ANEMIA AND TRANSFUSION DECISIONS IN THE INTENSIVE CARE UNIT

A dissertation submitted to the Tamil Nadu Dr. M.G.R. Medical University in partial fulfillment of the University regulations for the award of

M.D. Immuno Haematology & Blood Transfusion (Branch –XXI)

DEPARTMENT OF TRANSFUSION MEDICINE AND IMMUNOHAEMATOLOGY

CHRISTIAN MEDICAL COLLEGE, VELLORE

BONAFIDE CERTIFICATE

This is to certify that the work presented in this dissertation titled "**Anemia and Transfusion Decisions in the Intensive Care Unit** " done towards fulfillment of the requirements of the **Tamil Nadu Dr. M.G.R. Medical University, Chennai for the M.D. Immuno Haematology & Blood Transfusion (Branch– XXI)** exams to be conducted in April 2014, is a bonafide work of the candidate **Dr. Eliza Sherin Koshy**, Post graduate student in the Department of Transfusion Medicine and Immunohaematology, Christian Medical College, Vellore done under my supervision. This dissertation has not been submitted, fully or in part to any other board or University.

Dr. Sukesh C Nair,

Head of Department,

Department of Transfusion Medicine and Immunohaematology,

Christian Medical College, Vellore – 632004

BONAFIDE CERTIFICATE

This is to certify that the work presented in this dissertation titled "**Anemia and Transfusion Decisions in the Intensive Care Unit** " done towards fulfillment of the requirements of the **Tamil Nadu Dr. M.G.R. Medical University, Chennai for the M.D. Immuno Haematology & Blood Transfusion (Branch– XXI)** exams to be conducted in April 2014, is a bonafide work of the candidate **Dr. Eliza Sherin Koshy**, Post graduate student in the Department of Transfusion Medicine and Immunohaematology, Christian Medical College, Vellore done under my guidance and supervision. This dissertation has not been submitted, fully or in part to any other board or University.

Guide.:

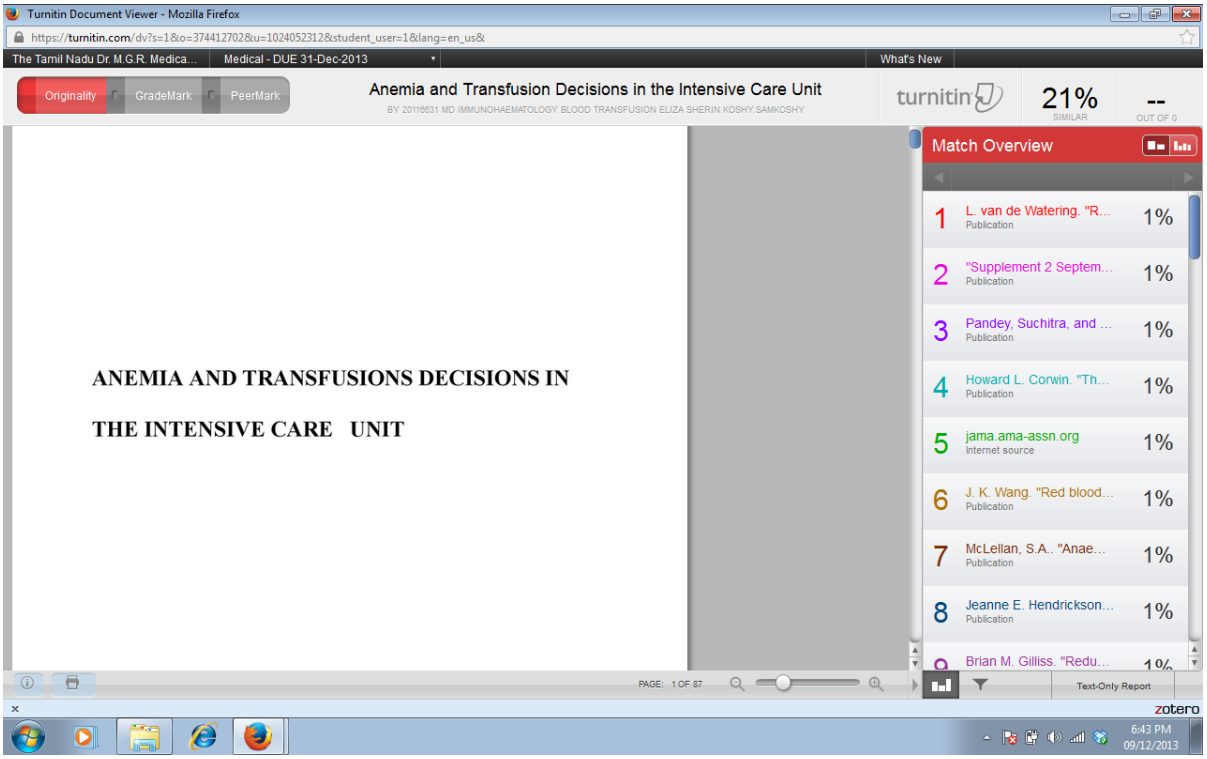
Dr. Dolly Daniel,

Professor,

Department of Transfusion Medicine and Immunohaematology,

Christian Medical College, Vellore – 632004

PLAGIARISM


Turnitin Document Viewer - Mozilla Firefox
https://turnitin.com/dv?s=1&o=374412702&u=1024052312&student_user=1&lang=en_us&
The Tamil Nadu Dr. M.G.R. Medica...
Medical - DUE 31-Dec-2013
What's New
Originality
GradeMark
PeerMark
Anemia and Transfusion Decisions in the Intensive Care Unit
BY 20116631 MD IMMUNOHAEMATOLOGY BLOOD TRANSFUSION ELIZA SHERIN KOSHY,SAMKOSHY
turnitin
21%
SIMILAR
--
OUT OF 0
ANEMIA AND TRANSFUSIONS DECISIONS IN
THE INTENSIVE CARE UNIT
Match Overview
1 L. van de Watering. "R... Publication 1%
2 "Supplement 2 Septem... Publication 1%
3 Pandey, Suchitra, and ... Publication 1%
4 Howard L. Corwin. "Th... Publication 1%
5 jama.ama-assn.org Internet source 1%
6 J. K. Wang. "Red blood... Publication 1%
7 McLellan, S.A.. "Anae... Publication 1%
8 Jeanne E. Hendrickson... Publication 1%
Brian M. Gilliss. "Redu... 1%
PAGE: 1 OF 87
Text-Only Report
zotero
6:43 PM
09/12/2013

Acknowledgments

Working on this thesis has been a very challenging, yet worthwhile experience. I am truly indebted to all the people who have helped me throughout this period.

First and foremost, I am extremely thankful to my Professor and my guide, Dr. Dolly Daniel for her encouragement, wisdom, knowledge and guidance at all stages of this thesis. Her constant support has made this dissertation a reality.

Next, I would like to thank my Co-Guide, Dr. J.V.Peter, for his valuable suggestions and insights during the time of my study. I would also like to thank him for compiling and analyzing all my data.

My sincere thanks to Dr.Sukesh Chandran, Professor and Head of Department, Dr. Joy Mammen, Professor, Dr.Mary Purna Chacko,Associate Professor and my colleagues' for their valuable advice.

I would also like to thank Ms.Gowri from Biostatistics for her help in analyzing the data.

I am also indebted to my husband for his help and support, and my son who had been very patient and understanding all through my work on this dissertation.

Above all, I thank God for His unmerited favor in every area of my life.

CONTENTS

ABBREVIATION

Hb	Haemoglobin
RBC	Red Blood Cell
MICU	Medical Intensive Care Unit
SICU	Surgical Intensive Care Unit
WHO	World Health Organization
PO2	Partial Pressure of oxygen
P50	Partial pressure of oxygen at 50% saturation
PCO2	Partial pressure of carbon dioxide
2,3-DPG	2,3 Di-phosphoglycerate
DO2	Oxygen delivery
CaO2	Arterial oxygen content
VO2	Oxygen consumption
EPO	Erythropoeitin
rHuEPO	Recombinant Human erythropoietin
TNF	Tumour Necrosis Factor
IL	Interleukin
SA	Sialic acid
IDA	Iron deficiency anemia
AI	Anemia of inflammation
APACHE	Acute Physiological and Chronic Health Evaluation Score
SOFA	Systemic Organ Failure Assessment Score

FNHTR	Febrile Non Haemolytic Transfusion Reactions
TRALI	Transfusion Related Acute Lung Injury
TACO	Transfusion Associated Circulatory Overload
TA-GVHD	Transfusion associated Graft vs Host Disease
TRIM	Transfusion Related Immunomodulation
HLA	Human Leukocyte Antigen
HPA	Human Platelet Antigen
ABC Trial	Anemia and Blood Transfusion in Critical Care
TRICC	Transfusion Requirements in Critical Care
SOAP	Sepsis Occurrence in Acutely Ill Patients
ATICS	Audit of Transfusion in Intensive Care Study

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

ANEMIA AND TRANSFUSION DECISIONS IN THE INTENSIVE CARE UNIT

Background: Anemia is frequently seen in critically-ill patients contributing to increased morbidity, mortality, and hospital stay.

In ICU, anemia is treated with allogenic blood transfusions. Audits show 95% of ICU patients have haemoglobin level below normal by ICU day-3 and 50% of critically-ill patients receive RBC transfusions, trigger being 8.5g/dl. Higher transfusion rates were associated with prolonged ICU stay.

Aim: To study transfusion thresholds and practices in Indian intensive care unit patients.

Materials and Methods: Patients admitted during October 2012-June 2013 to medical and surgical-ICU, with an anticipated stay greater than 48hrs were enrolled. Patients with known primary haematological disorder or who succumb to their illness or discharged within 24hrs of ICU admission were excluded. Patients were followed up throughout their stay until hospital discharge or if death occurred before that. Data collection included admission haemoglobin along with daily hemoglobin values, requirement of RBC transfusions with indication, pre-transfusion haemoglobin, length of ICU and hospital stay and outcome.

Results: 800 patients were enrolled. Overall, 38% of patients received transfusions with blood or blood products and 34% of patients received RBC transfusions. Most common indication for transfusion was a drop in haemoglobin without overt bleeding, mean pre

transfusion trigger was 7.0±1.6 g/dl. Mortality was 23% among those who did not receive transfusions and 30% among those who received packed cells. There was a statistically significant association between packed cell transfusions and prolonged ICU and hospital stay. Mean baseline haemoglobin was 10.7±2.8g/dl. Hemoglobin levels decreased throughout the duration of ICU stay. There was a statistically significant association between age, APACHE-II score, duration of ventilation and packed cell transfusion with mortality. After adjusting for other risk factors, APACHE-II score and ventilation duration remained significantly associated with increased mortality but no association was seen between receipt of RBC transfusion and mortality.

Conclusion: There was a significant drop in haemoglobin following admission to ICU. RBC transfusions were initiated below 7g/dl. Transfusion led to prolonged ICU and hospital stay and mortality in the transfused was significantly higher than in the non-transfused. Approaches should therefore be made to reduce RBC transfusions in critically-ill patients.

INTRODUCTION

INTRODUCTION

Anemia has been recognized as a frequent complication in the critically ill patient. It leads to a decrease in the oxygen carrying capacity of blood resulting in increased morbidity and mortality, and a prolonged length of stay in the hospital.

The causes for anemia are multifactorial and include hemodilution, frequent blood sampling for laboratory investigations, hemorrhage, bone marrow suppression, inadequate erythropoietin response and increased RBC destruction due to complement activation in critically ill patients.

In the intensive care setting, anemia is commonly treated with allogenic blood transfusion, the primary aim being to increase the oxygen delivery, determined by an increase in the cardiac output and oxygen content of arterial blood, which is in turn dependant on the haemoglobin level.

Several audits have found that approximately 95% of ICU patients have haemoglobin level less than normal by the third day of their ICU stay and roughly 50% of the patients receive packed cell transfusions during their ICU stay. Several landmark studies have examined anemia in the ICU setting and they reveal a number of similarities.

1. Majority of the ICU patients are anemic on admission.

2. The transfusion trigger in most studies was a hemoglobin level of about 8.5g/dl.

3. The commonest indication for initiating transfusion was anemia.

4. A more restrictive transfusion strategy was probably safer than a liberal transfusion strategy and hospital mortality was much lesser in the restrictive arm of patients.

5. Greater number of packed cell transfusions was associated with a lengthened ICU stay.

AIMS AND OBJECTIVES

AIM & OBJECTIVES

AIM

- To study thresholds for transfusion and transfusion practices in Indian intensive care unit patients.

OBJECTIVES

1. Prevalence of anemia at admission and haemoglobin trends during ICU stay

2. To ascertain transfusion thresholds and transfusion practices in ICU patients

3. To assess if transfusion is an independent factor associated with mortality.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Anemia is a prevailing problem and a frequent complication encountered in critically-ill patients. Its incidence from various observational studies is roughly 95% in patients who have for more than three days in the intensive care unit.(1)

DEFINITION:

Anemia is a state of pathological insufficiency in the oxygen-carrying capacity of blood. It can be calculated as unit volume concentrations of hemoglobin, volume of red blood cells, or the number of red blood cells.

The World Health Organization (WHO) defines anemia as a haemoglobin concentration that is below the normal range, which refers to the distribution of haemoglobin in a large representative set of individuals.

Historically, anemia is defined as a decrease in hemoglobin (less than 12 g/dl for women, less than 13 g/dl for men) and hematocrit (less than 36% for women and less than 39% for men) .In the ICU patient, typically quoted figures are a hemoglobin < 10 g/dl or a hematocrit < 30%.(2)

ERYTHROPOESIS:

Erythropoiesis is the process of development of mature red blood cells (erythrocytes). All blood cells including RBCs start as stem cells. The foremost cell recognized that specifically leads down the RBC path is called the proerythroblast. As it develops, the nucleus becomes

smaller, with the cytoplasm being more basophilic because of the appearance of ribosomes. As hemoglobin begins to be produced the cell, the cytoplasm of the cell attracts basic and eosin stains, hence the name, polychromatophilic erythroblast. Following this, the cytoplasm becomes more eosinophilic, and becomes an orthochromatic erythroblast. This erythroblast thereafter extrudes its nucleus and becomes a reticulocyte which enters the circulation. These reticulocytes contain small fragments and remnants of basophilic material including mitochondria and other organelles. When the reticulocytes lose these basophilic remnants (over 1 to 2 days), they become mature RBCs.

Older and damaged RBCs are constantly removed from the circulation primarily by the spleen, and the cell components (i.e., iron from hemoglobin) are further recycled to form new RBCs. Erythropoiesis requires a normal supply of substrates (vitamins B12 and folate), nutrients, and essential minerals (iron).

HAEMOGLOBIN

The major oxygen-carrying pigments of the body are hemoglobins. They are incorporated into red blood cells in adequate quantities to carry sufficient amount of oxygen from the lungs to the tissues to meet the needs of those cells for oxidative metabolism.

Hemoglobins are composed of globin polypeptide chain tetramer. This contains a pair of alpha - chains which are 141 amino acids long and a pair of beta chains which are 146 amino acids long. HbA is the chief adult hemoglobin with a structure $\alpha_2\beta_2$. The human hemoglobins are encoded in two gene clusters that are tightly linked. The alpha globin genes are present on chromosome 16, and the beta genes are present on chromosome 11.

Each globin chain encloses a single heme moiety, which consists of a protoporphyrin IX ring complexed with an iron atom in its ferrous state (Fe^{2+}). Every heme moiety has the capacity to bind to a single molecule of oxygen. Therefore one hemoglobin molecule transports up to four molecules of oxygen The hemoglobin molecule is highly soluble but the individual globin chains are not soluble. Unpaired globin usually precipitates, forming inclusions that damage the cell. The normal globin chain synthesis is well balanced in that each newly synthesized alpha or non-alpha globin chain has a partner with which to pair.(3)

The relationship between the oxygen content in the blood and the oxygen tension of blood is represented by the oxyhaemoglobin dissociation curve. Any given point on the sigmoid-shaped curve represents the haemoglobin oxygen affinity at that particular point. The P50 is usually used as a symbol of haemoglobin oxygen affinity. It is described as the oxygen tension at 50% haemoglobin saturation. The factors that increase haemoglobin oxygen affinity shifts the oxyhaemoglobin dissociation curve to the left thereby decreasing the P50, and factors which increase haemoglobin oxygen affinity will shift the curve to the right increasing the P50.

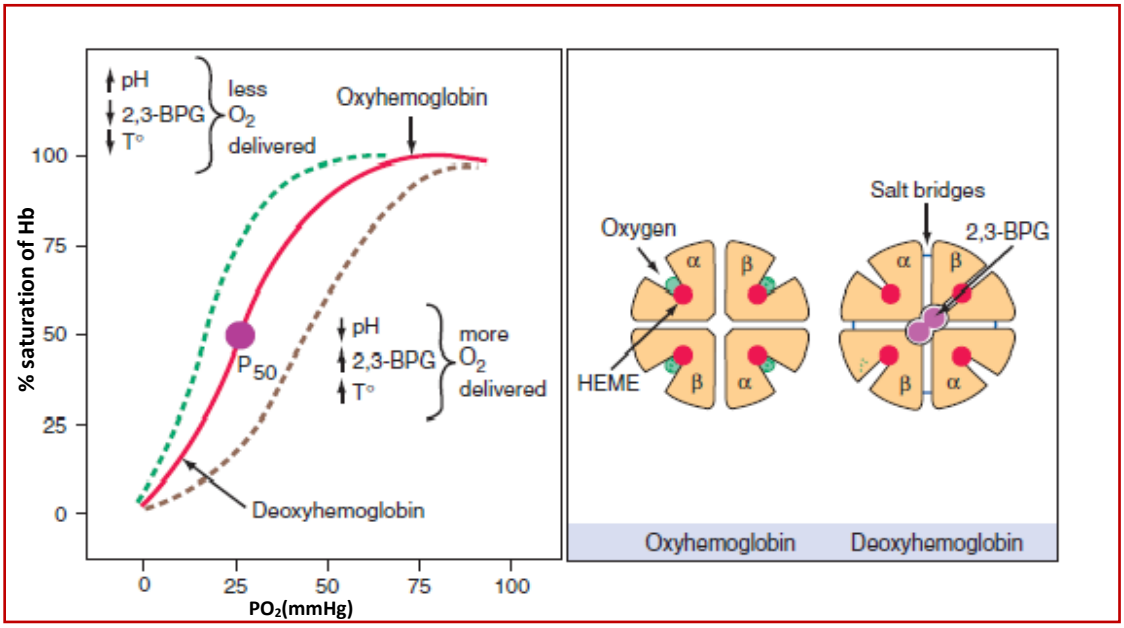


Fig 1 Oxyhaemoglobin dissociation curve. Harrison's Haematology and Oncology (4)

For oxygen transport, hemoglobin should bind to oxygen efficiently at the partial pressure of oxygen (PO_2) in the alveolus, thereafter retain and transport it to the tissues, and release it there at the PO_2 of tissue capillary beds. The hemoglobin tetramer is fully deoxygenated at low oxygen tensions. As O_2 tension rises oxygen binding begins slowly. But once some oxygen binds to the tetramer, there is an immediate increase in the slope of the curve. Therefore a higher oxygen affinity is exhibited by hemoglobin molecules that have bound some oxygen. This accelerates their ability of hemoglobin to combine with more oxygen. The affinity of hemoglobin for oxygen is also modulated by several other factors like pH, PCO_2 and temperature. The effects of pH on the haemoglobin-oxygen affinity is referred to as the Bohr effect, this describes the intrinsic property of hemoglobin where by a molecule of Hb can accept an H+ when it releases a molecule of oxygen. Deoxyhaemoglobin can hold on

to and accepts the H+ better than oxyhaemoglobin. The affinity of haemoglobin for oxygen is reduced by increased H+ concentration, when the blood reaches the tissues that have higher concentrations of lactic acid and CO_2. Oxygen is then released at these sites. Similarly an increase in temperature also decreases the affinity of haemoglobin for oxygen. 2,3-DPG a byproduct of the glycolytic pathway which occurs in the erythrocyte in similar concentrations as haemoglobin also lowers oxygen affinity when bound to hemoglobin.

