	Numeric value
ICU length of stay	Numeric value
ICU outcome	Alpha characters <30 characters
Hospital outcome	Alpha characters <30 characters
Sample request date	dd/mm/ccyy
Haematological results	
Haemoglobin concentration (g/L)	Numeric value
Mean red cell corpuscular volume	Numeric value
(MCV)	Numeric value
Mean red cell corpuscular haemoglobin	
concentration (MCHC)	Numeric value
White blood cell count	

Table (2.19): Different parameters used in the study and their definitions

Haematological result & dates	Definition (defined by sample request date)
Last haemoglobin concentration prior to discharge from ICU	The nearest haemoglobin record before ICU discharge date.
Last haemoglobin concentration prior to discharge from hospital	The nearest haemoglobin record before hospital discharge date.
Last mean cell volume (MCV) prior to discharge from hospital	The nearest MCV record before hospital discharge date
Last mean corpuscular haemoglobin concentration (MCHC) prior to discharge from hospital	The nearest MCHC record before hospital discharge date
Number of days before ICU discharge last haemoglobin was requested	ICU discharge date – last haemoglobin request date before ICU discharge.
Number of days before hospital discharge last haemoglobin was requested	Hospital discharge date – last haemoglobin request date before hospital discharge.
Number of days in hospital post ICU discharge	Hospital discharge date – ICU discharge date.
Mean haemoglobin concentration during post ICU stay	
Number of haemoglobin concentrations recorded during post ICU stay	
Minimum haemoglobin concentration during post ICU stay	

SPREADSHEET FOR ANALYSIS

From the study database, a spreadsheet was prepared for primary analysis.

Table (2.20) shows the final spreadsheet used for analysis. Figure (2.1) illustrates the management of the datsets used in the study to obtain final spreadsheet for analysis.

ANALYSIS

For analysis, tables were created summarising

- Patients' demographic data.
- Haemoglobin levels in bands for all patients at the time of ICU discharge.
- Haemoglobin levels in bands at the time of hospital discharge for all patients who discharged home.
- Different types of RBCs indices at the time of hospital discharge.

Table (2.20): The contents of the final spreadsheet used for analysis
ICU Key Number
Hospital Number
Date of admission to ICU
Date of discharge from ICU
Date of discharge from hospital
ICU outcome
Destination on discharge from ICU
Outcome on discharge from hospital
Destination on discharge from hospital
Type of destination on discharge from hospital
Age on admission to ICU (years)
Gender
APACHE II score
ICU stay (days)
Last haemoglobin concentration prior to discharge from ICU
Number of days before ICU discharge last haemoglobin was requested
Number of days in hospital post ICU discharge
Number of haemoglobin concentrations recorded during post ICU stay
Mean haemoglobin concentration during post ICU stay
Minimum haemoglobin concentration during post ICU stay
Last haemoglobin concentration prior to discharge from this hospital
Last mean cell volume prior to discharge from this hospital
Last mean corpuscular haemoglobin prior to discharge from this hospital
Number of days before hospital discharge last haemoglobin was requested

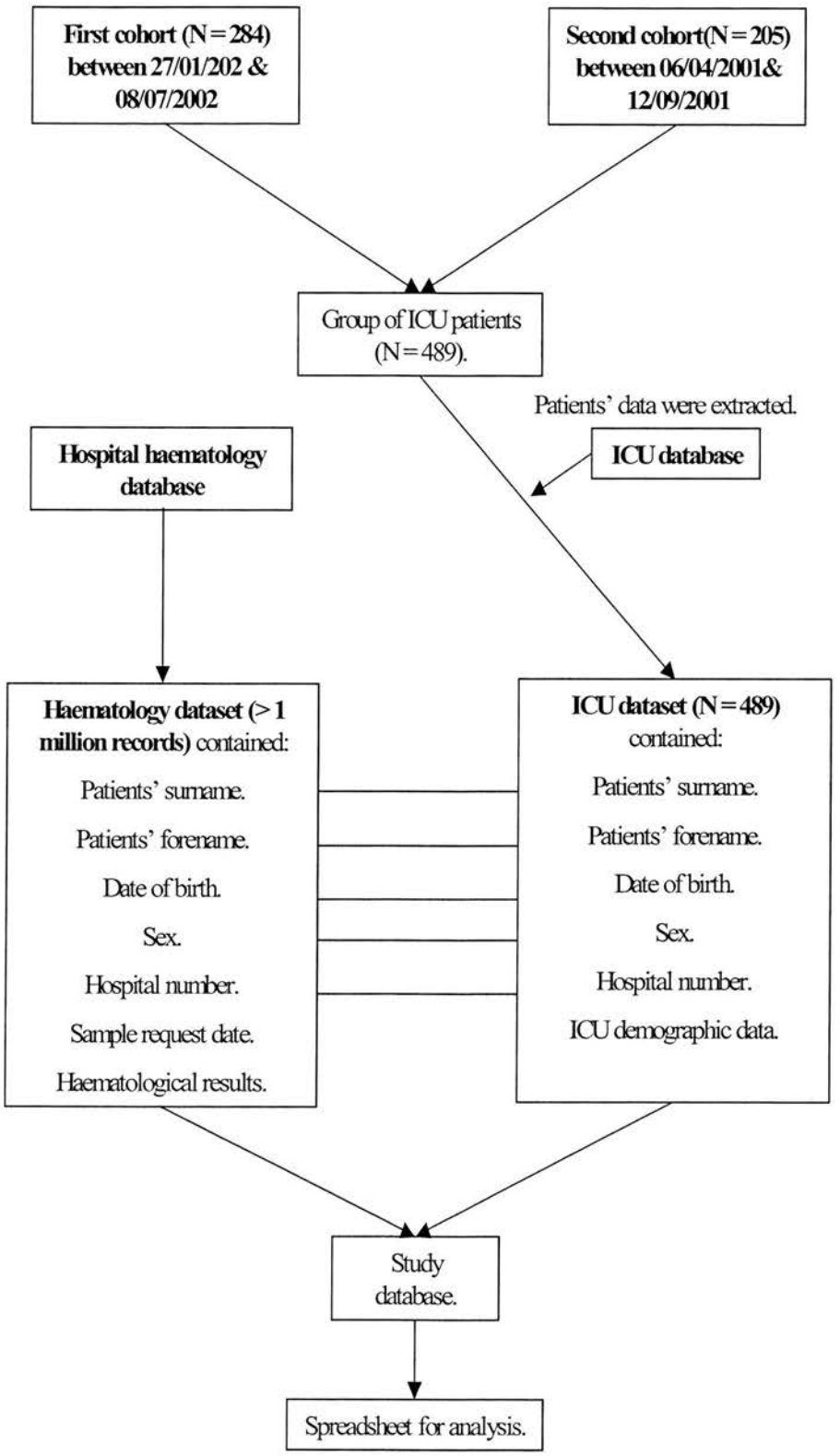


Figure (2.1): Flow chart illustrating management of the datasets used in the study

PROSPECTIVE STUDY INVESTIGATING THE PREVALENCE OF ANAEMIA AMONG SURVIVORS OF CRITICAL ILLNESS MANAGED WITH CONSERVATIVE TRANSFUSION TRIGGERS

AIMS

PRIMARY AIM

To determine the prevalence of anaemia during recovery from critical illness for patients who survived ICU and post-ICU hospital stays.

SECONDARY AIM

To describe the pattern of anaemia at hospital discharge.

To describe transfusion practice in patients after ICU discharge.

PATIENTS STUDIED

All patients who were discharged from the ICU between 19/11/2002 and 06/03/2003 were included in the study. During both periods, ICU clinicians used evidence based transfusion practice; the median pre-transfusion Hb concentration was 78 g/L (IQ: 74-84 g/L) during the first period and 78 (IQ: 73-85 g/L) during the second period.

EXCLUSION CRITERIA

- Patients who were discharged home directly from ICU.
- Patients who were discharged to another hospital (acute or rehabilitation) directly from ICU because final Hb at the time of discharge home was not known.
- Patients who died in ICU.

DATA COLLECTION

All patients who were discharged alive from ICU to a ward in the hospital were followed up daily during their hospital stay until death or discharge from the hospital.

Data that were collected during the follow up period:

- Patient's ICU demographic and outcome data.
- Haematological data.
- Transfusion-related data.

Patients' demographic and outcome data for all patients were collected from the Scottish Intensive Care Society (SICS) Audit Group database via dedicated ward-based desktop computer terminals.

Haematological data were collected daily from the case notes and the hospital haematology database via dedicated ward-based desktop computer terminals.

Transfusion data were collected from the case notes.

The data were collected in excel file.

Table (2.21) shows the data that were collected in different categories.

EXCLUDED DATA

All haematological and transfusion data during ICU stay were excluded from the study, because this was already well characterised for the first part of the study.

Readmissions: if a patient was readmitted to the ICU during the same hospital admission, all the haematological and transfusion data during this period were excluded from the study.

DATA QUALITY

After completion of the study spreadsheet, multiple checks were done to fill some missing Hb values from the haematology database terminals in the ICU.

ANALYSIS

Tables were created for analysis summarising

- Patients' demographic data and outcome.
- Haemoglobin levels in bands at the time of ICU discharge.
- Haemoglobin levels in bands at the time of hospital discharge.
- RBCs indices at the time of hospital discharge for patients who were discharged home.
- Transfusion data.

ANALYSIS OF COMBINED DATASET

We combined all data from the retrospective and prospective cohorts. From this dataset we calculated the overall prevalence of anaemia at hospital diacharge for all patients discharged home after survining ICU and hospital stay. We further explored the relationships between various characteristics of ICU stay, namely the last Hb before ICU discharge, gender, age, APACHE II score, and the length of ICU stay, and the probability of dicharge from hospital with a Hb < 100g/L. This analysis was done using logistic regression in collaboration with Mr Robert Lee, Medical Statistics Uint, Edinburgh University.

Multi-variable logistic regression was used to identify which variables were statistically significant independent predictors of a last Hb prior to hospital discharge <100 g/L. Two-way interactions between the potential predictors were examined in

addition to the main effects. A p-value <0.05 was regarded as statistically significant. As there was no evidence of non-linearity in the relationships between a logistic transformation of the response probability and APACHE II score, length of ICU stay, or last Hb before ICU discharge these were included as continuous variables in the logistic regression models. Since there was strong evidence of a non-linear relationship between a logistic transformation of the response probability and age on admission to ICU this variable was divided into quarters and included as a categorical variable in the logistic regression models.

Table (2.21): Collected data during the prospective study

Patient's demographic data and outcome
ICU key number (number to identify patients in ICU database).
Patient's surname.
Patient's forename.
Age.
Sex.
Date of birth.
ICU admission date.
ICU discharge date.
ICU length of stay.
APACHEII score.
ICU outcome
Destination after ICU discharge.
Haematological data
Last haemoglobin before ICU discharge.
Every Hb concentration measured during hospital stay after ICU discharge.
Last MCV and MCHC before hospital discharge for patients who discharged to their normal residency.
Transfusion data
Date of transfusion.
Number of red cell units transfused.
Pre-transfusion Hb level.
The number of the transfusion episode.
Specialty of the consultant who prescribed the transfusion.

RESULTS

RESULTS OF THE RETROSPECTIVE STUDY

ICU dataset

A total of 489 admissions were obtained from the SICS database for the two cohorts comprising all patients admitted to ICU between 27/1/2000-08/7/2000 (N=284) and 4/6/2001-12/9/2001 (N=205).

Of 489 ICU admissions: 321 were discharged alive (2 directly home), 77 patients were transferred to other hospitals after ICU discharge and 31 died, leaving 211 patients discharged home; of these 56.9% (N=120) were males and females 43.1% (N = 91). Figure (2.2) illustrates the outcome in all patients after ICU discharge until the time of hospital discharge.

The study group comprised 211 patients who were discharged home from hospital after surviving ICU and hospital stays. Demographic characteristics of these patients are shown in table (2.22).

Table (2.22): Patients' demographic data (N = 211)

Variable	Mean	SD	Median	Range	Q1	Q3
Age	52.2	19.1	55	13-92	35	66
APACHE II	17.8	6.4	18	3-35	14	22
ICU stay (days)	4.2	7.7	1.5	0-52.9	0.8	3.7
Number of days in ICU	5.2	7.8	2	1-54	2	5
Days in Hosp after ICU	18.4	17.4	13	1-119	7.5	24

Haematology dataset

A total of 1,102,676 haematological results from 220,717 blood samples in the period between 01/01/2000 and 31/10/2000 were obtained. A total of 688,522 results from 137,836 blood samples were obtained for the period between 01/06/2001 and 30/11/2000. These 2 datasets contained all patients who had been in the hospital during the study periods and were linked to patients' dataset.

Haemoglobin concentrations at the time of ICU discharge

4 patients (3 females & 1 male) had no haematological records at the time of ICU discharge. Last Hb values recorded in ICU were considered as ICU discharge Hb because these values were available to ICU physicians at the time of ICU discharge. Table (2.23) shows the number of patients in different Hb bands at the time of ICU discharge. Figure (2.3) shows the number of males and females in different Hb bands. Figure (2.4) shows Hb levels for all patients.

85.3% of all patients were discharged anaemic from ICU with a haemoglobin concentration below the hospital laboratory reference range (males <130 g/L & females <115 g/L). The prevalence of different degrees of anaemia ("mild" < laboratory reference range, "moderate" Hb< 100 g/L, and "severe" Hb< 90 g/L) at ICU discharge is shown in table (2.24).

Table (2.24): Prevalence of different degrees of anaemia at ICU discharge

Hb	Males 120	Females 91	Total 211
	N (%)	N (%)	N (%)
≤ 90g/L	35 (29.2%)	32 (35.2%)	67 (31.8%)
≤100g/L	66 (55%)	40 (44%)	116 (55%)
≤ Reference range	107 (89.2%)	73 (80.2%)	180 (85.3%)

Haemoglobin concentrations at the time of hospital discharge

Out of 211 patients (the study group), 20 patients had no haematological results after ICU discharge (during their post-ICU hospital stay). The median (IQR) and [range] of post-ICU hospital stay for these patients were 2 (1-4) and [1-18] respectively. For these patients, last Hb recorded in ICU was used as hospital discharge Hb. 191 had at least one haematological result during their post-ICU stay in the hospital.

81.5% of all patients who were discharged to their normal residency (n = 211) had a haemoglobin concentration below the hospital laboratory reference range at hospital discharge. Table (2.25) shows the number of patients in different Hb bands, figure (2.5) shows number of males and females, and figure (2.4) shows the distribution of all patients in different Hb bands at hospital discharge.

12.8% of the patients were discharged from the hospital to their normal residency with Hb <90g/L, and 35.5% with Hb <100g/L. Table (2.26) shows the prevalence of different degrees of anaemia at hospital discharge in detail.

Table (2.26): Prevalence of different degrees of anaemia at hospital discharge.

Hb	Males 120 N (%)	Females 91 N (%)	Total 211 N (%)
≤ 90g/L	10 (8.3%)	16 (17.6%)	27 (12.8%)
≤100g/L	40 (33.3%)	35 (38.5%)	75 (35.5%)
≤ Reference range	104 (86.7%)	68 (74.7%)	172 (81.5%)

Table (2.27) summarises Hb characteristics during ICU and post-ICU hospital stays.

Incidence of different types of anaemia at hospital discharge

83% of anaemic male patients and 80% of anaemic females, with Hb concentration < hospital laboratory reference range, had normocytic normochromic anaemia at

hospital discharge when using patients' MCV and MCHC to identify different types of anaemia (the hospital laboratory reference range for normal levels of MCV and MCHC are 76 to100 fl and 31 to 35g/dl, respectively). 2 MCHC values were missing for male patients with hospital discharge Hb < reference range and 4 values for females. Table (2.28) shows the prevalence of different types of anaemia for males and females with Hb < reference range at hospital discharge and figure (2.6) for all patients.

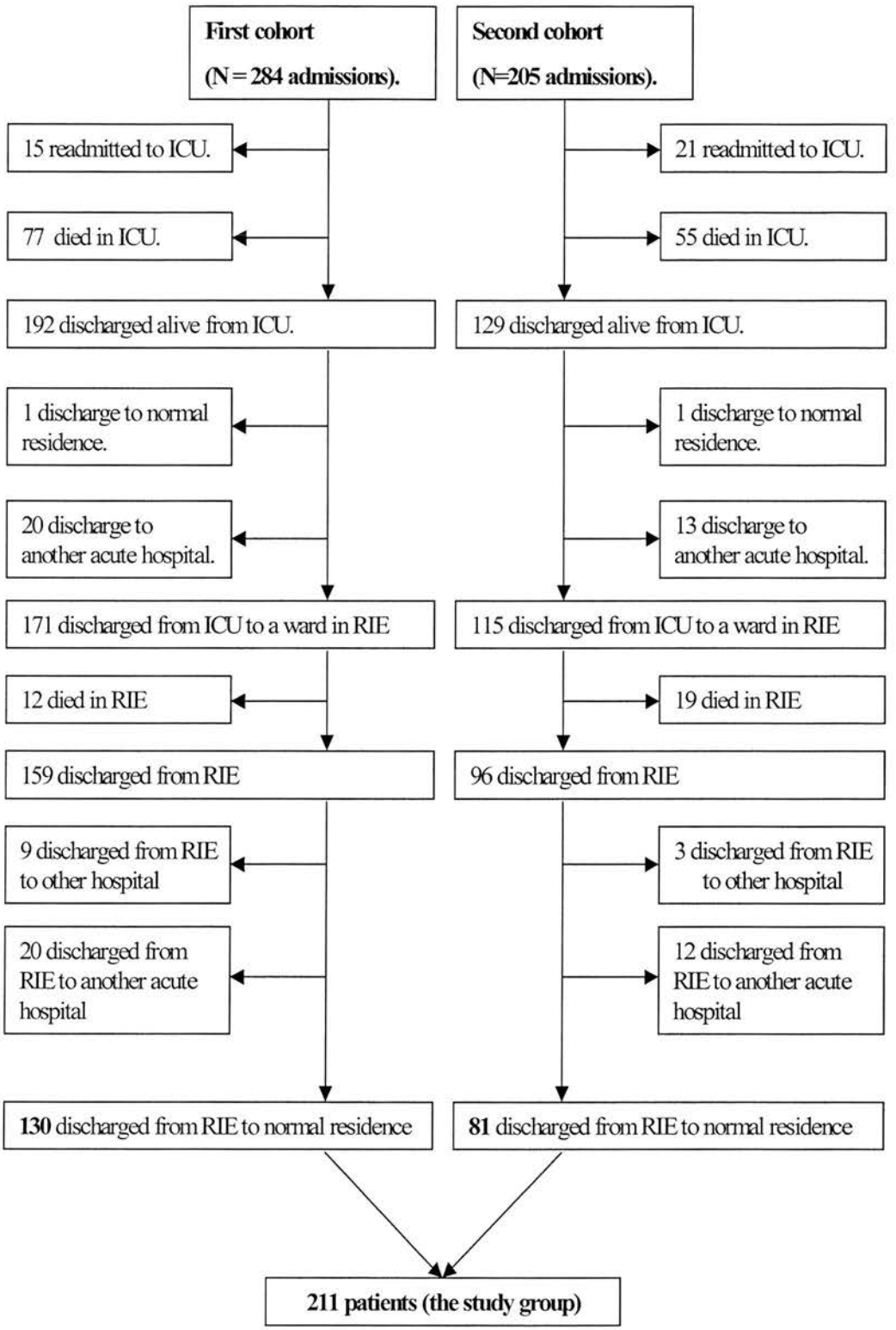


Figure (2.2): Flow chart illustrating outcomes in patients studied [retrospective cohort]

Table (2.23): Number of patients (males, females , and total) in different Hb bands at ICU discharge.

Haemoglobin level	Males	Females	Total
≥65-<70	1	1	2
≥70-<75	2	0	2
≥75-<80	7	5	12
≥80-<85	8	9	17
≥85-<90	11	14	25
≥90-<95	17	9	26
≥95-<100	18	9	27
≥100-<105	9	9	18
≥105-<110	12	6	18
≥110-<115	10	9	19
≥115-<120	4	7	11
≥120-<125	5	5	10
≥125-<130	3	3	6
≥130-<135	6	2	8
≥135-<140	1	0	1
≥140-<145	0	2	2
≥145-<150	3	0	3
>150	3	1	4

Figure (2.3): Number of males and females in different haemoglobin bands at ICU discharge

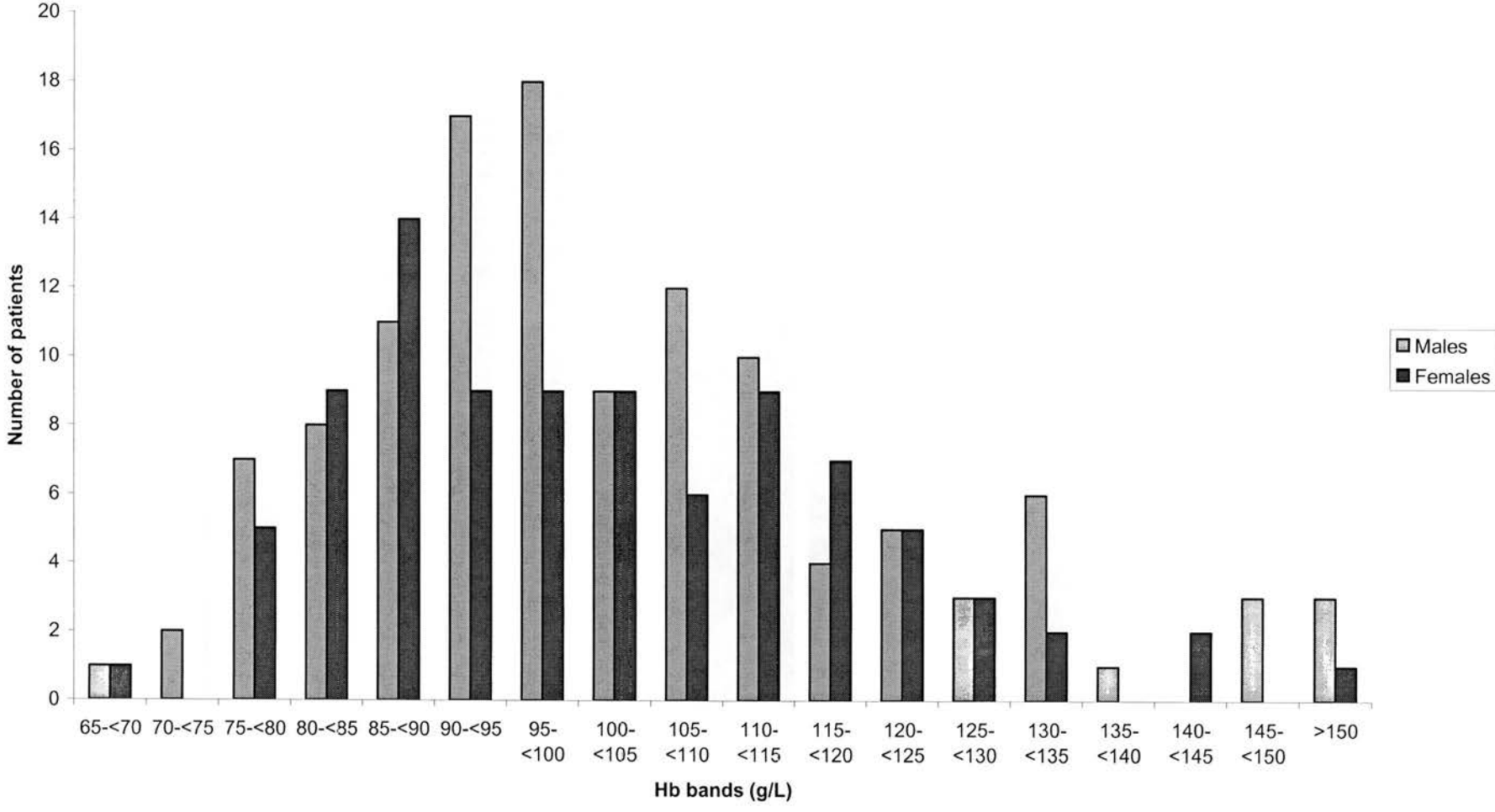
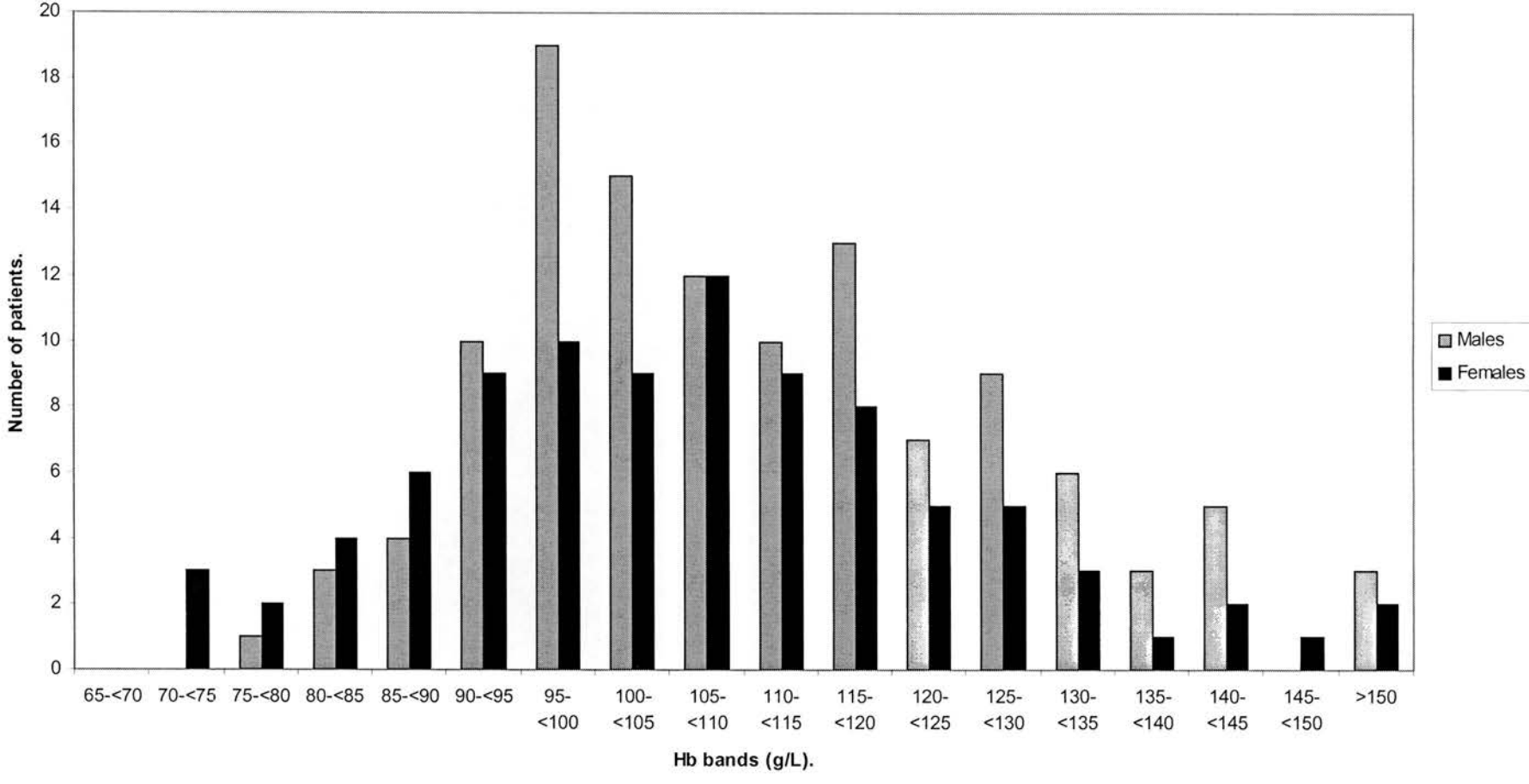
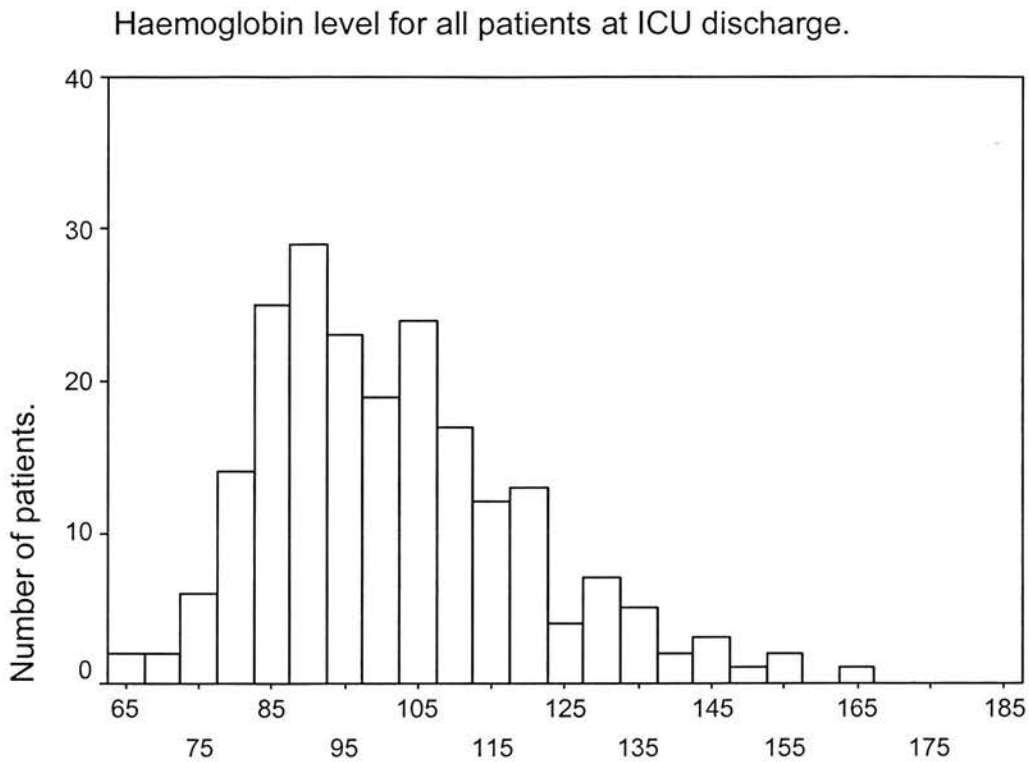


Table (2.25): Number of patients (males, females, and total) in different Hb bands at hospital discharge.

Haemoglobin level	Males	Females	Total
≥65-<70	0	0	0
≥70-<75	0	3	3
≥75-<80	1	2	3
≥80-<85	3	4	7
≥85-<90	4	6	10
≥90-<95	10	9	19
≥95-<100	19	10	29
≥100-<105	15	9	24
≥105-<110	12	12	24
≥110-<115	10	9	19
≥115-<120	13	8	21
≥120-<125	7	5	12
≥125-<130	9	5	14
≥130-<135	6	3	9
≥135-<140	3	1	4
≥140-<145	5	2	7
≥145-<150	0	1	1
>150	3	2	5

Figure (2.5): Number of males and females in different haemoglobin bands at hospital discharge.





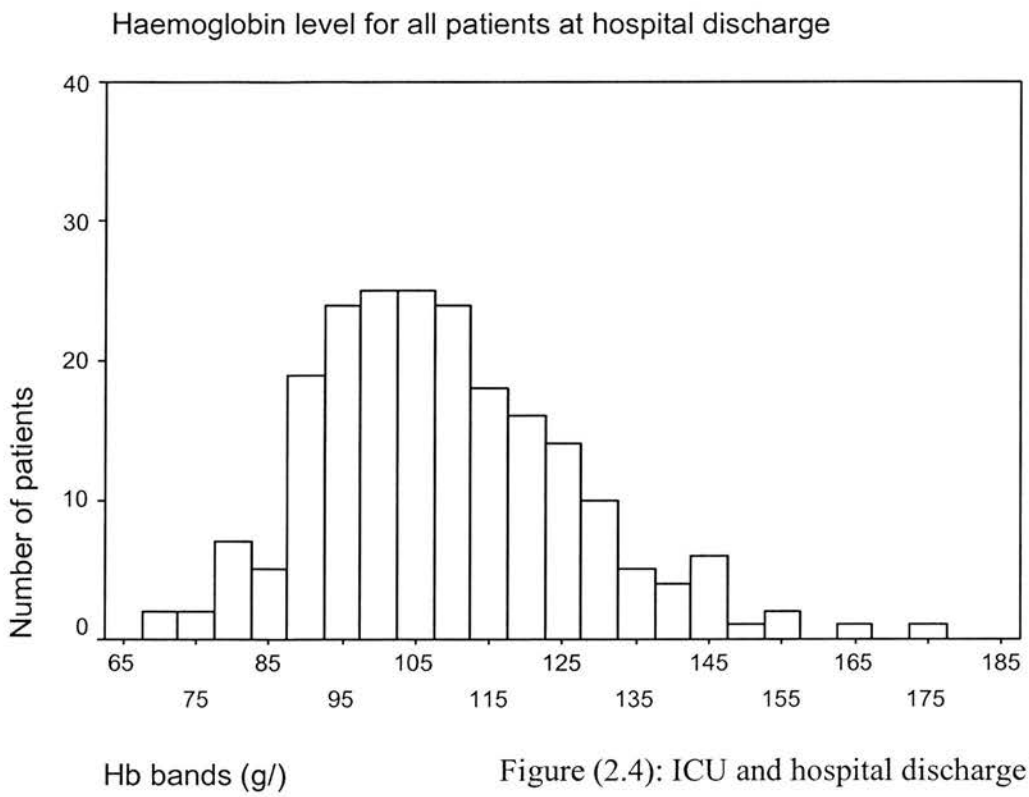


Figure (2.4): ICU and hospital discharge Hb

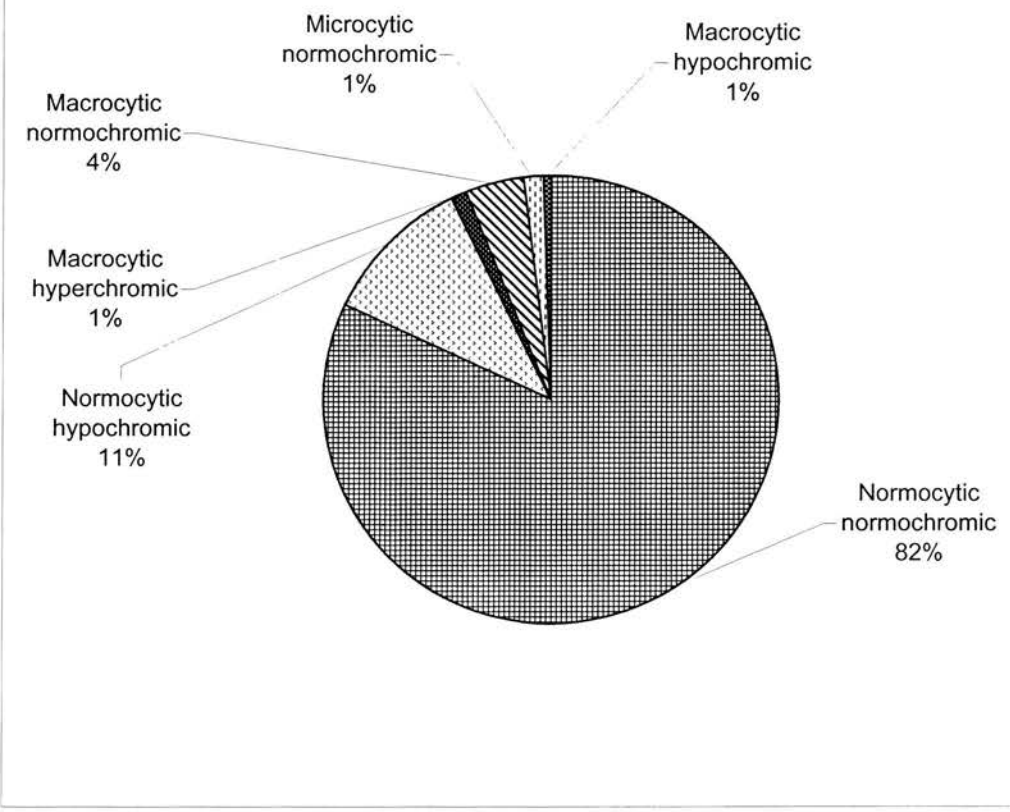
Table (2.27): Haemoglobin characteristics at the time of ICU and hospital discharge

Variable	Mean	SD	Median	Q1	Q3	Range
Number Hb values measured during ICU	5.7	7.8	3	2	6	0-54
First ICU Hb (g/L)	105.3	23.0	103	87	122	41-164
Mean ICU Hb (g/L)	101.4	17.5	98	88	113	63-163
Minimum ICU Hb (g/L)	92.1	21.1	89	77	106	31-163
Last ICU Hb (g/)	101.6	18.0	99	89	111	66-163
Number Days before last ICU Hb (days)	0.07	0.3	0	0	0	0-2
Number Hb values measured post ICU	10.5	10.8	8	3	16	0-76
Mean Hb post ICU (g/L)	104.0	15.0	100	93.5	113	76-157
Min Hb post ICU (g/L)	93.1	18.6	89	80	103	62-144
Number of days before last hospital Hb was requested (days)	2.6	3.8	1	0	3	0-24
Last Hb before hospital discharge (g/L)	108.9	17.7	107	96	119	70-174

Table (2.28): Prevalence of different types of anaemia for males and females with Hb< reference range at hospital discharge

Types of anaemia	Anaemic patients (n=166)	
	Male n=102	Female n=64
	N (%)	N (%)
Normocytic (MCV 76-100 fl) + normochromic (MCHC 31-35g/dl)	85(83.3)	51(79.7)
Normocytic + hypochromic	8 (7.8)	10 (15.6)
Macrocytic + hyperchromic	2 (1.9)	0
Macrocytic + normochromic	6 (5.9)	1 (1.5)
Microcytic + normochromic	1 (.9)	1 (1.5)
Macrocytic + hypochromic	0	1 (1.5)

Figure (2.6): Prevalence of different types of anaemia for all patients with Hb< reference range at hospital discharge.



RESULTS OF THE PROSPECTIVE STUDY

192 admissions (185 patients) were discharged alive from the ICU during the study period [19/11/2002 to 06/03/2003]; 117 males (61%) and 75 females (39%). Mean ± SD, median [Q1, Q3], and range of last Hb (g/L) result before ICU discharge were 101 ± 21, 99 [86, 117], and (63-151). Demographic characteristics of the 185 patients are shown in table (2.29).

Of the 192 admissions, 66 died in ICU, 7 and 4 were discharged directly from ICU to another acute hospital and home respectively. 7 patients were re-admitted to ICU during the same hospital stay; their haematological and transfusion data during the second ICU admission were not collected, but post-ICU data after the 2nd admission were included. 72 patients comprised the study group, 41 (57%) patients were males and 31 (43%) were females. These patients survived ICU and hospital stays and were discharged home. Figure (2.7) illustrates the outcome in all admissions after ICU discharge.

Table (2.29): Patients' demographic data (N= 185)

Variable	Mean	SD	Median	Range	Q1	Q3
Age	57.5	18.1	58	16-90	45	73
APACHE II	19.4	9.6	19	0-45	13	26
ICU stay (days)	5.5	11.8	1.3	0-88.9	0.8	4.2
Days in hospital after ICU	14.8	16.0	11	2-101	5	17.8

Haemoglobin concentrations at ICU discharge

Out of 185 patients who were discharged from ICU during the study period, 23 patients (12%) did not have ICU discharge Hb records. The last Hb values recorded

in the ICU were used as ICU discharge Hb.145 patients had their last ICU Hb measured on the day of ICU discharge, 13 patients one day before discharge, and 4 patients two days before discharge.

Out of the 72 patients (the study group), 2 patients had their last ICU Hb values were measured one day before ICU discharge. The study group demographic data and haemoglobin characteristics at the time of ICU discharge are shown in table (2.30). The numbers of patients in different Hb bands at the time of ICU discharge are shown in table (2.31). Figure (2.8) shows the numbers of males and females in different Hb bands and figure (2.9) shows the number of all patients at the time of ICU discharge.

87.5% of the patients had Hb concentration < reference range, 54.16% < 100, and 36.11% < 90g/L. Table (2.32) shows the prevalence of different degrees of anaemia at the time of ICU discharge.

Table (2.32): Prevalence of different degrees of anaemia at ICU discharge.

Hb	Males (n=41)	Females (n=31)	Total (n=72)
	N (%)	N (%)	N (%)
≤ 90 g/L	13 (31.7)	13 (41.9)	26 (36.1)
≤100g/L	18 (43.9)	21 (67.7)	39 (54.2)
≤ Reference range	37 (90.2)	26 (83.9)	63 (87.5)

Haemoglobin concentrations at the time of hospital discharge

2 patients out of 72 patients did not have post-ICU Hb results. For these patients, last ICU Hb values were used as hospital discharge Hb. Mean ± SD, and median (Q1, Q3) last Hb (g/L) before hospital discharge were 111 ± 18.7, and 110.5 (94, 122)

respectively. Table (2.33) shows the number of patients in different Hb bands at hospital discharge. Figure (2.10) shows the number of males and females in different Hb bands and figure (2.9) shows the number of all patients at the time of hospital discharge.

72.2%, 12.5%, and 33.3% had Hb concentration < reference range, <90g/L, and <100g/L respectively. Table (2.34) shows the prevalence of different degrees of anaemia at the time of hospital discharge.

Table (2.34): Prevalence of different degrees of anaemia at hospital discharge.

Hb	Males (n=41)	Females (n=31)	Total (n=72)
	N (%)	N (%)	N (%)
≤ 90 g/L	6 (14.6)	3 (9.7)	9 (12.5)
≤100g/L	13 (31.7)	11 (35.5)	24 (33.3)
≤ Reference range	34 (82.9)	18 (58.1)	52 (72.2)

Prevalence of different types of anaemia at hospital discharge for anaemic patients with Hb concentration < reference range

According to patients' last MCV and MCHC with Hb level < reference range, 80% of males and 88% of females had nomocytic normochromic anaemia at the time of hospital discharge. Table (2.35) shows the numbers and the percentages of males and females discharged to their normal residency with different types of anaemia and figure (2.11) shows the percentages of all patients.

Transfusion practice during post-ICU hospital stay

Of all patients discharged alive from ICU (N = 185), 21 (11%) patients received red blood cell transfusion (12 males and 9 females) during their hospital stay: 19

surgical, 1 medical and 1 renal. Of the 21 transfused patients, 11 (52%) patients were discharged from the hospital to their normal residency, 5 (24%) died, 2 (10%) were discharged to another acute hospital and 3 (14%) to a rehabilitation hospital.

4 patients had been re-admitted to ICU during the same hospital stay and they represented 57% of total re-admissions during the study period.

10 (48%) patients had one transfusion episode, 4 (19%) patients had two transfusion episodes, 4(19%) patients had three transfusion episodes and 3 (14%) had four transfusion episodes. Characteristics of patients during their first ICU admission who received red cell transfusion after ICU discharge are shown in table (2.36). The median pre-transfusion Hb for all patients was 74 g/L (inter-quartile range: 68-76 g/L). Characteristics of pre-transfusion Hb for each transfusion episode are shown in table (2.37).

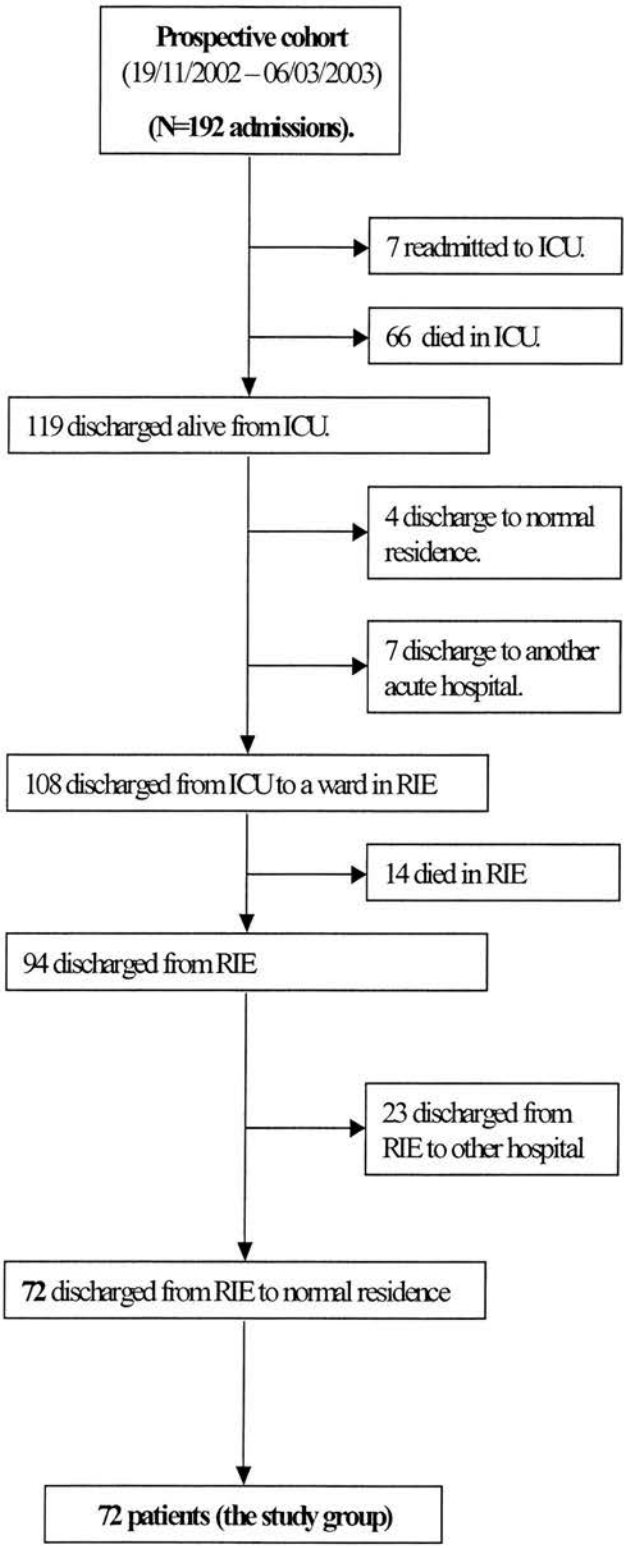


Figure (2.7): Flow chart illustrating outcomes in patients studied [prospective cohort]

Table (2.30): Demographic and haemoglobin characteristics for patients who survived ICU & post-ICU hospital stay and discharged home (N=72).

Variable	Mean	STD	Median	Q1	Q3	Range
Age (years)	51.3	15.9	52	34	63	21-85
APACHE II	15.8	6.8	15	11	20	4-41
ICU stay (days)	3.0	6.1	1.1	0.6	2.3	0.3-36.7
Last Hb level before ICU discharge (g/L)	101.1	20.9	99	86	117	63-151
Number of days before last ICU-Hb was requested (days)	0.1	0.3	0	0	0	0-2
Number of days in hospital post-ICU (days)	14.8	16.0	11	5	18	2-101
Number of Hb level recorded during post-ICU	8.1	8.5	6	2	11	0-50
Mean Hb post-ICU (g/L)	107.2	17.6	104	94	119	78-154
Minimum Hb level during post-ICU.	95.9	22.4	93	80	112	40-150
Last Hb level before Hospital discharge (g/L)	111	18.7	110.5	94	122	76-154
Numbers of days before hospital discharge Hb was requested (days)	1.5	2.5	1	0	2	0-16

Table (2.31): Number of patients in different Hb bands at ICU discharge

Haemoglobin level (g/L)	Males	Females	Total
≥60-<65	1	0	1
≥65-<70	0	1	1
≥70-<75	3	3	6
≥75-<80	2	0	2
≥80-<85	2	5	7
≥85-<90	4	4	8
≥90-<95	3	5	8
≥95-<100	2	2	4
≥100-<105	3	2	5
≥105-<110	3	2	5
≥110-<115	2	1	3
≥115-<120	5	3	8
≥120-<125	4	1	5
≥125-<130	3	0	3
≥130-<135	0	1	1
≥135-<140	1	0	1
≥140-<145	1	0	1
≥145-<150	1	1	2
≥150-<155	1	0	1
>155	0	0	0

Figure (2.8): Number of males and females in different haemoglobin bands at ICU discharge for patients who were discharged alive home after surviving ICU & Post-ICU hospital stay.

