DETECTION OF RED BLOOD CELL IMMUNIZATION AMONG TRANSFUSED CHRONIC KIDNEY DISEASE PATIENTS IN HOSPITAL UNIVERSITI SAINS MALAYSIA

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LIST OF ABBREVIATIONS

CKD	Chronic kidney disease
GFR	Glomerular filtration rate
eGFR	Estimated glomerular filtration rate
ESRF	End stage renal failure
DKD	Diabetic kidney disease
EPO	Erythropoietin
EDTA	Ethylenediamine tetra-acetic acid
RBC	Red blood cell
pmp	Per million population
PPUKM	Universiti Kebangsaan Medical Centre
GBD	Global burden disease
EBPG	European Best Practice Guideline
KDOQI	Kidney Disease Outcome Quality Initiative
EPO	Erythropoietin
TSAT	Transferrin receptor
IQR	Interquartile range
SD	Standard deviation
SHOT	Serious Hazard of Transfusion
HIV	Human immunodeficiency virus
HBV	Hepatitis B virus
HCV	Hepatitis C virus
TRALI	Transfusion related acute lung injury
PTP	Post transfusion purpura
HLA	Human leucocytes antigen

Rh D	Rhesus D
AIHA	Autoimmune haemolytic anaemia
IgG	Immunoglobulin G
IgM	Immunoglobulin M
HDFN	Haemolytic disease of new-born
HTR	Haemolytic transfusion reaction
DHTR	Delayed haemolytic transfusion reaction
DSTR	Delayed serologic transfusion reaction
TTI	Transfusion transmitted infection
HUSM	Hospital Universiti Sains Malaysia
IU	International unit
Fy	Duffy
Jk	Kidd
K	Kell
Le	Lewis
LISS	Low ionic strength saline

ABSTRAK

PENGESANAN ANTIBODI TERHADAP SEL DARAH MERAH DI KALANGAN PESAKIT YANG MENGALAMI KEGAGALAN BUAH PINGGANG YANG KRONIK DAN PERNAH MENERIMA TRANSFUSI DARAH, DI HOSPITAL UNIVERSITI SAINS MALAYSIA

Pengenalan : Penghasilan antibodi terhadap sel darah merah adalah salah satu komplikasi yang penting dan kerap berlaku kepada pesakit yang kerap menerima transfusi darah. Pesakit yang menghidap masalah kegagalan buah pinggang sangat mudah untuk mengalami kekurangan darah merah. Ini mennyebabkan pesakit-pesakit yang mempunyai kegagalan buah pinggang memerlukan pemindahan darah yang lebih kerap. Tujuan utama kajian ini dijalankan adalah untuk melihat kelaziman dan jenis antibodi terhadap sel darah merah dan juga faktor risiko yang terdapat dikalangan pesakit yang mengalami kegagalan buah pinggang dan pernah menerima transfusi darah.

Metodologi: Kajian keratan lintang ini telah dijalankan di Unit Perubatan Transfusi Darah Hospital Universiti Sains Malaysia selama satu tahun daripada Januari 2016 sehingga Januari 2017. Seramai 249 pesakit yang mengalami masalah kegagalan buah pinggang telah diambil sebagai subjek ujikaji. Semua pesakit ini telah menerima transfusi darah sekurang-kurangnya satu unit darah merah. Sampel darah pesakit telah diproses dan sera pula digunakan untuk ujian saringan antibodi. Sampel darah yang positif saringan antibodi akan diproses selanjutnya untuk penentuan jenis antibodi yang lebih spesifik.

Keputusan: Hasil daripada kajian ini, kami dapati kebanyakan subjek adalah lelaki (55.8%) dan golongan tua (55.8%) yang berumur lebih dari 60 tahun. Kebanyakan pesakit yang memerlukan pemindahan darah adalah pesakit yang mengalami kegagalan buah pinggang tahap akhir (tahap 4 dan tahap 5) iaitu 90.3%. Kekerapan pesakit yang mempunyai antibodi terhadap darah merah adalah 12.4%. Kebannyakannya adalah alloantibodi (96.8%) berbanding autoantibodi. Jenis antibodi yang paling kerap adalah antibodi anti Mia (40%) diikuti dengan anti E (22.8%). Penghasilan antibodi terhadap sel darah merah dipengaruhi sekiranya pesakit pernah mengandung sebelum pemindahan darah (p-value < 0.05). Faktor-faktor lain seperti sosiodemografi, tahap kegagalan buah pinggang, kekerapan hemodialisis, masalah penyakit yang lain dan jumlah bilangan darah yang diterima tidak mempengaruhi kepenghasilan antibodi terhadap sel darah merah.

Kesimpulan: Kesimpulan daripada kajian ini menunjukkan pesakit yang mengalami masalah kegagalan buah pinggang juga mempunyai risiko yang tinggi untuk menghasilkan antibodi terhadap cell darah merah yang diterima daripada pesakit lain. Risiko adalah lebih tinggi sekiranya pesakit pernah mengandung. Hasil daripada kajian ini kami menyarankan pesakit yang meghidapi kegagalan buah pinggang memerlukan darah yang sesuai untuk semua rhesus antigen.

ABSTRACT

DETECTION OF RED BLOOD CELL IMMUNIZATION AMONG TRANSFUSED CHRONIC KIDNEY DISEASE PATIENTS IN HOSPITAL UNIVERSITI SAINS MALAYSIA

Introduction: Red blood cell (RBC) immunization is a common complication in blood transfusion recipients. Patients with chronic kidney disease (CKD) eventually develop anaemia due to multifactorial and require regular blood transfusion which exposed patient for development of RBC antibody. The objectives of this study were to determine the incidence and specificity of RBC immunization and its risk factors among transfused CKD patients.

Study design and methodology: This is a cross-sectional study which was done over 1 year period from Jan 2016 until Jan 2017 in the Transfusion Medicine Unit Hospital Universiti Sains Malaysia. A total of 249 samples were collected from CKD patients who received at least one-pint blood transfusion which only match for ABO and RH(D) antigen. The blood samples (serum) were screened for the presence of antibody using antibody screening test. Samples with positive antibody screening will subjected for antibody identification.