The functional capacity of the erythrocyte, requires normal renal production of erythropoietin, a functioning erythroid marrow, and a sufficient supply of substrates for hemoglobin synthesis. A defect or deficiency in any of these vital components can lead to anemia.

Erythropoietin stimulation can lead to four to fivefold increase in production within a one to two week period, but this can happen only in the presence of adequate nutrients, especially iron.

Principles of oxygen transport

After dissolution in plasma, oxygen is transported after being bound to haemoglobin. Oxygen has a very low solubility in plasma and therefore transport by the process of diffusion is inefficient. In the non anemic patient with a Hb of about 14 gm/dl, most of the oxygen is haemoglobin-bound and a very small amount of approximately 2% only is found in a dissolved form in the plasma. On the contrary, in a patient with severe anemia, on 100% oxygen with a Hb of 5g/dl, we find that plasma contains almost 20% of dissolved oxygen.

Oxygen delivery (DO2) is a combined byproduct of cardiac output and measured arterial oxygen content represented as (CaO2). When represented as a formula:

DO_2=cardiac output × CaO_2 (where CaO_2=(Hb×1.34×SaO_2)+(PaO_2×0.003)

Likewise. oxygen consumption (VO2) is the byproduct of cardiac output and the arterio-venous (difference) in content of oxygen.(5)(6)

It is crucial to understand that a drop in the Hb level does not inevitably result in decreased DO2 because invariably cardiac output will increase. Alternate mechanisms which compensate are those which increase the oxygen extraction capacity thus lowering venous oxygen saturation and partial pressure.

These two mechanisms help patients who are normovolemic to tolerate Hb levels up to 5 g/dl with no decrease in oxygen consumption or other clinical evidence of oxygen deprivation. In addition the redistribution of blood flow which happens when Hb levels are low, to ensure oxygenation of vital organs such as heart, brain and vital tissues and organs helps optimize clinical situations.

Physiological adaptation to progressive normovolemic anemia

Progressive anemia leads to reduction in blood viscosity, favoring the venous return to heart and facilitating ejection of stroke volume. Moreover, normovolaemic anaemia increases sympathetic stimulation of the heart, thereby contributing to an increase in the cardiac output. DO2 decreases during progressive normovolaemic anaemia in spite of an increase in the cardiac output. Nevertheless, oxygen extraction also increases, thereby VO2 continues to be

constant in even at haemoglobin of 5 g/dL. Hence with acute normovolemic anemia, the decrease in arterial oxygen content is compensated for by

1) increasing the cardiac output,

2) redistribution of blood flow between and within organs, and

3) alteration in the amount of oxygen extracted from the blood.

Following exhaustion of these compensatory mechanisms, tissue injury occurs.

Microcirculatory changes take place as oxygen content decreases with progressive hemodilution, leading to a recruitment of capillaries. There is more time for gas exchange as the red cell capillary transit time increases. Moreover, anemia changes the oxygen extraction ratio from the RBC itself by increasing red cell 2,3-DPG and shifting the oxyhemoglobin dissociation curve to the right, so that more oxygen is released to the tissues.

Animal studies have constantly confirmed that healthy animals can tolerate acute, isovolemic hemodilution up to 5 g/dL without any untoward consequences.(7) Any further hemodilution results in cardiac ischemia and reduced oxygen uptake. When hemoglobin levels fall below 3g/dL, there is increased lactate production, decreased left ventricular function and cardiac output, and an increased risk of death. In healthy humans, the critical hemoglobin level is still unknown but studies have found that it is likely to be less than 5 g/dL.(6)

Etiology of anaemia in critical illness

The etiology of anaemia during critical illness can be due to a number of factors.(2) They can be divided into:

- Pre-existing chronic anaemia and
- Acquired anaemia.

Among the causes for acquired anemia include:

- **Haemodilution**

Critically ill patients often develop intravascular hypovolaemia that require some amount of fluid resuscitation. Heamoglobin/haematocrit has been considered as customary clinical measure for anemia, but it cannot be used as a reliable estimate of red cell mass as it is affected by an alteration in the plasma volume.

Haemodilution amounts for a considerable proportion of the fall in haemoglobin concentration that may occur rapidly during the early stages of critical illness, mostly during fluid resuscitation.

- **Blood loss/haemorrhage**

Studies in ICU patients have shown that the commonest cause for anemia in this setting is hemorrhage. Gastrointestinal bleeding or hemorrhage occurring due to trauma or surgery are

obviously evident but less overt hemorrhage, can occur due to various ICU interventions which may be not so easily detected.

Repetitive blood sampling for investigations has often been cited as a cause for significant blood loss. Corwin at al. noted that blood loss due to phlebotomy amounted for almost 30% of the total number of units transfused in 142 ICU patients, with an increased blood loss owing to phlebotomy in those patients who were transfused (8). It was estimated that in the anemic critically-ill patients, the median blood loss was as high as 128 ml per day.

The ABC trial estimated that the volume of blood lost through blood sampling, l amounted to 41 ml per 24 h.(9) When calculated as per day losses this could amount to iron losses of 64mg/day Considering that our daily iron ingestion is less than the estimated iron loss by 20 fold it is highly likely that in this group of critically ill patients, , iron deficiency could set in..

- **Anemia of inflammation**

Developing during states of inflammation, anemia of Inflammation (AI) is now the favored name for what was previously described as anemia of chronic disease. Following iron deficiency, it is the second most prevalent form of anemia seen The mechanisms responsible for this entity are four as mentioned below.: ***decreased production of erythropoietin (EPO), impaired response of the bone marrow to EPO, reduced iron levels, and increased clearance of RBCs.***(10)

A mild to moderate normocytic, normochromic anemia associated with abnormal iron utilization is caused due to inflammation.

The characteristic feature of this disorder is a combination of a raised ferritin in combination with a low serum iron and transferrin saturation.(11) An excess of hepcidin leading to inhibition of recycling of the iron by the macrophages is usually the cause. Inflammation (mediated by IL-1) leads to ferritin synthesis independent of the level of iron stores and hence elevated ferritin levels is no longer suggestive of iron stores in the presence of inflammation [10]. Thus, in spite of an iron profile mimicking iron overload, iron deficiency exists in these group of critically ill patients. Anemia owing to iron deficiency can also coexist with anemia of inflammation.

The level of hepcidin may be a useful marker of IDA in the presence of anemia of inflammation since hepcidin is the key regulator of iron homeostasis. Levels of hepcidin markedly decrease in the presence of IDA and they are elevated in anemia of inflammation.

- ***Reduced red cell production***

Erythropoiesis responds to changes in haemoglobin levels. And the process of erythropoiesis is a rather flexible one. It usually adapts to maintain a relatively constant Hb level. On a normal day only about 1% of circulating red cells are renewed. However, if there is an acute event such as acute blood loss, the rate of erythropoesis can compensate even up to 10 times. Sometimes, when the bone marrow does not produce adequate red cells, this response is blunted, and in addition there is a low reticulocyte response.

In addition, factors which affect haematopoiesis such as stimulatory and inhibitory mediators, many which are related to inflammation will also impact on anemia and response to the same. Inhibitory mediators act by suppressing the production and action of stimulatory

factors, which results in preventing hematopoietic stem cells growth or by causing programmed cell death. Interferon(IFNɣ) (12), tumor necrosis factor (TNF) (13), transforming growth factor (TGF)-b (14), and interleukin-1 all have shown to inhibit the growth of erythropoeitic progenitor cells, and injection of these cytokines into healthy humans or animals can result in anemia (15). An important role in mediating these effects is also seen with Nitric oxide (NO).(16)(17)(18)

The erythroid response to erythropoietin (EPO) administered exogenously is also blunted, as doses of EPO required to generate a response is much higher when compared to doses administered to patients with renal failure.

The chief regulator of erythropoiesis is the hormone erythropoietin which acts on specific target cells present in the marrow with the help of cell surface receptors. From the pool of erythroid colony-forming cells it stimulates the production and maturation of erythroid progenitor cells. (19). Furthermore, erythropoietin can lead to the premature release of cells, as well as reticulocytes, from the marrow. This may be counterbalanced by death signals, leading to impaired RBC production. In this context, it was seen that increased apoptosis of bone marrow erythroid precursors was demonstrated in sepsis patients. .

Anemia and hypoxia are the key stimulators of erythropoietin release. In the acute and chronic phases of anemia, the erythropoietin levels in the plasma are inversely related to plasma Hb levels.

In critically-ill patient presenting with long standing conditions like rheumatoid arthritis or cancer (20), the normal response to erythropoietin may be blunted , resulting in anemia. TNF

and IL-1, that are released during sepsis, surgery, certain infections and trauma, have been related to a decrease in the erythropoietin production, and this may also affect the erythropoietin receptor (21), hence affecting the release and function of erythropoietin.

Sufficient supplies of iron, vitamin B12 and folic acid are necessary for the normal erythrocyte development and a deficiency of any of these elements can lead to anemia.(22). Presence of increased oxidative stress, which occur in conditions like sepsis can also decrease the formation of EPO and inhibit erythroid precursor cells.

Iron deficiency can also result due to haemorrhage or repeated phlebotomy. Also, some ICU patients have nutritional deficiencies related with the present disease condition. These, however, are not likely to be the chief source of anemia in the ICU patient.

A number of drugs can also affect the production of RBCs like nitrous oxide, which causes megaloblastic changes thus limiting bone-marrow DNA synthesis.

- ***Reduced iron availability***

Hepcidin, the key regulator of iron metabolism acts by binding to the iron exporter ferroportin, thereby causing internalization of iron and inhibiting the release of iron from tissue macrophages. Thus, hepcidin reduces the concentration of iron in the blood. Its production is up regulated in response to increased serum iron levels.

Inflammation also induces hepcidin, which acts fast resulting in a drop in iron levels within hours (23)(11). Studies have shown that infusion of IL-6 into human volunteers induces increased hepcidin synthesis with a decrease in plasma iron levels (24).

Hepcidin levels are elevated in critically ill trauma patients, correlating with the duration of the anemia (25), and also in critically ill patients not suspected for iron deficiency.

- ***Increased red blood cell clearance***

The cause for decreased RBC lifespan in inflammation is thought to be due to an altered morphology of the RBCs, leading to increased adherence to the endothelium following which they are cleared from the circulation. The various mechanisms responsible for erythrocyte clearance in physiologic senescence and inflammation are:

- Phosphatidylserine (PS) exposure,
- Erythrocyte phagocytosis
- Reduced deformability and
- Adherence to endothelium.(10)

Sphingomyelinase is an enzyme which converts sphingomyelin into ceramide (26). Ceramide enhances the sensitivity of RBCs to raised intracellular Ca2+ concentration, and hence enhances PS exposure. A number of factors can lead to an increase in the plasma levels of sphingomyelinase, and some of these are also seen in sepsis, such as tumor necrosis factor-alpha and platelet activating factor (PAF) (27).

Moreover, bacteria such as *Staphylococcus aureus* also produces sphingomyelinase (28) which can convert sphingomyelin into ceramide . Ceramide thus enhances PS exposure which causes accelerated RBC clearance during inflammation.

Numerous factors decrease RBC deformability during sepsis like Reactive oxygen species (ROS) which causes RBC deformability in inflammation. ROS leads to protein degradation in RBCs in vitro (29), particularly of membrane proteins namely band 3 and spectrin.

NO is a mediator which acts as a vasodilator and is released by the vascular endothelial cells (30). Small amounts of NO is present in the blood under physiological conditions, but during inflammation and infection the concentration increases by 10-fold. NO also causes a decrease in the RBC deformability.

Another implicated factor in the clearance of RBCs during inflammation are sialic acid residues (SA) that are bound to glycophorin and is the reason for the negative charge of the RBC membrane (31). Due to this negative charge, RBCs manifest repellent property. SA content was reduced in the RBCs from critically ill patients when compared to RBCs from healthy volunteers. This was also associated with a decrease in RBC deformability.

Treatment of anemia

Therapy for anemia in the ICU was always directed to correcting the hemoglobin to an arbitrary “normal” because of the professed risk of anemia leading to increased morbidity and mortality.

The different modes of treatment of anemia in the ICUs are:

1) Blood transfusions

2) Erythropoietic agents

3) Iron therapy

4) Blood substitutes

Erythropoietic agents

Erythropoietic agents are used in critically ill patients in an attempt to reduce the need for blood transfusions. The rationale for recombinant erythropoietin (rHuEPO) therapy is that an increase in erythropoiesis results in higher Hb levels, thus reducing the need for RBC transfusions. This was confirmed by early experimental studies which demonstrated that rHuEPO when given in the perioperative period accelerated erythropoiesis resulting in drastically shorter times for haemoglobin to reach baseline levels. (32),(33)

In a study, long term treatment with a weekly dose of rHuEPO considerably reduces the exposure to RBC transfusions during the first 42 days of rHuEPO therapy. These patients achieved a higher hemoglobin level in spite of fewer packed cell transfusions.(34).The disadvantage of rHuEPO was that it was associated with an increased incidence of thrombosis.

Iron therapy

Iron therapy has a role for a subgroup of critically ill patients who have both anemia of inflammation (AI) and iron deficiency anemia (IDA). Patients are considered to have both IDA and AI if they have decreased hepcidin and serum transferrin/log ferritin ratios along with elevated CRP and ferritin levels. The tests available to discriminate the two are currently not available. Iron causes oxidative stress thereby increasing the risk of bacterial infection. Absorption of oral iron may be reduced in patients with AI due of the effect of hepcidin mediated decrease of ferroportin in duodenal enterocytes. Iron has to be supplemented in patients on erythropoietic agents for a favorable response.(1)

Blood substitutes

These are synthetic products that have oxygen-carrying capacity. Red cell substitutes include haemoglobin based oxygen carriers (HBOC), per fluorocarbon emulsions and liposome encapsulated haemoglobin.

The various modifications of HBOC s are unmodified cell-free haemoglobin, cross linked haemoglobin, polymerised haemoglobin and surface- conjugated HBOCs. All of them have longer shelf life with permissive storage conditions. There is no need for compatibility testing, and due to pathogen inactivation, there is absence of infectious risks. Majority of the oxygen carriers under development are based on either human or bovine hemoglobin.

Outdated RBCs are the source of human hemoglobin. They have increased oxygen carrying capacity, exert considerable oncotic properties thereby increasing the blood volume by an

amount which is greater than the transfused volume. They also have vasopressor effects due to the scavenging effects of nitrous oxide (35).

Blood transfusions

Blood transfusions are used in critically ill anemic patients to improve the oxygen delivery to tissues. Studies have shown that more than 50% of patients admitted to the ICU receive blood transfusions, the number increasing to 85% in those with longer length of stay (greater than seven days) (8)

Transfusion triggers

The historical and empirical '10/30 rule' was used for many years with regard to the transfusion practice. In 1942, Adams and Lundy initially recommended this as one amongst several perioperative suggestions to improve the result of surgical patients who have poor post anaesthetic outcome [8]. However, this rule has been misinterpreted and used roughly.

Many years later, the term 'transfusion trigger' came into existence. This term referred to a critical level of Hb level below which packed cell transfusions should be initiated.

Weiskopf et al (35) found that in the resting state,healthy individuals are able to withstand an state of acute state of isovolemic haemodilution uptil a drop of Hb to 5 g/dl, despite the fact that some amount of reduction in the mental functions may be present at this level of Hb. It was studied that in these individuals at rest, there was no considerable change in the venous oxygen saturation or the lactate concentration within the plasma even though the transport of oxygen was reduced throughout the phase of haemodilution (using 5% albumin

or plasma) uptil Hb dropped to 5 g/dl. However, the critical level of Hb increases in the presence of coronary artery disease.

In animal models, when 75% coronary artery stenosis was present, cardiac dysfunction was seen at a Hb level of 7 g/dl [24]. By challenging normovolemic anemic subjects or by measuring parameters of cardiovascular/cardiorespiratory function, (38) the consequences of oxygen deficit was seen at hemoglobin levels between 5 and 6 g/dL. These deficits were recoverable following the reinfusion of red cells.

A randomized controlled trial of 7 vs. 10 g/dL Hb thresholds in critically-ill patients, as well as patients with atherosclerotic disease , showed that lower threshold considerably reduced overall morbidity and mortality and it was not related to prolonged length of stay or morbidity in patients with cardiovascular disease, nor prolonged amount of ventilation for those requiring a respirator (39).

The authors concluded that a threshold of 7 g/dL Hb was apt across all critically-ill patients with the exception of those with unstable cardiac conditions in whom there was a statistically nonsignificant trend toward increased mortality when a more restrictive threshold was used.