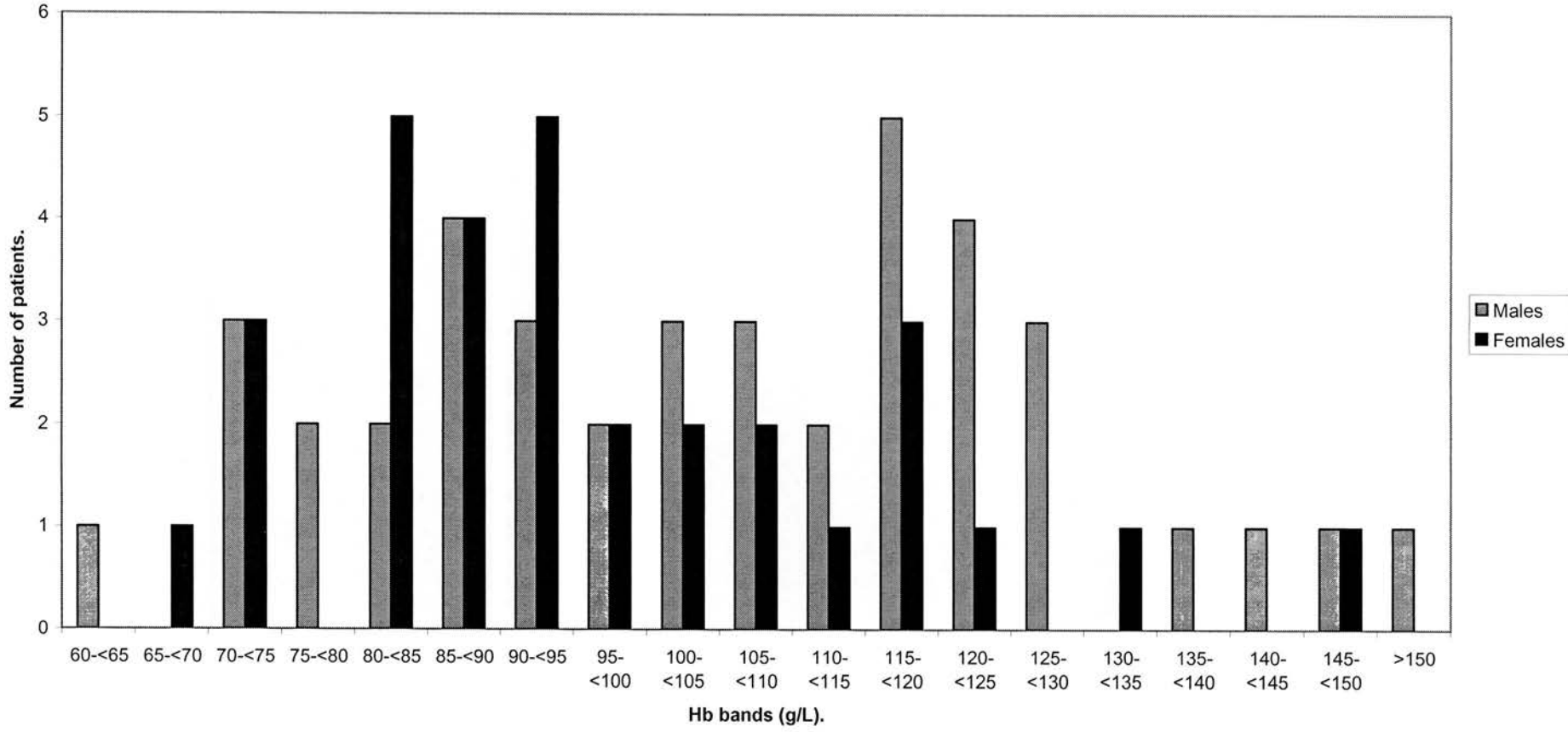
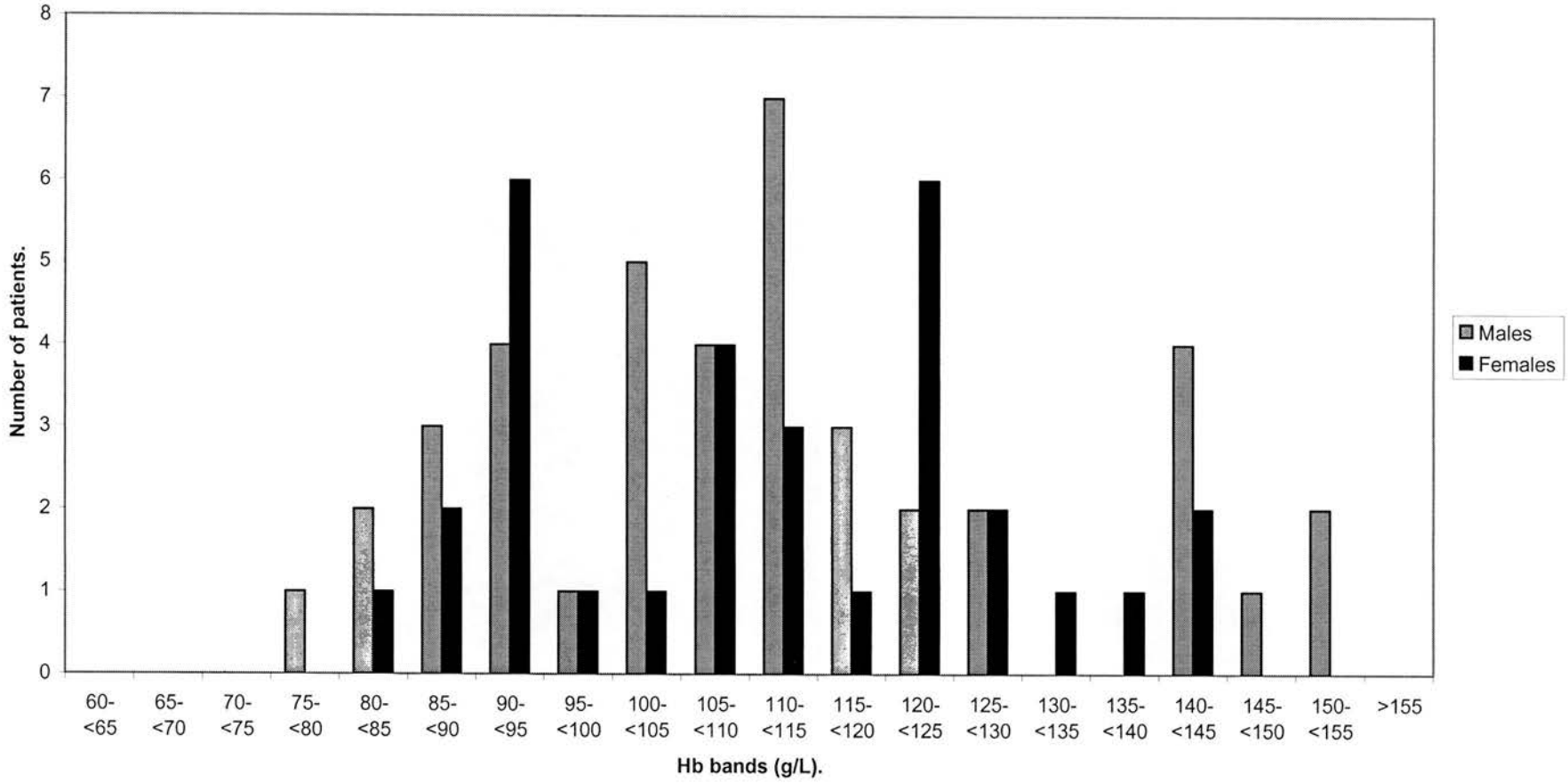
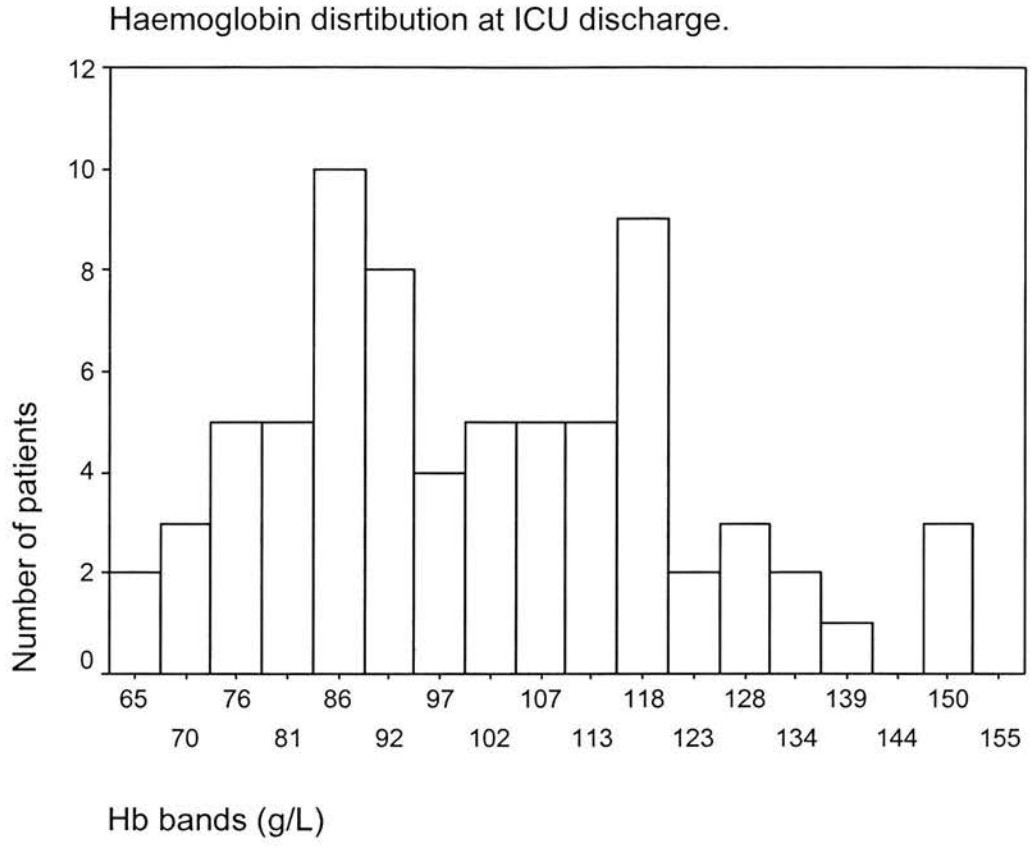


Table (2.33): Number of patients in different Hb bands at hospital discharge

Haemoglobin level	Males	Females	Total
≥60-<65	0	0	0
≥65-<70	0	0	0
≥70-<75	0	0	0
≥75-<80	1	0	1
≥80-<85	2	1	3
≥85-<90	3	2	5
≥90-<95	4	6	10
≥95-<100	1	1	2
≥100-<105	5	1	6
≥105-<110	4	4	8
≥110-<115	7	3	10
≥115-<120	3	1	4
≥120-<125	2	6	8
≥125-<130	2	2	4
≥130-<135	0	1	1
≥135-<140	0	1	1
≥140-<145	4	2	6
≥145-<150	1	0	1
≥150-<155	2	0	2
>155	0	0	0

Figure (2.10): Number of males and females in different haemoglobin bands for patients who were discharged home after surviving ICU.





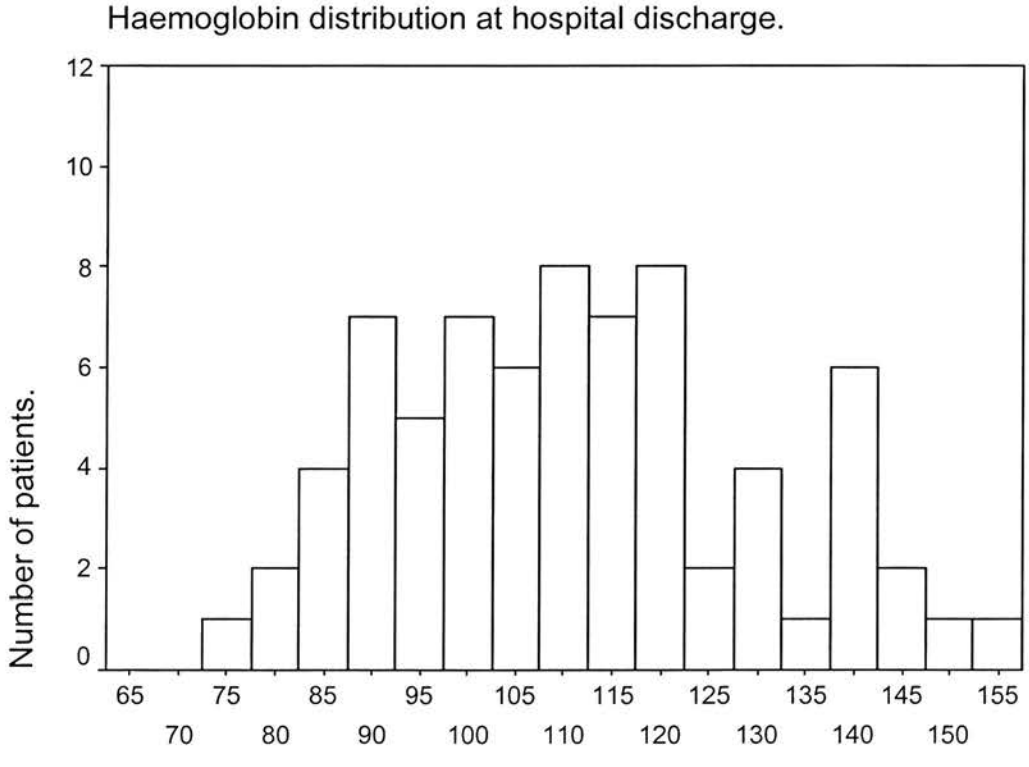


Figure (2.9): ICU and hospital discharge Hb

Table (2.35): Prevalence of different types of anaemia for males and females with Hb< reference range at hospital discharge

Types of anaemia	Anaemic patients (n= 52)	
	Male (n=34)	Female (n=18)
	N (%)	N (%)
Normocytic (MCV 76-100 fl) + normochromic (MCHC 31-35g/dl)	27 (79.4)	16 (88.9)
Normocytic + hypochromic	4 (11.8)	1(5.6)
Normocytic + hyperchromic	1(2.9)	0
Microcytic + hypochromic	0	1(5.6)
Macrocytic + normochromic	2 (5.9)	0

Figure (2.11): Prevalence of different types of anaemia for patiens with Hb< reference range at hospital discharge.

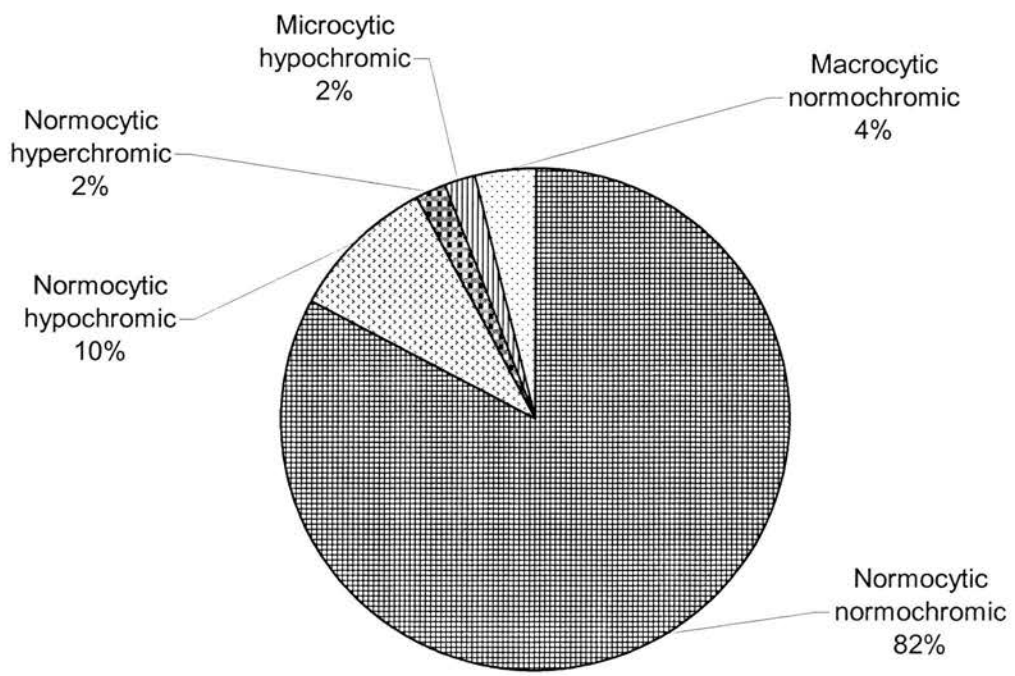


Table (2.36): Demographic and haemoglobin characteristics for transfused patients (n = 21).

Variable	Mean	STD	Median	Q1	Q3	Range
Age (years)	58.5	18.4	58	49	72	21-87
APACHEII	19.4	5.0	19	15	22	11-28
ICU stay (days)	9.3	13.2	2.2	0.9	12.8	0.5-45.5
Last Hb concentration before ICU discharge (g/L)	81.1	13.4	78	73	87	63-121
Last Hb before Hospital discharge (g/L)	93.9	3.3	95	91	96	87-98
Number of days in hospital post-ICU (days)	34.4	27.6	28.5	10.8	44.5	6-107

Table (2.37): Haemoglobin concentration (g/L) for each transfusion episode.

	1st episode (N =21 patients)	2nd episode (N =11 patients)	3rd episode (N =7 patients)	4th episode (N =3 patients)	All episodes
Mean	72	78	74	73	74.14
Median	75	73	74	70	74
STD	5.8	16.7	12.2	8.1	10.75
Q1	66	71.5	69.5	69	68.25
Q3	76	85.5	81	76.5	76
Range	62 to 84	49 to 111	53 to 90	68 to 83	49 to 111

SUMMARY FOR COMBINED COHORTS

There were a total of 681 admissions to ICU in the study cohorts. Of these 441 patients were discharged alive from ICU. Of these 283 patients were discharged from ICU to a ward in the hospital and then discharged to their normal residence. 122 (43%) were females and 161 (57%) were males.

The median (IQR) of last Hb before ICU discharge was 99 g/L (87 to 114 g/L). Table (2.38) shows the number (%) of patients with last Hb before ICU discharge below 90 g/L, 100 g/L, and < reference range.

The median (IQR) of last Hb before hospital discharge was 108 g/L (96 to 120 g/L). Table (2.39) shows the number (%) of patients with last Hb before hospital discharge below 90 g/L, 100g/L, and < reference range. Table (2.40) shows the number (%) of patients who were discharged home with different degrees of anaemia in different bands of age, APACHE II score, length of ICU stay, and last Hb before ICU discharge.

There were 219 patients who were discharged to their normal residence and had their last Hb before hospital discharge below the reference range. Table (2.41) shows the prevalence of different types of anaemia according to their last MCV and MCHC results.

MULTI-VARIABLE LOGISTIC REGRESSION FOR THE COMBINED RETROSPECTIVE AND PROSPECTIVE COHORTS

There was very strong evidence that a lower last haemoglobin before ICU discharge is associated with an increase in the odds of a last Hb prior to hospital discharge <100 g/L (table 2.42). There was also strong evidence of an association between age

on admission to ICU and a low Hb prior to hospital discharge. The 38-53 year olds and 54-65 year olds having considerably increased odds of a last Hb prior to hospital discharge <100 g/L compared to the under 38 year olds. The association between an increase in APACHE II score and an increase in the odds of a last Hb prior to hospital discharge <100 g/L was almost statistically significant. Although each one day increase in the length of ICU stay was estimated to increase the odds of a last Hb prior to hospital discharge <100 g/L by only a small amount this did approach statistical significance. Even though the odds of a last Hb prior to hospital discharge <100 g/L were greater for female patients than male patients, the unadjusted odds ratio was not statistically significantly different from one.

There was no evidence from the multi-variable analysis that, after considering the last haemoglobin before ICU discharge, any of the other variables were independent predictors of a last haemoglobin prior to hospital discharge <100 g/L (table 2.42). In addition there was no evidence that the relationship between the last haemoglobin before ICU discharge and a last Hb prior to hospital discharge <100 g/L was different for males and females (p=0.18), modified by age on admission (p=0.57), modified by APACHE II score (p=0.55), or modified by length of ICU stay (p=0.18). Some caution should be shown when interpreting the multi-variable analysis results since the confidence intervals for some of the adjusted odds ratios are quite wide. In particular the upper limits do not exclude what would be fairly strong independent associations between gender or age on admission and a last haemoglobin prior to hospital discharge <100 g/L.

Table (2.38): Prevalence of different degrees of anaemia at ICU discharge. All values in numbers (proportion, 95% CI of proportion).

Hb level	Males (n=161) N (%)	Females (n=122) N (%)	All patients (n=283) N (%)
Hb < 90 g/L (95% CI)	41 (25.5) (19.4% to 32.7%)	42 (34.4) (26.6% to 43.2%)	83 (29.3) (24.3% to 34.9%)
Hb < 100g/L (95% CI)	81 (50.3) (42.7% to 57.9%)	67 (54.9) (46.1% to 63.5%)	148 (52.3) (46.5% to 58.0%)
Hb < reference range. (95% CI)	144 (89.4) (83.7% to 93.3%)	96 (78.7) (70.6% to 85.0%)	240 (84.8) (80.2% to 88.5%)

Table (2.39): Prevalence of different degrees of anaemia at hospital discharge. All values in numbers (proportion, 95% CI of proportion).

Hb level	Males (n=161) N (%)	Females (n=122) N (%)	All patients (n=283) N (%)
Hb < 90 g/L (95% CI)	14 (8.7) (5.2% to 14.1%)	18 (14.8) (9.5% to 22.1%)	32 (11.3) (8.1% to 15.5%)
Hb < 100g/L (95% CI)	48 (29.8) (23.3% to 37.3%)	44 (36.1) (28.1% to 44.9%)	92 (32.5) (27.3% to 38.2%)
Hb < reference range. (95% CI)	137 (85.1) (78.8% to 89.9%)	82 (67.2) (58.5% to 74.9%)	219 (77.4) (72.2% to 81.9%)

Table (2.40): Last Hb before hospital discharge.

	Number of patients	Hb < reference range N (%)	Hb <100 g/L N (%)	Hb < 90 g/L N (%)
Gender:				
Males	161	137 (85)	48 (30)	14 (9)
Females	122	82 (67)	44 (36)	18 (15)
Age (years):				
< 38	71	42 (59)	14 (20)	7 (10)
38-53	70	55 (79)	32 (46)	10 (14)
54-65	68	63 (93)	25 (37)	9 (13)
≥ 65	74	59 (80)	21 (28)	6 (8)
APACHE II score:				
<14	76	56 (74)	20 (26)	6 (8)
15-17	66	55 (83)	20 (30)	5 (8)
18-21	63	42 (67)	20 (32)	6 (10)
≥ 22	63	51 (81)	27 (43)	15 (24)
Length of ICU stay (days):	70	44 (63)	15 (21)	5 (7)
<0.8	72	62 (86)	26 (36)	9 (13)
0.8-1.2	70	54 (77)	22 (31)	8 (11)
1.3-3.4	71	59 (83)	29 (41)	10 (14)
≥ 3.5				
Last Hb before ICU discharge (g/L):				
<90	83	76 (92)	47 (57)	20 (24)
90-99	65	62 (95)	26 (40)	9 (14)
100-114	68	54 (79)	13 (19)	2 (3)
≥ 115	67	27 (40)	6 (9)	1 (1)

Table (2.41): Types of anaemia for patients who were discharged home with Hb < reference range.

	Males (n = 137) N (%)	Females (n = 82) N (%)	All patients (n = 219) N (%)
Normochromic normocytic	133 (82)	67 (82)	180 (82)
Normochromic macrocytic	8 (6)	1 (1)	9 (4)
Normochromic microcytic	1 (<1)	1 (1)	2 (<1)
Hypochromic normocytic	12 (9)	11 (13)	23 (11)
Hypochromic macrocytic	0 (0)	1 (1)	1 (1)
Hypochromic microcytic	0 (0)	1 (1)	1 (<1)
Hyperchromic normocytic	1 (<1)	0 (0)	1 (<1)
Hyperchromic macrocytic	2 (1)	0 (0)	2 (<1)
Hyperchromic microcytic	0 (0)	0 (0)	0 (0)

Table (2.42): Univariate odds ratios for last Hb prior to hospital discharge <100 g/L and odds ratios after adjusting for last Hb in ICU

Variable	Unadjusted odds ratio (95% CI)	p-value	Adjusted odds ratio for last Hb in ICU (95%CI)	p-value
Gender:				
Males	1		1	
Females	1.33 (0.8 to 2.19)	0.27	1.20 (0.69 to 2.09)	0.51
Age (years)				
<38	1		1	
38-53	3.43 (1.65 to 7.43)	0.007	2.19 (0.95 to 5.20)	0.20
54-65	2.37 (1.12 to 5.19)		1.58 (0.68 to 3.75)	
≥ 65	1.61 (0.5 to 3.55)		1.12 (0.48 to 2.68)	
APACHE II score (5 point increase)	1.22 (1 to 1.49)	0.051	1.06 (0.85 to 1.33)	0.58
Length of ICU stay (1 day increase)	1.03 (1 to 1.07)	0.065	1.00 (0.97 to 1.04)	0.78
Last Hb before ICU discharge (10 g/L increase)	0.53 (0.43 to 0.64)	< 0.0001		

DISCUSSION

SUMMARY OF THE FINDINGS

A total of 681 ICU admissions were studied in both the retrospective and prospective cohorts. 441 (64.75%) patients were discharged alive from ICU. Of these 283 (64.17%) patients survived post-ICU hospital stay, were discharged to their normal residence, and comprised the final study group. 57% were males, the mean ± SD age was 52 ± 18.3 years, and mean APACHE II score was 17.2 ± 6.5.

84.8% of all patients in the study group were discharged anaemic from ICU with Hb levels below the normal reference range. 52.3% were discharged with Hb< 100 g/L and 29.3% with Hb <90 g/L. 77.4% were discharged to their normal residence from hospital with Hb levels below reference range. 32.5% were discharged with Hb <100 g/L and 11.3% with <90 g/L. Red cell morphology showed that normochromic normocytic indices were present in 82% of all anaemic patients with Hb level less than reference range when discharged home.

There was a strong association between last Hb before ICU discharge and the probability of discharge home with Hb <100g/L. After adjustment, there were no associations with age, sex, admission APACHE II score, or ICU length of stay. In a logistic regression model, after including last Hb in ICU, no other parameter was associated independently with a Hb <100g/L at discharge home.

In the prospective part of the study, 11% of patients received red cell transfusion after ICU discharge, comprising a total of 42 transfusion episodes (99 red cell units). For these transfusion episodes the median pre-transfusion Hb was 74 (IQR 68 - 76; range 49-111) g/L.

STRENGTHS AND WEAKNESSES

Strengths

The study took place in a large medico-surgical teaching ICU and included three cohorts of ICU patients. The cohorts accounted for 100% of ICU admissions during the study periods to obtain large numbers of patients. The cohorts included the entire ICU case mix so selection bias was unlikely. The cohorts were from three different years to compare clinicians' practice during different periods. The three cohorts were from different quarters of the year; therefore variation by time of year was unlikely to be a source of bias.

The retrospective cohort

The patient dataset for the retrospective cohort was obtained from the Scottish Intensive Care Society (SICS) Audit group database, which is accurate, contains all patients' demographic data, and includes 100% of ICU admissions. This cohort of patients was previously included in large national (Garrioch, Walsh, Maciver, McClellan 2002) and single centre (Chohan, McArdle, McClelland, Mackenzie, Walsh 2003) studies to investigate transfusion practice during critical illness. The data were prospectively collected at the time of these studies and have been previously reported, showing that a restrictive transfusion strategy was used during the ICU stay(Garrioch, Walsh, Maciver, McClellan 2002; Maciver, Walsh, Lee, MacKirdy, Garrioch, McClelland 2002; Chohan, McArdle, McClelland, Mackenzie, Walsh 2003; Walsh, Lee, Maciver, Garrioch, McClelland 2003a; Walsh, Lee, Maciver, Garrioch, McClelland 2003b).

During the above-mentioned prospective studies by Walsh et al., and Chohan et al., the daily ICU Hb values were recorded daily from the patients' notes. These Hb

values were compared to the linked values in our dataset and we found that there were no differences or missing values as a result of the linkage process when we compared both datasets (our dataset and the Walsh et al. and Chohan et al. datasets). Therefore, the accuracy of the linkage process was satisfactory and the chance of missing any haematological values as a result of the linkage process was small. Nearly 360,000 records were received from the haematology database. This large number generated few errors in the linkage process, which were resolved by repeating the linkage more than once in different ways until satisfactory linkage results were obtained. A few records in the haematology dataset contained differences in one or more character in one or more field was that used in the linkage process for some patients e.g. forename and/or surname, and/or date of birth. This created linkage errors, which were sorted by post-linkage manual checking. Therefore, errors in the linkage process were unlikely to cause any missing haematological data.

After completing and checking the linkage process, a manual search in the haematology database was carried out to find any missing haematological results to obtain complete records for analysis.

The linkage process was the key element of the study; all of the above-mentioned methodology in checking the linkage process provided the study with a high quality dataset for further analysis.

The prospective cohort

Haematological and transfusion data for the prospective cohort were collected daily. Patients were followed up on a regular baseis throughout the hospital after ICU discharge. All Hb results were recorded daily to avoid any missing values and results

were checked again in the haematology database to obtain a highly accurate dataset. It is therefore unlikely that significant inaccuracies occurred.

Transfusion data were collected from patients' notes. Reviewing blood transfusion forms for the number of red cell units transfused in each transfusion episode was carried out to increase the accuracy of this data. It is therefore unlikely that there were missing or inaccurate values.

Weaknesses

The retrospective cohort

It was not possible to define post-ICU transfusion practice for this cohort. However, a pilot trial to obtain this by linkage was carried out. A dataset was obtained from the blood bank database that contained the number of red cell units issued and returned to the blood bank. This dataset did not provide information as to whether these red cell units were actually received by the patients or not. It was also unclear from this dataset if ICU physicians or other hospital physicians prescribed the red cell units that were issued within short time after ICU discharge. We concluded that the data from this linkage were unreliable and did not report them. The only way to obtain post-ICU transfusion practice was to do a case note review, which was not feasible for this large number of records. Therefore, the analysis of this part of the study did not take place, but was included in the prospective cohort.

The prospective cohort

Haematological data were not collected during ICU stay as the prevalence of anaemia and transfusion practice during critical illness was already established in the unit from earlier studies.

After ICU discharge, a large number of patients were followed up in different wards. Manual checking from the hospital haematology database was done to check for any missing values that were not present in hospital notes at the time of visits.

Due to long hospital stay it was difficult to be sure that all post-ICU days were audited. The SICS database was used to check for missing days as hospital discharge date is routinely entered. Only one patient was found to have missing days (40 days) as he stayed in the hospital more than 100 days after ICU discharge. This missing data is unlikely to have affected the final results.

Transfusion practice was case note dependent; causes for blood transfusion were not documented and there were difficulties in tracing these causes. This is a well-known problem in auditing transfusion practice. It was not feasible to interview those clinicians making transfusion decisions.

The retrospective and the prospective studies

Both studies were observational. ICU discharge Hb and/or hospital discharge Hb for some patients were not always measured on the day of discharge. For these patients, the last Hb value recorded in ICU or in the hospital was used as discharge Hb. For most patients, these Hb values were measured 2-3 days before discharge so it was unlikely that Hb values changed significantly at the day of discharge. These values were the last values available and checked by physicians and reflected levels thought acceptable for discharge. If these levels were not acceptable, the physicians presumably would have checked them again on the day of discharge or delayed discharge to investigate or treat the anaemia. Therefore, using last recorded ICU and hospital Hb values as ICU or hospital discharge Hb were a reasonable reflection of what clinicians considered compatible with discharge home.

A small number of patients were readmitted to ICU during the study periods. Readmission generated a problem in defining the post-ICU period. Post-ICU period for the retrospective part of the study was defined as the period that followed the final ICU discharge. However, in the prospective part it was defined as the period that followed the first ICU discharge in order not to miss any transfusion data in post-ICU discharge. 7 patients were readmitted to ICU in this part of the study. Five of these patients died in the hospital and 2 were discharged home. It is unlikely that these readmissions influenced the estimates of the prevalence of anaemia that were obtained, or the regression analysis.

APACHE II score for both the prospective and the retrospective studies was obtained from the SICS database. This score is calculated from physiological variables in the first 24 hours in the ICU and also incorporates a weighting for age and chronic health. The SICS database is maintained by senior clinical staff in ICU, however, it is not externally validated and therefore there is a potential for inaccuracy in the dataset.

COMPARISON WITH OTHER STUDIES

The general characteristics of the cohorts were similar as regard the percentages of males and females, mean age, APACHE II score, ICU and hospital length of stays to other recent studies by Walsh et al. and Garrioch et al (Garrioch, Walsh, Maciver, McClellan 2002; Walsh, Lee, Maciver, Garrioch, McClelland 2003b) and are typical of UK ICUs with high illness severity at admission. Our estimates may not be applicable to ICU populations with lower illness severity.

Recent transfusion guidelines emphasise the evidence in favour of avoiding red cell transfusions during hospital treatment of critically ill and peri-operative patients by

using conservative transfusion triggers. Several cohort studies have confirmed that conservative transfusion triggers are being used in the ICU, and that anaemia is prevalent during critically illness(Hebert et al. 1998; Garrioch, Walsh, Maciver, McClellan 2002). Our data show that this approach results in a large number of anaemic patients who are recovering from critical illness being discharged in to the community. In most patients the anaemia has morphological characteristics similar to anaemia of chronic disease.

Several recent studies showed that the anaemia critical illness has similarities to the anaemia of chronic disease which is characterised by normocytic normochromic blood indices (Rodriguez, Corwin, Gettinger, Corwin, Gubler, Pearl 2001; Hobisch-Hagen et al. 2001; Corwin 2001b). Bone marrow suppression due to direct effect of circulating cytokines and inadequate EPO concentrations play an important role in the course of this type of anaemia(Rogiers et al. 1997; Goodnough 2001; Eckardt 2001; Corwin 2001a; Corwin 2001b). Our findings showed that most patients recovering from critical illness were discharged home with normocytic normochromic anaemia. This may indicate marrow suppression, inadequate/ineffective EPO, or functional iron deficiency. Rodriguez et al.(Rodriguez, Corwin, Gettinger, Corwin, Gubler, Pearl 2001), von Ahsen et al.(von Ahsen, Muller, Serke, Frei, Eckardt 2001), and Van Iperen et al.(van Iperen, Gaillard, Kraaijenhagen, Braam, Marx, van de 2000) showed that this is the case during ICU stay. However, there is no reported data investigating whether this continues after ICU discharge or for how long. Further studies are needed to define factors contributing to delayed recovery from anaemia after ICU discharge.

Our data suggest that anaemia associated with critical illness may persist for a considerable time. Improved understanding of the causes of this may enable the rational use of new treatments such as erythropoietin. Exogenous erythropoietin has been used in the treatment of the anaemia of critical illness. However, there are many arguments about its efficacy during the course of the disease. Krafte-Jacobs et al. (Krafte-Jacobs, Levetown, Bray, Ruttimann, Pollack 1994), and Van Iperen et al.(van Iperen, Gaillard, Kraaijenhagen, Braam, Marx, van de 2000) showed that there is a blunted response to exogenous EPO during critical illness. Other studies suggested that there is association between the level of circulating inflammatory mediators and bone marrow response to EPO. Defining which patients may benefit from treatment, and what stage in their illness, is important before EPO should be considered for widespread use.

POSSIBLE IMPLICATIONS OF ANAEMIA AFTER CRITICAL ILLNESS

Treatment of the anaemia of critical illness is potentially important. Some studies show that anaemia has adverse effects on different body organs. Deicher et al. (Deicher, Horl 2003), Hus et al.(Schuster, Rowley, Feinstein, McGue, Zuckerman 1984), and Astor et al. (Kovesdy, Astor, Longenecker, Coresh 2002)showed that anaemia is a risk factor for the progression of chronic renal disease. Ezekowitz et al.(Ezekowitz, McAlister, Armstrong 2003), Weiskopf et al.(Weiskopf et al. 1998; Weiskopf, Feiner, Hopf et al. 2003), Carson et al.(Carson et al. 1996), and Sandgren et al (2003, Abstracts of the American Geriatrics Society Annual Meeting, page 363) showed that anaemia is common in heart disease and associated with poor outcome. Granberg et al.(Granberg Axell 2002) and Weiskopf et al. (Weiskopf et al. 2000)

showed that anaemia can have adverse effects on the central nervous system functions.

It is unclear if anaemia of critical illness is a long-term disorder, can persist after ICU and hospital discharge, and whether it has similar negative impacts on different body organs and quality of life. Our literature review identified no studies investigating the prevalence of anaemia during recovery from critical illness, or its long-term effect on patients' health. There is also no information about whether there is an association between this type of anaemia and quality of life. However, there is clear evidence that anaemia is associated with poor quality of life in chronic renal failure(Seidenfeld, Piper, Aronson 2002), chronic inflammatory diseases(Peeters, Jongen-Lavrencic, Bakker, Vreugdenhil, Breedveld, Swaak 1999), cancer(Littlewood, Cella, Nortier 2002), and chronic heart failure(Horwich, Fonarow, Hamilton, MacLellan, Borenstein 2002).

Some UK studies showed that easy fatigability and poor quality of life are not uncommon after recovering from critical illness (Ridley, Wallace 1990; Eddleston, White, Guthrie 2000). Muscle weakness, cachexia and malnutrition are also common in patients recovering from critical illness (Elia, Stratton 2000). The presence of these factors, with anaemia, could worsen the quality of life after ICU and hospital discharge. Wright JC et al. showed that patients discharged from ICUs have excess mortality compared to population cohorts for 2-3 years following ICU discharge. The authors found 33.4% 5 years mortality after discharge from ICU (Wright, Plenderleith, Ridley 2003).

Factors that are associated with quality of life after critical illness are poorly understood. It has been shown that increasing Hb levels in another settings such as

chronic renal failure (1999 European best practice guidelines for the management of anaemia in patients with chronic renal failure, page 363), cancer (Littlewood, Bajetta, Nortier, Vercammen, Rapoport 2001), and chronic heart failure has improved quality of life and patients' outcome (Silverberg, Wexler, Sheps et al. 2001).

There is a need for further trials that can provide a clear idea about the prevalence of long-term anaemia after ICU discharge, its pathophysiology and causes, and its impact on quality of life. An interventional trial should follow to investigate the role of treating post-ICU anaemia in enhancing patients' recovery and improving quality of life after ICU and hospital discharge.

RED CELL 2,3-DIPHOSPHOGLYCERATE [RBC 2,3-DPG] AND P50 DURING CRITICAL ILLNESS: PROSPECTIVE OBSERVATIONAL STUDY.

The discovery of the role of red blood cell 2,3-diphosphoglycerate (RBC 2,3-DPG) in oxygen transport and its effect on the oxygen dissociation curve (ODC) has changed many thoughts in several areas of clinical medicine.

THE OXYHAEMOGLOBIN DISSOCIATION CURVE AND P50

The oxyhaemoglobin dissociation curve describes the relation between the oxygen saturation or content of haemoglobin and the oxygen tension at equilibrium. Affinity of haemoglobin for oxygen is expressed by P50 value. P50 represents the partial pressure of oxygen (Po_2) at which 50% of the haemoglobin is saturated under standard conditions of temperature and pH. Normally, the P50 is 27 mmHg for adult haemoglobin (figure: 3.1) (Eaton, Brewer, Schultz, Sing 1970; Hsia 1998).

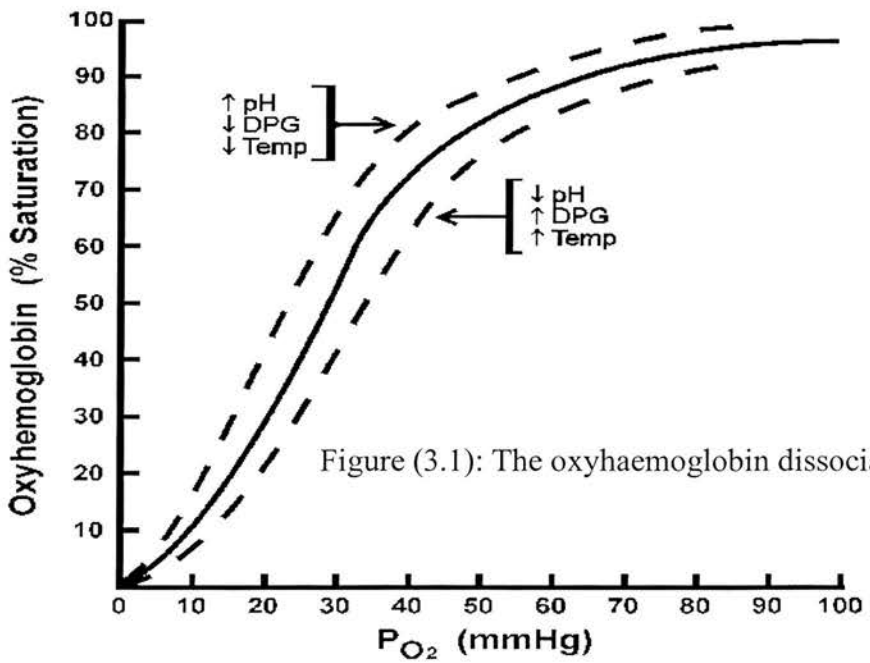


Figure (3.1): The oxyhaemoglobin dissociation curve

Arturson et al determined the oxygen affinity of human blood in vivo under standard conditions. The authors studied 12 males and 15 females. All the subjects were healthy with age range 20-48 years. A venous blood sample was taken from each individual while at rest. Immediately after the blood samples were drawn, Hb, oxygen saturation, HbCo, Po_2, Pco_2, pH, and RBC 2,3-DPG were measured. This study allowed the authors to plot the relation between Po_2, and oxygen saturation under actual condition conditions with respect to pH, Pco_2, RBC 2,3-DPG, and HBCo in apparently healthy humans. The authors suggested that it is possible to obtain measurements of the whole blood oxygen affinity by this technique (Arturson, Garby, Robert, Zaar 1974a).

DETERMINATION OF P50

There are two main approaches to measure P50: a) in vivo, this approach allows to estimate the P50 as it is in the real conditions in the body at the time of taking the blood sample, once the sample is drawn from the subject and maintained in anticoagulant, its pH, Pco_2, and temperature may change. This method adjusts for these variables, to estimate the in vivo state using ***special devices***, or can be ***calculated*** using Siggaard-Andersen or Samaja et al. equations (discussed later in detail), b) in vitro, P50 is estimated under standard conditions of pH, Pco_2, and temperature. Many equations have been derived and it is often called standard P50. The use of these equations as been to estimate a P50 value from arterial oxygen saturation (So_2) and partial pressure of oxygen Pao_2 alone, but assumes other factors (pH, Pco_2, and temperature) are within normal ranges.

Most studies concerning P50 used equations to calculate its value. Very few studies have measured the actual in vivo P50.

In vivo P50 (oxygen dissociation curve analyser)

Duvelleroy et al described a method of plotting the ODC from a sample of whole blood (5 – 10 ml). The method used a special device that allowed diffusion of a known volume of O_2 to a known volume of deoxygenated blood (deoxygenated using a tonometry for 30-45 min at 37 °C with humidified mixture of 95% N_2 and 5% CO_2) and measured blood Po_2 and O_2 content continuously using a closed chamber. pH and Pco_2 were determined with appropriate electrodes maintained at 37°C before and after the completion of the process. The device used allowed the authors to plot a graph showing the relationship between blood Po_2 mmHg (*y* axis) and O_2 content (*X* axis). After analysing the data, the authors found that the mean Po_2 at 50% saturation at pH = 7.40 was 26.53 ± 0.48 mmHg (Duvelleroy, Buckles, Rosenkaimer, Tung, Laver 1970).

In vivo P50 can be also ***calculated*** using the Siggaard-Andersen or the Samaja et al formulas.

Siggaard-Andersen described a very complex formula that contains measured and calculated values. A special software computer program is needed to estimate the in vivo P50 using this formula (Siggaard-Andersen, Wimberley, Fogh-Andersen, Gothgen 1988).

Smaja et al. described another formula to estimate in vivo P50 as follow (Samaja, Mosca, Luzzana, Rossi-Bernardi, Winslow 1981):

1) Log P50 was estimated from known values of Pco_2 and RBC 2,3-DPG/Hb (molar ratio) at definite value of pH using a special formula.

2) The authors suggested that because log P50 is always a linear function of pH in the range 6.9 to 7.6, its value at any pH in this range can be interpolated by using the formula:

$$\text{Log P50}_{(pH)} = [(pH - 7.0) \text{ X } (\text{log P50}_{(7.6)} - \text{log P50}_{(7.0)}] / 0.6 + \text{log P50}_{(7.0)}$$

3) This empirical relationship allowed the authors to estimate in vivo P50 at any given pH (range 6.9 to 7.0), Pco_2 (range 20 to 90 mmHg), and RBC 2,3-DPG/Hb ratio (range 0.3 to 2.5), with 0.73 mmHg SD.

In vitro P50 (standard P50 or $P50_{7.4}$)

The key equation used to calculate P50 was described by A.V. Hill.

The Hill equation is (Willford, Hill, Moores 1982):

$$S = Po_2^n / (Po_2^n + P50^n)$$

Where S is the fractional saturation, Po_2 is the partial pressure of oxygen, and n is the haem-haem interaction or a quantitative expression of the shape of the ODC (Hill factor = 2.7 for human normal blood).

Some authors have derived other formulas based on the same principles of Hill's formula such as Willford et al. and Samaja et al.

Willford et al. formula (Willford, Hill, Moores 1982):

$$P50 = Pao_2 \; X \; \frac{1 - (Sa - Sv)}{1 + (Sa - Sv)} \; X \; 1^{1/n}$$

Where Pao_2 is partial pressure oxygen, Sa is arterial oxygen saturation, Sv is venous oxygen saturation, and n is Hill factor (2.7).

Samaja et al. formula (Hill's transformation)(Samaja, Mosca, Luzzana, Rossi-Bernardi, Winslow 1981):

$$Log\ P50 = log\ Pao_2 + [log\ (SO_2 /(100 - SO_2)] / n$$

Comparison between in vivo versus standard P50

Ekeloef et al. compared 114 arterial or venous blood samples that were analysed using the Sigaard-Andersen oxygen status algorithm (in vivo P50) and Doyle's method based on Hill's equation (standard P50 or in vitro P50). The authors suggested that the Siggaard-Andersen oxygen status algorithm is the most clinically useful single-point method of P50 calculation. The authors added that Doyle's formula slightly overestimated the P50 by a value of 0.04 kPa (Ekeloef, Eriksen, Kancir 2001).

Samaja et al measured the actual in vivo P50 in 63 blood samples using a special tonometer. The authors calculated the in vivo P50 and the in vitro P50 using their own equations (mentioned above) and compared the results to the actual measured in vivo P50 using the special tonometer. They found that precision was slightly improved if the in vivo equation was used (r = 0.9938) compared to the in vitro standard P50 (r = 0.9853). They suggested that the calculated in vivo P50 was more accurate at lower and upper extremes (Samaja, Mosca, Luzzana, Rossi-Bernardi, Winslow 1981).

Factors affecting P50

The factors that are known to influence the dissociation curve and subsequently P50: a) arterial pH, b) Pco_2, c) body temperature and d) RBC 2,3-DPG (Stryer 1981; Beutler, Lichtman, Coller, Kipps, editors 1995).

Bohr effect (Pco_2 and pH)

Reducing the oxygen-binding affinity of haemoglobin by the addition of hydrogen ions or carbon dioxide to blood is known as Bohr effect. Conversely, oxygenation of haemoglobin reduces its affinity for carbon dioxide; this is known as Haldane effect. Because changes in pH rapidly affect the haemoglobin molecule's ability to bind oxygen, this mechanism has been postulated to be an important early adaptive response to anaemia. However, the equations describing the physical process indicate that a very large change in pH is required to modify the p50 by a clinically important amount ([almost equal to] 10 mmHg). As a result, the Bohr effect is unlikely to have important consequences (Hsia 1998; Hebert, Szick 2000).

There were early attempts to investigate the effect of the above-mentioned parameters on ODC. Winslow at al. analyzed 56 ODCs of fresh human blood, each from 0 to 150 Torr Po2. The data were collected over ranges of values for the 2,3-diphosphoglyceric acid-to-hemoglobin concentration ratio [DPG]/[Hb] of 0.2-2.7, for pH of 7.0-7.8, and for Pco_2 of 7-70 Torr. This study provided a tool to study the affinity of Hb for O_2 within the red blood cell and to predict the shape of the O_2 equilibrium curve in various physiological and pathological states. Other attempts to predict blood O_2 affinity have considered only P50 or have provided too little data for continuous simulations. The authors did not make a physical interpretation of the reactions of these variables within the red blood cell as they sated that more data were required (Winslow, Samaja, Winslow, Rossi-Bernardi, Shrager 1983).

Most of the studies that investigated the effect of different parameters on ODC and P50 are very early studies around 1930s and 1960s.Thomas et al. reviewed very early studies and noted that P50 corrected to pH 7.4 correlated with levels of RBC 2,3-

DPG, when 2,3-DPG was altered by in vitro or in vivo alterations of acid base status. The authors clarified that pH has dual effect on the position of the ODC by a direct effect and through alteration of RBC 2,3-DPG. In the same review the authors noted that carbon dioxide affects the ODC by changing pH and forming carbamino compounds (Thomas, III, Lefrak, Irwin, Fritts, Jr., Caldwell 1974).

Temperature

Reeves investigated the effect of temperature on the ODC. The author measured the oxygen affinity of human blood over six degree temperature intervals from 13 to 43°C. The results were expressed in standard pH and carbon dioxide. Table (3.1) shows P50 values that correspond to different values of temperature.

Table (3.1): P50 values that correspond to different vales of temperature Reeves results (Reeves 1980)

Temperature (°C)	P50 (Torr)
13	5.8
19	8.3
25	13.2
31	19.7
37	26.9
43	33.7

The author stated that the shape of the ODC was invariant with temperature. However, the measured CO_2-Bohr coefficient (Δ log P50/ Δ pH) ranged from –0.46 to –0.51 and was not temperature dependent (Reeves 1980).

RED BLOOD CELL 2,3-DIPHOSPHOGLYCERATE [RBC 2,3-DPG]

Red blood cell 2,3-Diphosphoglycerate (RBC 2,3-DPG) is the main acid-soluble constituent of the erythrocyte of most mammals [Table (1)], occurring in the human

at high concentration, compared with adenosine tri-phosphate (ATP) and orthophosphate (Pi) (Rose 1970). Table (3.2) summarises concentrations of the glycolytic intermediates in erythrocytes(Stryer 1981).

Table (3.2): Concentrations of the glycolytic intermediates in erythrocytes (Stryer 1981).

Intermediate	μM
Glucose	5000
Glucose 6-phosphate	83
Fructose 6-phosphate	14
Fructose 1,6 phosphate	31
Dihydroxyacetone phosphate	138
Glyceraldehyde 3-phosphate	19
1,3 Diphosphoglycerate	1
2,3-Diphosphoglycerate	4000
3-Phosphoglycerate	118
2-Phosphoglycerate	30
Phosphoenolpyruvate	23
Pyruvate	51
Lactate	2900
ATP	1850
ADP	138
Pi	1000

The discovery of the effect of red blood cell RBC 2,3-DPG and ATP on the oxygen dissociation characteristics of haemoglobin has greatly increased appreciation of the association between red cell metabolism and function. Recent evidence makes it clear that, through variations in the levels of ATP and RBC 2,3-DPG, the metabolism of the human red cell may help maintain normoxia (Eaton, Brewer, Schultz, Sing 1970).

RBC 2,3-DPG reduces the affinity of haemoglobin for oxygen by two mechanisms. First RBC 2,3-DPG binds preferentially to deoxyhaemoglobin and thereby alters the equilibrium between oxygen and haemoglobin. A second mechanism is by altering intraerythrocyte pH relative to plasma pH (Thomas, III, Lefrak, Irwin, Fritts, Jr., Caldwell 1974).

Glucose is the normal energy source of the red cell. It is metabolised by the erythrocyte along two major routes, the glycolytic pathway and the hexose monophosphate shunt. The steps in these pathways are essentially the same as those found in other tissues and in other organisms. Unlike most other cells, however the red cell lacks a citric acid cycle (Stryer 1981; Beutler, Lichtman, Coller, Kipps, editors 1995). Glucose metabolism of the erythrocyte for RBC 2,3-DPG synthesis (the direct glycolytic pathway) is shown in figure (3.2).

It has been hypothesised by some authors that red cell DPG may influence red cell deformability. Waugh et al studied the effect of RBC 2,3-DPG on the mechanical properties of the erythrocyte. From their results they confirmed the observations of early investigators that under some conditions RBC 2,3-DPG acts to destabilize the erythrocyte membrane. However, this effect is thought not to occur under physiologic conditions suggesting that the effects of RBC 2,3-DPG on the mechanical properties of erythrocyte membrane are not physiologically important (Waugh 1986).