Result: The result showed majority of our study population were male (55.8%) and elderly with age > 60 years (55.8%). Majority of patients also at late stage of CKD (stage 4 and 5) (90.3%) who require more blood transfusions. The prevalence of positive antibody screening among CKD patients were 12.4%. Among the patients who have

positive antibody screening, majority were alloantibody (96.8%). Anti Mia was the most common alloantibody (40%) followed by anti-E (22.8%). There was no significant association between sociodemographic background, stage of CKD, requirement of haemodialysis, underlying medical illness and number of packed cell transfusion with the development of RBC antibody. The only significant associated factor that we can prove was history of pregnancy (p-value < 0.05).

Conclusion: As a conclusion, prevalence of RBC immunization was common among CKD patients and the risk were increased in patients who had history of pregnancy, therefore we proposed for rhesus RBC phenotyping in CKD patients especially patients in reproductive age and to supply blood match rhesus antigen blood.

CHAPTER 1

INTRODUCTION

1.0 GENERAL INTRODUCTION

Chronic kidney disease (CKD) is one of the widespread health problem in the world and the prevalence is increasing worldwide. It is defined by an irreversible loss of renal function or decreased glomerular filtration rate for 3 or more months (Levey *et al.*, 2003). The estimated prevalence of CKD in the US was 16.8%, while in Asian population the prevalence ranged from12.1% to 17.5%. In Malaysia, the incidence and prevalence of patient with end stage renal disease (ESRD) on dialysis had increased significantly from 88 and 325 per million population (pmp) respectively in 2001 to 170 and 762 pmp respectively in 2009 (Ministry Of Health 2011).

The increased in ESRD was largely driven by the increasing incidence of diabetic kidney disease (DKD) globally over recent decades. The prevalence of people with diabetic is expected to reach 642 million people by 2040 and accounting about 40% will develop CKD (Perkovic *et al.*, 2016). It is reported that, Malaysia had the highest incident rate of patients with diabetes who entering renal replacement therapy (Huri *et al.*, 2015). The growing number of ESRD places an enormous human, economic and social burden on the health care system. It is also associated with significant morbidity and mortality.

One of the major medical issues facing by CKD population is anaemia. The causes of anaemia in patient with CKD are multifactorial. The most well-known cause is inadequate erythropoietin (EPO) production, which is often compounded by other factors including iron and folate deficiency, decreased red cell survival and accumulation of toxic inhibitors of erythropoiesis (Eschbach and Adamson, 1985; Hsu *et al.*, 2002). Low haemoglobin

levels around 5-7g/dl were common especially in haemodialysis patients thus require frequent blood transfusions to improve the symptoms of anaemia (Kliger *et al.*, 2012). Exposure to multiple blood transfusions inevitably lead to many undesirable outcomes, and one of the important and common outcome is development of RBC alloimmunization (Singer *et al.*, 2000).

Alloimmunisation is defined as development of antibodies in response to alloantigen after exposure to genetically different cells or tissue (Harmening, 2012). There are many factors which can influence the development of alloantibody in the blood. Many studies had showed that alloimmunisation formation are influence by the recipient's immune status, dose of blood transfused and immunogenicity of the antigen. Therefore, ensuring that the antigens of transfused blood cells are match with recipient blood cells are essential for safe blood transfusion. Previous study showed that the rate of RBC alloimmunizations among patients with chronic blood transfusion such as hemoglobinopathy, aplastic anaemia, myelogenous leukaemia, upper gastrointestinal bleeding and chronic kidney disease was 29%, 11%, 16%, 11% and 14% respectively (Blumberg *et al.*, 1983). So, these kinds of populations are at greater risk for developing red cell alloantibodies.

In view of the great concern regarding alloimmunisation among chronically transfused patients, many extensive studies were conducted involving thalassemia patients and sickle cell disease but only a few numbers of study assessing risk of RBC immunization among CKD patients. So, we chose CKD population for this study because this disease display pattern of increasing in trend thus patients are at risk for multiple blood transfusions. The dialysis acceptance and also prevalence rate in Malaysia have almost

double in recent years. For instance, dialysis acceptance was 1733 per million population (pmp) in 2004 and increasing to 3156 pmp in 2013. In National Renal Registry 2014, the total number of patients on dialysis has increased sharply from 6702 in 2000 to 31 637 in 2013, causing a significant strain on Malaysian health resources (Begum *et al.*, 2016).

Local data regarding the rate of RBC alloimmunisation has been reported in a study done in Universiti Kebangsaan Malaysia medical centre (PPUKM) which ranging from 5-30% among the multiply transfused patients (Yousuf *et al.*, 2013). Red cell alloimmunisation may lead to difficulty in finding the compatible blood for transfusion and might cause haemolytic transfusion reaction if the antibodies are not detected during pre-transfusion testing.

The presence of alloantibodies will create significant problems in transfusion therapy and can contributes to morbidity and mortality. This will result in difficulty for cross-matching of the blood especially during emergency. A clinically significant alloantibodies are capable of causing mild to severe red cell haemolysis (Poole and Daniels, 2007). Furthermore, autoantibody which present can mask the existence of alloantibody and this will complicate the situation. Autoantibody is an antibody that produced against the patient's own red cells. This autoantibody may prematurely destroy the RBC in certain circumstances and lead to immune haemolysis (autoimmune haemolytic anaemia, AIHA) (Petz and Garratty, 2004). These conditions are relatively unusual but may contribute to significant clinical problems which require attention of knowledge among the clinicians and laboratory personnel's. The most common form of AIHA is characterized by the presence of 'warm'-type autoantibodies which are immunoglobulin G (IgG) (Hoffbrand *et al.*, 2016). IgG type of immunoglobulin capable

to react optimally at 37 0C and causing extravascular RBC destruction by tissue macrophages (Petz and Garratty, 2004). The presence of autoantibody will mask the presence of alloantibody during antibody identification and may cause difficulty to provide compatible blood for these patients.

In view of very few data have been reported concerning alloantibody/autoantibody development in CKD patients, especially local data in Malaysia, this study is conducted to look for the incidence and risk factors in CKD patients for development of RBC immunization. Hopefully the result can be used for better comprehension of the problems and provide the ideal approach for transfusion policy in CKD population.