Transfusion is not normally indicated if anemia has persisted for weeks or months, as counter active mechanisms have had time to work. These anemias are best treated by addressing their etiologies, such as nutritional deficiency (e.g., iron) or by reducing the speed of autoimmune hemolysis. Any of these situations might necessitate a transfusion if the patient symptomatically requires a more rapid reversal than which may be achieved by correcting the underlying etiologies.

Clinical studies – towards finding a critical transfusion threshold

Retrospective analysis of medical and surgical patients belonging to Jehovah's Witness (40) recognized fifty deaths, of which twenty three of them were as a result of anemia. Apart from three patients who succumbed to their illness following a cardiac surgery, all the other patients who died owing to anemia had Hb levels of 5 g/dl or less. In spite of this, even when the Hb levels decreased to 5 g/dl or less, twenty five patients survived.

Another study comprising of 4470 critically-ill patients, those who presented with a cardiovascular disease showed a drift towards increased mortality as Hb levels decreased to below 9.5 g/dl (55% vs. 42%; P=0.09).

Wu et al. (41) studied that older patients with acute ischemic heart disease and who presented with decreased admission haematocrit showed greater 30-day mortality rates. They also document a short term survival advantage (as measured by a lower 30 day mortality rate) in patients who have received transfusions for haematocrits below 30-33%.

In a study by Carson et al. [53] analyzing the role of transfusions in an orthopedic setting of 8787 geriatric patients with hip fracture repair, the postsurgical transfusion did not influence the 30 and 90-day mortality in patients with Hb levels of 8.0 g/dl or higher, after adjusting for the critical Hb level, cardiac status and other risk factors, neither was there an impact of the preoperatively transfusion on the 30-day mortality in patients with Hb levels of 8.0 g/dl or higher, signifying that this might be a safe threshold in this subset of patients.

Hebert et al assessed (42) the 30-day mortality in 838 euvolemic ICU patients. In this study patients with Hb levels less than 9 g/dl were randomized to either a liberal or a restrictive arm. The 418 patients who were randomized to a restrictive arm had their Hb levels maintained between 7.0- 9.0 g/dl, and red cells were transfused only when Hb levels fell below 7.0 g/dl. Those assigned to the liberal arm were the remaining 420 and Hb was maintained between 10.0 -12.0 g/dl, and transfusions were initiated when Hb levels fell below 10.0 g/dl. Analysis of the results showed that the hospital mortality rate was significantly lower in the restrictive group than the liberal group (22.2% vs. 28.1%; P =0.05).

In the subset analyses, 30-day mortality rates were considerably decreased in patients who belonged to the restrictive arm who were younger and less acutely sick. In patients less than 55 years of age, the mortality rates were 5.7% and 13.0% in the restrictive and liberal arms, respectively, and in those with APACHE II scores of 20 or less, 30-day mortality rates were 8.7% and 16.1% , suggesting that in these subgroup of patients, a more restrictive transfusion strategy ought to be used.

Thus the choice to start packed cell transfusion is best guided by the patients' physiological want. However, the edge for treatment continues to be a debate and depends essentially on a combination of clinical signs and symptoms at the patients side in conjunction with laboratory data and available measures that best denotes variation in tissue oxygenation.

Drawbacks of transfusion:

RBC transfusions are used in the intensive care setting to increase oxygen delivery and improve tissue oxygenation. Hypothetically, an increase in Hb concentration increases the oxygen-carrying capacity of blood and delivering more oxygen to the tissues. However increasing evidence question the efficacy of RBC transfusion at improving outcomes in clinical situations other than acute haemorrhage.

Some of the non infectious and infectious hazards of transfusion are as follows:

1. Transfusion reactions

2. Transfusion related immunomodulation

3. Transfusion transmissible infections

TRANSFUSION REACTIONS

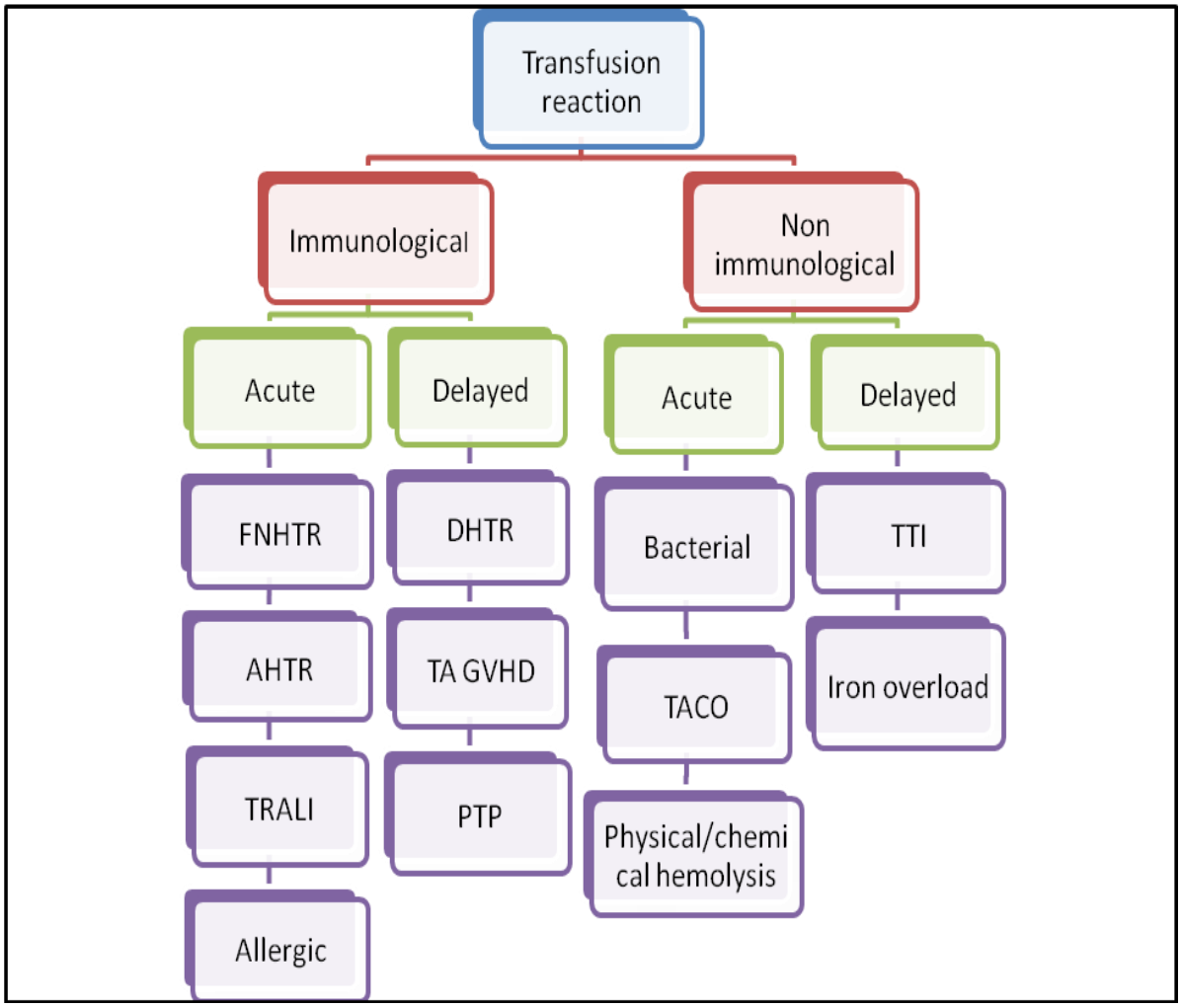


Fig 2 FNHTR-Febrile nonhemolytic transfusion reactions, **AHTR**-Acute hemolytic transfusion reactions, **DHTR**-Delayed hemolytic transfusion reactions, **TRALI**-Transfusion related acute lung injury, **TA GVHD**-Transfusion associated graft vs. host disease, **PTP**-Post transfusion purpura, **TACO**- Transfusion associated cardiac overload, **TTI**-Transfusion transmitted infections

HEMOLYTIC TRANSFUSION REACTIONS

Hemolytic transfusion reactions can be classified as acute or delayed reactions.

Acute hemolytic reactions can be defined as those reactions that occur within 24 hours of blood transfusion. They can be due to the presence of preexisting recipient alloantibodies against antigens on the donor erythrocytes. Mostly, the offending antibodies are immunoglobulin IgM which are naturally occurring like anti-A or anti-B), although complement fixing IgG alloantibodies could also be responsible.

The incidence of acute hemolytic transfusion reactions is estimated to be 1 in 40,000 transfused blood components (43). Immune-mediated hemolytic reactions, rarely can occur because of RBC antibodies in the plasma of the transfused product.

Delayed hemolytic transfusion reactions (DHTRs) usually occur between 24 hours and 1 week following the transfusion of apparently cross-match compatible RBCs. In these situations, the recipient has been previously sensitized to RBC antigens, either through pregnancy or transfusion. These alloantibodies which are usually IgG isotype typically against Rh and Kidd system and are present in such low levels that they are not detected during the pretransfusion antibody screen. However, following transfusion, there is a rapid anamnestic response leading to hemolysis. It has been estimated that DHTRs occur in approximately 1 in 7000 transfusions (43).

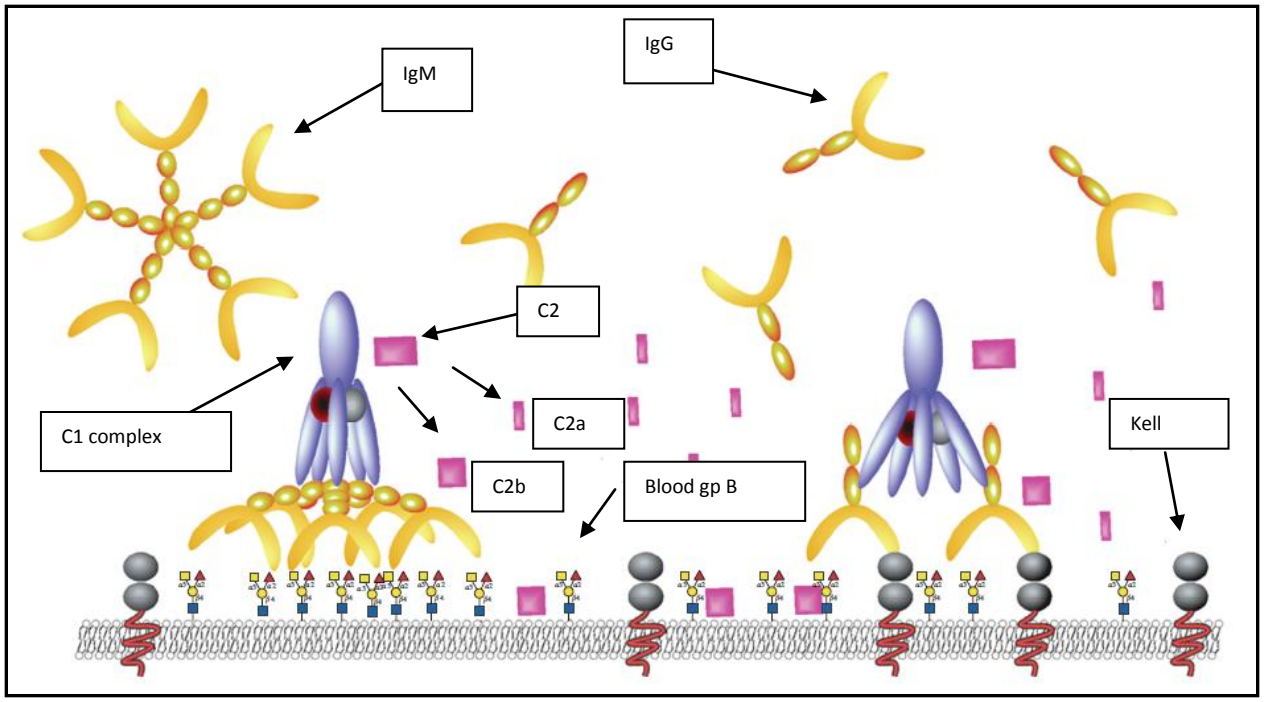


Fig 3. Complement activation during Hemolytic Transfusion Reactions. Stowel et al (44)

RBC ALLOIMMUNIZATION

It has been found that approximately 2%–8% of patients who are chronically transfused develop RBC alloantibodies(45). Contrary to other minor RBC antigens, the percentage of patients exposed to RhD who will develop an anti-D antibody is approximately between 30% and 80%. In the presence of RBC alloantibodies, locating compatible, antigen-negative RBCs are complex and moreover they may increase the risk of a delayed hemolytic transfusion reaction.

Rates of alloimmunization vary with the disease status such as sickle cell anemia patients in whom rates of alloimmunization are approximately 40%(46). In a recent study in China

done among chronically transfused thalassemia patients, rates of alloimmunization was found to be as high as 23% (47).

FEBRILE NONHEMOLYTIC TRANSFUSION REACTIONS

FNHTRs have been characteristically defined as a 1°C increase in temperature during or soon after commencement of a transfusion. Associated symptoms include chills, rigors, and discomfort. It is usually seen after platelet transfusions than RBC transfusions. The incidence rates ranges from 1% to <35%.

One mechanism is the interaction that occurs between the recipient's cytotoxic antibodies and HLA or WBC-specific antigens present on donor WBCs. This leads to the formation of antigen-antibody complexes in turn leading to complement binding with release of endogenous toxins. Different biological response modifiers including cytokines also has a role to play in these reactions. Another mechanism is mainly concerned with the storage of platelets which involves the continued production and release of cytokines from the residual WBCs present in the product (48).

It has been estimated that the risk of FNHTR is approximately 1 in 300 transfusions (45).

ALLERGIC REACTIONS:

Generalized pruritis and urticarial reactions are frequent, occurring in approximately 1–3% of all transfusions. They can be due to the presence of soluble antigens in the donor plasma which can produce a dose-dependent clinical response.

Allergic reactions occur in approximately 1 in 100 transfusions and anaphylactic reactions can be seen in approximately1 in 40,000 transfusions. (45).

IgE mediated reaction against protein hapten conjugates and complement mediated production of anaphylotoxins are the two projected mechanisms responsible for anaphylactoid reactions.

TRALI (TRANSFUSION RELATED ACUTE LUNG INJURY)

The incidence of TRALI is estimated to be 1 in 5000-10,000 red cell transfusions (45).

Fresh Frozen Plasma was the most commonly implicated product. According to The United Kingdom's Serious Hazards of Transfusion (SHOT) hemovigilance data, the risk of TRALI per component was 6.9 times higher for FFP than for RBCs.

TRALI is characterized by acute hypoxemia with non cardiogenic pulmonary edema that occurs during or within six hours of commencement of a transfusion.(49) Majority of patients usually recover in three days with the help of respiratory support, but approximately 5% to 25% of cases can be fatal.

The primary mechanism involved in the causation of TRALI is the accumulation and activation of neutrophils within the pulmonary endothelium. A two hit hypothesis has been proposed for TRALI. The first hit is a recipient factor that primes neutrophils on the pulmonary vasculature and the second hit is a mediator present in the transfused component which activates the primed neutrophils and causes increased permeability of the vascular endothelium.

Recipient factors includes a variety of factors like pre transfusion fluid balance, mechanical ventilation, patient in shock, following liver transplant surgery, IL-8 concentration pre-transfusion, end-stage liver disease, hematologic malignancy, sepsis etc. Antibodies that are present in donor plasma, when transfused to a patient who has the related antigen stimulates neutrophils within the pulmonary vascular endothelium which agglutinate and thereafter release enzymes, ROS, and inflammatory mediators that will injure the pulmonary microvasculature.(50),(51)

HLA Class II antibodies are most often the culprits than Class I antibodies and can indirectly activate primed neutrophils through monocyte activation and cytokine release. Human Neutrophil Antibodies, especially HNA 3a, are also known to be potent mediators of TRALI.

A nonimmune mediated mechanism for TRALI has also been postulated in which bioactive substances like lysophosphatidylcholine, nonpolar lipids and CD40 ligand accumulate in the blood products during storage and can act as the second hit to stimulate injury in the primed patients.(52)(53)

TACO (TRANSFUSION ASSOCIATED CIRCULATORY OVERLOAD)

TACO can be defined as acute respiratory distress causing hypoxia, and pulmonary edema temporally associated with the transfusion of packed cells.

The incidence is has been estimated to be approximately 1 in 700 red cell transfusions.(45)

TACO was found to be the second leading cause of mortality in the United States in 2010, following which its frequency has increased significantly over the years. Although TACO

has been reported after the transfusion of even a single unit of RBC, increased number of transfusions have been implicated as a risk factor independent of the cardiovascular status of the patient. The most common cause of TACO is the transfusion of a unit at a much faster rate. Hypervolemia associated with transfusion leads to congestive cardiac failure and pulmonary edema.

METABOLIC COMPLICATIONS

A number of metabolic complications can occur when blood products are transfused.

These complications are manly seen in neonatal patients or in circumstances wherein rapid, large-volume infusions occur. Such metabolic complications include effects of citrate toxicity, hyperkalemia, and hypothermia.

Citrate toxicity

The anticoagulant used during blood collection is sodium citrate. The final citrate concentration in blood components is found to be highest in the plasma products. Normally the liver rapidly metabolizes transfused citrate but during massive transfusion, the capacity of the liver to clear the citrate has been exceeded following which the citrate forms a complex with calcium, decreasing the ionized calcium resulting in a hypocalcemic state.

Hyperkalemia

When red cell products are stored, potassium leaks from the stored blood causing an increase in the concentration of potassium especially in the context of massive transfusion.

Studies have shown that there is an increase of roughly 1meq/day of extracellular potassium during the first 3 weeks of RBC storage in citrate phosphate dextrose adenine 1 (CPDA-1).

The potassium levels of units stored in additive solutions like Adsol are higher on Day 7 than on Day 0 (17 mmol/L and 1.6 mmol/L respectively).

Hypothermia

The rapid infusion of red cell and plasma products that are stored at cold temperatures may lead to hypothermia, especially in patients with major trauma who may already be vulnerable to hypothermia, depending on the nature and extent of their injuries.