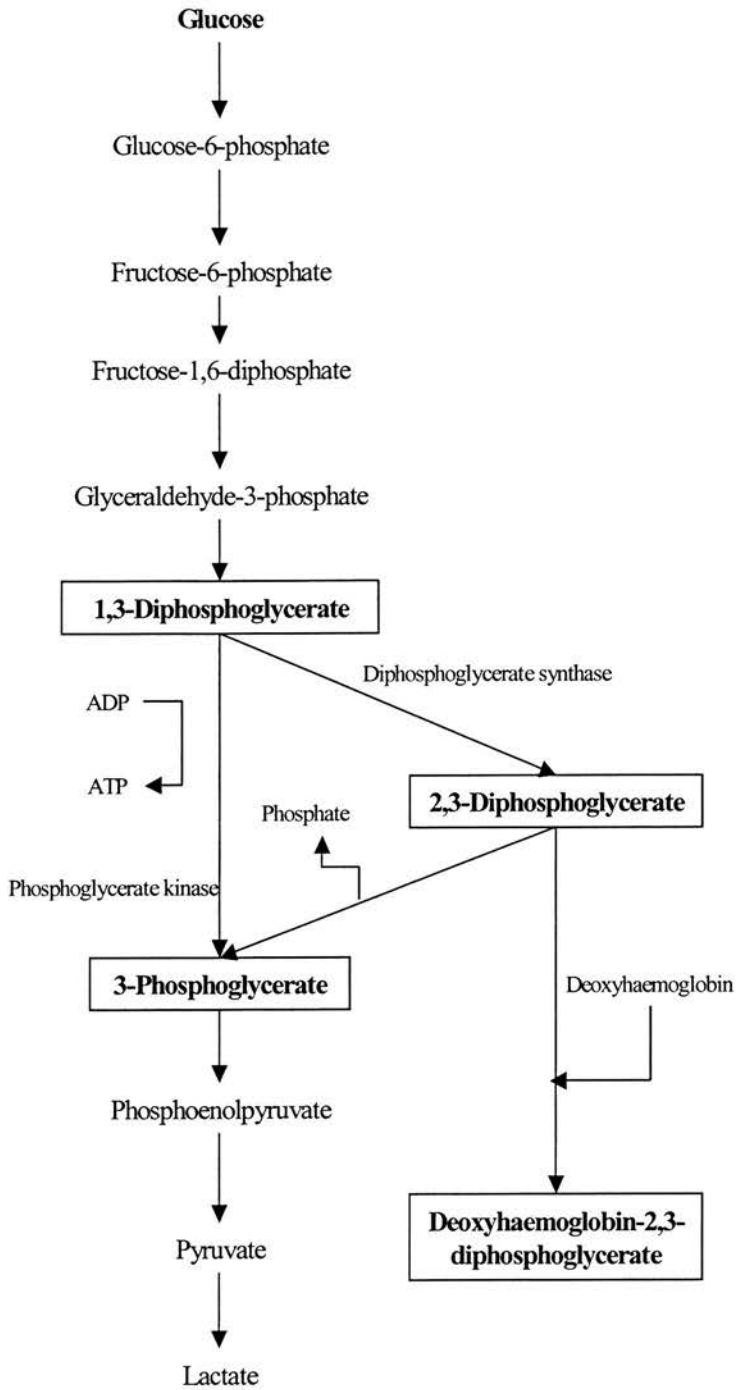


Figure (3.2): Flow chart illustrating red blood cell 2,3-DPG synthesis

ROLE OF RBC 2,3-DPG IN CONTROLLING RED CELL OXYGEN AFFINITY

As the usual P50 measurements are done under constant conditions of temperature, pH, and Po_2, observed variations in the oxygen affinity are usually related to the concentration of 2,3-DPG. The P50 of whole blood or haemoglobin solutions increases with increasing concentrations of 2,3-DPG (Mollison, Engelfriet, Contreras, editors 1993).

In human red cells DPG and Hb are nearly equimolar. DPG profoundly lowers the affinity of Hb for oxygen at concentrations commonly found in the red cells. One mol of DPG combines with one mol of deoxy-Hb to form a complex, which has a low oxygen affinity. If 2,3-DPG is displaced it becomes easier for the Hb molecule to undergo the allosteric transition to the tense (oxy) state with a higher oxygen affinity (Mollison, Engelfriet, Contreras, editors 1993) (Benesch, Benesch 1969).

2,3-DPG binds in an electrically charged pocket between the (beta) chains of hemoglobin, stabilizing the tense conformation and reducing oxygen affinity. The binding of RBC 2,3-DPG also lowers the intracellular pH and further enhances the Bohr effect. The P50 increases directly with the RBC 2,3-DPG concentrations. The RBC 2,3-DPG concentration is reduced in aging red cells and under conditions of hyperoxia or inhibition of glycolysis (by acidosis or hypophosphatemia). Other organophosphates and anions, such as chloride, also compete with RBC 2,3-DPG for binding sites on hemoglobin. Hence, their presence can reduce the regulatory effect of RBC 2,3-DPG on oxygen affinity (Hsia 1998).

Fairweather et al related the ODC to RBC 2,3-DPG in patients with acute and chronic respiratory failure. The authors studied 27 patients, in vivo P50 was

measured using a special tonometer [Edward and Martin] and RBC 2,3-DPG was measured using an enzymatic method. The authors found that the average values of RBC 2,3-DPG was 15.2 ± 3.5 x 10^{-6} mol/g Hb (normal reference range reported 8.5 to 20.5 μmol/g Hb) and P50 was 27.4 ± 2.3 mmHg (normal reference range reported 23-32 mmg). These values were within normal reference ranges despite hypoxia. The authors found that P50 values correlated positively with RBC 2,3-DPG ($r = 0.418$, $P < 0.05$). Multiple-regression analysis showed that the relationship between P50 and RBC 2,3-DPG can explain 17.9% of the variance in P50. The authors suggested that RBC 2,3-DPG was one of the most relevant variables that affected P50 in patients with respiratory failure (Fairweather, Walker, Flenley 1974).

FACTORS AFFECTING THE CONCENTRATION OF RBC 2,3-DPG IN RED CELLS IN VITRO

Most studies concerned with RBC 2,3-DPG in vitro were carried out to investigate different medias for storing blood (discussed in a later section in detail). Deuticke et al. found that RBC 2,3-DPG content of human red cells can be elevated in vitro by incubating the red cells in media containing inosine, pyruvate, and inorganic phosphate. The authors suggested that pH was the main factor that affected RBC 2,3-DPG through its effect on the synthetic pathway (Deuticke, Duhm, Dierkesmann 1971).

Bevington at al. showed that change in temperature over a short time had no significant effect on RBC 2,3-DPG in vitro. Blood samples were taken from normal healthy individuals. RBC 2,3-DPG was measured immediately after the blood samples were withdrawn, 30, and 60 minutes later. The samples were kept in ice. The authors did not mention the actual temperature of the blood samples. The mean

± SD of baseline RBC 2,3-DPG levels was 4.77 ± 0.29 mmol/l, after 60 min. 4.89 ± 0.27 mmol/l, and after 90 min. 4.86 ± 0.31 mmol/l (Bevington, Asbury, Preston, Russell 1985).

Rose et al did a laboratory study to investigate the enzymes controlling RBC 2,3-DPG in human erythrocytes by purifying the enzymes responsible for synthesis (diphosphoglycerate mutase) and breakdown (diphosphoglycerate phosphatase). They found that diphosphoglcerate mutase is inhibited by low levels of RBC 2,3-DPG by a competitive inhibition with 1,3 diphosphoglycerate. Inorganic phosphate inhibits the mutase enzyme as it acts as an inhibitor competitive with monophosphoglycerate. The activity of the phosphatase enzyme is stimulated by the presence of both chloride and phosphate. Phosphate in the absence of chloride has very little effect. Chlorides of Na, K, and Mg are equally effective as activators. The authors found very high concentrations of chloride and/or phosphate have equal inhibitory effects on the phosphatase enzyme (Rose 1970).

FACTORS AFFECTING THE CONCENTRATION OF RBC 2,3-DPG IN RED CELLS IN VIVO

FACTORS INFLUENCING ENZYME ACTIVITY

Studies of the physiology of red blood cells indicate that large increases or decreases in RBC 2,3-DPG concentrations can occur in response to changes in oxygen demands. The concentration of 2,3-diphosphoglycerate (DPG) depends on the balance between its rate of formation from 1,3-DPG by diphosphoglycerate mutase and its degeneration by diphosphoglycerate phosphatase (Rose, Liebowitz 1970; Eaton, Brewer, Schultz, Sing 1970).

Rose and Liebowitz performed another laboratory study, which confirmed that phosphate and phosphoglycerate levels are important for determining the rate of hydrolysis of RBC 2,3-DPG. The authors found that in human RBCs the maximum velocity of the enzyme occurred at pH 6.4. Hydrogen ions have been shown to inhibit the diphosphoglycerate mutase reaction and stimulate the phosphatase reaction. It appears that red cell 2,3-DPG levels are exquisitely sensitive to pH: a rise in pH causes a rise in 2,3-DPG levels, while acidosis results in RBC 2,3-DPG depletion. Rose proposed that end-product inhibition of RBC 2,3-DPG mutase is decreased by increased binding of RBC 2,3-DPG by deoxyhaemoglobin. It was suggested that decreased inhibition leads to an increase RBC 2,3-DPG levels (Rose, Liebowitz 1970; Thomas, III, Lefrak, Irwin, Fritts, Jr., Caldwell 1974; Beutler, Lichtman, Coller, Kipps, editors 1995).

Luque et al. did a study to compare factors that affect the concentration of RBC 2,3-DPG in human blood. 4ml samples were added to tubes with 1ml of a solution of citrate and glucose (pH 5.0). Two of these tubes also included tetrathionate or dithionate. A third sample included dipyridamole. The control sample added physiological saline. The final pH in all samples was 7.0. They found that the level of RBC 2,3-DPG is better maintained during storage in the presence of tetrathionate. They suggested that the variation of RBC 2,3-DPG under the influence of these compounds could be due to direct action on the enzymes responsible for its synthesis and degradation (Luque, Pinilla, Ventura, Santos-Ruiz 1972).

Jelkmann et al. did an animal study using foetal rabbit red blood cells. They found that RBC 2,3-DPG was increased during incubation in isotonic saline at 37°C, pH 7.4, glucose, inosine, and pyruvate (Jelkmann, Bauer 1978).

Table (3.3) summarises factors affecting the enzymatic pathway of RBC 2,3-DPG

Table (3.3) Factors affecting the enzymatic pathway for RBC 2,3-DPG synthesis (Rose 1970; Rose, Liebowitz 1970; Deuticke, Duhm, Dierkesmann 1971; Luque, Pinilla, Ventura, Santos-Ruiz 1972)

Factors decrease synthesis	Factors increase synthesis
2,3-DPG	Chlorides of Na, Mg, and Ca
Inorganic phosphate	Hydrogen ions
Very high levels of chloride	pyruvate
	Tetrathionate

THE EFFECT OF AGE AND SEX

There have been few reports suggesting that the chemical composition of the red blood cell changes during the life of an adult healthy human.

Purcell et al.showed that in a normal population, RBC 2,3-DPG decreased with advancing in age. They studied a total of 322 (176 males and 146 females) subjects from 18-97 years of age; anaemic patients were excluded from the study. The subjects were grouped according to age and RBC 2,3-DPG was measured. A significant inverse correlation between RBC 2,3-DPG concentration and age was found. RBC 2,3-DPG decreased significantly ($P< 0.05$) from a mean of 14.9μmol/gHb for the age group 18 to 24 years to 13.8μmol/gHb for the age group 65 to 74 years (table 3.4). Within each group the mean RBC 2,3-DPG concentrations in males and females were the same, except in the age group 35 to 44 years, where the difference between mean concentrations from males and females was significant (13.6 and 15.2μmol/gHb respectively) (Purcell, Brozovic 1974).

Table (3.4): The relationship between age and RBC 2,3-DPG concentration (Purcell, Brozovic 1974).

Age band in years (N)	Mean ± SD RBC 2,3-DPG (μmol/gHb)
18-24 (26)	14.9 ± 1.6
25-34 (35)	14.4 ± 1.3
35-44 (17)	14.3 ± 1.5
45-54 (17)	14.2 ± 1.5
55-64 (11)	14.9 ± 1.8
65-74 (56)	13.8 ± 1.9
75-84 (104)	13.9 ± 2.4
≥ 85 (16)	12.8 ± 2.0

In children, the mean haemoglobin concentration is lower than in adults. Howard et al measured RBC 2,3-DPG levels in 20 haematologically normal children, one to 12 years of age, and compared it to 15 normal adults. These authors found no differences between children and adults. Moreover, they determined p50 in conjunction with RBC 2,3-DPGlevels and the results were almost identical (table 3.5) (Pearson 1973).

Table (3.5): Mean ± SD of RBC 2,3-DPG and P50 in children and adults (Pearson 1973).

Subjects	RBC 2,3-DPG (μmol/gHb)	P50 (mmHg)
Children	12.98 ± 1.90	27.19 ± 0.61
Adults	13.00 ± 1.93	27.16 ± 0.72

THE EFFECT OF ALTITUDE

The erythropoietic system adapts to high altitude with two major responses: firstly, by increasing red cell production and, secondly, by shifting the oxygen dissociation

curve to the right by increasing RBC 2,3-DPG concentrations (Winslow, Samaja, West 1984; Beutler, Lichtman, Coller, Kipps, editors 1995).

Winslow et al. studied red cell function at extreme altitude on Mount Everest where they measured haemoglobin concentration, RBC 2,3-DPG, P50 (using special tonometer), and acid base status in the research team members (26 to 52 years old) at various altitudes. They found increases in RBC 2,3-DPG, which occurred in association with modest increases in P50. RBC 2,3-DPG increased to high levels when the members moved from sea level to 6,300 m, but further increased only slightly when they reached 8,050 m (table 3.6).

Table (3.6): Red cell function at extreme altitude on Mountain of Everest (Winslow, Samaja, West 1984)

Altitude	Hb	pH	P50	DPG	[DPG]/[Hb]
	g/L		Torr	mol/mol Hb	
Sea level	145 ± 23	7.399	28.1	0.84 ± 0.12	0.84
5400 m	178 ± 10	N/M	N/M	1.05 ± 0.09	N/M
6300 m	188 ± 15	7.467	29.8	1.04 ± 0.12	1.04
8050 m	N/M	7.552	29.6	N/M	1.18
8848 M	N/M	7.780	27.4	N/M	N/M

Mean ± SD [N/M = not mentioned in paper]

The authors suggested that the increase in RBC 2,3-DPG, P50 and left shift of the ODC was associated with respiratory alkalosis. The authors did not mentioned how quickly the response occured in relation to altitude, change in acid base balance or any other variables (Winslow, Samaja, West 1984).

Adams et al did a study to compare the concentration of RBC 2,3-DPG in 2 cretins (anaemic individuals due to a decreased red cell production because of low thyroid hormone production) and 2 normal subjects (control group) living at 3700 m. The

authors found that RBC 2,3-DPG values were higher in cretins than the controls despite similar levels of inorganic phosphate levels, and there was no correlation between Hb levels and RBC 2,3-DPG concentrations. Table (3.7) shows the absolute values of the results for all patients included in the study.

Table (3.7): Absolute valued of Hb and RBC 2,3-DPG in cretins (Adams 1976)

Subjects	Age (years)	RBC 2,3-DPG (μm/gHb)	Hb (g/L)
Cretin 1	27	12.07	115
Cretin 2	34	11.29	139
Control 1	52	10.29	144
Control 2	36	10.06	166

Normal reference range used in the study: RBC 2,3-DPG = 12 ± 1.2μm/gHb, Hb = not mentioned in the paper.

The number of individuals in the study was too small to make definite conclusions (Adams 1976).

Yoshino et al. did another study to measure RBC 2,3-DPG levels during high altitude hypoxia. Five untrained healthy males (20 – 25 years) were submitted to a 4000, 5000 and 6000m simulated altitude chamber. Blood samples from subjects before and after exposure to each stimulated altitude for 5 hours were analysed for RBC 2,3-DPG. A correlation was observed between the levels of RBC 2,3-DPG and the variation of arterial oxygen saturation. The results were shown in graphs without numerical values. The authors mentioned that the change in RBC 2,3-DPG concentration was rapid in response to high altitude and further maintained after returning to sea level. They suggested that the increase in RBC 2,3-DPG was an

indication of tissue hypoxia. The authors did not measure or adjust for acid base status (Yoshino, Hayashi, Katsumata, Mori, Mitarai 1980).

Mairbaurl H et al measured haematological and erythrocyte oxygen transport parameters in whole blood and density-separated erythrocytes in 11 mountaineers (mean ± SD age 36.8 ± 10.2 years) before and during 5 days of exposure to high altitude (4559 m). At high altitude, whole blood in vivo P50 (measured using a gas mixing pumps called Wosthoff device) remained almost unchanged, whereas standard P50 (calculated using Hill's equation) and RBC 2,3-DPG increased significantly. The Bohr coefficient for carbon dioxide was elevated significantly (the change of oxygen-binding affinity of haemoglobin by the effect of hydrogen ions or carbon dioxide). The authors suggested that during short-term exposure to high altitude, the oxygen affinity is decreased only under standard acid-base conditions. They explained their suggestion by increased levels of RBC 2,3-DPG as a result of low oxygen saturation and respiratory alkalosis (Mairbaurl, Schobersberger, Oelz, Bartsch, Eckardt, Bauer 1990).

THE EFFECT OF EXERCISE

Sports anaemia refers to a decrease in Hb and Hct in highly trained runners. Ricci et al measured Hb levels in athletes before and after exercise and in a control groups (healthy individuals) and the mean ± SD valuse were [138 ± 8, 148 ± 8, and 154 ± 6 g/L respectively] (Ricci, Masotti, De, V, Vedovato, Zanotti 1988). This type of anaemia may be due to increase in red cell destruction, increased iron loss in urine and sweat, or increased plasma volume due to overshoot re-hydration. Sports anaemia often occurs during intensive training; a compensatory mechanism was

demonstrated to increase oxygen delivery to tissues. Elevations in the levels of RBC 2,3-DPG seem to be involved in adaptation to the stress of prolonged exercise (Ricci, Castaldi, Masotti, Lupi, Bonetti 1984) (Ricci, Masotti, De, V, Vedovato, Zanotti 1988; Mollison, Engelfriet, Contreras, editors 1993)

Measuring RBC 2,3-DPG and P50 after exercise, Ricci et al. studied 24 non-smoking well-trained runners and compared them to healthy non-trained volunteers. They found that in runners, the mean RBC 2,3-DPG was significantly higher than in the control group (18.9 ± 3.7 and 15.4 ± 2.5μmol/gHb respectively), but the mean P50 values were not significantly different (24.5 ± 1.5 and 24.8 ± 1.2 mmHg respectively). The authors did not mentioned whether P50 values were in vivo or in vitro. As the authors found that mean MCV value was significantly higher in runners than in controls (86 ± 4 and 83 ± 4 fl respectively), they suggested that the increase of RBC 2,3-DPG was due to increase erythropoietic activity to compensate for mild haemolysis. The new red cells contained high levels of RBC 2,3-DPG (Ricci, Castaldi, Masotti, Lupi, Bonetti 1984).

Meen HD el al. examined changes in RBC 2,3-DPG after exercise. They measured RBC 2,3-DPG levels in 5 healthy men (25 – 50 years) over a prolonged period of time after heavy exercise in healthy men, on 3 separate days. They found with 6 min work, RBC 2,3-DPG was unchanged or slightly reduced immediately after and 15 min after the end of exercise, but then increased 8% above the initial level, 30 min after the exercise. With more work (12min), RBC 2,3-DPG increased 12% above its initial values. With an hour of work, RBC 2,3-DPG was increased 30 min after exercise, and rose continuously during the early recovery phase. Table (3.8) shows

RBC 2,3-DPG values and some other variables measured before and during exercise and table (3.9) shows the same variables after finishing exercise.

Table (3.8): Mean ± SD RBC 2,3-DPG, pH, lactate, and serum P before and during exercise (Meen, Holter, Refsum 1981).

Variable	Base line	30 min of work	End of work (60min)
RBC 2,3-DPG (mmol/l)	1.86 ± 0.11	2.08 ± 0.20	2.06 ± 0.20
pH	7.34 ± 0.04	7.35 ± 0.05	7.39 ± 0.04
Lactate (mmol/l)	1.18 ± 0.18	3.65 ± 1.55	3.07 ± 1.33
Serum P (mmol/l)	1.04 ± 0.07	1.26 ± 0.11	131 ± 0.06

Table (3.9): Mean ± SD RBC 2,3-DPG, pH, lactate, and Serum P after exercise (time in minutes) (Meen, Holter, Refsum 1981)

Variable	15	30	45	60	90	120	210	300
DPG	2.04 ± 0.17	2.02 ± 0.17	2.00 ± 0.15	2.02 ± 0.19	2.06 ± 0.19	2.00 ± 0.17	1.94 ± 0.17	2.00 ± 0.16
pH	7.37 ± 0.01	7.37 ± 0.02	7.37 ± 0.03	7.37 ± 0.01	7.34 ± 0.01	7.34 ± 0.04	7.38 ± 0.03	7.39 ± 0.03
Lactate	1.60 ± 0.49	1.25 ± 0.29	1.15 ± 0.23	1.08 ± 0.14	1.12 ± 0.18	1.06 ± 0.13	1.26 ± 0.36	0.95 ± 0.25
Serum P	1.24 ± 0.09	1.19 ± 0.09	1.15 ± 0.07	1.12 ± 0.07	1.11 ± 0.07	1.12 ± 0.08	1.16 ± 0.13	1.17 ± 0.09

The authors concluded from this study that heavy exercise was followed by a progressive increase in RBC 2,3-DPG, reaching a new level 30-40 min after exercise. They suggested that the main cause of the increase in RBC 2,3-DPG was due to

increase in serum phosphate. The authors suggested that lactate may have a role through a decrease in pH (Meen, Holter, Refsum 1981).

Boswart J et al., in a similar study found that after 6 months intense training there was a small, but significant increase in resting RBC 2,3-DPG. The authors studied 8 athletes (15 - 25 years). The authors showed the absolute values for all subjects included in the study at rest before and after 6 months intense exercise (table 3.10).

Table (3.10): RBC 2,3-DPG (mmol/l) values at rest before and after 6 months of training (Boswart, Kuta, Lisy, Kostiuk 1980).

Subjects	Before training	After 6 months
1	4.46	4.53
2	4.54	4.70
3	4.61	4.69
4	4.12	4.54
5	4.30	4.33
6	4.22	4.23
7	4.45	4.60
8	4.36	4.43

The authors concluded that physical exercise has an effect on RBC 2,3-DPG level, but did not suggest a mechanism (Boswart, Kuta, Lisy, Kostiuk 1980).

Ricci G et al. studied the effect of exercise on haematologic parameters, serum iron, serum ferritin, RBC 2,3-DPG, and serum erythropoietin in 8 long-distance runners during training (20 – 35 years) and compared them to 8 healthy volunteers (21-42 years). The authors found that there was no increase of RBC 2,3-DPG observed after exercise, but it was significantly higher than that in a control group. After exercise the athletes' erythropoietin was higher than that of controls. Interestingly, there was a

slight increase in neutrophil count after exercise. Table (3.11) summarises the results of the study.

Table (3.11): The effect of exercise on the haematological parameters (Ricci, Masotti, De, V, Vedovato, Zanotti 1988)

Group	2,3-DPG	Serum iron	Serum ferritin	EPO	Neutrophils
Runners					
Before exercise	19.2 ± 4.3	19.1 ± 4.9	115 ± 78	1.34 ± 0.44	3.49 ± 1.6
After exercise	19.6 ± 3.0	19.9 ± 5.4	130 ± 88	1.58 ± 0.59	4.83 ± 1.6
Controls	16.5 ± 1.1	21.7 ± 5.1	116 ± 63	1.00 ± 0.15	3.92 ± 0.6

2,3-DPG (µmol/gHg), serum iron (µmol/l), serum ferritin (µg/l), EPO (%), neutrophils 10^9/l

The authors did not find a correlation between red cell parameters and RBC 2,3-DPG in the study group. They did not suggest any explanations for the results (Ricci, Masotti, De, V, Vedovato, Zanotti 1988).

Katz A et al. studied the effect of intense interval training on RBC 2,3-DPG levels at rest and after maximal exercise. They found that training caused no significant difference in RBC 2,3-DPG levels at rest and concluded that the role of RBC 2,3-DPG in enhancing tissue oxygenation during increased metabolic demand remains obscure. The authors showed the results in graphs and not in numerical values (Katz, Sharp, King, Costill, Fink 1984).

The data of the studies investigating the effect of exercise on RBC 2,3-DPG are conflicting and most of them are unclear. Some studies suggested that exercise affects RBC 2,3-DPG through changes in pH or phosphate levels; other studies suggested that RBC 2,3-DPG can be affected through alteration in red cell production.

DIABETES

Very few studies looked at the oxygen affinity in subjects with diabetes mellitus. Arturson G et al. studied this relation where they recruited 28 diabetic patients, with an average duration of the disease of 14 years. No patients were acidotic; 27 healthy volunteers were used as controls. They determined oxygen affinity of whole blood, including measurements of pH, Pco_2 and RBC 2,3-DPG. They found that the values of RBC 2,3-DPG were higher in the diabetic patients than in the control subjects and no relation between RBC 2,3-DPG values and haemoglobin concentrations in either group (table 3.12).

Table (3.12): Oxygen affinity in subjects with diabetes mellitus (Arturson, Garby, Robert, Zaar 1974b)

Subjects	Hb	pH	RBC 2,3-DPG
Diabetics			
Males (12)	144 ± 1.4	7.39 ± 0.01	5.63 ± 0.67
Females (16)	140 ± 0.9	7.38 ± 0.02	5.71 ± 0.53
Controls			
Males (12)	147 ± 0.7	7.39 ± 0.03	4.67 ± 0.35
Females (15)	130 ± 0.9	7.38 ± 0.02	5.08 ± 0.49

Mean ± SD Hb (g/l), ph, RBC 2,3-DPG (mmol/l)

The authors did not show numerical values for P50, however they showed 2 figures for standard P50 and in vivo P50 and mentioned that oxygen affinity could not be evaluated on the basis of the available data. They referred the higher levels of RBC 2,3-DPG in the study group than that in the controls due to the presence of HbA_{Ic} which has lower oxygen affinity (Arturson, Garby, Robert, Zaar 1974b).

ANAEMIA

In anaemia the oxygen carrying capacity of blood is decreased and there are adaptive mechanisms to maintain adequate oxygen delivery to tissues such as increased cardiac output, hyperventilation, and increased RBC 2,3-DPG concentration (Beutler, Lichtman, Coller, Kipps, editors 1995; Szaflarski 1996).

Few studies have investigated the effect of acute and chronic anaemia on RBC 2,3-DPG, however, some studies have investigated in detail sports anaemia (discussed in a previous section).

Acute anaemia

Most studies that investigated the effect of acute anaemia were carried out in animal models. Anaemia was induced by normovolaemic haemodilution.

Cropp et al. studied the role of RBC 2,3-DPG in oxygen transport in experimental anaemia. The authors measured RBC 2,3-DPG levels before, during, and after inducing anaemia by isovolaemic exchange transfusions with plasma in 5 dogs. They found that when Hb concentration fell to half its normal values the RBC 2,3-DPG showed approximately double its base line levels. RBC 2,3-DPG reached approximately five times its base line levels at Hb concentration of 28 g/L. The authors showed a figure of the relationship between RBC 2,3-DPG levels and Hb concentrations and the relationship was a linear. Table (3.13) shows the observed mean ± SD values of RBC 2,3-DPG in different Hb concentrations.

Table (3.13): Oxygen transport in experimental anaemia (Cropp, Gee 1972)

Hb (g/l)	2,3-DPG (μmol/gHb)
133 ± 0.43	16 ± 0.8
73 ± 0.62	26 ± 1.4
37 ± 0.16	54 ± 4.0

The authors suggested that RBC 2,3-DPG increased as a compensatory mechanism to anaemia, however, they hypothesised that the increase in RBC 2,3-DPG values due to acute anaemia in dogs appear to be greater than in humans (Cropp, Gee 1972). Holter et al. studied the relationship between P50 and RBC 2,3-DPG during the early phase of the post-natal decrease in haemoglobin in rabbits. The authors mentioned that in these species Hb levels tend to decrease dramatically during the first ten days after birth. The authors found that there was a decline in Hb levels during the study period, gradual increase in the P50 value from 2.3 kPa to 3.2 kPa, and RBC 2,3-DPG increased markedly from 2.5 μmol/g to 17 μmol/g. They also showed a correlation between the rise in P50 and RBC 2,3-DPG ($r = 0.86$, $P<0.001$). The authors reported another negative correlation between P50 and Hb levels ($r = 0.63$, $P<0.001$). They suggested that this negative correlation, together with the correlation between P50 and RBC 2,3-DPG reflect an anti-hypoxic mechanism that occurs in acute anaemia (Holter, Halvorsen, Refsum 1982).

Fong et al. showed figures for RBC 2,3-DPG and the corresponding Hb levels in acutely bled rabbits and normal controls in one of their studies. Mean ± SD RBC 2,3-DPG was higher in the acutely bled group than the normal controls (31.1 ± 1.6 and 28.5 ± 0.9 μmol/gHb respectively) and the corresponding Hb levels were 92 ± 4 and 136 ± 4 g/L respectively (Fong, Ko, Streczyn, Westerman 1976).

Chronic anaemia

Few authors have investigated the relation between chronic anaemia and RBC 2,3-DPG. Most of these studies were carried out in relation to various types of anaemia such as anaemia associated with malignancy. Some were carried out in patients with

other types of anaemia such as sickle cell anaemia, folate deficiency and iron deficiency.

Fong et al. did an experimental study to investigate chronic anaemia, wound healing (as an indictor of oxygen delivery to the tissues), and RBC 2,3-DPG in rabbits. The authors included 42 rabbits with different types of anaemia; 13 iron deficiency, 10 water-induced anaemia, 10 phenylhydrazine anaemia, 9 iron deficient and received iron supplement, and 18 normal rabbits served as a control group. All the groups underwent a 5 inches skin incision, which was stitched in the same manner. Wound healing was measured by special formulas (tensile strength and energy absorption) that were derived from the wound characteristics. Table (3.14) summarises the result of the study.

Table (3.14): RBC 2,3-DPG in experimental chronic anaemia (Fong, Ko, Streczyn, Westerman 1976)

Subjects	Hb	pH	RBC 2,3-DPG
Controls	136 (108-170)	7.45 ± 0.01	28.5 ± 0.9
Iron deficiency anaemia	78 (68-97)	7.51 ± 0.01	31.5 ± 1.3
Water-induced anaemia	83 (72-93)	7.50 ± 0.01	30.0 ± 1.3
Phenylhydrazine anaemia	85 (77-90)	7.47 ± 0.03	28.2 ± 1.1
Iron deficient with iron supplement	134 (117-146)	Not mentioned	28.4 ± 1.3

Mean (range) Hb (g/l), mean ± SD pH, mean ± SD RBC 2,3-DPG (μmol/gHb)

The authors found that there was increased levels of RBC 2,3-DPG and wound healing was normal in rabbits with iron deficiency and water induced anaemia. They suggested that normal wound healing was associated with a decrease in oxygen affinity due to high levels of RBC 2,3-DPG. The Phenylhydrazine group showed decreased levels of RBC 2,3-DPG and normal wound healing. The authors suggested that the change in RBC 2,3-DPG was due to the effect of phenylhydrazine on the RBC 2,3-DPG metabolic pathway and that normal wound healing occurred due to other compensatory mechanisms for keeping normal tissue oxygen delivery such as increased cardiac output and vascularization (Fong, Ko, Streczyn, Westerman 1976). Haidas et al did a study to show RBC 2,3-DPG levels in children with nutritional iron deficiency (N = 7, age between 1.5 and 3 years), acute lymphoblastic leukaemia (N = 35, age between 2.5 and 13 years), and normal children served as a control group (N = 45, age between 1.5 and 14 years). The authors found a significant difference between RBC 2,3-DPG levels in patients with iron deficiency compared to controls. In the acute leukaemia group, 16 Children (no relapse) had normal levels of RBC 2,3-DPG and 19 children (with one or more relapse) had higher levels compared to controls. Table (3.15) summarises the findings of the study.

Table (3.15): RBC 2,3-DPG level in children with chronic anaemia (Haidas, Zannos-Mariolea, Matsaniotis 1976)

Subjects	Hb	RBC 2,3-DPG
Control group	126.9 ± 16.0	14.90 ± 0.68
Iron deficiency group	79.4 ± 12.0	20.87 ± 3.11
Acute leukaemic group		
No history of relapse	128.8 ± 13.5	14.11 ± 0.88
With history of relapse	118.0 ± 12.0	22.05 ± 2.75

Mean ± SD Hb (g/l), RBC 2,3-DPG (mol/gHb)

The authors suggested that the increase in RBC 2,3-DPG in the iron deficient group was a compensatory mechanism to low Hb levels, however, they did not give good explanations for the findings in the leukaemic group. They assumed that treatment in patients with no relapse might affect RBC 2,3-DPG levels. They recommended further studies to investigate the activities of enzymes responsible for the production and degeneration of RBC 2,3-DPG in these group of patients (Haidas, Zannos-Mariolea, Matsaniotis 1976).

Festa et al did a similar study. The authors studied the ODC (using an automatic apparatus) in children with anaemia and malignant disease. They studied 68 children with acute leukaemia and 27 with solid tumours. The authors included children who were anaemic at the time of diagnosis or developed anaemia during the course of the disease (Hb < 90 g/L). This group of children was compared to another 25 healthy adults, suggesting that RBC 2,3-DPG levels in children to be 3-12% higher than normal adults (normal adults 4.35 ± 0.33 μmol/ ml RBC). The authors found that RBC 2,3-DPG and P50 were elevated in children at the time of diagnosis and before starting treatment. They suggested that early before treatment, increased RBC 2,3-DPG was a compensatory mechanism to anaemia. There were a decrease in RBC 2,3-DPG and P50 levels in patients who received red cell transfusion and chemotherapy. The authors suggested that red cell transfusion decreased blood oxygen affinity and chemotherapy prevented the production of new red blood cells which have the ability to increase RBC 2,3-DPG levels (Festa, Asakura 1979).

Hjelm at al did similar study to measure RBC 2,3-DPG in 57 adult patients (age range from 21 to 83 years) with myelomatosis and different types of leukaemia and without cardiac, renal, or respiratory complications. The authors found that RBC 2,3-

DPG correlated inversely with Hb in all groups except acute leukaemia (r and P values are shown in table (3.16).

Table (3.16): The correlation between RBC 2,3-DPG and Hb in patients with myelomatosis and different types of leukaemia (Hjelm, Uden, Engstedt 1976)

Diagnosis	r^2	P value
Myelomatosis	-0.76	< 0.001
Chronic myelocytic leukaemia	-0.78	< 0.05
Chronic lymphoblastic leukaemia	-0.827	< 0.001
Acute leukaemia	-0.208	< 0.01

They suggested that RBC 2,3-DPG increased due to anaemia (Hb range was 70-90 g/L), but not in acute leukaemia because of disease-related factors such as drugs or red cell transfusion (Hjelm, Uden, Engstedt 1976).

Charche et al. investigated RBC 2,3-DPG in 32 patients with sickle cell anaemia and compared the results to 34 healthy individuals. The mean ± SD of RBC 2,3-DPG in healthy subjects was 4.47 ± 0.53 μmol/ml packed RBCs and in patients was 6.25 ± 0.91 μmol/ml packed RBCs. There was no correlation between RBC 2,3-DPG and either Hct ($r^s = 0.06$) or reticulocyte count ($r^s = 0.14$). The authors suggested that hypoxia due to the nature of the disease led to respiratory alkalosis, which increased RBC 2,3-DPG levels via increased pH (Charache, Grisolia, Fiedler, Hellegers 1970).

Opalinski et al studied RBC 2,3-DPG and creatine levels (as an indicator of red cell age) in 17 patients with different types of anaemia and compared the results to 13 healthy individuals (controls). The study included 3 patients with aplastic anaemia, 8 patients with haemolytic anaemia, and 6 patients with different types of anaemia such as iron, folate, or pot-haemorrrhagic anaemia.

The authors found that creatine levels were low and RBC 2,3-DPG was significantly increased in patients with aplastic anaemia compared to controls. In patients with haemolytic anaemia and other types of anaemia, both RBC 2,3-DPG and creatine were significantly increased. Table (3.17) summarises the results of the study.

Table (3.17): RBC 2,3-DPG and creatine levels in patients with different types of chronic anaemia (Opalinski, Beutler 1971)

Subjects	Hct	Creatine	RBC 2,3-DPG
Controls (13)	45.4 (40-49)	1.07 (0.83-1.35)	15.03 (13.64-16.21)
Aplastic anaemia (3)	16.8 (15-19)	0.19 (0.09-0.39)	16.45(15.57-17.73)
Haemolytic anaemia (8)	22.7 (13-29)	8.37 (3.7-15.12)	23.12 (17.43-29.73)
Other types of anaemia (6)	23.0 (14-31)	3.08 (1.58-4.24)	22.28 (16.87-27.71)

Mean (range) Hct (%), creatine (μmol/gHb), RBC 2,3-DPG (μmol/gHb)

The authors found a significant correlation between RBC 2,3-DPG and creatine. The authors suggested that younger red blood cells had higher levels of RBC 2,3-DPG. They concluded that anaemia was associated with increased RBC 2,3-DPG, but suggested an additional mechanism for this was the presence of young red cells that contained higher levels of RBC 2,3-DPG (Opalinski, Beutler 1971).

Espinos D et al. showed the relationship between RBC 2,3-DPG, anaemia, hypoxaemia and acid-base status in patients with liver cirrhosis. They compared 60 patients with hepatic cirrhosis, 33 patients with iron deficiency anaemia, and 86 healthy subjects. They found that RBC 2,3-DPG level was significantly higher in cirrhotic patients compared to the healthy subjects and the 33 patients with iron deficiency anaemia despite lower Hb concentration than observed in cirrhotic patients. Table (3.18) summarises the results.

Table (3.18): RBC 2,3-DPG level in cirrhotic and iron deficient patients (Espinos, Alvarez-Sala, Villegas 1982)

Variables	Cirrhotic	Iron deficient	Controls
Hb	7.31 ± 1.55	6.27 ± 0.98	9.02 ± 1.02
RBC 2,3-DPG	7.40 ± 1.23	5.86 ± 1.06	4.58 ± 0.59

Mean ± SD Hb (mmol/L), RBC 2,3-DPG (mmol/gHb)

The authors suggested that RBC 2,3-DPG level was affected by the acid-base disturbance accompanied liver cirrhosis more than the degree of anaemia, and they showed that alkalosis increased RBC 2,3-DPG ($r = 0.482$, <0.001). The authors did not mention pH levels for the other groups (Espinos, Alvarez-Sala, Villegas 1982). In summary, although clinical and animal studies do not all have consistent conclusions, the following factors have been associated with increase RBC 2,3-DPG in vivo:

- Low Hb level (acute and chronic) (Cropp, Gee 1972; Haidas, Zannos-Mariolea, Matsaniotis 1976; Fong, Ko, Streczyn, Westerman 1976; Hjelm, Uden, Engstedt 1976; Festa, Asakura 1979; Holter, Halvorsen, Refsum 1982)
- Alkalosis (Luque, Pinilla, Ventura, Santos-Ruiz 1972; Jelkmann, Bauer 1978)
- High altitude (Yoshino, Hayashi, Katsumata, Mori, Mitarai 1980; Winslow, Samaja, West 1984; Mairbaurl, Schobersberger, Oelz, Bartsch, Eckardt, Bauer 1990)
- Exercise (Meen, Holter, Refsum 1981; Katz, Sharp, King, Costill, Fink 1984; Ricci, Castaldi, Masotti, Lupi, Bonetti 1984)
- Lower age (Pearson 1973; Purcell, Brozovic 1974)

Part of confusion is data probably reflects the lack of consideration of all possible factors in the studies.

ANAESTHESIA AND SURGERY

Very few studies have investigated factors that affect RBC 2,3-DPG during anaesthesia and surgery. Schweizer et al. did a study in 50 patients undergoing major surgery such as thoracic, abdominal, and neurological. Anaesthetic technique and drugs used for all patients were similar. Blood transfusion was given as required. Pre-operative and post-operative RBC 2,3-DPG, Hb, pH, oxygen saturation, and Po_2 were measured. Blood samples were withdrawn immediately pre and post operatively. The authors found that 19 patients had no significant change between pre and post-operative RBC 2,3-DPG and blood lactate level. 31 patients showed a significant decrease of RBC 2,3-DPG post-operatively and a significant increase in blood lactate level. Table (3.19) summarises the findings.

Table (3.19): RBC 2,3-DPG before and after surgery (Schweizer, Howland 1973)

	Patients with no significant change in RBC 2,3-DPG (19)		Patients with significant change in RBC 2,3-DPG (31)	
Variables	Preoperative	Post-operative	Preoperative	Post-operative
RBC 2,3-DPG	15.14 ± 3.29	15.29 ± 3.57	15.92 ± 2.63	11.07 ± 2.80
Hb	124.9 ± 16.6	111.3 ± 16.8	119.6 ± 16.0	116.4 ± 12.1
SO_2	83.08 ± 10.18	79.43 ± 8.55	73.15 ± 24.76	78.85 ± 12.78
pH	7.36 ± 0.02	7.36 ± 0.05	7.36 ± 0.02	7.37 ± 0.05
Phosphate	1.64 ± 0.36	1.53 ± 0.44	1.71 ± 0.22	1.67 ± 0.20
Lactate	1.15 ± 0.45	1.78 ± 0.82	1.07 ± 0.22	2.17 ± 0.91
Blood transfused	108		1844	

Mean ± SD RBC 2,3-DPG (μmol/gHb), Hb (g/L), SO_2 (%), inorganic phosphate (meq/L), lactate (meq/L), blood (ml)

The authors suggested that the low levels of RBC 2,3-DPG was due to blood transfusion with RBC 2,3-DPG depleted blood. They hypothesized that the increased level of blood lactate was due to low concentration of RBC 2,3-DPG which shifted ODC to the left and induced an aerobic respiration with increased levels of lactate (Schweizer, Howland 1973). The authors did not consider the high lactate concentration of stored blood.

RBC 2,3-DPG IN STORED BLOOD

The decrease in the level of RBC 2,3-DPG and other phospho-compounds in blood stored for transfusion is well known. Depletion of DPG during the storage process has been a major concern because of the belief that this adversely affects oxygen unloading at the tissue level. The factors influencing RBC 2,3-DPG concentration during storage has been a major focus in transfusion medicine. All currently used systems for storage of RBCs result in loss of RBC 2,3-DPG and an associated increase in affinity for oxygen (Luque, Pinilla, Ventura, Santos-Rùiz 1972; Kahn, Zaroulis, Goetz, Howland 1986; Hogman, Knutson, Loof, Payrat 2002; Hess, Hill, Oliver, Lippert, Greenwalt 2002). Some key papers relating to advances in red cell storage are summarised in table (3.20). The authors of these studies have based their explanations on the fact that pH has a role in maintaining RBC 2,3-DPG in stored blood. The more alkalotic media used for preserving blood the more RBC 2,3-DPG would be preserved through the effect on its synthesis and degeneration.

Table (3.20): Advances in red cell storage.

Author	Aim of the study	Settings	Results and conclusions
Hogman et al.(Hogman, Knutson, Loof, Payrat 2002)	The authors aimed to improve the quality of stored blood collected in ordinary CPD.	A formulation of Erythro-sol (Erythro-sol 2), which more alkaline (pH 8.8) was compared with Erythro-sol (Erythro-sol 1) (pH 7.4). In vitro measures of ATP and RBC 2,3-DPG during 49 days of storage in the 2 additives were compared.	The maintenance of RBC ATP & RBC 2,3-DPG was significantly better in Erythro-sol 2 (more alkaline) than in Erythro-sol 1 (more acidic). Erythro-sol 2 was an improved additive solution for the storage of RBCs. The authors suggested that at alkaline pH the activity of hexokinase and phosphofructokinase involved in RBC 2,3-DPG synthesis showed higher concentration in alkaline pH.
Hess et al.(Hess, Hill, Oliver, Lippert, Greenwalt 2002)	The aim of the study was to investigate the role of alkaline CPD in preservation of RBC 2,3-DPG.	12 units of RBC were collected and divided into 4 groups; each group was preserved in CPD with different pH (pH= 5.7, 6.5, 7.5, or 8.7). Another 12 units were collected in alkaline CPD (pH = 8.7). The units were divided into 4 groups; an extra additive of disodium phosphate was added to each group (0, 9, 18, or 27 mmol).	Alkaline pH alone increased RBC 2,3-DPG concentration which decreased 2 weeks later, however, ATP concentration was decreased from the start. By adding more phosphate to alkaline CPD, both RBC 2,3-DPG and ATP were preserved for longer time (the authors did not mention the time). The authors suggested that alkaline pH alone increased RBC 2,3-DPG through activating the enzymatic process which utilized ATP. Alkaline CPD and phosphate provided better buffer for regenerating both RBC 2,3-DPG and ATP.

Author	Aim of the study	Settings	Results and conclusions
Hogman et al.(Hogman, Knutson, Loof 1999)	Study the effect of temperature on RBC 2,3-DPG and the accumulation of lactate on storage of whole blood.	Whole blood was collected in ACD-A and CPD. The blood was incubated at different temperatures (4, 10, 15, 20, 25, and 30 °C) for 24 hours. Blood gases, pH, bicarbonate, glucose, lactate and RBC 2,3-DPG were measured.	RBC 2,3-DPG decreased at 30 °C within 24 hours; 99% was lost within 18 hours. At 25 °C 94% was lost within 18 hours. At 20 °C, the decrease was 62% and that at 15 °C was 24%. No loss of RBC 2,3-DPG was observed at 4c and 10 °C. There was a significant positive correlation between time and lactate accumulation, and a negative correlation between storage temperature and blood pH. The authors suggested that storage of whole blood at 25-30 °C decreased RBC 2,3-DPG through accumulation of lactate and the change in pH (acidic).
Kurup et al.(Kurup, Arun, Gayathri, Dhanya, Indu 2003)	The aim of the study was to investigate the levels of RBC 2,3-DPG after modifying CPDA (citrate–phosphate–dextrose–adenine) and SAGM (saline–adenine–glucose–mannitol).	Modified CPDA was obtained by maintaining its pH at 7.6. Modified SAGM solution was obtained by maintaining its pH at 7.6, reducing adenine, and adding citrate. RBC 2,3-DPG concentration in the whole blood after 28 days of storage in modified CPDA, and in RBCs stored in modified SAGM, was compared with that stored in unmodified solutions.	RBC 2,3-DPG levels were maintained after 28 days in the modified formulations [10.63 ± 2.58 µmol/gHb in the case of modified CPDA and 12.07 ± 1.47 µmol/gHb in the modified SAGM], whereas in standard CPDA and SAGM solutions, the concentration of RBC 2,3-DPG decreased to very low levels (0.86 ± 0.97 µmol/g Hb for CPDA and 0.12 ± 0.008 for SAGM). The authors suggested that pH > 7.0 in favour of increase synthesis and decrease destruction of RBC 2,3-DPG.

Few studies have investigated the benefits of transfusing blood rich with RBC 2, 3-DPG and its impact on improving different organ functions.

Dennis et al. did a study to investigate myocardial performance after transfusing red cells with high RBC 2, 3-DPG concentrations. 22 patients undergoing coronary artery bypass surgery were included in the study. 11 patients received blood with a mean age of 5.5 days and contained 70% of normal RBC 2, 3-DPG concentration, this group served as a control group. 11 patients (the study group) received previously frozen, washed, concentrated blood with 150% of normal RBC 2, 3-DPG. There was a significant increase in cardiac index in patients who received blood enriched with RBC 2, 3-DPG compared to controls (2.95 L/min. versus 2.18 L/min. respectively). After transfusion and immediately off bypass, RBC 2, 3-DPG after transfusion was significantly higher in the study group compared to the control group (13.5 versus 10.6μmol/gHb respectively). Oxygen consumption was higher in the study group compared to the controls (135 versus 106 ml/min./square meter respectively). Hb level was similar in both groups. Patients in the study group were slightly more acidotic than the controls at the time of terminating the bypass process (pH = 7.26 versus 7.29 respetively). In vivo P50 (measured using a tonometer) was also elevated in the study group compared with controls (31.6 versus 28.3 torr respectively). After 24 hours, in vivo P50 and RBC 2, 3-DPG remained elevated in the study group compared with controls. Patients in the study group received less inotropes and showed a reduced frequency of use of intra-aortic balloon pumping. The authors suggested that high level of RBC 2, 3-DPG shifted the ODC to the right and improved tissue oxygenation. As a result of that, myocardial tissue oxygenation was improved with increase in myocardial work. The authors suggested that acidosis

in the study group was the reason of high level of in vivo P50 (Dennis, Vito, Weisel, Valeri, Berger, Hechtman 1975).

Proctor et al. described a case report of the effect of transfusing red blood cells rich with RBC 2, 3-DPG to a multiple traumatized unconscious 10 years old girl with severe hypoxia. Inosin, sodium pyruvate, and Na_2HPO_4 were added to the blood to increase its RBC 2, 3-DPG. A total of five units with high RBC 2, 3-DPG were transfused. The patients became fully alert after receiving the second unit and fully responded to simple commands. 92 hours later the patient died and brain autopsy revealed cerebral oedema and cortical petechiae. The authors suggested that high level of RBC 2, 3-DPG shifted the ODC to the right and improved tissue oxygen delivery including the brain tissues (Proctor, Parker, Fry, Johnson, Jr. 1973).

OXYGEN AFFINITY AND RBC 2,3-DPG IN CRITICALLY ILL PATIENTS

There have been a number of investigations into whether haemoglobin-oxygen affinity is increased, normal or decreased during critical illness, but the published data are inconclusive and conflicting.

Bevington et al investigated phosphate metabolism in erythrocytes of critically ill patients. The authors studied 46 critically ill patients within 24 hours of ICU admission. Red cell orthophosphate (P_i), adenosine 5-diphosphate (ADP), adenosine 5-triphosphate (ATP), and RBC 2,3-DPG were measured. The authors found that P_i varied widely in critically ill patients compared to a reference range (0.1 to 4.2 and 0.6 to 1.5 mmol/l respectively). The relationship between cellular organic phosphate and P_i was weak with marked depletion of ATP and this occurred only at very low levels of P_i. RBC 2,3-DPG did not show dependence on P_i levels. ATP/ADP ratio

was not significantly affected by P_i concentration (Bevington, Asbury, Preston, Russell 1985).

Myburgh JA et al studied P50 in critically ill patients with a mean age of 58 years. They compared standard P50 on venous blood in 20 critically ill patients and 20 normal individuals. The formula used in the calculation did not include RBC 2,3-DPG measurement. Arterial blood gas, lactate, haemoglobin, and phosphate levels were also measured. The authors found that P50 was significantly lower in critically ill patients compared with normal subjects. There was a strong positive correlation between P50 and arterial pH ($r = 0.79$) and base excess ($r = 0.69$). There were poor correlations between P50 and other variables measured in the study. They concluded that standard P50 is reduced in many critically ill patients, and correlats with arterial acid base status (Myburgh, Webb, Worthley 1991). However, the authors did not consider mathematical linkage as a confounder or the unknown true RBC 2,3-DPG.

Morgan et al did another study in the critical care setting. The authors investigated RBC 2,3-DPG and P50 in 20 critically ill male patients with APACHE II score > 20 and compared the RBC 2,3-DPG values to 20 healthy non-smoking males (controls). Venous blood samples were taken from patients and controls to measure RBC 2,3-DPG. Arterial blood samples were collected from patients only to calculate the in vivo P50 using the oxygen status algorithm of the Siggaard-Anderson formula. This formula of calculating P50 include arterial pH, arterial Po_2, arterial carboxy-haemoglobin, Hb, mean core temperature, and venous RBC 2,3-DPG. The authors found that the mean RBC 2,3-DPG concentration was lower in patients than that in controls (4.2 ± 1.3 and 4.9 ± 0.5 respectively). The mean in vivo P50 for patients was 28.0 ± 1.3 mmHg. Using multiple regression analysis, there was only a positive

significant correlation between RBC 2,3-DPG and pH. There was a weak positive correlation between RBC 2,3-DPG and Hb concentration. There was no correlation between RBC 2,3-DPG and calculated in vivo P50 (Morgan, Koch, Morris, Clague, Purdie 2001). A review of some clinical studies in other relevant sittings is shown in table (3.21)

Table (3.21): Published clinical studies that investigated different factors that affect RBC 2,3-DPG in different settings of patients

Author	Aim of the study	Settings	Results and conclusions
Umimoto et al.(Umimoto, Hirai, Hayashi, Tanaka 2000)	To investigate the effect of biofiltration (BF) on RBC 2,3-DPG and red cell-pH.	5 male patients (age range 52-60 years) were studied. 2 weeks crossover study was conducted with bicarbonate hemodialysis (BcHD) and biofiltration (a method in which bicarbonate is not used for dialysis). All patients were diabetic and did not receive red cell transfusion for 6 months before the study. DPG and red cell-pH were measured.	RBC-pH significantly increased during both types of treatments from the baseline. However, at the end of both types of treatments, there was no significant difference (7.19 ± 0.02 for BcHD and 7.20 ± 0.02 for BF). There was no significant difference between RBC-2, 3DPG values at the beginning of BcHD and BF (16.4 ± 1.2 and 16.0 ± 2.0 µmol/gHb respectively). RBC-2, 3DPG during BcHD remained unchanged while it showed a significant increase after 120 min and at the end of BF sessions. The authors suggested that BF corrected metabolic acidosis and bohr effect caused by an increase in RBC-pH during dialysis is compensated for by an increased in RBC-2, 3DPG.
Piccioni et al.(Piccioni, Cestari, Strunz, Auler 2003)	Investigate RBC 2,3-DPG in low cardiac output patients and its influence on In vivo P50.	The authors measured RBC 2,3-DPG and calculated the in vivo P50 for 18 patients with end-diastolic pressure > 20 mm Hg, ejection fraction < 40%, or valve pathology undergoing elective cardiac surgery and compare the results to 20 normal healthy volunteers.	The RBC-2, 3DPG was significantly lower in patients compared to healthy volunteers (6.89 ± 2.2 vs. 11.85 ± 2.4 µmol/gHb, respectively). There were no differences between the RBC-2, 3DPG pre-operatively, and early and late post-operatively. Before CPB, calculated in vivo P50 was significantly higher than standard P50 (22.4 ± 2.96 versus 29.1 ± 3.06 mmHg respectively) and remained significantly higher all throughout. The authors suggested that acidosis increased RBC-2, 3DPG and shifted ODC to the left. They added that the differences between standard and calculated in vivo P50 are similar to other studies.