CHAPTER 2

LITERATURE REVIEW

2.1 CHRONIC KIDNEY DISEASE

2.1.1 Definition, Prevalence and Classification

Chronic kidney disease is a term referred for heterogenous disorders affecting the structure and function of the kidney (Levey and Coresh, 2012). It is defined by the presence of kidney damage signs (including albuminuria, haematuria or proteinuria), decreased kidney function evidence by reducing GFR and abnormal imaging and histology lasting for three months or more (Ayodele and Alebiosu, 2010). Table 2.1 describe the criteria for definition of chronic kidney disease.

During the 20th century, infectious disease are the major health problems, however in this recent years, non-infectious disease has replaced the infectious disease and become the major cause of morbidity and mortality worldwide (Atkins, 2005; Nahas, 2005). It has been estimated about 80% of the disease burden occur in low and middle income countries and about 25% involve people less than 60 years old (Le Galès-Camus *et al.*, 2005). Chronic kidney disease has emerged as a global public health problem as the prevalence is increasing in trend. Based on World Health Report 2002 and Global Burden of Disease (GBD) project, the kidney disease and urinary tract cause deaths for approximately 850,000 every year (Schieppati and Remuzzi, 2005). It has high potential to progression to end-stage renal disease and cause a devastating medical, social and economic burden for the patient, family and country. In developed countries, ESRD is a major cost for health-care system and annual requirement of renal replacement therapy continue to increase between 6%-12%. This problem more prominent in developing countries. Over 2 million people now require haemodialysis to sustained life worldwide

and this estimation still lower than real numbers (Eggers, 2011). Effort to reduce the cost for management of the disease is difficult to achieved and many countries cannot afford renal replacement therapy. In 112 developing countries with combined population of over 600 million peoples, about 1 million patients died due to untreated kidney failure (Barsoum, 2006).

According to the 2010 US Renal Data System Annual Data Report, the primary causes of kidney disease are diabetes with incidence of ESRD 153 pmp in 2009, hypertension (99 pmp), glomerulonephritis (23.7 pmp) (Bakris and Collins, 2008) and cardiovascular disease. However about 28% of CKD patients who are elderly with age more than 60 have neither diabetic nor hypertensive (Vassalotti *et al.*, 2010). Same findings were also observed in developing countries but surprisingly glomerulonephritis and CKD of unknown origin have account for larger fraction of the total incidence, especially in young population (Sharma *et al.*, 2010).

Table 2.1: Criteria for definition of Chronic Kidney Disease

***Panel 1:* Criteria for definition of chronic kidney disease**

Duration >3 months on the basis of documentation or inference

Duration is necessary to distinguish chronic from acute kidney disease

- Clinical assessment can indicate duration
- Documentation of duration is not usually available in epidemiological studies

GFR <60 mL/min per 1·73 m²

- GFR is the best overall index of kidney function in health and disease
- Normal GFR in young adults is about 125 mL/min per 1·73 m²; GFR<15 mL/min per 1·73 m² is defined as kidney failure
- Decreased GFR can be detected by equations to estimate GFR that are based on serum creatinine (estimated GFR) but not by serum creatinine alone
- Decreased estimated GFR can be confirmed by measured GFR

Kidney damage as defined by structural abnormalities or functional abnormalities other than decreased GFR

Pathological abnormalities

- Clinical diagnosis is based on pathology and cause; markers of kidney damage might show pathology
- Glomerular diseases (diabetes, autoimmune diseases, systemic infections, drugs, neoplasia)
- Vascular diseases (atherosclerosis, hypertension, ischaemia, vasculitis, thrombotic microangiopathy)
- Tubulointerstitial diseases (urinary-tract infections, stones, obstruction, toxic effects of drugs)
- Cystic disease (polycystic kidney disease)

History of kidney transplantation

In addition to pathological abnormalities in native kidneys, common pathological abnormalities include:

- Chronic allograft nephropathy (non-specific findings of tubular atrophy, interstitial fibrosis, vascular and glomerular sclerosis)
- Rejection
- Drug toxic effects (calcineurin inhibitors)
- BK virus nephropathy
- Recurrent disease (glomerular disease, oxalosis, Fabry's disease)

Albuminuria as a marker of kidney damage

Increased glomerular permeability, urine ACR >30 mg/g*

- The normal urinary ACR in young adults is <10 mg/g. Urine ACR categories 10–29, 30–300 and >300 mg are high normal, high, and very high, respectively. Urine ACR >2000 mg/g is accompanied by signs and symptoms of nephrotic syndrome (low serum albumin, oedema, and high serum cholesterol)
- Threshold value roughly corresponds to urine dipstick values of trace or 1+, dependent on urine concentration
- High urinary ACR can be confirmed by urine albumin excretion in a timed urine collection

Abnormalities in urinary sediment as markers of kidney damage

- Red-blood-cell casts in proliferative glomerulonephritis
- White-blood-cell casts in pyelonephritis or interstitial nephritis
- Oval fat bodies or fatty casts in diseases with proteinuria
- Granular casts and renal tubular epithelial cells in many parenchymal diseases (non-specific)

Imaging abnormalities as markers of kidney damage (ultrasound, CT, and MRI with or without contrast, isotope scans, angiography)

- Polycystic kidneys
- Hydronephrosis due to obstruction
- Cortical scarring due to infarcts, pyelonephritis, or vesicoureteral reflux
- Renal masses or enlarged kidneys due to infiltrative diseases
- Renal artery stenosis
- Small and echogenic kidneys (common in late stages of CKD because of many parenchymal diseases)

Renal tubular syndromes as markers of kidney damage

- Renal tubular acidosis
- Nephrogenic diabetes insipidus
- Barrter and Gittelman syndromes
- Fanconi's syndrome
- Cystinuria
- Familial hypomagnesaemia with hypercalciuria and nephrocalcinosis

Excretion of urinary creatinine indicates muscle mass and varies with age, sex, race, diet, and nutritional status, and generally exceeds 1·0 g per day in healthy adults; therefore, the numeric value for urinary ACR (mg/g) is usually less than the rate of urinary albumin excretion (mg/day). Rates of 30–300 mg per day and >300 mg per day correspond to microalbuminuria and macroalbuminuria, respectively. Normal urine contains small amounts of albumin, low-molecular-weight serum proteins, and proteins that are from renal tubules and the lower urinary tract. In most kidney diseases, albumin is the main urine protein, comprising about 60–90% of total urinary protein when total protein is very high. Values corresponding to normal, high-normal, high, very high, and nephrotic-range total protein are about <50, 50–150, 150–500, >500, and >3500 mg/g, respectively. GFR=glomerular filtration rate. CKD=chronic kidney disease. *Conversion factor for albumin to creatinine (ACR) ratio: 1·0 mg/g=0·113 mg/mmol.