A number of metabolic impairments occur due to hypothermia including a decrease in the rate of citrate and lactate metabolism, increased oxygen affinity of hemoglobin, and increased potassium release from the red cells. The cardiac functions are also affected due to reduced core body temperature leading to morbidity and mortality. Hypothermia can be minimized by warming the blood products before transfusion with the help of a blood warmer.(54),(55)

TRANSFUSION ASSOCIATED GRAFT VS HOST DISEASE (TA-GVHD)

Transfusion-associated graft versus host disease occurs when immunocompetent allogeneic lymphocytes present in the transfused component engraft, proliferate and mount an immune response against the recipient tissues.

TA-GvHD usually occurs between 4 and 30 days following transfusion. The syndrome when it is full blown is associated with multiple organ system involvement leading to increased mortality approaching to almost 90%. TA-GvHD occurs in patients in whom the immunological system is not fully developed, such as following intrauterine transfusion, in premature infants following exchange transfusion and in immunocompromised patients.

TA-GvHD can occur when a donor who is homozygous at the HLA loci shares one of the HLA haplotypes of the patient. This has been reported in Japan in patients undergoing cardiac surgery probably due to the use of fresh blood from directed donations in populations having a high HLA homology.

The donor T lymphocytes are inactivated by irradiation to prevent GvHD resulting due to blood transfusion.. T-cell responses are undetectable after the exposure to at least 25 Gy.(56)

POST TRANSFUSION PURPURA

Post transfusion purpura is an infrequent complication characterized by purpura, epistaxis, gastrointestinal bleeding, and thrombocytopenia, classically seen 5–10 days following transfusion. It is presumed to result from antiplatelet antibodies most commonly against HPA 1a that reacts with the transfused platelets resulting in the formation of immune complexes or the release of complexes that are released after destruction of donor platelets.

In both these cases, the immune complexes adhere to the patient's platelets following which the platelets are sequestered. Glycoprotein GPIIb (HPA-3a, HPA-3b) and GPIIIa (HPA-1a, HPA-1b; HPA-4a) are the more commonly implicated platelet antigens.

In addition to anti-HPA-1a, another mechanism of secondary immune response, is the formation of cross-reactive antibodies against a common epitope common that is common to both to HPA-1a and -1b (57)

BACTERIAL CONTAMINATION

Sepsis related to the transfusion of bacterially contaminated red blood cells is also an uncommon event incidence rate being 1 in 50,000 (43) ,with fatality rate of 1 in 104,000 transfused RBC units. It has been found at the time of phlebotomy itself, one in 2000 units of blood may be contaminated from skin.

The most commonly implicated endotoxins are those produced by bacteria capable of growing in cold temperatures such as Pseudomonas species, Escherichia Coli , Yersinia enterocolitica, Serratia marcesans and Aeromonas species . They grow very slowly in cold blood, dividing about once a day, taking approximately 27 days to grow up to 10^8 organisms, hence resulting in an overwhelming infection and septic shock. (58)

Signs and symptoms of septic reactions typically appear rapidly during transfusion or within 30 minutes following transfusion.

RBC STORAGE DEFECTS

Storage of RBCs for long periods can also be responsible for the deleterious effects of transfusion. Metabolic, biochemical and molecular changes can be seen in RBCs stored over a 42 day period.

Membrane changes

RBCs undergo glycolytic metabolism secondary to which there is accumulation of lactic acid and H^+ over time. Secondary to this however, the pH in the blood bag drops , altering glycolysis, leading to a rapid decline in 2,3 DPG levels and a rapid increase in ATP production. Following this the rate of glycolysis is seen to decrease, and ATP production likewise due to accumulation of acid. By the 10^{th} day of storage, 2, 3 DPG decreases drastically while ATP concentrations increase during the initial days or remain stable for the first two to four weeks. Thereafter a decline is noted.

RBCs collected in CPDA- 1 show decreasing levels of RBC ATP content on storage. Studies show a decline from 4.18mmol/g Hb at collection to 2.40mmol/g Hb at 35 days of storage. Consequentially, red cell shape is affected by both acidification and lowering ATP concentrations (59). Echinocytes seen are a result of acidosis which causes the initial development of bumps leading to surface protrusions, hence forming echinocytes. These initial changes are reversible. However, as calcium concentration of the RBC increases and RBC ATP decreases, irreversible changes begin to set in. The changes include asymmetry of the phospholipid membrane being lost, the development of phospholipid rafts which are negatively charged on the surface of the cell, and the microvesicle formation The results of these changes is that with storage of red cells there is permanent membrane loss. With storage and over time it has also been noted that the rigidity of RBCs increases and they tend to become more adherent to vascular endothelium.(60),(61)

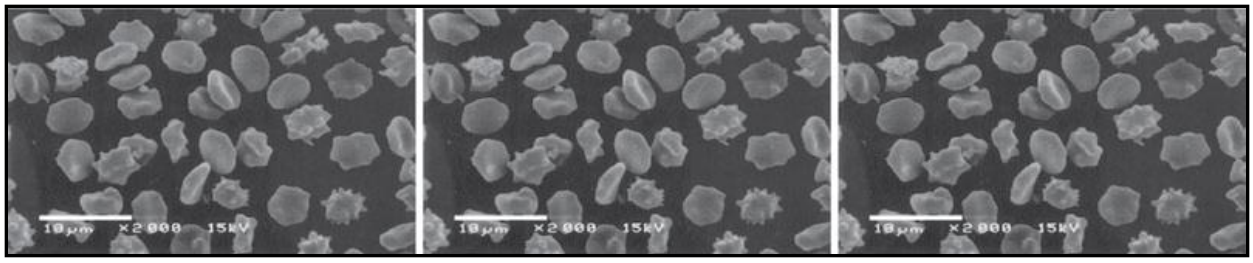


Fig 4. Electron microscopic pictures of RBCs showing the morphological changes from biconcave disc shaped to microspherocytes occurring as a result of storage for 42 days(62)

CD47, is a 50-kd glycoprotein, which is expressed on the surface and transmembrane in location. Widely expressed on all cells, it has been found that its expression decreases by 10–65% on RBCs when stored. It is used as a marker for self and therefore when expression falls to less than 50%, these red cells become susceptible for phagocytosis. It has been observed that approximately 20–25% of red cells are non viable at the end of the storage time and when transfused then are removed within a few hours from the circulation.(58)

In red cell products which are not leukocyte reduced, two weeks after storage Phosphatidyl serine (PS) becomes expressed. This sets off a procoagulant effect contributed by the inherent risk of increased adherence of these cells to vascular endothelium. This further result in compromise of blood flow, resulting in impaired oxygen delivery, and vasoocclusion of microvasculature in vivo. Situations in patients with high oxygen demands and impaired micro vascular perfusion should specifically take these changes into consideration.(63)

Inflammatory mediators like histamine, complements, lipids and cytokines accumulate in the supernatant of leukocyte containing RBCs. After storage for two weeks, there is increased expression of CD11b and CD16 on neutrophils in vitro, both of which are related to the priming of neutrophils. (64)

During red cell storage, residual platelets and red cells release factors like the CD40Ligand which is proinflammatory in nature and other lipid peroxidases which can be bioactive, respectively. These are capable of priming polymorph nuclear cells (PMN) and have been purported to be cofactors causing TRALI. (60)

2, 3-DPG

2,3-DPG is gradually depleted in the two week stored RBC leading to a shift of the O2-dissociation curve to the left thereby increasing oxygen affinity and impairing its delivery (65). There is considerable variability in the rate of in vivo restoration of 2, 3-DPG. One hour after transfusion, 25–30% of 2,3-DPG has been measured , after 24 hours there was 50% recovery but a full restoration was seen only after three days (63).

Sodium Potassium Pump

The Na+ / K+ pump is paralyzed at storage at 2-6°C when K+ leaves the cell and sodium enters the cell [36]. Following 3 days storage there is progressive leakage of potassium from the erythrocyte and the extra-cellular concentration can increase to 50 mEq/l.

Following transfusion, the red cell sodium content normalizes within 24 h, but it takes at least 4 days for complete K+ recovery [37]. High potassium levels is associated with the

danger of arrhythmias which is mainly seen with large volume transfusions in newborns and small infants, in whom lethal cardiac arrest have been reported (6).

Clinical trials based on storage of RBCs vs. mortality and lengths of ICU stay

It was reported in a study by Purdy et al, in a population of 31 transfused septic patients, a significantly longer mean storage time of transfused RBCs was seen in patients who died (25 days) when compared to those who survived (17 days),P <0.0001. (66)

Eikelboom et al (67) reported an independent association between the risk of hospital mortality and prolonged storage of red cells in patients with cardiovascular disease.

Similar association was seen between RBC storage time and ICU length of stay by Martin et al in 698 ICU patients. It was noted that the transfusion of RBCs stored for more than 14 days was independently associated with prolonged ICU length of stay.

A study by Murrell et al in trauma patients reported a statistically significant association between ICU length of stay and the dose of aged blood (defined as the average age of received RBC multiplied by the number of RBC received).

The duration of red blood cell (RBC) storage before transfusion alters RBC function and, leading to complications or even mortality, but this still remains a matter of debate. Lelubre and Vincent in a systematic study review found no definitive argument that was able to support the superiority of fresh RBCs over older RBCs.(68)

TRANSFUSION TRANSMITTED INFECTIONS

Transfusion transmitted disease (TTD) has been a major challenge in the field of transfusion medicine. In the blood donor community, the problem of TTD is directly proportional to its prevalence. Some of the main infections that are of concern in India include hepatitis B/C, HIV, malaria, syphilis, cytomegalovirus, parvo-virus B-19 and bacterial infections.

There is a 1% chance of transfusion associated problems including TTI with every unit of blood. In India, the overall prevalence of TTI is 0.6%. Data obtained from various studies on the overall seroprevalence of HIV, HBsAg, HCV and Syphilis in India were 0.44, 1.27, 0.23 and 0.28% respectively.(69)

Hepatitis B and C infections are prevalent in India and carrier rate is about 1-5% and 1%, respectively. Post transfusion hepatitis B/C has also been a major problem in India (about 10%) due to low viraemia and the presence of a mutant strain that is undetectable by routine ELISA.

A study done by Ali *et al*. showed post transfusion malaria incidence of 4.9% for multi-transfused patients. In another study the overall malaria antigen prevalence in blood donors was 0.09% and the malaria antibody prevalence was 6% in thalassemia patients and 15% in other multi transfused patients.(70)

The prevalence of anti CMV IgG in India is about 95%. Studies have found that about 5% of the donor population have IgM antibody which carries a considerable threat of transmitting CMV infection when transfused to the immune-compromised population. It is very difficult

to prevent post transfusion CMV but with the use of leukocyte filters, its incidence has reduced significantly.

Prevalence of parvo virus B-19 infection in blood donors is 39.9% which may contribute to increased morbidity when transfused to multitransfused or immunocompromised patients.(71)

Another emerging infection, i.e. chikungunya virus (CHIKV), has been a threat to the South Asian countries. Transfusion associated CHIKV is a problem during outbreaks and the high-titer viremia lasts for up to 6 days. During its outbreak, the estimated transfusion risk is about 150 per 10,000 transfusions. (68)

TRANSFUSION RELATED IMMUNOMODULATION

Allogeneic blood transfusion results in either alloimmunization or immune tolerance. Laboratory studies to demonstrate the effects of allogeneic transfusion on immunological function, have demonstrated reduced interleukin2 (IL-2) secretion, reduced natural killer cell activity, reduced delayed-type hypersensitivity responses with decreased CD4/CD8 ratios (72),(73) and decreased function of macrophages.

This was found to contribute to tumor recurrence, augmented rates of infections, rapid progression of viral diseases, and a decrease in the inflammatory bowel disease episodes.

The concept about immunomodulation came into existence when Opelz *et al.*in 1973 found better outcomes among recipients of cadaveric renal transplants who had received packed cell transfusions. This phenomena was recognized to be due to the immunomodulatory effect

of the transfused leukocytes, alterations in the circulating lymphocytes, function of Bcells, T helper cell /suppressor cell ratio and the number of circulating antigen presenting cells(APC) in the recipients of allogeneic blood transfusion. (74)

Thereafter, it was postulated that there may be two categories of immunosuppressive transfusion effect: one that is HLA dependent which is directed against the adaptive immunity and another non-specific effect which is against the innate immunity.

The nonspecific effect was presumed to be due to the transfusion of blood cells that undergo apoptosis during storage under refrigerated conditions. This immunosuppression following the transfusion of these cells may be attributed to the transforming growth factor beta (TGF-β).

With the transfusion of large amounts of fresh or stored blood, changes can be seen in the recipient's lymphocytes after a period of about 1 week. It was reported that atypical lymphocytes was seen to increase by a factor of five or more.

It is purported that TRIM may be due to any of the following:

- Allogeneic mononuclear cells (AMCs) present in blood stored for less than 2 weeks
- Pro-inflammatory soluble factors that are released from WBC granules which accumulate progressively in the supernatant of stored RBCs.
- Soluble, class I HLA molecules that circulate in the allogeneic plasma.

LANDMARK STUDIES FOCUSSING ON ANEMIA AND RED CELL TRANSFUSION IN THE INTENSIVE CARE UNIT.

In the ICU, transfusion must be viewed as any other intervention as it is associated with definite risks. The expected benefits and risks should be rightly weighed in the light of the goal of transfusion.

Several landmark studies have explored transfusion thresholds in critically ill patients and have evaluated the factors associated with the need for transfusion and the relationship between transfusions and outcomes.

CRIT Study (75) - A prospective multiple centre observational study was done in the United States which included 213 hospitals consisting of 284 ICUs. It was a nine month study period and 4,892 patients were enrolled.

In this study the mean baseline Hb on admission to ICU was 11g/dl, which significantly decreased to 9.8g/dl by the end of the study. Almost 70% of the patients admitted to the ICU had baseline haemoglobin less than 12g/dl by 48hrs after ICU admission, and half of these patients had a haemoglobin level of less than 10g/dl. It was seen that all through the duration of ICU and hospital stay, the anemia persisted regardless of the presence or absence of RBC transfusion. Individuals who had lower baseline haemoglobin were prone to receive an RBC transfusion. Almost 90% of patients with a baseline haemoglobin of less than 8g/dl received packed cell transfusion. On the contrary, merely 20% of patients with baseline haemoglobin more than 12g/dl received packed cell transfusion. Patients with a low haemoglobin level less than 8g/dl presented with more hemodynamic instability, sepsis and GI bleeding whereas

patients with a haemoglobin level greater than 12g/dl presented with more respiratory and cardiovascular problems. ICU or hospital length of stay was not statistically significantly associated with baseline haemoglobin.

44% of the ICU patients received transfusion of one or more than one RBC units while in the ICU with the average time to first transfusion being 2.3±3.7 days. More number of packed cells was transfused in the first week, when compared to the following weeks when patients received 1-2 RBC units per week. When compared to patients who had a shorter stay in the ICU those with a longer stay of seven or more days received transfusions more commonly (33.4% vs. 63%). The mean number of RBC units transfused was 4.6units.

Transfusion of packed cells was also independently associated with rates of mortality. It continued to be statistically significantly associated with an increased risk of death following adjustment for the propensity for receiving a transfusion, (adj OR-1.65, $p<0.001$).

Transfused patients had more complications and were also more likely to experience a complication. Those who received six or more RBC units had greater mortality rates than those who received no transfusions at all (25%vs10%). The number of units that were transfused was statistically significantly associated with prolonged ICU and hospital length of stay when compared to patients who had not received any transfusions.

The mean pretransfusion haemoglobin was 8.6g/dl and the indication for transfusions were primarily low haemoglobin in majority of the cases (90%), followed by active bleeding (24%), presence of ischemia (3%), increased cardiac output(2%), prior to or after a surgical procedure(19%) and hypotension(21%).

Mean baseline APACHE II score was 19.7±8.2 and this was significantly greater in those with baseline haemoglobin less than 10g/dl. Lower baseline APACHE II was found to be associated with a significantly decreased probability of RBC transfusion.

The average age of transfused RBC units was 21.2±11.4 days. No difference was seen in the clinical outcome and the average age of units that a patient received.

Anemia and Blood Transfusion in Critical Care (ABC) (9) study examined the incidence of anemia and use of transfusions in critically-ill patients. The study was a prospective, observational study including 146 Western European ICUs and consisting of 3534 patients.

In the study, the mean age of patients was 61years with 33.4% of patients being older than 70years.

The mean admitting haemoglobin was 11.3g/dl with mean admitting APACHE II score of14.8 and mean SOFA (Sequential Organ Failure Assessment Score) of 5.5.

Lower mean haemoglobin levels were associated with higher SOFA score, longer lengths of ICU and hospital stay and higher mortality rates.

The rate of RBC transfusions during ICU and post ICU period was 37% and 12.7% respectively. Almost 70% of the transfused patients received their initial transfusion during the first two days in the ICU. Those patients who remained in the ICU for more than seven days in general had a transfusion rate of 73.3%.

Those who were transfused were much older with higher admitting SOFA and APACHE II scores, lower admission Hb levels, and prolonged length of hospital stay.

The mean pre transfusion haemoglobin was 8.4g/dl. The mean age of the transfused RBC units was 16.2 days. Mean length of ICU stay was 4.5days(less than 1day-46.6% patients, 2-3days-21.4%, 4-7days-14.2% and more than 7days-17.8%). When the transfused groups were compared, the average number of ICU days was 7.2days for those who received transfusions when compared to 2.6 days for those who had not received any transfusion.

The overall ICU mortality was 13.5%. And it was found to be significantly higher for the transfused vs. non transfused patients (18.5% vs. 10.1%). Logistic regression showed a significant association between transfusion, APACHE II score, SOFA score, and age with mortality but such an association was not seen with the admission Hb.

There was a dose response relationship with increased mortality rates as the number of RBC units transfused increased and it was seen that receipt of a transfusion had increased the risk of dying by a factor of 1.4.

Transfusion Requirements in Critical Care (TRICC) (76) **Study**.

Over a three year period investigators of the TRICC study group and the Canadian Critical Care Trial groups had 838 patients enrolled into their study. There were 22 Canadian tertiary level intensive care units and 3 community level intensive care units which these patients had been admitted during this time.

The 838 patients enrolled, were assigned to either a restrictive or a liberal strategy of transfusion. 418 were assigned to the former, while 420 were assigned to the latter group.

Analyzing APACHE II score revealed an average score of 21. It was also observed that mechanical ventilation was required for more than 80% of the patients.