Author	Aim of the study	Settings	Results and conclusions
Keitt AS et al.(Keitt, Hinkes, Block 1974)	To compare factors regulating RBC 2,3-DPG in acute hypoxia.	12 chronic hypoxaemic patients, arterial oxygen was increased by chronic administration of oxygen by nasal cannula for 4 to 12 months, 7 of them became acute hypoxic by sudden withdrawal of the nasal cannula.	Before oxygen administration, RBC 2,3-DPG was correlated significantly with arterial Po_2 but not with arterial pH, however, during the first 24 hours after stopping giving oxygen by the nasal cannula, the acute change in RBC 2,3-DPG was highly correlated with a change in arterial pH. There was no correlation between RBC 2,3-DPG and Hb level. The authors suggested that change in pH was the most important factor that affected RBC 2,3-DPG level in acute hypoxia. Other factors such as oxygen saturation and Po_2 control RBC 2,3-DPG in chronic stable hypoxia.
Oski et al.(Oski, Gottlieb, Delivoria-Papadopoulos, Miller 1969)	Investigate RBC 2,3-DPG in subjects with chronic hypoxaemia.	20 normal non-smoking subjects, 10 hypoxaemic patients, 5 cyanotic heart disease patients, and 5 chronic heart disease patients were included in the study. RBC 2,3-DPG, pH, Po_2 and So_2 were measured.	RBC 2,3-DPG level was significantly elevated in patients with So_2 below 96%. In patients with cyanotic congenital heart disease, whose arterial saturation < 96%, the level of RBC 2,3-DPG was higher than in patients with chronic hypoxic lung disease. A direct relation between So_2 and RBC 2,3-DPG could not be observed. The authors did not explain the results and mentioned further analysis of the data were required.

Author	Aim of the study	Settings	Results and conclusions
Kahn et al.(Kahn, Zaroulis, Goetz, Howland 1986)	To evaluate oxygen transport and RBC 2, 3-DPG levels in patients with acute respiratory failure after red cell transfusion.	15 patients with respiratory failure ($Po_2 \leq 70$ torr) and Hct $\leq$ 35% were included in the study. 7-ml/kg red blood cells were transfused over 2 hours. All measurements were carried out before transfusion and 30 to 1 hour after transfusion.	There was a significant increase in Hb level after transfusion (109 ± 8 before transfusion and 125 ± 8 g/L after transfusion). RBC 2, 3-DPG decreased significantly after transfusion (14.5 ± 1.1 before transfusion and 13.1 ± 1.5µmol/gHb). There was no significant difference in P50 value before and after transfusion (29.9 ± 2.2 versus 28.8 ± 2.9 torr respectively). The authors excluded that change in pH could be the cause of RBC 2, 3-DPG levels; however, they did not suggest any reason for their results. They referred the insignificant change of P50 to the change in RBC 2, 3-DPG.
Edwards et al.(Edwards, Canon 1972)	To investigate RBC 2, 3-DPG levels in sever hypoxaemia of chronic lung disease.	50 blood samples were taken from 5 patients with severe arterial hypoxaemia (mean Po_2 = 38, Pco_2 = 54 torr and pH = 7.34). The authors measured RBC 2, 3-DPG and calculated standard P50 (in vitro P50).	The authors found that this group of patients had normal RBC 2, 3-DPG and in vitro P50, however, they did not report the values of the measured variables or the normal reference range. However, they showed the relationship between RBC 2, 3-DPG and in vitro P50 in a figure. The relationship appeared to be a linear. The authors did not provide clear explanations for their findings.

Author	Aim of the study	Settings	Results and conclusions
Lyons et al.(Lyons, Tabak 1972)	To determine the effect of chronic hypoxia on the oxygen affinity and to investigate the relation between the acid base status and the affinity of Hb for oxygen.	13 chronic hypoxaemic patients were included in the study.	There was no relationship between P50 and base excess values. There was a strong positive correlation between P50 and the sum of red blood cell Na and K. The authors explained this relation by change in intracellular pH. There was positive correlation between P50 and RBC 2, 3-DPG. There were no relationships between plasma pH, RBC 2, 3-DPG, oxygen tension, or haematocrit values. The authors suggested that acid base disturbance in patients with chronic hypoxia had different effect on RBC 2, 3-DPG compared to other subjects.

MATERIAL AND METHODS

RED CELL 2,3-DIPHOSPHOGLYCEREATE [RBC 2,3-DPG] AND P50 DURING CRITICAL ILLNESS: PROSPECTIVE OBSERVATIONAL STUDY.

AIMS

PRIMARY AIM

To measure red cell RBC 2,3-DPG concentration and P50 during critical illness.

SECONDARY AIM

To investigate factors that are associated with red cell RBC 2,3-DPG concentration and P50 during critical illness.

PATIENTS STUDIED

All patients who were admitted to ICU between 22/01/2002 and 14/07/2002 were considered for the study.

- ***INCLUSION CRITERIA***
    - Critically ill patients within 24 hours of ICU admission.
    - Patients who were expected to survive more than 24 hours after admission.
    - Informed assent was obtained from patient's relatives on the first day of admission.
- ***EXCLUSION CRITERIA***
    - Pregnant females.
    - Patients who were not expected to survive more than 24 hours after admission.

- Patients who received red cell transfusion within 24 hours of sampling.
- Patients who were expected to receive red cell transfusion within 24 hours of admission.
- Patients with known chronic haematological disorders.

STUDY DESIGN

Prospective observational study.

ETHICS

LREC reference number: LREC/2002/1/2.

No interventions were carried out and patient management was not altered in any way on the basis of the data collected and the extra blood tests that had been performed. Guidelines from the data protection act were followed and data were anonymised after completing the study. Patients' identifiers were deleted and ICU key number was used as a primary key in analyzing the data.

RECRUITMENT

All ICU admissions were screened daily during the study period for eligibility within 24 hours of ICU admission.

ORGANIZATION

During the study patients' demographic data were collected and some laboratory investigations were carried out.

PATIENTS' DEMOGRAPHICS

Demographic data were collected from the ICU database (Scottish Intensive Care Society (SICS) Audit Group database). Table (3.22) shows the data that were extracted from SICS database.

LABORATORY INVESTIGATIONS

All blood samples were taken from an indwelling arterial catheter (each blood sample = 2mL). During processing, blood samples were kept at ambient temperature. Table (3.23) shows the different investigations and calculated values that were done.

Table (3.22): Fields of patients' demographic data that were extracted from SICS database.

ICU key number
Forename
Surname
Age
Gender
Date of ICU admission
Date of ICU discharge
APACHE II score
ICU length of stay
ICU outcome

Table (3.23): Laboratory investigations, calculated, and recorded values.

Arterial blood gas	Biochemical	Electrolytes	Haematological	Calculated values	Recorded values
Hydrogen ions*	Urea**	Calcium*	Haemoglobin**	Calculated P50	FIO2
PCO2*	Creatinine**	Potassium*	RBC 2,3-DPG	PH	Body temperature
PO2*	Blood glucose level*	Sodium*			
Bicarbonate*	Serum lactate*	Chloride*			
Base excess*		Phosphorus**			
Anion gap*					

*Values measured using the blood gas machine. ** Values measured in the hospital laboratory. The methodology of measuring RBC 2,3-DPG will be discussed later.

EQUIPMENT

All blood samples for the study and control group, and reproducibility tests were treated in the same manner. Blood samples were collected in to a blood gas monovette (Sarstedt 05.1147.020 monovette 2ml LH).

The blood gas machine used in analysing the blood samples was a Bayer (Chiron) 865 analyser.

TIMING

Patient's demographic data were collected at the time of ICU admission.

Laboratory investigations: a total of up to 5 blood samples were taken from patients during the study:

- Baseline sample

  Immediately after ICU admission and obtaining assent from the patient's relatives, a 2 ml arterial blood sample was taken. The sample was analysed in the blood gas machine. The remaining volume of blood was taken to measure red cell RBC 2,3-DPG.

- Daily samples

  The remaining blood from the routine morning arterial blood gas samples was kept in ice after analysis. Samples were collected to measure RBC 2,3-DPG concentrations. Daily samples were collected up to 4 days after the first baseline blood sample.

Patients were withdrawn from the study in the following circumstances:

- Death.
- Discharge from ICU.
- Red cell transfusion.

- Patient's relative wanted to withdraw the patient from the study.

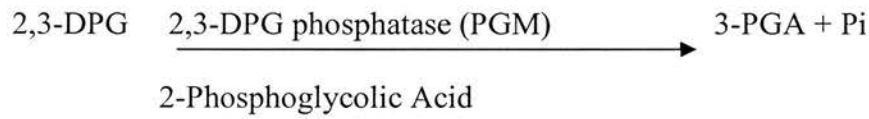
2,3-DIPHOSPHOGLYCERATE (RBC 2,3-DPG) ASSAY

KIT: Sigma Diagnostics RBC 2,3-DPG reagents, quantitative enzymatic determination of 2,3-diphosphoglyceric acid in blood at 340 nm (procedure No. 35-UV).

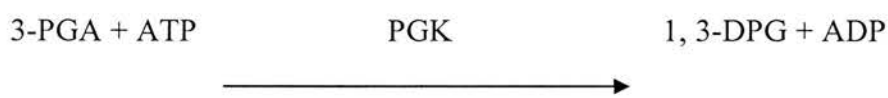
PRINCIPLE:

Three enzymatic reactions involved in the procedure are as follows:

1- RBC 2,3-DPG is hydrolysed to 3-PGA and inorganic phosphorus (Pi). The enzyme that catalyses this reaction is present in purified preparations of PGM and is termed 2,3-DPG phosphatase. 2-Phosphoglycolic acid is needed as a stimulator for this reaction.



2- 3-PGA reacts with ATP in the presence of PGK to form 1, 3-diphosphoglycerate (1, 3-DPG) and ADP.



3- 1,3-DPG oxidized NADH to NAD in the presence of GAPD and is reduced to G-3-P

Measuring the decrease in absorbance at 340 nm caused by the oxidation of NADH to NAD reflects the amount of RBC 2,3-DPG originally present.

Preparation and processing

1-Preparation of reagents.

Add 5mL Triethanolamine buffer to the ATP (This can be stored frozen).

Add 5mL H_20 to the phosphoglycolic acid (Store frozen).

Add 8.0 mL triethanolamine buffer to NADH vial. Cap and invert several times to NADH. (One vial will do 3 tests and can be stored in the fridge for up to 1 week.)

2-Preparation of supernatant (deproteinisation).

Add 3.0 mL ice-cold 8% TCA + 1.0 mL blood.

Shake vigorously for 10 seconds and keep cold for *5* min.

Centrifuge 3000 rpm for 10 mm at 4^0C.

This supernatant is stable for at least 1month when stored frozen.

3-From frozen.

a) To a cuvette add:

2.5 mL solution from vial

0.1 mL ATP solution

0.25 mL supernatant

Then mix by inversion.

b) Then add: 20µL GAPD/PGK

20µL phosphoglycerate mutase

Mix by inversion.

Wait *5* min.

Any delay beyond 5 min will cause an underestimation of DPG content.

c) Then read against H_20 at 340nm = Initial.

d) Then add 0.1 mL phosphoglyceric acid.

Mix by inversion.

Leave 30 min at room temperature (or 15 min at 37^0C) to allow the reaction to go to completion.

Read against H_20 at 340 nm = Final

4-Calculate

[(Initial – Final) – 0.03] x 7.7 2,3-DPG μmol/mL

Multiply by 100 to give μmol/dL.

Divide by Hb g/dL to give RBC 2,3-DPG μmol/gHb.

Samples were kept frozen after step (2) and were analysed in batches within one month of preparation.

CALCULATION OF IN VIVO P50

The *in vitro* method used by commercial blood gas analysers uses the Hill equation to calculate P50 from the PaO_2 and venous haemoglobin oxygen saturation alone, and does not include actual values for RBC 2,3-DPG, $PaCO_2$, pH, or temperature. It is therefore subject to assumptions that are not met by many sick patients. Direct *in vivo* assessment of P50 uses tonometry was not feasible. We used the only published method derived specifically to estimate *in vivo* P50 from directly measured values for pH, RBC 2,3-DPG, PCO_2, and body temperature. We considered this the best method of estimating the true position of the haemoglobin oxygen dissociation curve. P50 values for arterial blood were calculated from the equation described in the study by Samaja M et al. (Equations and nomogram for the relationship of human blood P50 to 2,3-Diphosphoglycerate, CO2, and H+) (Samaja, Mosca, Luzzana, Rossi-Bernardi, Winslow 1981).

The input variables for this calculation are arterial pH, PCO2, body temperature, RBC 2,3-DPG levels, and carboxy haemoglobin level. Microsoft excel program was used to calculate P50 values. The detail of this calculation was described previously.

REFERENCE GROUP

We aimed to construct a control group that matched the study group in number, age, and sex. All subjects were healthy volunteers who worked for the Scottish National Blood Transfusion Service. 2mL blood was taken from each volunteer for measuring RBC 2,3-DPG. Hb concentration was measured for every volunteer as a part of measuring RBC 2,3-DPG.

REPRODUCIBILITY

Number of patients: 10 critically ill patients.

3 mL blood sample was taken from each patient. Each sample was analysed for measuring 2,3-DPG concentration three times. Hb concentration was measured in each sample as part of measuring 2,3-DPG. Reproducibility was explored by calculating the within patient standard deviation using analysis of variance (ANOVA) from the analysed samples. This calculation allowed us measurement error between the measured RBC 2,3-DPG and the actual RBC 2,3-DPG in the blood samples. This difference could happen due to errors in pipetting and reading the results.

FINAL SPREADSHEET

Data were collected in an excel file. After completing the study, patients' identifiers (surname, forename, and date of birth) were deleted. ICU key numbers were used to identify the patients. Table (3.24) shows the fields of the final dataset that was used for analysis.

DATA ANALYSIS

Data were analysed in two steps:

FIRST STEP

Examination of all patients' data on day one of the study was carried out.

Regression analysis for day 1 DPG data

We further explored the relationship between the measured physiological variables on day one in the ICU and the red cell RBC 2,3-DPG concentration using regression analysis. We included variables that had correlations with RBC 2,3-DPG on univariate analysis using correlation coefficients, namely pH, creatinine, K^+, calcium, and chloride. We did not include derived acid-base variables. We also entered Hb and Pco_2 in to the model as this can influence RBC 2,3-DPG concentrations in other settings.

SECOND STEP

Examination of various factors that affected RBC 2,3-DPG and P50 for patients who completed ≥ 3 days in the study. For this group of patients, all available values for days 1, 2, 3, 4, and 5 were averaged. The relationships between the average RBC 2,3-DPG and other measured and derived variables were explored. These relationships allowed us to examine the relationships over a longer period of critical illness.

Table (3.24): Fields of the study spreadsheet.

ICU key number
Age
Gender
APACHEII score
ICU length of stay
ICU outcome
H+ (Hydrogen ions)
PCO2
PO2
HCO3-std (bicarbonate level)
BE (base excess)
sO2 (oxygen saturation)
FIO2
Na
K
Ca
Cl
Ph
An Gap (anion gap)
Glucose
Haemoglobin concentration (Hb)
Lactate
Serum urea
Serum Creatinine
Body temperature
RBC 2,3-DPG
Calculated in vivo P50
PH

RESULTS

STUDY GROUP

The intensive care unit was screened daily from 22/01/2002 until 14/07/2002. A total of 313 critically ill patients were admitted to ICU during the study period, of these 111 matched the inclusion criteria and were included in the study.

64 (58%) patients were males and 47 (42%) were females. Patients' demographic data are shown in table (3.25).

Table (3.25): Patients' demographic data

Variable	Mean	SD	Median	Range	Q1	Q3
Age (years)	52.3	17.1	53	14-86	41	65
APACHE II	17.5	8.7	17	0-37	12.5	23
ICU stay (days)	6.3	9.4	3.3	0.2-52.4	1.4	7.1
Days in Hospital after ICU (days)	11.8	17.6	5	0-101	0	16

REFERENCE GROUP

There was a technical problem in the company producing the commercial kit for RBC 2,3-DPG measurement (Sigma No. 35-UV), which occurred midway through the study. It was not possible to obtain enough kits to construct the aimed reference group to match the study group in age, sex, and number, because the company stopped manufacture.

We were able to include 34 patients in the reference group. 18 (53%) were males and 16 (47%) were females. The mean ± SD of their age was 48 ± 14 years. It was not feasible to calculate the in vivo P50 from the reference group because arterial blood samples were not collected from this group.

REPRODUCIBILITY

A total of 10 blood samples were collected from 10 critically ill patients. Each blood sample was analysed 3 times for RBC 2,3-DPG concentration. Table (3.26) shows the results of this analysis.

Table (3.26): Absolute values for RBC 2,3-DPG (μmol/gHb) in blood samples used for measuring reproducibility.

Sample	First analysis	Second analysis	Third analysis
1	12.94	13.84	15.51
2	9.82	11.74	10.59
3	13.26	12.92	12.75
4	5.82	5.82	5.82
5	17.27	17.59	17.59
6	10.09	9.74	9.03
7	10.53	10.61	10.46
8	14.63	12.80	13.96
9	11.83	11.37	11.37
10	5.94	5.50	5.72

Reproducibility: within patient standard deviation was 0.63 μmol/gHb.

FIRST DAY ANALYSIS:

Mean ± SD, median, inter-quartile range, minimum, and maximum values for all the investigations that were carried out are shown in table (3.27).

RBC 2,3-DPG RESULTS

The mean ± SD of 2,3-DPG for male patients was 14.9 ± 6.2 μmol/g Hb and 13.0 ± 6.4 μmol/g Hb for females (mean difference = 1.97, 95%CI of the mean difference =

4.39 to – 0.44%, t = 1.63, and p = 0.11). Table (3.28) shows the mean 2,3-DPG values for the study group and reference group. Figures (3.3 & 3.4) show the distribution of RBC 2,3-DPG levels for females and males in the study and reference groups.

Table (3.28): Mean ± SD of RBC 2,3-DPG for the study and reference group (μmol/g Hb).

	Males	Females
Study group (N = 111)	14.9 ± 6.2	13.0 ± 6.4
Reference group (N = 34)	15.3 ± 3.6	17.8 ± 3.5

All patients (males and females) in the study group: 14.1 ± 6.3 μmol/gHb
All subjects (males and females) in the reference group: 16.7 ± 3.7 μmol/gHb.

A comparison between first day RBC 2,3-DPG for the study group and the reference group, males, females and all patients [mean difference, 95% CI, t test, and p values], are shown in table (3.29). Figure (3.5) shows the distribution of first day RBC 2,3-DPG values for all patients in the study group and RBC 2,3-DPG values for the reference group.

Table (3.29): Comparison between RBC 2,3-DPG in the study group and the reference group [first day RBC 2,3-DPG values (μmol/g Hb)].

Category	Mean difference	95% CI of the mean difference	t test	p value
Males	0.68	2.94 to –1.58	0.60	0.55
Females	4.87	7.50 to 2.24	3.72	0.001
All patients	2.56	4.29 to 0.83	2.94	0.004

There was no significant difference between mean RBC 2,3-DPG in males in the study group and the reference group. The mean RBC 2,3-DPG level in females in the study group was lower compared to females in the reference group. For all patients

(males and females), the mean RBC 2,3-DPG level was significantly lower in the study group compared to the reference group.

Correlations

Correlations between RBC 2,3-DPG and primary and derived measures were explored.

Primary measures

Highly significant correlation was found between RBC2,3-DPG and arterial pH (r^2 =0.47, p<0.0001), but not with $Paco_2$ (r^2 =-0.07, p=0.45). Weaker correlations were found with creatinine (r^2 =-0.35, p<0.0001), potassium (r^2 =-0.20, p=0.032), phosphate (r^2 =-0.20, p=0.046), chloride (r^2 =-0.19, p=0.044), glucose (r^2 =0.19, p=0.044) and urea (r^2 =-0.18, p=0.073). There were no significant correlations between 2,3-DPG and haemoglobin concentration, blood lactate, oxygenation indices, other blood electrolyte concentrations, age or temperature.

Derived measures

Highly significant correlations were found between RBC 2,3-DPG and standard bicarbonate (r^2 =0.46, p<0.0001), base excess (r^2 =0.44, p<0.0001), and P50 (r^2 =0.80, p<0.0001). There was a weak correlation between RBC 2,3-DPG and anion gap (r^2 =-0.29, p=0.002).

Table (3.30) shows r^2 and P values [uncorrected for multiple tests] for all recorded variables in relation to first day RBC 2,3-DPG. Figures (3.6, 3.7, 3.8, 3.9, 3.10) show the relationships between 2,3-DPG and some of these variables.

Regression analysis

Using regression analysis, our final model included only pH and Cl concentration. The B coefficients were not significant for creatinine, calcium, Hb, phosphate, or K^+ after adding pH to the model. The final B coefficients were:

PH: B 32.15 (95% CI 19.07 to 46.22), $P < 0.001$

Cl: B –0.196 (95% CI: -0.39 to –0.01), $P = 0.044$

R^2 for the model was 0.293

These data suggest that pH had the strongest relationship with red cell DPG with a weak association with plasma CL. The model explained about 29% of the variation in RBC 2.3-DPG concentration observed.

FIRST DAY CALCULATED IN VIVO P50 RESULTS

The mean ± SD (range) of first day calculated in vivo P50 for all patients who were included in the study was 28.6 ± 4.5 (15.2 – 41.8) mmHg. The mean ± SD first day P50 for males was 29.2 ± 4.6 mmHg and for females was 27.9 ± 4.3 mmHg. There was no significant difference in mean calculated in vivo p50 between males and females. Table (3.31) shows the mean difference between males and females, 95%CI of the mean difference, t test, and p values.

Table (3.31): Comparison between P50 values in males (N = 64) and females (N = 47).

Mean difference	95% CI of the mean difference	t test	p value
1.26	2.97 to –0.45	1.46	0.15

First day P50 values (mmHg)

Figure (3.11) shows the distribution of first day calculated in vivo P50 values for males and females in the study group. Figure (3.12) shows the distribution of calculated in vivo P50 values for all patients in the study.

Correlations between measured variables and in vivo p50

The correlation coefficients between p50 and factors known to influence it were calculated, namely pH, Pco_2, temperature, and RBC 2,3-DPG. Table (3.32) shows r^2 and p values for these correlations.

Table (3.27): Results for variables measured on day 1 of ICU stay (N = 111 patients).

Variables	Mean	SD	Median	Q1	Q3	Range
PH	7.35	0.11	7.37	7.29	7.41	6.99-7.56
$Paco_2$ (kPa)	5.7	1.4	5.6	4.8	6.2	3.6-10.1
Pao_2 (kPa)	16.8	6.6	15.0	12.6	19.2	7.2-50.5
HCO_3 (mmol/L)	22.7	5.1	22.8	19.5	25.3	9.0-37.4
BE (mmol/L)	-2.5	6.5	-2.0	-6.8	1.3	-21.6-15.2
SO_2 (%)	98.0	2.3	98.6	97.8	99.1	86.7-99.8
Na (mmol/L)	136.6	6.3	136.9	134.3	140.2	114.2-154.3
K (mmol/L)	4.1	0.6	4.0	3.7	4.4	2.9-6.1
Ca(mmol/L)	1.1	0.1	1.1	1.0	1.1	0.8-1.7
CL (mmol/L)	104.1	5.8	104.0	101.0	108.0	87.0-118.0
Anion gap (mmol/L)	13.4	5.1	13.2	10.4	16.3	4.7-36.5
Glucose (mmol/L)	8.0	3.0	7.6	6.3	9.1	2.8-22.9

Variables	Mean	SD	Median	Q1	Q3	Range
Lactate (mmol/L)	2.7	2.7	1.8	1.3	2.9	0.6-19.4
Temperature (°C)	36.7	1.0	36.7	36.2	37.2	33.7-39.8
FIO2 (%)	0.5	0.2	0.4	0.4	0.6	0.21-1.0
Hb (g/L)	103.3	22.3	100.0	87.5	117.0	62.0-167.0
Urea (mmol/L)	10.4	8.0	7.7	4.4	13.0	1.5-40.0
Creatinine (µmo/L)	142.5	99.6	99.0	77.0	179.0	47.0-663.0
Phosphate (mmol/L)	1.1	0.6	1.0	0.7	1.3	0.2-3.1
RBC 2,3-DPG (µmol/gHb)	14.1	6.3	13.2	9.9	8.0	3.2-32.5
P50 (mmHg)	28.6	4.5	27.2	24.7	30.2	15.5-41.8

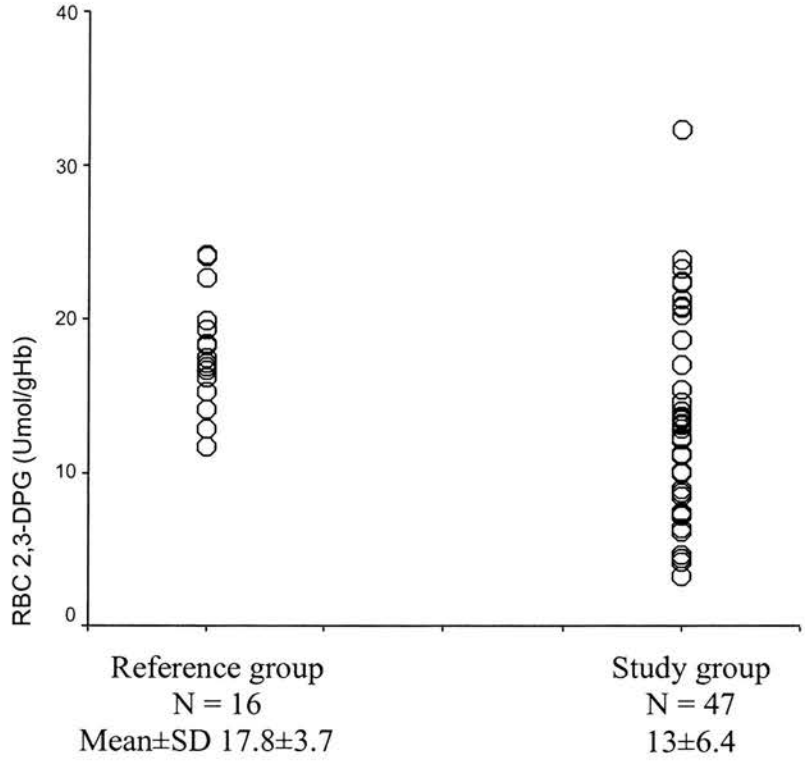


Figure (3.3) the distribution of first day RBC 2,3DPG for females in the study and reference groups.

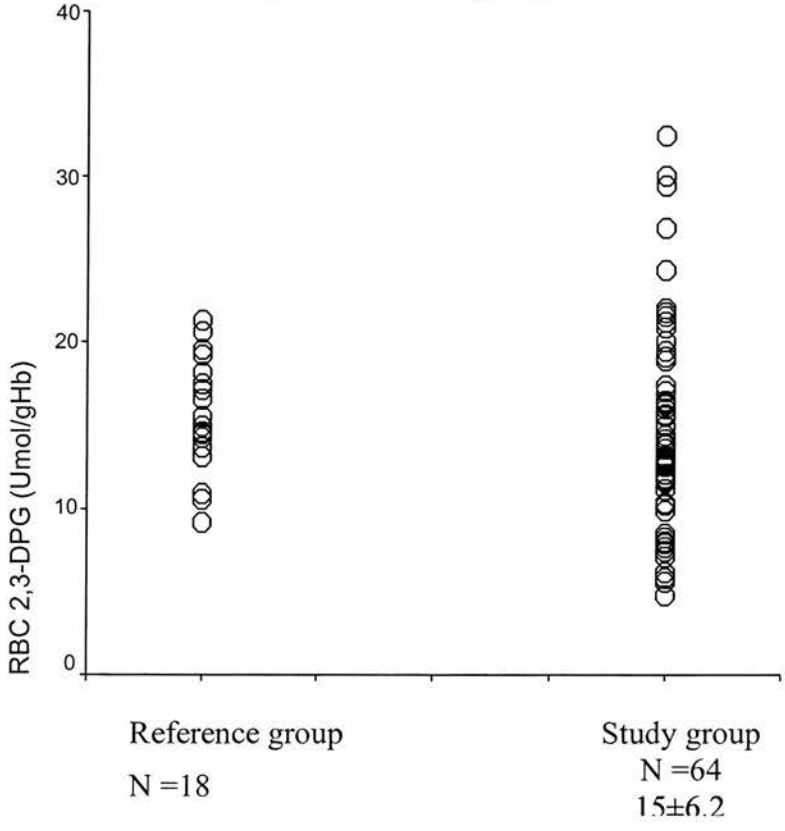


Figure (3.4) the distribution of first day RBC 2,3DPG for males in the study and reference groups.

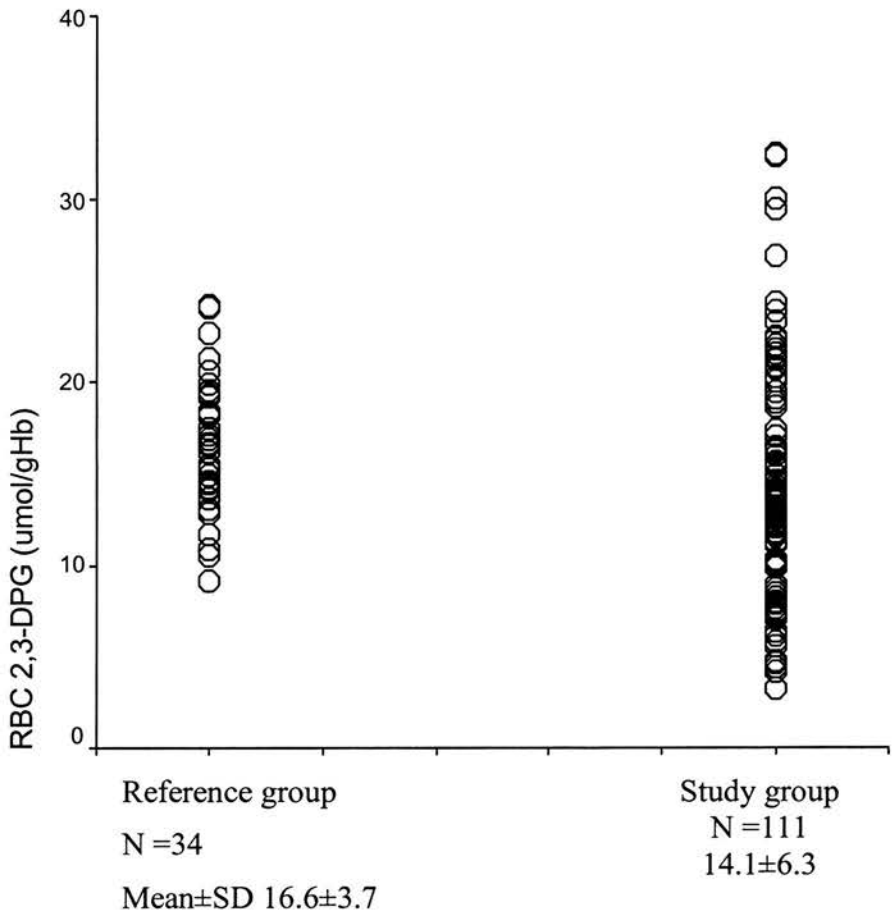


Figure (3.5) the distribution of first day RBC 2,3DPG for all patients in the study and reference groups.

Table (3.30): Correlations coefficient for the different values recorded in the study in relation to first day 2,3-DPG level (N = 111).

Variables	**r^2 value**	**P value**
Primary measures		
Age	0.05	0.599
PH	0.47	<0.0001
Pco_2	-0.07	0.454
Po_2	-0.01	0.965
SO_2	0.09	0.338
Na	-0.04	0.660
K	-0.20	0.032
Ca	0.02	0.813
CL	-0.19	0.044

Variables	r^2 values	P value
Glucose	0.19	0.044
Lactate	-0.14	0.140
Temperature	-0.06	0.561
FIO_2	-0.06	0.539
Hb	-0.14	0.157
Urea	-0.18	0.073
Creatinine	-0.35	<0.0001
Phosphate	-0.20	0.046
Derived measures		
P50	0.80	<0.0001
Anion gap	-0.29	0.002
Hco_3	0.46	<0.0001
BE	0.44	<0.0001

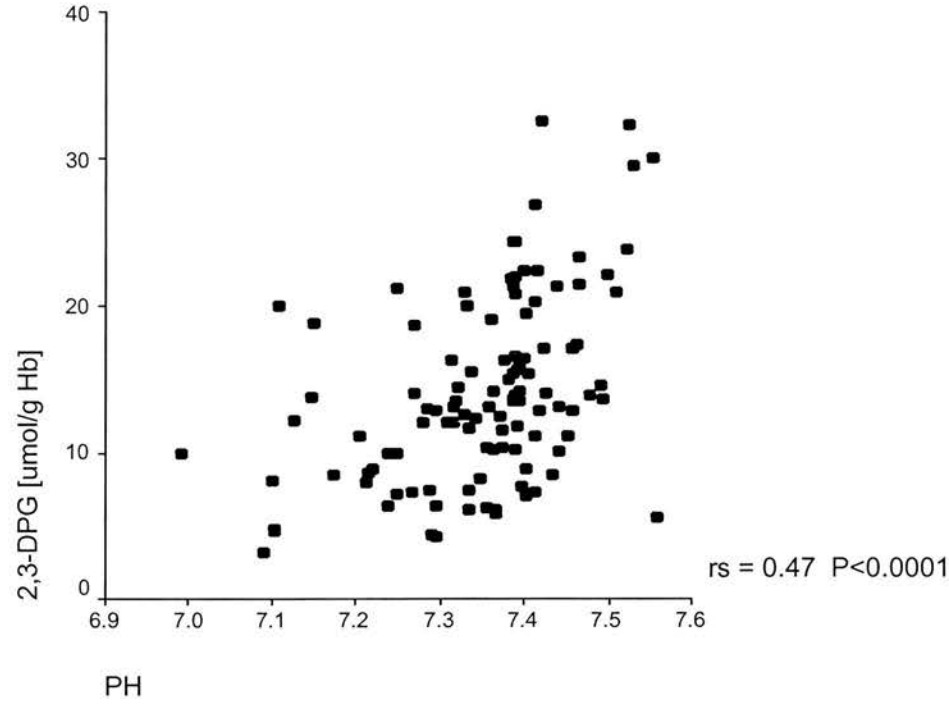


Figure (3.6): The correlation between first day RBC 2,3-DPG and pH

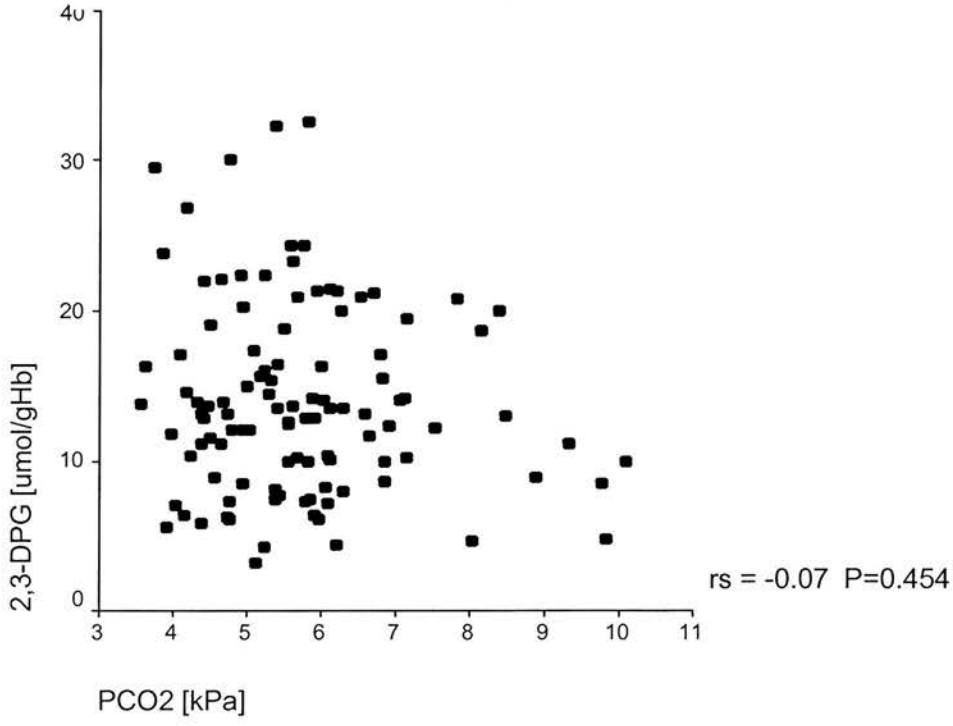


Figure (3.7): The correlation between first day RBC 2,3-DPG and Pco_2

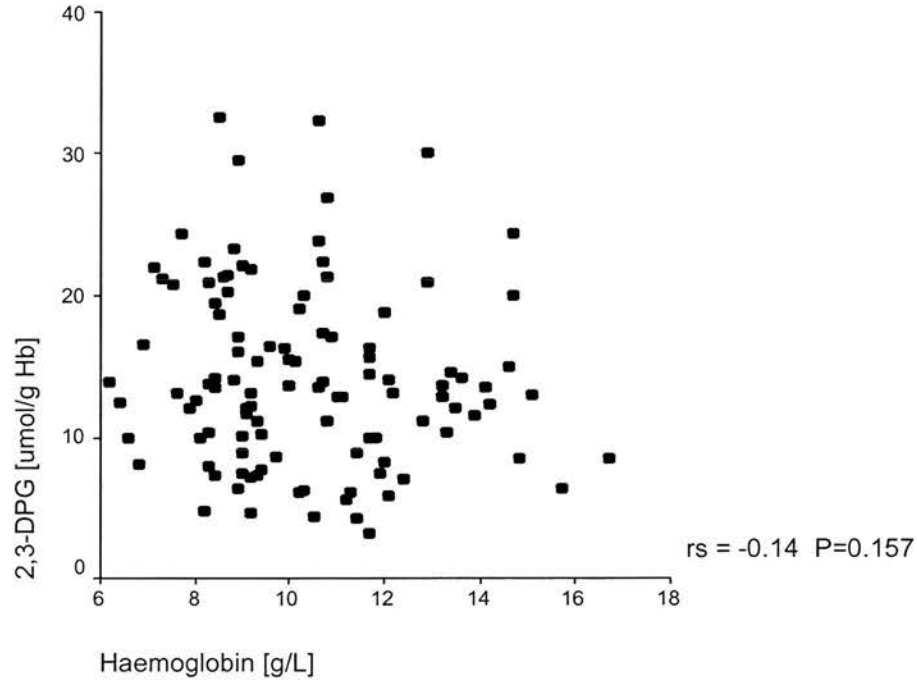


Figure (3.8): The correlation between first day RBC 2,3-DPG and haemoglobin

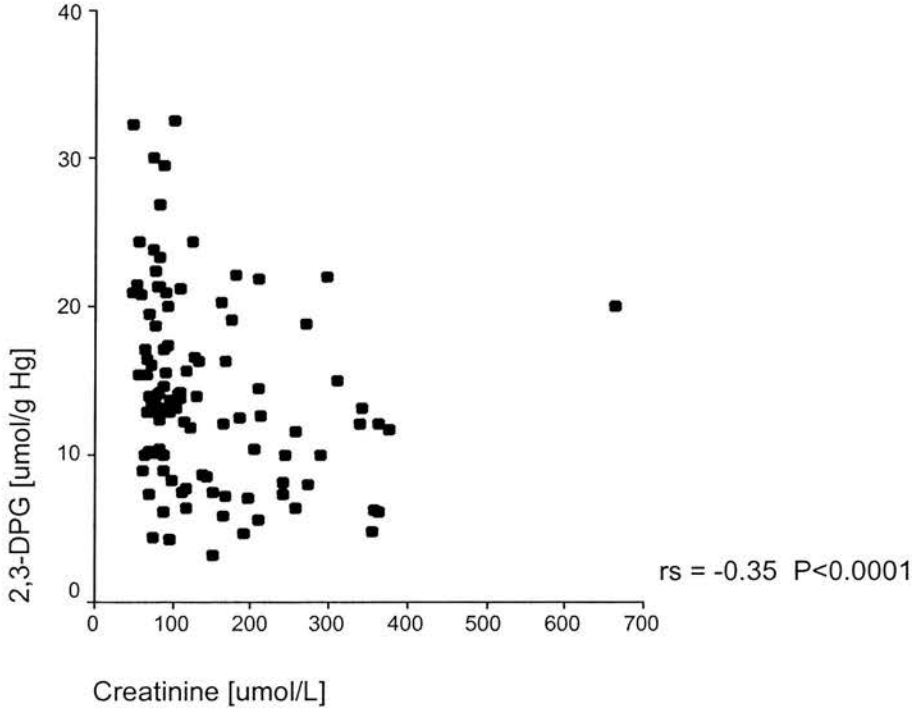


Figure (3.9): The correlation between first day RBC 2,3-DPG and creatinine

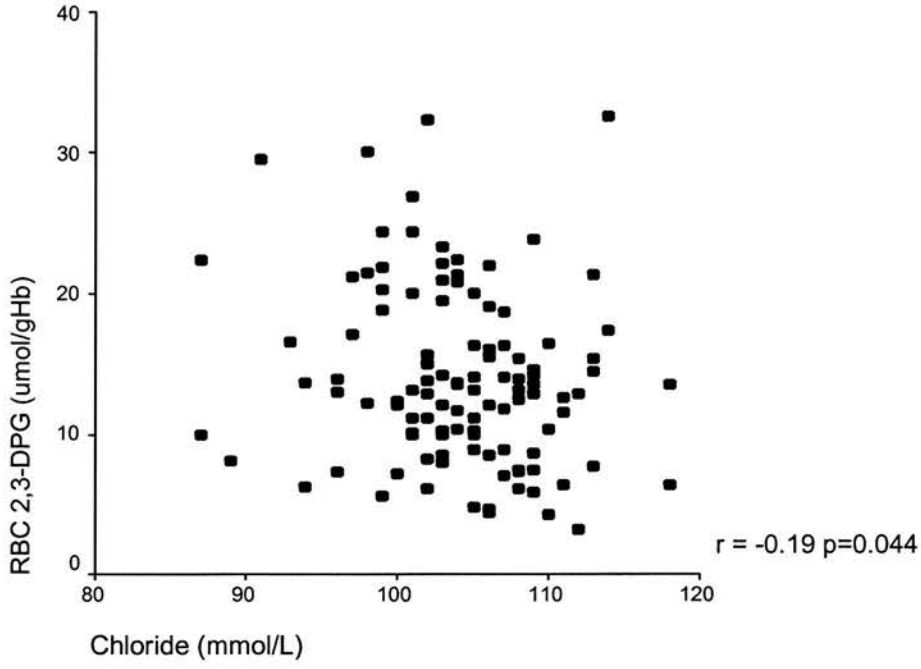


Figure (3.10): The correlation between first day RBC 2,3-DPG and chloride

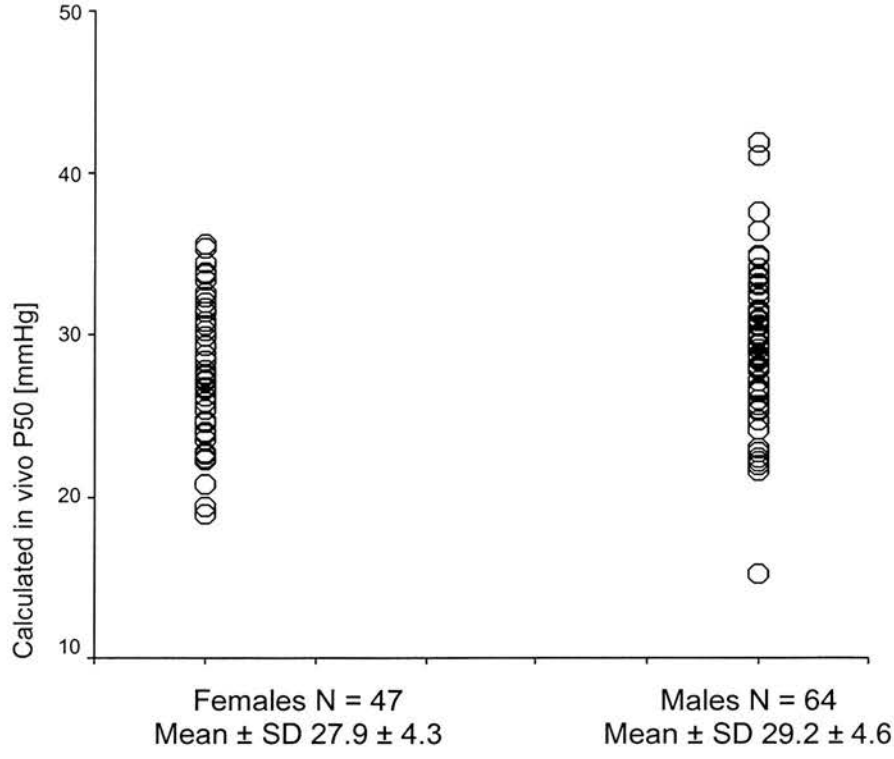


Figure (3.11): The distribution of calculated in vivo P50 values for both male and female patients.

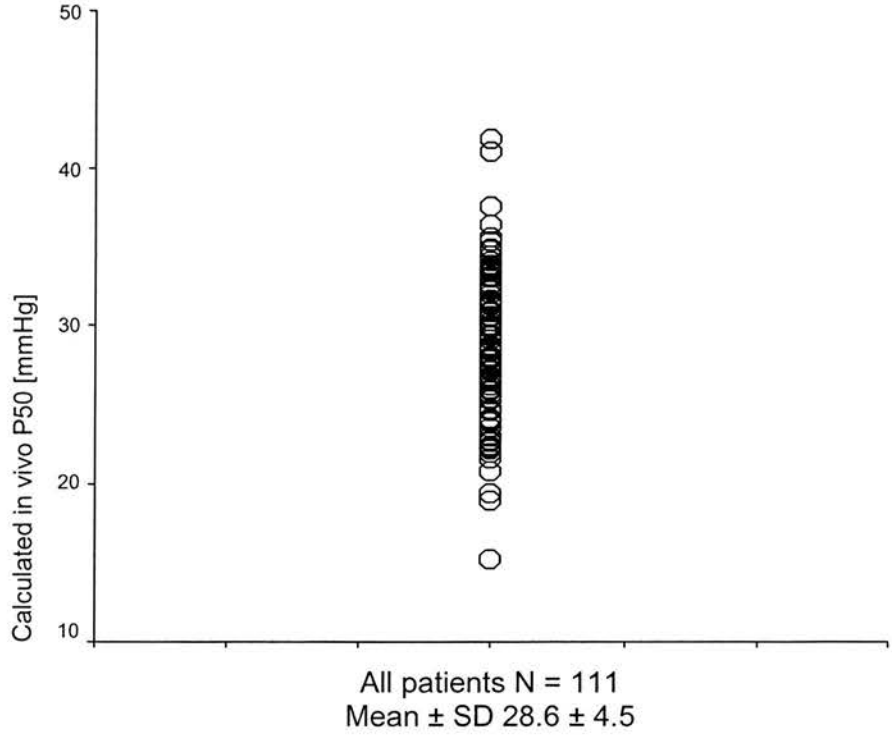


Figure (3.12): The distribution of calculated in vivo P50 values for all patients included in the study.

Table (3.32): Correlations coefficient for the different measurements of first day that are known to affect P50 (number of patients = 111).

Variables	**r^2 value**	**P value**
pH	-0.01	0.915
Pco_2	0.21	0.029
Temperature	0.08	0.428
RBC 2,3-DPG	0.80	<0.0001

PATIENTS WHO STAYED ≥ 3 DAYS

55 patients completed 3 days or more in the study. 33 (60%) were males and 22 (40%) were females. Patients' characteristics and demographic data are shown in table (3.33).

Table: (3.33) Patients' demographic data (N = 55)

Variable	Mean	SD	Median	Range	Q1	Q3
Age (years)	51.6	17.6	52	14-86	39	65
APACHE II	16.8	8.5	18	0-33	12.5	22
ICU stay (days)	10.5	11.7	6.7	3.0-52.4	4.5	11.5
Days in Hospital after ICU (days)	13	21	4	0-101	0	16

RBC 2,3-DPG ANALYSES

RBC 2,3-DPG levels for patients who completed ≥ 3 days were averaged. The mean ± SD of the average RBC 2,3-DPG for males and females was 16.7 ± 6.0 and 14.6 ± 5.8 μmol/gHb respectively and for all patients was 15.8 ± 6.0 μmol/gHb. There was no significant difference between the mean RBC 2,3-DPG level in male and female patients in the study group [the mean difference was 2.09 μmol/gHb, 95% CI of the mean difference = 1.2 to – 5.4, t test = 1.29, and p value = 0.205]. There was no significant difference between the mean RBC 2,3-DPG in both the reference and the study groups [the mean difference was 0.84 μmol/gHb, 95% CI of the mean difference = 2.9 to – 1.2, t test = .83, and p value = 0.41].

Correlations

Correlations between RBC 2,3-DPG and primary and derived measures were explored for patients who completed ≥ 3 days (N = 55).

Primary measures

Highly significant correlation was found between RBC2,3-DPG and arterial pH (r^2 =0.43, p = 0.001). Weaker correlations were found with creatinine (r^2 = -0.25, p = 0.075), calcium (r^2 =-0.26, p=0.055), and $Paco_2$ (r^2 = -0.23, p=0.093). There were no significant correlations between 2,3-DPG and haemoglobin concentration, blood lactate, oxygenation indices, other blood electrolyte concentrations, or temperature.

Derived measures

Highly significant correlations were found between RBC 2,3-DPG and P50 (r^2 =0.67, p<0.0001). There were weaker correlations between RBC 2,3-DPG and standard bicarbonate (r^2 =0.23, p = 0.099), and base excess (r^2 =0.24, p = 0.074).

Table (3.34) shows r^2 and P values [uncorrected for multiple tests] for all recorded variables in relation to first day RBC 2,3-DPG. Figures (3.13, 3.14, 3.15, 3.16, and 3.17) show the relationships between 2,3-DPG and some of these variables.