Adapted from Chronic Kidney Disease, Levey 2012

Chronic kidney disease is classified into five stages. This classification is divided according to the glomerular filtration rate and albuminuria. GFR is the best measurement of overall kidney function. It is estimated from serum creatinine measurements by using the Modification of Diet in Renal Disease study equation based on age, sex, race and calibration for serum creatinine. Normal range for young adult is approximately 120 to 130mL/min per 1.73m2. Level of less than 60 mL/min per 1.73m2 will represent loss of half of the normal renal function. GFR level more than 90 mL/min per 1·73 m^2 (stage 1), 60–89 mL/min per 1·73 m^2 (stage 2), 30–59 mL/min per 1·73 m^2 (stage 3), 15–29 mL/min per 1·73 m^2 (stage 4), and less than 15 mL/min per 1·73 m^2 (stage 5) (Levey and Coresh, 2012). Table 2.2 showed stages of CKD patients (stage 1 to 5) based on GFR level.

The worldwide prevalence of CKD and end stage renal failure are increasing in trend. The prevalence are somehow varies in each countries such as in U.S (13.1%), Taiwan (9.8-11.9%), Norway (10.2%), Japan (12.9-15.1%), China (3.2-11.3%), Korea (7.2-13.7%), Thailand (8.45-16.3%), Singapore (3.2-18.6%), and Australia (11.2%) (HWANG *et al.*, 2010). The discrepancies of the prevalence were attributed by multiple factors such as age, ethnic groups, survey policies and equations of estimated glomerular filtration rate calculation. For instance, National Kidney Foundation, US has standardized the diagnosis and staging of CKD based on eGFR.

Table 2.2: Classification of CKD based on GFR level, Prevalence and Action Plan for each Stages of Chronic Kidney Disease.

Stage	Description	GFR, mL/min per 1.73m^2	Prevalence,n (%)‡	Action§
-	At increased risk	≥60 (with chronic kidney disease risk factor	-	Screening chronic kidney disease risk reduction
1	Kidney damage with normal or increased GFR	≥90	5900 000 (3.3)	Diagnosis and treatment
2	Kidney damage with mild decreased GFR	60-89	5300 000 (3.0)	Estimating progression
3	Moderate decreased GFR	30-59	7 600 000 (4.3)	Evaluating and treating complication
4	Severely decreased GFR	15-29	400 000 (0.2)	Preparation for kidney replacement therapy
5	Kidney failure	≤15 (or dialysis)	300 000(0.1)	Kidney replacement (if uraemia present)

* GFR glomerular filtration rate.
‡ Prevalence for stage 5 is from the U.S. Renal Data System (1998); it includes approximately 230 000 patients treated with dialysis and assumes 70 000 additional patients not receiving dialysis. Prevalence for stages 1 to 4 is from the Third National Health and Nutrition Examination Survey (1988 to 1994). Population of 177 million adults age 20 or more years.
§ Includes actions from preceding stages
Table adapted from National Kidney Foundation Kidney Disease Outcome Quality Initiative Classification, Prevalence, and Action Plan for Stages of Chronic Kidney Disease, 2003

In Malaysia, the incidence and prevalence of end stage renal disease patients on dialysis have dramatically increased with estimated prevalence around 9%. The prevalence of ESRD on dialysis has risen markedly from 352 pmp in 2001 to 975 pmp in 2012 (Salman

et al., 2015). The main contributing factors to the disease are hypertension, primary kidney disease, diabetes and ischaemic heart disease. Other risk factors that also involve such as ethnic group, age, obesity, high cholesterol, family history and smoking.

2.1.2 Anaemia in Chronic Kidney Disease

a) Definition of anaemia and background

European Best Practice Guidelines (EBPG) has defined anaemia in CKD patients are based on their gender and age. The haemoglobin value of <11.5 g/dl in women and <13.5 g/dl in men (<12 g/dl in those aged >70 years) is considered below normal limit. Anaemia work-up should be commenced when haemoglobin levels fall below these limits (Collins *et al.*, 2003). In the 2013 update, Kidney Disease Outcome Quality Initiative (KDOQI) has modified this definition, and the new criteria for diagnosing of anaemia in adult males are haemoglobin <13.5 g/dl (Haemoglobin ≤ 13.2g/dl in men > 70 years of age) and <12 g/dl in adult females all ages (Locatelli *et al.*, 2013).

In chronic kidney disease, anaemia is the most important haematological abnormality and its management has been improvised by recombinant human erythropoietin (EPO). However, the used of EPO may lead to variable adverse clinical outcomes and also debilitating symptoms such as tiredness, lethargy, muscle fatigue, intolerance to cold, breathlessness on exertion and poor exercise activity. It also associated with high prevalence of cardiovascular disease and cause high morbidity. Mortality as a result of cardiovascular disease was reported approximately 50% in patients with chronic renal disease (London, 2003). Based on study done by McClellan *et al*, the prevalence of

anaemia is strongly associated with declining of GFR. Percentage of patients with haemoglobin ≤ 12 g/dL increased from 26.7% to 75.5% when GFR decreased from ≥ 60 mL/min/1.73 m2 to < 15 mL/min/1.73 m2 (McClellan *et al.*, 2004). In other report described the prevalence of anaemia was significantly increased as the creatinine clearance falls to < 70mls/min in males and < 50mls/min in females (Hsu *et al.*, 2002).

b) Pathophysiology of anaemia in chronic kidney disease

Chronic anaemia is almost invariably consequences of chronic renal disease. The causes for development of anaemia in renal disease are multifactorial. As renal function deteriorates, there is progressive reducing in haemoglobin level and the features will be more evidence once the GFR level reduce to less than 30 ml/min (Astor *et al.*, 2002). The type of anaemia is commonly normochromic normocytic anaemia which will associate with the presence of echinocytes (burr cells) in the blood film (Figure 2.1). The reticulocyte count is usually normal or slightly low, and the bone marrow will show normoblastic erythropoiesis without the erythroid hyperplasia which are expected at that level of anaemia. Patients who have undergone nephrectomy are prone to be more severely anaemic compared to patients with polycystic disease (Hoffbrand *et al.*, 2016). Blood volume studies will show a reduced RBC mass, but normal total blood volume.