Analysis of threshold for red cell transfusion between both groups revealed some differences. The Hb of patients assigned to the restrictive strategy was maintained in the range of 7.0 to 9.0 g/dl. A haemoglobin concentration below 7gm/dl was the threshold for initiating a transfusion. On the contrary, among patients assigned to the liberal strategy Hb levels were maintained at 10.0 to 12.0 g/dl, and transfusions were initiated at a threshold of 10.0 g/dl.

Differences were observed in the mean number of red cell units transfused between these two groups as well. The mean number of RBC units transfused per patient in the restrictive group was 2.6±4.1 whereas in the group with the liberal strategy it was 5.6±5.3 units per patient.

While differences were noted in ICU and hospital mortality rates – with rates being lower in the restrictive strategy group when compared to the liberal strategy group(22.2%vs28.1%), there was no difference in the 30-day mortality –values being similar in the two groups (18.7% vs. 23.3%, P= 0.11).

Comparing the two groups and looking at the unadjusted odds ratio for mortality within 30 days showed a value of 0.75 (P=0.09). Adjusting for variables such as age, APACHE II score, other significant diagnosis, and coexisting illnesses, there was no significant change in the Odds ratio.(odds ratio, 0.72; P=0.07).

A significant finding was the lower mortality rates in the restrictive strategy group who were also less acutely ill as evidenced by an APACHE score <20 (8.7% in the restrictive-strategy group vs. 16.1% in the liberal-strategy group, P=0.03). This was also replicated in younger patients – less than 55 years of age (5.7% mortality vs. 13.0% mortality, respectively; P=0.02). However when the patient population had cardiac disease that was of clinical significance, this trend did not hold (20.5%vs22.9%, respectively; P=0.69).

This study reflected a trend that maintaining Hb levels between 7.0 to 9.0g/dl and adopting a restrictive strategy of transfusion practice was advantageous as it appeared to decrease 30 day mortality and alongside also decrease mean number of red cell transfusions by almost 54%.

Sepsis Occurrence in Acutely Ill Patients (SOAP) Study (77) – This was a multicentre, observational study comprising of adult patients admitted to 198 European ICUs.

Of the 3,147 patients, 1040 patients (33%) received an RBC transfusion. Older patients (mean age 62years) received more transfusions and were more likely to have liver pathology/surgical admission/infection/sepsis.

Comparing transfused and those not transfused in the study revealed that the transfused patients had a longer ICU stay (5.9vs2.5days) when compared to the non transfused with a higher ICU mortality rate (23%). However these patients were more acutely ill with a higher baseline APACHE II score (40.2) at admission than other patients. It was observed that those who received more transfusions also had a longer ICU stay. Analyzing relationship between number of units transfused and mortality rate, there appeared to be a direct relationship.

However in multivariate analysis, mortality rate and receipt of blood transfusion was not significantly associated Using the extended Cox proportional hazard analysis, adjusting for red cell transfusion as a time-dependent variable, it was found to be associated with a decreased relative hazard of death at 30 days in the entire population.

Audit of Transfusion in Intensive Care (ATICS)Study (78) – This was a prospective study comprising 1042 patients admitted successively to 10 Scottish adult ICUs during a 100 day study period.

The admission haemoglobin was less than 9g/dl in 21% of patients and approximately 27% of patients developed a haemoglobin concentration of less than 9g/dl on at least one occasion during ICU stay.

The percentage of patients who received RBC transfusions in the ICU was 39.5% and the RBC requirement in the ICU was approximately 1.87 RBC units per admission.

Transfusion episodes due to clinically significant haemorrhage (an estimated loss around 300ml during a 24 hour period) accounted for 40% of all transfusions with a mean of 3.1 units per episode. Transfusion episodes not associated with clinically significant haemorrhage amounted to 60% of all transfusion episodes with a mean of 1.9 units per episode. The median transfusion threshold haemoglobin level for RBC transfusions that were not associated with haemorrhage was 7.8g/dl. The mean ICU length of stay was 2.2 days and the mean APACHE II score was 19.8.

MATERIALS AND METHODS

MATERIALS AND METHODS

Study Design and Settings:

This prospective observational study was conducted in the Medical and Surgical ICU of Christian Medical College Hospital, Vellore. This is a 2500 bedded tertiary referral centre with a 12 bedded Medical ICU, 12 bedded Medical HDU and 13 bedded Surgical ICU. The enrollment period was from October 2012 through June 2013.The study protocol was approved by the Institutional Review Board.

Study participants: A total of 800 patients were enrolled throughout the study period. Patients were enrolled within 48hrs of ICU admission.

Inclusion criteria: All patients admitted to the medical and surgical ICU, with an anticipated stay of more than 48hrs were enrolled in the study.

Exclusion criteria included:

1. Patients with known primary haematological disorder

2. Patients who succumb to their illness or are discharged within 24hrs of ICU admission.

Patients were followed up throughout the length of their ICU stay until hospital discharge or if death occurred before that.

Data collection:

Data was collected exclusively by the principal investigator using pre printed case report forms (CRF's) (Annexure II). All patients who met the inclusion criteria were recruited into the study. Data collection included demographics, primary diagnostic category, type of surgery(if applicable), co morbidities, admission haemoglobin and APACHE II score.

Follow up of these patients included daily data collection of hemoglobin, requirement of RBC transfusions, indication for transfusion, pre transfusion haemoglobin, number of RBCs transfused, ventilator days, ICU and hospital length of stay and outcome.

The daily haemoglobin values were obtained either from the Arterial blood gas analyzers (Gem 4000, from Instrumentation Laboratories used in MICU and ABL 800, Radiometer Copenhagen used in SICU) or from Beckman-Coulter DXH 800 Haematology Analyzer.

Statistical analysis:

The study size was calculated according to the patient turnover in both the Medical and Surgical ICU- roughly a sample size of 1500 for a period of six months.

The primary endpoint of the study was to evaluate the RBC transfusion practice with respect to transfusion thresholds and indications for transfusion in critically ill patients.

The secondary endpoints were to determine the prevalence of anemia in ICU patients and to assess the time course of haemoglobin concentrations during their ICU stay.

A fall in haemoglobin was defined as a reduction in haemoglobin due to overt or covert bleed, hemolysis or due to illness per se. The fall in haemoglobin in critically ill is due to a multiplicity of factors that include dilutional (due to fluid resuscitation), anemia of illness, repeated blood tests, with or without clinical bleeding or hemolysis.

We also analyzed the effect of anemia and transfusions on the clinical outcome, to assess if transfusion is an independent risk contributing to mortality in critically ill patients.

The CRF's were analyzed using STATA 10 with the data base created in EXCEL. Descriptive statistics were computed for all data variables. Difference testing between groups was performed using the two sample *t* test and associations were checked using Chi square test.

Logistic regression was conducted to assess determinants of mortality. Adjustment was made for potential confounding factors including age, APACHE II score, baseline haemoglobin, RBC transfusion and need for ventilation.

Repeated measures ANOVA was used to detect significant difference in the decrease in haemoglobin between the transfused and non transfused groups following admission to the ICU.

RESULTS

RESULTS

Patients who were admitted to the Medical and Surgical ICU during the period from October 2012 to June 2013 were included in this study.

All those patients with a primary haematological diagnosis and those who died or were discharged within 24 hrs of ICU admission were excluded from the study.

After exclusion, eight hundred patients who fulfilled the inclusion criteria were recruited in the study.

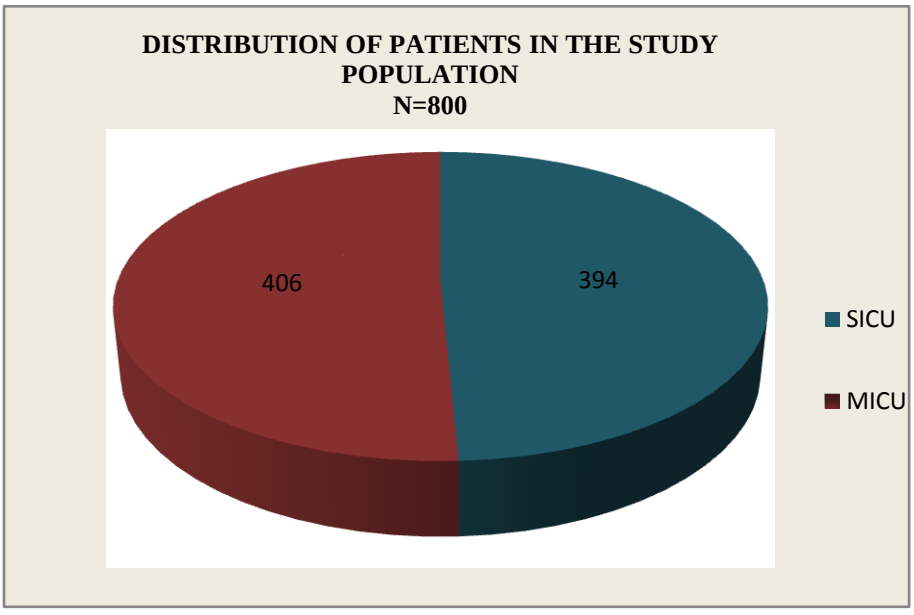


Figure 5 . Distribution of patients in the study population

BASELINE CHARACTERISTICS:

Table 1. Overall characteristics of patients in the study population

	Mean ± Standard deviation(SD)	***Lower quadrant(LQ) Median(Mdn) / Upper Quadrant(UQ)***
Age, years	*45.0 ± 16.8*	*30.0/45.0/57.0*
Gender,		
Male	*467(58%)*	
Female	*333(42%)*	
Baseline APACHE II score	*18.30± 7.14*	*13.0/17.0/23.0*
Baseline haemoglobin level, g/dl	*10.7± 2.8*	*8.6/10.3/12.5*

Comparison of baseline characteristics between MICU and SICU patients

When assessed individually, the mean age of patients admitted in MICU and SICU was not statistically different (Table 2). The mean APACHE II score was also not statistically different among the patients in both the ICUs. The average admission haemoglobin in patients admitted to the SICU was far lesser when compared to the medical cohort in MICU and the difference was statistically significant.

The most common indication for admission to the medical ICU in the decreasing order of frequency was organophosphorus poisoning(13%), Acute respiratory distress syndrome(11%), scrub typhus(11%), acute kidney injury(11%), septic shock(10.5%) and DIC(9%).

The most common admission indications in the surgical ICU were abdominal surgery(29%), polytrauma(24%), pelvic surgery(15%), limb ischemia(12%), obstetric emergencies(7%) and thoracic surgery(6%).

The prevalence of co morbid conditions varied in both the ICUs. Diabetes and Hypertension were the most frequent co morbid conditions seen in 34% and 28% respectively in the MICU and 24% and 26% respectively in the SICU.

Table 2. Baseline characteristics of MICU and SICU patients

	MICU (N=406)	*SICU (N=394)*	*P value*
Age, years	*44.3±17.3*	*45.5±16.2*	*0.31*
APACHE II score	*18.3±6.2*	*18.4±7.8*	*0.92*
Hb at admission, g/dl	*11.4±3.0*	*9.9±2.4*	*0.00*
Co morbidities (%):			
Diabetes	*(136)34%*	*(94)24%*	
Hypertension	*(114)28%*	*(102)26%*	
Ischemic heart disease	*(31)8%*	*(15)4%*	
Chronic kidney disease	*(29)7%*	*(22)6%*	

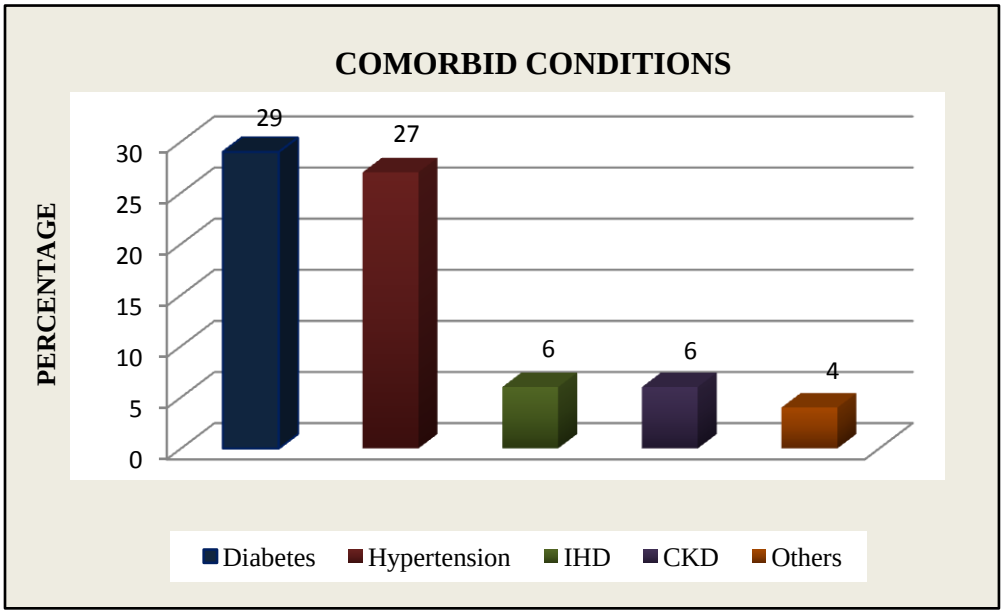


Fig 6. Percentage of co morbid conditions in both ICUs.

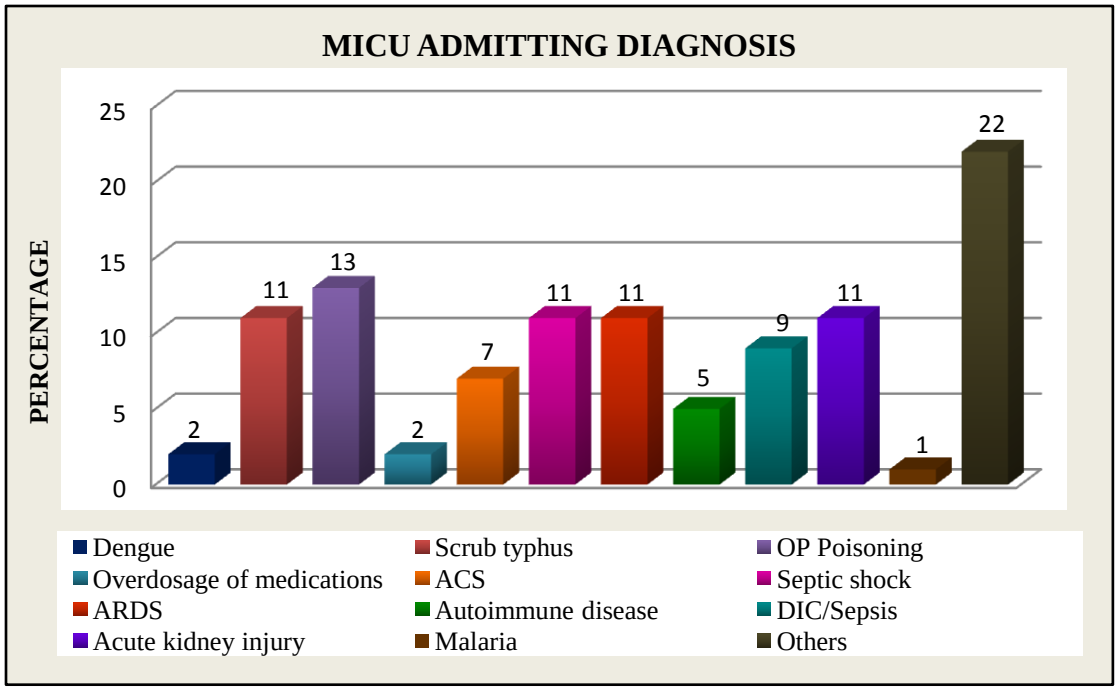


Figure 7. Admitting diagnosis in MICU

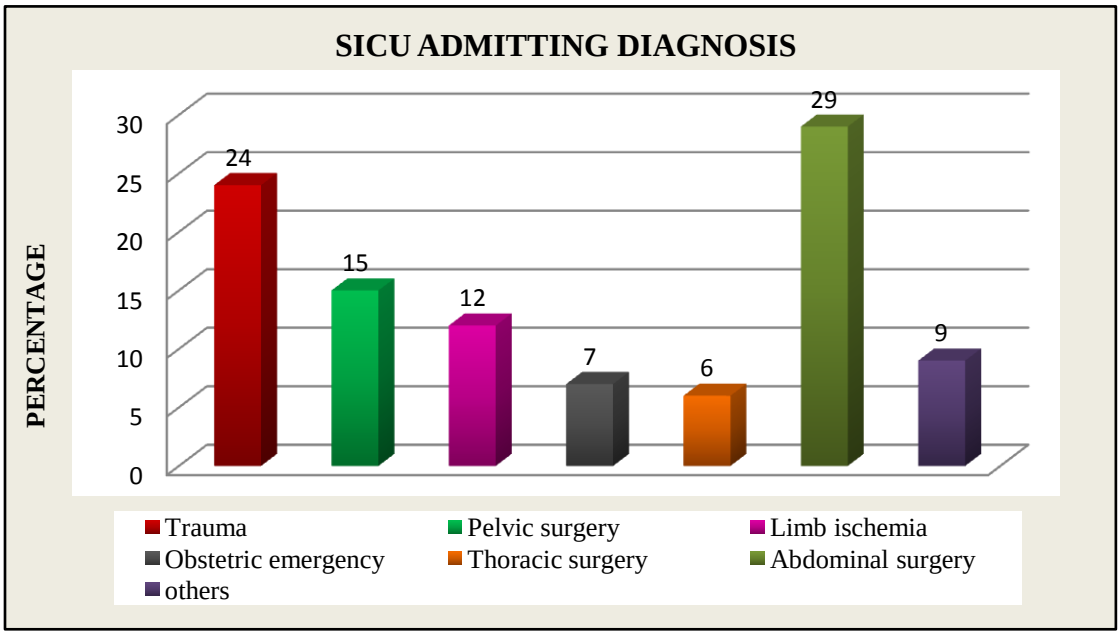


Figure 8. Admitting diagnosis in SICU

OUTCOME OF ICU STAY

Table 3. Overall outcome of ICU stay

	Mean± SD	***LQ/Mdn/UQ***
ICU length of stay, days	*7.3 ± 5.4*	*4.0/6.0/9.0*
Hospital length of stay, days	*15.8± 11.0*	*9.0/13.0/20.0*
Mechanical ventilation%	*81*	
Ventilation duration, days	*5.3± 5.7*	*2.0/4.0/7.0*
Mortality %	*25*	

Eighty one percent of patients admitted to SICU and eighty percent of patients admitted to MICU required ventilatory support during their ICU stay and the duration of ventilation was significantly different in both the groups. Mortality rates and ICU length of days were significantly higher for the medical cohort when compared to the surgical cohort (Table 4).