CALCULATED IN VIVO P50 RESULTS

The mean ± SD of calculated in vivo P50 for all patients who completed ≥ 3 days was 29.2 ± 3.8 mmHg. The mean ± SD for males was 29.6 ± 3.8 mmHg and for females was 28.7 ± 3.9 mmHg. There was no significant difference in mean calculated in vivo p50 between males and females.

Correlations between measured variables and in vivo p50

The correlation coefficients between calculated in vivo p50 and factors known to influence it were calculated, namely pH, Pco_2, temperature, and RBC 2,3-DPG. Table (3.35) shows r^2 and p values for these correlations.

Table (3.35) Correlations coefficients for mean measurements that are known to affect P50 in relation to mean P50 values for patients who completed ≥ 3 days (N = 55).

Variables	**r^2 value**	**P value**
pH	-0.15	0.292
Pco_2	-0.02	0.879
Temperature	0.25	0.075
RBC 2,3-DPG	0.67	<0.0001

Table (3.34) Correlations coefficients for mean values recorded in relation to mean RBC 2,3-DPG levels for patients who completed ≥ 3 days in the study (N = 55).

Variables	**r^2 value**	**P value**
Primary measures		
Age	0.24	0.084
PH	0.43	0.001
$Paco_2$	-0.23	0.093
Pao_2	-0.12	0.400
So_2	0.11	0.421
Na	-0.09	0.526
K	-0.14	0.311
Ca	-0.26	0.055
CL	-0.15	0.265
Glucose	0.12	0.404

Variables	r^2 values	P values
Lactate	-0.04	0.792
Temperature	0.07	0.636
FIO2	-0.02	0.875
Hb	-0.18	0.198
Urea	0.00	0.986
Creatinine	-0.25	0.075
Phosphate	0.03	0.830
Derived measures		
P50	0.67	<0.0001
Anion gap	-0.22	0.114
HCO3	0.23	0.099
BE	0.24	0.074

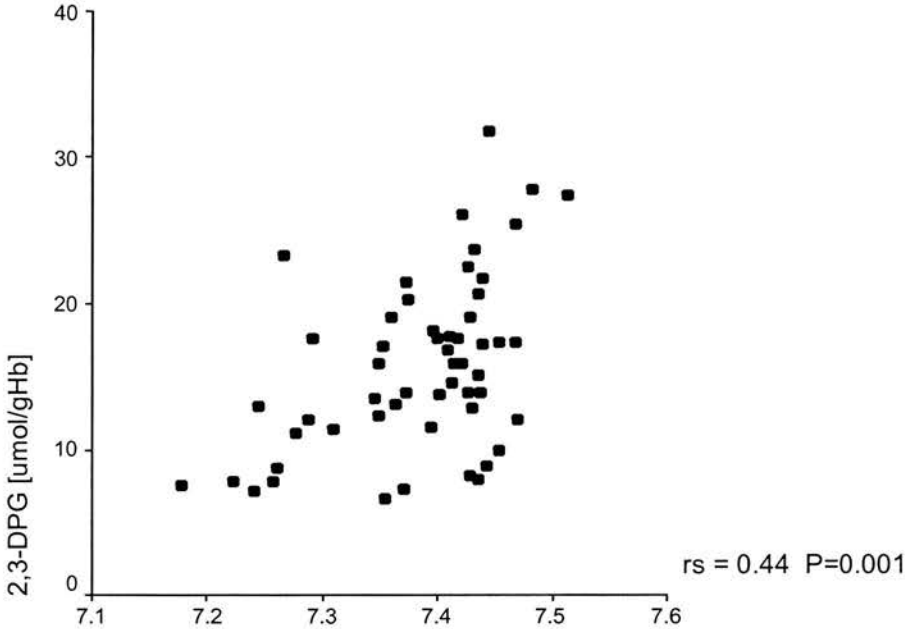


Figure (3.13): The correlation between mean RBC 2,3-DPG and mean pH for patients completed ≥ 3 days in the study

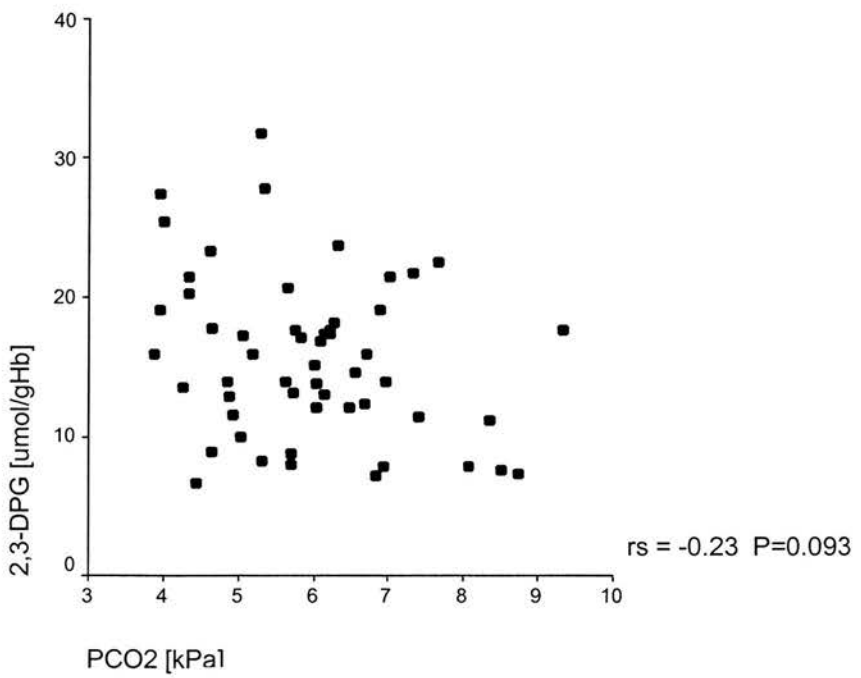


Figure (3.14): The correlation between mean RBC 2,3-DPG and mean Pco_2 for patients completed ≥ 3 days in the study

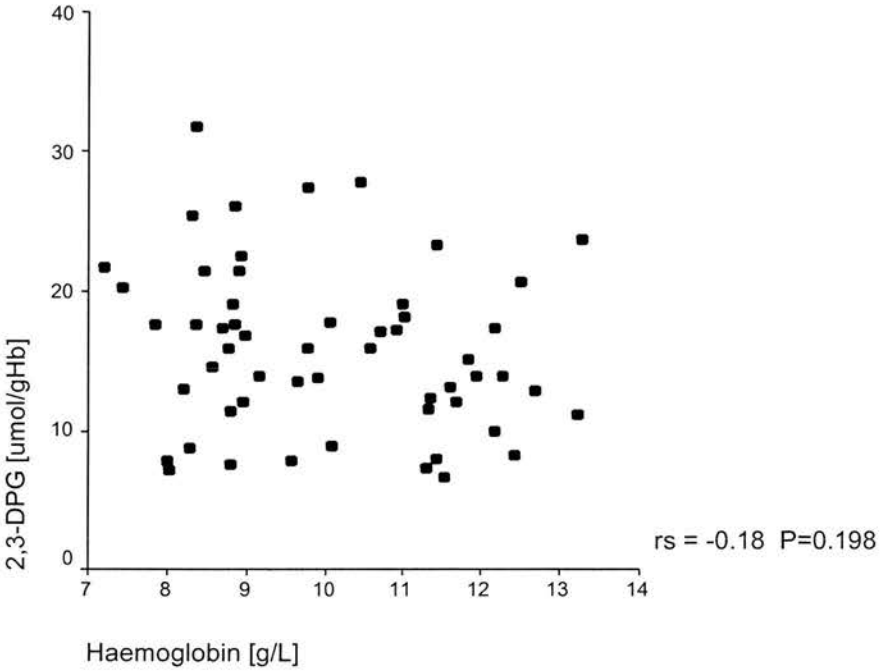


Figure (3.15): The correlation between mean RBC 2,3-DPG and mean haemoglobin for patients completed ≥ 3 days in the study

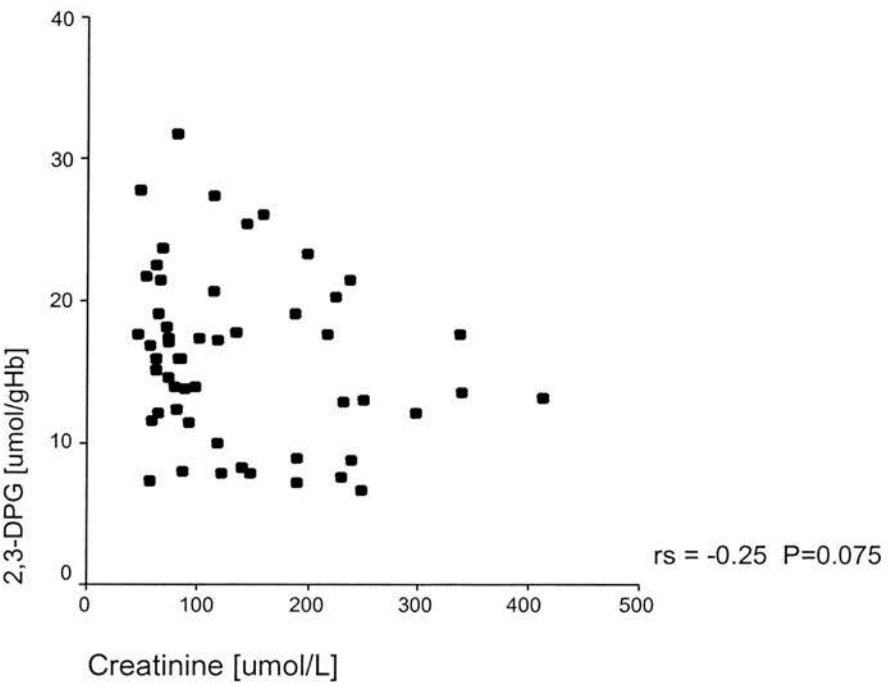


Figure (3.16): The correlation between mean RBC 2,3-DPG and mean creatinine for patients completed ≥ 3 days in the study

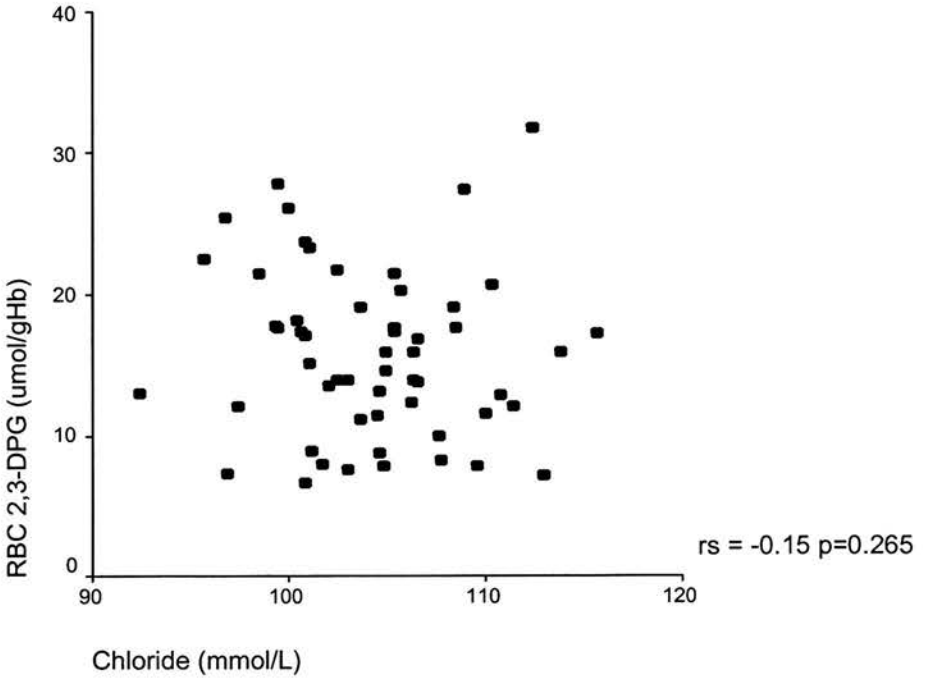


Figure (3.17): The correlation between mean RBC 2,3-DPG and mean chloride for patients completed ≥ 3 days in the study

DISCUSSION

SUMMARY OF THE RESULTS

111 critically ill patients were included in the study. 58% were males and 42% were females. Mean age was 52 ± 17 years and mean APACHE II score was 17.5 ± 8.7. Mean first day RBC 2,3-DPG value as 14.9 ± 6.2 μmol/gHb for males and 13.0 ± 6.4 μmol/gHb for females. There was no statistically significant difference between the mean first day RBC 2,3-DPG in male patients compared to males in the reference group. Females in the study group had a significantly lower first day RBC 2,3-DPG level than females in the reference group. There was a much wider variation in RBC 2,3-DPG values in critically ill patients compared with reference healthy subjects, which was present in males and females.

Highly significant correlations were found between first day RBC 2,3-DPG and arterial pH. Strong correlations were, not surprisingly, also found with derived acid base variables, but not with $Paco_2$. Weaker correlations were found with creatinine, potassium, phosphate, chloride, glucose, and urea. There were no significant correlations between 2,3-DPG and Hb, blood lactate, oxygenation indices, other blood electrolyte concentrations, age or temperature.

The mean ± SD first day calculated in vivo P50 for all patients was 28.6 ± 4.5 mmHg. There was no significant difference between mean first day calculated in vivo P50 values between male and female patients. P50 correlated strongly with RBC 2,3-DPG, but not Pco_2, pH or temperature.

55 critically ill patients completed 3 days or more in the study. The mean age for this group of patients was 51 ± 17 years and the mean APACHE II score was 16.8 ± 8.5.

The mean ± SD of the 3-5 day average RBC 2,3-DPG for males was 16.7 ± 6.0 and 14.6 ± 5.8 μmol/gHb for females. There was no significant difference in mean RBC 2,3-DPG between male and female patients.

There were a highly significant correlation between RBC2,3-DPG and arterial pH, and P50. Weaker correlations were found with creatinine, calcium, standard bicarbonate, base excess, and $Paco_2$. There were no significant correlations between 2,3-DPG and haemoglobin concentration, blood lactate, oxygenation indices, other blood electrolyte concentrations, or temperature.

The mean ± SD of the 3-5 day average calculated in vivo P50 for males was 29.6 ± 3.8 and 28.7 ± 3.9 mmHg for females. There was no significant difference between males and females.

Using multiple regression analysis, our data suggest that pH had the strongest relationship with red cell RBC 2,3-DPG with a weak association with plasma CL. The model explained about 29% of the variation in DPG concentration observed.

WEAKNESSES AND STRENGTHS IN THE STUDY

The study took place in a large medical-surgical teaching hospital ICU. The study group included a wide case mix, with typical ICU illness severity, and included males and females, so selection bias was unlikely.

Transfused patients were excluded from the study, so the effect of red cell transfusion on RBC 2,3-DPG did not affect the results. A wide range of physiological variables were included in the study to allow us to explore the effect of these factors on RBC 2,3-DPG. It is possible that a different relationship would have been found if concentrations within red cells were measured. However, we believed

that, for factors such as pH and plasma abnormalities probably reflected acid-base disturbances within red cells. Specific measurements of red cell abnormalities would be needed to confirm this assumption, but were not feasible in this study. Patients were followed up for five days during the study period, if they remained in the ICU. We were therefore able to examine data both from the day of ICU admission and averaged over 3 to 5 days of critical illness.

We constructed a reference group comprising a number of healthy volunteers in whom RBC 2,3-DPG was measured, so results were compared to subjects who represented the healthy population in Scotland. We aimed to construct a reference group with sex and age match with the study group. The company producing the commercial kits for measuring RBC 2,3-DPG stopped producing them during the study, so we were unable to study as many reference subjects as intended. However, the age distribution of the volunteers examined was similar to the patient sample.

We established the reproducibility for RBC 2,3-DPG assays. Within sample standard variation of RBC 2,3-DPG in the measured samples was small, demonstrating low measurement errors. Differences between patients were therefore likely to indicate true physiological variations.

It was unclear the best way to estimate P50 in the critically ill patients. The blood gas machine [Bayer (Chiron) 865 analyser] can provide P50 values from a venous blood sample by calculating it from po_2 and oxygen saturation using an equation based on Hill's equation (discussed in detail on page 148). This method of estimating the in vitro P50 does not include the actual values of RBC 2,3-DPG, pH, and temperature of the patient [all of these values are standardised by the machine].

Siggaard-Andersen et al used an equation to calculate the in vivo P50 that includes all the variables that can affect in vivo P50 (pco_2, pH, RBC 2,3-DPG, and body temperature). However, they used a calculated value for RBC 2,3-DPG not the actual measured RBC 2,3-DPG value in the patient's blood (Siggaard-Andersen, Siggaard-Andersen 1990). We used the only available method of estimating in vivo P50 from directly measured values for pH, RBC 2,3-DPG, Pco_2, and body temperature.

The equation by Samaja et al. used in the study (Samaja, Mosca, Luzzana, Rossi-Bernardi, Winslow 1981) is based on a calculation that includes physiological factors (pco_2, pH, RBC 2,3-DPG, and body temperature) that are known to affect the in vivo P50 value. These factors can vary widely during the course of critical illness. However, it is likely that the correlation between 2,3 DPG and the calculated P50 are due to mathematical linkage as both appear in the Samaja equation. Mathematical linkage can cause artificial relationships in medical research. Because of this uncertainty, the significance of the strong correlation between RBC 2,3-DPG and P50, but lack of correlations between pH, Pco_2, temperature, and P50 is unclear. Samaja et al validated their formula and found that calculated in vivo P50 is more accurate than the standard in vitro P50 when comparing both to the measured in vivo P50 using tonometry (Samaja, Mosca, Luzzana, Rossi-Bernardi, Winslow 1981).

It was not possible to directly measure P50 in the blood of critically ill patients. Our method therefore represented the best available technique for measuring oxygen-binding characteristics of Hb in a large sample of critically ill patients.

COMPARISON WITH OTHER STUDIES

We have shown that the mean RBC 2,3-DPG level in the 111 critically ill patients [males and females] was on average slightly lower and had larger variability than its level in the normal reference group. Morgan et al showed similar results, in a small study of 20 male critically ill patients (Morgan, Koch, Morris, Clague, Purdie 2001). In our 64 male patients, there was no significant difference between the mean RBC 2,3-DPG compared to the normal reference group. Our literature review found no other studies that measured RBC 2,3-DPG specifically in critically ill ICU patients. We found a strong correlation between RBC 2,3-DPG and blood pH in the critically ill. Acidosis was associated with lower levels of RBC 2,3-DPG and alkalosis was associated with higher RBC 2,3-DPG levels. Morgan et al showed similar results (Morgan, Koch, Morris, Clague, Purdie 2001).

There is some evidence suggesting that pH has a strong effect on RBC 2,3-DPG level. Rose et al., Luque et al, and Jelkman et al. showed that pH can affect the rate of RBC 2,3-DPG production as pH affects the activity of mutase and phosphatase enzymes responsible for the synthesis and the destruction of RBC 2,3-DPG (Rose, Liebowitz 1970; Luque, Pinilla, Ventura, Santos-Ruiz 1972; Jelkmann, Bauer 1978). There is strong evidence that pH affects RBC 2,3-DPG level in vitro. Most of these studies were carried out to show the effect of pH in maintaining RBC 2,3-DPG in stored blood. Hogman et al. and Hess et al. showed that alkaline media maintained RBC 2,3-DPG level in stored blood (Hogman, Knutson, Loof, Payrat 2002; Hess, Hill, Oliver, Lippert, Greenwalt 2002).

We found no correlations between RBC 2,3-DPG and Pco_2 and Po_2. Some studies have found correlation between hypoxia, hypercapnia and increased RBC 2,3-DPG

level. Most of these studies were carried out in hypoxic non-critically ill patients with abnormally high Pco_2 and low Po_2 levels (Oski, Gottlieb, Delivoria-Papadopoulos, Miller 1969; Keitt, Hinkes, Block 1974). We suggest that this correlation was not present in our patients as none of them were left hypoxic in the ICU and all patients were well oxygenated [the mean Pco_2 was 5.7 ± 1.4 kPa and Pao_2 was 16.8 ± 6.6 kPa]. Hypercapnia was present early in a few patients.

In the present study there was no correlation between RBC 2,3-DPG and the acute anaemia that characterises early critical illness (Hb level). In the 20 critically ill patients studied by Morgan et al, there was a negative correlation between Hb level and RBC 2,3-DPG (Morgan, Koch, Morris, Clague, Purdie 2001). However, Hb level was lower in our study, compared to the Morgan et al. study [median (range) Hb g/L = 100 (62 to 167) g/L and 113 (89 to 154) g/L respectively] as we used more evidence based transfusion triggers in our ICU.

Most studies that showed a correlation between acute anaemia and increased RBC 2,3-DPG were carried out in animal models (Cropp, Gee 1972; Fong, Ko, Streczyn, Westerman 1976; Holter, Halvorsen, Refsum 1982). It is unclear if red cell physiology in these animals is similar to human red cells.

Other studies have shown a negative correlation between Hb level and RBC 2,3-DPG in patients with chronic anaemia. Most of these studies were carried out in anaemia associated with malignancy (Haidas, Zannos-Mariolea, Matsaniotis 1976; Hjelm, Uden, Engstedt 1976; Festa, Asakura 1979). In these settings it has been argued that increasing RBC 2,3-DPG concentration in RBCs is a compensatory mechanism that increases oxygen unloading when oxygen content is decreased, therby improving tissue oxygen delivery (Opalinski, Beutler 1971; Hjelm, Uden, Engstedt 1976).

Our data indicate that during early critical illness, and over the first 3 to 5 days, anaemia is not associated with compensatory high or supernormal RBC 2,3-DPG concentrations within red cells. In fact, in these patients with strongest evidence of tissue hypoxia, namely systemic acidosis, RBC 2,3-DPG concentration is decreased. It is unclear from our data whether this is of clinical relevance to tissue oxygen delivery.

The mean calculated in vivo P50 in our study using the Samaja et al equation was 28.6 ± 4.5 mmHg. In a similar study by Morgan et al, the mean calculated in vivo P50 using the modified Siggaard-Andersen equation [the original formula contains a calculated value for RBC 2,3-DPG, but a modification included the actual measured venous value for RBC 2,3-DPG], was similar to our result [28.0 ± 1.3 mmHg] (Morgan, Koch, Morris, Clague, Purdie 2001).

In the present study and Morgan et al study, P50 values of the study group were not compared to normal subjects. However, Myburgh et al showed that the mean calculated in vitro P50, in 20 critically ill patients, was 24.5 ± 2.9 mmHg and this value was significantly lower than 20 normal subjects [26.2 ± 2.2 mmHg] (Myburgh, Webb, Worthley 1991). Our results did not show similarity to the data of Myburgh et al results. This variation in the results may be due to: a) Myburgh et al calculated the vitro P50 and we calculated the in vivo P50 (a different method), b) differences in the characteristics of the patients and illness severity. Our data suggest that overall P50 values are similar to quoted normal values in critically ill patients. However, as with RBC 2,3-DPG, we observed a wide range of values.

We found a strong positive correlation between RBC 2,3-DPG and calculated in vivo P50. This was not found in the study by Morgan et al for calculated in vitro P50. This

may be due to the difference in the formulae used to calculate the in vivo P50 and the position of RBC 2,3-DPG in these formulae. However, in addition the correlation in our study should be considered with caution, as it is possible that it results, at least in part, from mathematical linkage. Walsh et al showed that if a relationship is investigated between a derived variable and another variable that is either a component of the derived variable or shares a component with it, the relation might be affected by mathematical coupling. As a result, part or all the relation may be spurious (Walsh, Lee 1998). Mathematical coupling might be the case in the relation between RBC 2,3-DPG, Pco_2, pH, temperature, and P50, as P50 is a derived variable from a formula that included all of these variables. Our data suggest that RBC 2,3-DPG has the strongest correlation with in vivo P50, but we can not adjust for mathematical linkage in our conclusion.

IMPLICATIONS OF OUR FINDINGS

The clinical importance of RBC 2,3-DPG levels in critically ill patients is unclear, but it is widely believed that low RBC 2,3-DPG values could have adverse effects, particularly in relation to stored blood. A few studies showed that high RBC 2,3-DPG was associated with improved clinical condition and outcome. Dennis et al. showed that there was a significant increase in cardiac index after transfusing red cells with high RBC 2, 3-DPG concentrations for patients undergoing coronary artery bypass surgery (Dennis, Vito, Weisel, Valeri, Berger, Hechtman 1975). In another experimental study, Bakker et al. perfused isolated rat livers with fresh and RBC 2,3-DPG-depleted human erythrocytes at different levels of hypoxia and found that perfusion with fresh erythrocytes showed higher venous Po_2 values. By using

bile flow rate as an indirect measure for the rate of oxygen consumption, a favourable effect of perfusion with fresh erythrocytes was found (Bakker 1977). These studies supported the fact that higher levels of RBC 2,3-DPG could improve tissue oxygen delivery. If these studies are translatable to critical illness, our finding of lower RBC 2,3-DPG concentrations in sicker patients could be clinically relevant. Old stored blood has lower RBC 2,3-DPG concentration than fresh blood. With the current leucodepleted product RBC 2,3-DPG is absent after 10 days (Walsh et al. 2004). If acidosis decreases RBC 2,3-DPG regeneration, which is usually thought to take 4 to 24 hours, then this process could delay regeneration of older stored blood in acidotic patients. In theory, this could mean older transfused blood takes longer to recover in acidotic patients, who would paradoxically benefit most from augmented oxygen delivery. The hypothesis that older stored blood is ineffective or even harmful in the critically ill was supported by a study using gastric tonometry as an oxygenation endpoint(Marik, Sibbald 1993; Marik 1993), although a more recent double blind randomised study found no differences between RBCs aged < 5 and >20 days in changing oxygenation indices (Walsh et al. 2004).

In conclusion, RBC 2,3-DPG concentration was slightly lower in critically ill patients. Female critically ill patients had lower values than males. Lower RBC 2,3-DPG values were most likely secondary to acid-base disturbance, possibly through enzymatic effect on RBC 2,3-DPG metabolism. Calculated in vivo P50 values were normal overall, but there was wide variation that correlated most closely with RBC 2,3-DPG.

Further studies need to establish whether these observations translate in to clinically importance effects on oxygen transport.

CHAPTER FOUR

ANAEMIA, BLOOD LOSS, AND TRANSFUSION IN ELECTIVE ORTHOPAEDIC SURGERY

INTRODUCTION

Most patients attending for elective orthopaedic surgery (EOS) are elderly and a proportion have a haemoglobin concentration (Hb) below the normal reference range. Preoperative anaemia increases the risk of peri-operative transfusion. If it could be shown that pre-operative anaemia could be corrected by simple, safe and inexpensive treatment, this might be an effective way of reducing transfusion needs and so could be included among the interventions that could be recommended to reduce the needs for transfusion. This chapter reviews the relevant evidence from clinical studies, concentrating on the two commonest major elective orthopaedic procedures, Total Hip Replacement (THR) and Total Knee Replacement (TKR). In some tables these procedures are grouped together and referred to as Major Joint Surgery (MJS).

RATIONALE FOR THE STUDY

Lower pre-operative Hb or haematocrit levels (Hct) appear to be strongly associated with peri-operative blood transfusion [Scottish Intercollegiate Guidelines Network (SIGN): Preoperative Blood Transfusion for Elective Surgery] (Feagan, Wong, Kirkley et al. 2000; 2004b, SIGN guideline, page 363). Although it may seem self-evident that an anaemic patient is more likely to be transfused, the nature of the relationship between pre-operative Hb and the probability of blood transfusion is not entirely clear. An obvious explanation is that if a patient is already anaemic, surgical

blood loss will more rapidly cause a critically low Hb level, but other factors are probably also at work, for example:

- Lower Hb is associated with prolongation of the bleeding time (Baskurt, Meiselman 2003).
- Many surgical units routinely order blood if the pre-operative haemoglobin concentration is below a predefined level: the immediate availability of blood may lower the clinician's threshold for administering blood transfusion.

A number of authors and guidelines recommend that pre or peri-operative haematinics be given to anaemic surgical patients to improve their Hb concentrations and so decrease the incidence of red cell transfusion [Scottish Intercollegiate Guidelines Network (SIGN): Pre-operative Blood Transfusion for Elective Surgery] (Taylor, Mallen, Lind 1982; Zauber, Zauber, Gordon et al. 1992; Andrews, Lane, Bradley 1997; Weisbach, Skoda, Rippel et al. 1999; 2004b, SIGN guideline, page 363). Nevertheless, there is little published evidence that this measure is effective in treating pre-operative anaemia or reducing red cell transfusion.

At the surgical pre-admission clinic, determination of Hb level can identify anaemic patients and give the opportunity to investigate and correct this anaemia before surgery (Andrews, Lane, Bradley 1997). However, treating anaemia during pre-operative assessment without going further to investigate its cause may mean a lost opportunity to diagnose and treat a major underlying cause.

PRE-OPERATIVE HAEMOGLOBIN OR HAEMATOCRIT LEVELS IN EOS PATIENTS

A few studies were identified on the prevalence and type of anaemia in patients attending for Elective Orthopaedic Surgery (EOS). Most commonly, the laboratory findings are those typical of "anaemia of chronic disease" which is characterized by normocytic normochromic red cell indices.(Beutler, Lichtman, Coller, Kipps, editors 1995; Andrews, Lane, Bradley 1997; Towheed, Shea, Wells, Hochberg 2002)

Goodnough et al. reported that of 385 patients undergoing EOS in the USA, 25% were anaemic (defined as a Hct below 39%) (Goodnough, Vizmeg, Marcus 1993). In a similar study of 281 EOS patients 26% were anaemic (Goodnough, Vizmeg, Sobecks, Schwarz, Soegiarso 1992b).

Andrews et al. in the UK showed that 18% of 100 EOS patients were anaemic (Hb below 120 g/L) (Andrews, Lane, Bradley 1997). Some studies report pre-operative Hb or Hct levels for patients undergoing EOS as a baseline values before a study intervention. Table (4.1) shows the mean ± (SD) values for pre-operative Hb or Hct recorded in these studies.

Table (4.1): Reported pre-operative Hb and Hct values for patients undergoing EOS.

Author	Number of patients	Mean ± SD Hb (g/L)	Mean ± SD Hct (%)
Weisbach et al.(Weisbach et al. 1999)	90	143 ± 11	Not mentioned
Laupacis et al.(Laupacis, Feagan, Wong 1993)	208	137 ± 12	Not mentioned
Olijhoek et al.(Olijhoek, Megens, Musto et al. 2001)	110	123 ± 80	36.9 ± 2.3
Faris et al.(Faris, Ritter, Abels 1996)	200	131 ± 16	39 ± 4.1
Feagan et al.(Feagan et al. 2000)	78	125.7 ± 7	Not mentioned
Jarolem et al.(Jarolem, Scott, Jaffe, Stein, Jaffe, Atik 1995)	56	126	Not mentioned
Biesma et al.(Biesma, Kraaijenhagen, Poortman, Marx, van de 1992)	34	142 ± 7	43 ± 2
Larocque et al.(Larocque, Gilbert, Brien 1998)	299	138.3 (SD not mentioned)	Not mentioned

CAUSES OF PRE-OPERATIVE ANAEMIA IN EOS POPULATION

Causes of anaemia can be broadly categorised as: (a) Decreased red cell production, and (b) Increased erythrocyte destruction or loss (chapter 1) (Beutler, Lichtman, Coller, Kipps, editors 1995; Cuervo, Mahomed 2002). While anaemia is extremely common worldwide, the causes of anaemia that are more likely to affect EOS patients in a developed country are: i) medications, ii) nutritional insufficiency, and iii) other associated medical conditions that are more common in older patients such as renal insufficiency and malignancy.

In 30 anaemic EOS patients reported in the study cited above, Goodnough et al. found that 30% of them had evidence of iron deficiency anaemia and 53% had unclassified anaemia of chronic disease (Goodnough, Vizmeg, Sobecks, Schwarz, Soegiarso 1992a). In another study of elderly surgical patients, Kiyama et al. studied 32 patients with mean age $\geq$ 68 years who underwent open-heart surgery. They found that 18 (56.25%) of these patients had iron deficiency anaemia, 2 (6.25%)had anaemia of chronic renal failure, and 12 (37.5%) had unexplained anaemia (Kiyama, Ohshima, Imazeki, Yamada 1999).

Increasing age in the absence of disease is not a cause of anaemia: it has little effect on the circulating red blood cell mass, white blood cell count, the number or function of platelets, or coagulation. Red blood cell fragility increases by age but it does not affect erythrocyte life span in vivo. Therefore, clinically significant anaemia or disorders of haemopoiesis in the elderly as in younger individuals should always be considered first as due to disease and not attributed to age (Millar, editor 2000).

The use of drugs may be a cause of anaemia in EOS patients. Many patients have chronic joint pain and been taking aspirin-containing and/or non-steroidal anti-

inflammatory drugs for many years. These are known to cause increased gastrointestinal blood loss often leading to iron deficiency (Beutler, Lichtman, Coller, Kipps, editors 1995; Andrews, Lane, Bradley 1997; Towheed 2002). Dietary insufficiency of vitamins and essential minerals is common in elderly patients who represent the majority of EOS population (Zauber et al. 1992; Avenell 2002). Moreover, malabsorption is not uncommon in this group of patients (Beutler, Lichtman, Coller, Kipps, editors 1995). There is some evidence that in elderly people; significant non-symptomatic low serum vitamin B12 levels may be found in individuals who do not have clinical findings of vitamin B12 deficiency. Low levels of B12 may be due to mal-absorption of protein-bound vitamin B12, but not due to mal-absorption of free vitamin B12 (Beutler, Lichtman, Coller, Kipps, editors 1995). A few studies, summarised in table (4.2), report ferritin levels as an indication of iron deficiency anaemia and vitamin B12 and folate levels in elective surgical patients including EOS patients.

Table (4.2): Mean ± SD of baseline ferritin, vitamin B12, and folate levels in surgical patients.

Author	Procedure, patients	Number of patients	Mean ± SD Ferritin (R.R)	Mean ± SD B12 (R.R)	Mean ± SD Folate (R.R)
Kasper et al.(Kasper, Lazansky, Stark, Klimek, Laubinger, Borner 1998)	Open heart, aortic surgery, craniotomy, prostatectomy, and THR (36% of patients in the study).	108	N M	309 ± 123 (200-950) ng/L	6.3 ± 2.2 (3-17) µg/L
Weisbach et al.(Weisbach et al. 1999)et al.	70%Orthopaedic and 30%cardiac.	123	For both genders156 ± 102 (females 10-130 & males27-300) µg/L	N M	N M
Rohling et al.(Rohling, Zimmermann, Breymann 2000)	Surgical patients	12	2.3 ± 13.9 µg/L (N M)	414 ± 142 ng/L (N M)	7.4 ± 3.3 ng/L (N M)
Sowade et al.(Sowade, Messinger, Franke, Sowade, Scigalla, Warnke 1998)	Cardiac	76	Median 115 (N M)	N M	N M
Feagan et al.(Feagan et al. 2000)	Hip surgery	201	113 ± 113.5 (N M)	N M	N M

Author	Procedure	Number of patients	Mean ± SD Ferritin (R.R)	Mean ± SD B12 (R.R)	Mean ± SD Folate (R.R)
Biesma et al.(Biesma, Kraaijenhagen, Poortman, Marx, van de 1992)	Hip replacement	34	90.6 ± 59.3 (10-200) µg/L	N M	N M
Laupacis et al.(Laupacis, Feagan, Wong 1993)	Hip replacement	208	151 ± 154 (N M)	N M	N M
Faris et al.(Faris, Ritter, Abels 1996)	Major orthopaedic	200	161.3 ± 339 (>20) µg/L	N M	N M
Olijhoek et al.(Olijhoek et al. 2001)	EOS	110	123.2 ± 86.9 (N M)	N M	N M

R.R = normal reference range used in the study. N M = not mentioned in paper.

BLOOD LOSS IN EOS

Peri-operative blood loss in patients undergoing EOS, especially during major joint surgery can be substantial and much of the blood loss is into the tissues. Bleeding is significantly greater during revision surgery (Bierbaum, Callaghan, Galante, Rubash, Tooms, Welch 1999; Grosvenor, Goyal, Goodman 2000; Millar, editor 2000; Parker, Rajan 2002).

There are many ways to estimate intra-operative blood loss during surgery, however, none of them has been proven to be an accurate or "gold standard" method and none can measure blood loss into tissues surrounding the operative site. Intra-operative blood loss may be estimated by: (a) visual assessment of the amount of blood coming out from the operative field (qualitative estimation), (b) the volume of the blood in the operating suction, (c) swab-weighing, as 1 ml of blood weights approximately 1 g, (d) such values may be recorded in real time or retrospectively recorded from anaesthetics charts, and (e) calculating red cell mass pre and post-operatively (Rawle, Seeley 1987).

A widely used method of estimating post-operative blood loss is to measure the amount of blood in the operative drains. However, this does not give an accurate measurement of red cell loss as the haematocrit (Hct) in the drained blood falls with time after surgery. Majkowski et al. compared the mean post-operative Hct level in venous blood and drained blood for patients following TKR. The authors found that Hct was 37% in the venous blood versus 31% in the drained blood (Majkowski, Currie, Newman 1991).

Another approach is to estimate the blood loss from the fall in Hct and an estimate of blood volume, taking account of any blood that been transfused and of haemodilution (Toy, Kaplan, McVay, Lee, Strauss, Stehling 1992).

Table (4.3) summarizes published data on surgical blood loss during major joint surgery, estimated by, use of one or other of these methods.

Table (4.3) published studies that showed data on intra-operative blood loss during EOS.

Author	Study design & aims	Surgical procedures	Study intervention	Mean intra-operative blood loss	Mean reduction in Hb or Hct from pre to post-operative
Berman AT et al.(Berman, Geissele, Bosacco 1988)	Retrospective study to investigate the degree of blood loss associated with TKR. *	140	Procedures that did not require blood transfusion (n = 70).	1.8 units*/procedure.	16%
			Procedures that required blood transfusion (n = 70).	Not mentioned.	Not mentioned.
				The mean blood loss for both groups was 2.2 units* per arthroplasty.	
Kurdy NM(Kurdy 1996)	A retrospective study to assess the transfusion needs for hip and knee arthroplasty. *	103	THR (n = 44)	758 (range: 200-2250) ml	Not mentioned
			TKA (n = 59)	There was no intra-operative blood loss (tourniquet was used).	Not mentioned

* Blood loss expressed as equivalent number of units of units of blood for transfusion (the authors did not mention the volume of the units).

Author	Study design & aims	Surgical procedures	Study intervention	Mean ± SD intra-operative blood loss	Mean reduction in Hb or Hct from pre to post-operative
Toy PT(Toy, Kaplan, McVay, Lee, Strauss, Stehling 1992)	A multi-central retrospective study to determine blood loss, the number of transfusion episodes and the haemoglobin levels achieved in transfused primary THR patients. **	324	*a) Blood loss recorded from anaesthetics records*:		
			Primary THR (n = 235).	738± 549 ml.	Not mentioned.
			Revision THR (n = 70).	943± 604 ml.	Not mentioned.
			b) Calculated blood loss: (total blood volume lost = change in blood volume + transfused red blood cell volume). Red blood cell body volume was calculated from the equation: (red cell volume = preoperative haematocrit × assumed blood volume × 0.91).		
			Primary THR (n = 239).	635± 259 ml.	Not mentioned.
			Revision THR (n = 70).	797±416 ml. [Median (IQR)]	Not mentioned.
Lisander et al.(Lisander, Ivarsson, Jacobsson 1998)	Prospective observational study to investigate the efficacy of red cell salvage in reducing the need for allogeneic blood. ***	96	Primary cemented THR (n = 49).	885 (456-1215) ml	Not mentioned.
			Primary non-cemented THR (n = 11).	855 (649-1074) ml	Not mentioned.
			Revision THR (n = 36).	1474 (976-2079) ml	Not mentioned.

Author	Study design & aims	Surgical procedures	Study intervention	Mean intra-operative blood loss	Mean reduction in Hb or Hct from pre to post-operative
Knight JL(Knight, Sherer, Guo 1998)	Non- randomised prospective study to suggest alternative strategies to minimize autologous blood wastage, the risk of homologous blood transfusion, and cost associated with blood product usage after TKA.[++]	177 (155 patients)	Unilateral TKA and did not pre-donate and admission Hct was ≤ 40% (n = 19/155 patients).	957 ml	12.3%
			Unilateral TKA and did not pre-donate and admission Hct was > 40% (n = 28 patients).	1013 ml	8.5%
			Unilateral TKA and pre-donated one unit (n = 47 patients).	833 ml	7.9%
			Unilateral and pre-donated 2 units (n = 20 patients).	820 ml	6%
			Bilateral and pre-donated 2 units (n = 21 patients).	1936ml (968 ml per TKA)	7.7%
			Revision surgery and pre-donation was optional (n = 14 patients).	973 ml	10.2%

Author	Study design & aims	Surgical procedures	Study intervention	Mean ± SD intra-operative blood loss	Mean reduction in Hb or Hct from pre to post-operative
Shulman (Shulman, Grecula, Hadjipavlou 2002)	Prospective control trial to investigate the efficacy of acute iso-volaemic haemodilution combined with intra-operative red salvage and red cell salvage alone.$^{+++}$	80	Primary THR (n = 47)	1356 ± 1415 ml	Not mentioned
			Revision hip replacement (n = 33)	2174 ± 1655 ml	Not mentioned

Methods used to estimate the volume of blood loss:

- *Case note review (not specified)
- ** Case note review (anaesthetics records) and calculated volume of blood loss.
- *** Calculated from Hct levels total amount of red cell transfused.
- $^{++}$ Measured blood loss (the authors did not mention the method)
- $^{+++}$ Measured using autotransfusion device.

BLOOD TRANSFUSION IN EOS

The blood components most often administered during or after orthopaedic surgery are red blood cells or whole blood (Lemos, Healy 1996; Knight, Sherer, Guo 1998). The volume and red cell content of these components vary, but typical values are shown in table (4.4) (2004a, guidelines for the blood transfusion services in the UK).

Table (4.4): Volume and red cell content of a red cell and a whole blood unit (2004a, guidelines for the blood transfusion services in the UK)

	Red cells (with additive solution)	Whole blood
Volume (ml)	220 – 420	513 ± 45
Haemoglobin (g/pack)	40 – 66	40 – 66
Haematocrit (%)	50 – 75	46 – 66

Studies on transfusion report either the volume of blood (ml) or number blood pack units -transfused during different EOS procedures.

Studies of the effectiveness of the different strategies to reduce the need for blood transfusion report blood also report their data several different ways such as: a) percentage of operated patients who get transfused, b) units of blood per patient who is transfused, and c) units of blood per patient operated. This leads to difficulty in comparing the results of studies.

QUANTITY OF BLOOD TRANSFUSED

Several studies show that the frequency and amount of blood transfusion to patients having similar orthopaedic procedures (e.g. primary unilateral hip arthroplasty) vary widely (Sirchia, Giovanetti, McClelland, Fracchia, editors 1994). Table (4.5) summarises published studies that reported amount of blood loss during EOS.

In summary, it is likely that the variation in blood use that is observed among surgical teams is due to the combined effect of 3 broad categories of influence:

(1) *Differences in the patient population*: age profile, socio-economic status, proportion of males and females, proportion of anaemic patients, and level of co-morbidity

(2) *Types of surgical procedure performed* e.g. primary or revision, hip or knee replacement, and associated technical details of procedure.

(3) *Differences in patient management* these may include: a) use of blood ordering schedules and transfusion protocols or thresholds, b) variants of surgical technique, c) variants of anaesthetic technique, d) use of specific blood conserving measures such as pre-operative autologous blood donation intra or post operative blood salvage or anti fibrinolytic agents (EACA or aprotininin).

In large observational studies, analysis of the variables associated with blood use show that the two strongest predictors of transfusion are low preoperative Hct (confounded with female gender) and an effect linked to the hospital or surgical team performing the procedures (Sirchia, Giovanetti, McClelland, Fracchia, editors 1994). These factors are explored in more detail in the next section.

Table (4.5): Amount of blood loss during EOS.

Author	Study design and aims	Surgical procedures	Study intervention	Number of units transfused
Toy et al.(Toy, Kaplan, McVay, Lee, Strauss, Stehling 1992)	Retrospective study to estimate blood loss and number of red cell units transfused in patients undergoing THR.	324	Primary THR [made autologous donation] (n = 189).	2.1 ± 1.2
			Primary THR [did not make autologous donation] (n = 60).	1.7 ± 2.9
			Revisions THR [made autologous donation] (n = 51).	3.3 ± 2.3
			Revision THR [did not make autologous donation] (n = 24).	2 ± 2.2 [Mean ± SD of total number of units transfused (both homologous and autologous) *per transfused patient*]
Berman et al.(Berman, Geissele, Bosacco 1988)	Retrospective study to determine the amount of blood loss in TKA.	140 (108 patients)	70 patients received blood transfusion and 70 did not.	2.6 (range: 1-11) *per transfused arthroplasty*.

Author	Study design and aims	Surgical procedures	Study intervention	Number of units transfused
Helm et al.(Helm, Karski, Parsons, Sampath, Bale 2003)	Prospective study to estimate the amount blood loss and transfusion in patients undergoing MJS.	161	79 patients before introducing a clinical/laboratory protocol for reducing blood-transfusion requirements	1.3 (0-6)
			82 patients after introducing transfusion protocol.	0.6 (0-4)
				[Mean (range) of number of units transfused *per operated patient*].
Goodnough et al.(Goodnough, Vizmeg, Marcus 1993)	Case note review to determine the amount of red cell loss and transfusion in pre-donated and non pre-donated patients who undergoing MJS.	385	Autologous donors, 56 primary THR	3 ± 1.3
			Non-autologous donors, 27 primary THR	2.3 ± 1.5
			Autologous donors, 23 revision THR	4.1 ± 1.8
			Non-autologous donors, 21 revision THR	4 ± 4
			Autologous donors, 73 TKA	2.5 ± 1.6
			Non-autologous doors, 28 TKA	1.2 ± 1.5
				[Mean ± SD of all blood transfused (autologous and homologous) *per operated patient*].

Author	Study design and aims	Surgical procedures	Study intervention	Number of units transfused
Warner et al.(Warner, Warner, Schroeder, Offord, Maxson, Santrach 1998)	Medical records review for MJS patients in three different periods in which different transfusion practices were used to evaluate the success of these straegies in reducing blood transfusion.	899 (808 patients).	Pre-AIDS era (n = 232). Autologous donation program (n =269). Current practice during the study (n = 398).	2.1 ± 1.6 2.3 ± 2.1 1.8 ± 2.2 [Mean ± SD of all blood transfused (autologous and allogeneic) *per procedure*]
Jarolem et a.(Jarolem, Scott, Jaffe, Stein, Jaffe, Atik 1995)	Retrospective study to determine intra-operative blood loss and transfusion in patients undergoing TKA.	106	65 with tourniquet. 50 without tourniquet.	1.71(1-8) 1.66(1-4) [Mean (range) *per operated patient*].
Kurdy et al.(Kurdy 1996)	Retrospective study to assess transfusion needs for patients undergoing MJS.	103	Intra-operative THR (n = 44) Post-operative THR (n = 44) Intra-operative TKR (n = 59) Post-operative TKR (n = 59)	69 (0.7%) 21 (0.2%) 66 (0.6%) 15 (0.1%) [Total units transfused peri-operatively per group (*per patient operated)*]

STRATEGIES USED TO REDUCE THE NEED FOR BLOOD TRANSFUSION

Many authors and guidelines recommended particular techniques to reduce the need for red blood transfusion during EOS. The evidence for clinical effectiveness of these specialised methods is limited although there is evidence that attention to general aspects of management, and the use of transfusion protocols may be effective in reducing transfusion needs. Table (4.6) summarizes some of the techniques that may be used with intention to decreasing red cell transfusion requirements for patients undergoing EOS.

Table (4.6): Summary of approaches to reducing the need for blood transfusion in EOS

Pre-operative management.	Manage pre-operative anaemia.
	Blood ordering schedule
Intra-operative management.	Anaesthetic techniques.
	Surgical techniques.
	Transfusion protocol
Specific transfusion sparing strategies.	Pre-operative autologous donation.
	Acute isovolaemic haemodilution.
	Red blood cell salvage.
	Antifibrinolytic agents.
Post-operative.	General measures e.g. body temperature control.
	Transfusion protocol
	Diet and haematinics.

General aspects of management

Transfusion practices have significantly changed in the surgical setting in recent times. A Hb level below 100g/L was accepted as an indication for peri-operative

blood transfusion for many years but many recent guidelines recommended transfusion at a Hb range between 60g/L and 100g/L, depending on the patient's physiological reserves and the degree of anaemia. Transfusion at these lower Hb levels in the surgical setting reduces the use of red cell transfusion (Goodnough, Marcus 1998; Faris, Spence, Larholt, Sampson, Frei 1999; Hill et al. 2002). Hill SR et al. performed a Cochrane systematic review on the effect of transfusion thresholds and protocols on the use of red blood cell and on patients' clinical outcomes. This review did not only include studies of patients undergoing EOS. The authors included randomised controlled trials from 1966 to 2000 in which defined levels of Hb or haematocrit were used as trigger for red cell transfusion and blood was transfused below these levels. Ten trials comprising 1780 patients (838 critically ill patients from one trial) matched the inclusion criteria. The review authors found that restrictive transfusion strategies reduced the probability of red cell transfusion by 42%. Post-operatively, haematocrit or Hb levels were 5.6% lower in the restrictive strategy than that in the liberal. The authors showed that reduction in the use of allogeneic blood was not associated with increase in morbidity or mortality. Mortality was one fifth lower in patients who received less allogeneic blood compared to that among patients transfused liberally. It must be noted that this conclusion was obtained mainly from the study of Hebert et al.(Hebert et al. 1999), which contributed 83% of the weight in the meta-analysis. The authors reported that the *relative risk of getting exposed to blood transfusion is 0.58 and 95% CI (0.47 to 0.71)*(Hill et al. 2002).

Helm et al. have subsequently reported a study to evaluate post-operative transfusion practice before and after introducing a clinical and laboratory protocol for reducing blood transfusion in patients undergoing EOS. Before establishing the protocol, patients were transfused when their 24 hours post-operative Hb was < 90g/L. The protocol depended on calculating the maximum allowable blood loss (MABL) for each patient using the formula:

MABL = estimated blood volume x Hct level pre-operatively – minimum Hct / pre-operative Hct.

This formula was combined with replacing intra and post-operative blood loss with crystalloids until the MABL has been reached. Following introduction of the protocol the percentage of patients transfused decreased from 66% to 24% (Helm, Karski, Parsons, Sampath, Bale 2003).

Preoperative considerations

Management of pre-operative anaemia

Iron therapy

Body iron stores can be restored by oral iron therapy. This is simple, safe, and inexpensive but compliance may be poor. Ferrous sulphate is the most commonly used preparation. 200 mg contains 60 mg iron. Other preparations contain different amounts of metallic iron e.g. 300 mg ferrous gluconate, which provides 36 mg iron (Hoffbrand, Lewis, Tuddenham, editors 1999).

The few studies that were identified on the effect of oral iron alone on pre-operative Hb in patients awaiting EOS are summarised in table (4.7). More data are available about the effects of iron with erythropoietin: also shown in the table.

Table (4.7): Pre-operative iron level in surgical patients (orthopaedic and other surgical procedures).

Author	Aim	Number of subjects	Study intervention	Results	
				Before treatment	***After treatment***
Andrews C et al.(Andrews, Lane, Bradley 1997)	Randomised controlled study to investigate the role of iron pre-load in optimising Hb levels and iron stores pre-operatively in patients undergoing MJS.	100	Patients divided into 3 groups according to their pre-operative Hb level:		
			Hb < 120 g/L + 4week course of oral ferrous sulphate (N = 18).	Mean Hb = 108 g/L	119 g/L
			Hb > 120 g/L + 4 weeks course of oral ferrous sulphate group (N = 35).	Mean Hb = 138 g /L	No increase in pre-operative Hb level. Hb dropped by a mean of 4g/L at one week after surgery.
			Hb > 120 g/L + no iron [control group] (N = 40).	Mean Hb = 140 g/L	Hb dropped by a mean of 13g/L at one week after surgery.

Author	Aim	Number of subjects	Intervention	Results	
Kasper S et al.(Kasper, Lazansky, Stark, Klimek, Laubinger, Borner 1998)	Randomised Double blind study to compare the efficacy of oral iron combined with intravenous iron to that of oral iron alone in increasing pre-operative Hb in autologous blood donation in surgical patients with adequate iron stores (orthopaedic and other elective surgical procedures)	108	Patients who pre-donated 3 or more units of blood at weekly intervals were divided into 3 groups according to the treatment that was given *21 days* pre-operatively:	***Before per-donation***	***After donation and before surgery***
			Placebo (no iron) (N = 22)	Mean Hb = 144 g/L Mean Hct = 44% Mean Ret. Count = 69 x 10^9 /L Mean ferritin = 166 µg/L	115 g/L 36% 71 x 10^9 /L 116 µg/L
			Daily oral iron alone (N = 45)	Mean Hb = 147 g/L Mean Hct = 45% Mean Ret. Count = 76 x 10^9 /L Mean ferritin = 161 µg/L	122 g/L 38% 126 x 10^9 /L 89 µg/L
			Daily oral iron + a weekly dose of intravenous iron (N = 41).	Mean Hb = 144 g/L Mean Hct = 44% Mean Ret. Count = 70 x 10^9 /L Mean ferritin = 159 µg/L	118 g/L 37% 119 x 10^9 /L 224 µg/L

Author	Aim	Number of subjects	Study intervention	Results	
Feagan BG et al.(Feagan et al. 2000)	Randomised, double blind, multi-centre trial to investigate the efficacy of EPO with iron therapy in reducing red cell transfusion for patients undergoing THR.	201	*4 weeks* before surgery all patients received oral iron and were divided into 3 groups:	***Before treatment***	***After treatment and before surgery***
			High EPO dose (N = 44).	Mean Hb = 114 g/L	Hb increase by 19.5 g/L and reticulocyte count by 58.8 x 10^9/L.
			Low EPO dose (N = 79).	Mean Hb = 108 g/L	Hb increased by 17.2 g/L and reticulocyte count by 37 x 10^9/L.
			Placebo (N = 78).	Mean Hb = 128 g/L	Hb increased by 1.2g/L and reticulocyte count by 1.8 x 10^9/L.

Author	Aim	Number of subjects	Intervention	Results	
Olijhoek G et al.(Olijhoek et al. 2001)	A randomised, double blind, multi-centre, placebo-controlled trial to investigate the role of oral versus intra-venous iron in erythropoietic response to EPO therapy in patients undergoing EOS.	110	*14 days* before surgery, patients were divided into 4 groups:	***Before treatment***	***After treatment and before surgery***
			EPO + oral iron (N = 29).	Mean Hb = 123g/L. Mean Hct = 37% Mean Ret.% = 1.3%	Hb increased by 16 g/L. Hct and Ret%significantly increased.
			EPO + intravenous iron (N = 29).	Mean Hb = 120g/L. Mean Hct = 35.7% Mean Ret.% = 1.6%	Increased by 15 g/L. Hct and Ret.% significantly increased
			Placebo + oral iron (N = 27).	Mean Hb = 125g/L. Mean Hct = 37.5% Mean Ret.% = 1.5%	Decreased by 1g/L. Hct and Ret.% showed non-significant increase.
			Placebo + intravenous iron (N = 25).	Mean Hb = 126g/L. Mean Hct = 37.5% Mean Ret.% = 1.6%	Increased by 2g/L. Hct and Ret.% showed non-significant increase.

Author	Aim	Number of subjects	Study intervention	Results	
Rohling et al.(Rohling, Zimmermann, Breymann 2000)	Randomised trial to assess the effect of different pre-operative iron treatment modalities to increase erythropoiesis.	12 patients undergoing elective surgery.	*3 weeks* before surgery all the patients received EPO 200 U/kg and were divided into 2 groups.	***Before treatment***	***After treatment and before surgery***
			Iron sucrose group (iv) (N = 6).	Mean Hb = 128 g/L Mean Hct = 39.6 % Mean Ret.% = 8.6 % Mean ferritin = 27.3 µ mol/L	154 g/L 48.4 % 35 % 266.8 µ mol/L
			Iron sulphate group (oral) (N = 6).	Mean Hb = 128 g/L Mean Hct = 39.5% Mean Ret.% = 7.3% Mean ferritin = 39 µmol/L	149 g/L 48.2 % 18.6 % 34µ mol/L

Author	Aim	Number of subjects	Study intervention	Results	
Sowade O et al. (Sowade, Messinger, Franke, Sowade, Scigalla, Warnke 1998)	Double blind, placebo controlled trial to estimate the efficacy of oral iron during treatment with erythropoietin in patients undergoing cardiac surgery.	76	Treatment stared *14 days* before surgery. Patients were divided into 2 groups, both of them received oral iron:	***Before treatment***	***After treatment and before surgery***
			EPO group (n = 36).	Median Ret.% =1.3%	4.7%
				Median Hb = 143 g/L	160 g/L
				Hct = 42%	49%
			Placebo group (n = 36).	Median Ret.% =1.3%	1.4%
				Median Hb = 140 g/L	139g/L
				Hct = 41%	41%

Author	Aim	Number of subjects	Study intervention	Results	
Weisbach V et al.(Weisbach et al. 1999)	Randomised controlled trial to investigate the role of iron therapy as an adjuvant to autologous blood donation in elective surgery (EOS and cardiac surgery).	90	Patients who pre-donated blood 3 times were divided into 3 groups *35 days* before surgery:	***At the time of first donation.***	***At surgery.***
			Oral iron group (n = 23).	Mean Hb = 141 g/L Mean Ret.% = 1.2 Mean ferritin = 139 µg/L	128 g/L % = 2.4 97 µg/L
			Intravenous iron group (n = 20).	Mean Hb = 141 g/L Mean Ret.% = 1.1 Mean ferritin = 217 µg/L	127 g/L % = 2.3 224 µg/L
			No iron [control group] (n = 17).	Mean Hb = 142 g/L Mean Ret.% = 0.9 Mean ferritin = 164 µg/L	125 g/L 1.4 109 µg/L

Intra-operative techniques

Anaesthetic techniques

Regional anaesthesia such as spinal or epidural is a common type of anaesthesia used for patients undergoing THR and TKR. There is a widely held view that there are certain advantages of regional over general anaesthesia for these patients including a reduction in the incidence of thrombotic episodes and a reduced operative blood loss. However, the published evidence does not fully support this opinion and why this should occur is unclear. These effects may be a consequence of an increased peripheral limb blood flow in combination with reduced venous tone. Alternatively they may arise from an alteration of blood viscosity and coagulability, as a result of changes in the metabolic and neurohumoral responses to surgery (Aitkenhead, Smith, editors 1996; Millar, editor 2000; Parker, Handoll, Griffiths 2002).

Some studies of the effect of different anaesthetic techniques on peri-operative blood loss during major joint surgery are reviewed below.

Niemi et al. compared spinal with hypotensive epidural anaesthesia on blood loss and coagulation in a randomised controlled trial for patients undergoing THR. They studied 30 patients, 15 patients in each group. All patients received low molecular weight heparin once before anaesthesia. Epidural blockade was done up to reach T1-T4 and spinal blockade at least to T10. The mean arterial blood pressure was 62 mmHg in hypotensive epidural group and 84 mmHg in the spinal anaesthesia group. In contrast to Parker et al, the authors found a significant difference in the amount of blood loss and the number of red cell units transfused in the two groups. Table (4.8) shows the amount of blood loss and numbers of red cell units transfused in both

groups. The authors did not find significant difference in the coagulation profile in both groups (Niemi, Pitkanen, Syrjala, Rosenberg 2000).

Table (4.8): Blood loss and transfusion in THR with spinal and epidural anaesthesia (Niemi, Pitkanen, Syrjala, Rosenberg 2000)

	Blood loss (ml)		Blood transfusion (units) per patient transfused.	
	Epidural group (n = 15)	Spinal group (n = 15)	Epidural group (N = 15)	Spinal group (N = 15)
End of surgery	400 (163-575)	900 (663-1100)	0	1.1 (0-2)
3 h post-operatively	600 (300-775)	1100 (763-1338)	0.5 (0-3)	1.7 (0-3)
Next morning	850 (500-1350)	1500 (1025-1838)	1.7 (0-5)	2.9 (1-6)
Total	**1850**	**3500**	**2.2**	**5.7**

Estimated median cumulative blood loos (IQR). Median cumulative number of transfused red cells (range)

Juelsgaard P et al. measured the amount of blood loss and red cell transfusion in 43 patients underwent hip surgery under general and spinal anaesthesia. The patients were divided into 3 groups: the first group received incremental spinal anaesthesia, the second received single-dose spinal anaesthesia, and the third received general anaesthesia. The authors did not compare the mean arterial blood pressure levels of the three groups. There was no significant difference in the amount of blood loss or the number of red cell units transfused intra or post-operatively [table (4.9)] (Juelsgaard, Sand, Felsby et al. 1998).

Table (4.9): Blood loss and transfusion in THR with incremental spinal, single dose spinal, or general anaesthesia (Juelsgaard et al. 1998).

	Blood loss (ml) (N = 43)			Blood transfusion (ml) per patient transfused. (N = 43)		
Type of anaesthesia	Increment-al spinal (N = 14)	Single dose spinal (N = 15)	General (N = 14)	Increment-al spinal (N = 14)	Single dose spinal (N = 15)	General (N = 14)
Intra-operative	300 (100-1000)	463 (100-1700)	396 (40-2000)	107 (0-500)	117 (0-600)	71 (0-700)
Post-operative	124 (40-370)	170 (25-550)	129 (0-340)	107 (0-450)	120 (0-300)	186 (0-600)
Total	**424**	**633**	**525**	**214**	**237**	**257**

Values are mean (range)

Dauphin et al. compared the use of combined general with epidural anaesthesia to general anaesthesia alone in the amount of blood loss during hip arthroplasty in a randomised control trial. The authors studied 37 patients underwent THR. Patients were divided into 2 groups, 17 patients received general anaesthesia alone (group A) and 20 patients received combined general with epidural anaesthesia (group B). The mean intra-operative blood loss was significantly less in group B compared to group A patients. There was no statistically significant difference in the mean amount of post-operative blood loss in the two groups. The total mean blood loss was statistically significant between both groups. Table (4.10) shows the amount of peri-operative blood loss in each group. The authors suggested that combined general with epidural anaesthesia decreased intra-operative blood loss (Dauphin, Raymer, Stanton, Fuller 1997).

Table (4.10) Blood loss in THR with combined general with epidural anaesthesia to general anaesthesia (Dauphin, Raymer, Stanton, Fuller 1997)

	General anaesthesia.	Epidural + general anaesthesia.
Mean ± SD intra-operative blood loss (ml).	1259.2 ± 366	663.8 ± 299
Mean ± SD post-operative blood loss (ml).	600.8 ± 390.8	444 ± 300.8
Total mean ± SD blood loss (ml).	**1860 ± 616.6**	**1107.8 ± 387.6**

Borghi B et al. showed in a prospective, randomised, multi-centre study the amount of peri-operative blood loss and red cell transfusion in 210 patients underwent THR under different anaesthetic techniques. The patients were randomised to receive either general anaesthesia alone, epidural anaesthesia alone, or combined general and epidural anaesthesia, 70 patients were in each group. In each group, patients were similar in their demographic data, history of hypertension, and the duration of surgery. There were no significant differences in the amount of peri-operative blood loss and red cell transfusion in the three groups [table (4.11)]. However, clinically relevant hypotension (systolic blood pressure decreased > 30% from baseline) and bradycardia (heart rate < 45 beats/ minute) occurred in patients who received combined general and epidural anaesthesia at the time of inducing general anaesthesia (Borghi, Casati, Iuorio et al. 2002).

Table (4.11): Blood loss in THR with general anaesthesia, epidural anaesthesia, and combined general anaesthesia with epidural (mean values ± SD) (Borghi et al. 2002)

	General anaesthesia.	Epidural anaesthesia.	General + epidural.
Intra-operative	479 ± 107	547 ± 99	465 ± 102
Post-operative	545 ± 110	502 ± 129	593 ± 106
Total blood loss	**1024**	**1049**	**1058**
Patients transfused	22%	13%	18%

Urwin et al. did a meta-analysis for randomised trials to compare general versus regional anaesthesia in patients undergoing hip surgery. The analysis included 2162 patients. They clarified that regional anaesthesia had some advantages over general anaesthesia in reducing early post-operative mortality and the incidence of deep vein thrombosis. Intra-operative blood pressure was statistically significant lower in the regional anaesthesia group compared to the general anaesthesia group. However, there was a significant lower amount of blood loss in the general anaesthesia group. Table (4.12) shows summary of the study results (Urwin, Parker, Griffiths 2000).

Table (4.12) Mortality rate, blood loss and transfusion, and operative complications in THR with regional and general anaesthesia (Urwin, Parker, Griffiths 2000)

	Regional anaesthesia.	General anaesthesia.
Mortality-1 month.	6.4%	9.4%
Mortality-3 months.	12.1%	12.8%
Mortality-6 months	16.8%	16.1%
Mortality-12 months.	22.5%	21%
Patients received transfusion per operated patients.	58.3%	56.7%
Blood loss per operated patient (ml).	152	6
Transfusion per operated patient (ml).	103	100
Deep vein thrombosis.	30.2%	46.9%
Operative hypotension.	34.3%	26%
Operative time (min).	189	187

Other technique of anaesthesia for hip surgery is the local nerve block. This may be supplemented with sedatives or analgesics. A lumber plexus block can be used in hip surgery (Millar, editor 2000). Only the plexus on the side of the fracture needs to be blocked, which may reduce the incidence of anaesthetics complications such as hypotension. This technique may markedly increase the incidence of other complications such as peripheral nerve injury. There are no studies compared the use of local anaesthetics techniques with other techniques in measuring the amount of peri-operative blood loss and transfusion in patients undergoing joint surgery.

The choice of the anaesthetic technique whether regional, general, or local should be determined by the patient's medical status (Aitkenhead, Smith, editors 1996; Millar, editor 2000).

Surgical techniques

It is widely stated / believed that blood loss and transfusion requirements during EOS can be influenced by many different techniques that can be applied during surgery such as: a) application of a limb tourniquet, b) aggressive warming, c) temporary balloon occlusion of the main arterial supply, d) the use of diathermy, e) experienced surgical team, and f) operative position. However, the published evidences did not fully support these beliefes. The techniques are reviewed below.

Application of a limb tourniquet

Bloodless operative field during EOS can be done in certain operations where a limb tourniquet can be used e.g. knee surgery. The limb should first elevated and squeezed using a bandage before inflation. The inflation pressure of the tourniquet should be approximately 100 mmHg above the systolic blood pressure of the patient for lower limb surgery and 50 mmHg for upper limb surgery. Towards the end of the procedure, the tourniquet should be deflated temporarily to check proper haemostasis within the operative field (Emmanuel, McClelland, Page, editors 1997).

Jarolem KL et al. did a retrospective study to compare blood loss and transfusion requirements in 106 patients who underwent total knee arthroplasty with and without the use of arterial tourniquet. During the procedure, 56 had a lower limb arterial tourniquet and 50 did not. The authors found that the mean intra-operative blood loss was significantly higher in patients without tourniquet compared with patients with

tourniquet (445 ml versus 300ml respectively). One hour post-operatively, there was a significant difference in the mean change of Hb level (pre-operative and post-operative) between the two groups. There was no significant difference between the numbers of patients who received blood transfusion. From these results, the authors clarified that the only clear benefit from using a tourniquet during TKA is the bloodless field, however, it might not decrease the need for blood transfusion. Table (4.13) summarises blood loss and transfusion in TKA with and without arterial torniquate (Jarolem, Scott, Jaffe, Stein, Jaffe, Atik 1995).

Table (4.13): Blood loss and transfusion requirements in TKA with and without the use of arterial tourniquet (Jarolem, Scott, Jaffe, Stein, Jaffe, Atik 1995)

	With tourniquet (n = 56)	Without tourniquet (n= 50)
Mean intra-operative blood loss.	300 ml.	445 ml.
Mean decrease in early post-operative Hb.	14.4 g/L	20.4 g/L
Percentage of patients transfused per operated patient.	64%	61%

Aggressive warming

Hypothermia is a common complication during surgery. In anaesthetized patients, heat loss exceeds production as a result of an alteration in hypothalamic functions due to the effect of the anaesthetics agents, wound exposure, and inappropriate intravenous fluids' temperature. The risk of hypothermia is greater in patients with

limited metabolic rate such as elderly patients (Aitkenhead, Smith, editors 1996; Millar, editor 2000).

Hypothermia leads to serious complications such as alteration in the clotting system due to inhibition of platelets functions and coagulopathy. This can lead to increase the incidence of blood loss during and after surgery (Aitkenhead, Smith, editors 1996; Millar, editor 2000; Winkler, Akca, Birkenberg et al. 2000).

Watts et al. investigated the effect of different degree of hypothermia on platelets functions and fibrinolytic activity. They studied 112 severely traumatized patients, 40 were normothermic (temperature = 37°C) and 72 patients were hypothermic (temperature < 37°C). The authors found when used multivariate analysis that 34°C was the critical point at which enzyme activity slowed significantly, and at which significant alteration in platelet activity was occurred. Fibrinolysis was not significantly affected at any temperature (Watts, Trask, Soeken, Perdue, Dols, Kaufmann 1998).

Winkler M and colleagues investigated the effect of aggressive warming and maintenance of normothermia on the amount of blood loss and the need of red cell transfusion during hip arthroplasty. The authors studied 150 patients undergoing hip arthroplasty procedures under spinal anaesthesia. Patients were randomly divided into two groups, 75 patients each, aggressive warming group (tympanic membrane temperature was maintained at 36.5°C) or conventional warming group (tympanic membrane temperature was maintained near 36.0°C). The authors found that intra-operative blood loss was significantly higher in the conventional warming group compared to the aggressive warming group. However, there was no significant difference in post-operative blood loss in both groups. The total median amount of

blood loss during surgery and 2 days pos-operatively showed statistically significant difference in the 2 groups. Table (4.14) summarises the study results. The authors suggested that aggressive warming to maintain normothermia during hip surgery reduced the amount of blood loss (Winkler et al. 2000).

Table (4.14): Effect of aggressive warming and maintenance of normothermia on the amount of blood loss and the need of red cell transfusion during hip arthroplasty (Winkler et al. 2000)

	Aggressive warming (N = 75).	Conventional warming (N = 75).
Median (IQR) intra-operative blood loss (ml).	488 (368 – 721)	618 (480 – 864)
Median (IQR) early post-operative blood loss (ml).	No significant difference.	
Total median (IQR) blood loss 2 days post-operatively (ml).	600 (400 – 820) 1531(1055 – 1746)	600 (480 – 864) 1678 (1366 – 1965)

Widman et al. investigated the effect of warming using amino acid infusion for patients underwent hip arthroplasty. They studied 46 patients, 22 received amino acid infusion one hour before and during spinal anaesthesia, 24 patients received Ringer's lactate and served as a control group. The authors calculated the mean change in the core body temperature before and after anaesthesia and the amount of blood loss prei-operatively. They found that the mean pre-anaesthetic core temperature increased in the amino acid group and it did not change in the control group. Post-operatively, the mean core temperature decreased in both groups by different level. The authors found that the mean intra-operative blood loss was significantly higher in the control group compared to the amino acid group. However, there was no

significant difference in the amount of post-operative blood loss in both groups. Table (4.15) summarises the study results (Widman, Hammarqvist, Sellden 2002).

Table (4.15): Effect of warming using amino acid infusion on blood loss in hip arthroplasty (Widman, Hammarqvist, Sellden 2002)

	Amino acid group (N = 22).	Ringer's lactate group (N = 24).
The change in mean pre-anaesthetic core temperature	+ 0.4°C	No change.
The change in mean post-anaesthetic core temperature	-0.4°C	-0.9°C
Mean ± SD peri-operative blood loss.	516 ± 271 ml	702 ± 344 ml
Mean ± SD post-operative blood loss.	No significant difference.	

Schmied et al. studied 60 patients undergoing primary THR. The authors investigated blood loss and blood transfusion in 30 normothermic patients (core temperature 36.6 ± 0.4°C) and 30 hypothermic patients (core temperature 35.0 ± 0.5°C). They found that the mean peri-operative blood loss was significantly greater in the hypothermic patients compared to normothermic patients (2.2 ± 0.5 L vs 1.7 ± 0.3 L respectively). Seven hypothermic patients required 8 units of allogenic blood and one normothermic patient required a unit (Schmied, Kurz, Sessler, Kozek, Reiter 1996).

Temporary balloon occlusion of the main arterial supply

Temporary arterial balloon occlusion has been used in controlling intra-operative bleeding in many major abdominal procedures. Ullmark G et al. studied the

effectiveness of the temporary balloon occlusion of the iliac or femoral in reducing blood loss in major joint surgery.15 hip and knee (mixture of primary and revision) procedures in 13 patients were included in the study. The authors found that the use of balloon occlusion during surgery reduced the peri-operative bleeding when they compared the amount of blood loss to a matched retrospective group of 12 patients (control group). The mean peri-operative blood loss was 0.5 L for the balloon group and 1 L for the matched control group. However, two complications in patients with balloon occured, one patient had bleeding due to catheter dislocation and another patient developed postoperative necrosis of the tip of a toe. (Ullmark, Hovelius, Strindberg, Wallner 2000).

Diathermy

Electrocautery is commonly used in many surgical procedures. Scarring and poor wound healing are frequent complications. Kearns SR et al. did a randomised clinical study to compare the use of electrosurgical incision with the traditional scalpel incision. They studied 100 patients underwent elective laparotomy. The patients were randomised to either scalpel or diathermy incision. There was a significant difference between both groups in the mean wound related blood loss (105.5 ± 8.7 ml in the scalpel group versus 64.4 ± 6.7 ml in the diathermy group). There was no significant difference in the mean total peri-operative blood loss in the two groups (915 ± 88 ml in the scalpel group versus 927 ± 110 ml in diathermy group). The authors also found that the diathermy group showed significant lower pain scores than the scalpel group and there was no difference between both groups in wound complications (Kearns, Connolly, McNally, McNamara, Deasy 2001).

Widman et al. investigated the efficacy of the diathermy knife in reducing the amount of intra-operative blood loss in patients undergoing primary THR. They studied 67 patients prospectively. Patients were divided into two groups, group A where the diathermy knife was used in all dissections and group B where the scalpel was used. Bleeding points were coagulated with diathermy in both groups. The authors found that there was no significant difference in the mean intra-operative blood loss in the two groups (Widman, Isacson 1999).
The use of diathermy might reduce intra-operative blood loss and keep a clear operative field, which facilitates the operative procedure.

Operative position

Few studies have investigated the role of operative position on the amount of blood loss during THR. Widman et al. studied 74 patients underwent THR. They compared the amount of peri-operative blood loss when the surgery was carried out in the lateral position with that in the supine position. There was a significant less blood loss peri-operatively in the lateral group, on average 201 ml (Widman, Isacson 2001).

Schneeberger et al. compared the amount of blood loss in primary THR in lateral position with preservation of the joint capsule (20 patients) with that in supine position without preservation of the capsule (20 patients). There was a significant less intra-operative blood loss in the lateral group than that in the supine group. The mean volume of intra-operative blood loss was 830 ml in the lateral group and 1165 ml in the supine group. The authors concluded that the intra-operative blood loss in patients undergoing primary THR can be reduced significantly in the lateral position

and combined that with preservation of the joint capsule (Schneeberger, Schulz, Ganz 1998).

Experienced surgical team and operative technique

The training, experience and care of the surgeon performing the procedure are the most crucial factor in reducing blood loss. Surgical technique and careful pre-operative planning, meticulous attention to bleeding points and careful haemostasis, and appropriate use of different intra-operative haemostatic techniques such as collagen pads, thrombin powder, and fibrin glue can reduce intra-operative blood loss (Lemos, Healy 1996; Emmanuel, McClelland, Page, editors 1997).

Surgical approach may decrease bleeding during EOS (Keating 1998). Parker et al. reported the percentage of patients who underwent hip surgery and received red cell transfusion under different surgical techniques. The authors mentioned that 79% of the patients who had hip arthroplasty required blood transfusion of at least 400ml. On the other hand, 57% of patients who had extra-capsular procedures required blood transfusion (Parker, Handoll 2000).

SPECIFIC TRANSFUSION SPARING INTERVENTIONS

There are transfusion sparing interventions have been mentioned in the literatures to reduce the need of red cell transfusion in patients undergoing EOS [table (4.16)].

Table (4.16): transfusion-sparing interventions for patients undergoing EOS.

Pre-operative autologous blood donation with or without erythropoietin.
Acute isovolaemic haemodilution.
Intra-operative blood salvage.
Post-operative blood salvage.
Drugs to reduce the need of blood transfusion.

PRE-OPERATIVE AUTOLOGOUS DONATION

Pre-operative blood donation is used for patients undergoing elective surgery and likely to have significant peri-operative blood loss which needs blood transfusion. This process involves donation of a number of red cell units by the patient few weeks before surgery and keep the units within the blood bank to be used by the same patient when needed (Thomas 1996; Henry, Carless, Moxey, O'Connell 2002). Patients can donate 10.5ml/kg blood once a week until 72 hours before surgery. In some cases patients may donate twice a week. Iron supplements and/or EPO in many settings can be given to decrease the incidence of phlebotomy-induced anaemia (Biesma, Kraaijenhagen, Poortman, Marx, van de 1992; Baudoux 1996; Desmond, Thomas, Gillon, Fox 1996).

Henry et al. did a Cochrane review to evaluate the effectiveness of autologous blood donation in decreasing the need of allogeneic blood in surgical patients. The authors reviewed 19 randomised control trials, however, only nine of these studies matched the selection criteria. Other studies were rejected because of weakness in their methods, particularly inadequate randomisation techniques, un-blinded observations, and the subjective nature of the outcome variables. Accepted studies reported blood transfusion data but other clinical outcome data could be analysed.

Of the nine accepted studies, four were carried out in the EOS setting. A total of 1119 patients were studied, of whom 566 were randomised to pre-operative autologous blood donation. The authors found that the overall risk of allogeneic blood transfusion was decreased by 63% in patients who underwent pre-operative autologous blood donation. Most of patients (80%) who donated autologous blood pre-operatively received peri-operative blood transfusion (i.e. autologous and/or

allogeneic). Although pre-operative autologous blood donation decreased the use of allogeneic blood, the risk of receiving any blood transfusion (i.e. allogeneic and/or autologous) was increased in patients whom donated blood pre-operatively. The authors did not achieve a definite conclusion about the actual benefits of pre-operative blood donation. The authors reported that the *relative risk of getting exposed to allogeneic blood is 0.37 and 95% CI (0.26 to 0.54)* (Henry, Carless, Moxey, O'Connell 2002).

Table (4.17) summarises other published studies that investigated the role of autologous pre-operative blood donation in reducing the need of allogeneic blood transfusion.

Table (4.17) published studies that investigated the role of autologous pre-operative blood donation in reducing the need of allogeneic blood transfusion.

Author	Aim of the study	Number of subjects	Study intervention	Results
Sculco et al.(Sculco , Gallina 1999)	A retrospective study to show the effectiveness of pre-operative autologous blood donation program as a blood management tool in patients undergoing EOS.	1405	All patients received daily oral iron supplementation several weeks pre-operatively and were divided into 2 groups:	Percentages of patients who were transfused with autologous and/ or allogeneic blood (total blood transfusion) were expressed from the numbers of operated patients.
			Pre-donated blood group (n = 1162).	84% of THR were transfused. 80% of TKA were transfused. The need of allogeneic blood was ranging from 4% to 17% (correlated inversely with pre-donation Hb).
			Non pre-donated group (n = 243).	52% of THR were transfused. 48% of TKA were transfused. The need of allogeneic blood was ranging from 31% to 83% (correlated inversely with pre-operative Hb).

Author	Aim of the study	Number of the subjects	Study intervention	Results		
Julius et al.(Julius, Purchase, Isham, Howard 1994)	To investigate whether the availability of autologous blood would change the transfusion practice in patients undergoing EOS.	236		Mean ± SD estimated intra-operative blood loss (ml).	Mean autologous + allogeneic blood transfusion (units/patients/hospitalisation).	% Patients received allogeneic blood.
			Autologous blood donors (n = 134).	899 ± 981	2.49	22.4%
			Non-autologous donors (n= 102).	976 ± 1.085	3.6	100%
				The authors concluded that the availability of autologous blood resulted in conservative transfusion practice.		
Kiyama H et al.(Kiyama, Ohshima, Imazeki, Yamada 1999)	To investigate the effectiveness of pre-operative blood donation with EPO treatment in reducing the need of allogeneic blood transfusion in anaemic cardiac surgery patients.	32	Patients were divided equally into two groups according to the pre-donation treatment:	Mean number of allogeneic units transfused during surgery.	Mean number of allogeneic units transfused after surgery.	% Patients were not transfused.
			EPO group (n = 16).	0.59 ± 1.12	0.49 ± 1.32	75%
			Iron group (n = 16).	5.01 ± 2.63	0.98 ± 1.01	0%

Author	Aim of the study	Number of subjects	Study intervention	Results
Hatzidakis et al.(Hatzidakis, Mendlick, McKillip, Reddy, Garvin 2000)	A retrospective analysis to identify the risk factors for allogenic blood transfusion and to define the indications for preoperative autologous blood donation in patients undergoing MJS.	489	264 (54%) patients pre-donated blood. Oral iron was given before donation.	The average number of units donated was 2 units per patient (total 527 units were collected). 56% were discarded. 50% of patients who donated blood did not need blood transfusion. From 489 patients, 22% received autologous blood, 12% received allogeneic, and 4% received both autologous and allogeneic. 8% of patients who underwent primary procedures received allogenic blood compared to 33% for patients who had revision surgery.
Toy et al.(Toy, Strauss, Stehling et al. 1987)	A multi-centre retrospective study to evaluate the extent to which pre-donated blood was used and its role in decreasing the demand of allogeneic blood in patients underwent elective surgery.	4996	The authors searched for patients who were eligible for autologous blood donation at the time of their surgery (n = 590).	162 (28%) of the eligible patients underwent EOS. 32 patients (5%)of the eligible patients actually pre-deposited blood, of these 21 (66%) were EOS patients. 13% of patients, who pre-donated blood, received allogeneic blood, as compared with 36% of those who did not pre-deposited. In patients underwent THR & TKA and did not pre-donate blood the rates of homologous transfusion were 72% and 55% respectively compared to 0% of whom pre-donated blood.

Author	Aim of the study	Number of subjects	Study intervention	Results		
Churchill W.H. et al.(Churchill, McGurk, Chapman et al. 1998)	A multi-centre retrospective study to compare transfusion practice in patients undergoing EOS.	2655	2590 patients were eligible for pre-operative blood donation.	% Transfused of pre-donated patients.	Mean units/ transfused patients.	Mean length of hospital stay (days).
			Pre-donated THR (n = 345)	62	2.91 ± 2.29	9.7 ± 6.3
			Non-pre-donated THR (n = 657)	79	2.13 ± 1.12	7.3 ± 2.5
			Pre-donated TKA (n = 315)	35	2.30 ± 1.46	8.3 ± 4.0
			Non-pre-donated TKA (n = 611)	63	1.75 ± 0.99	7.6 ± 3.2

Few studies have shown any evidence that autologous blood offers other benefits to the patient over allogeneic blood. Avall et al did a controlled randomised trial to investigated post-operative inflammatory response after autologous blood transfusion in 31 patients underwent THR. Patients either received allogeneic red blood cells (n = 15) or predeposited autologous whole blood transfusion (n = 16). Plasma concentrations of some inflammatory mediators were measured before, during, and after surgery. The authors found that there was significant increase in plasma concentrations of IL-6 and IL-8 in both groups, with a significantly greater increase in the autologous blood group. The authors suggested that the lower response in the allogeneic group might result from transfusion-induced suppression of cellular immunity (Avall, Hyllner, Bengtson, Carlsson, Bengtsson 1997).

Difficulties for autologous blood transfusion

Pre-operative autologous blood donation can be logistically difficult due to: a) low degree of public interest to donate blood before major surgery (Thomas 1996). Goodnough et al mentioned that up to 25% of hip and 35% of knee replacement patients do not pre-donate autologous blood even in aggressively promoted programs(Goodnough, Vizmeg, Sobecks, Schwarz, Soegiarso 1992a), b) patient's medical condition such as pre-operative anaemia, pregnancy, cardiac patients, active bacterial infection, and coagulation disorders(Thomas 1996; Desmond, Thomas, Gillon, Fox 1996; Hartmann, Winkler, Preis, Germann, Donner, Muller 1997), c) time consuming and expensive(Bierbaum, Callaghan, Galante, Rubash, Tooms, Welch 1999), d) transfusion reactions are likely to occur(Hatzidakis, Mendlick,

McKillip, Reddy, Garvin 2000), and e) errors in labelling and storage (Desmond, Thomas, Gillon, Fox 1996).

ACUTE ISO-VOLAEMIC HAEMODILUTION

Acute iso-volaemic haemodilution is a form of autologous blood donation that can be defined as removal of one or four units of blood from a patient immediately before operation, either before or shortly after induction of anaesthesia, and maintain the circulating volume by crystalloid and/or colloid fluids. This process leads to 20% to 30% decrease in patient's baseline haematocrit level (Lemos, Healy 1996; Napier, Bruce, Chapman et al. 1997; Callaghan 2000).

It is hard to find studies that investigated the role of acute isovolaemic haemodilution in reducing the incidence of allogeneic blood transfusion in EOS setting. Schmied H et al. evaluated the effect of red scavenging, haemodilution and active warming in reducing allogeneic blood transfusion. The authors retrospectively studied 821 patients underwent THR (601 patients) and TKR (220 patients). During the studied period, the authors' institute launched three different transfusion-sparing programs; red cell scavenging, haemodilution, and active warming. As an overall view, the authors found that the presence of such programs decreased the requirement of allogeneic blood significantly. They also found that acute iso-volaemic haemodilution by its own showed a statistically significant effect on reducing the amount of allogeneic blood transfusion. They also found that there was a significant positive relation between the mean pre-operative Hb and the use of iso-volaemic haemodilution technique. Table (4.18) shows the amount of allogeneic blood used before and after these transfusion-sparing programs (Schmied, Kurz, Sessler, Kozek, Reiter 1996).

Table (4.18) The mean (SD) number of allogeneic red cell units per transfused patients (Schmied, Kurz, Sessler, Kozek, Reiter 1996)

	Mean ± SD blood requirement before the program.	Mean ± SD blood requirement after the program.
Transfusion sparing programs (cell salvage, active warming, and haemodilution).	1.3 ± 1.7 units	0.6 ± 1.7 units
Haemodilution only.	1.1 ± 0.9 units	0.4 ± 0.9 units

Some authors suggested that the use of these techniques is impractical in many orthopaedic procedures as most of them are for short duration. During these short blood-loosing procedures, there is great concern of sudden decrease in the patient's Hb level (Napier et al. 1997; Keating 1998). However, Sculco et al mentioned that blood collected by this technique is fresh; its oxygen carrying capacity and coagulation functions are well preserved (Sculco 1995).

Goodnough et al. did two randomised trials to compare acute isovolaemic haemodilution with pre-operative autologous donation in reducing the need for allogeneic red cell transfusion. One of these trials involved patients undergoing TKA and the other trial involved patients who underwent THR. Table (4.19) summarises these studies.

Table (4.19): Published studies to compare the effect of acute isovolaemic haemodilution with pre-operative autologous donation in reducing the need for allogeneic red cell transfusion.

Number of subjects	Study intervention	Results		
32 patients undergoing TKA.(Goodnough, Monk, Despotis, Merkel 1999)	Patients were randomised into 2 groups:	Number of allogeneic red cell transfused.	Number of autologous red cell transfused.	Mean ± SD total number of red cell transfused.
	Patients who underwent autologous donation (n = 17)	11	14	0.6 ± 1.4
	Patients who underwent acute isovolaemic haemodilution (n = 15)	14	30	0.9 ± 1.2
		The authors suggested that there were no differences in using any of these techniques in reducing the need of red cell transfusion.		
48 patients undergoing THR.(Goodnough, Despotis, Merkel, Monk 2000)	Patients were randomised into 2 groups:	Mean ± SD number of allogeneic red cell transfusion.	Mean ± SD number of autologous red cell transfusion.	
	Patients who underwent autologous donation (n = 25)	0	1.1 ± 1.1	
	Patients who underwent acute isovolaemic haemodilution (n = 23)	0.4 ± 0.9	1.65 ± 0.9	
		The authors suggested that acute isovolaemic haemodilution is safe and is as effective as pre-operative autologous donation in reducing the need of allogeneic red cell transfusion.		

INTRA-OPERATIVE BLOOD SALVAGE

Intra-operative blood cell salvage can be used in procedures with acute blood loss of more than 1000 ml (Sculco 1995; Thomas 1996). This process involves collection of autologous blood from the operative site that can be re-infused with or without washing with saline (Mertes, Booke, Van Aken 1997). In a short time 250 ml of autologous blood with haematocrit between 40% and 60% can be re-infused intra-operatively (Lisander, Ivarsson, Jacobsson 1998; Catling, Williams, Fielding 1999). Carless et al explored in a Cochrane review the effectiveness of red cell salvage as a tool of autologous blood transfusion. They reviewed 30 trials comprised 2125 patients. 1073 patients were included in randomised controlled trials. 12 trials involved EOS procedures. The authors concluded that the methodology and the quality of the trials were poor. For example methods of randomisations were inadequate in every study. The authors found that over all the studies the use of red cell salvage reduced the rate of exposure to allogeneic blood by 42%. However, when the relative risk ratio of allogeneic blood transfusion was compared between cardiac surgery (N = 12 trials) and EOS, there was a significantly larger effect of blood salvage when used in EOS procedures (relative risk of allgeneic transfusion was 0.35% in EOS versus 0.82% in cardiac surgery. The authors reported that the *relative risk of getting exposed to allogeneic blood is 0.75 and 95% CI (0.41 to 1.37)* (Carless, Henry, Moxey, O'Connell, Fergusson 2003).

Other studies investigated the effectiveness of this method in reducing the need of allogeneic blood transfusion are summarised below.

Lisander et al. did a prospective observational study to investigate the role of intra-operative red cell salvage in reducing the incidence of allogeneic blood transfusion in

patients undergoing THR. 96 patients were included in the study and intra-operative red cell salvage device was used for all patients. Table (4.20) shows the volume of salvaged red blood cell, number of salvaged red cell units, number of allogeneic blood units transfused, number of patients who did not receive allogeneic blood transfusion, and the baseline and last Hct before hospital discharge for all the study groups.

Table (4.20): Prospective observational study to investigate the role of intra-operative red cell salvage in reducing the incidence of allogeneic blood transfusion in patients undergoing TRH (Lisander, Ivarsson, Jacobsson 1998)

	Primary cemented (N = 49)	Primary non-cemented (N = 11)	Revision hip surgery (N = 36)
Volume of salvaged red cells (mL).	159 (104-23)	166 (119-319)	299 (191-417)
Number of salvaged units (units).	1.1 (0.7-1.6)	1.1 (0.8-2.1)	2 (1.3-2.8)
Number of allogeneic units transfused (units).	0 (0-1)	0 (0-2)	2 (0.5-3)
Percentage of patients not receiving allogeneic blood (%).	69	55	9
Baseline Hct (%).	40	39	40
Hospital discharge Hct (%).	34 (31-37)	35 (32-36)	32 (29-33)

Values are the median (IQR). All red cell unit (i.e. salvaged and allogeniec) = 150 ml.

The authors suggested from this study that blood salvage has limited effectiveness in avoiding allogeneic blood transfusion (Lisander, Ivarsson, Jacobsson 1998).

Shulman et al compared the use of both acute iso-volaemic haemodilution and intra-operative blood salvage in 40 patients underwent THR with another 40 patients who had intra-operative blood salvage alone (control group). Table (4.21) summarises the main findings in the study (Shulman, Grecula, Hadjipavlou 2002).

Table (4.21): Acute isovolaemic haemodilution with intra-operative blood salvage versus intra-operative blood salvage alone in THR (Shulman, Grecula, Hadjipavlou 2002)

	Blood salvage + haemodiultion. (N = 40)	Blood salvage alone. (N = 40)
Total red cell units transfused.	183	146
Mean number of total red cell units transfused per transfused patients.	4.6 ± 2.5 units	3.9 ± 3.7 units
Per patient autologous red cell units (mean)	4 ± 1.4 units	1.9 ± 1.8 units
Percentage of patients who received at least one autologous unit (330ml).	58%	42%
Salvage blood		
Haematocrit level	36% ± 4.4 %	
2,3-diphospholycerate	2.1 ± 0.2 µmol/dL	
Adenosine tri-phosphate	51.8 ± 3.5 µmol/dL	
Mean hospital stay.	6.2 ± 1.2 days	8.4 ± 4.6 days

Abildgaard et al investigated the effectiveness of intra-operative red cell salvage in patients undergoing major EOS. They studies retrospectively 43 patients. 58 % of intra-operative blood loss and 24 % of the total peri-operative blood loss were salvaged, however, 36 of the patients needed allogeneic blood transfusion. The authors suggested that the efficiency of the intra-operative autotransfusion in reducing allogeneic blood transfusion was relatively low mainly because the postoperative blood loss is substantial in patients undergoing major EOS (Abildgaard, Aaro, Lisander 2001).

POST-OPERATIVE BLOOD SALVAGE

Postoperative blood loss can be significant after major joint surgery, particularly after knee arthroplasty where a tourniquet is usually used throughout the procedure (Majkowski, Currie, Newman 1991; Sculco 1995; Millar, editor 2000). In such cases the average post-operative blood loss may exceed 1000 mL (Kurdy 1996; Lotke, Barth, Garino, Cook 1999). In post-operative blood salvage procdure, post-operative blood loss can be drained from the wounds by special drainage system, washed with saline or unwashed, and re-infused to the to the patient (Lemos, Healy 1996; Callaghan 2000).

There are few studies of the efficacy of post-operative red cell salvage alone and its role in reducing the need of allogeneic blood transfusion in patients undergoing EOS. Most of the studies investigated the role of this method combined with pre-operative autologous donation. Table (4.22) summarises some of these studies.

Table (4.22): Published studies of the efficacy of post-operative blood salvage in reducing allogeneic transfusion in patients undergoing MJS.

Author	Study design& aims	Number of patients	Study intervention	Result
Majkowski et al. (Majkowski, Currie, Newman 1991)	Randomised controlled trial to assess the safety and efficacy of postoperative autologous blood salvage and re-infusion in patients undergoing TKA.	40	Allogeneic blood was given post-operatively if Hb fell below 95 g/L or if there were signs of haemodynamic instability. Patients were divided according to the use of post-operative blood salvage device into two groups:	There was no significant difference in the mean total amount of blood drained in the two groups. The authors found that the use of post-operative red cell salvage reduced the need of allogeneic blood by 64%.
			Patients with device (n = 20).	35% required allogeneic blood. Total allogeneic units transfused = 18 units (mean 0.9/patients transfused).
			Patients without device (n = 20).	95% required allogeneic blood. Total allogeneic units transfused = 50 units (mean 2.5/ patients transused.
Rizzi L.et al.(Rizzi, Bertacchi, Ghezzi, Bellavita, Scudeller 1998)	Prospective study to assess the efficacy and limitations of post-operative blood salvage in patients undergoing MJS.	161	155 THR and 26 TKA.	63% of the post-operative collected blood was re-infused. 39 patients (24%) needed an allogeneic blood transfusion, of these, 24 underwent primary THA, 13 revisions and 2 unilateral TKA.

Author	Study design& aims	Number of patients	Intervention	Result				
Berman et al.(Berman, Levenberg, Tropiano, Parks, Bosacco 1996)	Prospective study to evaluate the quality of salvaged blood, safety of auto-transfusion, and the role of pos-operative red salvage in minimizing allogeneic blood transfusion in patients undergoing TKA.	100	The authors compared 2 groups of patients:	The control group needed an average of 1.8 units of allogeneic blood per TKA. The authors did not mention the numbers in the study groups. However, they suggested that post-operative red cell salvage decreased the need of allogeneic blood transfusion. Characteristics of the salvaged blood were compared in the study groups (mean values). The authors did not report these parameters for the control group.				
				Hb	Hct	Free Hb	Deferential WBCs	Fibrin split products
			25 patients had calcium binding resin or acid citrate dextrose anti-coagulant in the collection canister.	118.5	36.9	214.8	59.7	<50
			25 patients had acid citrate dextrose anti-coagulant in the collection canister.	77	23.3	65.5	69.0	516
			Student T tests to compare statistical difference of both groups (P< 0.05 when T > 2.02; T<-2.02).	-5.79	-6.2	-2.43	3.11	-15.84
			50 patients (control group) had slandered drains.					

Author	Study design& aims	Number of patients	Intervention	Result
Grosvenor et al.(Grosvenor, Goyal, Goodman 2000)	Retrospective, case control study to evaluate the effect of post-operative blood salvage on the need for allogeneic transfusion in patients undergoing THR.	156	2 groups of patients:	
			82 patients had post-operative blood salvage devices.	10% received allogeneic blood transfusion.
			74 did not have post-operative blood salvage devices.	23% received allogeneic blood transfusion.
				Multi-variate analysis showed that post-operative blood salvage decreased the risk of allogeneic blood transfusion (approximately 10 times).
Xenakis et al.(Xenakis, Malizos, Dailiana et al. 1997)	Prospective study to determine the effect of post-operative red salvage alone and in combination of with pre-operative autologous donation on the transfusion requirements in patients undergoing MJS.	375	Patients were divided into 3 groups:	The mean number of allogeneic blood transfused were as follow:
			117 patients received allogeneic blood (control group).	4.5 units per THR transfused, 3.5 units per TKA transfused, and 4.2 units per all patients transfused.
			208 patients were managed with postoperative blood salvage.	2.7 units per patients transfused.
			50 patients pre-donated blood and had pos-operative blood salvage devices.	1.7 units per patients transfused. The combination of red cell salvage and pre-donation were associated with lower need of allogeneic blood.

Author	Study design& aims	Number of patients	Intervention	Result			
Ayers et al.(Ayers, Murray, Duerr 1995)	A prospective randomised study to assess the efficacy of post-operative red cell salvage in reducing the need of allogeneic blood in patients undergoing THR.	232	Patients were randomised into one of two groups, both of them were advised to pre-donate blood:	% Of patients who received allogeneic blood transfusion:			
				Primary with pre-donation.	Primary & no pre-donation	Revision with pre-donation.	Revision & no pre-donation.
				(N = 124)	(N = 32)	(N = 63)	(N = 13)
			103 had post-operative red cell salvage.	0%	10%	32%	60%
			129 patients did not have post-operative red cell salvage devices.	1%	63%	19%	100%
Dalen et al.(Dalen, Skak, Thorsen, Fredin 1996)	Prospective control trial to show the efficacy of post-operative red cell salvage in reducing the need for allogeneic blood transfusion in patients undergoing TKA.	117	Patients were divided into 2 groups:	The mean number of allogeneic red cell units transfused per transfused patients were:			
			58 patients had post-operative red salvage devices (prospective group).	2.1 units.			
			59 patients had standard drains studied (retrospective group).	2.5 units.			

Hazards of red cell salvage

Some authors showed some adverse effects of re-infusing the salvaged blood to the patients during or after surgery. These adverse effects are: a) Anaemia due to infusion of diluted blood, b) thrombocytopenia and coagulopathy due to loss of platelets during salvage, c) sepsis bacterial contamination is likely to occur with such devices, and d) microemboli due to the ineffective filtering system (Desmond, Thomas, Gillon, Fox 1996; Huet, Salmi, Fergusson, Koopman-van Gemert, Rubens, Laupacis 1999).

DRUGS USED TO DECREASE THE NEED FOR ALLOGENIC BLOOD

Henry et al did a Cochrane review to investigate the role of anti-fibrinolytic drugs such as aprotinin, tranexamic acid, and epsilon aminocaproic acid, on peri-operative blood transfusion. The authors reviewed 61 trials of aprotinin compromised 7027 patients. They found that aprotinin reduced the rate of blood transfusion by 30% and decrease post-operative bleeding. The authors also reviewed 18 trials of tranexamic acid comprising 1342 patients. They found that tranexamic acid decreased the rate of blood transfusion by 34%. However, the authors reviewed four trials of epsilon aminocaproic acid comprising 208 patients and they found that the use of epsilon aminocaproic acid had non-significant reduction in blood transfusion. The authors concluded that aprotinin could reduce the need for blood transfusion and decrease post-operative bleeding without side effects. The authors mentioned that there were similar trends for the other two drugs (Henry, Moxey, Carless, O'Connell 2002a).

Henry et al did another Cochrane review to investigate the role of desmopressin in minimising peri-operative allogeneic blood transfusion in patients who do not have

congenital bleeding disorders. The authors reviewed 14 randomised controlled parallel group trials comprised 1034 patients. The authors found that there was no evidence that desmopressin can decrease peri-operative allogeneic blood transfusion in patients who do not have congenital bleeding disorders (Henry, Moxey, Carless, O'Connell 2002b).

Some other studies investigated the use of Tranexamic acid and Aprotinin. Hiippala et al. did a randomised control study to investigate the use of short-term anti-fibrinolytic (Tranexamic acid) therapy in reducing post-operative blood loss in patients undergoing TKA. The authors hypothesised that application of a limb tourniquet enhances local fibrinolysis and the use of such treatment may reduce blood loss through the inhibition of the fibrinolytics. They studied 77 patients, 39 patients received the treatment intra-operatively and 38 received normal saline and served as a control group. Peri-operative blood loss was measured and the number of red cell units transfused was recorded. The authors found that the amount of peri-operative blood loss in the control group was a significantly greater compared with the study group. They also found that the number of red cell units transfused was significantly higher in the control group than that in the study group. The authors suggested that the use of such treatment had a significant effect on reducing the need of red cell transfusion. Table (4.23) summarises the results of the study (Hiippala, Strid, Wennerstrand et al. 1997).

Table (4.23): The effect of the use of short-term anti-fibrinolytic (Tranexamic acid) therapy in reducing post-operative blood loss in patients undergoing TKA (Hiippala et al. 1997)

	Treatment group (N = 39).	Control group (N = 38).
Mean Peri-operative blood loss.	689 ± 289 ml	1509 ± 643 ml
Mean number of red cell units transfused/ patients transfused.	1 ± 1.2	3.1 ± 1.6

Veien et al also studied Tranexamic acid in 30 patients underwent TKR in a randomised control trial. The patients were divided into two groups, 15 patients each. One group received Tranexamic acid and the other did not and served as a control group. The number of patients who were transfused with allogeneic blood was higher in the control group. There was a significant reduction in mean total blood loss in the study group compared with the control group. Table (4.24) summarises the results of the study (Veien, Sorensen, Madsen, Juelsgaard 2002).

Table (4.24): The effect of the use of Tranexamic acid in reducing total blood loss in patients undergoing TKR(Veien, Sorensen, Madsen, Juelsgaard 2002)

	Treatment group (n = 15)	Control group (n = 15)
% Of patients who received allogeneic blood.	0%	13%
Mean total blood loss.	409.7 ± 174.9ml	716.7 ± 313.1 ml

Aprotinin has also been studied to investigate its role in reducing blood loss and transfusion in patients undergoing major EOS. Aprotinin is a proteinase inhibitor derived from bovine lung that has anti-fibrinolytic properties. Aprotinin acts as an

inhibitor of human trypsin, plasmin, plasma-kallikrein and tissue kallikrein, thus inhibiting fibrinolysis. It also inhibits the contact phase activation of coagulation that both initiates coagulation and promotes fibrinolysis.

Murkin et al. studied 53 patients underwent bilateral or revision THR. They divided the patients into two groups. Treatment group received Aprotinin and a placebo group who served as a control group. The authors found that the mean peri-operative blood loss was significantly lower in the study group compared with the control group [table (4.25)] (Murkin, Shannon, Bourne, Rorabeck, Cruickshank, Wyile 1995).

Table (4.25): The role of Aprotinin in reducing blood loss for patients undergoing THR (Murkin, Shannon, Bourne, Rorabeck, Cruickshank, Wyile 1995)

	Treatment group (n = 29).	Control group (n = 24).
Mean peri-operative blood loss.	1498 ± 110 ml	2069 ± 223 ml
% Of patients transfused.	62.1%	70.8%
Mean number of units transfused/patients transfused	2 ± 0.2	2.9 ± 0.4

Capdevila V et al studied the role of Aprotinin in reducing the need of allogeneic blood transfusion in patients undergoing major EOS. The authors studied 23 patients, 12 patients received Aprotinin and 11 patients did not and used as a control group. Total blood loss was lower in the study group by 65% than in the control group. The study group had 67% lower intra-operative blood transfusion requirements than the control group [table (4.26)]. There were no adverse effects of the drug such as deep vein thrombosis. The authors suggested that Aprotinin reduced bleeding and

allogeneic blood transfusion dramatically in patients undergoing major EOS without adverse effects (Capdevila, Calvet, Biboulet, Biron, Rubenovitch, d'Athis 1998).

Table (4.26): The role of Aprotinin in decreasing blood loss in patients undergoing major EOS (Capdevila, Calvet, Biboulet, Biron, Rubenovitch, d'Athis 1998)

	Treatment group (N = 12).	Control group (N = 11).
Median (range) total blood loss.	5305 (3000-9770) ml	1783 (1140-4955) ml
Median (range) number of red cell units transfused/ patients transfused.	7 (4-16)	3 (2-5)

POST-OPERATIVE CARE FOR PATIENTS UNDERGOING EOS.

There are many issues which should be considered during post-operative period in patients undergoing EOS that can minimize the need for red cell transfusion.

TEMPERATURE CONTROL

As mentioned before hypothermia may aggravate bleeding by impairing platelet function and directly reducing clotting factor enzyme function. Optimising patients' body temperature may decrease post-opeative bleeding and the need for blood transfusion (Rohrer, Natale 1992; Schmied, Kurz, Sessler, Kozek, Reiter 1996).

POST-OPERATIVE ANAEMIA

Anaemia after surgery could be explained by blood loss during and after operation. However, some authors referred it as a manifestation of post-operative acute phase inflammatory response. Others mentioned that the mechanism of postoperative anaemia remains unknown (Biesma, van de, Beguin, Kraaijenhagen, Marx 1995; van Iperen, Kraaijenhagen, Biesma, Beguin, Marx, van de 1998).

Patients undergoing EOS especially major joint replacement are often malnourished at the time of surgery. Post-operative period, the actual amount of iron absorption from food may be limited by poor dietary intake and impaired intestinal function and could further contribute to iron deficiency anaemia (Zauber et al. 1992; Rock, Jr., Meeks 2001).

Avenell et al. did a Cochrane review to assess the effects of nutritional interventions in elderly people recovering from hip fracture. The authors reviewed 15 randomised trials comprising 1054 participants. Overall the quality of trials was poor; specifically in terms of allocation concealment, assessor blinding and intention to treat analysis. Some of the studies suggested that high protein diet might decrease the period of stay in rehabilitation hospitals. On the other hand, few trials investigated the role of vitamin B1 and vitamin D supplements on the rate of recovery. The authors found no evidence of benefit for either vitamin supplement. They concluded that there is very weak evidence that high protein diet might affect the rate of recovery in elderly patients recovering from hip fracture (Avenell 2002).

Little information was found on the causes of post-operative anaemia and its pathophysiology. Biesma et al investigated the pathophysiology of post-operative anaemia in 48 patients who underwent THR and had normal levels of folic acid, vitamin B12, and iron indices. Patients had no signs of post-operative fever or inflammation were only included in the study. During the study they measured different variables of erythropoiesis, iron metabolism, and markers of inflammation during recovery from surgery for the patients who received blood transfusion and who did not. Most of patients were females (43 patients) with a mean age of 71.6 years. The authors found that C-reactive protein (CRP) increased significantly on day

4 post-operatively from a mean of 4 mg/L to 82 mg/L (the maximum level during the follow up period). CRP fell after day 4 post-operatively, was high until day 14, but returned to its baseline level on day 42.

IL-6 was undetected pre-operatively, however, peaked on first day post-operatively and was undetected again by day 7.

Hb, iron, transferrin, and ferritin concentrations were not influenced by iron therapy during the postoperative period. There were no differences of erythropoietic and iron variables between transfused and non-transfused patients. The authors suggested that post-operative anaemia is a form of "anaemia of chronic disease" due to the presence of post-operative inflammatory mediators. They hypothesized that there is decreased response of erythropoietin. This leads to a doubt about the effectiveness of using either erythropoietin and/or iron therapy in treating this type of anaemia. Table (4.27) summarizes laboratory findings for patients included in the study (Biesma, van de, Beguin, Kraaijenhagen, Marx 1995).

Table (4.27): Mean variables of post-operative erythropoiesis and iron metabolism for patients who received blood transfusion and who did not (Biesma, van de, Beguin, Kraaijenhagen, Marx 1995)

Variables (mean values)	Day -1	Day 1	Day 4	Day 7	Day 10	Day 14	Day 42
Hb (g/L)							
Transfused	13.7	11.3	11.4	11.6	11.3	11.6	13
Not transfused	14.3	11.1	11.1	11.3	11	11.8	13.4
Serum erythropoietien (U/L)							
Transfused	5	12	12	17	9	7	7
Not transfused	5	16	17	18	13	9	4
Reticulocyte count (x10*9/L)							
Transfused	43	49	62	78	71	64	48
Not transfused	58	49	66	136	97	94	53
Serum transferring receptors (µg/L)							
Transfused	4.2	2.9	4.1	5.3	3.9	4.7	5.6
Not transfused	3.8	2.7	3.2	3.3	3.5	3.8	4.3
Serum ferritin (µg/L)							
Transfused	84	188	175	177	182	192	100
Not transfused	92	145	152	135	124	168	67
Serum iron (µmol/L)							
Transfused	15	5	6	9	8	10	11
Not transfused	17	4	7	6	7	7	13
Serum transferring (d/L)							
Transfused	2.7	1.9	2	2.2	2.2	2.4	2.8
Not transfused	2.9	1.9	2.1	2.2	2.4	2.4	2.8
Transferrin saturation (%)							
Transfused	22	11	11	16	14	16	16
Not transfused	24	7	12	11	11	12	19

Management of post-operative anaemia.

Very few studies have investigated different managements of post-operative anaemia in patients undergoing EOS. Zauber et al. did a randomised controlled trial to assess the role of oral iron in treating post-operative anaemia following hip surgery. All patients had normal iron stores and normoblastic erythropoiesis (revealed from the surgically removed femoral head). Patients were randomised to receive either oral ferrous sulphate during their hospital stay (N = 37) or placebo (N = 42). The authors did not find statistically significant differences in the rate of Hb or reticulocyte recovery. They suggested that administration of oral iron postoperatively to elderly orthopaedic patients with adequate tissue iron stores did not hasten the recovery of haemoglobin levels. Table (4.28) summarises laboratory findings during the follow up period (Zauber et al. 1992).

Table (4.28): The role of oral iron in treating post-operative anaemia in patients undergoing hip surgery (Zauber et al. 1992)

	Females		Males		Reticulocyte count	
	Iron group	Control group	Iron group	Control group	Iron group	Control group
Baseline	133 ± 14	135 ± 9	144 ± 15	144 ± 15	17 ± 7	13 ± 6
Week 1	109 ± 9	110 ± 11	113 ± 17	107 ± 7	21 ± 9	21 ± 9
Week 2	109 ± 10	111 ± 10	114 ± 10	112 ± 8	27 ± 10	34 ± 17
Week 3	111 ± 14	112 ± 8	107 ± 3	108 ±8	27 ±19	34 ± 17

Mean Hb (g/L), reticulocyte count (x 10^{-3})

In another surgical setting, Crosby et al. evaluated the role of iron in treating acute blood loss anaemia in elderly patients undergoing coronary artery bypass surgery. 121 elderly patients were included in the study. Patients were randomised to one of four groups: control group, placebo group, low iron dose group, and usual iron dose

group. Treatment started post-operatively and for 8 weeks. The authors found that both Hb and Hct decreased 6 days post-operatively for all patients to a mean of 95 ± 12 g/L and 28% ±2.3%. Mean Hb and Hct levels returned to near normal values, 136 ± 9.7 g/L & 40.6% ± 3% at the end of the study. Iron stores remained within normal ranges for most subjects regardless of randomisation. The authors suggested that the use of iron in treating acute blood loss anaemia did not help in Hb recovery or change iron stores post-operatively (Crosby, Palarski, Cottington, Cmolik 1994).

DEFINITIONS

- *Patients administration system (PAS):* A database maintained by the hospital's records department that covers all of hospital admissions and contains patients' identifiers, hospital admission date, hospital discharge date, and the surgical procedure.
- *Patient's identifiers & fields of linkage:* Patient's forename, surname, gender, date of birth, and hospital number.
- *Hospital Laboratory database:* A database that contains haematological results, samples request date, and a code that indicates the source of the blood sample (hospital and ward) all are linked to patients' identifiers.
- *Preoperative /Pre-admission Hb:* Hb value that had been requested within one month before admission and including the day of admission. If there were more than one Hb value within that month, the pre-admission Hb is defined as the Hb value with a sample request date closer to the date of admission.
- *Pre-operative / Pre-admission haematology results:* Haemoglobin *[Hb]*, mean red cell volume *[MCV]*, and mean red cell corpuscular haemoglobin *[MCH]* values that had been requested within one month before admission. If there were more than one result within that month, the pre-admission haematology results is defined as the result with a sample request date closer to the date of admission.
- *Anaemia:* Haemoglobin concentration <130 g/L for males and <115 for females.
- *SNBTS Blood Bank Patient Database:* A database, for the study hospital, that contains patients' identifiers, records of blood samples received for group and

save procedure, and number of blood products assigned for identified patient, issued to and returned unused by that patient.

- *Blood Bank Patient record:* any records of an event in which a patient's blood sample is received for group and save, or one or more red cell units are assigned.
- *Group and save [G&S]:* An event in which a blood sample is received by the blood bank for blood grouping and detection of red cell antibodies and is saved for 3 days maximum in the blood bank (no red cell units assigned).
- *Red cell units assigned:* An event in which one or more individually identified red cell units are assigned to an identified patient: (often referred to as "Issue" or "Cross match").
- *Transfusion episode:* Any admission record with a number of (red cell units issued) – (number of red cell returned) > 0.
- *Transfused patient:* A patient who appeared in the blood bank database with one or more admissions in which the number of red cell units issued is more than the number returned.
- *Princess Margaret Rose Hospital [PMR]:* The main teaching orthopaedic Hospital (Edinburgh).
- *EOS:* Elective orthopaedic surgery.
- *THR:* Total hip arthroplasty or replacement.
- *TKA - TKR*: Total knee arthroplasty or replacement.
- *MJS:* Major joint surgery (THR + TKA).
- *Other Procedure:* Any orthopaedic procedure rather than MJS

MATERIAL AND METHODS

AIMS

PRIMARY AIM

To determine the prevalence and type of pre-operative anaemia in patients undergoing elective orthopaedic surgery.

SECONDARY AIM

To determine the prevalence of peri-operative red cell transfusion

To determine the association between the rate of red cell transfusion and pre-operative Hb level.

PATIENTS STUDIED

A large cohort of patients from a specialized orthopaedic hospital performing only elective surgical procedures [PMR] was used for this study, for which haematological and transfusion data were retrospectively extracted from existing databases.

STUDY PERIOD

All inpatients admitted to PMR from 1/8/2000 to 31/7/2001.

STUDY DESIGN

The study was designed to use only data that had been recorded during routine patient management and was available in the hospital's computerized information systems.

Three datasets were accessed:

- Patient dataset [from PAS database].
- Haematology dataset [from the Hospital Laboratory database].
- Transfusion dataset [from the Blood Bank Patient database].

ETHICS AND CONFIDENTIALITY

A senior consultant anaesthetist in the clinical unit sponsored the study. It was carried out in the form of a retrospective audit. No interventions were carried out and patient management was not altered in any way on the basis of the data collected. We obtained guidance from the Local Regional Ethics Committee that ethical review was not required. Guidelines from the data protection act were followed and anonymous data after linkage were done. Patients' identifiers were deleted and each linked record was assigned a key number that was used as a primary key in analyzing the data.

DATASET FORMAT

Each dataset was obtained in Excel files.

DATA MANAGEMENT

Datasets were imported to Microsoft Access for linking.

PAS DATASET

From the orthopaedic hospital PAS database, patients' identifiers were obtained. The extracted fields for each patient are shown in table (4.29).

Addition of haematology laboratory data to PAS data

This process involved the linking of the each patient record to an extract from the haematological laboratory database, using the patient identifier.

The extract from the laboratory database contained all the specified haematological results (Hb values and red cell indices) on patients' samples submitted from PMR between 1/7/2000 and 31/7/2001 (1 month before study period).

The process of linkage is described in detail in appendix (1). The extracted fields are shown in table (4.30)

Addition of Transfusion data to PAS data

This process involved linking of each patient record, using the patient identifiers to make an extract from the blood bank patients database.

From the blood bank database we obtained all transfusion records for all patients who had any request forms originating from PMR between 1/7/2000 and 31/8/2001. The extract from the blood transfusion database for each patient is shown in table is shown in table (4.31)

Table (4.29): Extracted fields from PAS

Field	Content
Surname	Alpha characters <30 characters
Forename	Alpha characters <30 characters
Sex	M/F
Date of birth	dd/mm/ccyy
Age	Numeric value
Hospital ID	ISD Hospital code
Date of hospital admission	dd/mm/ccyy
Date of hospital discharge	dd/mm/ccyy
ICSD code	
Operation code	

Table (4.30): Extracted fields from the hospital laboratory database

Field	Content
Hospital ID	ISD Hospital code
Surname	Alpha characters <30 characters
Forename	Alpha characters <30 characters
Sex	M/F
Date of birth	dd/mm/ccyy
Sample request date	dd/mm/ccyy
Haematological results	
Haemoglobin concentration (g/L)	Numeric value
Mean red cell corpuscular volume (MCV)	Numeric value
Mean red cell corpuscular haemoglobin (MCH)	Numeric value

Table (4.31): Extracted fields from the blood bank patients database

Field	Content
Hospital ID	ISD Hospital code
Surname	Alpha characters <30 characters
Forename	Alpha characters <30 characters
Sex	M/F
Date of birth	dd/mm/ccyy
Sample request date	dd/mm/ccyy
Group and save	G&S
Units cross-matched	Numeric value
Number of red blood units cell issued	Numeric value
Number of red cell units returned	Numeric value

LINKAGE PROCESS

Figures (4.1, 4.2, and 4.3) illustrate the linkage procss of different datasets to obtain the final database for analysis.

STUDY DATABASE

From the linked PAS dataset, the laboratory dataset, and the hospital blood bank dataset, a study database was prepared consisting of, for each admission, the PAS data with any linked haematology and transfusion data. Patients' identifiers were deleted and a unique key number was assigned for each admission for further analysis. Table (4.32) shows the fields that were included in study dataset.
After the linkage process was completed, some patients had more than one haematological record. For the purpose of the study, the pre-admission haematological record was defined as a haematological result, with sample request date within one month before the hospital admission date [if there was more than one

result, the one closest to admission date was used in the analysis]. The rest of the haematological records were ignored (deleted).

After linking the blood transfusion dataset, some patients had more than one transfusion record. For the group and screen records, the events related to the study were defined as (1) the records of G&S requests submitted within one month before admission and during hospital stay (the period between hospital admission and discharge dates). (2) Red Cell Units Assigned (RCUA) and (3) Red Cell Units "Transfused" (RCU "T") were defined as the records created by the Blood Bank within one week before hospital admission and during the hospital stay.

Table (4.32): Fields of the study dataset

Fields	Contents
Study Index number	Numeric value
Sex	M/F
Age	Numeric value
Hospital ID	ISD Hospital code
Date of hospital admission	dd/mm/ccyy
Date of hospital discharge	dd/mm/ccyy
Haematological results	
Sample request date	dd/mm/ccyy
Haemoglobin concentration (g/L)	Numeric value
Mean red cell corpuscular volume (MCV)	Numeric value
Mean red cell corpuscular haemoglobin (MCH)	Numeric value
Blood transfusion data	
Sample request date	dd/mm/ccyy
Group and screen	G&S
Units of red cells crossmatched	Numeric value
Number of red cell units issued	Numeric value
Number of red cell units returned	Numeric value
Number of red blood cell units "transfused" (number of units issued – number of units returned)	Numeric value

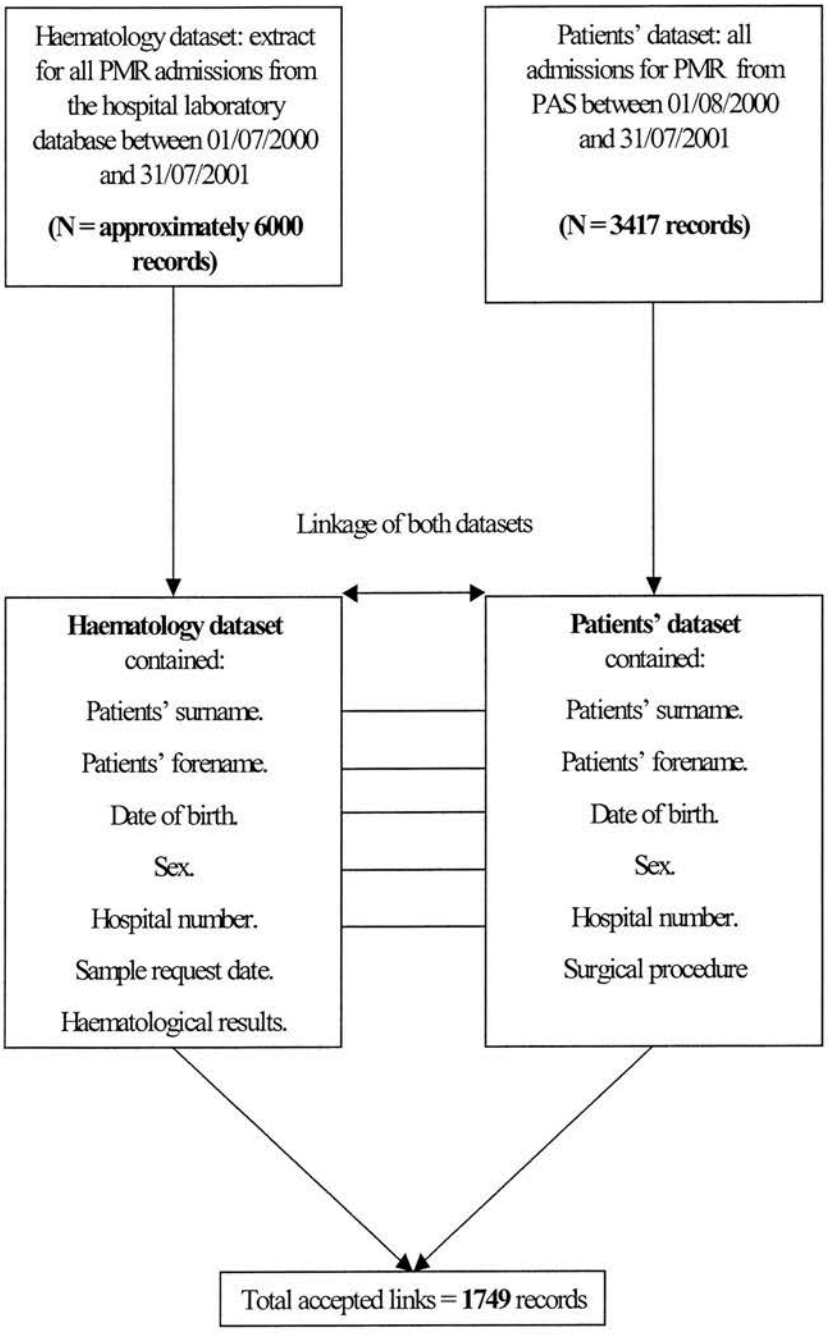


Figure (4.1): Flow chart to illustrate the linkage between PAS dataset and the hospital laboratory dataset

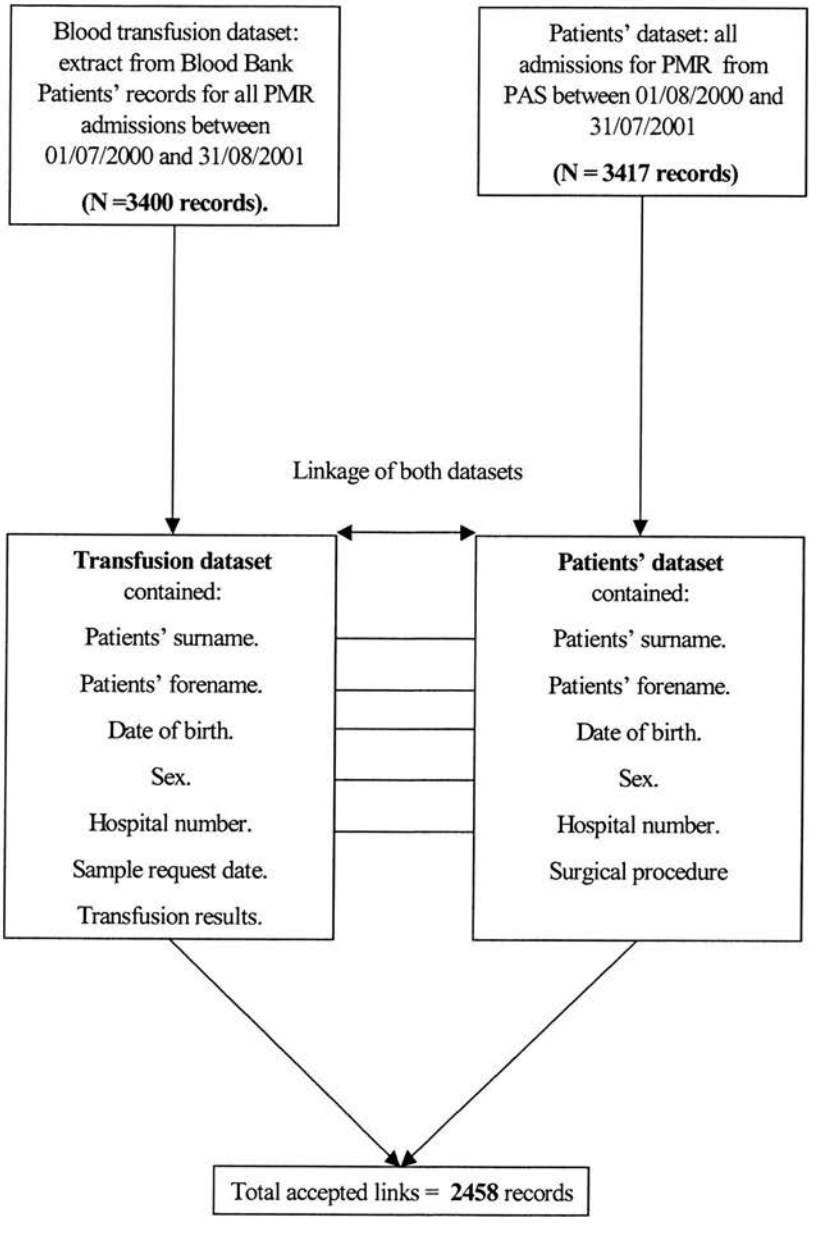


Figure (4.2): Flow chart to illustrate the linkage between PAS dataset and the patients blood bank dataset

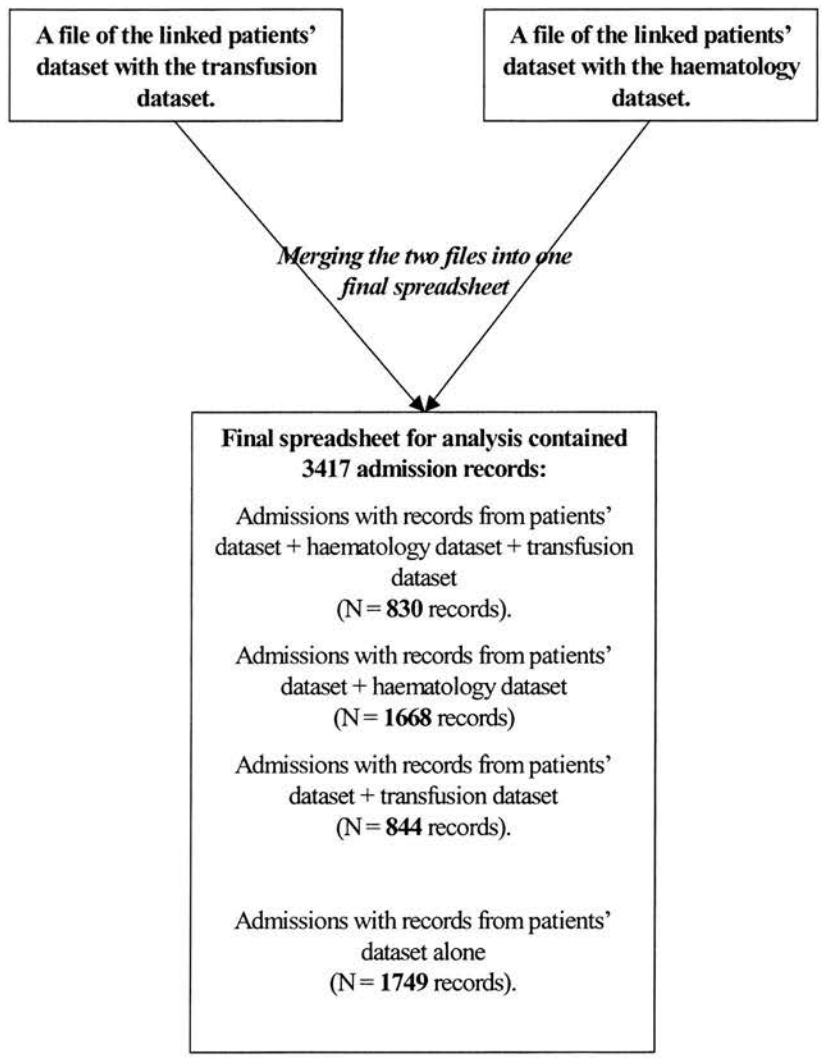


Figure (4.3): Flow chart to illustrate the final spread sheet used for analysis

RESULTS

PATIENTS' CHARACTERISTICS: THE PATIENTS' DATASET.

3417 admissions were recorded in PAS that comprised 3257 patients between 01/08/2000 and 31/07/2001. Numbers of patients who were admitted more than once are shown in table (4.33). 1696(49.6%) of admissions were males and 1721(50.4%) were females. The mean (SD) and median [IQR, (range)] age (years) for all admissions during the study were 54.6 (18.4) and 57 [40 to 70, (12 to 93). There were no admissions in age band 21 to 30 years old; table (4.34) shows the number of admissions in different age bands.

1322 (38.6%) admissions underwent major joint surgery (MJS). 2095 (61.4%) underwent other different orthopaedic procedures. Table (4.35) shows the number (%) of admissions in different EOS procedures. Table (4.36) shows the general characteristics of admissions undergoing different procedures. Table (4.34) shows the characteristics of admissions in which MJS was performed.

Table (4.33): Total number of patients who had different numbers of admissions during the study period.

	Number of patients who had one admission	Number of patients who had two admissions	Number of patients who had three admissions	Number of patients who had four admissions	Total number of patients included in the study.
Hip	580	20	1	0	601
Knee	503	26	0	0	529
Revision hip	89	8	0	0	97
Revision knee	37	1	0	0	38
Other	1898	77	10	2	1987
Revision other	5	0	0	0	5
Total	**3112**	**132**	**11**	**2**	**3257**

1610 (49.4%) of these patients were males and 1647 (50.6%) were females.

Table (4.34): Number (%) of all admissions in different age bands.

Age Bands (years)	Males (n= 1696) N (%)	Females (n= 1721) N (%)	Total admissions (n= 3417) N (%)
10-20	67/1696 (4)	50/1721 (3)	117/3417 (3)
21-30	0/1696	0/1721	0/3417
31-40	491/1696 (29)	265/1721 (15)	756/3417 (22)
41-50	249/1696 (15)	199/1721 (12)	448/3417 (13)
51-60	270/1696 (16)	312/1721 (19)	582/3417 (17)
61-70	321/1696 (19)	392/1721 (23)	713/3417 (21)
71-80	246/1696 (14)	388/1721 (22)	634/3417 (19)
81-90	51/1696 (3)	110/1721 (6)	161/3417 (5)
>90	1/1696 (<1)	5/1721 (<1)	6/3417 (<1)

Table (4.35): Number (%) of admissions in which different EOS procedures were carried out.

Procedures	Males (n= 1696) N (%)	Females (n= 1721) N (%)	Total (n= 3417) N (%)
THR	249/1696 (15)	374/1721 (23)	623/3417 (19)
TKR	245/1696 (14)	310/1721 (18)	555/3417 (16)
Revision hip	46/1696 (3)	59/1721 (3)	105/3417 (3)
Revision knee	19/1696 (1)	20/1721 (1)	39/3417 (1)
Revision other	3/1696 (<1)	2/1721 (<1)	5/3417 (<1)
Other:	1134/1696 (67)	956/1721 (55)	2090/3417 (61)
Soft tissue	132	145	277
Spine	79	113	192
Construction	168	230	398
Other joints	195	236	431
Endoscopy	512	218	730
Miscellaneous	48	14	62

Table (4.36): General characteristics of all admissions.

	Variables	Mean ± SD	Median	Q1	Q3	Range
MJS	Age (years)	68 ± 11	69	62	76	15-91
	LOS (days)	8.3 ± 5.2	7	6	9	0-99
	Pre-admission Hb (g/dl)	131 ± 13.8	132	123	141	76-167
Spine surgery	Age (years)	49 ± 17.4	49	36	61	13-85
	LOS (days)	3.6 ± 3.7	3	0	5	0-17
	Pre-admission Hb (g/dl)	139 ± 13.3	142	129	148	104-160
Construction surgery	Age (years)	44 ± 18	50	29	57	12-93
	LOS (days0	3 ± 7.5	2	0	3	0-120
	Pre-admission Hb (g/dl)	135 ± 13.3	136	126	143	96-164
Other joint surgery	Age (years)	53 ± 18.1	54	39	68	15-92
	LOS (days)	3.7 ± 8	2	1	5	0-99
	Pre-admission Hb (g/dl)	130 ± 15.9	132	121	141	76-167
Endoscopy	Age (years)	41 ± 14.1	40	29	51	15-86
	LOS (days)	0.4 ± 1.9	0	0	0	0-34
	Pre-admission Hb (g/dl)	139 ± 11.8	139	131	146	110-184
Miscellaneous	Age (years)	50 ± 19.5	54	36	64	16-84
	LOS (days)	3.6 ± 5.9	1	0	3	0-21
	Pre-admission Hb (g/dl)	129 ± 18.2	129	120	144	82-152

Table (4.37): General characteristics of admissions that underwent MJS.

	Variables	Mean ± SD	Median	Q1	Q3	Range
Hip	Age (years)	66 ± 12.3	68	60	75	15-91
	LOS (days)	7.8 ± 3.7	7	6	8	1-45
	Pre-admission Hb (g/dl)	131 ± 13.4	131	123	139	84-175
Knee	Age (years)	70 ± 8.8	71	64	76	26-91
	LOS (days)	7.9 ± 3.1	7	6	9	0-36
	Pre-admission Hb (g/dl)	133 ± 13.5	133	125	141	82-170
Revision hip	Age (years)	68 ± 11.8	71	61	76	34-88
	LOS (days0	12.8 ± 10.7	8	7	14	1-64
	Pre-admission Hb (g/dl)	130 ± 15.4	131	122	141	90-161
Revision knee	Age (years)	70 ± 9.4	71	65	76	46-87
	LOS (days)	12.8 ± 10.7	9	7	111	1-86
	Pre-admission Hb (g/dl)	130 ± 15.4	126	110	143	87-159

Linkage results [haematology data]

Of the linked haematology records, 1631 were complete matches and 118 were partial matches but were accepted as true matches on visual inspection.

Ten patients had two haematological results one month before hospital admission date. The result with the nearest request date to hospital admission date was used in the analysis. 12 anaemic female patients (Hb $\leq$ 115 g/L) had no MCV or MCH records in the haematology database. The linkage process and the numbers of linked records are described previously in figure (4.3).

HAEMATOLOGICAL RESULTS

1749 (51.2%) admissions had pre-admission haematological results. Of these 1142 (63.7%) admissions were for MJS. Table (4.38) shows the number and percentage of admissions with and without pre-admission haematological results that underwent different procedures.

The mean (SD) and median [IQR, range] pre-admission haemoglobin (g/L) for all admissions with haematology records were [132 (14.38) and 133 [124 to 142, (76 – 184)]. Table (4.39) and figure (4.5) show the numbers of males and females in different Hb bands. Figure (4.6) shows the number of all admissions in different Hb bands. Figure (4.7) shows mean pre-admission Hb levels in different procedures. Figure (4.8) shows mean pre-admission Hb levels for different age bands.

299 (17%) admissions were anaemic [Hb < hospital laboratory reference range: males <130g/L and females <115g/L]. Of these 154 (51.5%) were males and 145 (48.5%) were females. Table (4.40) shows number (%) of anaemic admissions in different procedures.

For all anaemic admissions that had Hb results, 64% had normocytic normochromic anaemia before hospital admission. The prevalence of different types of anaemia for all admitted anaemic males and females is shown in table (4.41). Figure (4.9) shows the percentage of all anaemic admissions with different types of anaemia.

MCV and MCH results were available for 211 anaemic admissions that underwent MJS. Table (4.42) shows the number (%) of males and females admissions with different types of anaemia that underwent MJS. Figure (4.10) shows the percentage of all MJS admissions with different types of anaemia.

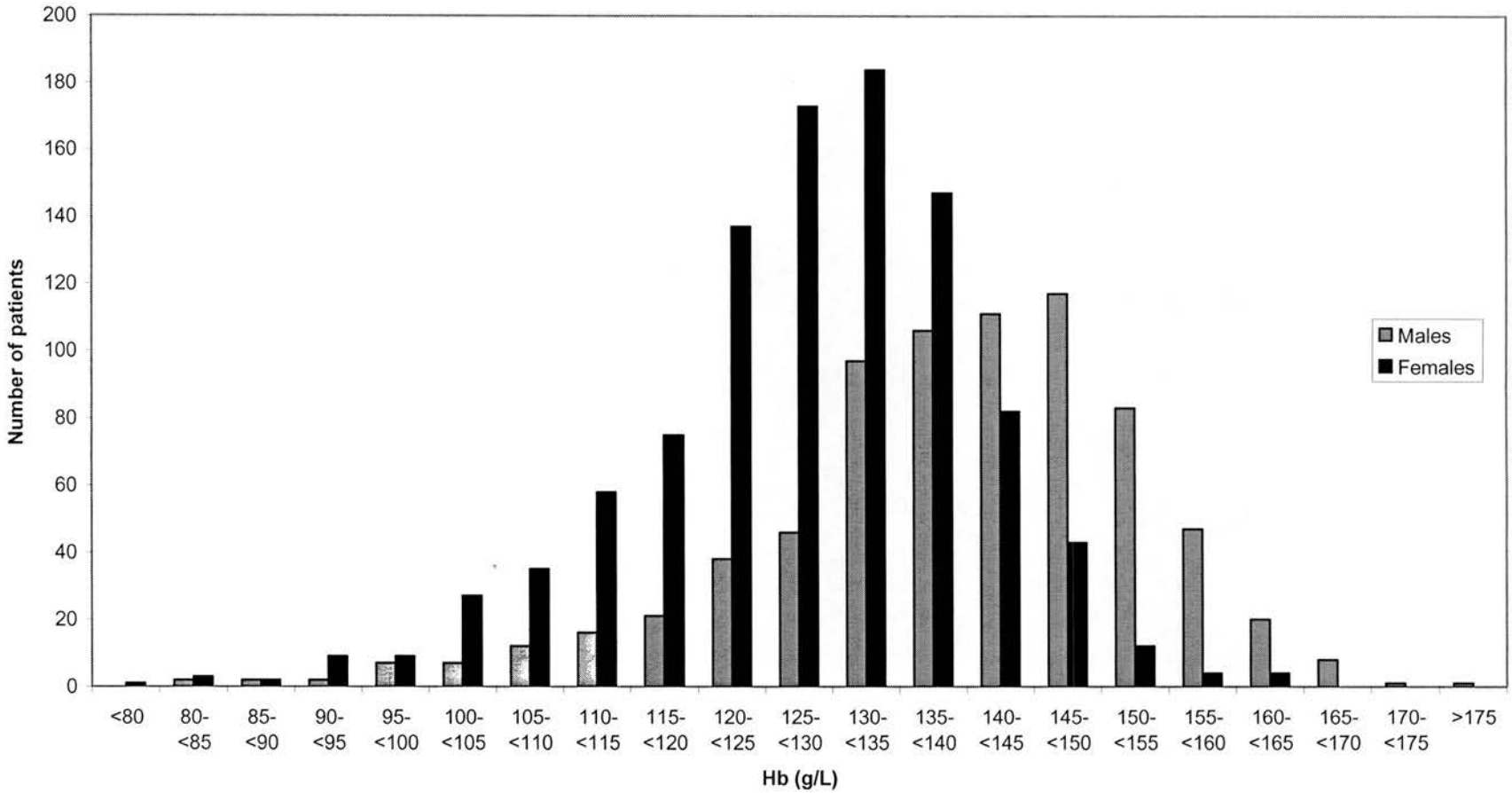
Table (4.38): Number (% of the total number of admissions in each procedure) of admissions with and without available pre-admission haematological results in different EOS procedures.

Procedures	With pre-admission haematological results (N = 1749). N (%)	Without pre-admission haematological results (N = 1668). N (%)
THR (N = 623)	544/623 (87)	79/623 (13)
TKR (N = 555)	490/555 (88)	65/555 (12)
Revision hip (N = 105)	77/105 (73)	28/105 (27)
Revision knee (N = 39)	31/39 (80)	8/39 (20)
Other (N = 2090)	603/2090 (29)	1487/2090 (71)
Soft tissue	96	181
Spine	54	138
Construction	119	279
Other joints	207	224
Endoscopy	112	618
Miscellaneous	15	47
Revision other (N = 5)	4/5 (80)	1/5 (20)

Table (4.39): Number of male, female, and total admissions in different Hb (g/L) bands

Hb bands	Males	Females	Total
<80	0	1	1
≥80-<85	2	3	5
≥85-<90	2	2	4
≥90-<95	2	9	11
≥95-<100	7	9	16
≥100-<105	7	27	34
≥105-<110	12	35	47
≥110-<115	16	58	74
≥115-<120	21	75	96
≥120-<125	38	137	175
≥125-<130	46	173	219
≥130-<135	97	184	281
≥135-<140	106	147	253
≥140-<145	111	82	193
≥145-<150	117	43	160
≥150-<155	83	12	95
≥155-<160	47	4	51
≥160-<165	20	4	24
≥165-<170	8	0	8
≥170-<175	1	0	1
>175	1	0	1

Figure (4.5): Number of male and femal admissions in different pre-admission Hb bands



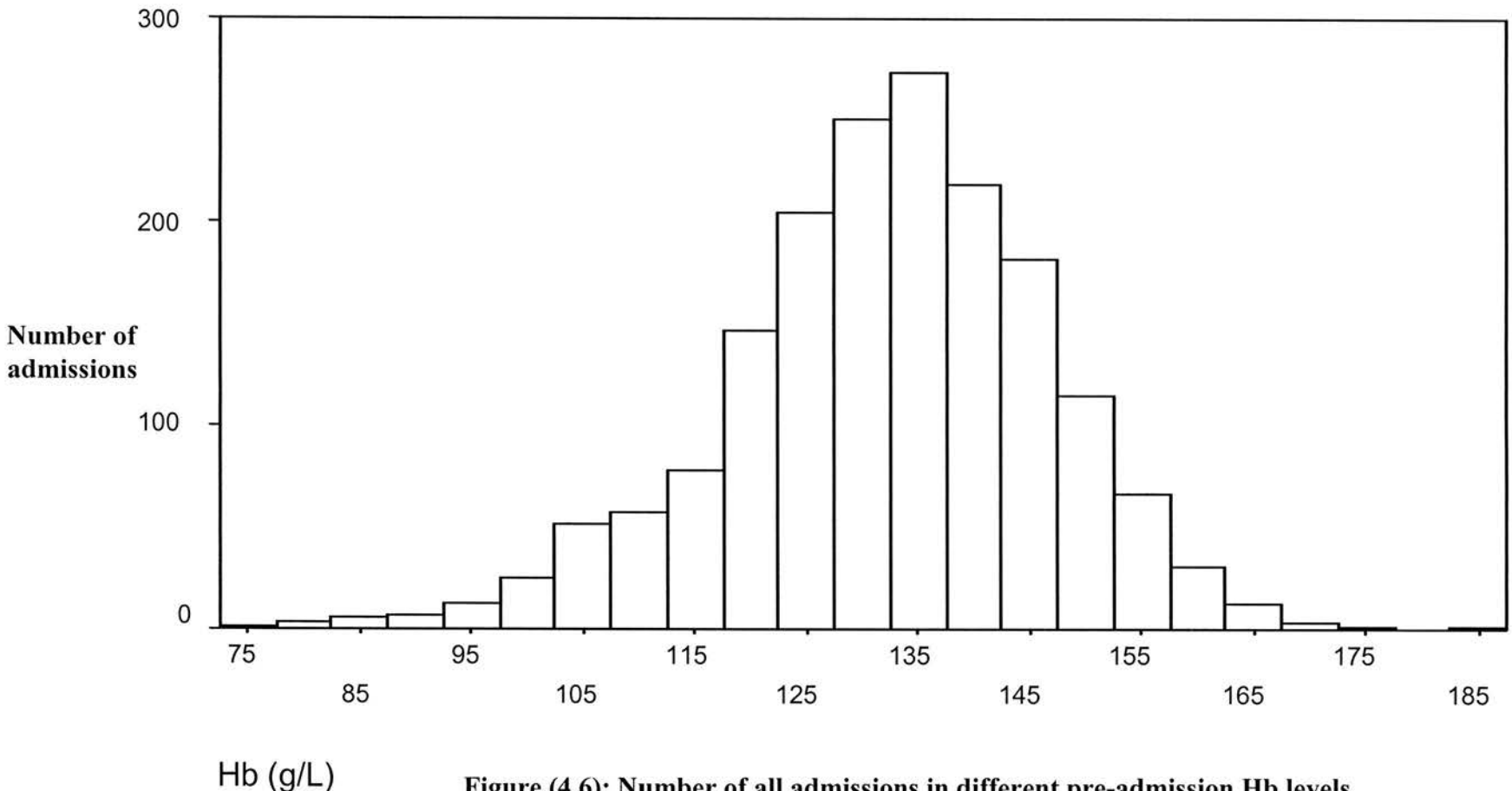


Figure (4.6): Number of all admissions in different pre-admission Hb levels

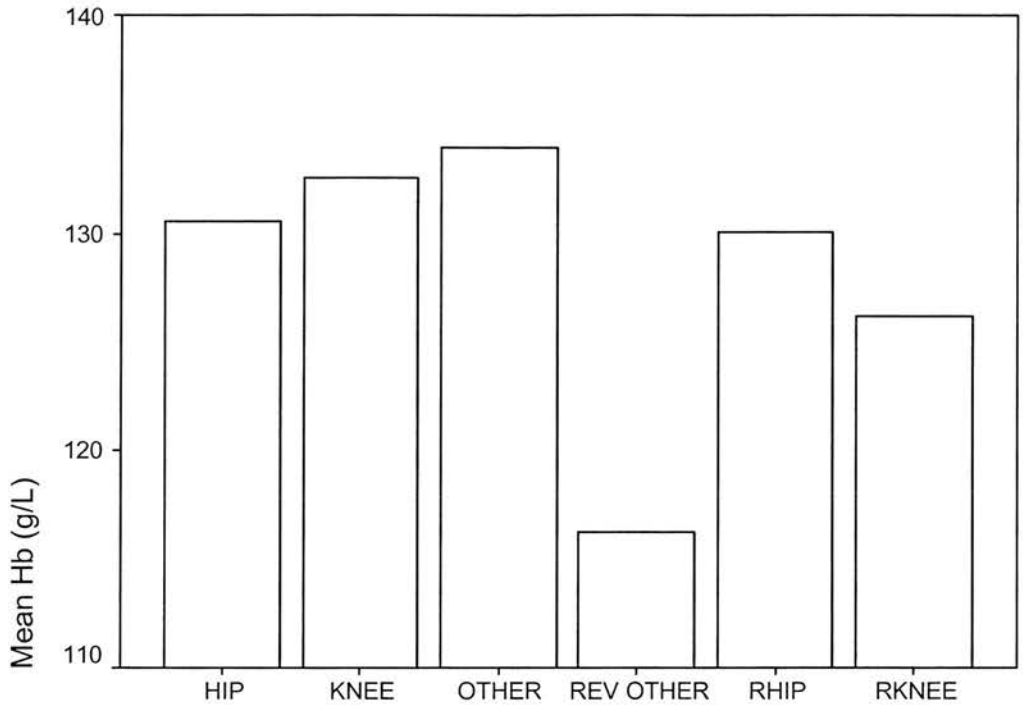


Figure (4.7): Mean pre-admission Hb levels in different procedures

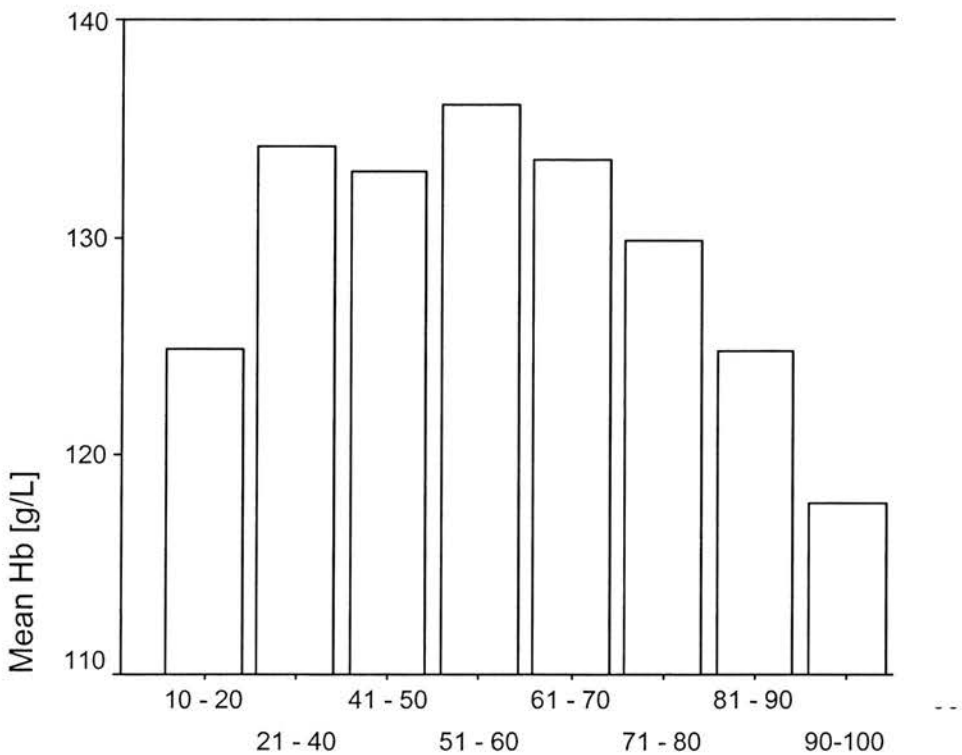


Figure (4.8): Mean pre-admission Hb levels in different age bands

Table (4.40): Number (%) of anaemic admissions that underwent different EOS procedures.

Operation	Males (n =154) N (%)	Females (n = 145) N (%)	Total (n = 299) N (%)
THR	51/154 (33)	47/145 (32.5)	98/299 (32.5)
TKR	50/154(32.5)	35/145 (24)	85/299 (28)
Revision hip	8/154 (5)	9/145 (6)	17/299 (6)
Revision knee	4/154 (2.5)	7/145 (5)	11/299 (4)
Other	39/154 (25.5)	47/145 (32.5)	86/299 (29)
Revision other	2/154 (1.5)	0	2/299 (0.5)

Table (4.41): Prevalence of different types of anaemia for all anaemic admissions.

	Male (n = 744) N (%)	Female (n = 1005) N (%)
Prevalence of anaemia (Hb ≤ 130 for males and ≤ 115 for females).	154/744 (20.7)	133/1005 (13.2)
Normocytic (MCV 76-100fl) + normochromic (MCHC 27-32pg)	112/154 (72.7)	71/133(53.4)
Normocytic + hypochromic	23/154 (15)	38/133 (28.6)
Normocytic + hyperchromic	10/154 (6.5)	5/133 (3.8)
Microcytic + hypochromic	5/154 (3.3)	9/133 (6.7)
Macrocytic + hyperchromic	4/154 (2.5)	9/133 (6.7)
Macrocytic + normochromic	0	1/133 (0.8)

Figure (4.9): Prevalence of different types of anaemia for all anaemic admissions (n = 299)

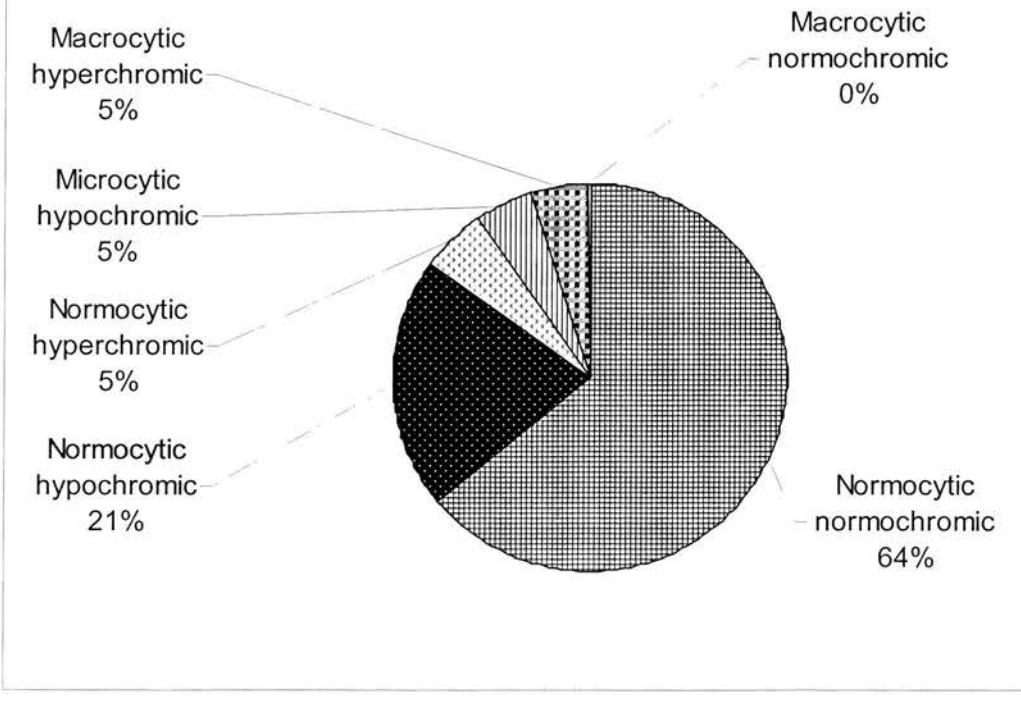
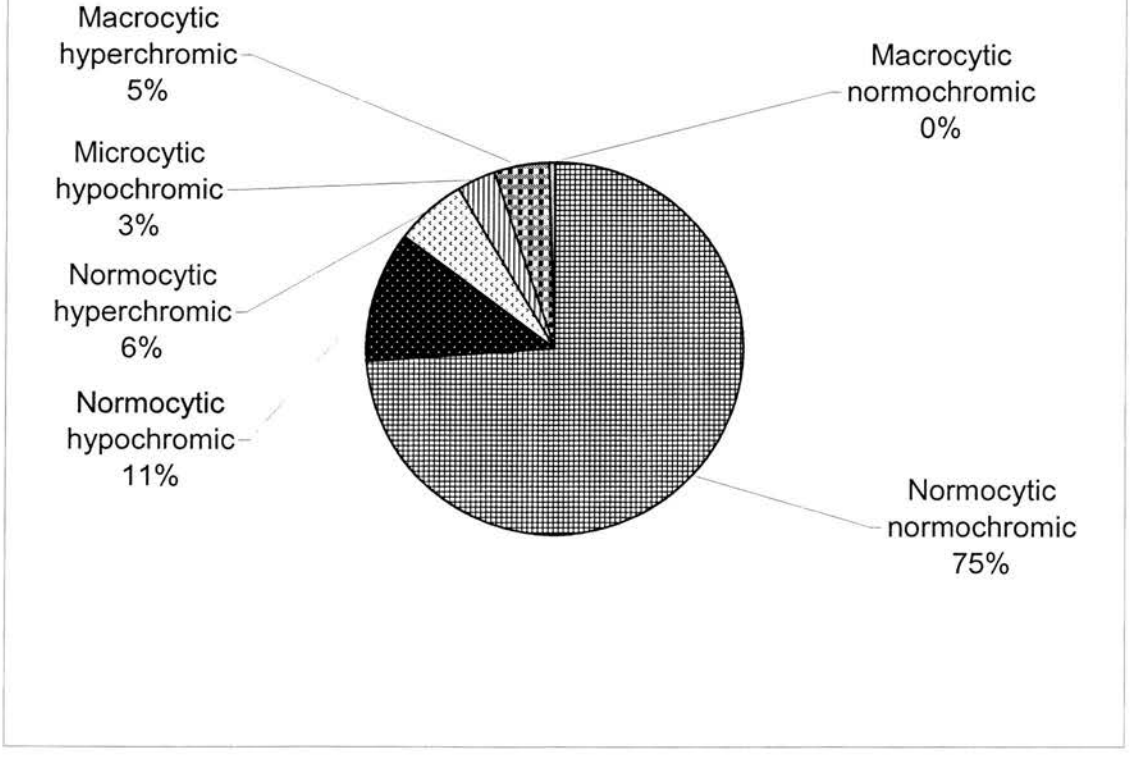


Table (4.42): Prevalence of different types of anaemia for all anaemic admissions that underwent MJS.

	Male (n = 491) N (%)	Female (n = 651) N (%)
Prevalence of anaemia (Hb ≤ 130 for males and ≤ 115 for females).	113/491 (27.1)	98/651 (15)
Normocytic (MCV 76-100fl) + normochromic (MCHC 27-32pg)	77/113(68.1)	79/98(80.6)
Normocytic + hypochromic	20/113 (17.7)	4/98 (4.1)
Normocytic + hyperchromic	9/113 (8)	4/98 (4.1)
Microcytic + hypochromic	3/113 (2.7)	4/98 (4.1)
Macrocytic + hyperchromic	4/113 (3.5)	6/98 (6.1)
Macrocytic + normochromic	0	1/98 (1)

Figure (4.10): Prevalence of different types of anaemia for anaemic admissions who underwent MJS (n = 1142)



TRANSFUSION RESULTS

3400 records were received from the blood bank patient database. These had been selected as all request records originating from the PMR and covering the period between 01/07/2000 and 31/08/2001. The linkage process and the numbers of the linked records are described previously in figures (4.2 & 4.3).

3095 (86.5%) BTS records fully matched with the entire fields of linkage and 460 (13.5%) of BTS records were not linked. Of the linked records, 2458 were related to the study period as a decision was made to include only G&S records that had been requested one month before hospital admission date and cross-match records one week before that date. 1049 records in which red cell units were assigned related to 923 admissions and 1409 records of group and save only related to 1368 admissions (the reasons for obtaining a period of 14 months data and accepting the full matches only are discussed in detail in the discussion section). Table (4.43) shows number (%) of admissions in G&S group. Table (4.44) shows number (%) of admissions with different numbers of instances on which one or more red cell units were assigned during the same hospital stay.

305 admissions had one or more transfusion episode comprising a total of 367 episodes. Of these episodes 128 (35%) were for males and 239 (65%) were for females. General characteristics of admissions that had transfusion episodes are shown in table (4.45). Table (4.46) shows number of transfused admissions in different numbers of transfusion episodes.

838 red cell units were used for all the admissions in the cohort during the study period. Table (4.47) shows number of admissions in different procedures that were transfused with different numbers of red cell units.

83% of total units of red cell units during the study period were used for admissions undergoing MJS and 17% were used for admissions undergoing other EOS procedures. Table (4.48) shows numbers of red cell units transfused for different procedures. Table (4.49) shows the mean (SD), median (IQR) and range of the number of red cell units transfused per transfused admission in each procedure. 59% of admissions that were transfused were in the age range between 61 to 80 years. Table (4.50) shows number of admissions transfused in different age bands in different procedures. Figure (4.11) shows the number of all transfused admissions in different age bands.

BLOOD BANK REQUEST AND TRANSFUSION IN RELATION TO HAEMATOLOGICAL RESULTS

Pre-operative red cell transfusion in relation to availability of Hb results

271(73.8%) of the transfusion episodes occurred during admissions for which a pre-admission Hb records had been linked and 96 (26.2%) episodes occurred during admissions for which no pre-admission Hb records could be linked. Table (4.51) shows number of transfusion episodes in different procedures with and without available pre-admission Hb records.

631 red cell units were transfused for episodes in which there was a linked pre-admission Hb records and 207 units transfused for admissions with no linked pre-admission Hb records.

Of the transfusion episodes for admissions with a linked Hb results, 73 % occurred in cases with pre-admission Hb value between 100 g/L and 135 g/L. For these episodes, 72% of the transfused red cell units were used. Table (4.52) shows the number of

transfusion episodes and red cell units used in different Hb bands. 31% of the transfusion episodes occurred with pre-admission Hb level ≤ 124 g/L. Figure (4.12) and table (4.53) show the total number of transfusion episode in different Hb bands.

Table (4.43): Number (%) of admissions with different numbers of G&S records during the same hospital stay.

Procedure	G&S once	G&S twice	G&S three times	Total
THR (N = 623)	504/623 (81%)	15/623 (2%)	0	519/623 (83%)
TKR (N = 555)	493/555 (89%)	13/555 (2%)	1/555 (<1%)	507/555 (91%)
Revision hip (N = 105)	83/105 (79%)	3/105 (3%)	0	86/105 (82%)
Revision knee (N = 39)	29/39 (74%)	1/39 (3%)	0	30/39 (80%)
Other (N =2090)	216/2090 (10%)	7/2090 (<1%)	0	223/2090 (11%)
Revision other (N = 5)	3/5 (60%)	0	0	3/5 (60%)
Total (N = 3417)	**1328/3417 (39%)**	**39/3417 (1%)**	**1/3417 (<1%)**	**1368/3417 (40%)**

Table (4.44): Number (%) of admissions with different numbers of instances on which one or more red cell units were assigned during the same hospital stay.

Procedure	Red cell units assigned on one occasion	Red cell units assigned on two occasions	Red cell units assigned on three occasions	Red cell units assigned on four occasions	Red cell units assigned on five occasions	Total
THR (N = 623)	527/623 (85%)	50/623 (8%)	2/623 (<1%)	0	0	579/623 (93%)
TKR (N = 555)	81/555 (15%)	6/555 (1%)	0	0	0	87/555 (16%)
Revision hip (N = 105)	65/105 (62%)	22/105 (21%)	3/105 (3%)	0	0	90/105 (86%)
Revision knee (N = 39)	27/39 (69%)	4/39 (10%)	0	1/39 (3%)	0	32/39 (82%)
Other (N =2090)	109/2090 (5%)	23/2090 (1%)	2/2090 (<1%)	0	1/2090 (<1%)	135/2090 (7%)
Revision other (N = 5)	0	0	0	0	0	0
Total (N = 3417)	**809/3417 (24%)**	**105/3417 (3%)**	**7/3417 (<1%)**	**1/3417 (<1%)**	**1/3417 (<1%)**	**923/3417 (27%)**

Table (4.45): General characteristics of admissions that included all transfusion episodes.

Variable	Mean	SD	Median	Q1	Q3	Range
Age (years)	66	17.7	71	60	78	13-90
Pre-admission Hb (g/L)	122	15.4	123	110	132	82-162
Length of hospital stay (days)	11	9.1	9	7	12	1-86
Number of red cell units transfused	2	0.8	2	2	3	1-6

Table (4.46): Number (%) of admissions with different numbers of transfusion episodes (% per admission).

Procedure	One episode N (%)	Two episodes N (%)	Three episodes N (%)	Four episodes N (%)	Five episodes N (%)	Total number of admissions transfused N (%)
THR (N = 623)	139/623 (23)	21/623 (3)	2/623 (<1)	0	0	162/623 (26)
TKR (N = 555)	40/555 (7)	1/555 (<1)	0	0	0	41/555 (7)
Revision hip (N = 105)	42/105 (40)	10/105 (9)	1/105 (1)	0	0	53/105 (50)
Revision knee (N = 39)	4/39 (10)	3/39 (8)	1/39 (2)	0	0	8/39 (20)
Spine (N = 192)	16/192 (8)	8 (4)	1 (<1)	0	1 (<1)	26 (14)
Construction (N = 398)	4/398 (1)	3/398 (<1)	0	0	0	7/398 (2)
Other joint (N = 431)	3/431 (<1)	2/431 (<1)	0	0	0	5/431 (1)
Soft tissue (N = 277)	3/277 (1)	0	0	0	0	3/277 (1)
Endoscopy (N = 730)	0	0	0	0	0	0
Miscellaneous (N = 62)	0	0	0	0	0	0
Total (N = 3417)	**251/3417 (7)**	**48/3417 (1)**	**5/3417 (<1)**	**0**	**1/3417 (<1)**	**305/3417 (9)**

Table (4.47): Number (%) of admissions that received different numbers red cell units [transfused] (% per transfused admissions).

Procedure	1 unit N (%)	2 units N (%)	3 units N (%)	4 units N (%)	5 units N (%)	6 units N (%)	7 units N (%)	8 units N (%)	9 units N (%)	10 units N (%)	11 units N (%)
THR (N = 162)	13/162 (8)	99/162 (61)	36/162 (22)	4/162 (3)	8/162 (5)	0	1/162 (<1)	0	0	1/162 (<1)	0
TKR (N = 41)	1/41 (2)	34/41 (83)	6/41 (15)	0	0	0	0	0	0	0	0
Revision hip (N = 53)	3/53 (6)	23/53 (43)	13/53 (25)	4/53 (8)	4/53 (8)	4/53 (8)	1/53 (2)	0	1/53 (2)	0	0
Revision knee (N = 8)	0	6/8 (75)	1/8 (13)	0	0	0	1/8 (13)	0	0	0	0
Spine (N = 26)	2/26 (8)	10/26 (39)	5/26 (19)	3/26 (11)	3/26 (11)	0	0	1/26 (4)	0	1/26 (4)	1/26 (4)
Construction (N = 7)	0	2/7 (29)	2/7 (29)	0	3/7 (43)	0	0	0	0	0	0
Other joint (5)	1/5 (20)	2/5 (40)	0	1/5 (20)	0	1/5 (20)	0	0	0	0	0
Soft tissue (3)	0	3/3 (100)	0	0	0	0	0	0	0	0	0
Endoscopy (0)	0	0	0	0	0	0	0	0	0	0	0
Miscellaneous (0)	0	0	0	0	0	0	0	0	0	0	0
Total (N = 305)	**20/305 (7)**	**179/305 (59)**	**63/305 (21)**	**12/305 (4)**	**18/305 (6)**	**5/305 (2)**	**3/305 (1)**	**1/305 (<1)**	**1/305 (<1)**	**2/305 (<1)**	**1/305 (<1)**

Table (4.48): Number (%) of red cell units transfused in different procedures.

Procedure	Total number of units transfused (n = 838) N (% of all red cell units used during the study period)
THR	409/838 (49)
TKR	89/838 (11)
Revision knee	28/838 (3)
Revision hip	172/838 (20)
Other	140/838 (17)
Revision other	0

Table (4.49): Mean (SD), median (IQR), and range of the number of red cell units transfused per transfused admission in different procedures.

Procedure	Mean	SD	Median	Q1	Q3	Range
THR	2.2	0.69	2	2	3	1 to 10
TKR	2.1	0.39	2	2	2	1 to 3
Revision hip	2.7	1.05	2	2	3	1 to 9
Revision knee	2.2	0.9	2	2	2	1 to 7
Other	2.3	1.04	2	2	3	1 to 6

Table (4.50): Number (%)of admissions transfused in different age bands in different procedures (% of all transfused admissions).

Age band	THR	TKR	Revision hip	Revision knee	Other	Revisio n other	Total (N = 305) N (%)
10-20	0	0	0	0	14	0	14/305 (5)
21-40	3	1	0	0	2	0	6/305 (2)
41-50	10	2	2	0	1	0	15/305 (5)
51-60	20	3	7	0	7	0	37/305 (12)
61-70	46	6	15	2	6	0	75/305 (25)
71-80	50	21	21	3	9	0	104/305 (34)
81-90	33	8	8	3	2	0	54/305 (18)
>90	0	0	0	0	0	0	0

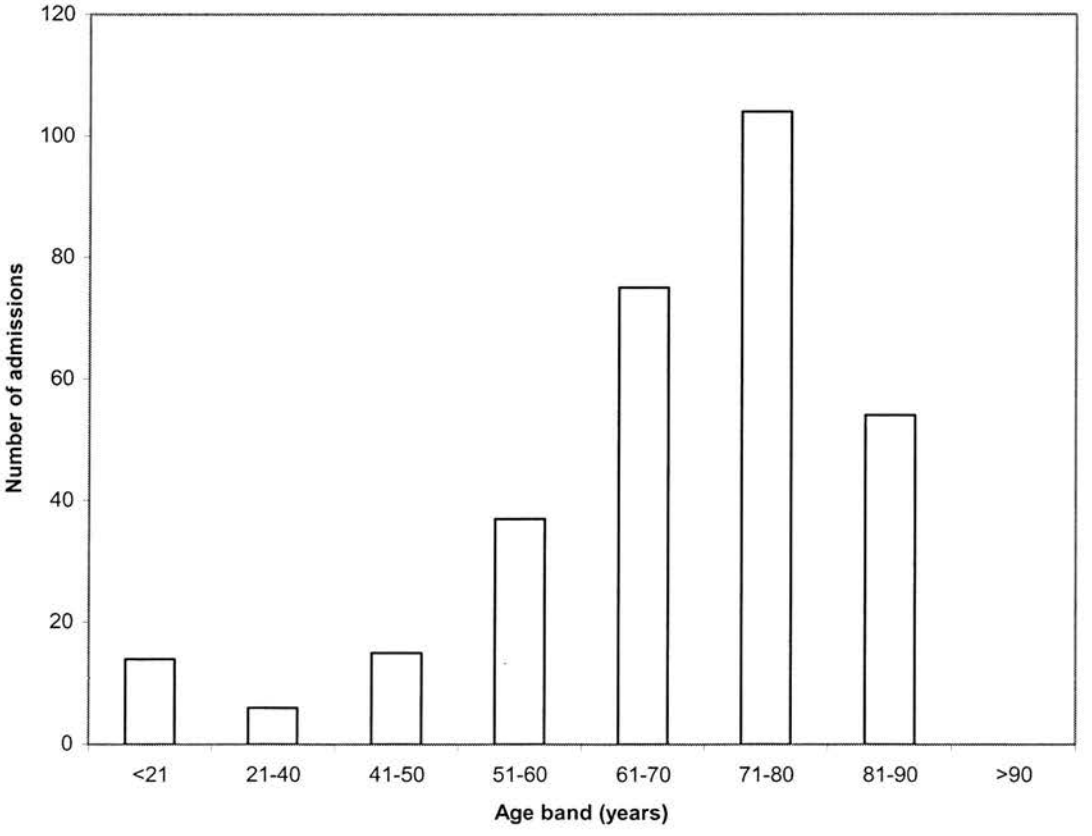


Figure (4.11): Number of admissions transfused in different age bands

Table (4.51): Number (%) of transfusion episodes that occurred with or without available pre-admission Hb records in different procedures (% of all admissions in each group).

Procedure	Transfusion episode with known pre-admission Hb (N = 271) N (%)	Transfusion episode with unknown pre-admission Hb (N = 96) N (%)	Total numbers episodes (N = 367) N (%)
THR	162/271 (59.8)	25/96 (26)	187/367 (51)
TKR	38/271 (14)	4/96 (4.1)	42/367 (11.5)
Revision hip	50/271 (18.5)	15/96 (15.6)	65/367 (17.5)
Revision knee	8/271 (2.9)	5/96 (5.2)	13/367 (3.5)
Other	13/271 (4.8)	47/96 (49)	60/367 (16.5)
Revision other	0	0	0

Table (4.52): Number (%) of transfusion episodes and red cell units transfused in different Hb bands (% of all transfusion episodes and the total number of red cell units transfused for admissions with available Hb results).

Haemoglobin level	Number of transfusion episode (n = 271) N (%)	Number of units transfused (n = 631) N (%)
<80	0	0
≥80-<85	3 (1)	5 (1)
≥85-<90	3 (1)	9 (1)
≥90-<95	2 (1)	4(1)
≥95-<100	10 (4)	25 (4)
≥100-<105	21 (8)	44 (7)
≥105-<110	24 (9)	49 (8)
≥110-<115	25 (9)	57 (9)
≥115-<120	21 (8)	45 (7)
≥120-<125	37 (14)	88 (14)
≥125-<130	41 (15)	97 (16)
≥130-<135	32 (12)	70 (11)
≥135-<140	20 (7)	46 (8)
≥140-<145	15 (6)	34 (6)
≥145-<150	6 (2)	16 (3)
≥150-<155	4 (1)	7 (1)
≥155-<160	5 (2)	11 (2)
≥160-<165	2 (1)	6 (1)
≥165-<170	0	0
≥170-<175	0	0
>175	0	0

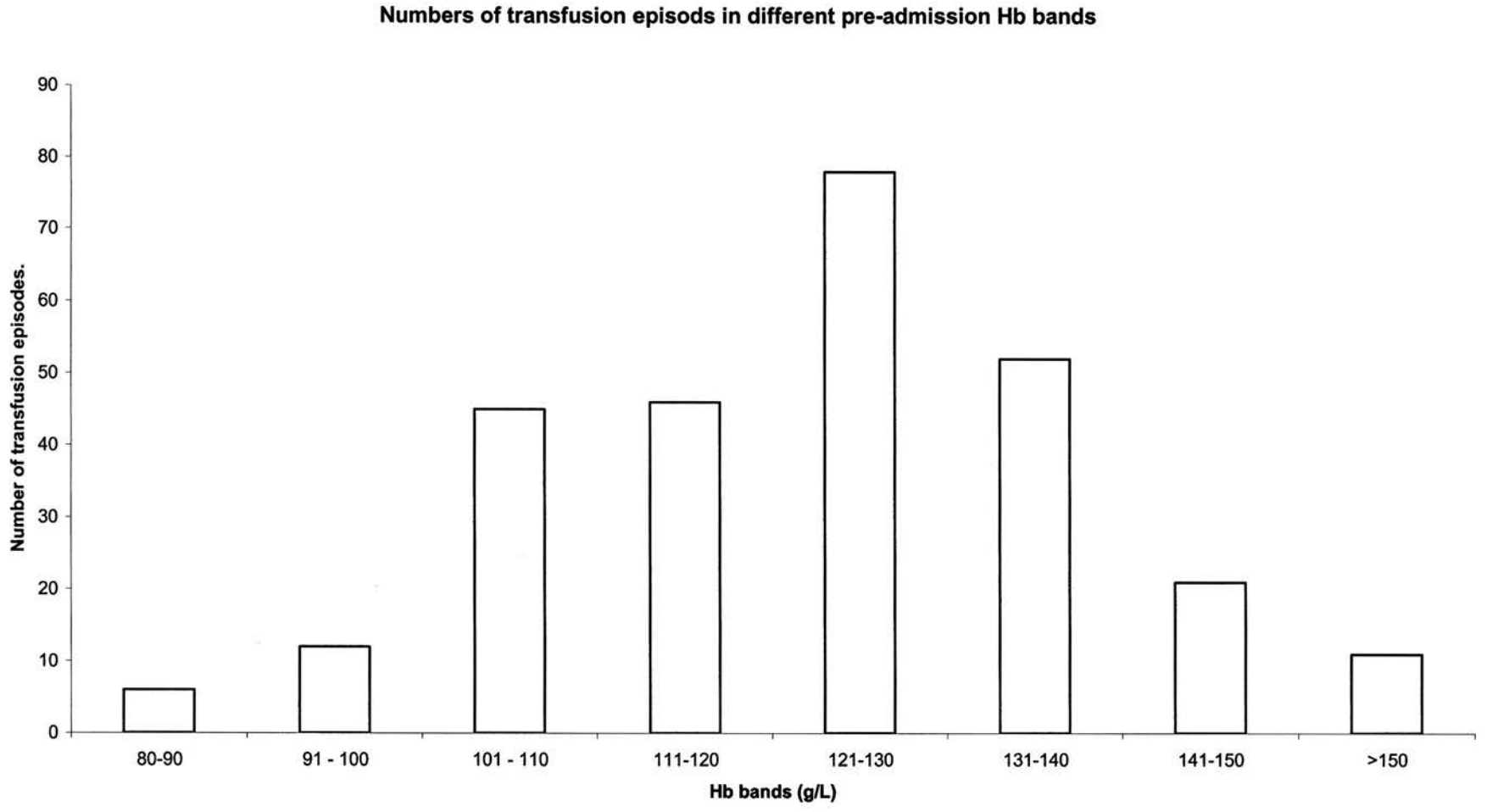

Numbers of transfusion episods in different pre-admission Hb bands
Number of transfusion episodes.
90
80
70
60
50
40
30
20
10
0
80-90
91 - 100
101 - 110
111-120
121-130
131-140
141-150
>150
Hb bands (g/L)

Table (4.53): Number (%) of transfusion episodes in Hb bands (% per total number of admissions in each Hb band).

Hb bands (g/L) (N = number of admissions).	Number (%) of transfusion episodes.
80 – 90 (10)	6 (60%)
91 – 100 (27)	12 (44%)
101 – 110 (81)	45 (55%)
111 – 120 (170)	46 (27%)
121 – 130 (394)	78 (20)
131 – 140 (534)	52 (10%)
141 – 150 (353)	21 (6%)
>150 (180)	11 (5%)

DISCUSSION

SUMMARY OF THE RESULTS

3417 EOS admissions [3257 patients] were admitted to the main teaching orthopaedic hospital [PMR] during the study period and were included in the study. For 51.2% of these admissions a full blood count result within one month before hospital admission was available. The prevalence of anaemia before hospital admission in those admissions that had a full blood count result was 17%. Of these anaemic patients 64% had normocytic normochromic blood indices.

1322 (38.6%) admissions underwent major joint surgery (THR & TKA). Of these 1142 (86.4%) patients had pre-admission full blood count results, 211 (18.5%) were anaemic. 74% of these anaemic admissions had pre-admission normocytic normochromic blood indices.

305 admissions received red cell transfusion during the study period of which 59% were elderly patients between 61 and 80 years. A total of 838 red cell units were transfused to these patients. The mean ± SD [median, (IQR), and range] of pre-admission Hb (g/L) for admissions that were transfused was 121.5 ± 15.4 [13, (110,132), and (82 – 162)] respectively.

31% of the transfused admissions had pre-admission Hb level ≤ 124g/L and these anaemia patients used 72% of all the transfused red cell units. 83.5% of all transfusion episodes were associated with total hip or knee replacement.

STRENGTHS AND WEAKNESSES

STRENGTHS

The study was carried out in a large elective orthopaedic teaching centre, the case mix comprising all admissions to the hospital for a complete year.

Patients' demographic data were obtained from the Patient Administration System [PAS] database, which covers all of hospital admissions. This is the most complete and accurate source of demographic and procedure information that should be routinely available for any hospital in the UK. It was unlikely to miss any admission during the study period.

The haematology dataset was obtained for a period of 13 months to include an extra-month before the study period. This was to capture the haematological results for patients who had their pre-admission visit one month before the start of the study period. Therefore, it was unlikely to miss any available pre-admission haematological results. However, we showed that only 51.2% of the admissions had pre-admission haematological values. Possible explanations for this are: a) the haematological tests were not requested before and or the day of hospital admission (possible flaw in the pre-admission clinic protocol), b) there were no records in the hospital laboratory database (possible flaw in data entry in labs), c) record in the database but with wrong identifiers, so not liked to PAS dataset, d) result date could be after admission date (possible flaw in the study protocol), e) Hb was requested the next day after the day of hospital admission, f) error in the linkage process, this was unlikely to occur as the process was carried out in steps and manual checks were carried out for the fuzzy matches. Therefore, it was unlikely to miss any data because of a faulty in entering of one or two characters of one or more linkage fields in any of

the linked datasets (see pages 314, 315, 316 and appendix I). The methodology of the linkage and the manual checks that followed provided the study with a high quality dataset for further analysis.

The transfusion dataset contained extra two months period was obtained [one month before and one month after the study period] to capture transfusion records for admissions that had their pre-admission visit one month before the start of the study period and to capture records until the time of hospital discharge [last admission was discharged one month after the study period]. Therefore, it was unlikely to miss any transfusion records for all admissions in the study.

86.5% the transfusion records were fully linked with PAS dataset, possible explanations: a) dates' format in the blood bank dataset was different than that in PAS dataset and full matches only were accepted for analysis after reformatting the dates, b) records were in the dataset but with wrong identifiers, so not linked, c) for some admissions, some G&S and assigned records were not related to the study period (> one month before of hospital admission date or after the hospital discharge date). These records were linked automatically to PAS dataset as a part of the linkage process. These events were identified from their dates and were excluded from the analysis.

All transfusion records related to the study period were linked to PAS and used in the analysis, however, only the pre-admission haematology results was linked and used in the analysis. This explains the higher proportion of records that had been linked from the transfusion dataset to PAS dataset comparing it to the proportion of the haematology records that had been linked.

The linkage process of the patients' dataset was carried out under the supervision of an expert in that field in a specialized unit with secure computers and procedures that comply with data protection guidelines.

The study population contained a large number of patients who underwent major joint surgery; this allowed us to construct a subgroup of patients typical of those who are more likely to be older, anaemic, and thus more at risk of red cell transfusion.

The transfusion dataset was extracted from the Scottish National Blood Transfusion Service [SNBTS] patient database. This database accounts for all patient blood bank requests that occur in the PMR and its parent Trust. This dataset provides the same patients' identifiers and transfusion data that can be linked to PAS dataset. Therefore, it was unlikely to miss transfusion events in our study group.

WEAKNESSES

With the experience gained from this study, some aspects of the design could be improved.

The PAS database does not record the exact date of the pre-admission clinic visit. We therefore had to assume that hospital guidelines were complied with, i.e. that patients preadmission clinic [PAC] attendances took place within the month preceding their admission date. It would be preferable to have the PAC attendance date recorded in the PAS dataset.

Some patients had more than one haematological result within one month before hospital admission date. The result dated closest to the hospital admission date was used in the analysis. It might be considered that a case note review should have been

done to validate a sample of these dates, but this was not in the study design and was not feasible with resources available.

During the linkage process of the patients' dataset with the haematology dataset any haematological results during the period of admissions were excluded as the purpose of the study was specifically to study the relationship between PAC Hb value and blood use. It may have been better to include in the analysis the haematological results for the whole period of the patients' admission. Obtaining these values would decrease the numbers of patients without Hb values in the study and would help in estimating the clinical thresholds for transfusion during the hospital admission.

The numbers of red cell units transfused was calculated from the numbers of red cell units that the blood transfusion service assigned to individual patients and the numbers of these units of red cells that were returned to BTS. The assumption had to be made that these units were transfused to the patient, as there is no database that records the actual administration of the blood units. Case note review to confirm the numbers of red cell units transfused was not part of the protocol and could not be undertaken with the available resource. Furthermore, previous work in the same hospital has indicated that the case notes do not provide a reliable complete record of all units transfused. [Palmer J (EUB, SNBTS) personal communication]

CRITIQUE OF THE STUDIES (included in literature review)

PRE-OPERATIVE ANAEMIA IN PATIENTS UNDERGOING EOS

The present study is the first large-scale survey of pre-operative anaemia in a representative large population of patients admitted for elective orthopaedic surgery.

Some studies reported baseline Hb and/or Hct values for these patients as a part of the recorded data; however, most of these studies did not report the normal reference ranges (Biesma, Kraaijenhagen, Poortman, Marx, van de 1992; Laupacis, Feagan, Wong 1993; Faris, Ritter, Abels 1996; Weisbach et al. 1999; Olijhoek et al. 2001).

MEASURMENT AND REPORTING BLOOD LOSS DURING EOS

There is no gold standard method in estimating the amount of blood loss in surgical patients. In most studies, intra-operative blood loss was mainly recorded from anaesthetic charts and/or the volume of fluid in the suction device. Post-operative blood loss was mainly recorded from the wound drains or calculated by different equations (Berman, Geissele, Bosacco 1988; Toy, Kaplan, McVay, Lee, Strauss, Stehling 1992; Kurdy 1996). None of the studies have mentioned tissue or unseen blood loss.

The reported blood loss volumes for the same procedures were vary widely in different studies. Table (4.54) shows the range of methods used in estimating operative blood loss in published studies.

Table (4.54): Different methods used in estimating and reporting blood loss in EOS.

	Case note review	Calculated	Measured (the method not mentioned)	Measured from suction device
Berman et al.(Berman, Geissele, Bosacco 1988)	✔			
Kurdy et al.(Kurdy 1996)	✔			
Toy et al(Toy, Kaplan, McVay, Lee, Strauss, Stehling 1992).	✔	✔		
Lisander et al.(Lisander, Ivarsson, Jacobsson 1998)		✔		
Knight et al (Knight, Sherer, Guo 1998)			✔	
Shulman et al.(Shulman, Grecula, Hadjipavlou 2002)				✔

Some authors reported the volume of blood loss in ml; however, some others expressed as the equivalent number of red cell units. Not all the authors mentioned the volume or Hct of these units (Berman, Geissele, Bosacco 1988).

BLOOD TRANSFUSION IN EOS

The amount of blood transfusion varied widely in different studies that were similar in their operative technique and surgical procedures (Sirchia, Giovanetti, McClelland, Fracchia, editors 1994).

Most of the studies reported the numbers of red cell units transfused without clarifying the volume of the units transfused. Some of these studies did not clarify whether the transfusion was with whole blood or red blood cells only (Berman, Geissele, Bosacco 1988; Toy, Kaplan, McVay, Lee, Strauss, Stehling 1992).

The amount of red cell transfused was variously expressed as units per operated patient, per transfused patient, or per procedure. A further inconsistency in reporting is that some studies that investigated the amount of allogeneic and autologous red cell transfusion have recorded the total amount of red cell transfusion without reporting the amount of each type of red cell used separately.

Table (4.55) shows the inconsistency in methods of reporting the amount of blood transfused.

Table (4.55): Different ways of expressing the amount of blood transfused in published studies.

	Per transfused patient	Per transfused procedure	Per operated patient	Per group of patients
Toy et al.(Toy, Kaplan, McVay, Lee, Strauss, Stehling 1992)	✔			
Berman et al.(Berman, Geissele, Bosacco 1988)		✔		
Warner et al.(Warner, Warner, Schroeder, Offord, Maxson, Santrach 1998)		✔		
Helm et al.(Helm, Karski, Parsons, Sampath, Bale 2003)			✔	
Goodnough et al.(Goodnough, Vizmeg, Marcus 1993)			✔	
Jarolem et al.(Jarolem, Scott, Jaffe, Stein, Jaffe, Atik 1995)			✔	
Kurdy et al.(Kurdy 1996)				✔

STUDIES ON TRANSFUSION SPARING OR BLOOD CONSERVATION TECHNIQUES

The methodological quality of most of the trials that investigated different transfusion sparing techniques was poor and the majority of these trials were un-blinded. Most of these studies lacked randomisation and the number of subjects included in the study were too small.

Pre-operative iron therapy

Very few studies have investigated the role of pre-operative iron therapy in treating pre-operative anaemia and reducing the need for red cell transfusion. Most of these were commercially supported studies that investigated the role of EPO and included a group of patients who received iron therapy alone whether given by oral or parenteral route as a control group. These studies have shown that iron alone appears to be rather ineffective in erythropoiesis pre-operatively (Kasper, Lazansky, Stark, Klimek, Laubinger, Borner 1998; Feagan et al. 2000; Olijhoek et al. 2001).

Pre-operative autologous donation

Some authors have studied the role of pre-operative autologous blood donation in reducing the need for allogeneic blood transfusion; many have shown the impracticality of this technique. Pre-operative autologous blood donation can be logistically difficult due to: a) low degree of public interest to donate blood before major surgery (Thomas 1996). Goodnough et al mentioned that up to 25% of hip and 35% of knee replacement patients do not pre-donate autologous blood even in aggressively promoted programs (Goodnough, Vizmeg, Sobecks, Schwarz, Soegiarso 1992a), b) patient's medical condition such as pre-operative anaemia, pregnancy, cardiac patients, active bacterial infection, and coagulation disorders

(Thomas 1996; Desmond, Thomas, Gillon, Fox 1996; Hartmann, Winkler, Preis, Germann, Donner, Muller 1997), c) time consuming and expensive(Bierbaum, Callaghan, Galante, Rubash, Tooms, Welch 1999), d) transfusion reactions are likely to occur(Hatzidakis, Mendlick, McKillip, Reddy, Garvin 2000), and e) errors in labelling and storage (Desmond, Thomas, Gillon, Fox 1996). f) substantial wastage of the pre-donated blood units.

Moreover, most of the studies showed that patients who pre-donate autologous blood are likely to be anaemic by the time of operation and receive higher volumes of red cell transfusion (when both allogeneic and autologous units counted) than patients who do not.

Red cell salvage

Some studies report a reduction in allogeneic red cell transfusion but a recent systematic review concludes that there is little convincing evidence for the effectiveness of the technique in reducing red cell use (Carless, Henry, Moxey, O'Connell, Fergusson 2003). Furthermore there are reports of adverse effects of re-infusing the salvaged blood to the patients during or after surgery. These adverse effects are: a) Anaemia due to infusion of diluted blood, b) thrombocytopenia and coagulopathy due to loss of platelets during salvage, c) sepsis due to bacterial contamination, d) microemboli due to the ineffective filtering system (Desmond, Thomas, Gillon, Fox 1996; Huet, Salmi, Fergusson, Koopman-van Gemert, Rubens, Laupacis 1999).

Management of post-operative anaemia

There are not enough data showing the prevalence of post-operative anaemia in patients undergoing EOS or the impact of using different transfusion sparing

strategies on patients' Hb levels. Biesma et al. are the only authors who have reported studies on post-operative anaemia in patients undergoing EOS (Biesma, van de, Beguin, Kraaijenhagen, Marx 1995).

There is a need for a randomised controlled trial to show the effectiveness of peri-operative anaemia management to reduce the need for allogeneic blood transfusion and on patients' clinical outcome.

Anaesthetic and surgical techniques that may reduce blood loss

Some studies have investigated different anaesthetic and surgical techniques that may reduce operative blood loss and the need of red cell transfusion. Results are very inconsistent. Juelsgaard et al and Borghi et al showed that there was no significant difference in the amount of blood loss in patients undergoing EOS under different anaesthetic techniques namely general, spinal, epidural, and combination of epidural and general anaesthesia (Juelsgaard et al. 1998; Borghi et al. 2002). However, Niemi et al and Dauphin et al showed that epidural anaesthesia reduced the amount of operative blood loss significantly (Dauphin, Raymer, Stanton, Fuller 1997; Niemi, Pitkanen, Syrjala, Rosenberg 2000).

Blood loss measurement methods varied in the studies, as there is no gold standard technique to estimate the amount of peri-operative blood loss.

Blood losses were extremely variable between these studies, for example Niemi et al reported that the total amount of blood loss [peri-operative] in patients who underwent EOS under spinal anaesthesia was 3500 ml and in a similar study by Juelsgaard et al the amount of blood loss was 633 ml (Juelsgaard et al. 1998; Niemi, Pitkanen, Syrjala, Rosenberg 2000).

Very few studies have investigated the role of different surgical techniques as the use of tourniquet, operative position, and aggressive warming in reducing operative blood loss in EOS. Surprisingly, there was no significant difference in the amount of intra-operative blood loss and transfusion in patients who underwent TKR with and without tourniquet, there was a little reduction of blood loss in patients underwent THR in lateral position (Jarolem, Scott, Jaffe, Stein, Jaffe, Atik 1995; Schneeberger, Schulz, Ganz 1998).

None of these studies have shown consistent evidence that any of these techniques (anaesthetics or surgical) are effective in reducing blood loss and transfusion.

The literature reviewed suggested that not all of the "blood sparing" interventions that are widely used are highly effective in reducing the need of red cell transfusion.

IMPLICATIONS OF THE RESULTS

The main study question was to investigate the extent to which correction of pre-operative anaemia might be expected to reduce the need for red cell transfusion in view of the published evidence that pre-operative anaemia is associated with an increased risk of peri-operative red cell transfusion. The study confirms this relationship. Furthermore it shows that the 31% of transfused admissions that had pre-admission Hb level $\leq$ 124g/L used 72% of all the transfused red cell units.

In this patients population we found that 83.5% of all transfusion episodes were associated with total hip or knee replacement.

However, it is less clear to what extent a simple preoperative intervention to give haematinics would impact on red cell transfusion needs, for the following reasons.

(a) The majority of anaemic patients had normocytic normochromic anaemia and may fail to respond to haematinic therapy. Only 25% (in total 75) of anaemic patients have hypochromic red cell indices. (b) The timing of the preadmission clinic, i.e. less than a month before admission, leaves inadequate time for an optimum response to haematenics. (c) Review of the literature does not provide much support for the view that preoperative iron (oral or parenteral) is effective in raising Hb prior to surgery in this type of patient populations concerned unless used in conjunction with erythropoietin.

It is possible that there is a population of non-anaemic patients with marginal iron stores who could benefit from preoperative iron therapy, but the routine clinical data available for analysis did not include the parameters needed to assess this possibility. The present study raises doubts about the potential for reducing peri-operative red cell transfusion by correction of pre-operative anaemia through early intervention during the pre-admission clinic visit

CHAPTER FIVE

CONCLUDING REMARKS

Anaemia is commonly found in critically ill patients. Compensatory physiological effects of anaemia are governed by the extent and the rate of development of the anaemia as well as by chronic or acute disease that limits physiological reserves. Peri-operative blood loss and transfusion in elective orthopaedic patients is common. It has been shown that the rate of red cell transfusion is associated with pre-operative haemoglobin level. Audits and systematic reviews of the use of blood transfusion have been shown that peri-operative patients and critically ill patients in intensive care units (ICUs) receive a significant number of units of red cells as these patients are more likely to develop anaemia during hospital stay. The themes of this thesis were to investigate the prevalence of anaemia and red cell transfusion in critically ill and elective orthopaedic patients.

In chapter [2], prevalence of anaemia among survivors of critical illness managed with conservative transfusion triggers, I established that anaemia is prevalent after critical illness in a group of patients managed with conservative transfusion triggers. This anaemia has blood indices similar to anaemia of chronic disease. The exact nature, causes and duration of this anaemia are still unclear. The prevalence and effects of long-term post-ICU anaemia on different body organs and quality of life (QOL) are also unclear. There is clear evidence for an association between anaemia and quality of life in other settings such as chronic renal failure, cancer, and chronic heart failure. Treating anaemia in these setting improves QOL and patients' outcome. It is unclear if active intervention in treating post-ICU anaemia with drugs such as

iron, folate, and EPO could improve rates of recovery. The optimum time to start a drug intervention and the doses are also unclear. Further studies are needed to: a) further understand the prevalence of long term post-ICU anaemia, b) fully understand the factors preventing normal recovery of erythropoiesis, c) investigate the association of this type of anaemia with QOL and physical function, and d) intervention studies to investigate different methods of treatment.

The studies that should follow my work should include:

1) A cohort study to understand the pathophysiology of anaemia during and at the recovery from the critical illness. Nutritional status, inflammatory markers, erythropoietin level, and functional iron status should be investigated.

Measuring vitamin B12, folate, and serum iron can assess nutrition status. CRP and IL-6 can assess the extended of the inflammatory process. Serum transferrin, ferritin level, and serum iron can be measured to revile functional iron deficiency.

These measures can be carried out at fixed times during the course of the critical illness and for a respectable time during the recovery phase.

2) A study to establish the prevalence of anaemia at fixed time after ICU and hospital discharge. Unlike our observational studies, where we were unable to follow up patients at fixed times after ICU and hospital discharge, there was no source of data after hospital discharge.

Patients should be recruited after ICU discharge and followed up weekly during the first month, every 2 weeks during the second month, once during the third month, and once at 6^{th} month period.

During the study blood samples should be taken every time (mentioned above) to assess the following functions:

(a) Assess bone marrow production of red blood cells by measuring

- Reticulocyte count
- Reticulocyte percent
- Soluble transferrin receptors

(b) Assess bone marrow stimulation by measuring

- Erythropoietin level

(c) Assess bone marrow suppression by measuring.

- Interleukin-6 (IL-6)
- C-reactive protein (CRP)

(d) Assess the type of anaemia by measuring

- Reticulocyte haemoglobin concentration (CHR)
- Folate & B12
- MCH (FBC)

Haemoglobin level can be measured every time during the follow up period. QOL assessment can be recorded during the period of the study by using different questionnaires.

4) Randomised controlled trials to examine rates of recovery of Hb post-ICU with EPO/iron treatment for a target population e.g. patients with Hb levels < 100 g/L at the time of ICU discharge.

In chapter [3], we have been shown that there are changes occurred to patients' RBCs as indicated by RBC 2,3-DPG during critical illness. It has been shown that the anaemia of critical illness is not associated with abnormal RBC 2,3-DPG levels, overall. In particular, increases in RBC 2,3-DPG, which could augment oxygen unloading in tissues, do not occur. It is unclear from the present study if this lack of

compensation affects oxygen unloading to the tissues. We have shown that acidosis is associated with low RBC 2,3-DPG level. This paradoxical finding raises interesting questions about the clinical importance of RBC 2,3-DPG concentrations for oxygen unloading in sick patients. Further studies should address function / quality of RBCs and investigate whether specific treatments could improve oxygen transport by patients own blood.

The literature review showed several established animal models to study oxygen delivery in relation to RBC 2,3-DPG. Further work should focus on exploring this association. It is difficult to carry out such studies in critically ill patients, as there are many abnormalities in factors controlling RBC 2,3-DPG level such as acid base status, however, animal studies can be designed to explore this relation more before starting a study that involves patients.

We have shown that there was a strong correlation between RBC 2,3-DPG level and calculated in vivo P50. It was unclear if this relation due to mathematical linkage or not. Further studies should be carried out to investigate this correlation. A laboratory study where a special tonometer with a facility to control pH, PCO_2, PO_2, and temperature during the plotting process should be used to plot the oxygen haemoglobin dissociation curve in blood samples with known different concentrations of RBC 2,3-DPG. All other variables such as pH, PCO_2, PO_2, and temperature should be standardised for all the samples through out the plotting processes.

In chapter [4], our work has shown that a considerable number of patients are anaemic before admission for elective orthopaedic surgery. The nature of this type of anaemia is usually similar to the anaemia of chronic disease. We have shown that

low pre-admission Hb values are associated with red cell transfusion. The findings emphasise the need for more studies to define the nature of pre-operative anaemia, the prevalence of iron deficiency and the potential benefits from pre-operative haematinic treatment, which may decrease the need of red cell transfusion. My data, based on a large population, should be used to design intervention studies in targeted population in whom expensive therapies such as EPO are most likely to be coast effective.

The studies that showed follow my work should include;

1) A cohort study to understand the pathophysiology of pre-operative anaemia for patients scheduled for major elective orthopaedic procedures. Anaemic patients at the time of the pre-admission can be identified. This group of patients can be investigated for their nutritional status by measuring B_{12}, folate, and serum iron levels. Measuring ferritin level should assess iron status.

2) Identifying patients for a randomised control trial of pre-operative haematinics administration. Patients should be divided in two groups at the time of pre-admission clinic [treatment group, receive iron, folate, and B_{12}, and placebo group]. Outcomes from this study could be assessed by the rate of Hb rise from post-operative day 1 to day 6, Hb rise from post-operative day 1 to day 30, percentage of patients receiving red cell transfusion, units of red cells for 100 patients operated, and indicators of rehabilitation e.g. return of mobility.

Improved understanding in these areas could further decrease the need for blood transfusion.

The present work was carried out in two settings of patients, critically ill and elective orthopaedic surgery patients. Most of these patients are elderly, presenting with

anaemia at admission or develop it during the course of their medical care and use clinically important amount of allogeneic blood. The majority of these patients have a similar type of anaemia, which is normocytic normochromic anaemia [similar to the anaemia of chronic disease].

Data in this thesis illustrate some of the major outstanding questions in two key blood user areas, namely, intensive care and elective orthopaedic surgery. Similar questions are likely to apply to most other types of patients requiring red cell transfusion. Increasing public awareness of the risks of blood transfusion and political issues arising from variant CJD will require improved evidence based transfusion practice. The data in my thesis raise many important questions to answer the many outstanding questions in the area of anaemia and blood transfusion. Appropriately designed well-powered clinical trials are needed to address these questions.

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APPENDICES

APPENDIX I

THE METHODOLOGY OF THE LINKAGE PROCESSES

A number of data files were obtained from the hospital laboratory database, one file for each month that contained the haematological records. For each blood sample record, in addition to the specified results, there was a unique identifier (specimen number) for the sample, the sample request date, and the patient's identifiers [patient's forename, surname, gender, date of birth, hospital number, and location]. Patients' dataset included for each admission a unique identifier (index number), patients' identifiers [patients' forename, surname, gender, date of birth, and hospital number], and patients' demographic data.

The blood sample records in each time period were linked to the admission records using a multi-stage process. This first involved looking for complete or partial matches in forename, surname, gender, and date of birth between hospital laboratory dataset and patients' dataset as follows:

1) All blood sample records were considered and those with identical forename, surname, gender and date of birth to an admission record were identified, i.e. a complete match on these four fields.

2) The blood sample records without complete matches were considered and those with identical forename, surname and date of birth but different gender to an admission record were identified, i.e. a partial match on these four fields.

3) The blood sample records without a complete or partial match were considered and those with identical forename, surname and gender but different date of birth to an admission record were identified, i.e. a partial match on these four fields.

4) The blood sample records without a complete or partial match were considered and those with identical forename, gender and date of birth but different surname to an admission record were identified, i.e. a partial match on these four fields.

5) The blood sample records without a complete or partial match were considered and those with identical surname, gender and date of birth but different forename to an admission record were identified, i.e. a partial match on these four fields.

6) The blood sample records without complete or partial matches were considered and those with identical forename and surname but different gender and date of birth to an admission record were identified, i.e. a partial match on these four fields.

7) The blood sample records without a complete or partial match were considered and those with identical gender and date of birth but different forename and surname to an admission record were identified, i.e. a partial match on these four fields.

Listings of all the partial matches identified in stages 2) to 7) above were then checked manually. In particular the non-matching fields and hospital numbers from the blood sample record and the admission record were reviewed.

Those blood sample records where this review identified the partial match as producing an incorrect linkage between the blood sample and the admission were removed from the linked blood samples dataset.

It was only possible to utilise the hospital number field when reviewing the partial matches between blood sample records and the admissions rather than as part of the identical fields matching process. This was due to inconsistent formats being used for

hospital numbers and the use of multiple hospital numbers for different blood samples within a single hospital stay.

Finally the sample request date was used to exclude those blood samples taken before or after the study period from the dataset of linked blood samples. Patients' identifiers were deleted as a part of the data protection rules and were replaced by unique identifiers (index number).

APPENDIX II

PRESENTATIONS MADE TO LEARNED SOCIETIES RELATED TO PRESENT WORK

September 2002

Pre-operative anaemia in patients undergoing elective orthopaedic surgery: could pre-operative haematinics help to reduce the need for transfusion?

XX Annual Scientific Meeting of the British Blood Transfusion Society, Edinburgh, UK.

September 2002

Pre-operative anaemia in patients undergoing elective orthopaedic surgery: Retrospective single centre study.

The 4th Arab Congress and the 3rd African Congress of Blood Transfusion, Tunis, Tunisia.

December 2002

Red blood cell 2,3- diphosphoglygerate concentration in early critical illness.

The UK Intensive Care Society, 'State of the art' Meeting, London, UK.

April 2003

Prevalence of anaemia among survivors of critical illness managed with conservative transfusion triggers: a single centre cohort study.

The UK Anaesthesia Research Society Meeting, Glasgow, UK.

June 2003

Anaemia & Transfusion in the contexts of elective orthopedic surgery & Intensive Care Units (ICUs).

Biannual Inter-country Meeting for Directors of Blood Transfusion Services in Member States, World Health Organization - Regional Office for the Eastern Mediterranean, Cairo, Egypt

October 2003

How prevalent is anaemia after ICU discharge: a prospective single centre cohort study?

16th Annual Congress of The European Society of Intensive Care Medicine, Amsterdam, The Netherlands.

January 2004

Anaemia at hospital discharge among survivors of critical illness.

Scottish Intensive Care Society, Stirling, Scotland – UK.

APPENDIX III

PUBLICATIONS TO DATE RELATED TO PRESENT WORK

Saleh EED, McClelland DBL, Picken M, Williams K, Semple D, Walsh TS. Pre-operative anaemia in patients undergoing elective orthopaedic surgery: could pre-operative haematinics help to reduce the need for transfusion? Transfus Med 2002;12(Supplement 1):24.

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