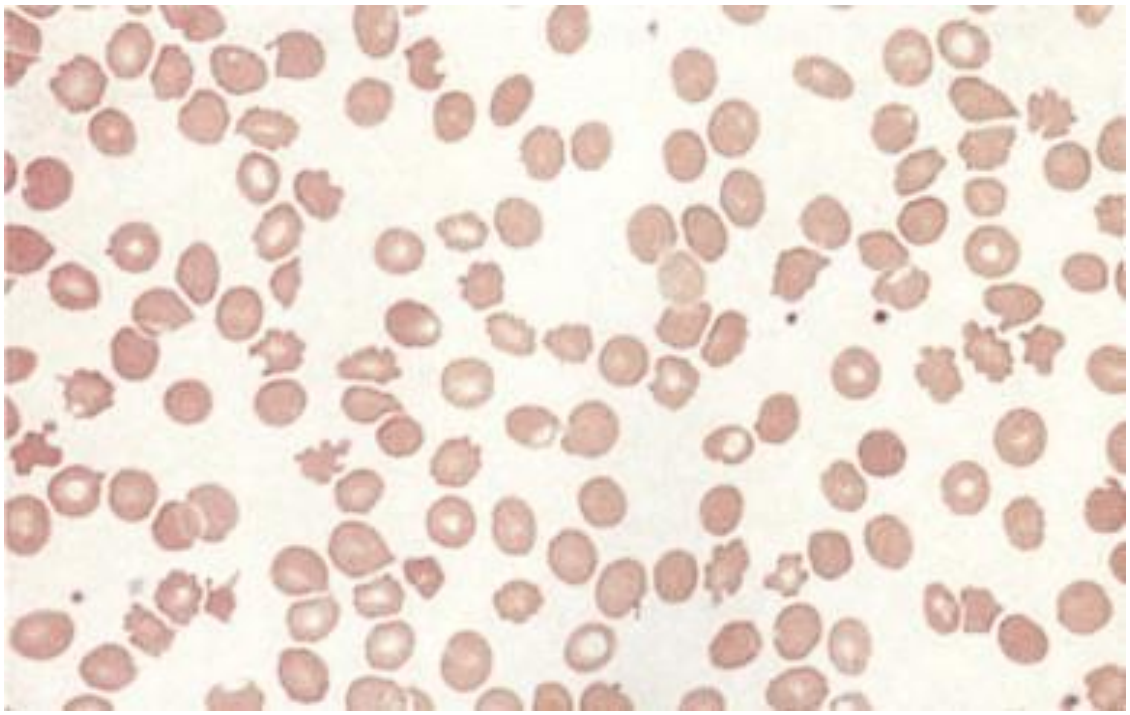

Figure 2.1: Peripheral blood film in chronic kidney disease, showing acanthocyte and 'burr' cells. Figure adapted from journal Anaemia of chronic kidney disease, Macdougall 2007.

The reduction of erythropoietin levels which occur in renal disease is the dominant cause of anaemia. This is result from loss of peritubular cells in the kidney which responsible for synthesis and secretion of EPO (Caro *et al.*, 1979). The EPO level will be inappropriately low for the degree of anaemia in renal anaemia (Figure 2.2). EPO is the hormone which responsible for maintaining the proliferation and differentiation of erythroid precursor cells in the bone marrow. An increase of serum creatinine above 133 μmol/L is significantly cause reduction of the plasma EPO and haemoglobin concentration, however there is no direct correlation between reduction of GFR and impairment of renal EPO production. Presence of circulating inhibitors of erythropoiesis also have been demonstrated, but they are not clinically significant. Red cell survival also shown to be diminished in renal disease patient, but this is also a minor factor (Hoffbrand *et al.*, 2016).