Table 4. Comparison of outcome among the ICUs

	MICU (N=406	***SICU (N=394)***	***P value***
Ventilation duration, days	*6.9±6.7*	*3.6±3.8*	*0.00*
ICU length of stay, days	*9.0±6.0*	*5.5±3.9*	*0.00*
Hospital length of stay, days	*15.6±10.9*	*16.3±11.4*	*0.33*
Mortality%	*34*	*16*	*0.00*

***RBC TRANSFUSIONS*:**

Overall, 307 patients (38%) admitted to the ICU received transfusions with blood or blood products while in ICU. Of those who received any transfusion, 269 (34%) patients received one or more RBC units with or without blood products out of which, 177 (22%) patients received only packed cell transfusions and 92(12%) patients received packed cells along with other blood products like FFP, PRC and cryoprecipitate.

As expected, the proportion of patients who received transfusions was significantly higher in the surgical cohort (44%) when compared with the medical patients (23%), [p<0.0001]. The proportion of patients who received only packed cells without blood products was higher in the surgical patients(29%) when compared to the medical patients(16%) as well as those

patients who received packed cells along with any blood product was higher in the SICU(25%) vs. MICU(8%).

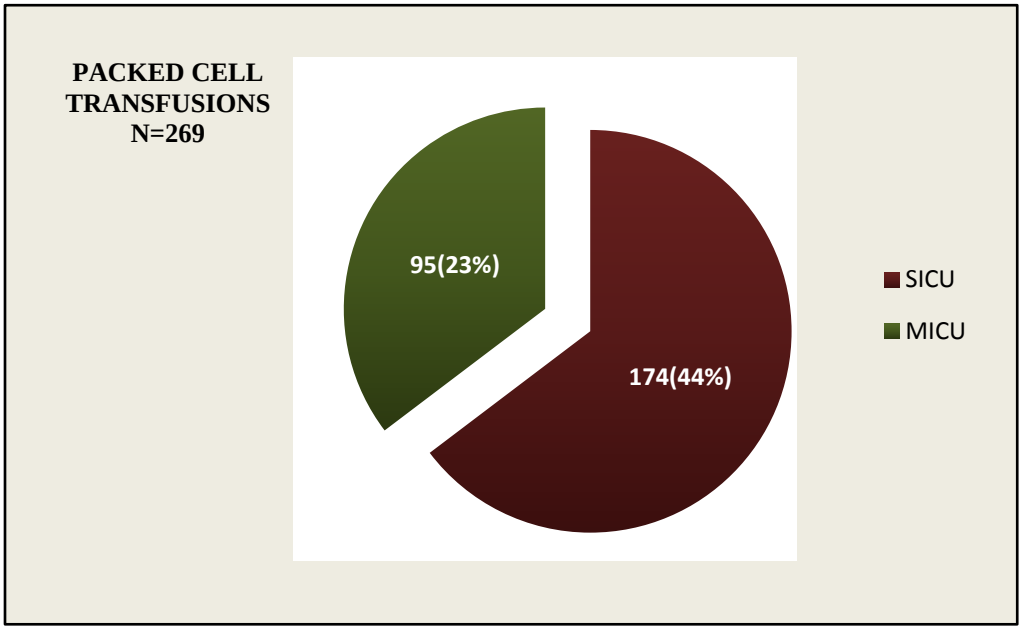
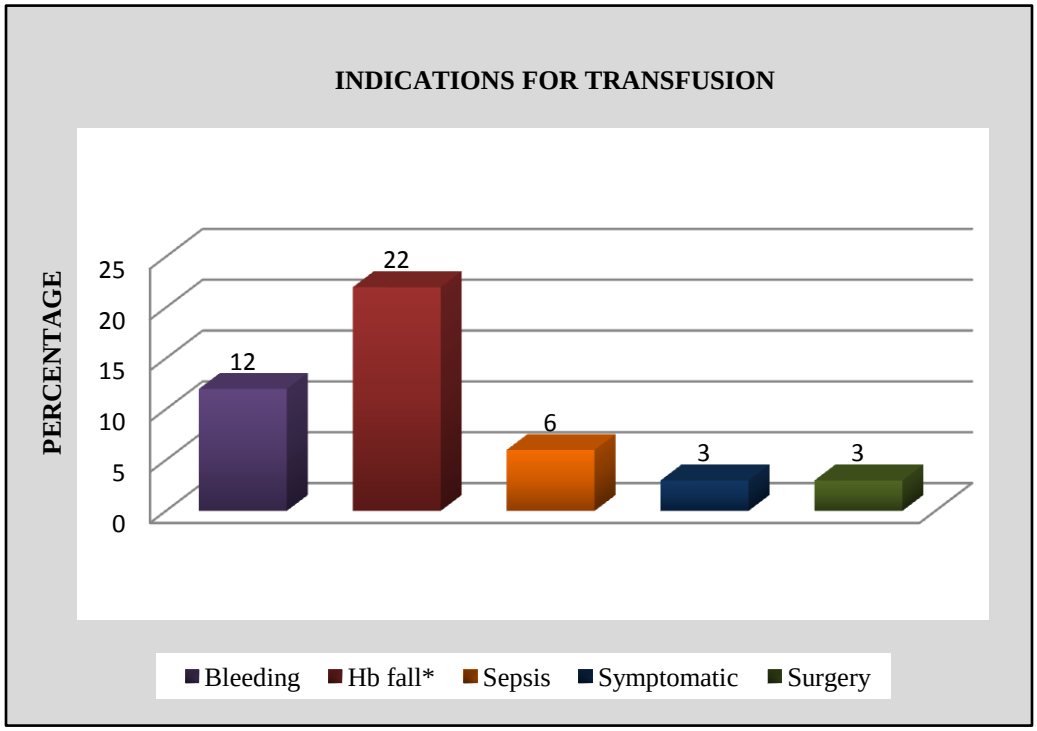


Figure 9. Packed cell transfusions in MICU and SICU

Indications for transfusions



*As defined in material and methods

Fig 10. Overall indications for packed cell transfusions

Although the most common overall indication for transfusion in the ICUs was a drop in haemoglobin, when analyzed individually among the ICU, 25% of patients in the SICU were transfused due to fall in haemoglobin(as defined in Materials and Methods) in the absence of overt bleeding whereas an equal proportion of patients(23%) were transfused due to post surgical bleeding. In contrast, majority(19%) of patients received packed cell transfusions in the MICU due to fall in haemoglobin in the absence of overt bleeding when compared to a minor proportion of patients who received transfusion following bleeding(1%).

Pre Transfusion Trigger

The mean pre transfusion haemoglobin was 7.0±1.6 g/dl (95%CI: 6.8-7.2). However the mean Hb pre transfusion in Medical ICU was 6.3gm% compared to 7.4gm% in the Surgical ICU. This difference is statistically significant (P value 0.000).

Table 5. Mean pre transfusion haemoglobin trigger in both ICU's.

	MICU		*SICU*		
	Mean(SD)	**95%confidence**	**Mean(SD)**	**95%confidence**	**p value**
Hb trigger	*6.3(0.9)*	*6.1-6.5*	*7.4(1.8)*	*7.1-7.7*	*0.000*

Of the 269 patients who received packed cell transfusions, 167(62%) patients had a pretransfusion trigger less than 7g/dl and 102(38%) patients were transfused with a trigger of 7g/dl and greater. Of these, 89(87%) patients were from the surgical cohort and 12(12%) were from the medical cohort. The indications for transfusion in patients who were transfused at a higher threshold were bleeding(43%), drop in haemoglobin(14%), symptomatic(11%), surgery(9%) and sepsis(5%).

Association between transfusion and mortality

Overall mortality inclusive of both ICUs was 23% among those who did not receive transfusions and 30% among those who received one or more RBC units with or without

blood products across their stay in the ICU and this difference was statistically different (OR-*1.5*, 95%CI=*1.08-2.09*, pvalue-*0.016*).

When patients were separately categorized as medical or surgical patients, the association between transfusion and mortality continued to be significant.

Table 6. Association between transfusion and mortality in MICU and SICU

	MICU			***SICU***		
Mortality	**OR**	**95%confidence**	**p value**	**OR**	**95%confidence**	**p value**
Transfusion	*2.08*	*1.30-3.32*	*0.002*	*2.01*	*1.17-3.48*	*0.012*

Association of Transfusion with ICU and Hospital length of stay

Transfusion with blood or blood products was found to be significantly associated with a prolonged ICU and hospital length of stay.

Table 7. Association of transfusion with any blood or product with ICU and hospital stay

Any transfusion	***OR***	***95%confidence***	***p value***
ICU length of stay	*1.05*	*1.03-1.08*	*0.000*
Hospital length of stay	*1.02*	*1.01-1.03*	*0.001*

There was a statistically significant association between receipt of packed cell transfusions (with or without any blood product) and prolonged ICU and hospital stay in overall ICU patients (Table 8)

Table 8. Association of packed cell transfusions with ICU and hospital stay

Transfusion	*OR*	*95%confidence*	*p value*
ICU length of stay	*1.06*	*1.03-1.09*	*0.000*
Hospital length of stay	*1.02*	*1.01-1.03*	*0.002*

In the Medical ICU, a trend of longer ICU stays was noted in patients who had greater number of red cell transfusions (Fig 11). However this analysis could not be done in the surgical ICU setting as most patients had a uniformly short stay averaging 5.5± 3.9 days.

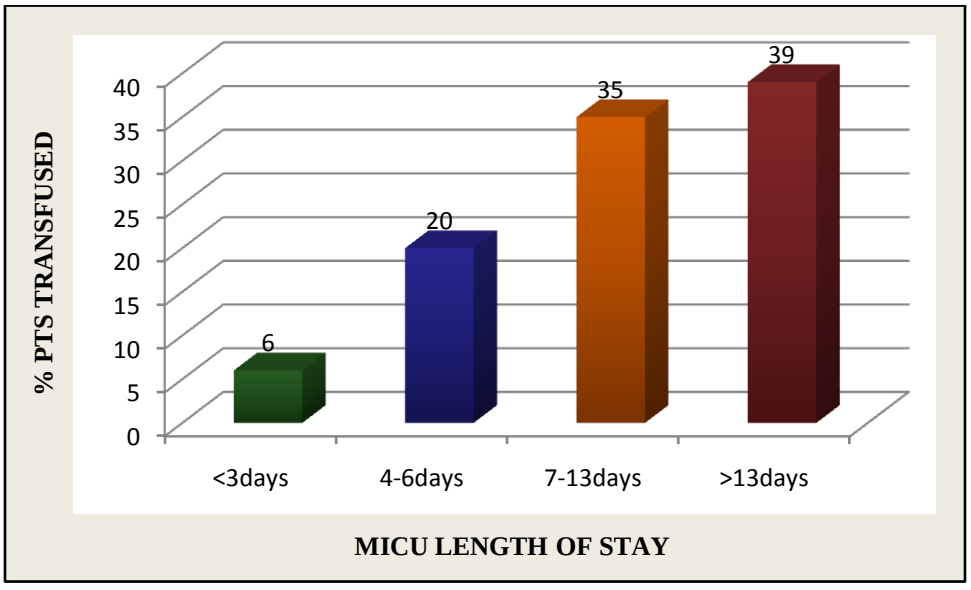


Fig 11. RBC transfusions across MICU stay

When analyzed individually, a significant association was found between packed cell transfusion and prolonged hospital length of stay among the medical ICU patients. However, such a correlation was not seen in the surgical cohort of patients, as shown in the table below.

Table 9. Association of packed cell transfusions with hospital length of stay in MICU and SICU patients

Transfusion	*OR*	*95%confidence*	*p value*
Hospital length of stay in MICU patients	*1.04*	*1.02-1.06*	*0.000*
Hospital length of stay in SICU patients	*1.00*	*0.98-1.02*	*0.762*

Association of Transfusion with Age and APACHE score

There was no significant association between age and APACHE II score between the transfused and non transfused groups.

Table 10. Association of age and APACHE II score with transfusion

AGE(yrs)			
	Mean(SD)	**95% Confidence**	**p value**
No Transfusion	*45±17*	*44-47*	*0.06*
Transfusion	*43±17*	*41-45*	

APACHE II score			
	Mean(SD)	**95% Confidence**	**p value**
No Transfusion	*17.6±6.8*	*16.9-18.2*	*0.99*
Transfusion	*19.7±7.6*	*18.8-20.6*	

BASELINE HAEMOGLOBIN

Overall the mean baseline haemoglobin was 10.7±2.8g/dl (Table 1). Hemoglobin level decreased throughout the duration of the stay in the ICU.

The trends were similar when the ICUs were independently analyzed – as shown in the graphs below. The drop in haemoglobin from day 1 to day 12 in patients who were

transfused and not transfused were compared. The difference between these two groups of patients in both ICUs showed similar trends and statistically significant differences (p value <0.001) in both MICU and SICU.

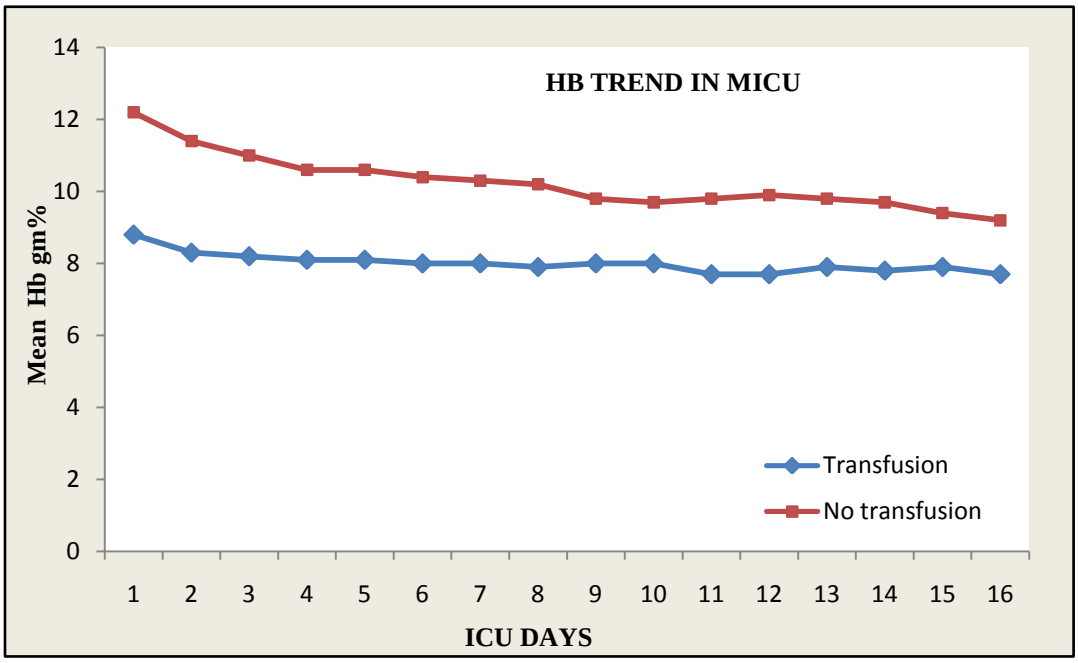


Figure 12. Haemoglobin levels from Day 1 through Day 16. Trend for remaining days of admission were omitted due to small number

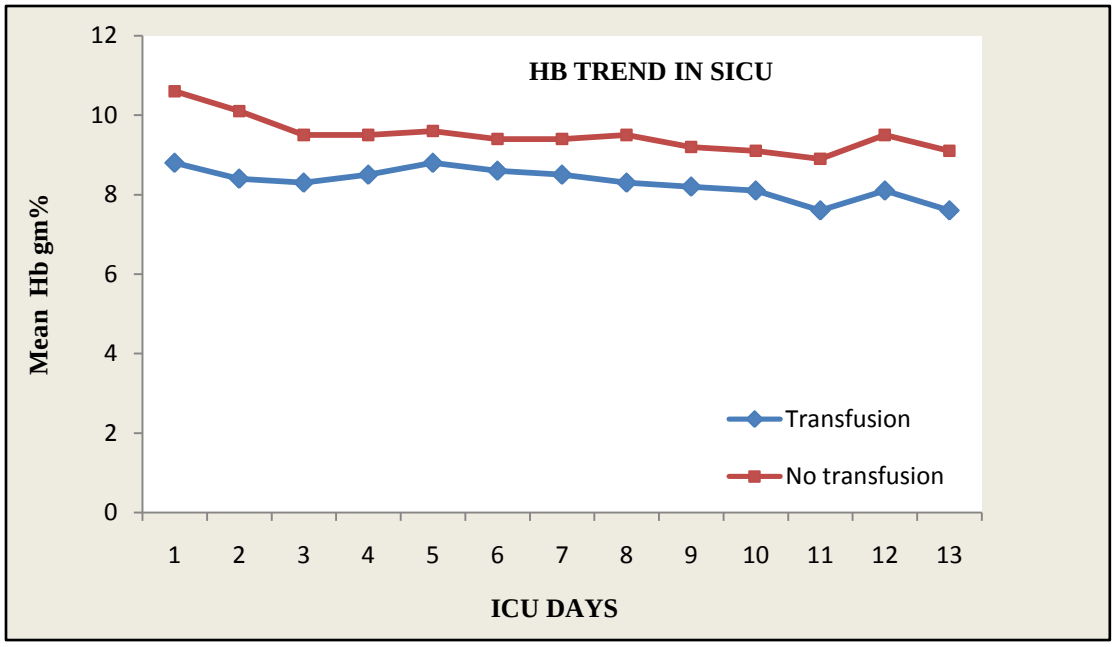


Figure 13. Haemoglobin levels from Day 1 through Day 13.

Individuals with lower baseline haemoglobin were more likely to receive an RBC transfusion and the difference was statistically significant.

Table 11. Baseline haemoglobin among the transfused and non transfused.

	HAEMOGLOBIN AT ADMISSION		
	Mean(SD)	**95% Confidence**	**p value**
No Transfusion	*11.6(2.5)*	*11.4-11.8*	*0.000*
Transfusion	*8.8(2.5)*	*8.5-9.1*	

MORTALITY

There was a statistically significant association between age, APACHE II score, duration of ventilation and receipt of packed cell transfusion with overall mortality. However, there was no association between admission haemoglobin and mortality.