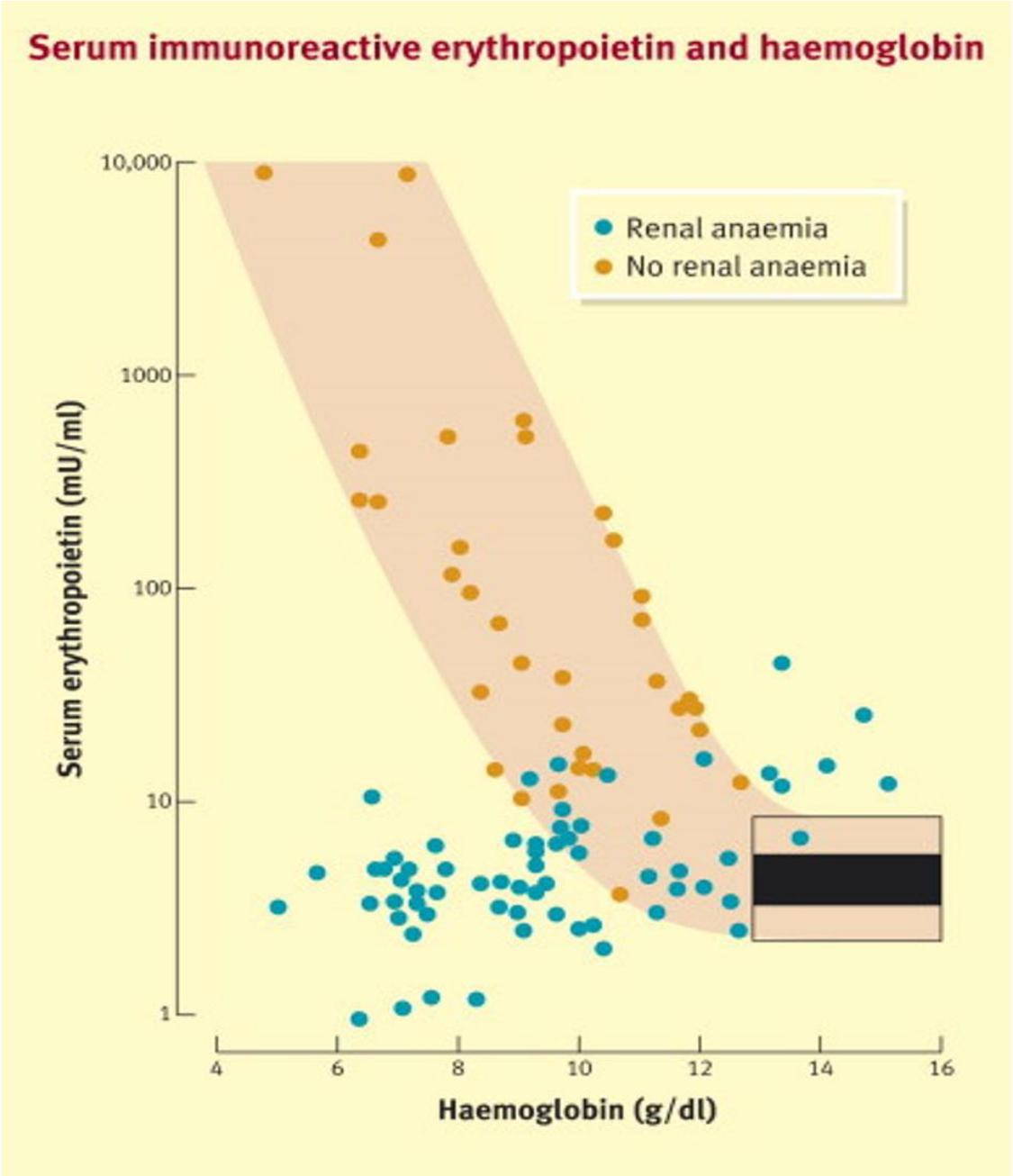

Figure 2.2: The graph shows the relationship between haemoglobin and serum erythropoietin levels in non-renal anaemia and in patients with chronic kidney disease. The rectangle indicates the interquartile range and 95% confidence range of erythropoietin levels in non-anaemic healthy adults.
Adapted from journal Anaemia of chronic kidney disease, Macdougall 2007.

Many other factors can also contribute to aggravation of anaemia in chronic kidney disease. Iron deficiency is one of the common factors due to poor oral intake and increased iron losses. The latter may be caused by increased in blood loss through

gastrointestinal tract and exacerbate by frequent haemodialysis procedures. In addition, chronic immune activation arise from contact activation of immune cells by dialysis membrane and also infection (Weiss and Goodnough, 2005). Hyperparathyroidism which is a complication of renal disease may worsen the anaemia by inhibiting the erythropoiesis and fibrosis of the bone marrow. Uraemia state will reduce the RBC life span by causing low grade haemolysis. Folate deficiency also occur which mainly due to poor intake or excessive lose during haemodialysis (Table 2.3) (Macdougall, 2007).

Table 2.3: Factors which contributing to renal anaemia.

Factors for anaemia in chronic kidney disease
Relative deficiency of erythropoietin
Reduced RBC survival as a result of haemolysis
'Uraemic inhibitors' of erythropoiesis
Hyperparathyroidism with marrow fibrosis
Aluminium toxicity
Iron/folate deficiency
Blood loss

Adapted from Anaemia of chronic disease, Macdougall 2007.

2.1.3 Management of anaemia and role of blood transfusion in CKD patients

Management of renal anaemia is very crucial as this problem is very common among CKD patients. Multiple clinical practice guideline for the management of anaemia in this patients have been established such as in UK, NICE Guideline (Conditions, 2006), European Best Practice Guidelines in the Europe (Vaage-Nilsen, 2005) and in the US,

K/DOQI Guidelines (National, 2006). All these guidelines have offer similar recommendation regarding EPO therapy, monitoring of iron status and iron supplement. The European Renal Best Practice has organized the Kidney Disease: Improving Global Outcome (KDIGO) group which has produced a comprehensive guideline for the management of anaemia in CKD patients. Based on this guideline, it addressed all the important points including therapy with erythropoiesis stimulating agent (ESA), iron therapy, ESA resistance and also blood transfusion. It has been proved that correction of anaemia in CKD patients will improve the cardiac function (Hayashi *et al.*, 2000), cognitive function (Pickett *et al.*, 1999), quality of life and physical activity (Lim *et al.*, 1989). Based on the EBPG, the suggested haemoglobin target in CKD patients are ≥ 11g/dl. The level of ≥14g/dl is undesirable in general, and the target for patients with concomitant cardiovascular disease is 12g/dl (Collins *et al.*, 2003). Other randomized clinical trials have suggested that haemoglobin level of > 13 g/dl may increase risk of cardiovascular events (Besarab *et al.*, 1998).

a) Non-transfusion therapy

Iron therapy is an important step in the treatment of anaemia in CKD. This is due to absolute or functional iron deficiency are common. Based on TREAT study, CKD patients also have element of iron deficiency despite of chronic disease itself. This finding has been proved as all patients in the control group able to maintain a relatively high mean haemoglobin level during follow up when receiving minimal darbepoetin alfa and iron therapy. This is concluded as many of these patients are not fully iron repletion (median transferrin saturation (TSAT) 23%, IQR 18-29%) (Pfeffer *et al.*, 2009). Based on this

result, role of iron therapy is re-evaluated and not only be used for patients who are iron deficient but also in those who are apparently adequate iron stores.

Guideline also recommend the possibility to perform a trial of iron therapy (intravenous or oral) when tolerated in anaemia CKD patients if an increase in haemoglobin level is desired. This treatment will be helpful in reducing the need for blood transfusion. Oral iron therapy should be used as first-line therapy for a minimum of 3 months in the absence of gastrointestinal intolerance for patients with mild to moderate anaemia. Intravenous iron is the first choice in severe anaemia or when oral iron is ineffective. This is especially in the condition with presence of absolute iron deficiency (TSAT <20% and serum ferritin <100ng/ml) or increase in haemoglobin level without starting erythropoietin is desired and TSAT is <25%, serum ferritin < 300ng/ml in dialysis patient (Locatelli *et al.*, 2013).

Erythropoietin therapy is effective in correcting the anaemia in patients with chronic kidney disease about 90-95%. Current recommendations suggesting erythropoietin therapy should be started if haemoglobin level falls below 10-11 g/dl (Macdougall, 2007). However, the initiation of ESA treatment needs to justify the potential benefit of reducing blood transfusion and anaemia related symptoms against the unfavourable risk in individual patient (such as stroke, vascular access loss and hypertension). A great caution must be done in case of CKD patients with active malignancy, had history of stroke or previous history of malignancy. Study has shown that targeting higher haemoglobin level in CKD population have association with higher risk for thrombosis, stroke and death (Palmer *et al.*, 2010). In case of non-dialysis CKD patients with haemoglobin level ≥ 10 g/dl, ESA initiation is not recommended (Pfeffer *et al.*, 2009).

b) Red cells transfusion therapy

Red cell transfusions are required to promote the oxygen-carrying capacity of the blood by increasing the haemoglobin level of patient with acute or chronic anaemia. However, blood transfusion for managing chronic anaemia is not recommended when possible, to minimize the related risk for their used (Murphy *et al.*, 2001). This is especially, in case of patients who are eligible for kidney transplantation. This is related to the many unwanted complications of red cell transfusion particularly alloimmunization. Based on the guideline, blood transfusion in CKD patients are indicated for management of chronic anaemia if the benefit of red cell transfusion may outweigh the risk, such as in the condition that ESA therapy is ineffective, or risk of ESA therapy outweigh it benefit (Streja *et al.*, 2008).

Decision for blood transfusion in CKD patients should not be based on the haemoglobin level alone but must be based on occurrence of symptoms which caused by the anaemia (Locatelli *et al.*, 2013). AABB guideline is also agreeable with this concept (Carson *et al.*, 2011). Study done in FOCUS trial which has include the symptoms into the decision for blood transfusion. This trial has comparing patients in the restrictive group received transfusion if haemoglobin level was less than 8 g/dl or if patient were symptomatic. The result shows, overall outcome examined were not statistically significant (Carson *et al.*, 2012).

. 2.2 COMPLICATIONS OF BLOOD TRANSFUSION

2.2.1 Background

Blood transfusion which originate from an unrelated donor are known as allogenic red blood cell transfusions. It is certainly beneficial in specific situation but the used are accompanied by many risk and side effects. The risk of death from transfusion are very low and as estimated from Serious Hazard of Transfusion (SHOT) data in 2012 is 1 in 322 580 components issued while major morbidity occur 1 in 21 413 blood components issued (Bolton-Maggs *et al.*, 2013). The risk of transfusion-transmitted infection (TTI) by viruses like human immunodeficiency virus (HIV), hepatitis B (HBV) and hepatitis C (HCV) is much lower since modern blood banking has used highly sensitive assay for screening of blood donors. Nowadays, implementation of nucleotide testing for viral screening has further reduced the residual risk of transfusion acquired HIV, HBV and HCV to approximately 1 in 500,000 to 750, 000 blood exposures (Perrotta and Snyder, 2001).

In view of the incidence for TTI is low, non-infectious complication of transfusion therapy become relatively more important (Figure 2.3). Acute transfusion reactions and transfusion-associated circulatory overload carry the highest risk for morbidity and death(Bolton-Maggs and Cohen, 2013). More common complication is immune-mediated such as alloimmunization to red cells, platelet and plasma protein which can lead to red cells destruction, febrile non-haemolytic and allergic reaction (Taylor *et al.*, 2008). Based on the cumulative result from all reports in SHOT shows that, the commonest unpredictable transfusion reaction is an acute transfusion reaction such as

allergic, severe febrile or anaphylactic reaction. Ha(Bolton-Maggs *et al.*, 2013)emolytic transfusion reaction (HTR) are the commonest among the potentially preventable complication. This type of hazard can be prevented by better pretransfusion assessment and monitoring. However, overall most common adverse events are caused by errors, resulting from the transfusion an incorrect component (Bolton-Maggs *et al.*, 2013). Complications of blood transfusion were summarized in Table 2.4.

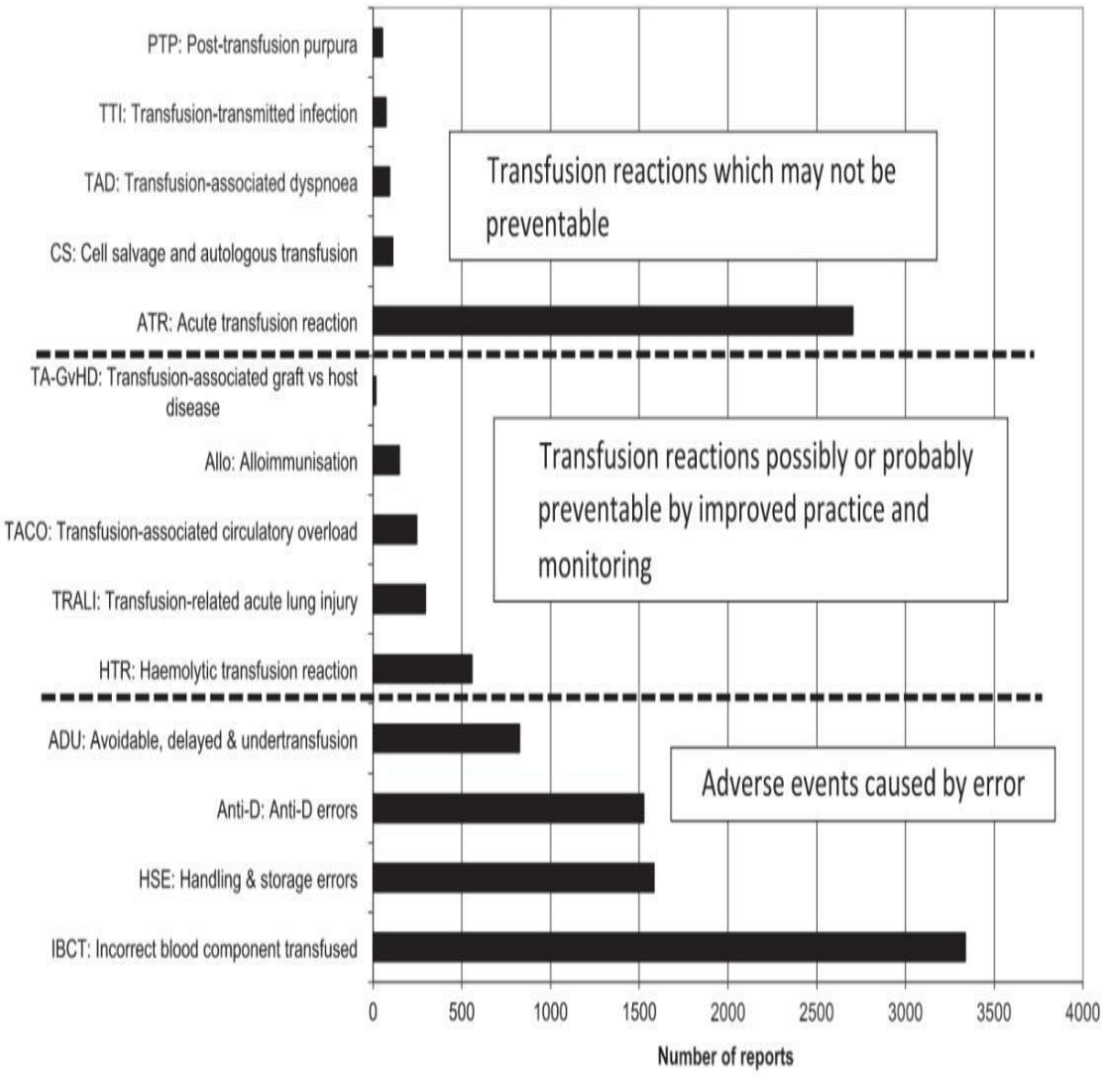

Figure 2.3: Cumulative data for SHOT categories from 1996/7 to 2012 (n= 11570). Figure adapted from Bolton-Maggs and Cohen 2013.

Table 2.4: Non-infectious complication of blood transfusion

Immune mediated	Non-immune mediated
Haemolytic transfusion reactions	Septic transfusion reaction
RBC Alloimmunization	Nonimmune haemolysis
Febrile nonhemolytic transfusion reaction	Miss transfusion
Allergic/Urticarial/ Anaphylactic reaction	Transfusion-associated circulatory overload
Transfusion-related acute lung injury (TRALI)	Metabolic derangement
Post-transfusion purpura (PTP)	Coagulation complication from massive transfusion
Transfusion-associated graft versus host disease	Iron overload.
Microchimerism	

Table adapted and modified from Hendrickson and Hillyer, 2009

2.2.2 Red Blood Cell Alloimmunization

Individuals who had exposed to RBC alloantigen through transfusion, pregnancy or transplantation will eventually produce antibodies against the alloantigen that expressed by the RBCs. The incidence of development of antibodies is still debated and ranges from 1 to 6% in single transfusion and in certain condition can be up to 30% in multi-transfused patient such as in sickle cell disease, thalassemia and myelodysplastic syndrome

(Schonewille *et al.*, 2006). Furthermore, about 30 to 80% of rhesus (D) negative individual will eventually develop anti-D upon exposure towards Rh (D) positive antigen (Frohn *et al.*, 2003). The presence of RBC alloantibodies can pose serious clinical problems such as HTRs as well as logistic problems. It is difficult and laborious to prepare the compatible blood with antigen negative to that particular antibody in timely and properly matched for transfusion in the presence of significant antibodies. This may result in delay blood transfusion in emergency condition and have high risk of transfusion reaction. Antigenic differences, immunogenicity, dose and frequency of transfusion and also recipient immune status are the factors that have been suggested to influence the rates of alloimmunization (Zalpuri *et al.*, 2012a).

Red cell immunogenicity refers to the ability of an antigen which present on the surface of red blood cell to stimulate an immune response. This immune response is characterized by development of antibody. These RBCs antigens will elicit immune response when they are processed in conjunction with human leucocytes antigen (HLA) molecules on antigen presenting cells and are presented to the T-cell receptor on T-lymphocytes (Banchereau and Steinman, 1998). The derived peptide is bound to the HLA groove by a series of hydrogen bonds and also by protrusion of the side chains into a small cavities along the peptide-binding site (Chelvanayagam and Easteal, 1997). These cavities are essential for HLA class II binding. The recognition of RBC-derived peptide which displayed by the HLA class II molecule will eventually activates a specific CD4+ T cell and in turn will activates a specific B cells to produces a clone of antibody-producing cells (Texier *et al.*, 1999). This activated B cells will produce antibody towards specific antigen.

The immunogenicity of the antigen will depend on the polymorphism of the HLA proteins. The rhesus (Rh) blood group system was reported to be the most polymorphic of human blood groups. It consisting of at least 45 independent antigens and antibody towards these antigens are the most clinically significant in transfusion medicine (Avent and Reid, 2000). Among the rhesus group antigens, the D is known to be the most immunogenic whereby Kell, Kidd and Duffy antigen also implicated in alloimmunization with lower immunogenicity (Giblett, 1961). More than 80% of immunocompetent rhesus negative patients will develop anti-D once exposed to rhesus positive blood (Schonewille *et al.*, 2000). The exposure can occur during pregnancy or blood transfusion. Because of this reason, Rh D system are included in the blood group screening and prophylactic Rh D negative is given to rhesus negative patients. However, this practice will not prevent formation of alloantibody towards other types of RBC antigen. RBC alloimmunization towards other group of RBC antigen can occur in up to 40% depending on the patient-related factor and number of transfusions (Schonewille and Brand, 2005).

Besides Rhesus group, the International Society of Blood Transfusion has recognized 302 blood group antigens which belong to 1 of 29 genetically discrete blood group system. Antibodies to many of these antigen were potentially have clinical significant by causing destruction of RBC (Poole *et al.*, 1991). Table 2.5 shows the blood group systems with the immunogenicity and their clinical significant.