Table 12. Univariate analysis for risk factors of overall mortality

	MORTALITY		
	OR	**95% Confidence**	**p value**
Age	*1.01*	*1.01-1.03*	*0.002*
APACHE II score	*1.09*	*1.06-1.12*	*0.000*
Ventilation duration	*2.51*	*1.54-4.11*	*0.000*
Hb at admission	*0.96*	*0.90-1.01*	*0.135*
Transfusion	*1.50*	*1.08-2.09*	*0.016*

On comparison of the risk factors in both the ICUs, there was a statistically significant correlation between age, APACHE II score, admission haemoglobin, duration of mechanical ventilation and packed cell transfusion with mortality in the medical cohort of patients. In the surgical cohort, a significant association was found between APACHE II score, duration of ventilation and packed cell transfusions with mortality but there was no association between age and haemoglobin at admission (Table 13). Admission haemoglobin was therefore seen to be significantly associated with mortality in the medical patients but not in the surgical cohort.

Table 13. Univariate analysis for risk factors of mortality in MICU and SICU

	MICU			*SICU*		
Mortality	**OR**	**95%confidence**	**p value**	**OR**	**95%confidence**	**p value**
Age	*1.02*	*1.00-1.03*	*0.001*	*1.01*	*0.99-1.02*	*0.186*
APACHE II	*1.06*	*1.03-1.10*	*0.001*	*1.15*	*1.10-1.20*	*0.000*
Hb at admission	*0.88*	*0.82-0.95*	*0.001*	*0.97*	*0.87-1.09*	*0.609*
Ventilation duration	*2.66*	*1.45-4.87*	*0.002*	*2.55*	*1.06-6.15*	*0.038*
Transfusion	*2.08*	*1.30-3.32*	*0.002*	*2.01*	*1.17-3.48*	*0.012*

After adjusting for other risk factors, APACHE II score and duration of mechanical ventilation remained statistically significantly associated with an increased risk of overall mortality. On the other hand, there was no significant association after adjustment with receipt of packed cell transfusion and mortality(Table 14a) or receipt of a transfusion with blood or any blood product and mortality(Table 14b).

Table 14a) Multivariate analysis for risk factors of overall mortality with RBC transfusion

	MORTALITY		
	Adj.OR	**95% Confidence**	**p value**
Age	*1.01*	*0.99-1.02*	*0.149*
APACHE II score	*1.08*	*1.05-1.11*	*0.000*
Ventilation duration	*2.00*	*1.19-3.36*	*0.009*
Hb at admission	*0.98*	*0.92-1.05*	*0.614*
RBC Transfusion	*1.21*	*0.81-1.82*	*0.348*

Table 14b) Multivariate analysis for risk factors of overall mortality with any transfusion

	MORTALITY		
	Adj.OR	**95% Confidence**	**p value**
Age	*1.01*	*0.99-1.02*	*0.149*
APACHE II score	*1.08*	*1.05-1.11*	*0.000*
Ventilation duration	*2.00*	*1.19-3.36*	*0.009*
Hb at admission	*0.98*	*0.92-1.05*	*0.614*
Any Transfusion	*1.39*	*0.93-1.06*	*0.825*

Comparison between ICUs, after adjustment for risk factors, revealed a statistically significant association between age, admission haemoglobin, duration of ventilation and receipt of any transfusion with blood or blood products and mortality in the Medical ICU.

In contrast, only APACHE II score remained statistically significantly associated with mortality after adjustment in the Surgical ICU patients. The association between receipt of any transfusion and mortality was not seen following adjustment for other risk factors as shown in the tables below.

Table 15a). Multivariate analysis for risk factors of mortality with RBC transfusion in MICU and SICU

	MICU			***SICU***		
Mortality	**Adj. OR**	**95% confidence**	**p value**	**Adj. OR**	**95% confidence**	**p value**
Age	*1.02*	*1.00-1.03*	*0.005*	*0.98*	*0.96-1.00*	*0.154*
APACHE II	*1.03*	*0.99-1.07*	*0.080*	*1.15*	*1.10-1.21*	*0.000*
Hb at admission	*0.89*	*0.81-0.98*	*0.019*	*1.02*	*0.90-1.16*	*0.738*
Ventilation duration	*2.57*	*1.33-4.97*	*0.005*	*1.44*	*0.56-3.71*	*0.448*
RBC Transfusion	*1.40*	*0.77-2.57*	*0.270*	*1.71*	*0.89-3.28*	*0.108*

Table 15b). Multivariate analysis for risk factors of mortality with any transfusion in MICU and SICU

	MICU			*SICU*		
Mortality	**Adj. OR**	**95% confidence**	**p value**	**Adj. OR**	**95% confidence**	**p value**
Age	*1.02*	*1.00-1.03*	*0.005*	*0.98*	*0.96-1.00*	*0.154*
APACHE II	*1.03*	*0.99-1.07*	*0.080*	*1.15*	*1.10-1.21*	*0.000*
Hb at admission	*0.89*	*0.81-0.98*	*0.019*	*1.02*	*0.90-1.16*	*0.738*
Ventilation duration	*2.57*	*1.33-4.97*	*0.005*	*1.44*	*0.56-3.71*	*0.448*
Any Transfusion	*1.92*	*1.07-3.45*	*0.028*	*1.81*	*0.94-3.52*	*0.078*

SUMMARY OF RESULTS

In this study 800 patients who fulfilled the inclusion criteria were recruited.

Of these patients, 58% were males and 42% were females. The mean age of the patients was 45±16.8years, with baseline APACHE II score of 18.30± 7.14 and a baseline haemoglobin of 10.7±2.8. When analyzed separately, the average admission haemoglobin in patients admitted to the SICU was far lesser when compared to the medical cohort in MICU and the difference was statistically significant.

Following admission to ICU, our study showed that 50% of the patients had haemoglobin levels below 10g/dl by 48hours of admission. Hemoglobin level decreased throughout the duration of the stay in the ICU.

The most common indication for admission to the medical ICU in the decreasing order of frequency was organophosphorus poisoning(13%), Acute respiratory distress syndrome(11%), scrub typhus(11%), acute kidney injury(11%), septic shock(10.5%) and DIC(9%).

The most common admission indications in the surgical ICU were abdominal surgery(29%), polytrauma(24%), pelvic surgery(15%), limb ischemia(12%), obstetric emergencies(7%) and thoracic surgery(6%).

Diabetes and Hypertension were the most frequent co morbid conditions seen in 34% and 28% respectively in the MICU and 24% and 26% respectively in the SICU.

81% of patients admitted to SICU and 80% of patients admitted to MICU required ventilatory support during their ICU stay and the duration of ventilation was higher in the medical cohort and it was statistically significant.

Overall mortality rate in the ICU was 25% with mortality rates and ICU lengths of stay being significantly higher for the medical cohort when compared to the surgical cohort.(34%vs 16% and 9±6 days and 5.5±3.9 days respectively, p=0.00)

Overall, 38% of patients received transfusions with blood or blood products and 34% of patients received RBC transfusions. The proportion of patients who received transfusions was significantly higher in the surgical cohort (44%) when compared with the medical patients (23%), [$p<0.0001$]. Also,the proportion of patients who received packed cells along with any blood product was also higher in the SICU(25%) vs. MICU(8%).

The overall indication for transfusion was a drop in the haemoglobin in the absence of overt bleeding.

The mean pre transfusion haemoglobin was 7.0±1.6 g/dl. However the mean pre transfusion haemoglobin in Medical ICU was 6.3gm% compared to 7.4gm% in the Surgical ICU.

The overall mortality inclusive of both ICUs was 23% among those who did not receive transfusions and 30% among those who received one or more RBC units with or without blood products across their stay in the ICU and this difference was statistically different.

There was a statistically significant association between receipt of packed cell transfusions (with or without any blood product) and prolonged ICU and hospital stay.

There was a statistically significant association between age, APACHE II score, duration of ventilation and receipt of packed cell transfusion with overall mortality. This was particularly observed among the medical cohort whereas in the surgical cohort, a significant association was found between APACHE II score, duration of ventilation and packed cell transfusions with mortality.

After adjusting for other risk factors, APACHE II score and duration of mechanical ventilation remained statistically significantly associated with an increased risk of overall mortality. On the other hand, there was no significant association after adjustment with receipt of packed cell transfusion and mortality.

Comparison between ICUs after adjustment for risk factors revealed a statistically significant association between age, admission haemoglobin, duration of ventilation and receipt of any transfusion with blood or blood products and mortality in the Medical ICU. There was no association between receipt of a packed cell transfusion and mortality following multivariate analysis. In contrast, only APACHE II score remained statistically significantly associated with mortality after adjustment in the Surgical ICU patients.

Even though transfusion was not an independent predictor of mortality in our study, it contributed to increased mortality in the presence of other risk factors in the critically ill.

DISCUSSION

DISCUSSION

Worldwide, anemia is a significant problem and especially in developing countries it is very widespread yet the most neglected issue. India is one of the countries with highest prevalence of anemia in the world. It is estimated that over 50% of the Indian population is anemic. In the context of this, anemia in the critically ill remains an important issue.

Many landmark studies have quantified the incidence of anemia and the use of packed cell transfusions in the critically ill patients.

In our study, the mean age of the study population was 45±17years which was far lesser than the mean age of the patients in other studies like CRIT study (60±18years) and the ABC trial(61years)(75),(9).

The majority of the patients were males which was similar to the previous studies.

The mean APACHE II score was 18.30± 7.14, and it was similar across both the ICU's in the study population.

The average duration of ICU stay was 7.3±5.4 days with a mean of 9.0±6.0 days in MICU and 5.5±3.9 days in SICU, which was similar to that observed in the CRIT study.

Our study found that 70% of critically ill patients had a haemoglobin concentration less than 12g/dl on admission to the ICU. This was similar to the ABC trial which showed that 63% of critically ill patients had a haemoglobin concentration less than 12g/dl on admission to the ICU. Following admission to ICU, our study showed that 50% of the patients had

haemoglobin levels below 10g/dl by 48hours of admission. Another audit of transfusion practice in the United Kingdom following the TRICC study found haemoglobin concentration less than 9g/dl in 55% of all patients who stayed more than 24hours in the ICU and it also occurred early, on the first and second ICU days in 52% and 77% of these patients.(79)

The anemia in these patients persisted throughout the duration of their ICU stay. This finding was consistent with previous studies like The CRIT Study wherein by 48hours after ICU admission, 70% of patients had a baseline haemoglobin less than 12g/dl and half of these patients had a haemoglobin less than 10g/dl.

Transfusion practice in response to anemia was consistent with other studies. RBC transfusions during the ICU period was 34%, which was similar to the ABC trial (37%) and the SOAP study (33%) (75).

In our study population, the surgical ICU patients received more RBC transfusions when compared to their medical cohort (44% vs. 23%) which was observed in the study by Groeger et al(80).

The mean pre transfusion trigger observed was 7.0±1.6g/dl, a value that is comparable to the TRICC study(42). This was lower than that observed by the CRIT(8.6±1.7g/dl) and ABC trial(8.4±1.3g/dl).

The most common indication for packed cell transfusion was low haemoglobin which was comparable to the CRIT study and ATICS study(76).

Individuals with lower baseline haemoglobin were more likely to receive an RBC transfusion when compared to those who did not receive transfusion and the difference was statistically significant (11.6±2.5 vs. 8.8±2.5g/dl, p value-0.000).

There was no significant association between age and receipt of transfusion across ICUs.

The association between transfusion rates and degree of organ failure, as assessed by the APACHE II score, was also analyzed. The baseline APACHE II score was not statistically different between the transfused and non transfused patients.

Transfusion was associated with a longer duration of ICU stay (OR-1.06, 95%CI:1.03-1.09).

It was also associated with a prolonged hospital length of stay when analyzed overall but on comparison within ICUs, it was noticed that packed cell transfusions were associated with a prolonged hospital stay only in the medical cohort of patients (OR-1.04, 95%CI:1.02-1.06, p value-0.000), whereas such a correlation was not seen among the surgical ICU patients (OR-1.00, 95%CI:0.98-1.02, pvalue-0.762).

Transfused patients had a higher ICU mortality rate than the non transfused patients (30% vs. 23%, p value- 0.015).

Receipt of a transfusion in the ICU increased a patient's odds of dying by a factor of 1.50 (95% confidence interval, 1.08-2.09). This fact was observable in the other studies like the ABC trial (OR-1.37, 95% CI: 1.02-1.84) and the TRICC study where the mortality rates during ICU and hospitalization were lower in the restrictive strategy when compared to the liberal strategy group. In contrast, the SOAP study showed that blood transfusions were not

associated with increased mortality by multivariate analysis or propensity matching and an extended Cox proportional hazard analysis showed that patients who received a transfusion had a better survival.

A logistic regression model was used to adjust for differences in all observed background characteristics in the estimation of the effects of transfusion.

Variables chosen were age, APACHE II score, admission haemoglobin, ventilation duration and receipt of transfusion. The model showed that blood transfusion was not an independent risk factor associated with a worse mortality rate (OR-1.21, 95%CI:0.81-1.82,p value-0.348), an observation similar to the SOAP study.

This model also showed that the associations of APACHE II score and ventilation duration with mortality were statistically significant but age and admission hemoglobin level was not.

When the model was created independently for each ICU, it was observed that that the association between age, admission haemoglobin, ventilation duration and receipt of any transfusion with mortality remained statistically significant in the MICU patients whereas only APACHE II score remained significantly associated with mortality in the SICU patients.

Table16 : Comparison of data of current study with other studies

	Year conducted, Country	***No of patients and number of ICUs***	***Percentage transfused in ICU***	***Commonest indication for transfusion***	***Pre transfusion haemoglobin level***	***Percentage ICU mortality***
Current Study	2012-2013, India	800 patients in 2 ICUs	34.0%	Low haemoglobin	7.0±1.6g/dl	25%
CRIT Study (Corwin et al)	2000-2001 USA	4,892 patients in 284 ICUs	44.0%	Low haemoglobin	8.6±1.7g/dl	18%
ABC trial (Vincent et al)	1999, Western Europe	3,534 patients in 146 ICUs	37.0%	Acute bleeding	8.4±1.3g/dl	13.5%
ATICS Study (Walsh et al)	2001, Scotland	1,023 patients in 10 ICUs	39.5%	Acute bleeding	7.8g/dl	25%
SOAP Study (Vincent et al)	2002, Europe	3,147 patients in 198 ICUs	33%	NS*	NS*	23.7%
Australasian study (Westbrook et al)	2008, Australia and New Zealand	5,128 patients in 47 ICUs	14.7%	Improve oxygen delivery	7.7g/dl	NS*

****NS: not specified***

CONCLUSION

CONCLUSION

Anemia is a common finding in the critically ill patients.

The most common indication for RBC transfusion is to treat the low haemoglobin rather than acute bleeding.

As demonstrated in other studies, we also found a significant drop in the haemoglobin during ICU stay both in the surgical ICU as well as in the medical ICU when analyzed individually.

Many studies including ours have shown that most critically ill patients can be managed with a haemoglobin threshold of 7g/dl, below which packed cell transfusions can be initiated.

Although transfusions can be lifesaving, many studies including ours have reported that the risks of transfusion outweigh the benefits.

Mortality in the transfused was significantly higher than in the non transfused and receipt of a transfusion in the ICU increased the odds of dying by a factor of 1.5 when analyzed overall. However, comparison between ICUs after adjustment of other risk factors revealed a statistically significant association in the medical ICU between receipt of any transfusion with blood or blood products with mortality. In contrast, such an association was not seen in the surgical cohort.

Transfusion also led to prolonged ICU and hospital length of stay. There was a significant association in the medical patients but it was not observed with their surgical counterparts.

Even though transfusion was not an independent predictor of mortality in our study, it contributed to increased mortality in the presence of other risk factors in the critically ill.

Therefore approaches should be made to reduce RBC transfusions and the need for transfusions should be individualized based on the patient's clinical circumstances rather than a subjective haemoglobin concentration.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Prakash D. Anemia in the ICU: anemia of chronic disease versus anemia of acute illness. Crit Care Clin. 2012 Jul;28(3):333–343, v.

2. Walsh TS, Saleh E-E-D. Anaemia during critical illness. Br J Anaesth. 2006 Sep;97(3):278–91.

3. Longo D. Harrison's Haematology and Oncology. 17th ed.

4. Longo D. Harrison's Haematology and Oncology pg 82. 17th edition.

5. Harder L, Boshkov L. The optimal hematocrit. Crit Care Clin. 2010 Apr;26(2):335–354, table of contents.

6. Klein HG, Spahn DR, Carson JL. Red blood cell transfusion in clinical practice. Lancet. 2007 Aug 4;370(9585):415–26.

7. Hsia CC. Respiratory function of hemoglobin. N Engl J Med. 1998 Jan 22;338(4):239–47.

8. Corwin HL, Parsonnet KC, Gettinger A. RBC transfusion in the ICU. Is there a reason? Chest. 1995 Sep;108(3):767–71.

9. Vincent JL, Baron J-F, Reinhart K, Gattinoni L, Thijs L, Webb A, et al. Anemia and blood transfusion in critically ill patients. JAMA J Am Med Assoc. 2002 Sep 25;288(12):1499–507.

10. Straat M, van Bruggen R, de Korte D, Juffermans NP. Red blood cell clearance in inflammation. Transfus Med Hemotherapy Off Organ Dtsch Ges Fuï Transfusionsmedizin Immunhaïatologie. 2012 Oct;39(5):353–61.

11. Weiss G, Goodnough LT. Anemia of chronic disease. N Engl J Med. 2005 Mar 10;352(10):1011–23.

12. Taniguchi S, Dai CH, Krantz SB. Specific binding of interferon-gamma to high affinity receptors on human erythroid colony-forming cells. Exp Hematol. 1997 Mar;25(3):193–8.

13. Roodman GD, Bird A, Hutzler D, Montgomery W. Tumor necrosis factor-alpha and hematopoietic progenitors: effects of tumor necrosis factor on the growth of erythroid progenitors CFU-E and BFU-E and the hematopoietic cell lines K562, HL60, and HEL cells. Exp Hematol. 1987 Oct;15(9):928–35.

14. Dybedal I, Jacobsen SE. Transforming growth factor beta (TGF-beta), a potent inhibitor of erythropoiesis: neutralizing TGF-beta antibodies show erythropoietin as a potent stimulator of murine burst-forming unit erythroid colony formation in the absence of a burst-promoting activity. Blood. 1995 Aug 1;86(3):949–57.

15. Tracey KJ, Wei H, Manogue KR, Fong Y, Hesse DG, Nguyen HT, et al. Cachectin/tumor necrosis factor induces cachexia, anemia, and inflammation. J Exp Med. 1988 Mar 1;167(3):1211–27.

16. Schobersberger W, Hoffmann G, Fandrey J. Nitric oxide donors suppress erythropoietin production in vitro. Pflügers Arch Eur J Physiol. 1996 Oct;432(6):980–5.

17. Yoshioka K, Thompson J, Miller MJ, Fisher JW. Inducible nitric oxide synthase expression and erythropoietin production in human hepatocellular carcinoma cells. Biochem Biophys Res Commun. 1997 Mar 27;232(3):702–6.

18. NATA > Network for Advancement of Transfusion Alternatives [Internet]. [cited 2013 Aug 7]. Available from: http://www.nata-online.com/index.php?NumArticle=443

19. Adamson JW. Regulation of red blood cell production. Am J Med. 1996 Aug 26;101(2A):4S–6S.

20. Boyd HK, Lappin TR, Bell AL. Evidence for impaired erythropoietin response to anaemia in rheumatoid disease. Br J Rheumatol. 1991 Aug;30(4):255–9.

21. Jelkmann WE, Fandrey J, Frede S, Pagel H. Inhibition of erythropoietin production by cytokines. Implications for the anemia involved in inflammatory states. Ann N Y Acad Sci. 1994 Apr 15;718:300–309; discussion 309–311.

22. Rodriguez RM, Corwin HL, Gettinger A, Corwin MJ, Gubler D, Pearl RG. Nutritional deficiencies and blunted erythropoietin response as causes of the anemia of critical illness. J Crit Care. 2001 Mar;16(1):36–41.

23. Hayden SJ, Albert TJ, Watkins TR, Swenson ER. Anemia in critical illness: insights into etiology, consequences, and management. Am J Respir Crit Care Med. 2012 May 15;185(10):1049–57.

24. Nemeth E, Rivera S, Gabayan V, Keller C, Taudorf S, Pedersen BK, et al. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. J Clin Invest. 2004 May;113(9):1271–6.

25. Sihler KC, Raghavendran K, Westerman M, Ye W, Napolitano LM. Hepcidin in trauma: linking injury, inflammation, and anemia. J Trauma. 2010 Oct;69(4):831–7.

26. Huwiler A, Kolter T, Pfeilschifter J, Sandhoff K. Physiology and pathophysiology of sphingolipid metabolism and signaling. Biochim Biophys Acta. 2000 May 31;1485(2-3):63–99.

27. Lang PA, Kempe DS, Tanneur V, Eisele K, Klarl BA, Myssina S, et al. Stimulation of erythrocyte ceramide formation by platelet-activating factor. J Cell Sci. 2005 Mar 15;118(Pt 6):1233–43.

28. Kempe DS, Akel A, Lang PA, Hermle T, Biswas R, Muresanu J, et al. Suicidal erythrocyte death in sepsis. J Mol Med Berl Ger. 2007 Mar;85(3):273–81.

29. Uyesaka N, Hasegawa S, Ishioka N, Ishioka R, Shio H, Schechter AN. Effects of superoxide anions on red cell deformability and membrane proteins. Biorheology. 1992 Jun;29(2-3):217–29.

30. Marín J, Rodríguez-Martínez MA. Role of vascular nitric oxide in physiological and pathological conditions. Pharmacol Ther. 1997 Aug;75(2):111–34.

31. Mohandas N, Gallagher PG. Red cell membrane: past, present, and future. Blood. 2008 Nov 15;112(10):3939–48.

32. Corwin HL. The role of erythropoietin therapy in the critically ill. Transfus Med Rev. 2006 Jan;20(1):27–33.

33. McCook O, Georgieff M, Scheuerle A, Möller P, Thiemermann C, Radermacher P. Erythropoietin in the critically ill: do we ask the right questions? Crit Care Lond Engl. 2012 Sep 26;16(5):319.

34. Silver M, Corwin MJ, Bazan A, Gettinger A, Enny C, Corwin HL. Efficacy of recombinant human erythropoietin in critically ill patients admitted to a long-term acute care facility: a randomized, double-blind, placebo-controlled trial. Crit Care Med. 2006 Sep;34(9):2310–6.

35. Weiskopf RB, Viele MK, Feiner J, Kelley S, Lieberman J, Noorani M, et al. Human cardiovascular and metabolic response to acute, severe isovolemic anemia. JAMA J Am Med Assoc. 1998 Jan 21;279(3):217–21.

36. Weiskopf RB, Kramer JH, Viele M, Neumann M, Feiner JR, Watson JJ, et al. Acute severe isovolemic anemia impairs cognitive function and memory in humans. Anesthesiology. 2000 Jun;92(6):1646–52.

37. Weiskopf RB, Feiner J, Hopf H, Lieberman J, Finlay HE, Quah C, et al. Fresh blood and aged stored blood are equally efficacious in immediately reversing anemia-induced brain oxygenation deficits in humans. Anesthesiology. 2006 May;104(5):911–20.

38. Geha AS, Baue AE. Graded coronary stenosis and coronary flow during acute normovolemic anemia. World J Surg. 1978 Sep;2(5):645–51.

39. Lieberman JA, Weiskopf RB, Kelley SD, Feiner J, Noorani M, Leung J, et al. Critical oxygen delivery in conscious humans is less than 7.3 ml O2 x kg(-1) x min(-1). Anesthesiology. 2000 Feb;92(2):407–13.

40. Viele MK, Weiskopf RB. What can we learn about the need for transfusion from patients who refuse blood? The experience with Jehovah's Witnesses. Transfusion (Paris). 1994 May;34(5):396–401.

41. Wu WC, Rathore SS, Wang Y, Radford MJ, Krumholz HM. Blood transfusion in elderly patients with acute myocardial infarction. N Engl J Med. 2001 Oct 25;345(17):1230–6.

42. Hébert PC, Wells G, Blajchman MA, Marshall J, Martin C, Pagliarello G, et al. A multicenter, randomized, controlled clinical trial of transfusion requirements in critical care. Transfusion Requirements in Critical Care Investigators, Canadian Critical Care Trials Group. N Engl J Med. 1999 Feb 11;340(6):409–17.

43. McIntyre L, Tinmouth AT, Fergusson DA. Blood component transfusion in critically ill patients. Curr Opin Crit Care. 2013 Aug;19(4):326–33.

44. Stowell SR, Winkler AM, Maier CL, Arthur CM, Smith NH, Girard-Pierce KR, et al. Initiation and Regulation of Complement during Hemolytic Transfusion Reactions. Clin Dev Immunol [Internet]. 2012 Oct 16 [cited 2013 Aug 11];2012. Available from: http://www.hindawi.com/journals/cdi/2012/307093/abs/

45. Heddle NM, Soutar RL, O'Hoski PL, Singer J, McBride JA, Ali MA, et al. A prospective study to determine the frequency and clinical significance of alloimmunization post-transfusion. Br J Haematol. 1995 Dec;91(4):1000–5.

46. Aygun B, Padmanabhan S, Paley C, Chandrasekaran V. Clinical significance of RBC alloantibodies and autoantibodies in sickle cell patients who received transfusions. Transfusion (Paris). 2002 Jan;42(1):37–43.

47. Cheng CK, Lee CK, Lin CK. Clinically significant red blood cell antibodies in chronically transfused patients: a survey of Chinese thalassemia major patients and literature review. Transfusion (Paris). 2012 Oct;52(10):2220–4.

48. King KE, Shirey RS, Thoman SK, Bensen-Kennedy D, Tanz WS, Ness PM. Universal leukoreduction decreases the incidence of febrile nonhemolytic transfusion reactions to RBCs. Transfusion (Paris). 2004 Jan;44(1):25–9.

49. Pandey S, Vyas GN. Adverse effects of plasma transfusion. Transfusion (Paris). 2012 May;52 Suppl 1:65S–79S.

50. Sachs UJH, Hattar K, Weissmann N, Bohle RM, Weiss T, Sibelius U, et al. Antibody-induced neutrophil activation as a trigger for transfusion-related acute lung injury in an ex vivo rat lung model. Blood. 2006 Feb 1;107(3):1217–9.

51. Takahashi D, Fujihara M, Azuma H, Miyazaki T, Uchimura D, Wakamoto S, et al. Stimulation of human neutrophils with sera containing HLA Class I alloantibody causes preferential degranulation of azurophilic granules and secretory vesicles. Vox Sang. 2010 May;98(4):560–6.

52. Khan SY, Kelher MR, Heal JM, Blumberg N, Boshkov LK, Phipps R, et al. Soluble CD40 ligand accumulates in stored blood components, primes neutrophils through CD40, and is a potential cofactor in the development of transfusion-related acute lung injury. Blood. 2006 Oct 1;108(7):2455–62.

53. Silliman CC, Paterson AJ, Dickey WO, Stroneck DF, Popovsky MA, Caldwell SA, et al. The association of biologically active lipids with the development of transfusion-related acute lung injury: a retrospective study. Transfusion (Paris). 1997 Jul;37(7):719–26.

54. Hendrickson JE, Hillyer CD. Noninfectious serious hazards of transfusion. Anesth Analg. 2009 Mar;108(3):759–69.

55. Harmening D. Modern Blood banking and Transfusion Practices. 5th ed.

56. Klein HG, Anstee D. Mollison's Blood Transfusion in Clinical Medicine Pg 674-675. 11th edition.

57. Klein HG, Anstee D. Mollison's Blood Transfusion in Clinical Medicine, pg 676-677. 11th ed. Blackwell Publishing;

58. Zimrin AB, Hess JR. Current issues relating to the transfusion of stored red blood cells. Vox Sang. 2009;96(2):93–103.

59. Hess JR, Greenwalt TG. Storage of red blood cells: new approaches. Transfus Med Rev. 2002 Oct;16(4):283–95.

60. Relevy H, Koshkaryev A, Manny N, Yedgar S, Barshtein G. Blood banking-induced alteration of red blood cell flow properties. Transfusion (Paris). 2008 Jan;48(1):136–46.

61. Yedgar S, Koshkaryev A, Barshtein G. The red blood cell in vascular occlusion. Pathophysiol Haemost Thromb. 2002 Dec;32(5-6):263–8.

62. Tinmouth A, Fergusson D, Yee IC, Hébert PC, ABLE Investigators, Canadian Critical Care Trials Group. Clinical consequences of red cell storage in the critically ill. Transfusion (Paris). 2006 Nov;46(11):2014–27.

63. Van de Watering L. Red cell storage and prognosis. Vox Sang. 2011 Jan;100(1):36–45.

64. Anniss AM, Sparrow RL. Storage duration and white blood cell content of red blood cell (RBC) products increases adhesion of stored RBCs to endothelium under flow conditions. Transfusion (Paris). 2006 Sep;46(9):1561–7.

65. Bennett-Guerrero E, Veldman TH, Doctor A, Telen MJ, Ortel TL, Reid TS, et al. Evolution of adverse changes in stored RBCs. Proc Natl Acad Sci U S A. 2007 Oct 23;104(43):17063–8.

66. Purdy FR, Tweeddale MG, Merrick PM. Association of mortality with age of blood transfused in septic ICU patients. Can J Anaesth J Can Anesthésie. 1997 Dec;44(12):1256–61.

67. Eikelboom JW, Cook RJ, Liu Y, Heddle NM. Duration of red cell storage before transfusion and in-hospital mortality. Am Heart J. 2010 May;159(5):737–743.e1.

68. Lelubre C, Vincent J-L. Relationship between red cell storage duration and outcomes in adults receiving red cell transfusions: a systematic review. Crit Care Lond Engl. 2013 Apr 8;17(2):R66.

69. Pallavi P, Ganesh CK, Jayashree K, Manjunath GV. Seroprevalence and trends in transfusion transmitted infections among blood donors in a university hospital blood bank: a 5 year study. Indian J Hematol Blood Transfus Off J Indian Soc Hematol Blood Transfus. 2011 Mar;27(1):1–6.

70. Dubey A, Elhence P, Ghoshal U, Verma A. Seroprevalence of malaria in blood donors and multi-transfused patients in Northern India: Relevance to prevention of transfusion-transmissible malaria. Asian J Transfus Sci. 2012;6(2):174–8.

71. Choudhury N. Transfusion transmitted infections: How many more? Asian J Transfus Sci. 2010 Jul;4(2):71–2.

72. Bordin JO, Heddle NM, Blajchman MA. Biologic effects of leukocytes present in transfused cellular blood products. Blood. 1994 Sep 15;84(6):1703–21.

73. Simon T, Snyder E, Solheim B, Stowell C, Strauss R, Petrides M. Rossi's principles of Transfusion Medicine. 4th edition. Blackwell Publishing;

74. Blajchman M a. Immunomodulatory Effects of Allogeneic Blood Transfusions: Clinical Manifestations and Mechanisms. Vox Sang. 1998;74(S2):315–9.

75. Corwin HL, Gettinger A, Pearl RG, Fink MP, Levy MM, Abraham E, et al. The CRIT Study: Anemia and blood transfusion in the critically ill--current clinical practice in the United States. Crit Care Med. 2004 Jan;32(1):39–52.

76. Hébert PC, Wells G, Blajchman MA, Marshall J, Martin C, Pagliarello G, et al. A multicenter, randomized, controlled clinical trial of transfusion requirements in critical care. Transfusion Requirements in Critical Care Investigators, Canadian Critical Care Trials Group. N Engl J Med. 1999 Feb 11;340(6):409–17.

77. Vincent J-L, Sakr Y, Sprung C, Harboe S, Damas P, Sepsis Occurrence in Acutely Ill Patients (SOAP) Investigators. Are blood transfusions associated with greater mortality rates? Results of the Sepsis Occurrence in Acutely Ill Patients study. Anesthesiology. 2008 Jan;108(1):31–9.

78. Walsh TS, Garrioch M, Maciver C, Lee RJ, MacKirdy F, McClelland DB, et al. Red cell requirements for intensive care units adhering to evidence-based transfusion guidelines. Transfusion (Paris). 2004 Oct;44(10):1405–11.

79. McLellan SA, McClelland DBL, Walsh TS. Anaemia and red blood cell transfusion in the critically ill patient. Blood Rev. 2003 Dec;17(4):195–208.

80. Groeger JS, Guntupalli KK, Strosberg M, Halpern N, Raphaely RC, Cerra F, et al. Descriptive analysis of critical care units in the United States: patient characteristics and intensive care unit utilization. Crit Care Med. 1993 Feb;21(2):279–91.

ANNEXURES

ANNEXURE I

INSTITUTIONAL REVIEW BOARD (IRB)
CHRISTIAN MEDICAL COLLEGE
VELLORE 632 002, INDIA

Dr.B.J.Prashantham, M.A.,M.A.,Dr.Min(Clinical)
Director, Christian Counseling Centre
Editor, Indian Journal of Psychological Counseling
Chairperson, Ethics Committee, IRB

Dr. Alfred Job Daniel, MS Ortho
Chairperson, Research Committee &
Principal

Dr. Nihal Thomas
MD, MNAMS, DNB(Endo), FRACP(Endo), FRCP(Edin)
Secretary, Ethics Committee, IRB
Additional Vice Principal (Research)

December 17, 2012

Dr. Eliza Sherin Koshy
PG Registrar
Department of Transfusion Medicine and Immunohaematology
Christian Medical College
Vellore 632 002

Sub: **FLUID Research grant project NEW PROPOSAL:**
Anemia and transfusion decisions in the intensive care unit.
Dr. Eliza Sherin Koshy, Post Graduate Registrar, Transfusion Medicine and Immunohaematology, Dr. Dolly Daniel, Transfusion Medicine and Immunohaematology, Dr. J V Peter, Division of Critical Care, Dr. Gijoe George Jacob, Surgical intensive Care Unit.

Ref: IRB Min. No. 7933 dated 02.08.2012

Dear Dr. Eliza Sherin Koshy,

I enclose the following documents:-

1. Institutional Review Board approval
2. Agreement

Could you please sign the agreement and send it to Dr. Nihal Thomas, Addl. Vice Principal (Research), so that the grant money can be released.

With best wishes,

Dr. Nihal Thomas
Secretary (Ethics Committee)
Institutional Review Board

Dr Nihal Thomas
MBBS MD MNAMS DNB (Endo) FRACP(Endo) FRCP(Edin)
Secretary (Ethics Committee)
Institutional Review Board

CC: Dr. Dolly Daniel, Transfusion Medicine and Immunohaematology

TEL : 0416 - 2284294, 2284202 FAX : 0416 - 2262788, 2284481 E-mail : research@cmcvellore.ac.in

ANNEXURE II

ANEMIA AND TRANSFUSION DECISIONS IN INTENSIVE CARE UNITS

Name:		**Age:**		**Sex:**		**Hospital No:**	

Primary diagnosis:

Type of surgery:

APACHE Score:

Comorbidities:	(**√**)If present
Diabetes	
Hypertension	
IHD	
C Kidney Disease	
Sepsis	
Others(Mention)	

H/O any recent treatment with recombinant erythropoietin/haematinics/blood transfusion:

Haemoglobin levels:

	Admission	**Day1**	**Day2**	**Day3**	**Day4**	**Day5**	**Day6**	**Day7**	**Day8**	**Day9**
Hb levels:										

Indication for transfusion: (√)If present

Bleeding	**Drop in Hb**	**Sepsis**	**Ischemic**	**Hypotension**	**Symptomatic**	**Surgery**

Transfusion trigger and Number of RBC units transfused:

Date of Transfusion													
Time of Transfusion													
Transfusion Trigger													
Type of Unit[†]													
No. of units													

[†]Whole Blood- WB, LDRC-leukocyte depleted red cells, RC-Rejuvenated cells

Other components transfused:

Date of Transfusion									
Type of Component[‡]									
No. of units Transfused									
Indication									

[‡] FFP(Fresh Frozen plasma) , PRC(Platelet rich concentrate), SDP(Single donor platelets), CP(Cryoprecipitate)

Outcome of ICU stay: (√) If present

Discharged	
Death	

Number of ventilated days:

Number of days of ICU stay:

Total length of hospital stay: