THE CLINICAL AND HAEMATOLOGICAL EFFECTS OF HORMONAL CONTRACEPTION ON WOMEN WITH SICKLE CELL DISEASE

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I, Asma Adam Eissa, confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis.

ABSTRACT

Sickle cell disease (SCD) is known to be a prothrombotic condition; this is also true for combined hormonal contraceptives (HC), which increases the thrombotic risks in their users. Recently, Sickle Cell Trait (SCT) has been reported to carry increased risks of thrombosis Nonetheless, HC methods are efficacious and widely used while, pregnancy carries major risks for SCD women. Hence, there is a need for robust evidence about the safety or risks of HC in SCD and SCT to aid in the choice of contraceptive methods for these women.

This study aimed to test the hypothesis that there are no additional clinical or haematological risks to SCD patients and women with SCT using hormonal contraceptive methods that is over and above those inherent in their SCD and SCT status.

This is a multi-centre, prospective cohort study, which looked at and compared clinical complications, haemostatic and haematological markers in 68 women with SCD, 22 women with SCT and 27 similar women with normal haemoglobin.

In conclusion a two year follow-up of women with SCD using Combined Oral Contraception (COC) found no incidence of Venous Thrombo Embolism (VTE) in these women and the occurrence of other clinical complications, such as sickle-cell crises, the need for blood transfusion and hospital admissions were minimal. It also demonstrated that these complications are comparable to women with normal haemoglobin using COC. Also the use of COC in women with SCD did not significantly alter the haemostatic markers studied, nor did it adversely affect their liver function or exacerbate any inflammatory changes. Progestogen only contraception (POC) use is associated with an increased incidence of menstrual irregularities which are not significantly different from those noted in women with normal haemoglobin taking POC. SCT women manifested increased prothrombotic tendencies, which are more marked in women using COC, while women with

normal haemoglobin showed increased inflammatory and endothelial activation markers regardless of the type of contraception used.

AbBREVIATIONS

ADAMTS13	A Disintegrin And Metalloproteinase with Thrombospondin Motifs Number 13
ANOVA	Analysis of Variance
ATP	Adenosine Triphosphate
cGMP	cyclic guanosine monophosphate
COC	Combined (i.e. oestrogen and progestogen) oral contraceptives
CRP	C-reactive protein
DMPA	Depomedroxy progesterone acetate
DNA	Deoxyribonucleic Acid
DVT	Deep vein thrombosis
DVVT	Dilute Viper Venom time
EDTA	Ethylenediaminetetraacetic acid
EE	Ethinyl oestradiol
FSH	Follicular stimulating hormone
GnRH	Gonadotrophin releasing hormone
Hb	Haemoglobin
HbS	sickle Hb
HC	Hormonal contraceptive
HRT	Hormone replacement therapy (i.e. oestrogen and progestogen for postmenopausal women)
ICAM-1	intercellular adhesion molecule

IS	Ischaemic stroke
ISC	Irreversibly sickled cells
IUDs	Intra uterine contraceptive devices
IUS	Mirena® Intra Uterine System (i.e. slow release levonorgestrel)
LH	Luteinising hormone
LNG	Levonorgestrel
MEC	Medical Eligibility Criteria
MI	Myocardial Infarction
MP	Microparticles
NETA	Norethindrone acetate
NO	Nitric Oxide
OR	Odds Ratio
PE	Pulmonary embolism
POC	Progestogen-only contraceptives
POP	Progestogen-only pills
PPP	Platelet poor plasma
PS	Phosphatidylserine
RBC	Red blood cell
RR	Relative risk
SCD	Sickle cell disease (i.e. HbSS, HbSC, HbSβ° thalassaemia)
SCD40-L	Soluble cell differentiation-40 ligand
SCS	Sickle Cell Society

SCT	Sickle cell trait
SD	Standard deviation
sVCAM	Soluble vascular cell adhesion molecules
TF	Tissue factor
TG	Thrombin generation
VCAM	Vascular cell adhesion molecules
VTE	Venous thromboembolism
vWF	von Willebrand factor
WBC	White blood cell

CONTENTS

TABLE OF FIGURES

LIST OF TABLES

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CHAPTER 1

INTRODUCTION

1.1 THE EPIDEMIOLOGY OF SICKLE CELL DISEASE

Sickle cell disease (SCD) is a genetic disease affecting the haemoglobin (Hb) molecule leading to different changes in its characteristics and functions. It is the most common genetically inherited disorder worldwide. Its gene carriers were estimated in 2001 to be approximately 7% of the world population (Weatherall and Clegg, 2001). Approximately 300,000- 400,000 babies are born each year with SCD worldwide (Weatherall and Clegg, 2001). SCD has its origins in sub-Saharan Africa and the Middle East. However, due to population migration, it is now of increasing importance worldwide (Petrou and Modell, 1995; Stuart and Nagel, 2004a; Weatherall and Clegg, 2001). In the United States, sickle cell disease affects an estimated 70,000 to 100,000 people, and two million people are carriers of the disease (Hassell, 2010). In the UK SCD is now the most common serious inherited genetic disorder, affecting one in every 2,000 births (Streetly et al., 2009). The NHS Sickle Cell and Thalassaemia Screening Programme identifes about 350 newborn babies with SCD annually in the UK. The most recent estimate indicates 12 000–15 000 SCD sufferers in the UK (Streetly et al., 2009). Nevertheless, this number is small, and individual clinicians may not encounter many SCD patients in their career, and particularly not in the context of pregnancy and family planning.

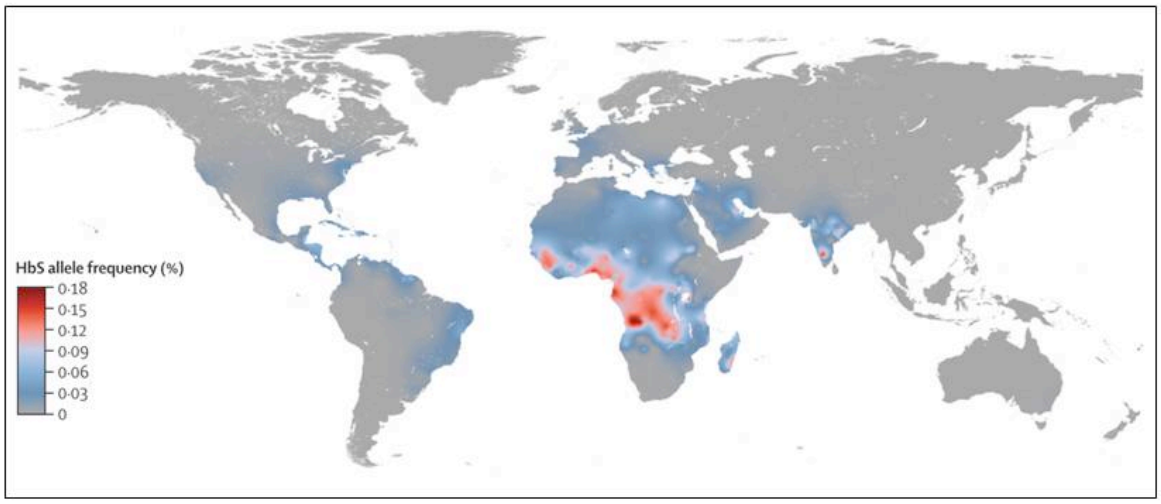

Figure 1-1 Global distribution of the HbS allele, 2012

The map shows high allele frequencies across most of sub-Saharan Africa, the Middle East, and India, as well as gene flow following migrations to Western Europe and the eastern coast of the Americas. Reproduced from (Piel et al., 2013)

1.2 THE PATHOPHYSIOLOGY OF SCD

Current understanding of the pathophysiology of sickle cell disease has evolved over many years, and many scientists have contributed to this (Frenette and Atweh, 2007) The first scientifically published description of sickle cell disease was made in 1910, by James B Herrick, who described the findings of haemolytic anaemia and elongated sickle (or crescent)- shaped red blood cells in a dental student from Grenada (Herrick, 2001). However, it was not until 1949, that Itano and Linus Pauling described for the first time, the molecular abnormality of Haemoglobin S, characterised by the use of electrophoresis (Itano and Neel, 1950; Itano, 1951; Pauling, 1964) After a further ten years, Ingram found, more

specifically, that the abnormal haemoglobin results from the substitution of a single glutamic acid residue with valine in the β globin polypeptide chains of the molecule (Ingram et al., 1962a). It took the scientific world another decade to discover that haemoglobin S polymerises, in conditions of low oxygen tension, to form pseudo-crystals, which cause the red blood cells to have the rigid "sickle" shape (Murayama, 1966). New understanding of the pathophysiology of SCD continues to emerge in the 21st century.

The concept that the phospholipid membrane of the sickle red blood cell (RBC) also plays an important role in the clinical manifestations of the disease and its complications is relatively new, and has mainly unfolded in the last two decades (Frenette and Atweh, 2007). With this has come an appreciation of the interaction of the sickle RBC membrane with other cellular and non-cellular components of the blood, and with the endothelium of blood vessels (Embury *et al*., 2004; Frenette and Atweh, 2007; Kaul *et al.*, 2000; Spring *et al*., 2001; Stuart and Nagel, 2004a). This feature is responsible for the increased coagulability of blood in SCD sufferers, and the related clinical complications of this, which are in addition to those directly caused by the sickled RBC's themselves (Ataga and Key, 2007; Porter *et al*., 1993; Singer and Ataga, 2008).

1.3 THE DIFFERENCE BETWEEN NORMAL HAEMOGLOBIN AND SICKLE HAEMOGLOBIN

Human haemoglobin (Hb) synthesis is a multi step process. In the first step, the haem ring, the protein that binds the iron ions, which transport oxygen, is synthesised by the RBC mitochondria and cytosol. Then, the haem binds to the iron molecule, and finally, it is surrounded by two pairs of globin (polypeptide) chains, which are synthesised by the ribosome, to "protect" the haem ring and give a stable structure to the molecule (Lal and Vichinsky, 2010). One of the two globin pairs is always of the same amino acid sequence and structure, and is known as alpha globin. The second pair of globin chains is formed from one of three other polypeptide sequences, designated as beta, gamma or delta chains (Lal and Vichinsky, 2010). The difference between these globin chains lies in the sequence of their amino acids. The molecule which forms from the joining of the haem with the globin is called haemoglobin, which is the functional tetramer unit which carries oxygen around the body. Normal adult Hb, known as HbA, is formed from two alpha chains and two beta chains (Lal and Vichinsky, 2010). Fetal haemoglobin (HbF) is formed from two alpha chains and two gamma chains, whilst Hb made from two alpha and two delta chains is known as HbA2, which is functionally essentially the same as HbA.

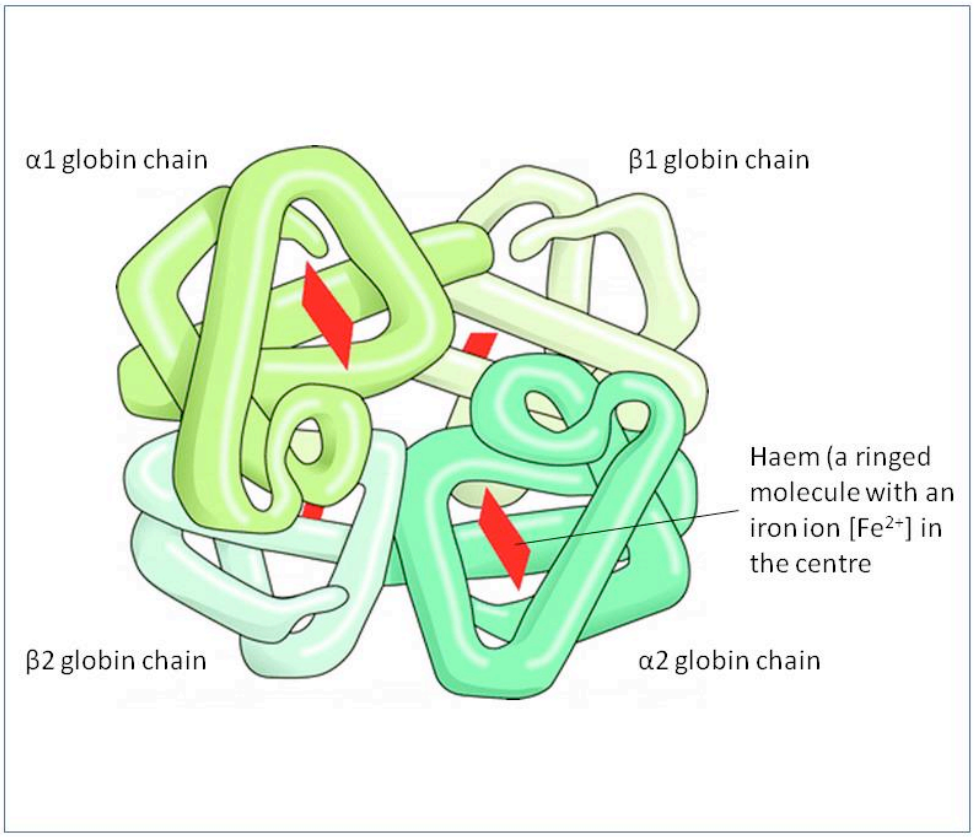

Figure 1-2 Molecular Structure of adult haemoglobin molecule

The diagram shows the quaternary structure of a human adult haemoglobin molecule with 4 haem rings bound to iron. Two haem rings are surrounded by 2 α chains and the other two are surrounded by β globin chains. Adapted from(Loukopoulos, 2001).

In SCD there is a faulty beta globin chain. The fault arises from the substitution of the amino acid glutamate by a valine residue (Ingram *et al.*, 1962a). Physiologically, glutamic acid is a polar residue, and hence normal Hb circulates without sticking to the endothelium, or to other blood components. Valine is hydrophobic and tends to stick to the adjacent amino acids in the globin chain,

making HbS stickier, and leading to various clinical and pathological complications (Ataga and Key, 2007). Other single amino acid substitutions result in a range of haemoglobin variants, most of which are of no clinical significance. One which does have clinical manifestations is known as HbC, in which lysine is substituted for glutamic acid at the same position in the beta chain as that affected in HbS (Allison, 2009; Allison AC, 1957; Takahashi *et al*., 1987).

There is another category of haemoglobinopathies, the thalassaemias, in which the globin chains are structurally normal, but their production is not achieved in balanced quantities, and different tetramers are therefore formed. If alpha globin chain production is affected, these are known as alpha thalassaemias, and when the production of beta globins is affected, these are known as beta thalassaemias (Flint *et al*., 1993; Orkin *et al*., 1982).

1.4 MODE OF INHERITANCE OF SCD

The production of HbS results from a single gene mutation affecting position six of chromosome 11. It follows a simple Mendelian autosomal recessive mode of inheritance (Ingram *et al*., 1962b). Although the causes of these mutations are broadly unknown, the mutation producing HbS is known to confer some beneficial effects, since the heterozygous state (known as sickle cell trait) improves survival

in areas where Falciparum malaria is endemic and thus has a major impact on population survival (Allison, 1957a, 1957b).

The specific mechanism whereby the heterozygous state confers relative protection is unclear, and, in contrast, the mortality rate from malaria in patients with SCD is particularly high (Tan *et al*., 2011; Timmann *et al*., 2012). A recent study suggested that the malaria parasite uses the actin mono-filaments beneath the RBC membrane to traverse and live inside the cell. In SCD these filaments are broken, as a consequence of RBC sickling, which may explain the reduced malaria parasite transmission in carrier status (Cyrklaff *et al*., 2011) However, it does not explain why the disease is severe in SCD. Whatever the mechanism, the consequence has been that the distribution of the SCD genes have paralleled malaria distribution for many years, until it became modified by population migration (Flint *et al*., 1998, 1993; Lal and Vichinsky, 2010; Stuart and Nagel, 2004a).

1.5 TYPES OF SICKLE CELL DISEASE

1.5.1 Genotypes in SCD

SCD comprises many genetic entities (Lal and Vichinsky, 2010). All of them contain HbS. SCD occurs either with the homozygous inheritance of the HbS mutation (when it is designated as HbSS), or when the HbS mutation is inherited in

combination with another haemoglobin variant (Lal and Vichinsky, 2010). The most important combinations are with HbC, which is designated as HbSC disease, and with another variant known as HbO Arab, designated HbSO. Another clinically significant combination is that of HbS with β thalassaemia. The most severe β thalassaemia mutations result in the complete failure of beta chain production, and the combination is known as HbS β° thalassaemia (Lal and Vichinsky, 2010). Other thalassaemia mutations result in reduction rather than absence of beta chain production, and combinations are designated, for example, as HbSβ+ thalassaemia (Orkin *et al*., 1982; Stuart and Nagel, 2004b). In this thesis the term SCD is used to encompass HbSS, HbSC and HbS β° thalassaemia.

1.5.2 Phenotypes in SCD

SCD phenotypes and disease severity vary considerably. Published literature suggests that there are secondary effecter genes in SCD (epistatic genetic factors) which produce confounding features, such as the increase or decrease in the severity of RBC destruction, the continued production into adult life of HbF, and increased endothelial adhesion (Flanagan *et al*., 2011; Mendonça *et al*., 2010; Nagel and Steinberg, 2001; Nagel, 2005; Sankaran *et al*., 2010).

1.6 PATHOPHYSIOLOGY OF SCD AND ITS CLINICAL CORRELATIONS

In conditions of low oxygen tension valine sticks to the adjacent amino acids and leads to haemoglobin S polymerisation, which is the first step in a cascade of abnormal events (Bookchin *et al*., 1977; Noguchi and Schechter, 1985; Stuart and Nagel, 2004a). The crystals formed from this process both damage the RBC membrane and give rise to the characteristic sickle shape of the RBC (Eaton and Hofrichter, 1990, 1987; Roth *et al*., 1979). Factors which increase the likelihood of HbS polymerisation are increased intracellular haemoglobin S concentration, low blood pH, and intracellular deoxygenation, whilst it is lessened by the presence of high HbF concentrations (Nagel *et al*., 1979; Noguchi *et al*., 1993; Steinberg and Brugnara, 2003; Stuart and Nagel, 2004a). The resultant HbS crystals impede normal RBC deformability, which is essential for the passage of RBC's through capillaries. This gives rise to many adverse effects (Adachi and Asakura, 1978; Adachi et al., 1980a, 1980b; Eaton and Hofrichter, 1990; Harrington et al., 1997).

The natural history of SCD includes a chronic state of anaemia and haemolysis, punctuated by acute episodes of painful crises, and an accumulated legacy of organ damage (Stuart and Nagel, 2004a). By convention, the condition of a person with SCD when they are not suffering an acute sickling crisis is designated as their "steady state".

1.6.1 Role of RBC membrane phospholipid disruption

Initially, it was thought that the main problem in SCD was rheological in nature, as sickled RBCs tend to move slowly, causing stasis and vaso-occlusion, because of their impaired deformability (Chien *et al*., 1970, 1970). However growing evidence suggests that there is a more complex set of processes, which starts when repeated polymerisation of HbS, and its reversal, injure the RBC membrane and alter its normal phospholipid orientation (Apovo *et al*., 1989; Dzandu and Johnson, 1980; Fathallah *et al*., 1997; Nagel *et al*., 1979; Ramachandran *et al*., 1987; Siciliano *et al*., 2011, 2010; Stuart and Nagel, 2004a).

Normally, the phospholipid distribution in the RBC membrane is asymmetrical, with sphingomyelin and phosphatidylcholine being mainly on the outer side of the cell membrane, while phosphatidylserine (PS) is exclusively situated on the inner surface (An and Mohandas, 2008; Boon and Smith, 2002; Daleke, 2008; Devaux, 2000, 1991; Traïkia *et al*., 2002). This asymmetry is usually maintained by an active pump system, the aminophospholipid translocase pump, which transfers PS consistently to the inner aspect of the membrane, thus keeping the phospholipids in an asymmetrical arrangement (Sulpice *et al*., 1994). The exposure of negatively charged phospholipids contributes to the pro-inflammatory and pro-thrombotic state of sickle cell blood (Frenette and Atweh, 2007; Manodori *et al*., 2000). Manodori *et al* (2000) suggest that PS adherence to the endothelium of micro-vessels is mediated through a matrix protein, thrombospondin. However, more

recent evidence indicates that PS adhesion involves a specific receptor, which is present in the microvascular endothelial cells, identified as PSR. These receptors mediate a direct interaction between PS-positive erythrocytes and the micro-endothelium (Setty and Betal, 2008). The same authors also found that these receptors can be induced and up-regulated by hypoxia and inflammatory mediators.

The time taken for polymerisation of HbS is usually longer than the transit time taken by RBC's to traverse the microcirculation. Thus, in normal circumstances, many HbS RBCs escape without undergoing polymerisation (Eaton and Hofrichter, 1990; Mozzarelli *et al*., 1987; Steinberg and Brugnara, 2003); Steinberg *et al*, 2001). However, under circumstances of slow microcirculation e.g. immobility, dehydration or acidosis, polymerisation will occur readily. The crystals formed from the polymerisation of HbS disrupt the ATP-dependent aminophospholipid translocase pump and this results in PS exposure on the outer surface of the RBC membrane (De Jong *et al*., 2001; Stuart and Nagel, 2004a). PS exposure may also result from oxidative stresses in the sickle RBC and from the rapid influx of extra cellular calcium into the cell (Devaux, 2000, 1991; Kuypers et al., 1998; Sulpice et al., 1994; Traïkia *et al*., 2002). PS exposure contributes to all the pathological and clinical manifestations of SCD, by making the sickle RBC adhere to the vascular endothelium (Hebbel *et al*., 1980; Wood *et al*., 1996).

1.6.1.1 PS exposure and vaso-occlusive complications

Hebbel and Joneckis (Hebbel *et al*., 1980; Joneckis *et al*., 1993) reported that sickle RBCs adhere to the endothelium of blood vessels in vitro. As previously discussed, the mechanism behind this adhesiveness is the presence of PS on the outer surface of the sickle RBC, which docks with the vessel endothelium (Setty *et al*., 2001, 2002). This in turn, results in endothelial injury and RBC haemolysis, which results in the release of free haemoglobin in the plasma compartment. The injured endothelium suffers an oxidative stress, with resultant decreased levels of vaso-dilators such as Nitric Oxide (NO), leading to vasoconstriction (Akinsheye and Klings, 2010; Allen and Piantadosi, 2006; Gladwin *et al*., 2001; Reiter *et al*., 2002). Normally, NO is produced in the endothelium from its precursor L-arginine. Its main role is to cause vascular smooth muscle relaxation, by increasing cyclic guanosine monophosphate (cGMP) levels. The cGMP dephosphorylates myosin light chains, and hence relaxes the vascular smooth muscle, causing vaso-dilatation (Murad, 1986). The decrease in NO levels found in SCD is thought to be a consequence of the combination of decreased L-arginine levels (Gladwin *et al*., 2001; Morris, 2008; Morris *et al*., 2000a, 2000b), increased NO scavenging by free haemoglobin (Reiter *et al.*, 2002) and NO scavenging by oxygen free radicals (Aslan *et al*., 2001). The resultant vasoconstriction impedes the microcirculation further, and leads to more hypoxia, giving rise to further RBC sickling and reduced end organ perfusion (Reiter and Gladwin, 2003). This is recognised clinically as an acute vaso-occlusive "sickling" crisis (Mendonça *et al*., 2010; Stuart and Nagel, 2004a).

1.6.1.2 PS exposure and RBC haemolysis

PS exposure on the outer surface of the RBC membrane predisposes the cell to apoptosis and phagocytosis by the spleen, leading to splenomegaly, hypersplenism, haemolytic anaemia and jaundice (Van Engeland *et al.*, 1998). It also releases cell - free haemoglobin, which plays a part in thrombosis (Porter et al., 1993). With RBC destruction and haemolysis, the released Hb forms complexes with its scavenger, haptoglobin. It is then mainly cleared by macrophages (Philippidis *et al*., 2004; Schaer *et al*., 2008, 2007)

Haptoglobin has the capacity to bind up to 150 micrograms of free Hb (Langlois and Delanghe, 1996);Langlois *et al*,1996). The amount of free Hb released during the steady state in patients with Hb SS ranges from 20 to 330 µg/ml but can exceed 410 µg/ml during sickling crises (Naumann et al., 1971; Zhou *et al*., 2011, 2009a). When the carrier capacity of haptoglobin is exceeded free Hb accumulates in the plasma (Buehler and D'Agnillo, 2010; Langlois and Delanghe, 1996). This free Hb scavenges NO, leading to vasoconstriction, pulmonary hypertension, cellular damage (Minneci *et al*., 2005; Reiter and Gladwin, 2003; Reiter *et al*., 2002), causes platelet activation and initiates thrombosis (Brill et al., 2012; Buehler and D'Agnillo, 2010; Fuchs *et al*., 2010; Zhou *et al*., 2011). Free Hb has been demonstrated *in vitro* to inhibit the enzyme, A Disintegrin And Metalloproteinase with Thrombospondin Motifs (ADAMTS13), which limits platelet activation

(Sadler, 2002; Studt *et al*., 2005; Zhou *et al*., 2011). It is also been suggested that free Hb can inhibit the cleavage of von Willebrand Factor (Zhou *et al.*, 2009b). In addition, scavenging of NO by free Hb reduces cGMP, which inhibits platelet aggregation (Crawford *et al*., 2006; Kayanoki *et al*., 1999a; Radomski *et al*., 1987). NO also usually interferes with clotting factors such as Factor XIII thus preventing clot formation (Kayanoki *et al*., 1999b; Radomski *et al*., 1987; Shao *et al*., 2001; Zhou *et al*., 2011).

1.6.1.3 PS exposure, endothelial activation and the SCD chronic inflammatory state

The increased endothelial activation seen in SCD, was first noted by Solovey and colleagues in 1997, when they described the circulating endothelial cells in SCD as showing pro-inflammatory, pro-coagulant, and pro-adhesive properties (Solovey *et al*., 1997). It is largely understood now, that this endothelial activation is started by the sickle RBC, with exposed PS on its surface, adhering to the endothelium and injuring it (Kuypers *et al*., 1998; Setty and Betal, 2008; Sulpice *et al*., 1994; Zwaal and Schroit, 1997). This invites the adhesion of leucocytes to the endothelium (mainly neutrophils) and leads to the formation of multi-cellular aggregates, from leucocytes and irreversibly sickled cells (ISC) (Brill *et al*., 2012; Frenette, 2002; Fuchs *et al*., 2010; Turhan *et al*., 2002). These produce the chronic inflammatory state noted in SCD (Lard et al., 1999; Setty *et al*., 2008; Stuart and Setty, 2001; Turhan *et al*., 2002). Studies conducted in transgenic mouse models also suggest that vascular inflammation and heterocellular aggregates contribute to the cascade

of events causing both vaso-occlusive crises of SCD and thrombosis (Chiang and Frenette, 2005; Frenette and Atweh, 2007; Frenette, 2002; Turhan *et al*., 2002).

Decreased NO levels also contribute to the development of the chronic inflammatory process in SCD (Aslan *et al.*, 2001; Gladwin *et al*., 2001, 1999; Morris, 2008; Reiter and Gladwin, 2003). Naturally, NO inhibits the formation of pro-adhesive and pro-inflammatory molecules such as endothelial Vascular Cell Adhesion Molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), E-selectin, P-selectin, and also inhibits leucocyte migration and adhesion (Kim-Shapiro *et al*., 2006; Stuart and Nagel, 2004a). Hence, in situations of reduced NO production or abnormal consumption, there is a tendency for the circulation of higher levels of these molecules, and this plays a further role in the vascular inflammation characteristic of SCD (Bunn *et al*., 2010; Solovey *et a*l., 2004, 1998, 1997, 2001). Clinically, an abnormally increased white blood cell (WBC) level in SCD is an indicator of disease severity and correlates with mortality (Platt *et al*, 1994; Miller *et al*, 2000).

1.6.2 The mechanism of thrombosis

Thrombi are formed of fibrin and platelets (Mackman, 2008, 2007). The initiation of a thrombosis starts with what is traditionally known as the extrinsic pathway, while the propagation of this process follows the intrinsic pathway (Mackman,

2008, 2007). In the general population thrombo-embolic disease is multi-factorial, caused by a combination of genetic and acquired risk factors .

The main physiological activator of haemostasis is tissue factor (TF), a cell surface glycoprotein with a high affinity for coagulation Factor VII (Almus *et al*., 1990; Moosbauer et *al.*, 2007). Physiologically, TF is contained within the vascular adventitial cells (Bouchard *et al*., 1997; Drake *et al*., 1989a, 1989b; Fleck *et al*., 1990; Mackman *et al*., 2007; Morel *et al*., 2006; Müller *et al*., 2003). In pathological conditions, TF is displayed by endothelial cells, monocytes, neutrophils and platelets, leading to an increase in total amount of circulating microparticles (Gregory *et al*., 1989; Müller *et al*., 2003; Siddiqui *et al*., 2002; Solovey *et al*., 1998; Zillmann *et al*., 2001).

Following injury or damage to the vessel wall TF activates coagulation FVII to FVIIa forming [TF:FVIIa] complex, which then immediately activates FX to FXa and FIX to FIXa (comprises the initiation phase of coagulation). As soon as the [TF:FVIIa] complex is formed, it is immediately inhibited by tissue factor pathway inhibitor (TFPI). The small amount of FXa produced activates prothrombin (FII) to thrombin (IIa) which is enough to activate FXI to FXIa which then further activates FIX to FIXa, together with cofactors FVIII and FV who are activated to FVIIIa and FVa respectively (amplification phase of coagualtion) (figure 1.3). Thrombin is a protease enzyme which is formed from the cleavage of prothrombin by prothrombinase (Fenton, 1986; Fenton et al., 1993, 1991). Prothrombinase is a complex formed from the binding of activated Factor V (Va) with activated Factor X (Xa) (Dahlbäck, 1997; Kalafatis and Mann, 2001; Lawson et al., 1993; Shen and

Dahlbäck, 1994). Once thrombin is formed, it converts soluble fibrinogen into insoluble fibrin. The initial unstable fibrin mesh is converted to stable fibrin by Factor XIII (Coughlin *et al.*, 1992; Fenton, 1986; Vu *et al.*, 1991) and at the same time platelets form plugs at the site of injury , this is usually activated by collagen and thrombin.

Inorder for the platelets to function, von Willebrand Factor (VWF) is needed, a glycoprotein, which traps platelets and "glues" them together (Crawley *et al*., 2005). ADAMTS13 is the enzyme responsible for cleaving the macro-polymer of vWF, resulting in the disaggregation of platelets (Crawley et al., 2011). The completion of thrombus formation is achieved when fibrin is formed (Lämmle and Griffin, 1985).

Thrombin potentiates its own secretion through a positive feedback mechanism, by virtue of activating Factor V and Factor VIII (Van Veen *et al*., 2008). Conversely it exerts a negative feedback on the clotting system, by binding to thrombomodulin (an endothelial membrane protein) to form a compound which activates Protein C, giving rise to activated Protein C (APC) along with co-factor Protein S, which in turn inhibits activated Factor V and activated Factor VIII (Va and VIIIa) (Bajzar *et al*., 1996; Guillin, 1998; Jakubowski and Owen, 1989; van Veen *et al*., 2008). However, thrombin has some harmful effects, as it is a potent vaso-constrictor, and has pro-inflammatory characteristics. Furthermore it potentiates oxidative stress in endothelial cells, and promotes cell apoptosis (Borissoff *et al*., 2010; Fenton *et*

al., 1993; Ofosu, 2002). Thrombin is inactivated by another protease inhibitor designated as anti-thrombin (Hatton *et al*., 1978; Lawson *et al*., 1993).

Thrombin generation assays (TGAs) are thought to correlate better with hyper-coagulable states and are considered to be more informative than traditional coagulation tests, such as Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APPT) (Van Veen *et al*., 2008). TGAs are global haemostasis assays carried out on platelet poor plasma or platelet rich plasma that can evaluate the clotting system, whilst the PT and APPT tests only measure the end point of the formation of a clot which is the end product of the process, therefore only evaluating approximately 5% of the activated coagulation system (Hemker and Béguin, 1995; Rand *et al*., 1996). TGAs are still mainly used as research tools, and will be described in more detail in Chapter 7.

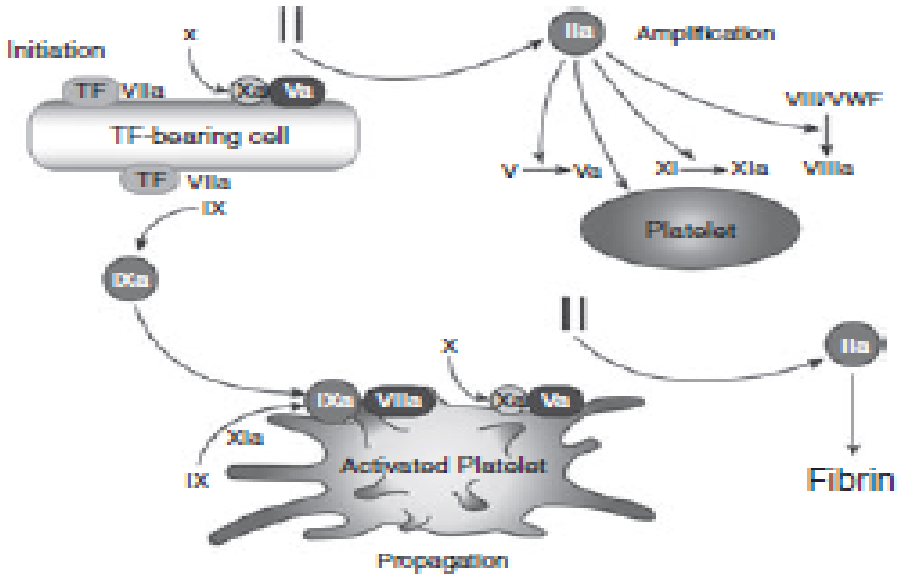

Figure 1-3. The modern cell-based model of haemostasis , reproduced from Hoffman & Dargaud, 2012.

1.6.3 Venous thrombosis in SCD

Though there is wide consensus about the increased risk of arterial thrombosis and strokes in SCD (Ganesan *et al*., 2002; Prengler *et al.*, 2002), there is less agreement with regard to venous thrombo-embolism (VTE) i.e. pulmonary embolus and deep vein thrombosis (PE and DVT) (Key and Derebail, 2010). The incidence of clinical VTE in SCD sufferers in the steady state is not well researched (Key and Derebail, 2010). James *et al* (2006) reported that pregnancy in SCD is associated with an increased incidence of VTE (with an Odds Ratio of 6.7 and Confidence intervals between 4.4 and 10.1). However, their study did not look separately at the antenatal and postnatal periods, and they did not differentiate between PE and DVT (James et al., 2006). Stein *et al* (2006) looked at VTE in

hospitalised SCD patients and found that whereas the incidence of DVT was the same in SCD patients as in controls of black African ethnic origin with normal Hb, the incidence of PE was higher in patients with SCD than in their control patients (Stein *et al*., 2006).

There is an impression that, despite the hyper-coagulable state in SCD, clinical VTE may not occur in the steady state without additional risk factors, such as the presence of other co-morbidities, prolonged hospitalisation, or pregnancy (Key and Derebail, 2010).

1.6.3.1 Pathogenesis of thrombus in SCD

SCD creates a hyper-coagulable state (Ataga and Key, 2007; Francis, 1991; Stuart and Nagel, 2004a; Stuart and Setty, 2001). There is evidence that the markers of thrombin generation and tissue factor expression are increased in SCD, with a reduction in the natural anti-coagulant proteins, abnormal activation of the fibrinolytic system and activation of platelets, even in the steady state (Ataga and Key, 2007; Setty *et al*., 2001; Stuart and Nagel, 2004b; Stuart and Setty, 2001; Westerman *et al.*, 2008). The factors contributing to this pro-thrombotic tendency are numerous, and the mechanisms responsible for this hypercoagulability share many similarities with the mechanisms causing vaso-occlusive crises, described

previously. In fact, it is sometimes not clear if the hyper-coagulable state is a result of the vaso-occlusive complications or the cause of them (Ataga and Key, 2007).

The core abnormality in the pro-thrombotic cascade is the loss of the RBC membrane asymmetry, described earlier, leading to the formation of red cell membrane microvesicles, with PS exposed on the outer surface of the lipid bilayer (Ataga and Key, 2007; Westerman *et al*., 2008). This loss of natural cell membrane asymmetry occurs only in the RBC in SCD, and there is no evidence that platelet cell membranes show any similar PS exposure (Ataga and Key, 2007; Ataga et al., 2007). The exposed PS on SCD RBC's increases the tendency to thrombosis in four ways. Firstly, directly, because exposed PS forms an attachment port for coagulation and anticoagulation enzymatic complexes (Zwaal and Schroit, 1997). Secondly, indirectly, due to endothelial injury caused by adhesiveness of the sickle RBC's and the subsequent formation of a multi-cellular aggregate of WBCs and platelets. The fact that circulating endothelial cells with increased tissue factor (TF) expression and chronically activated platelets are consistently found in SCD, even in the steady state, signifies that this inflammatory response also plays a role in coagulation activation (Setty *et al*., 2002; Solovey *et al*., 2004). Thirdly, the presence of PS on the outer surface of RBC's serves as a detection signal for cell apoptosis (Fadok *et al*., 1992) leading to chronic haemolysis, which contributes further to the pro-thrombotic state by releasing free circulating haemoglobin (Cappellini, 2007). This is evident by the fact that RBC's with greater PS exposure cause a two-fold increase in endothelial tissue factor (TF) expression, compared

with RBCs with less PS exposure (Setty *et al*., 2008, 2002); Setty *et al*, 2006). Lastly, there is also some evidence that SCD is associated with increased levels of circulating cell membrane-derived vesicles, or microparticles (Morel et al., 2006; Porter *et al*., 1993) .These microparticles are released by RBC's, platelets, endothelial cells and monocytes, either following their activation or as a result of apoptosis. Microparticles thus play an active role in producing the hyper-coagulable state (Westerman *et al*., 2008).

1.6.3.2 Risk of VTE in Sickle Cell Trait (SCT)

Asymptomatic people with SCT exhibit a lesser degree of the same coagulation activation which is described in patients with SCD (Westerman *et al*., 2002). It is thought that the mechanism is similar to those in SCD, but with lesser degrees of PS exposure on the surface of RBC's (Chiu *et al*., 1981). Dowling *et al* (2003) looked at the incidence of VTE in a black American population and found that the incidence of VTE is doubled in people with SCT compared with people with no haemoglobinopathy (Dowling *et al*., 2003).

A recent study in the USA has also reported that sickle cell trait is associated with clinical VTE. Despite a prevalence of less than 1% as shown in Figure 1-1, the sickle mutation contributes to approximately 7% of clinical episodes of VTE among people of Afro-Caribbean ethnic origin (Austin *et al*., 2007; Austin *et al*., 2009). The

authors draw attention to the fact that this is a stronger correlation than that of the Factor V Leiden mutation (Austin *et al*., 2007). In addition, it has been suggested that there is potentiation of the risk of VTE in SCT women by the use of the combined oral contraceptive (COC) (Austin *et al*., 2009).

1.7 HORMONAL CONTRACEPTION

The naturally occurring oestrogen, 17 beta oestrogen (E2) is secreted by the granulosa cells of the ovary. Secretion starts early in the follicular phase of the menstrual cycle and reaches its peak at the time of ovulation (Channing *et al*., 1980). Most of the E2 is bound to the carrier protein, Sex Hormone Binding Globulin (SHBG), however its effects are exerted by the free form (Channing *et al*., 1980). There are E2 receptors in almost all body organs and E2 supports diverse physiological processes, including aspects of the cardiovascular system (Kuiper *et al*., 1998, 1996; Moriarty *et al*., 2006; Toran-Allerand, 2004). For example, E2 is proven to stimulate nitric oxide synthase (NOS) production by vascular endothelial cells (Hisamoto and Bender, 2005; Simoncini, 2009).

After ovulation the follicle transforms into the corpus luteum, which secretes progesterone, and thus progesterone levels peak in the mid-luteal phase of the cycle (Channing, 1980). The main actions of progesterone are confined to the reproductive system. Progesterone is mainly bound to the carrier protein,

Corticosteroid Binding Globulin (CBG) (Hammond *et al*., 1984). Again, the physiological actions are exerted by the free form, which is rapidly metabolised (Channing *et al*., 1980).

The synthetically derived oestrogen, ethinyl oestradiol (EE), has an ethinyl residue, which has a higher receptor affinity, and it is more durable during transport, giving it a longer half-life than E2 (Djerassi, 2006; Djerassi *et al.*, 2006). Because of this, EE is the most commonly used oestrogen in contraceptive medication. Unlike E2, progesterone has many synthetic analogues, known collectively as progestogens (Hammond *et al*., 2001; Raynaud and Bercovici, 1979). The first manufactured progestogens are identified in clinical practice as "first generation" progestogens and include preparations such as Norethindrone acetate (NETA) and Ethynodiol diacetate (Hammond *et al*., 2001). These first generation preparations are derived from 19 nor-testerone and they exhibit low to medium progestogenic activity and slight oestrogenic effects (Hammond et al., 2001). The so called "second generation" progestogens are now the most commonly used in contraceptive medications. They are derived from 17-α-hydroxy-progesterone and include Levonorgestrel (LNG) and Norgestrel. They have progestotogenic and androgenic effects; hence Levonorgestrel adversely affects serum lipoproteins (Hammond et al., 2001; Blumenthal and Edelman, 2008). However, in combinations with oestrogen, both NETA and LNG reduce androgen production (Thorneycroft *et al*., 1999). The "third generation" progestogens (e.g. desogestrel, gestodene) have less androgenic and oestrogenic effects compared with the older progestogens. Thus

they show less negative impact on serum lipids, body metabolism and weight gain, and they have positive effects on acne (Hammond *et al*., 2001; Practice Committee of American Society for Reproductive Medicine, 2008).

Hormonal contraceptives (HC) can be divided into the combined hormonal contraceptives (CHC) containing oestrogen and progestogens, and the progestogen only contraceptive (POC) (Practice Committee of American Society for Reproductive Medicine, 2008).

1.7.1 Combined Hormonal Contraceptives (CHC)

CHC's work by suppressing ovulation (Rivera *et al*., 1999). They decrease the pulsatility of GnRH release from the hypothalamus, decrease the pituitary responsiveness to GnRH stimulation, and suppress LH and FSH production, with the result of inhibiting the mid-cycle LH surge, thus preventing ovulation (Graziottin, 2008; Kastin *et al*., 1972; Killick *et al.*, 1987; Mishell *et al*., 1977; Mulders and Dieben, 2001). Combined hormonal contraceptives can be administered orally or topically as a vaginal ring or as transdermal patches. All the women in my study using CHC were taking a combined oral contraceptive pill (COC). Other routes of administration will not be discussed here.

COC's are known to be well tolerated and highly effective (Foidart *et al*., 2000; Huber *et al*., 2000). It has been reported that approximately 100 million women worldwide are using COC's (WHO Scientific Group, 1998). COC's are extensively used worldwide, in the USA it is estimated that 25% of women of reproductive age have used them (Practice Committee of American Society for Reproductive Medicine, 2008; Odlind *et al*., 2002). In the UK, it has been estimated that a similar percentage of women have used COC (Lader, 2009). It is the preferred method of young women in the UK, in particular. 53% of women aged 20-24 years have used the combined oral contraceptive pills in their life time (Lader, 2009). COC's are popular with women because of their ease of use, effectiveness, low rate of side effects, and the predictable return of subsequent fertility (within three months) (Cerel-Suhl and Yeager, 1999; Blumenthal and Edelman, 2008).

COCs are also known to reduce the incidence of endometrial and ovarian cancer, to reduce chronic pelvic pain, ovarian cysts, and to reduce menstrual blood loss (Practice Committee of American Society for Reproductive Medicine, 2008). There is also a considerable role for COC in the control of symptoms of dysmenorrhea and endometriosis (Harada et al., 2008).

However COC's are found to increase the risk of venous and arterial thrombosis (Van Hylckama Vlieg and Middeldorp, 2011). The increased VTE risk is noted mainly with the preparations containing third generation progestogens, especially

gestodene (Bloemenkamp et al., 1995; WHO Scientific Group," 1998; Jick et al., 1995; Kemmeren et al., 2001a, 2001b; Spitzer, 1996). However, the VTE risk with norgestimate-containing pills is considered similar to that of users of levonorgestrel-containing OCs.(Jick et al., 2006)

Recently, drospirenone, a progestogen derived from 17α-spironolactone has been used in COC medications. It exerts progestogenic and anti-androgenic effects (Krattenmacher, 2000).

Current COCs contain EE and any of the progestogens mentioned earlier, except one preparation (Qlaira®), which contains estradiol valerate and dienogest (Keder, 2011). The first manufactured COC preparations (1960) consisted of 150μg mestronol and 9.85mg norethyndriol (Colton, 1992; Junod and Marks, 2002; Practice Committee of American Society for Reproductive Medicine, 2008). Subsequently there was a progressive reduction in the EE component of COC's to 35μg, 30μg, and even 20 μg (Speroff, 1999). COCs are also produced in bi-phasic and tri-phasic preparations, where the progestogen dose varies to mimic the phases of the menstrual cycle, with the added consequence of minimising the total progestogen dose.

1.7.1.1 COC and VTE risk

Only two years after COC's were approved for use in the USA, Jordan wrote "because Enavid may produce uncontrollable vomiting and provoke pulmonary embolism and infarction, I would counsel caution in the use of this widely advertised drug" (Jordan and Anand, 1961). Later, Inman wrote about the increased occurrence of VTE in COC users, and attributed this increase to the oestrogen component of COC (Inman and Vessey, 1968; Inman, 1970).

Five decades have passed since those initial reports and, despite the reduction in the EE dose in COC medication, from 50 μg to 30 and to 20 μg, and changes in the progestogen components used, still the evidence is that COC's are associated with significantly increased rates of VTE (WHO Scientific Group," 1998, WHO, 1995; Jick et al., 1995; Kemmeren et al., 2001a, 2001b; van Hylckama Vlieg et al., 2009). 1995, the "pill scare year", was an important mile stone in the history of COC's, when initial reports were published suggesting that third generation progestogens (desogestrel and gestodene) in COC's increase the risk of VTE more than second generation progestogens such as levonorgestrel and norgestrel (Bloemenkamp *et al*., 1995; Jick *et al*., 1995; Spitzer, 1996; WHO, 1995). These studies stimulated a series of further studies looking at VTE risk in COC in more detail. All of them reached the conclusion that there is an increased risk of VTE, which is greater with those containing third generation progestogens (except norgestimate) than first

generation (ie norethindrone acetate and ethynodiol diacetate) or second generation progestogens (Kemmeren *et al*., 2001a, 2001b).

The Royal College of General Practitioners' ("New oral contraception study," 1986) Oral Contraception Study, which has been an ongoing study since 1968, initially suggested an increased risk of adverse vascular events among women who had ever used COC's. This was noted mainly among older women and those who smoked ("New oral contraception study," 1986). A twenty five year follow up report from the same study, however, suggested that all the adverse effects of oral contraceptives occurred in current or recent users, with few effects persisting beyond 10 years after stopping use (Hannaford and Kay, 1998; Hannaford et al., 1994). The most recent reports from this study indicate that long term use of COC is not associated with increased mortality in the studied cohort (Hannaford et al., 2010). Further epidemiological studies have confirmed this finding, and, the current consensus is that vascular risks are maximal within the first three months of starting COC's (Van Hylckama Vlieg et al., 2009; Vessey et al., 1989). Currently the estimate of absolute VTE risk in COC users is 6.29 per 10,000 woman-years, for users of COC's containing less than 50μg EE, which compares with the background risk in non-users of 3 per 10,000 woman-years (Lidegaard et al., 2009).

Further studies have stratified the VTE risk according to the progestogen component of the pills. It is estimated that the risk associated with the third

generation progestogens is 1.5 to 3 times the risk with second generation COC preparations (Jick et al., 1995; WHO, 1995). Lidegaard (2011) found that the relative risk (RR) of VTE in users of COC preparations containing less than 50 μg EE was 2.9 with levonorgestrel use, 6.6 with desogestrel, 6.2 with gestodene and 6.4 with drospirenone use (95% CI of 2.2 to 3.85, 5.6 to 7.8; 5.6 to 7.0 and 4 to 7.5, respectively) (Lidegaard et al., 2011).

COC formulations containing drospirenone are found by some studies to increase VTE risk more than COC's containing third generation progestogens (Parkin et al., 2011), whilst other studies have not found this (Dinger et al., 2007). Parkin et al, (2011) in data derived from the RCGP study, concluded that the incidence of VTE is 23.0 per 100 000 woman years (95% confidence interval 13.4 to 36.9) in current users of COC's containing drospirenone, and 9.1 per 100 000 woman years (6.6 to 12.2) in current users of COC's containing levonorgestrel (Parkin et al., 2011). Similar findings are derived from a study conducted by Sidney et al (Sidney et al., 2012).

1.7.1.1.1 Mechanisms for increased VTE risk in COC use

It is evident now that COCs interfere with all haemostatic parameters (Tchaikovski and Rosing, 2010). Firstly, COCs increase the pro-coagulant factors. They consistently elevate prothrombin, fibrinogen and FVII, while factors such as FVIII,

FX and FV may or may not be increased (Kluft and Lansink, 1997; Middeldorp et al., 2000; van Rooijen et al., 2002). Secondly, COC use is associated with a decrease in natural anti coagulants. Several studies suggest that there is a decrease in anti-thrombin, protein S and Tissue Factor Inhibitor (TFPI). This in turn leads to activated protein C resistance (APCR). The APCR noted in COC users is both APTT assay based and the endogenous thrombin potential based (Mackie *et al*., 2001; Rosing et al., 1999; van Rooijen et al., 2002). The resistance to APC is mainly due to decreases in protein S and TFPI levels, with a small effect from the decrease in anti thrombin levels (Rosendaal and Reitsma, 2009). Thirdly, there is activation of the fibrinolytic system in COC users with increased Plasminogen, and tissue plasminogen (tPA) activity (Kluft and Lansink, 1997; Meijers et al., 2000). Van Rooijen et al (2006) suggested that SHBG is a surrogate marker for the prothrombotic risk of COC and the greater the increase in SHBG the greater the VTE risk (Van Rooijen et al., 2006). All these thrombotic tendencies are seen even with the low dose EE pills, but are more noticeable with COCs containing higher EE doses and "third generation progestogens" (Rosing et al., 1999; Tans et al., 2000; van Rooijen et al., 2002).

1.7.1.2 COC and arterial disease

While, there is a general consensus that COC increases VTE risks in its users, the risk of arterial disease, i.e. myocardial infarction (MI) and ischaemic stroke (IS) is less agreed upon, with some studies suggesting that there no increased risk at all

(Petitti et al., 1996; Rosenberg et al., 1990; Schwartz et al., 1998; Siritho et al., 2003). However, other studies suggest that COC does indeed increase the risks of IS and MI (Baillargeon et al., 2005; Croft and Hannaford, 1989; Gillum et al., 2000; La Vecchia, 1992; Lidegaard and Kreiner, 2002).

Recently Lidegaard *et al*,(2012), in an epidemiological study, with 1.7 million participants, concluded that COC use increases the risks of thrombotic stroke and myocardial infarction. Furthermore, they found that the risk increases with increase in the EE dose, with the risk increased by a factor of 0.9 to 1.7 with COC preparations that contain 20 μg of EE and by a factor of 1.3 to 2.3 if the dose is 30μg or more (Lidegaard et al., 2012).

In contrast to VTE risk, most of the studies that looked at IS and MI risk suggest that there is no difference in the incidence of these complications according to the type of progestogen used, with second generation progestogen preparations carrying the same risk as the third generation progestogens (Kemmeren et al., 2002; Lidegaard et al., 2012). There is also no reported added risk of IS and cardiovascular disease in users of drospirenone-containing COC's, compared with those containing second- and third-generation progestogens, as is the case with regard to VTE risk (Gronich et al., 2011). When comparing risk of IS to the risk of MI, Lidegaard et al (2012) found that the IS risk was higher than MI risk, quoting

this risk to be 21.4 per 100,000 person-years for IS and 10.1 per 100,000 person-years for MI (Lidegaard et al., 2012).

Some studies have attempted to explore whether the increased arterial risks are due to confounding factors, or arise de novo. They have reached different conclusions, with some suggesting that conditions such as cigarette smoking, migraine, obesity, and hypertension are the pertinent causes of MI or IS development in COC users (Curtis et al., 2002; Petitti, 2003). However, Baillargeon et al, (2005) found that even after correcting for these confounding factors, there is still an increased incidence of IS and MI with COC use (Baillargeon et al., 2005).

Finally, the issue of the increased risk in current COC users versus ever users, has also stimulated some studies, which agree that the risk is higher in current users. (Baillargeon et al., 2005) have indicated an overall adjusted odds ratio (OR) for IS in current COC users, to be approximately 2.08 (95% CI of 1.55 to 2.80) compared with past users. Nonetheless, despite these proven risks, the incidence is so low that even if a small increase is there, it is likely to have no impact in young women. Hence, the contraceptive benefits of COC are calculated as outweighing these risks, in numerical terms, except in women with co morbidities (Baillargeon et al., 2005; Kemmeren et al., 2002; Lidegaard and Kreiner, 2002).

1.7.2 Progestogen Only Contraception (POC)

POCs comprise the oral preparations, progestogen only pills (POP), and the long-acting parenteral reversible progestogen-only contraceptive medications (LARC) ie injectable Depot medroxyprogesterone acetate (DMPA), the subcutaneous contraceptive implants and the levonorgestrel intra-uterine system (IUS).

1.7.2.1 Progesteron Only Pills

The current evidence is that POC carries negligible risk of VTE (Kuhl, 1996; Mantha et al., 2012; Vasilakis *et al*., 1999). However, the number of studies in this area is quite small. A recent meta analysis found only eight studies looking at VTE risk in POP users (Mantha *et al*., 2012). Five of these studies were case-control studies (Vasilakis et al , 1995; WHO, 1998; Heinemann et al,1999; Barsoum, 2010; Van Hylckama Vlieg et al, 2010), while three were retrospective cohort studies. (Conard et al., 2004a; Lidegaard and Kreiner, 2002; Vaillant-Roussel et al., 2011). These studies reported a RR of VTE of 1.03, with POP use (95% CI of 0.76 to 1.39) (Mantha et al., 2012). Kemmeren et al, (2004) even suggested that POCs containing Levonorgestrel confer anti thrombotic effects (Kemmeren et al., 2004).

1.7.2.2 Long Acting Reversible Contraceptives (LARC)

Depo Medroxyprogesterone Acetate (DMPA) acts mainly by preventing ovulation (Bhathena, 2001; Power and Guillebaud, 2002). However, it acts also by increasing the thickness of cervical mucus, and changing the quality of the endometrium,

hence hindering fertilisation and implantation (Bhathena, 2001; Fraser and Weisberg, 1981; Hickey and Fraser, 2000). In 2004 sub-cutaneous injection of 104mg of medroxy-progesterone acetate was approved for contraceptive use in the USA, however, the most widely used preparation currently is the intra-muscular injection of 150mg of medroxy-progesterone acetate. The main side effects of DMPA are amenorrhoea and irregular bleeding (Arias et al., 2006; Bhathena, 2001). DMPA is recommended to be given every 12 weeks (FSRH, 2008). DMPA is also associated with a delay in return of fertility after cessation of use, with an average delay of 10 months (Arias et al., 2006; Garza-Flores et al., 1985; Jain et al., 2004; Pardthaisong, 1984).

1.7.2.3 Levonorgestrel intra uterine system (Mirena®)

The Mirena IUS was approved for contraceptive use in the UK in 1995 (Kailasam and Cahill, 2008). Its reservoir contains 32mg of levonorgestrel and it is licensed, for contraceptive purposes, to be in situ for five years (Kailasam and Cahill, 2008). Besides the very low contraceptive failure rate of 0 - 0.2 pregnancies per 100 woman-years of use, levonorgestrel has the added benefit of reducing menstrual blood loss by approximately 75%, due to its anti proliferative effects on the endometrium (Hidalgo et al., 2002; Luukkainen et al., 2001).

1.7.2.4 Contraceptive implants

The contraceptive subcutaneous implants comprise a heterogeneous group which includes different progestogen preparations and dosages, and with different intended durations of use (Croxatt, 2002; Meirik et al., 2013; Mommers et al., 2012). However, all of them act by inhibiting ovulation and to a lesser extent by increasing the viscosity of cervical mucus and thinning the endometrium (Mäkäräinen et al., 1998). The first implant to be used was Norplant (Leiras Oy Pharmaceuticals) which consisted of six silastic capsules, each filled with 36mg levonorgestrel and intended for five years of use (Shoupe and Mishell, 1989). This was followed by Norplant II (Norplant-2, Jadelle) which consisted of two rods and was manufactured by Schering (Brache et al., 2006, 2003)and the Implanon which contains 68 milligrams of etonorgestrel and is licensed for three years' use (Graesslin and Korver, 2008). Nexplanon (Organon), which replaced Implanon in the UK in 2010, is similar to it, but has the advantage of being radiopaque and therefore less problematic to remove (Mommers et al., 2012). The main side effect with all contraceptive implants is irregular vaginal bleeding (Abdel-Aleem et al., 2007; d' Arcangues, 2000; Mainwaring *et al*., 1995) particularly in the first few months after insertion.

1.8 REPRODUCTIVE HEALTH IN SCD

The essence of this study is to explore the safety of effective and reversible methods of contraception, which would enable women with SCD to plan their pregnancies optimally.

1.8.1 Pregnancy in SCD

Worldwide, the prevalence of sickle cell disease has increased over the past few decades, and this is also the case in the UK. The number of people with SCD in the UK, albeit low, has doubled in two decades, from estimates of 5,000 in 1986 to 12,000 in 2009 (Streetly et al., 2009). Pregnancies in SCD in the UK number approximately 60 per year (Author's unpublished data, Appendix 1). Nonetheless, it is difficult for many doctors in areas of particularly low prevalence to develop expertise in treating patients with SCD, and this may be a confounding factor in the outcome of some SCD pregnancies. Great developments in the paediatric and haematological care of SCD patients mean that the majority of affected individuals now live into adulthood. If it is presumed that fertility is normal in SCD, many adult women with SCD potentially could experience pregnancy. However, because SCD is an incurable condition, with multiple chronic complications, pregnancies in women with SCD are particularly complex. The haematological and cardiovascular nature of the disease makes SCD complications particularly relevant to pregnancy, because pregnancy imposes significant strains on these two important body systems. Maternal sickle cell disease is consistently reported to be associated with

an increased complication rate compared to pregnant women without this disease (Howard and Oteng-Ntim, 2012; Howard *et al*., 1995; Rahimy *et al*., 2000; Serjeant et al., 2004a; Tuck *et al.*, 1983; Villers et al., 2008). Despite some studies suggesting that SCD pregnancy-associated complications have fallen over recent decades (Chase et al., 2010; Smith et al., 1996; Yu et al., 2009) still substantial maternal mortality occurs: 2·1% in Jamaica, 1.6% in the U.K and 0.07% to 1.7% in the USA (Serjeant et al., 2004a; Villers et al., 2008). There is also a high perinatal mortality rate in these pregnancies, i.e. still births and deaths in the first week of life. In the UK this is reported for the years 2000 to 2004, to be 53/1000 (Author's unpublished data, Appendix 1), which compares with the overall contemporaneous perinatal mortality rate in the UK of 7.9/1000 (NOS, 2010). Morbidity is also increased, with severe sickle crises occurring in about 40% of pregnancies, severe pre eclampsia and deliveries by emergency caesarean section, being amongst the most significant (Howard *et al.*, 1995; Rahimy *et al*., 2000; Serjeant *et al*., 2004b; Tuck *et al*., 1983; Villers *et al.*, 2008). Recently, the UK Obstetric Survellance System Sixth Annual Report found no reporting of maternal mortality, but a high morbidity rate, with 52% of women experiencing antenatal painful crises, 6% of them having acute chest syndrome and 24% of these women receiving antenatal blood transfusion (UKOSS, 2012).

It is therefore particularly important that pregnancies in women with SCD are planned, as much as is possible, so that their health and care can be optimised prior to conception, and plans for their care in pregnancy in place, since there is

evidence that this can improve the outcomes (Biermann *et al*., 2006). Effective planning of pregnancies would give women and their doctors a chance to time pregnancy when they are in optimal health and their medication has been adjusted appropriately, for example, stopping Hydroxycarbamide (previously known as Hydroxyurea). This medication has the beneficial effect of increasing the levels of HbF, which mitigates the adverse effects of HbS. However, it is an anti-neoplastic agent, inhibits the enzyme ribonucleotide reductase, thus leading to DNA synthesis inhibition. Hence, it is potentially teratogenic and the USA's Food and Drug Administration (FDA) advises that women contemplating pregnancy should avoid its use (USA FDA, 2012).

1.8.2 Contraception in SCD

Although women with SCD attain menarche late, compared with women with normal haemoglobin, once they start menstruating there is no further reduction in fertility (Serjeant *et al*., 2005). Their need for good contraceptive advice is generally met with a lack of knowledge and understanding about appropriate contraceptive methods for SCD women from the majority of doctors and nurses they are likely to encounter (Howard and Tuck, 1994; Howard *et al*., 1993).

Widely held instinctive concerns about the prescription of combined hormonal contraceptives for women with SCD arise from the fact that SCD alone has many haemostatic complications, as described earlier, with proven prothrombotic tendencies, whilst the combined contraceptive pills are also known to carry

thrombotic and cardiovascular risks. Hence, there is an understandable concern that the two together will bring particularly deleterious risks for these women, with regard to VTE, strokes and acute sickling crises. There is however, a lack of scientific evidence for this assumption. In consequence SCD women may potentially be deprived of the most commonly used and efficacious contraceptive methods available, which is the issue being pursued in this thesis.

In practice, in the SCD population, DMPA use is ten times the rate in the general population, with approximately 38% of women with SCD using it, compared with 3% for both DMPA and implants in the general population (FSRH, 2008).

There has also been a marked increase in the use of POP in women with SCD, which is used by 15% of them, compared with 5% of the general population and in the levonorgestrel-releasing intra uterine system (Mirena®), used by 9% of SCD women compared with 1% of the general population. This increase in use is probably understandable, on the basis that POC carries negligible added risk of thrombosis *(*Conard *et al.*, 2004b). In addition, DMPA has been reported in two small studies to be beneficial to SCD women, with a reduced frequency of painful crises (De Abood *et al*., 1997; De Ceulaer *et al*., 1982). This has led to wide recommendation in haematology circles, of this method of contraception for women with SCD. It should be noted, however, that DMPA and other POPs are associated with more side effects than COC's, and are not particularly liked by

young women, as is reflected by their relatively low rate of use in the general population (FSRH, 2008).

The unplanned pregnancy rate in SCD women is approximately 60% (Howard et al., 1993) and 53% in 2010 (Author's unpublished data, Appendix 2). This clearly implies that these women are not being given effective advice.

1.8.2.1 COC and SCD

In contrast to the general population, the use of COC in women with SCD is low, despite the World Health Organisation's guidelines for contraceptive use now acknowledging that the use of COC in women with SCD has more benefits than risks (UKMEC, 2009). A recent study conducted by the author found a significant decline in the use of COC by women with SCD from 1993 to 2010, from 45% to 31 % with a marked increase in POC use from 37% to 67. % (Appendix 2).

COC use requires careful consideration in women with SCD because of three main issues. Firstly, COC's are known to increase the thrombotic risk in its users and this thrombotic risk may be considered to be potentially additive to the increased thrombotic risk conferred by SCD itself. Secondly, women with SCD are at increased risk of arterial disease and COC is also proven to increase the risk of

ischaemic strokes (IS) and myocardial infarctions. Yet on the other hand, SCD is characterised by painful occlusive crises and there are some studies suggesting that hormonal steroids decrease the risk of sickle crises. In addition, the non contraceptive effects of COC, such as the decrease in menstrual blood loss could be beneficial for SCD patients. COC use also is known to relieve dysmenorrhoea, which is especially beneficial for women with SCD, as it is reported there is greater incidence of dysmenorrhoea in SCD, and some women experience a clustering of sickling crises with menstruation (Samuels-Reid and Scott, 1985).

COC use in SCD, has attracted much less research compared with COC use in the general population. The assumption that COC would definitely be harmful for SCD patients and the fact that there is a small minority of SCD women using COCs could have contributed to this. The author identified only four studies looking at COC use in SCD. These studies found no major adverse side effects. Howard et al (1993) reporting on 67 SCD women using COC, noted irregular bleeding in three of his participants, increased crises in four, and eight who discontinued their COC use. De Abood et al.(1997) compared the effects of COC (Microgynon, with 30µg ethinyl oestradiol and levonorgestrel 150µg) on the intensity and frequency of painful crises, with DMPA and a control group of sterilised SCD women. They found no adverse effects from any contraceptive method in the study and no change in the haematological parameters that they studied. Yoong *et al* (2003) compared changes in red cell deformability in COC users with a group using POC and with a third group of non contraceptive users. No changes in red blood cell deformability

were found in his in vitro studies. Yoong et al (2003) also looked at the markers of thrombin generation, platelet activation and fibrinolysis in women with sickle cell disease on the COC, POC and in women not on any hormonal preparations, and found no significant variations in these haematologic markers studied.

1.8.2.2 Progestogen only Contraception and SCD

Despite the fact that POCs are used by a greater proportion of SCD women compared to COC medications, still the studies which have looked at them are scarce and many of them have involved only small numbers of participants (Haddad *et al*., 2012).

Adadevoh and Isaacs (1973) measured the effects of oral megestrol acetate on the numbers of irreversible sickled cells (ISC) and of sickle crises in seven women and one man with SCD in a randomised controlled cross over study. Each participant received megestrol acetate and the placebo (vitamins) in succession over a six week period. The researchers did not report the wash out period, nor the randomisation process they had followed and used different progestogen doses for the individuals studied They found higher overall sickle crisis rates among the group whilst they were taking megestrol acetate, although the individual sickling rates were the same in the two groups and there was no effect on the number of ISCs. Extrapolation of this study to clinical contraceptive use should be made with

caution, because of the very small sample size, and the fact that the doses administered here were not those relevant to normal contraceptive use (Adadevoh and Isaacs, 1973). In another study 30 SCD women who had used POP were interviewed and none of them reported serious side effects nor increased sickle crises, although six reported irregular bleeding and two discontinued use because of side effects (Howard *et al*., 1993).

1.8.2.2.1 Depot MedroxyProgesterone Acetate (DMPA) and SCD

DMPA is generally perceived to be useful in SCD for three main reasons. Firstly, since there is a contention that there is clustering of painful crises around menstruation in SCD women (Yoong and Tuck, 2002) and DMPA usually induces amenorrhoea, DMPA use could be associated with a reduced incidence of painful crises (De Abood e al., 1997; De Ceulaer et al., 1982). Secondly, because of the chronic anaemia usually experienced by SCD women, those who experience heavy blood loss during menstruation or prolonged bleeding could incur exacerbation of this problem, and DMPA-induced amenorrhoea would be beneficial for this issue. A study by Samuels-Reid and Scott (1985) found that patients with SCD bleed for longer and their periods are heavier than a control group of women. However, this finding was not supported by other researchers, for example Yoong and Tuck, 2003 found that nearly 90% of women with SCD reported light or moderate menstrual bleeding and the duration of bleeding was a mean of 4.69 days +/- SD (4.65).

DMPA was one of the first contraceptive methods to be studied in SCD women, however, there are, to date, only five studies of its use, and the number of subjects in each has been small. There are also considerable differences in the study designs and in their measured outcomes, both clinical and haematological.

The first study by De Ceulaer *et al*, 1982**,** compared the use of DMPA with placebo injections in a randomised cross-over study, which involved 25 women with Hb SS disease. Each participant received both the DMPA and the placebo injections. The women who initially received DMPA for nine months, had a six month "wash out" period before receiving the placebo injection for a further 9 months, and vice versa for the other group. The main outcome measures were painful crises and some haematological markers i.e. total Hb, HbF%, reticulocyte count and total bilirubin concentration. They found a statistically significant reduction in the incidence of painful crises, and a concurrent improvement in most of the haematological indices. Although this study design has been appraised to be level I (Mohllajee *et al*., 2005) with a fair internal validity (Haddad *et al*, 2012**)**, however, the sample size was small, and the six month "wash out" period after DMPA might not have been enough to clear its effects, which can last up to 18 months after the last injection. (Garza-Flores *et al.*, 1985). The time periods involved might also raise concerns regarding the tendency to seasonal variation in the frequency of sickling

crises, although this study was conducted in Jamaica, where this is unlikely to be a significant factor.

The second study including DMPA was carried out in 1993 by Howard et al who interviewed a total of 164 women with sickle cell disease in North London. This was a cross sectional, questionnaire-based study, which looked at whether women with SCD experience complications from different contraceptive methods. In this study, 26 women reported DMPA use. Approximately a third of them experienced irregular bleeding, 11.5 % had stopped DMPA because of side effects, but none experienced any increase in sickle crises, or any episode of VTE. This study relied on self reporting and there were no laboratory or objective clinical data included in the analysis.

The third study was a non randomised controlled study done by De Abood *et al*, in 1997. They compared DMPA use in 13 SCD women with Microgynon (30 μg ethinyl estradiol with 150 μg levonorgestrel) in 14 SCD women, with a third group acting as a control comprising 16 women who had had surgical sterilisation. The researchers were interested in the occurrence and severity of painful crises, and haematological parameters such as haemoglobin and haematocrit levels, reticulocyte count, and coagulation markers including prothrombin time and thromboplastin time. Their study found that the DMPA group reported a significant decrease in painful episodes over the period of the study (50% at 3

months, 70% at one year; p=.014) compared to a lesser decrease in the COC and the sterilisation groups at one year follow up. No adverse side effects were reported in either group and no change in the studied blood parameters occurred at anytime during the follow-up period. This is also a small sized study, which used DMPA in non- contraceptive regimens, (DMPA was given every month for a total of three months in contrast to the normal three months interval used in contraception); hence the extrapolation to contraceptive use again requires caution (De Abood *et al* 1997).

The fourth and fifth studies were performed by Yoong et al, 1999 and 2003. Both these studies were laboratory based, and cross-sectional, which looked at various haematological parameters, and included 30 and 44 participants, respectively. The first study principally compared red cell deformability, in three groups: SCD women using progestogen-only contraceptives, SCD women using COC a third group of SCD women not taking any exogenous hormones. The 2003 study looked at platelet activation and thrombin generation in three similar groups. Both studies concluded that there were no significant differences in these parameters among the three groups (Yoong *et al*., 1999).

O'Brien *et al*. (2011) conducted a cross sectional study to look at rates of use, types of contraception used and the frequency of pregnancy, VTE and osteopenia in women aged 13-21 years in Michigan, USA. They used the International

Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) codes to collect information from the Michigan Medicaid program between 2000- 2003. They identified 250 women with SCD, of whom only 20 were hormonal contraceptive users (8%), with 12 using DMPA. The authors did not state what the contraceptive methods used by the other eight women were. The study reported that VTE and osteopenia were infrequently recognised; however, the exact number is not specified. Within the inherent limitations of this study design, still one would comment that the rate of hormonal contraceptive use in this population of women with SCD was small.

1.8.2.2.2 Contraceptive implants and SCD

Studies looking at contraceptive implants in SCD are few, small in size, and look at different outcome measures. However, all have found no adverse effects. Firstly, Ladipo *et al*. (1993) carried out an observational study looking at the safety of Norplant in 25 SCD women. They looked at adverse clinical outcomes, haematological parameters, including reticulocyte counts, HbF and total bilirubin, and biochemical markers including serum albumin and serum creatinine concentrations, HDL cholesterol, and aspartate transaminase (AST). They found no unexpected or serious side effects, haematological or biochemical changes after the use of Norplant. This was again a small size study which did not look specifically at the incidence of sickle crises, and its practical relevance is limited, since Norplant

was withdrawn from use in the UK in 1999 (Gbolade, 1999) and in the USA in 2002 (Wysowski, 2005).

The second study was conducted by Nascimento et al. (1998). This was a non-randomised, controlled study intended to assess safety, clinical, haematological, and biochemical outcomes in 30 women with SCD. They compared Uniplant use in 20 SCD participants with a control group of ten non-users of hormonal contraceptives. Clinically, they found a significant decrease in the incidence of painful crises and other symptoms, e.g. headache and body weakness, in the Uniplant group. They also found no serious side effects during one year of follow up, and all participants chose to continue with Uniplant use after the study. Haematologically, the study also found significant increases in HbF levels in the study group. Yet again, the sample size was not powered enough to detect any clinical change and there were no paired statistical tests done.

The third study was done by Barbosa et al. (2001), who carried out uncontrolled descriptive study included ten SCD women and looked at unintended pregnancies, abnormal weight gain and increase in blood pressure values associated with Uniplant use. They also studied biochemical changes in carbohydrate metabolism by undertaking Glucose Tolerance Tests (GTTs), measuring fasting insulin and glycosylated haemoglobin levels. There were no pregnancies during the study period. In addition, there were no significant changes in weight or blood pressure, glycosylated hemoglobin and serum insulin levels before and after insertion of the

Uniplants. As with the previous studies the small sample size is a drawback, as well as the lack of a comparison group**.** The biochemical markers studied in this research (i.e. of carbohydrate metabolism) are not known to change with SCD or with progestogen implant use, hence their significance in this study is unclear

1.8.2.2.3 Mirena Intra Uterine System (IUS) use in SCD

The author believes that the use of the Mirena IUS use has not been studied in women with SCD as yet.

1.8.2.3 SCD and Intra uterine devices (IUDs)

Howard *et al*, in their 1993 cross-sectional study, also assessed complications in women with SCD using standard (non-hormonal) IUDs. The study included 28 women with a total of 140 years of use. Nearly 40% reported having menorrhagia and 18% experienced infections (the nature of which was not identified), but there were no serious adverse events reported, and no IUD users discontinued use because of side effects. As commented previously the study relied on self reporting and there was no confirmation of the specific type of IUD used.

1.9 SICKLE CELL TRAIT AND HORMONAL CONTRACEPTION

The evidence that SCT is a prothrombotic condition has already been discussed (Austin *et al*, 2007). The increased risk of VTE with the use of COCs has also been discussed previously. There has been only one study attempting to to assess the combined effects of these two factors on the incidence of VTE (Austin *et al*. 2009). This was a case controlled study of 60 African-American women with a first episode of otherwise unprovoked VTE and the control group were 196 African-American women without VTE. The OR of being a current HC user in the VTE cases was 3.8 (95% CI 1.7-8.1), which was statistically significant. The OR of being a current HC user and having SCT in the VTE cases was apparently doubled at 6.7, although the confidence interval here was very wide , 1-43, and the calculation did not reach statistical significance. No conclusion can therefore be drawn from this as to whether the VTE risk of SCT and the HC use is multiplicative or simply additive, and there has been no study published to date looking at arterial events (MI and IS) with this combination of SCT and HC use.

1.10 Summary of literature review

Hadad *et al*, in 2012, carried out a systematic review to examine the safety of hormonal contraceptives and intrauterine contraceptive device use among women with sickle cell disease, and concluded that the lack of evidence on the risk of VTE among COC users with sickle cell disease, and on IUD use among women with sickle cell disease, represented major gaps in the research literature**.**

At the same time, many authors identify sickle cell disease as a condition that exposes women to increased risk of unintended pregnancy, with 38-63% of pregnancies being unplanned. There has been a tendency to be less cautious with the prescription of HCs for women with sickle cell disease, solid evidence to underpin this remains lacking. Furthermore, no research has concluded clearly that any one hormonal contraceptive method poses lesser or higher risks for SCD women than any other. Recommendations from official bodies have been relaxed, but this is mainly based on the fact that the risks of pregnancy are so high (UK MEC, 2009).

This lack of evidence leads to considerable confusion in the contraception advice given to women with SCD. There is some tendency to favour certain contraceptive methods such as the long acting progestogen-only injections (principally DMPA) for their presumed beneficial effect on SCD crises and lesser thrombotic risks. Meanwhile, the combined oral contraceptive pills (COCP) are regarded by many clinicians as unsuitable for SCD women, on the grounds of the known increased thrombotic risks associated with their use. This is despite the much greater preference for COCPs rather than POC methods in the general population of women. The extremely limited research evidence available makes it difficult for medical personnel or women with SCD to make well informed contraceptive decisions.

Some laboratory work looking at the effect of the hormones used in contraceptive preparations on blood samples from women with SCD has been produced with encouraging findings (Yoong *et al.*, 1998). Therefore there is a clear need to study the use of COCP in patients with sickle cell disease in vivo. Should such studies show benefit, or at least the absence of any added risks, this will help to strengthen the basis of contraceptive advice available for these women.

CHAPTER 2

HYPOTHESIS AND AIMS

2.1 INTRODUCTION

While the haematological tests used in this study are intended to improve the understanding of the factors which are known to influence the risk of thrombosis, this project also studies the clinical effects of hormonal contraceptives on women with sickle cell disease and sickle cell trait and their relationships. The practical importance of being able to give complete and well founded guidance to women with SCD is to enable them to achieve their family planning aims optimally, with the minimum of unplanned (as opposed to planned) pregnancies. The benefit of this should permit improved pregnancy outcomes.

2.2 STUDY HYPOTHESIS

There are no additional clinical or haematological risks to Sickle Cell Disease patients and women with Sickle Cell Trait using hormonal contraceptive methods, over and above those inherent in their SCD and SCT.

2.3 AIMS OF THE STUDY

1. To characterise any differences in the occurrence of clinical complications i.e. sickle crises, the need for blood transfusion, episodes of VTE, menstrual irregularities, and hospital admissions.

2. To explore the differences in the contraceptive side effects, discontinuation of contraceptive method, accidental interruptions and the number of planned and unplanned pregnancies in women with SCD and SCT.
3. To evaluate any differences in haemostatic markers i.e. pro- and anti-coagulant markers, haematological parameters, coagulation activation markers, platelet activation markers, markers of endothelial activation, markers of tissue damage, markers of thrombin generation, markers of haemolysis and liver function, both metabolic and synthetic.

These features are assessed in the following groups

- Women with SCD using different methods of hormonal contraception
- Women with SCT using different methods of hormonal contraception
- Women with normal Hb using different methods of hormonal contraception
- Women with SCD, women with SCT and women with normal haemoglobin using Combined (oestrogen and progestogen) Oral Contraceptives
- Women with SCD, women with SCT and women with normal haemoglobin using Progestogen-Only Contraception
- Women with SCD, women with SCT and women with normal haemoglobin not using any form of hormonal contraception.

CHAPTER 3

MATERIALS AND METHODS

3.1 STUDY DESIGN

This was a multi-centre, prospective cohort study involving six different hospitals in London. The study was set up to investigate the possibility of additional clinical or haematological risks of hormonal contraceptive methods in women with Sickle Cell Disease (SCD) and Sickle Cell Trait (SCT), over and above those risks associated with the haematological disorders themselves.

Eligible consenting patients with SCD were recruited over a period of 25 months from September 2006 to October 2008. Women with SCT were recruited between December 2009 and August 2010. Inclusion criteria were: women between 18 and 54 years of age, not pregnant nor planning a pregnancy within a year from recruitment, as well as not suffering from pulmonary hypertension and not receiving anticoagulant therapy. A third comparable group was recruited between December 2009 and August 2010 from women of black African ethnic origin, in the same age range and with the same exclusion criteria. The recruitment process is described in more detail in Chapter 4.

3.2 STUDY GROUPS

Study participants were recruited from the Sickle Cell Society (SCS) activities or were past obstetric patients at the RFH. Interviews were conducted and blood samples collected either at the Royal Free Hospital (RFH) or their local hospitals. All women had their travel expenses reimbursed.

Women were divided into three main groups according to their chosen contraception method or if they were not using any hormonal contraception. They were also divided into a further three main categories, depending on their haemoglobin type, giving a total of nine subgroups, shown in Table .3-1. The study comparisons were made across each column and down each row of the table.

	Type of contraception			
Type of haemoglobin		SCD	SCT	AA
	COC	SCD+ COC	SCT+ COC	AA + COC
	POC	SCD+ POC	SCT +POC	AA + POC
	No hormonal contraception	SCD + No HC	SCT + No HC	AA + No HC

Table .3-1 Study groups

Nine study groups emerged from dividing the women by type of haemoglobin (normal i.e. AA, SCD and SCT) and type of contraception (COC, POC and no hormonal contraception).

3.3 ETHICAL APPROVAL

Ethical approval was obtained from the Cambridge 4 Ethics Committee, reference number: 06/MRE05/59 (Appendix 3). I also obtained site specific assessment (SSA) approvals from the local Research Ethics Committees of all the participating centres, in addition to separate approvals from the Research and Development offices of the participating centres. All women participating in the study gave informed written consent prior to their enrolment in the study (the consent form is shown in Appendix 9).

3.4 POWER CALCULATION

Dr Richard Morris (Reader in Medical Statistics & Epidemiology, Department of Primary Care & Population Sciences, UCL) has calculated the required sample size in this study to be 55 women in each group. This was calculated using the standard deviation (SD) of the thrombin generation (which is the main test in my study). This SD is calculated by reference to a previous study carried out by Yoong et al, 2003 which looked at the coagulation markers in SCD and found a thrombin generation SD (TGSD) of 10 units.

The TGSD between the women in my study is likely to be smaller than that quoted by Yoong et al (9) since Yoong used only one measurement per woman.

Assuming a SD of 7 for Beta –TG it will be possible to detect a difference of 4.3 units between the hormonal contraceptive users and non users, with 90% power at a 5% significance level.

The formula below is used to calculate the sample size:

$$n = 2x \frac{(z\,\alpha/2 + z\beta)^2 \sigma^2}{\delta^2}$$

(σ = standard deviation of the TG in both groups, α = type 1 error (False positive), β = type 2 error (False negative) and δ = the difference between the mean of TG parameters in the HC users and non-users).

3.5 DATA

3.5.1 Interviews

All women who consented to take part in the study were interviewed by me during their first visit. Information about the woman's demographic and clinical characteristics, past medical history, previous and current contraceptive use, and haemoglobin type was obtained during the interview. Full details of the information collected during the interview are given in Appendix 2. All the women were given a diary to note prospectively any relevant events, such as hospital admission, sickle crisis or other complications, blood transfusion, contraceptive problems or failures and any medication changes. These were the clinical events, side effects and complications used in the study analysis. Some retrospective

information given by the women at their enrolment interview will be mentioned in Chapter 7, but none of this information is included in the study results.

3.5.2 Contraceptives used

Oral contraceptive preparations used in all women except two are monophasic preparations containing E2 and levonoregestrel or norethisterone. The other two women used preparations containing E2 and Drospirenone (one woman has SCT and the other woman has normal Hb).

The Progestogen only contraception methods used were DMPA in one woman, POP in 11 women, implants in seven women, and eight women used Mirena IUS. The distribution of the Mirena IUS is even between women with SCD, SCT and normal Hb.

3.5.3 Physical examination

A physical assessment was undertaken at each visit, namely blood pressure check, weight and BMI calculation.

3.5.4 Data collection schedule

The schedule of data collection during the study is shown in the Table 3-2 below. The women with SCD were studied for a period of 22 to 26 months. The women with SCT and the women with normal Hb were studied for a period of 20 to 25 months.

	INITIAL	3 MONTHS	END
DEMOGRAPHIC CHARACTERISTICS			
AGE	✓		
ETHNICITY	✓		
SMOKING HISTORY	✓		
HAEMOGLOBIN TYPE	✓		
CLINICAL CHARACTERISTICS			
HEIGHT	✓		
WEIGHT	✓	✓	✓
BMI	✓	✓	✓
BP	✓	✓	✓
CLINICAL EVENTS	✓	✓	✓
SICKLE CRISIS	✓	✓	✓
BLOOD TRANSFUSION	✓	✓	✓
HOSPITAL ADMISSION	✓	✓	✓
STROKES	✓	✓	✓
VTE (PEs AND DVTs)	✓	✓	✓
CONTRACEPTIVE DATA			
SIDE EFFECTS, COMPLICATIONS		✓	✓
DISCONTINUATION, ACCIDENTAL		✓	✓
PLANNED AND UNPLANNED		✓	✓
BLOOD SAMPLES		✓	

Table 3-2 Data collection schedule

3.6 BLOOD COLLECTION

3.6.1 Timing of blood collection

The first blood collection took place at least 3 months after the enrolment process to ensure that the contraceptive methods used were well established, and a steady state was reached. It is acknowledged that this delay between recruitment and drawing of blood samples, does carry the disadvantage that any intial surge of hyper-coagulability will be missed. In the general population the highest incidence of VTEs is in the first three months of use. My current study is unable to investigate whether this is the case in women with SCD.

Blood collection was planned to be at least four weeks after the last sickle cell crisis or six weeks after an exchange transfusion, or any blood transfusion. Collections were carried out during the luteal phase of each woman's menstrual cycle. However, this aspect of timing was not relevant in women with prolonged use of Medroxy progesterone Acetate (Depo-Provera injections) who were amenorrhoeic and also not applicable in some of the Mirena IUS users and in the women using contraceptive implants. Thus, blood samples were obtained from these women without reference to previous cycles.

3.6.2 Precautions for blood collections

Physical factors that could lead to sample discrepancies such as smoking, eating and drinking, as well as general stress, were taken into account during the blood taking process. For these reasons, the women did not undertake any vigorous physical activity (including rushing up the stairs), and were asked to rest in the

waiting area for a minimum of 30 minutes prior to venesection. Furthermore, haemostasis from prolonged tourniquet induced pressure is known to contribute to haemoconcentration. Thus, wherever possible, the use of a tourniquet was kept to a minimum. When deemed necessary, a tourniquet was used to aid with the identification of vein location, and was released promptly when the needle was inserted into the vein. Due to the frequent thrombosis of peripheral veins in sickle cell patients, as a result of their repeated use for blood transfusions and blood taking, veins other than those in the ante-cubital fossa were also used. Occasionally these involved peripheral veins in the anterior aspect of the arm or dorsum of the hand. A (green) wide needle (size 21 gauge) was preferred; however in limited cases, when veins were particularly fibrosed, a smaller gauge (blue) needle was used.

Specimen collection, storage and transmission of the samples to the laboratory were all carried out according to the International WHO guidelines on drawing blood: best practices in phlebotomy, in order to minimise flaws in the specimens (WHO, 2010). All patients were identified appropriately, associated request forms were completed and specific precautions were taken for any hazardous specimens. All samples were transported by me to the appropriate laboratory within four hours of collection, in specially supplied containers, at room temperature. Appropriately validated training was undertaken to allow for this process.

3.7 LABORATORY INVESTIGATIONS

3.7.1 Laboratory markers studied

Pro and anti coagulant markers	Coagulation activation markers	Platelet activation markers	Endothelial activation markers	Markers of tissue damage
PT/INR	TAT	sP selectin	sVCAM	Microparticles
INR	Prothrombin Fragments 1+2	sCD40 ligand	slCAM-1	Tissue Factor
APTT	D dimer	PF4	sEselectin	
Thrombin Time				
Fibrinogen				
VWF Ag				
Lupus Screen (DRVVT)				
Antithrombin acrivity Protein C activity, Protein S free & APCR with FV deficient plasma				
Factors II, V,VII, X, VIII, IX, XI, XII & XIIIa				
LT (5pM)	Free Hb	ALT	CRP	Hb
ETP (5pM)	Haptoglobin	AST	WBC	Platelets
PH (5pM)	LDH	Bilirubin	Neutrophils	HCT
TP (5pM)		Albumin	Lymphocytes	
Slope		ALP	Monocytes	
ST (5pM)			Eosinophil	
			Basophils	

Table .3-3 Laboratory markers studied

PT: Prothrombin time, **INR**: international normalised ratio, **APTT**: Activated Partial Thrombopastin Time, **TT**: Thrombin Time, **Fib:** Fibrinogen, **VWFAg**: von Willebrand Factor Antigen, **DRVVT**: Dilute Russell Viper Venom time, **AT:AC**: anti-thrombinactivity**, PC:AC**: Protein C activity, **APCR V**: Activated Protein C resistance, **Factors II, V,VII, VIIIC, IX, X, XI, XII, XIIIa:** Clotting factors**, TAT:** Thrombin Anti-thrombin complexes, **F1+2:** Prothrombin fragments 1 and 2, **sP selectin:** Soluble Platelet selectin, **sCD40 ligand:** Soluble Cell Differentiation molecule 40 ligand, **PF4**: platelet factor 4, **sVCAM**: soluble vascular cell adhesion molecule-1, **slCAM**: soluble intercellular adhesion molecule-1, **sEselectin**: soluble Endothelial-leukocyte adhesion molecule-1, **Mp**: Micro particles, **TF**: Tissue Factor. **LT (5pM)**: lag time (initiated with 5 pMol tissue factor), **ETP (5pM)**: endogenous thrombin potential (initiated with 5 pMol tissue factor), **PH (5pM)**:Peak height (initiated with 5 pMol tissue factor), **TP (5pM)**: time to peak (initiated with 5 pMol tissue factor), **Slope**: thrombin generation slope, **ST (5pM)**: Starting time (initiated with 5 pMol Tissue factor), **LDH:** Lactate dehydrogenase, **ALT :** Alanine aminotransferase, **AST**: Aspartate aminotransferase, **ALP**: Alkaline phosphatase, **CRP**: Complement-Reactive Protein**, WBC:** White Blood Cells, **HCT**: Haematocrit.

(Table of Abbreviations)

As alluded to previously (Chapter 1) all the pro- and anti-coagulant markers listed above, are known to be abnormal in SCD in the steady state, showing a change of the order of 5 to 30%. Most of these markers are known also to be changed by taking hormonal contraception, although by a much smaller order of magnitude, and are strongly correlated with the incidence of clinical VTE (Auerbach *et al*.,2004; Heijboer *et al*.,1990) The markers of coagulation activation, platelet activation and tissue damage, which I have chosen to study, are also recognised as significant indicators of the pathological features of sickle cell disease and to correlate with the incidence of VTE in the general population. Furthermore, D dimer levels are known to correlate strongly with arterial thrombosis, although they are also known to rise markedly in any condition involving inflammation or

malignancy(Gershlick,1999) The validity of the endothelial activation molecules I have chosen to measure in my study, as markers of clinically significant endothelial damage, and hence correlating with arterial and venous thrombosis, will be discussed further in Chapter 7. The markers of thrombin generation which I have studied are less well established as predictors of clinical VTE, and the value of these will also be discussed further in Chapter 7. The markers of haemolysis measured are chosen as indicators of erythrocyte damage, and are thus expected to be relevant to sickle cell disease only.

3.8 SAMPLE HANDLING

3.8.1 Samples for free Haemoglobin (Hb), full blood count (FBC), Lactate dehydrogenase (LDH), and bilirubin

All blood samples were transported in a Blue Lid Bio Bottle® container (Bio-packaging Ltd, Coventry) to the Royal Free Hospital laboratories. Samples needed for Free Hb, FBC, were collected into a 4 ml violet BD Vacutainer® bottle containing 7.2mg ethylenediaminetetraacetic acid (EDTA) (Beckton Dickerson, Poole, UK), and samples for bilirubin, ALT, AST and LDH were collected into a 5 ml yellow BD Vacutainer® Serum Separator Tube (SST™). Each bottle was connected to the distal end of the blood collection system (BD Vacutainer® Safety Lok™) in the subject's vein and filled up to the reference line.

3.8.2 Sample collection for haemostatic markers

After collecting the above samples, the Vacutainer® cap was removed and a multi-adapter (Sarstedt Aktiengesell-Shaft and Co, Germany) was connected to the distal end of the butterfly system to collect a citrated whole blood sample for haemostatic testing. 30 mls of venous whole blood was taken from an antecubital fossa vein into a bottle with 0.106Mol/L sodium citrate (S-Monovettes®) (Sarstedt, Leicester UK). Platelet poor plasma (PPP) was derived from this sample by double centrifugation (12 minutes each spin) at 2000g, separated, divided into aliquots and frozen at -85°C until testing was undertaken. For each sample received from a subject requiring double centrifugation, centrifuge tubes were labelled with the specimen number and patient name, as well as the centre of origin. An aliquot of double spun plasma for the thrombin generation assay was further centrifuged for two minutes at 6000g in a micro-centrifuge (Mini-spin®, VWR UK, East Grinstead), and divided again into aliquots and frozen at -85°C. An excessive negative pressure on the blood sample when drawing on the plunger of the syringe can cause haemolysis and thus the plungers were handled gently and drawn slowly to avoid over creation of negative pressure and frothing. Using the wrong anticoagulant, insufficient or conversely excess anticoagulant, as well as inadequate mixing of the blood with the anticoagulant, can give rise to flawed specimens, therefore all blood samples were mixed promptly and gently with the citrate. Specimens were only accepted for testing if they were up to ≤20% under-filled. Any specimens under or over filled by more than 20% were rejected for haemostasis testing.

All blood samples were kept and centrifuged at room temperature within four hours of collection. All sample analyses was carried out at the Royal Free Hospital laboratories: in the Katharine Dormandy Haemophilia Centre & Thrombosis Unit Laboratory, Special Haematology laboratory and the Clinical Biochemistry laboratory.

3.8.3 Blood sample preparation and analysis

3.8.3.1 Free Hb sample analysis

These samples were processed in the Special Haematology laboratory at the Royal Free Hospital. The analysis followed a standard protocol (given in Appendix 4) This protocol was prepared by Dr S Sulkar, and the actual analysis was done by the Special Haematology staff.

3.8.3.2 Thrombin Generation Tests: sample analyses

The thrombin generation test (TGT) is an *in-vitro* global function test of the haemostatic system. All patients in this study had TGT measurement using triple spun platelet poor plasma (PPP) triggered with PPP reagent that containing 5 pMol tissue factor (TF) and 4μMphospholipid, using the Calibrated Automated Thrombinoscope (CAT) method (Thrombinoscope B.V. Maastricht, The

Netherlands). Briefly, 20µL of 5 pM reagent (activator) was added to three wells of an ELISA plate (Immunolon 2HB™, VWR UK, East Grinstead). Then 20µL of Thrombin Calibrator reagent with a known thrombin concentration, was added to the two other wells, to take into account the inter filter effect from the colour of the patient's sample (Hemker *et al*, 2003). 80µL of PPP derived from the subject's blood sample is then added to all five wells followed by 20µL of FluCa substrate (a fresh fluorogenic substrate containing 2.5 mMol Z-Gly-Gly-Arg-AMC and 100 mMol $CaCl^2$). Fluorescence was measured using a Fluoroskan Ascent fluorimeter (Thermolab Systems, Helsinki, Finland). The endogenous thrombin potential (ETP) was generated by dedicated software (Thrombinoscope™ B.V., Maastricht, The Netherlands) (Hemker et al, 2003). Thrombinoscope BV, the developer of the Calibrated Automated Thrombinoscope (CAT) was founded in 2004 by the University of Maastricht.

3.8.4 Other haemostatic markers

3.8.4.1 Techniques to assay platelet and endothelial activation markers

Plasma levels of the following adhesion molecules were determined by Enzyme-linked Immunosorbent Assay (ELISA) techniques, as per the manufacturers' inserts: soluble CD40 ligand (Bender Med Systems, Vienna, Austria), soluble Intercellular adhesion molecule-1 (s ICAM) (Bender Med Systems, Vienna, Austria), Soluble Vascular cell adhesion molecule-1 (sVCAM-1) (Bender Med Systems, Vienna, Austria), Soluble Endothelial-leukocytes adhesion molecule-1 (s E-

selectin) (Bender Med Systems, Vienna, Austria) and Soluble Platelet selectin (sP Selectin) (Bender Med Systems, Vienna, Austria). Laboratary reference ranges are given in Appendix 4.

3.8.4.2 Techniques to assay clotting factors and pro-coagulant markers

Frozen double spun platelet-poor plasma (PPP) was used to measure clotting factors VIII, IX, XI and XII by a one stage APTT-based clotting assay, using an APTT lyophilized silica reagent. (Instrumentation Laboratory, (IL) Warrington, UK) and deficient plasma from Pathway Diagnostics, (Epsom, UK) on an ACL 3000 analyser. Clotting factors FII, VII, V and X were measured by a one stage PT-based clotting assay on an ACL TOP 700 analyser (IL, Warrington, UK) with Recombiplastin 2G reagent and deficient plasmas all from IL.

Single spun plasma was also used to measure Prothrombin times (PT), Activated Partial Thromboplastin Time (APTT), and Fibrinogen on an ACL TOP coagulometer (IL, UK) using Recombiplastin 2G (IL, Warrington, UK), APTT SynthasIL reagent (IL, UK) and QFA reagent (IL, Warrington, UK). An APTT 50/50, PT 50/50 was also measured in samples with prolonged PT or APTT results. Thrombin time measurement was also made on an ACL TOP 700 coagulometer using 3 unit/mL bovine thrombin.

von Willebrand factor antigen (VWF:Ag) was determined by an in-house ELISA (Riddell et al., 2002). Thrombin anti-thrombin (TAT) complexes (Siemens, Marburg, Germany), prothrombin fragment 1+2 (F1+2) (Siemens, Marburg, Germany), D-dimers (IL, Warrington, UK), and FXIII:A measurements were also by ELISA, using paired matched antibody sets (Affinity Biologicals, Ancaster, Ontario, Canada). Protein C (PC) and Protein S (PS) were measured on an ACL TOP coagulometer using the Prochrom ™ protein C chromogenic activity kit (IL, Warrington, United Kingdom) and the Free protein S kit (IL, Warrington, United Kingdom). Antithrombin activity levels (AT:Ac) were measured on a CS2000i (Sysmex, Milton Keynes, UK) using the AT activity assay (Siemens, United Kingdom). Tissue factor (TF) levels were measured using the Actichrome TF assay (American Diagnostica, US), Platelet Factor 4 was measured by ELISA (American Diagnostica Inc, Stamford, USA), and Microparticles were measured in PPP samples using the Zyumphen ELISA (Hypen Bio Med, Quadratech, UK) according to the manufacturers' instructions.

3.8.4.3 Haptoglobin assay

The Haptoglobin assay was from Siemens, Marburg, Germany, using the No-Partigen Haptoglobin measurement by radial diffusion. The method of measurement was according to the manufacturer's instructions,

3.8.4.4 C-Reactive protein (CRP) assay

The CRP-HS assay (Hyphen Biomed, Neuville-Sur-Oise, France) was used, which is a highly sensitive ELISA method for the measurement of human CRP in plasma. The method used was again by the manufacturer's instructions, which give a reference range of 0.2 -10 ug/mL.

The thrombin generation tests, haemostatic and thrombotic marker assays, and the CRP and haptoglobin measurements were done by Ms A Riddell, Chief Biomedical Scientist, Dr William Pickering, Tejas Gandhi, Saravanan Vinayagam and Saman Aghighi (Biomedical Scientists) in the laboratories of the Katherine Dormandy Haemophilia and Thrombosis Unit at the Royal Free Hospital. I undertook the sample collection and transport and was trained in the laboratory techniques.

3.8.4.5 Techniques to assay FBC, Bilirubin, LDH, AST, ALT, ALP, albumin

All FBC and liver function test measurements were carried out at the Royal Free Hospital Haematology and Biochemistry laboratories, using widely used conventional apparatuses and standardised methods. The tests were carried out by the Haematology and Biochemistry laboratory biomedical scientists. A list of laborotary reference ("normal") ranges for these markers is also given (Appendix 5).

3.9 STATISTICAL ANALYSIS

Numerical variables were summarised using the mean and standard deviation or the median and range, depending on the data distribution i.e. normal or skewed distributions. Categorical variables were summarised using counts and percentages.

One-way analysis of variance (anova) was used to investigate differences in numerical biomarkers by type of contraception in the study subjects and differences in numerical biomarkers by haemoglobin type.

Two-way analysis of variance was used to investigate differences in numerical biomarkers by type of contraception and haemoglobin type.

All the assumptions for the tests were checked, and, where appropriate, the non-parametric equivalent test was also used.

Linear Regression testing (LRT) was used as the main statistical test to examine all the results in this thesis. .Logistic regression analysis was also undertaken, to see if

it might be useful. .This was meant to ellicit any possible relationship between a particular outcome and a particular variable. In the event, this exercise proved uninformative, with the one exception of demonstrating that high D-dimer level is linked to VTE risk, which is already a well known finding. It is also acknowledged that findings with logistic regression analysis can be misleading with very small numbers, which is another reason why this was not used further and has not been presented in my thesis. Therefore, all the results presented in this thesis are those analysed by linear regression testing.

CHAPTER 4

THE STUDY GROUPS

4.1 THE STUDY GROUPS

This chapter describes the characteristics of the nine study groups, defined by their types of haemoglobin and by their method of contraception used. The recruitment process and sequence of assessments will also be described.

4.1.1 Recruitment and selection of sickle cell women

Firstly, I obtained the lists of haematologists and sickle cell counsellors in the Greater London area with the help of the UK Forum for Haemoglobin Disorders. Subsequently, I contacted these haematologists and counsellors and most agreed to be named as "principal investigators" with me in the project. Hence, a Multi-Centre Research Ethics (MREC) approval was required and was obtained for the study (as described in Chapter 3).

In some of the relevant hospitals, I was able to gain registration for the project with the hospital's Research and Development (R&D) offices. However in the remaining hospitals this process was not successful, despite investing considerable time and effort in negotiating with the relevant staff. The processes from applying for MREC approval to the completion of study registration with the final centre successfully recruited took more than two years (from September 2006 to October

2008). There was also a further delay in some of the hospitals involved, before I was able to arrange appointments to attend the relevant haematology clinics in order to recruit potential participants for the study. The initial intention was to include all hospitals with known sickle cell patients in the Greater London area in the study, however due to either difficulties in securing an identified "principal investigator" for the project at some hospitals, or, as mentioned earlier, due to failure to obtain Site Specific Assessment (SSA) and local R&D approvals, this did not materialise. This was not because of any actual objection to my study, but due to slow and unresponsive bureaucratic procedures at some hospitals.

At each of the participating hospitals I made a list of all sickle cell women who fitted the inclusion criteria for my study i.e. they were aged between 18 and 54 years, not pregnant and not intending to be pregnant within a year of recruitment. Thier names were obtained from the clinical and laboratory data bases of the haematologists, counsellors and haemoglobinopathy nurses and from the local representatives of the Sickle Cell Society. All these women were contacted by letter, together, with the Patient Information sheet (PIS) (Appendix 6) and a stamped and addressed envelope. They were asked to reply using a detachable slip from the covering letter if they are interested in participating. Women who did not reply initially were later contacted by telephone, in case they had not received my letter of invitation.

I attended several meetings of the Sickle Cell Society (SCS) and distributed PIS's to women with sickle cell disease, and talked to them in person to explain the study further, if they wished. I also gave a formal presentation about the study at the SCS 30th anniversary meeting which was attended by many SCD women, and the response and interest they showed was very positive and encouraging. Many of the women I met in this context told me about their own personal difficulties in obtaining clear contraceptive advice from their own doctors, which reinforced to me the relevance of what I was attempting to achieve with my project

In addition, I sat repeatedly in haematology clinics at the participating hospitals to talk to potentially eligible women on a one-to-one basis. All these women were given the PIS to read and I answered their questions. Some of them discussed the project with their haematologist and haemoglobinopathy counsellor before deciding to participate. Interested women were then asked to sign the consent form

4.1.2 Recruitment and selection of women with sickle cell trait

I obtained a list of all women who were found to have SCT as part of their antenatal screening from the Haemoglobinopathy midwife at the Royal Free Hospital (RFH). Women who delivered during the three year period 2007- 2009 where contacted by letter and I asked them to state their current method of contraception, if they

were pregnant at the time of contact or if they were intending to become pregnant during the proposed time of the study. I also invited the NHS staff at the different participating hospitals and colleagues at the RFH with SCT to participate. In total I made initial contact with 80 women. Women who responded positively and who fitted the inclusion criteria for the study were then interviewed individually and their informed consent obtained. These women were given the PIS intended for SCD (Appendix 6) as well as the PIS and consent forms designed by the Katherine Dormandy Haemophilia Centre at the RFH for their Plasma Bank (Appendix 7 & 8). All the participating SCT women were seen and their blood samples taken at the RFH except for three of them, who were seen at their own local hospitals.

4.1.3 Recruitment and selection of women with normal Haemoglobin

Potentially eligible women with normal haemoglobin were chosen from all the hospital staff who are ethnically either black African or Afro-Caribbean with the same age group and other inclusion criteria as the SCD and SCT women. They were given the same information and documentation about the study, and asked to sign the study consent form, if they agreed to participate. All these women were given time to consider the details of the study, and it was made clear to them that they could opt to withdraw from the study at any time, if they wished.

4.2 PARTICIPATION OF RECRUITED WOMEN

A total of 130 women consented to take part in this study, of whom thirteen didnot complete all aspects of the study. One died during a visit to Nigeria, one emigrated to the United States of America, one became pregnant during the time of the study, and ten women were lost to follow up.

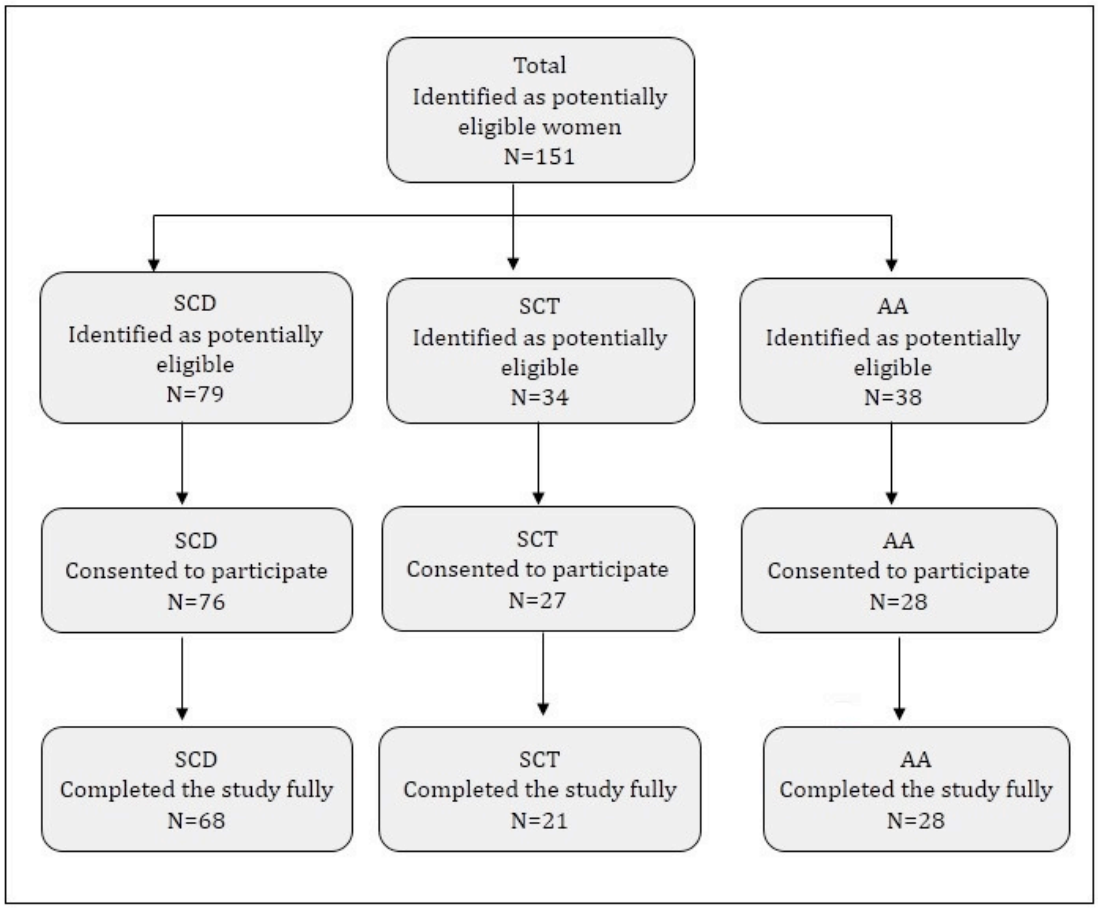

Figure 4-1 Flow chart of recruitment of all participants by haemoglobin type

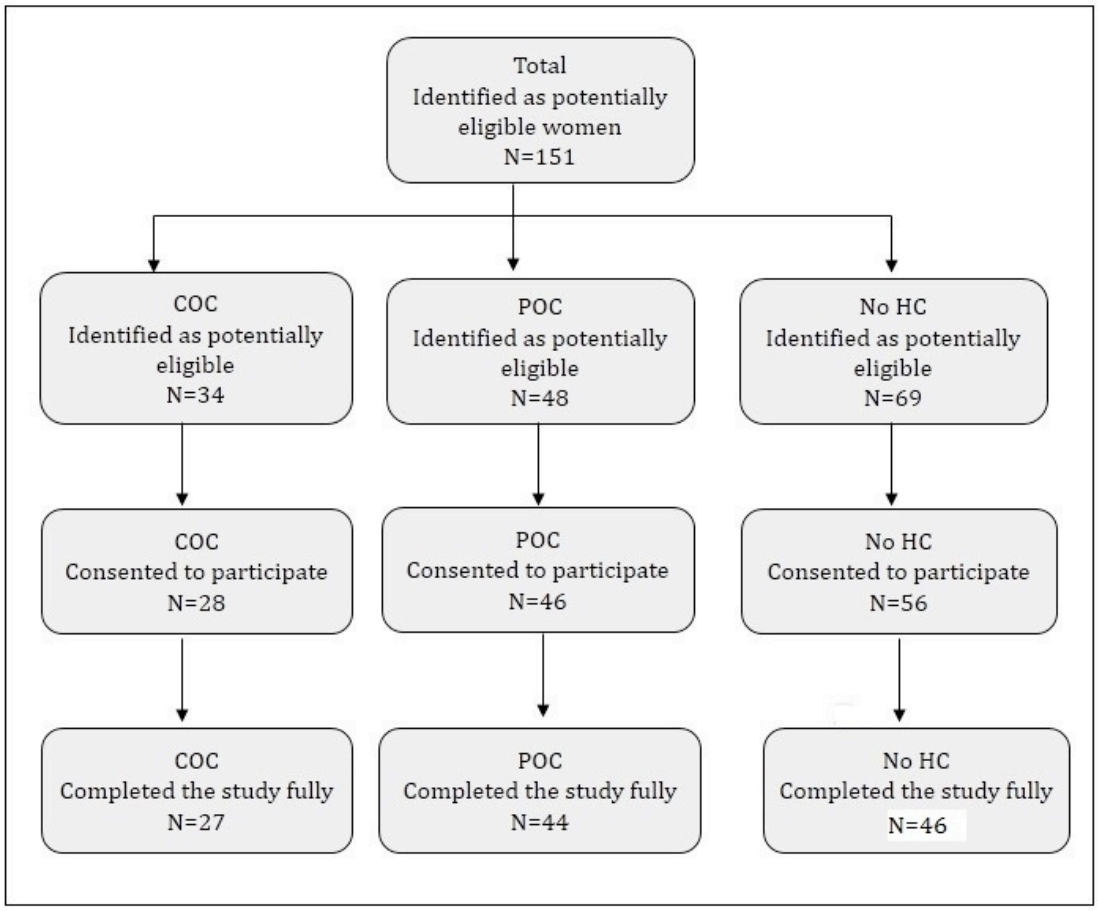

Figure 4-2 Flowchart of recruitment of women by contraceptive type

Figure 4-3 summarises the recruitment process for women with SCD with each type of contraceptive method, Figure 4-4 for women with SCT with each type of contraceptive method and Figure 4-5 for women with normal Hb.

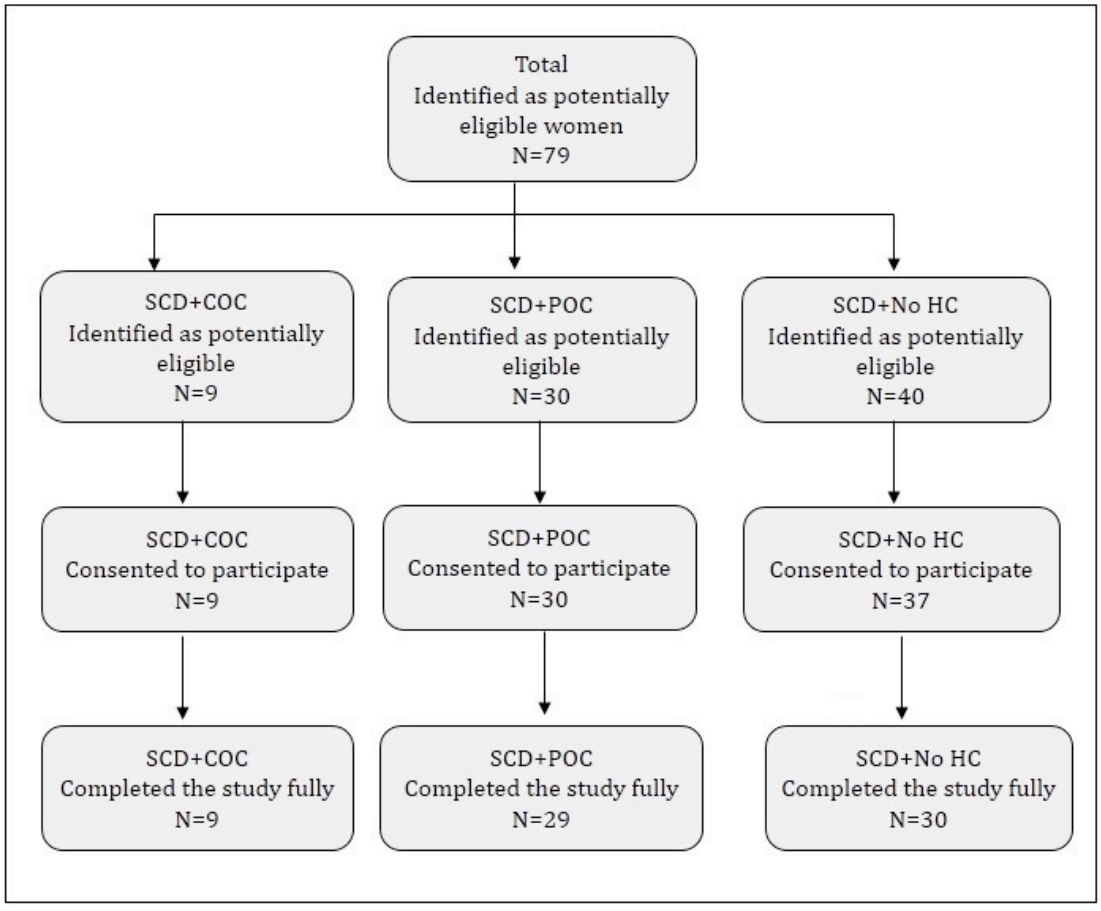

Figure 4-3 Flowchart of recruitment of SCD patients

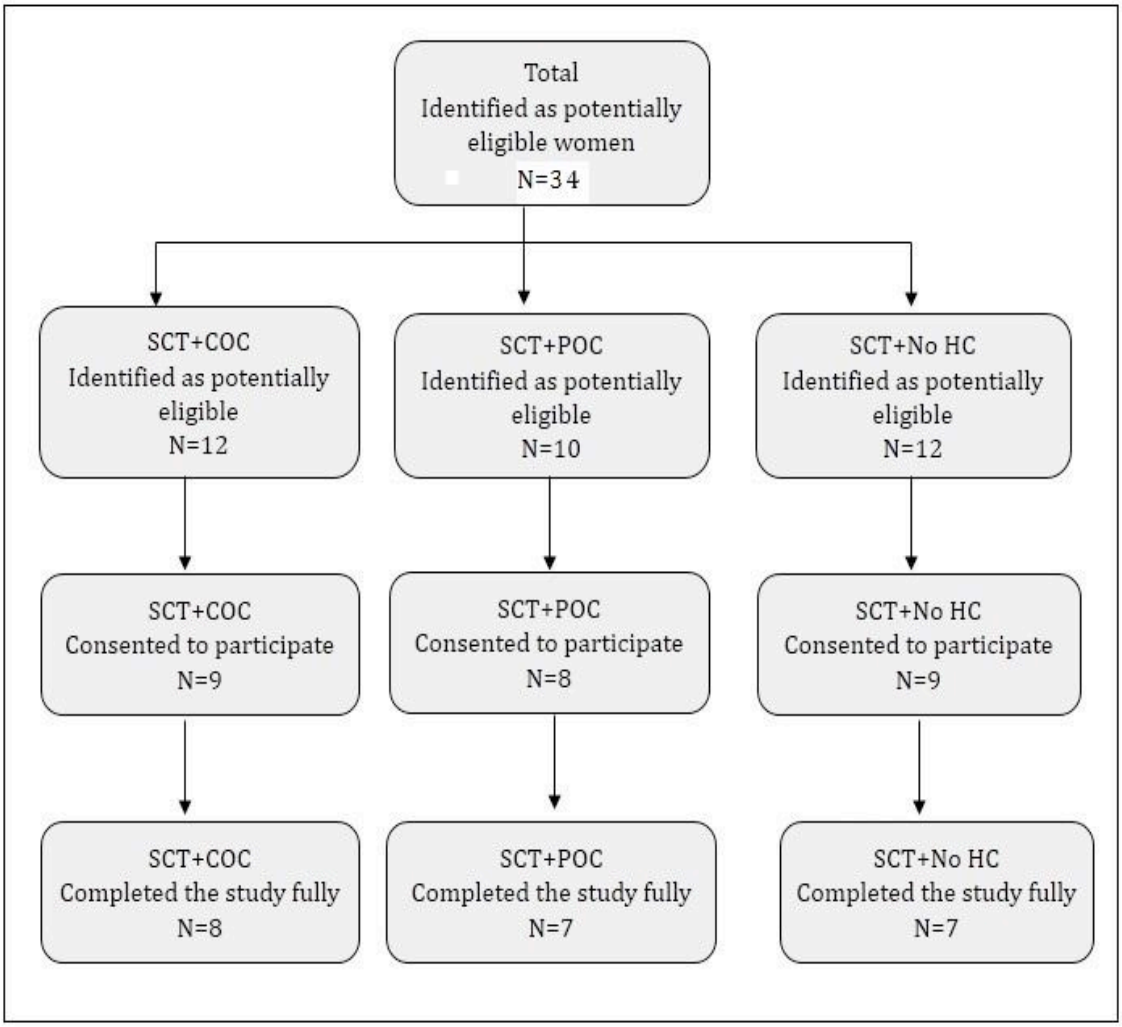

Figure 4-4 Flowchart of recruitment of SCT women

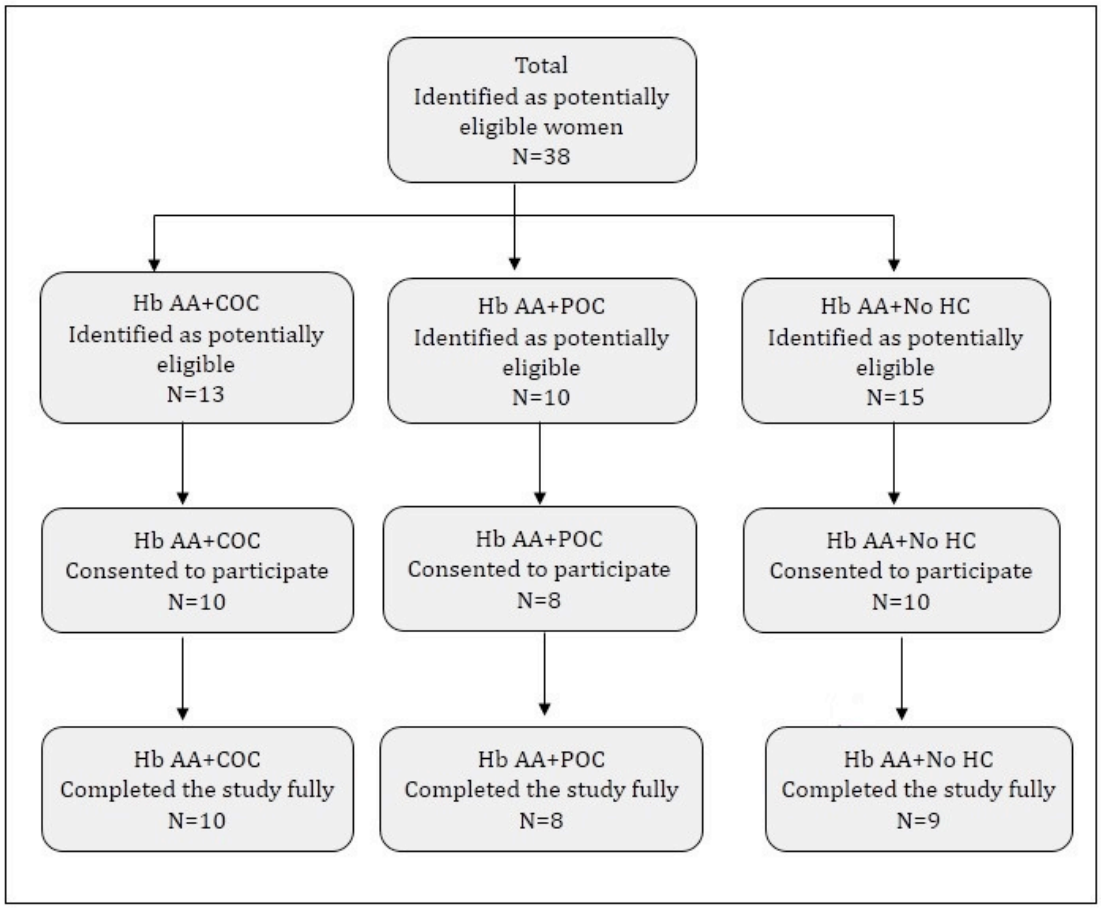

Figure 4-5 Flowchart of recruitment of participants with normal Hb (AA)

Figure 4-6 summarises the recruitment process for women on the combined oral contraceptive pills (COC), Figure 4-7 for women on progesterone only contraception (POC) and Figure 4-8 for women not using any hormonal contraception.

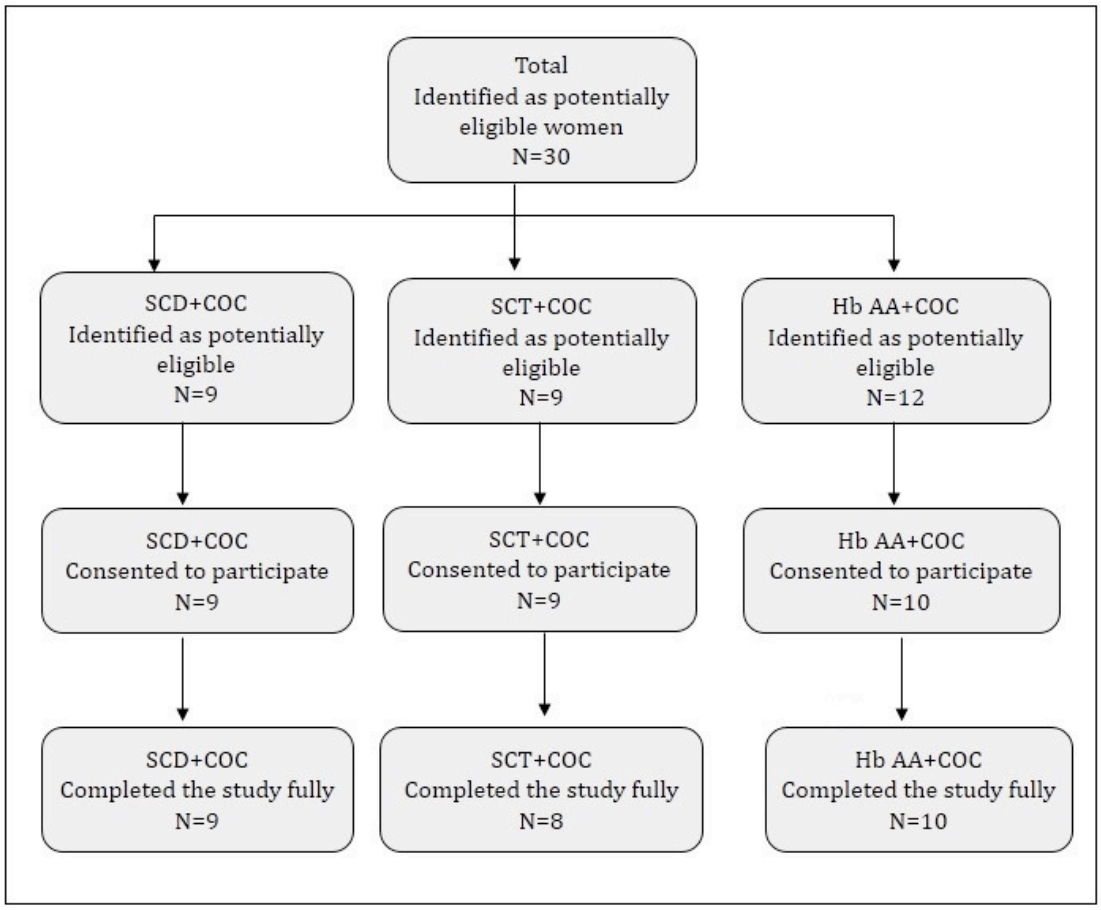

Figure 4-6 Flowchart of recruitment of women using COC

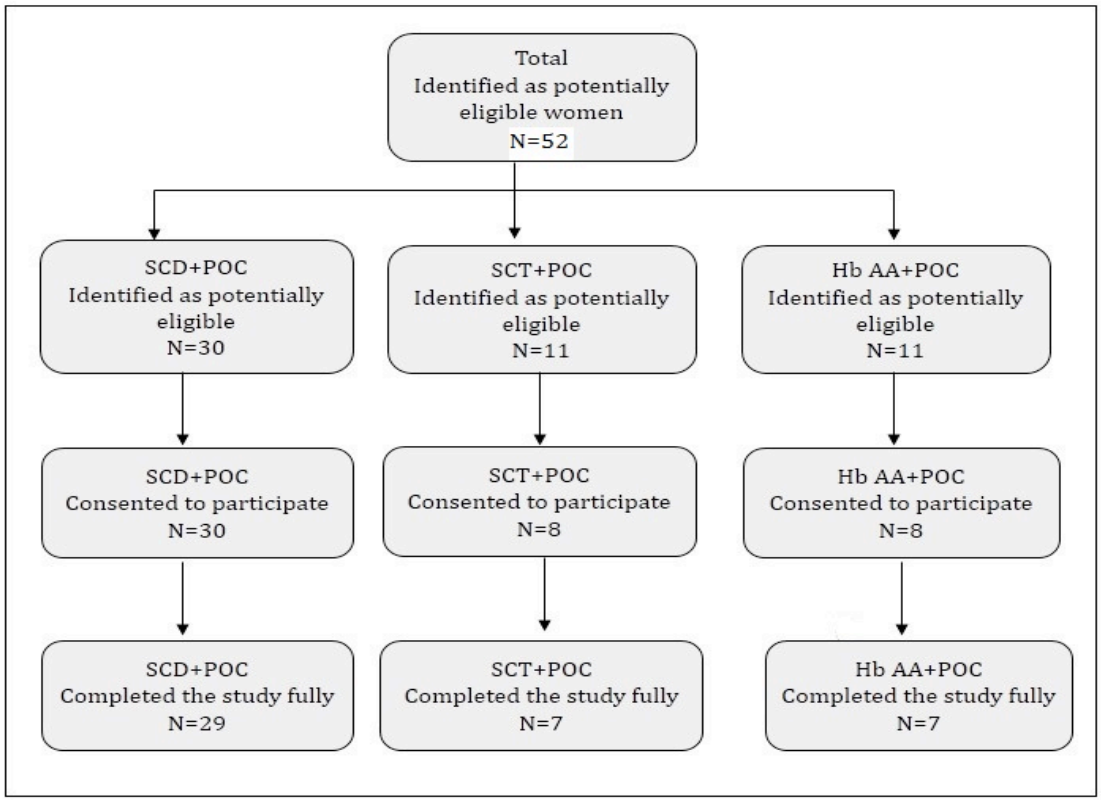

Figure 4-7 Flowchart of recruitment of women using POC

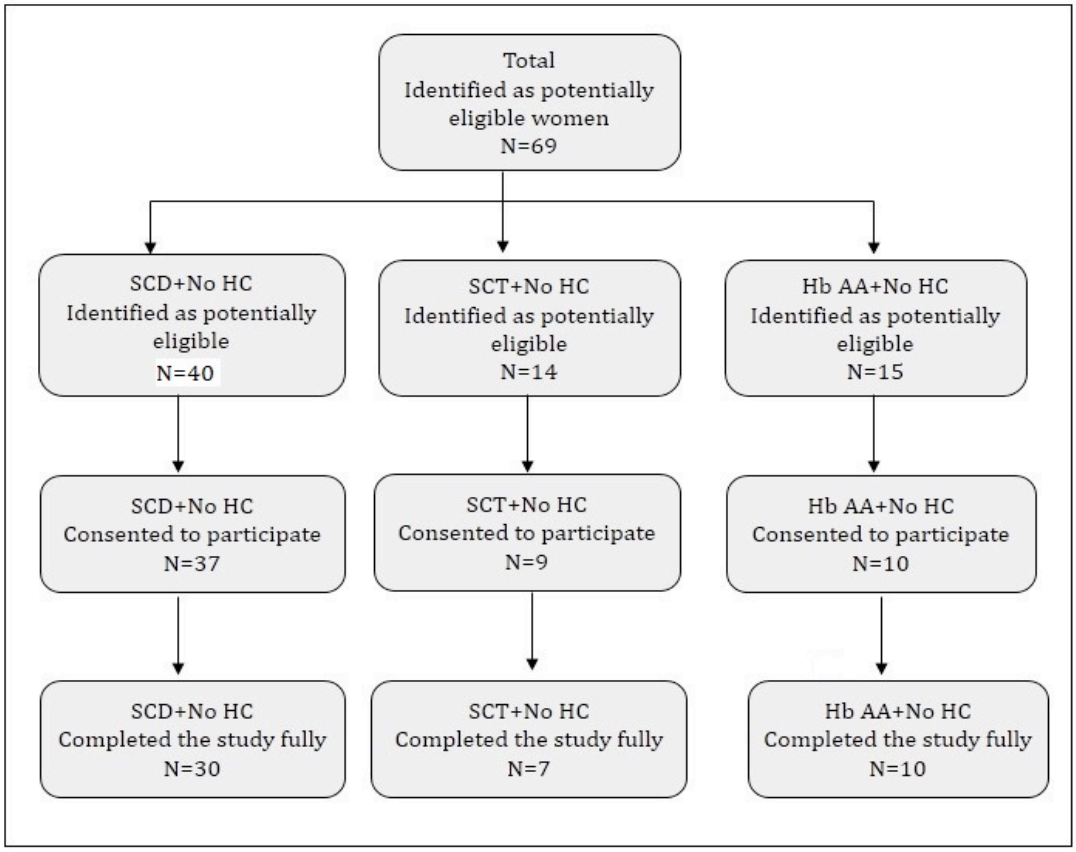

Figure 4-8 Flowchart of recruitment of women not using any hormonal contraception (HC)

Centre	SCD (n=68)	SCT (n=22)	Hb AA (n=27)	Overall% (n=117)
Camden and Islington Sickle cell and Thalassaemia Centre	3(4.41)	0	0	2.56
Central Middlesex Hospital and Brent Sickle and Thalassaemia Centre.	21(30.88)	0	4	21.36
Homerton University Hospital	7(10.29)	2	0	9.40
Royal Free Hospital (including women recruited from the SCS membership)	17(25.00)	20	23	51.28
University College Hospital	7(10.29)	0	0	5.98
Whittington Hospital	13(19.11)	0	0	11.11

Table 4-1 Number of study participants from each centre for each haemoglobin type

4.3 DEMOGRAPHIC ANALYSIS ACROSS SCD WOMEN

Population demographics for the 3 groups of contraception for women with SCD, were compared to each other in Table 4-2, where the non-hormonal contraceptive group served as the reference population. The following variables were compared between the groups: age, BMI, smoking status and cigarettes smoked per day. There were no statistically significant differences in these variables between the 3 groups.

Demographic variables	No HC (n = 30)	POC (n = 29)	COC (n =9)	P value
Mean age in years (SD)	37.56(9.75)	35.62(7.18)	25.33(5.65)	0.005
Difference (95% CI)		-1.94 (-6.25 to 2.37)	-12.23 (-17.72 to -1.6.73)	
BMI mean (SD)	25.62(4.42)	24.34(4.36)	24.23(4.79)	0.942
Difference (95% CI)		-1.28(-6.76 to 4.20)	-1.39 (-5.5 to 2.72)	
Smoking (%)	2 (6.66)	6 (20.68)	1 (11.11%)	
Difference (95% CI)		4(-0.11 to8.31)	-1(-5.11 to 3.11)	
No of cigarettes mean (SD)	0.46(1.94)	1.89(4.83)	0.44(1.33)	0.876
Difference (95% CI)		1.43 (-2.68 to 5.53)	-0,02(-4.33 to 4.29)	

Table 4-2 Demographics of the women with SCD

Using Bonferroni adujsment, there is a significant difference in age between the group of women using COC s and the others**.**

It is interesting to note that the number of cigarettes smoked per day is small in all the three groups. Also the prevalence of smoking among my study groups (except in the group using POC) is considerably lower than in the general population of young women. Anecdotally this seems to reflect a common cultural characteristic of black women of African origin, although this does not seem to be so noticeable in black women displaying Carribean cultural norms. Given the known association between smoking and thrombosis, this is clearly a favourable behaviour in the context of my study.

4.4 DEMOGRAPHIC ANALYSIS ACROSS SCT WOMEN

As for women with SCD, population demographics for the 3 groups of contraception for women with SCT were compared to each other in, where the non-hormonal contraceptive group served as the reference population in Table 4-3. The same variables were compared between the groups: age, BMI, smoking status and cigarettes smoked per day, although none of the women with SCT smoked. There were no statistical differences between COC users, POC users and the reference group, non-hormonal contraceptive users.

Demographic variables	No HC (n = 30)	POC (n = 29)	COC (n =9)	P
Mean age in years (SD)	32.28 (8.03)	36.57(4.54)	32.12(4.38)	0.232
Difference between means (95% CI)		4.29 (1.74 to 6.83)	-0.16 (-2.7 to 2.38)	
BMI mean (SD)	32.28(8.03)	25.07(2.38)	24.62(3.02)	0.747
Difference (95% CI)		-7.21(-11.13 to -3.29)	-7.66 (-11.58 to -3.74)	
Smoking (%)	0	0	0	

Table 4-3 Demographics of the women with SCT

ANOVA analysis, comparing the demographics of SCT POC and COC users to the SCT non-hormonal contraceptive users, showed no significant difference between the three groups.

4.5 DEMOGRAPHIC ANALYSIS ACROSS NORMAL HB WOMEN

Women with normal haemoglobin, AA, were similarly grouped by contraceptive use into, COC, POC and non-hormonal contraceptive users (no HC). The demographics of age, BMI, smoking status and cigarettes smoked per day were compared across these groups, where the no HC group served as the reference population in Table 4-4. As for women with SCD and SCT, there were no statistically significant differences in age, BMI and smoking between the three groups.

Demographic variables	No HC (n = 9)	POC (n = 8)	COC (n =10)	P
Mean age in years (SD)	37(9.68)	37.5(10.75)	31.8(9.41)	0.930
Difference between means (95% CI)		0.5 (-2.04 to 2.85)	-5.2 (-7.74 to -2.84)	
BMI mean (SD)	26.11(3.29)	27.62(2.77)	26.6(3.47)	0.829
Difference (95% CI)		1.51(-1.03 to 4.05)	-1.02 (-3.56 to 1.52)	
Smoking	1	0	0	
No of cigarettes mean (SD)	0.55(1.66)	n/a	n/a	

Table 4-4 Demographics of the women with Hb AA

ANOVA analysis, comparing the demographics of Hb AA POC and COC users to the Hb AA non-hormonal contraceptive users, showed no significant difference between the three groups.

4.6 DEMOGRAPHIC ANALYSIS OF COC USERS

The COC users recruited to the study were grouped according to their haemoglobin type into SCD, SCT and normal haemoglobin. The demographics of these 3 groups were compared, using the normal haemoglobin group, Hb AA as the reference population in Table 4-5. The 3 groups were comparable with regard to age, BMI and smoking habits.

Demographic variables	Hb AA (n = 10)	SCT (n = 8)	SCD (n =9)	P
Mean age in years (SD)	31.8(9.41)	32.12(4.38)	25.33(5.65)	0.100
Difference (95% CI)	Reference	0.32 (-2.42 to 3.06)	-6.47 (-8.82 to -4.11)	
BMI mean (SD)	26.6 (3.47)	24.62 (3.02)	24.23(4.79)	00.428
Difference (95% CI)	Reference	-1.98 (-6.09 to 2.13)	-2.37 (-4.72 to -0.018)	
Smoking (%)	0	0	1 (11.11%)	
No of cigarettes mean (SD)	0	0	0.44(1.33)	

Table 4-5 Demographic characteristics of COC users

4.7 DEMOGRAPHIC ANALYSIS OF POC USERS

Similarly the POC users recruited to the study were also grouped according to their haemoglobin type into SCD, SCT and normal haemoglobin. The demographics of these 3 groups were compared, using the normal haemoglobin group, Hb AA as the

reference population in Table 4-6. There were also no statistically significant differences in age, BMI and smoking between the three groups.

Demographic variables	Hb AA (n = 8)	SCT (n = 7)	SCD (n =29)	P
Mean age in years (SD)	37.5(10.75)	36.57(4.54)	35.62(5.65)	0.110
Difference (95% CI)	Reference	-0.93 (-3.67 to 1.61)	-0.95 (-3.69 to 1.79)	
BMI mean (SD)	27.62 (2.77)	25.07 (2.38)	24.34(4.35)	0.137
Difference (95% CI)	Reference	-2.55 (-5.87 to 1.37)	-0.73 (-3.27 to 1.81)	
Smoking (%)	1(12.50)	1(14.28)	6 (20.68%)	
Difference (95% CI)	Reference	0.06 (-2.09 to 2.21)	-0.02 (-2.33 to 2.37)	0.691
No of cigarettes mean (SD)	0	0	0.44(1.33)	

Table 4-6 Demographic characteristics of POC users

4.8 DEMOGRAPHIC ANALYSIS OF USERS OF NON-HORMONAL CONTRACEPTIVES

Women in the study who were not using hormonal contraception (non-HC) were also grouped according to their haemoglobin type into SCD, SCT and normal haemoglobin. The demographics of these 3 groups were compared, using the normal haemoglobin group, Hb AA as the reference population in Table 4-7 . There were also no statistically significant differences in age, BMI and smoking between the three groups

Demographic variables	Hb AA (n = 9)	SCT (n = 7)	SCD (n =30)	P
Mean age in years (SD)	32.28 (8.03)	37 (9.68)	37.56 (9.75)	0.848
Difference between means (95% CI)		4.72 (0.60 to 8.40)	5.28 (2.92 to 7.63)	
BMI mean (SD)	26.11 (3.29)	24.14 (3.28)	25.62 (4.41)	0.489
Difference between means (95% CI)		-1.97 (-5.10 to 0.57)	1.48 (-0.87 to -4.02)	
Smoking (%)	1 (10)	0	2 (22.22%)	
No of cigarettes mean (SD)	0.55 (1.66)	0	0.466 (1.94)	

Table 4-7 Demographic characteristics of non-HC users

CHAPTER 5

RESULTS OF THE COMPARISONS BY TYPE OF HAEMOGLOBIN

Figure 5-1: This is a picture of a twenty two year old patient with SCD (inserted with full permission of the participant).

5.1 INTRODUCTION

The study results for women with SCD will be presented first in the form of tables and the most important findings will be summarised at the end of these tables. This will be followed by tables and a summary of the results for the women with SCT. Finally, the findings in the women with normal Hb will be presented, again in the form of tables, and the key findings will be summarised.

5.2 CLINICAL EVENTS IN SCD WOMEN

Clinical variables	No HC (n = 30) Mean (SD)	POC (n = 29) Mean (SD)	COC (n = 9) Mean (SD)	P
Number of previous pregnancies	1.2 (1.09)	1.79 (1.47)	0.77 (0.83)	0.104
95% C.I.	Reference	0.59 (-0.06 to 1.24)	-0.42 (-1.37 to .052)	
Duration of use of contraceptive method prior to enrolment (months) mean (SD)	18 (4.72)	33.44 (7.53)	14.7 (3.65)	0.005
95% C.I.	Reference	15.54 (11.03 to 21.08)	-3.3 (-7.80 to -1.20)	
Type of SCD				
Hb SS	19	24	4	
Hb SC	9	2	5	
Hb S βThalassaemia	2	3	0	

Table 5-1 The obstetric history and duration of use of their contraceptive method prior to the enrolment interview in the SCD women

Clinical events	No HC (n=30)	POC (n=29)	COC (n=9)
Severe sickle crises (total)	14	12	2
Number of women with severe sickle crises	10	5	1
Hospital admissions (total)	16	13	3
Number of women with hospital admission.	11	7	2
Blood transfusions (total number of sessions)	5	3	0
Number of women receiving transfusion	3	3	0
VTE (DVT & PE)	0	0	0
Strokes & heart attacks	0	0	0
Arterial thrombosis	0	0	0

Table 5-2 Clinical events during the study in the SCD women

Clinical events	No HC (n=30)	POC (n=29)	COC (n=9)
Side effects (i.e. headaches, nausea, mood swings, bloatedness, breast tenderness)	0	4	1
Discontinuation	0	0	0
Unintended interruptions	0	1	1
Planned pregnancies	1	0	0
Unplanned pregnancies	1	0	0
Amenorrhoea	0	11	0
Menstrual irregularities	4	8	2

Table 5-3: Contraceptive side effects and problems during the study in the SCD women

5.3 LABORATORY RESULTS FOR SCD WOMEN

Laboratory marker (reference values)	No HC (n=30) Mean (SD)	POC (n=29) Mean (SD)	COC (n=9) Mean (SD)	P
Prothrombin time (12 – 15 secs)	14.93 (1.57)	15.35 (1.77)	14.83 (1.65)	0.814
Difference (95% CI)	Reference	0.42 (-0.44 to 1.29)	0.1 (-1.37 to 1.16)	
International normalised ratio (INR)(0.8-1.2)	1.16 (0.134)	1.19 (0.147)	1.15 (0.11)	0.652
Difference (95% CI)	Reference	0.03 (-0.04 to .1)	0 (-0.11 to .1)	
Activated partial thromboplastin time (25-35 seconds)	27.98 (2.35)	29.24 (3.55)	29.41 (2.68)	0.090
Difference (95% CI)	Reference	1.26 (-0.28 to 2.81)	1.43 (-0.82 to 3.68)	
Thrombin Time (12-14 seconds)	13.8 (1.00)	13.12 (1.28)	12.85 (0.67)	0.099
Difference (95% CI)	Reference	-0.68 (-1.25 to -0.1)	- 0.95 (-1.79 to -0.12)	
Fibrinogen (2.14-3.51 g/dL)	3.09 (0.61)	3.33 (0.56)	3.46 (0.80)	0.423
Difference (95% CI)	Reference	0.24 (-0.08 to 0.56)	0.37 (-0.1 to .84)	
Factor VIII Coagulant (50-150 iu/dL)	168.93 (51.58)	154.14 (41.74)	149.11 (38.16)	0.418
Difference (95% CI)	Reference	-14.79 (-38.98 to 9.4)	-19.82 (-54.81 to 15.16)	
von Willebrand Factor antigen (46 – 153 (iu/dL)	181.96 (68.43)	171.33 (64.39)	164.22 (66.28)	0.950
Difference (95% CI)	Reference	-10.64 (-45.2 to =23.93)	-17.74 (-68.19 to -32.7)	
Dilute Viper Venom Time (ratio) (<1.16)	0.92 (0.17)	0.92 (0.12)	0.91 (0.07)	0.025
Difference (95% CI)	Reference	-0.01 (-0.08 to -0.07)	-0.01 (-0.12 to -0.09)	

Table 5-4: Pro-and anti-thrombotic biochemical markers in SCD (1)

Laboratory marker (reference values)	No HC (n=30) Mean (SD)	POC (n=29) Mean (SD)	COC (n=9) Mean (SD)	P
Anti-thrombin activity (85-113 iu/dL)	98.56 (10.89)	95.58 (9.18)	92.02 (13.40)	0.356
Difference (95% CI)	Reference	-2.98 (-8.47 to 2.51)	-6.54 (-14.55 to 1.47)	
Protein C activity (70-135 iu/dL)	85.03 (15.49)	82.00 (12.53)	75.53 (20.23)	0.188
Difference (95% CI)	Reference	-3.03 (-10.84 to 4.77)	-9.5 (-20.89 to 1.89)	
Free Protein S (75-125 iu/dL)	56.3 (13.30)	59.06 (14.95)	59.20 (16.87)	0.655
Difference (95% CI)	Reference	2.77 (-4.78 to 10.31)	2.9 (-8.11 to 13.91)	
Activated Protein C Resistance (Factor V Leiden) (>2.0)	2.84 (0.35)	2.87 (0.31)	2.80 (0.14)	0.030
Difference (95% CI)	Reference	0.04 (-0.14 to 0.21)	-0.04 (-0.28 to -0.2)	
Factor II (84.3-120.0 iu/dL)	90.33 (14.27)	91.27 (9.99)	91.00 (11.93)	0.179
Difference (95% CI)	Reference	0.94 (-5.46 to 7.34)	0.67 (-8.68 to 10.01)	
Factor V (63.85-120.5 iu/dL)	100.30 (18.62)	95.93 (16.33)	98.88 (12.46)	0.404
Difference (95% CI)	Reference	-4.37 (-13.21 to 4.47)	-1.41 (-14.31 to 11.49)	
Factor VII (77.5-150.5 iu/dL)	92.40 (22.70)	78.17 (22.40)	83.11 (21.30)	0.976
Difference (95% CI)	Reference	-14.23 (-25.88 to -2.57)	-9.29 (-26.3 to 7.72)	

Table 5-5: Pro-and anti-thrombotic biochemical markers in SCD (2)

Laboratory marker (reference values)	No HC (n=30) Mean (SD)	POC (n=29) Mean (SD)	COC (n=9) Mean (SD)	P
Factor IX (77.7-130.0 iu/dL)	121.16 (29.69)	120.65 (41.70)	121.88 (22.28)	0.060
Difference (95% CI)	Reference	-0.51 (-18.56 to 17.53)	0.72 (-25.61 to 27.06)	
Factor X (71.8-130.0 iu/dL)	94.83 (22.96)	89.62 (16.62)	102.55 (19.63)	0.243
Difference (95% CI)	Reference	-5.21 (-15.64 to 5.21)	7.72 (-7.49 to 22.94)	
Factor XI (68-144 iu/dL)	92.36 (20.84)	86.03 (15.95)	89.44 (14.58)	0.265
Difference (95% CI)	Reference	-6.33 (-15.78 to 3.11)	-2.92 (-16.7 to 10.86)	
Factor XII (46.7-181.0 iu/dL)	99.76 (24.16)	83.68 (30.19)	97.77 (32.82)	0.392
Difference (95% CI)	Reference	-16.08 (-30.66 to -1.5)	-1.99 (-23.27 to 19.29)	
Factor XIIIa (97.4-179.0 iu/dL)	66.10 (29.90)	66.39 (18.26)	67.55 (26.06)	0.391
Difference (95% CI)	Reference	0.29 (-10.76 to 11.34)	1.46 (-14.52 to 17.44)	

Table 5-6 Pro and anti-thrombotic biochemical markers in SCD (3)

In tables 5.4, 5.5 and 5.6 I used ANOVA to compare the Pro- and anti-thrombotic biochemical markers in women with SCD using COC and POC with SCD women who are not using-hormonal contraception. The 95% CI are derived for these comparisons. The results from SCD women not using hormonal contraception, were used as the reference point for the analyses

Laboratory marker (reference ranges)	No HC (n = 30) Mean (SD)	POC (n = 29) Mean (SD)	COC (n = 9) Mean (SD)	P
Thrombin:anti-thrombin III complex (2 - 4.2 mcg/l)	19.70 (40.28)	5.45 (3.06)	8.15 (4.29)	<0.001
Difference (95% CI)	Reference	-14.24 (-28.89 to -0.41)	-11.55 (-33.43 to -10.33)	
Prothrombin fragment 1 + 2 (69 – 229 pmol/L)	492.72 (380.72)	374.37 (277.51)	244.62 (114.91)	0.005
Difference (95% CI)	Reference	-118.35 (-288.2 to 51.49)	-248.1 (-501.73 to -5.53)	
D dimer (<130 ng/ml)	943.76 (1019.4)	762.17 (728.76)	614.44 (283.46)	0.001
Difference (95% CI)	Reference	-181.59 (-617.44 to -254.26)	-329.32 (-965.42 to -306.77)	

Table 5-7: Coagulation activation markers in the SCD women

Laboratory marker(reference ranges)	No HC (n = 30) Mean (SD)	POC (n = 29) Mean (SD)	COC (n = 9) Mean (SD)	P
Soluble Platelet selectin(92-212 ng/ml)	84.89 (32.91)	81.77 (26.71)	72.00 (20.47)	0.249
Difference (95% CI)	Reference	-3.12 (-18.64 to 12.4)	-12.9 (-35.04 to 9.25)	
Soluble Cell Differentiation 40 ligand(0.03-3.98 ng/ml)	1.32 (3.19)	1.52 (1.69)	1.06 (0.92)	<0.001
Difference (95% CI)	Reference	0.19 (-1.11 to -1.49)	-0.26 (-2.12 to -1.59)	
Platelet factor 4 (<10I.U./ml)	140.62 (74.09)	147.51 (67.63)	150.38 (66.90)	0.884
Difference (95% CI)	Reference	6.89 (-31.89 to 45.68)	9.76 (-47.06 to 66.59)	

Table 5-8: Platelet activation markers in the SCD women

Laboratory marker (reference values)	No HC (n=30) Mean (SD)	POC (n=29) Mean (SD)	COC (n=9) Mean (SD)	P
Soluble vascular cell adhesion molecule (131-1223 ng/ml))	1095.10 (563.89)	1162.03 (917.68)	1003.22 (604.20)	0.036
Difference (95% CI)	Reference	66.93 (-327.32 to 461.18)	-91.88 (-654.39 to 470.62)	
Soluble inter-cellular adhesion molecule-1 (41-669 ng/ml)	555.06 (162.63)	528.03 (128.44)	445.06 (119.85)	0.377
Difference (95% CI)	Reference	-27.03 (-103.98 to 49.91)	-110 (-219.78 to -.21)	
Soluble endothelial-leucocyte adhesion molecule-1 (1.5 – 88.1ng/ml))	86.86 (34.49)	52.66 (29.58)	89.77 (52.84)	0.091
Difference (95% CI)	Reference	-16.2 (-36.18 to -3.78)	20.92 (-7.59 to -49.42)	

Table 5-9: Endothelial activation markers in the SCD women

Laboratory marker (reference values)	No HC (n=30) Mean (SD)	POC (n=29) Mean (SD)	COC (n=9) Mean (SD)	P
Micro particles (<10nMol/ml)	34.94 (20.36)	39.03 (27.10)	25.41 (13.46)	0.061
Difference (95% CI)	Reference	4.09 (-7.94 to 16.12)	-9.53 (-27.01 to 7.94)	
Tissue Factor(<2pMol/ml)	12.07 (5.96)	8.83 (6.94)	13.72 (5.40)	0.626
Difference (95% CI)	Reference	-3.24 (-6.75 to .27)	1.65 (-3.19 to 6.48)	

Table 5-10: Markers of tissue damage in the SCD women

Laboratory marker (reference values)	No HC (n=30) Mean (SD)	POC (n=29) Mean (SD)	COC (n=9) Mean (SD)	P
Lag Time (initiated with 5pMol Tissue Factor)(2.0 - 3.2 minutes)	1.66 (0.66)	1.59 (0.53)	1.36 (0.34)	0.095
Difference (95% CI)	Reference	-0.06 (-0.37 to 0.24)	-0.29 (-0.73 to 0.15)	
Endogenous Thrombin Potential (initiated with 5pMol Tissue Factor)(1159 – 2168 nMol)	1345.43 (245.32)	1480.67(269.79)	1482.88 (348.20)	0.431
Difference (95% CI)	Reference	135.25 (-6.72 to 277.21)	137.46 (-67.87 to 342.78)	
Peak Height (initiated with 5pMol Tissue Factor)(147 – 359 nM)	305.64 (85.92)	319.07 (52.31)	342.66 (51.52)	0.792
Difference (95% CI)	Reference	13.43 (-15.61 to 42.46)	37.02 (-4.98 to 79.02)	
Time to Peak (initiated with 5pMol Tissue Factor)(4.6 – 7.4 min)	3.90 (0.96)	3.91 (0.82)	3.43 (0.38)	0.028
Difference (95% CI)	Reference	0.02 (-0.43 to 0.47)	-0.47 (-1.12 to -0.18)	
Slope (33 – 142 nM/min)	142.72 (40.36)	143.25 (39.32)	168.51 (35.63)	0.914
Difference (95% CI)	Reference	0.53 (-20.13 to 21.19)	25.79 (-4.1 to 55.67)	
Start Time (initiated with 5pMol Tissue Factor)(19 28.3 min)	17.48 (2.01)	18.57 (2.75)	17.50 (2.29)	0.263
Difference (95% CI)	Reference	1.09 (-0.16 to 2.34)	0.02 (-1.79 to 1.83)	

Table 5-11: Markers of thrombin generation in the SCD women

Laboratory marker (reference values)	No HC (n=30) Mean (SD)	POC (n=29) Mean (SD)	COC (n=9) Mean (SD)	P
Free Hb (0- 1/l)	0.10 (0.09)	0.09 (0.10)	0.10 (0.05)	0.120
Difference (95% CI)	Reference	-0.01 (-0.06 to .03)	0 (-0.07 to .07)	
Haptoglobin (< 1.22 g/l)	1.54 (0.46)	1.56 (0.66)	1.58 (0.70)	0.154
Difference (95% CI)	Reference	0.02 (-0.31 to 0.35)	0.05 (-0.4 to 0.49)	
Lactate dehydrogenase (240-480 units/l)	734.17 (321.20)	637.23 (227.28)	555.50 (210.22)	0.148
Difference (95% CI)	Reference	-96.94 (244.73 to 50.84)	-178.67 (397.19 to 39.85)	
Bilirubin (< 21 µMol/l)	39.74 (27.72)	38.11 (32.63)	32.00 (20.80)	0.326
Difference (95% CI)	Reference	-1.63 (-17.52 to 14.26)	-7.74 (-30.22 to 14.74)	

Table 5-12: Markers of haemolysis in the SCD women

Laboratory marker (reference values)	No HC (n=30) Mean (SD)	POC (n=29) Mean (SD)	COC (n=9) Mean (SD)	P
Alanine amino transferase (<33 units/l)	22.07 (9.240	19.96 (11.89)	23.11 (12.46)	0.364
Difference (95% CI)	Reference	-2.11 (-7.89 to 3.68)	1.04 (-7.32 to 9.4)	
Aspartate amino transferase (<31 units/l)	35.19 (19.55)	29.78 (11.91)	34.00 (14.26)	0.045
Difference (95% CI)	Reference	-5.41 (-14.03 to 3.21)	-1.19 (-13.43 to -0.11)	
Alkaline phosphatase (< 129 units/l)	88.13 (36.43)	86.86 (48.12)	89.66 (65.29)	0.078
Difference (95% CI)	Reference	1.28 (-25.47 to 22.92)	1.53 (-33.62 to 36.68)	
Serum albumin (35-50g/l)	45.82 (2.91)	45.79 (3.56)	44.66 (5.83)	0.028
Difference (95% CI)	Reference	-.03 (-1.97 to 1.9)	-1.16 (-3.97 to -0.164)	

Table 5-13: Biochemical markers of liver function in the SCD women

Laboratory marker (reference values)	No HC (n=30) Mean (SD)	POC (n=29) Mean (SD)	COC (n=9) Mean (SD)	P
Haemoglobin (11.5-15-5 g/dl)	9.07 (1.44)	9.22 (1.55)	10.37 (1.73)	0.788
Difference (95% CI)	Reference	0.16 [-.64 to .95]	1.31 [.14 to 2.47]	
Platelet count (140-400 10^9/l)	331.40 (144.39)	323.39 (134.12)	310.77 (109.49)	0.652
Difference (95% CI)	Reference	-8.01 [-79.48 to 63.46]	-20.62 [-123.99 to 82.75]	
Haematocrit (0.35-0.47 l/l)	0.26 (0.03)	0.26 (0.04)	0.27 (0.05)	0.305
Difference (95% CI)	Reference	0.01 [-.02 to .03]	0.02 [-.02 to .05]	

Table 5-14: Blood count values in the SCD women

Laboratory marker (reference values)	No HC (n=30) Mean (SD)	POC (n=29) Mean (SD)	COC (n=9) Mean (SD)	P
Complement reactive protein (0.2-10.0 mcg/ml)	35.39 (22.07)	35.58 (19.20)	42.11 (31.31)	0.205
Difference (95% CI)	Reference	0.23 (-1.34 to 1.79)	1.67 (-0.57 to 3.91)	
White blood cells (3.5-11.0 10^9/l)	8.22 (2.69)	8.45 (3.42)	9.89 (2.04)	0.190
Difference (95% CI)	Reference	0.28 (-0.86 to 1.43)	1.32 (-0.27 to 2.91)	
Neutrophils (1.7-8.0 10^9/l)	4.22 (1.70)	4.50 (2.38)	5.54 (2.06)	0.261
Difference (95% CI)	Reference	0.27 (-0.27 to 0.8)	0.18 (-0.56 to 0.92)	
Lymphocytes (1.0-3.510^9/l)	2.57 (1.07)	2.83 (0.89)	2.75 (0.86)	0.686
Difference (95% CI)	Reference	0 (-0.13 to 0.12)	0.11 (-0.07 to 0.28)	
Monocytes (0.1-1.010^9/l)	0.70 (0.37)	0.82 (0.36)	0.91 (0.55)	0.271
Difference (95% CI)	Reference	0.12 (-0.1 to 0.34)	0.21 (-0.1 to 0.52)	
Eosinophils (0.0-0.46 10^9/l))	0.23 (0.22)	0.22 (0.19)	0.33 (0.31)	0.210
Difference (95% CI)	Reference	0 (-0.13 to 0 .12)	0.11 (-0.07 to 0.28)	
Basophils (0.0-0.20 10^9/l)	0.04 (0.03)	0.04 (0.04)	0.04 (0.02)	0.036
	Reference	0 (-0.02 to .02)	0 (0.03 to .03)	

Table 5-15: Inflammatory markers in the SCD women

5.4 SUMMARY OF RESULTS FOR THE SCD WOMEN

It is noticeable that SCD women who are using POC had been using their method for a significantly longer time period of time prior to enrollment in the study. This partly results from the women using Mirena IUS® , which is usually regarded as a longer time scale method (5 years between changes).Of the women who are using any hormonal contraception method, those using POC were 3 times as many as those using COC.

In terms of serious clinical events there were fewer severe sickle cell crises in the women on COC, who also had fewer hospital admissions and fewer episodes of blood transfusion.The woman who suffered VTE during the study was not using hormonal contraception. There were no episodes of stroke and heart attack during the study period.

The women most frequently experiencing method specific side effects during the study were those on POC, in whom the main issues were menstrual irregularities and amenorrhea.

In the pro and anti- coagulant markers studied, the only abnormally high marker was VWF which showed mild elevation in all three groups without any statistically

significant difference. On the other hand although, the mean values for APCR lie within the reference range the differences between the groups did reach statistical significance.

All the coagulation activation markers studied show levels higher than the reference range, which is an adverse result. However, these levels are also significantly increased in the group of SCD women, who are not using any hormonal contraception.

The platelet activation marker PF4 is markedly increased (an adverse result) without any statistically significant difference between the three groups. The sCD40 ligand mean level is increased in the group using POC compared to other SCD women and this increase reached statistical significance, although the actual levels are still within the reference range.

Endothelial activation markers showed no marked differences between SCD women on different methods of contraception, except the level of sVCAM which was increased in the group using POC.

Markers of tissue damage showed marked elevation. MP is about 2.5 to 3 times higher than the reference range, while TF 4 was 2 to 6 times higher in all the three

groups. There is no statistically significant difference between the groups though. Thrombin generation tests are all within the reference range in all three groups.

LDH and bilirubin showed marked elevation compared to the reference range in all three groups.

Liver function tests showed abnormal results, with AST levels higher in SCD women not using any hormonal contraception and in women using COC The difference in its mean values reached statistical significance and CRP was 3 to 4 times higher than the reference range. This elevation occurred in all the three groups. The blood count values are not markedly different between the three groups. The interpretation of these results will be discussed in detail in Chapter 7.

Figure 5-2 This is a picture of a thirty two year old woman with SCT (inserted with full permission of the participant).

The study results for women with SCT will be presented in this section first in the form of tables and the most important findings will be summarised at the end of these tables, as mentioned previously.

5.5 CLINICAL EVENTS IN SCT WOMEN

Clinical variables	No HC (n=7) Mean (SD)	POC (n=7) Mean (SD)	COC (n=8) Mean (SD)	P
Number of previous pregnancies (mean+/-SD)	1.42 (1.13)	1.71 (0.95)	1.12 (0.640)	0.904
Difference (95% CI)	Reference	0.29 (-0.74 to 1.31)	-0.3 (-1.3 to 0.69)	
Duration of use of contraceptive method prior to enrolment (months) (mean+/-SD)	16 (5.12)	12 (6.80)	11.5 (6.48)	0.781
Difference (95% CI)	Reference	-0.4 (-9.48 to 1.49)	-4.5 (-10.8 to 1.18)	

Table 5-16 The obstetric history and duration of use of their contraceptive method prior to the enrolment interview in the SCT women

Clinical variables	No HC (n=7)	POC (n=7)	COC (n=8)
Severe sickle crises (total)	0	0	0
Hospital admissions (total)	2	1	0
Blood transfusions (total number of sessions)	0	0	0
VTE (DVT & PE)	0	0	0
Strokes & heart attacks	0	0	0
Arterial thrombosis	0	0	0

Table 5-17 Clinical events during the study in the SCT women

Clinical variables	No HC (n=7)	POC (n=7)	COC (n=8)
Side effects (i.e. headaches, nausea, mood swings, bloatedness, breast tenderness)	0	1	2
Discontinuation	0	0	0
Unintended interruptions	2	0	0
Planned pregnancies	0	0	0
Unplanned pregnancies	0	0	0
Amenorrhoea	0	1	0
Menstrual irregularities	2	1	0

Table 5-18: Contraceptive side effects and problems during the study in the SCT women

5.6 LABORATORY RESULTS FOR SCT WOMEN

Laboratory marker (Reference values)	No HC (n=7) Mean (SD)	POC (n=7) Mean (SD)	COC (n=8) Mean (SD)	P
Prothrombin time (12 – 15 secs)	11.02 (0.77)	11.51 (0.94)	11.73 (1.28)	0.459
Difference (95% CI)	Reference	0.49 (-0.68 to 1.65)	0.71 (-0.42 to 1.84)	
International normalised ratio (INR)(0.8-1.2)	1.03 (0.07)	1.03 (0.02)	1.06 (0.04)	0.106
Difference (95% CI)	Reference	0 (-0.06 to 0.05)	0.02 (-0.03 to .08)	
Activated partial thromboplastin time (25-35 seconds)	32.05 (4.44)	30.27 (1.30)	29.53 (1.78)	0.009
Difference (95% CI)	Reference	-1.79 (-4.94 to 1.37)	-2.52 (-5.57 to -0.53)	
Thrombin Time (seconds)(12-14)	15.38 (1.88)	14.22 (0.83)	14.02 (1.22)	0.163
Difference (95% CI)	Reference	-1.16 (-2.7 to 0.38)	-1.36 (-2.85 to 0.13)	
Fibrinogen (2.14-3.51 g/dL)	4.93 (3.59)	3.25 (0.09)	2.94 (0.66)	<0.001
Difference (95% CI)	Reference	-1.68 (-4.06 to 0.7)	-1.99 (-4.29 to -0.31)	
Factor VIII Coagulant (50-150 iu/dL)	148.00 (19.00)	154.57 (80.06)	121.00 (34.05)	0.004
Difference (95% CI)	Reference	6.57 (-50.1 to 63.24)	-27 (-81.87 to -27.87)	
von Willebrand Factor antigen (46 – 153 iu/dL)	124.32 (36.75)	86.92 (32.00)	105.87 (33.40)	0.944
Difference (95% CI)	Reference	-37.4 (-75.52 to 0.72)	-18.45 (-55.37 to -18.46)	
Dilute Viper Venom Time (ratio) (<1.16)	0.94 (0.21)	1.13 (027)	0.99 (0.16)	0.466
Difference (95% CI)	Reference	0.19 (-0.06 to 0.43)	0.04 (-0.19 to 0.28)	

Table 5-19 Pro-and anti-thrombotic biochemical markers in SCT women (1)

Laboratory marker (Reference values)	No HC (n=7) Mean (SD)	POC (n=7) Mean (SD)	COC (n=8) Mean (SD)	P
Anti-thrombin : Anti-coagulant ratio (85-113)	108.08 (8.74)	108.26 (6.43)	100.45 (9.39)	0.648
Difference (95% CI)	Reference	0.17 (-9.17 to 9.52)	-7.63 (-16.67 to 1.42)	
Protein C : Activity (70-135 iu/dL)	100.28 (21.62)	102.80 (28.03)	99.80 (15.46)	0.368
Difference (95% CI)	Reference	-0.49 (-25.82 to 24.85)	2.51 9-22.02 to 27.04)	
Free Protein S (75-125 iu/dL)	70.28 (14.63)	74.94 (11.78)	87.12 (38.13)	0.009
Difference (95% CI)	Reference	4.66 (-23.81 to 33.12)	16.84 (10.72 to 44.4)	
Activated Protein C Resistance (Factor V Leiden) (>2.0)	2.71 (0.12)	2.85 (0.11)	2.75 (0.24)	0.087
Difference (95% CI)	Reference	0.14 (-0.06 to 0.34)	0.01 (0.18 to 0.2)	
Factor II (84.3-120.0 iu/dL)	95.71 (20.87)	104.71 (7.34)	107.12 (8.32)	0.018
Difference (95% CI)	Reference	9 (-6.02 to 24.02)	11.41 (3.13 to 25.95)	
Factor V (63.85-120.5 iu/dL)	81.71 (23.02)	97.00 (17.22)	98.00 (28.33)	0.493
Difference (95% CI)	Reference	15.29 (-11.12 to 41.69)	16.29 (9.28 to 41.85)	
Factor VII (77.5-150.5 iu/dL)	83.14 (21.11)	93.42 (16.68)	76.75 (12.18)	0.409
Difference (95% CI)	Reference	10.29 (-8.55 to 29.12)	-6.39 (-24.63 to -11.84)	

Table 5-20: Pro-and anti-thrombotic biochemical markers in SCT women (2)

Laboratory marker (Reference values)	No HC (n=7) Mean (SD)	POC (n=7) Mean (SD)	COC (n=8) Mean (SD)	P
Factor IX (77.7-130.0 iu/dL)	101.14 (16.75)	108.57 (28.40)	95.75 (23.11)	0.474
Difference (95% CI)	Reference	7.43 (-18.58 to 33.43)	-5.39 (-30.57 to -19.79)	
Factor X (71.8-130.0 iu/dL)	91.71 (10.64)	102.57 (21.43)	98.25 (14.20)	0.247
Difference (95% CI)	Reference	10.86 (-7.01 to 28.73)	6.54 (-10.77 to -23.84)	
Factor XI (68-144 iu/dL)	91.42 (21.46)	102.42 (13.95)	100.87 (18.31)	0.603
Difference (95% CI)	Reference	11 (-9.34 to 31.34)	9.45 (-10.24 to -29.14)	
Factor XII (46.7-181.0 iu/dL)	85.71 (36.11)	103.14 (23.51)	95.87 (20.37)	0.329
Difference (95% CI)	Reference	17.43 (-13 to 47.85)	10.16 (-19.3 to -39.62)	
Factor XIIIa (97.4-179.0 iu/dL)	97.85 (14.62)	81.57 (30.50)	78.37 (27.80)	0.225
Difference (95% CI)	Reference	-16.29 (-44.73 to 12.15)	-19.48 (-47.02 - to 8.06)	

Table: 5-21 Pro-and anti-thrombotic biochemical markers in SCT women (3)

Laboratory marker (Reference range)	No HC (n = 7) Mean (SD)	POC (n = 7) Mean (SD)	COC (n = 8) Mean (SD)	P
Thrombin:anti-thrombin III complex (2-4.2 mcg/l)	3.12 (0.57)	4.04 (1.49)	10.61 (19.97)	<0.007
Difference (95% CI)	Reference	0.92(-18.68 to 20.52)	7.49(04.15 to 25.13)	
Prothrombin fragment 1 + 2 (69 – 229 pmol/L)	142.71 (109.00)	139.00 (51.36)	210.00 (219.45)	0.005
Difference (95% CI)	Reference	-3.71(-101.71 to -94.29)	67.29 (34.32 to 95.39 to	
D dimer (<130 ng/ml)	126.20 (70.58)	196.00 (106.69)	184.37 (200.72)	0.079
Difference (95% CI)	Reference	69.8 (89.55 to 143.55)	58.16 (33.55 to 143.55)	

Table 5-22: Coagulation activation markers in SCT women

Laboratory marker (Reference values)	No HC (n=7) Mean (SD)	POC (n=7) Mean (SD)	COC (n=8) Mean (SD)	P
Soluble Platelet selectin(92-212 ng/ml)	52.57 (14.05)	47.14 (20.60)	63.50 (16.22)	0.652
Difference (95% CI)	Reference	-5.43 (-24.59 to 13.73)	10.93 (-7.63 to 29.48)	
Soluble Cell Differentiation 40 ligand(0.03-3.98 ng/ml)	0.48 (0.32)	1.25 (1.36)	1.24 (1.05)	0.011
Difference (95% CI)	Reference	0.77 (-0.37 to 1.9)	0.76 (-0.35 to 1.86)	
Platelet factor 4 (<10 I.U./ml)	77.00 (6.28)	78.50 (17.67)	137.00 (97.58)	0.004
Difference (95% CI)	Reference	1.5 (-82.05 to 85.05)	60 (23.55 to 143.55)	

Table 5-23: Platelet activation markers in the SCT women

Laboratory marker (Reference values)	No HC (n=7) Mean (SD)	POC (n=7) Mean (SD)	COC (n=8) Mean (SD)	P value
Soluble vascular cell adhesion molecule (131-1223 ng/ml))	486.42 (206.99)	606.42 (505.56)	419.75 (178.04)	0.018
Difference (95% CI)	Reference	120 (-244.11 to 484.11)	-66.68 (-419.23 to 285.87)	
Soluble inter-cellular adhesion molecule-1 (41-669 ng/ml)	281.44 (70.19)	271.87 (41.29)	281.62 (69.55)	0.404
Difference (95% CI)	Reference	-9.57 (-79.23 to 60.09)	0.18 (-67.27 to 67.63)	
Soluble endothelial-leucocyte adhesion molecule-1 (1.5 – 88.1ng/ml))	51.14 (22.98)	81.00 (35.18)	136.12 (138.41)	<0.001
Difference (95% CI)	Reference	29.86 (-67.78 to 127.49)	84.98 (9.55 to 179.52)	

Table 5-24: Endothelial activation markers in SCT women

Laboratory marker (Reference values)	No HC (n=7) Mean (SD)	POC (n=7) Mean (SD)	COC (n=8) Mean (SD)	P
Micro particles (nMol/ml) (< 10)	8.34 (5.15)	8.32 (4.02)	9.96 (5.36)	0.769
Difference (95% CI)	Reference	-0.01 (-5.5 to 5.48)	1.62 (-3.7 to 6.94)	
Tissue Factor(< 2 pMol/ml)	5.48 (7.54)	2.12 (1.89)	5.67 (5.43)	0.015
Difference (95% CI)	Reference	-3.36 (-9.49 to 2.77)	0.19 (0.05 to 6.12)	

Table 5-25: Markers of tissue damage in SCT women

Laboratory marker (Reference values)	No HC (n=7) Mean (SD)	POC (n=7) Mean (SD)	COC (n=8) Mean (SD)	P
Lag Time (initiated with 5pMol Tissue Factor) (2.0 - 3.2 minutes)	2.38 (1.38)	2.24 (0.56)	1.76 (0.26)	0.001
Difference (95% CI)	Reference	-0.14 (-1.13 to 0.85)	-0.62 (-1.61 to -0.37)	
Endogenous Thrombin Potential (initiated with 5pMol Tissue Factor) (1159 – 2168 nMol)	1484.71 (332.92)	1303.00 (230.56)	1608.14 (282.05)	0.691
Difference (95% CI)	Reference	-181.71 (-501.69 to 138.26)	123.43 (-196.54 to 443.4)	
Peak Height (initiated with 5pMol Tissue Factor)(147 – 359 nM)	283.85 (59.41)	233.85 (86.88)	272.51 (23.38)	0.021
Difference (95% CI)	Reference	-50 (-119.91 to 19.91)	-11.34 (-81.25 to -5.85)	
Time to Peak (initiated with 5pMol Tissue Factor)(4.6 – 7.4 min)	4.93 (1.63)	4.12 (0.80)	4.40 (0.95)	0.198
Difference (95% CI)	Reference	-0.8 (-2.14 to .53]	-0.52 (-1.86 to 0.81)	
Slope (33 – 142 nM/min)	114.90 (33.82)	132.41 (63.94)	114.57 (39.86)	0.278
Difference (95% CI)	Reference	17.51 (-36.04 to 71.07)	-0.33 (-53.88 to 53.22)	
Start Time (initiated with 5pMol Tissue Factor)(19 - 28.3 min)	20.92 (2.22)	24.21 (5.50)	23.14 (3.91)	0.128
Difference (95% CI)	Reference	3.29 (-1.33 to 7.9)	2.21 (-2.4 to 6.83)	

Table 5-26: Markers of thrombin generation in SCT women

Laboratory marker (Reference values)	No HC (n=7) Mean (SD)	POC (n=7) Mean (SD)	COC (n=8) Mean (SD)	P
Free Hb (0-1/l)	0.14 (0.19)	0.12 (0.09)	0.05 (0.05)	0.194
Difference (95% CI)	Reference	-0.02 (-0.16 to 0.13)	0.09 (-0.23 to 0.05)	
Haptoglobin (< 1.22 g/l)	1.11 (0.43)	1.15 (0.44)	0.88 (0.34)	0.776
Difference (95% CI)	Reference	0.03 (-0.42 to 0.49)	-0.23 (-0.68 to 0.21)	
Lactate dehydrogenase (240-480 units/l)	417.83 (76.16)	467.28 (57.29)	406.71 (62.45)	0.803
Difference (95% CI)	Reference	49.45 (-26.99 to 125.9)	-11.12 (-87.56 to 65.33)	
Bilirubin (< 21 µMol/l)	5.28 (1.70)	7.00 (3.46)	9.62 (5.620	0.028
Difference (95% CI)	Reference	1.71 (-2.81 to 6.24)	4.34 (0.05 to 8.72)	

Table 5-27: Markers of haemolysis in SCT women

Laboratory marker (Reference values)	No HC (n=7) Mean (SD)	POC (n=7) Mean (SD)	COC (n=8) Mean (SD)	P
Alanine amino transferase (<33 units/l)	20.14 (13.35)	14.71 (3.90)	19.12 (5.61)	0.820
Difference (95% CI)	Reference	-5.43 (-14.97 to 4.12)	-1.02 (-10.26 to 8.22)	
Aspartate amino transferase (<31 units/l)	23.71 (9.17)	23.14 (4.87)	19.75 (2.49)	0.219
Difference (95% CI)	Reference	-0.57 (-7.32 to 6.18)	-3.96 (-10.5 to 2.57)	
Alkaline phosphatase (< 129 units/l)	54.57 (22.99)	64.14 (17.78)	56.62 (18.89)	0.844
Difference (95% CI)	Reference	9.57 (-12.76 to 31.9)	2.05 (-19.57 to 23.68)	
Serum albumin (35-50g/l)	42.42 (1.61)	43.71 (2.13)	45.12 (6.08)	0.206
Difference (95% CI)	Reference	1.29 (-3.17 to 5.75)	2.7 (-1.62 to 7.02)	

Table: 5-28 Biochemical markers of liver function in SCT women

Laboratory marker (Reference values)	No HC (n=7) Mean (SD)	POC (n=7) Mean (SD)	COC (n=8) Mean (SD)	P
Haemoglobin (11.5-15-5 g/dl)	11.65 (0.54)	12.47 (1.03)	11.75 (1.17)	0.265
Difference (95% CI)	Reference	-0.62 (-93.31 to 92.07)	26.38 (-66.31 to 119.07)	
Platelet count (140-400 10^9/l)	252.33 (84.91)	251.71 (70.91)	278.71 (81.43)	0.913
Difference (95% CI)	Reference	0.92 (-0.22 to 2.06)	0.21 (-0.94 to 1.35)	
Haematocrit (0.35-0.47 l/l)	0.35 (0.02)	0.38 (0.03)	0.36 (0.03)	0.514
Difference (95% CI)	Reference	0.03 (-0.02 to .07)	0.01 (-0.03 to -0.05)	

Table 5-29: Blood count values in SCT women

Laboratory marker (Reference values)	No HC (n=7) Mean (SD)	POC (n=7) Mean (SD)	COC (n=8) Mean (SD)	P value
Complement reactive protein (0.2-10.0 mcg/ml)	13.28 (10.79)	13.57 (16.29)	11.12 (8.55)	0.270
Difference (95% CI)	Reference	0.29 (-13.31 to 13.88)	-2.16 (-15.32 to 11)	
White blood cells (3.5-11.0 10^9/l))	5.78 (2.59)	4.16 (1.26)	6.90 (1.99)	0.724
Difference (95% CI)	Reference	-1.62 (-5.73 to 2.5)	1.13 (-1.92 to 4.18)	
Neutrophils (1.7-8.0 10^9/l))	3.21 (1.62)	1.90 (0.94)	3.99 (1.79)	0.813
Difference (95% CI)	Reference	-1.3 (-4.29 to 1.68)	0.79 (-1.42 to 3)	
Lymphocytes (1.0-3.510^9/l))	1.93 (1.28)	1.75 (0.07)	2.29 (0.37)	0.023
Difference (95% CI)	Reference	-0.18 (-1.89 to 1.53)	0.36 (0.19 to 1.63)	
Monocytes (0.1-1.010^9/l)	0.31 (0.15)	0.42 (0.13)	0.34 (0.08)	0.827
Difference (95% CI)	Reference	0.03 (-0.23 to 0.28)	0.11 (-0.08 to 0.3)	
Eosinophils (0.0-0.46 10^9/l))	0.22 (0.17)	0.15 (0.15)	0.17 (0.10)	0.637
Difference (95% CI)	Reference	-0.07 (-0.35 to 0.2)	-0.04 (-0.25 to 0.16)	
Basophils (0.0-0.20 10^9/l))	0.02 (0.19)	0.01 (0.00)	0.03 (0.02)	0.609
Difference (95% CI)	Reference	-0.01 (-0.05 to 0.02]	0 (-0.02 to 0.03)	

Table 5-30: Inflammatory markers in SCT women

5.7 SUMMARY OF RESULTS FOR THE SCT WOMEN

The prior enrollment contraception period is more evenly distributed between the three groups of SCT women. There are minimal serious clinical events noted in SCT women.

The pro and anti- coagulant markers showed some interesting differences: Free protein S level is lower than the reference range in the group of women using POC and women not using any HC methods. This adverse result is statistically significantly different from the levels in women using COC. There was an increase in Factor VIII levels in SCT women using POC, with again the group using COC showing the best results in this comparison. The SCT women not using any HC have APTT and Fibrinogen levels which are higher than the reference range and this was statistically significantly different from the levels in the other two groups. FII levels were higher than the reference range in SCT women using COC. The difference is statistically significant.

Coagulation activation markers showed also were found to have some interesting values and differences. TAT levels weremore than 2.5 times higher than the reference range in the women using COC. The differenece between the groups was statistically signficant. Prothrombin fragment F1+2 level were statistically significantly high in the COC group, although the level itself was well within the reference range.

Similar to the coagulation activation markers, two of the platelet activation markers, sCD40 and PF4, were statistically significantly higher than the reference group in women using COC. However, whilst, PF4 is elevated in all three groups, the elevation in women using COC is twice as high as in the other two groups (13 times higher than the normal range). SCD40 ligand is increased in the women using POC in addition to the women using COC, but still in the normal range.

All the endothelial activation marker levels, except sE selectin were within the reference range (however, it should be noted that the reference range is wide). SE selectin was elevated in women using COC and this increase was statistically significant. The differences in sVCAM levels were also statistically significant, with the group taking POC showing the highest levels.

Markers of tissue damage in SCT women showed two different directions. While, MPs were within the reference range in all three groups. TF, in contrast was raised two above the reference range in women taking COC and women not taking any contraception. The difference in TF values between the three groups was statistically significant.

Two of the thrombin generation test parameter (PH (5pM) and LT (5pM)) were statistically significantly higher in women not using HC.

Parameters of liver function and blood cell counts were normal in all three groups.

Figure 5-3: This is a twenty six year old woman with normal haemoglobin type (This picture is inserted with full permission of the participant)

5.8 CLINICAL EVENTS IN WOMEN WITH NORMAL HAEMOGLOBIN

Clinical variables	No HC (n=9) Mean (SD)	POC (n=8) Mean (SD)	COC (n=10) Mean (SD)	P
Number of previous pregnancies Mean (SD)	1.40 (0.88)	1.75 (1.16)	1.10(0.87)	0.670
Difference (95% CI)	Reference	0.35(-2.0 to-2.89)	-0.3(-6.18 to -3.64)	
Duration of use of contraceptive method prior to enrolment (months) Mean(SD)	20 (7.68)	14 (16.36)	15.2 (10.07)	0.543
Difference (95% CI)	Reference	-6(-8.54 to-3.45)	-4.8(-7.15 to -2.25)	

Table 5-31: Obstetric history and duration of use of their contraceptive method prior to the enrolment interview in women with normal Hb

Clinical variables	No HC (n=9) Mean (SD)	POC (n=8) Mean (SD)	COC (n=10) Mean (SD)
Severe sickle crises (total)	0	0	0
Hospital admissions (total)	3	1	2
Blood transfusions (total number of sessions)	0	0	0
VTE (DVT & PE)	0	0	0
Strokes & heart attacks	0	0	0
Arterial thromboses	0	0	0

Table 5-32: Clinical events during the study in SCT women

Clinical variables	No HC (n=9) Mean (SD)	POC (n=8) Mean (SD)	COC (n=10) Mean (SD)
Side effects (i.e. headaches, nausea, mood swings, bloatedness, breast tenderness)	0	3	1
Discontinuation	0	1	0
Unintended interruptions	0	2	0
Planned pregnancies	0	1	0
Unplanned pregnancies	1	0	0
Amenorrhoea	0	2	0
Menstrual irregularities	2	3	0

Table 5-33: Contraceptive side effects and problems during the study in the women with normal Hb

5.9 LABORATORY RESULTS FOR WOMEN WITH NORMAL HAEHOGLOBIN

Laboratory marker (reference values)	No HC (n=9) Mean (SD)	POC (n=8) Mean (SD)	COC (n=10) Mean (SD)	P
Prothrombin time (12 – 15 seconds)	10.77 (0.72)	11.23 (0.57)	11.16 (0.97)	0.346
Difference (95% CI)	Reference	0.47 (-0.5 to 1.44)	-0.97 (-2.09 to .14)	
International normalised ratio (INR)(0.8-1.2)	1.00 (0.06)	1.04 (0.05)	1.03 (0.08)	0.398
Difference (95% CI)	Reference	0.03 (-0.02 to .08)	-0.03 (-0.09 to .04)	
Activated partial thromboplastin time (25-35 seconds)	31.08 (2.18)	30.15 (2.13)	30.00 (2.62)	0.810
Difference (95% CI)	Reference	0.36 (-0.87 to 1.6)	0.46 (-0.96 to 1.88)	
Thrombin Time (12-14 seconds)	15.01 (0.76)	14.05 (1.29)	14.19 (1.07)	0.384
Difference (95% CI)	Reference	-0.8 (-1.33 to -0.27)	-0.59 (-1.19 to 0.02)	
Fibrinogen (2.14-3.51 g/dL)	2.82 (0.51)	3.14 (0.63)	3.11 (0.52)	0.813
Difference (95% CI)	Reference	-0.04 (-0.5 to 0.43)	-0.14 (-0.68 to 0.39)	
Factor VIII:C (50-150 iu/dL)	143.55 (42.86)	152.37 (62.81)	155.10 (39.99)	0.400
Difference (95% CI)	Reference	-6.9 (-26.78 to 12.98)	-17.78 (-40.5 to 4.94)	
von Willebrand Factor antigen (46 – 153 iu/dL)	143.13 (45.89)	129.66 (53.53)	109.77 (35.18)	0.512
Difference (95% CI)	Reference	-15.27 (-41.37 to 10.83)	-38.83 (-68.84 to -8.82)	
Dilute Viper Venom Time (ratio) (<1.16)	0.91 (0.04)	1.09 (0.24)	1.16 (0.19)	<0.001
Difference (95% CI)	Reference	0.06 (-0.02 to 0.14)	0.1 (0.01 to 0.19)	

Table 5-34: Pro and anti-coagulant markers in the women with normal Hb (1)

Laboratory marker (reference values)	No HC (n=9) Mean (SD)	POC (n=8) Mean (SD)	COC (n=10) Mean (SD)	P
Anti-thrombin : Activity (85-113 iu/dL)	105.87 (5.82)	109.07 (9.34)	102.33 (7.64)	0.458
Difference (95% CI)	Reference	-1.39 (-5.85 to 3.06)	-3.1 (-8.23 to 2.02)	
Protein C : Activity (70-135 iu/dL)	100.38 (23.48)	105.20 (19.57)	98.10 (23.52)	0.861
Difference (95% CI)	Reference	-1.31 (-9.79 to 7.17)	1.61 (-8.14 to 11.36)	
Free Protein S (75-125 iu/dL)	73.22 (10.60)	66.87 (20.15)	78.60 (25.60)	0.067
Difference (95% CI)	Reference	1.27 (-6.87 to 9.42)	12.92 (3.56 to 22.28)	
Activated Protein C Resistance (Factor V Leiden) (>2.0)	2.71 (0.03)	2.71 (0.09)	2.78 (0.07)	0.024
Difference (95% CI)	Reference	0.05 (-0.07 to 0.16)	-0.03 (-0.01 to -0.15)	
Factor II (84.3-120.0 iu/dL)	101.66 (26.41)	107 75 (16.85)	117.80 (18.74)	0.429
Difference (95% CI)	Reference	3.04 (-3.81 to 9.89)	12.33 (4.46 to 20.21)	
Factor V (63.85-120.5 iu/dL)	80.55 (8.88)	94.87 (10.77)	97.70 (17.07)	0.160
Difference (95% CI)	Reference	2.3 (-5.26 to 9.86)	4.58 (-4.11 to 13.27)	
Factor VII (77.5-150.5 iu/dL)	86.88 (24.79)	83.62 (11.81)	95.50 (20.30)	0.176
Difference (95% CI)	Reference	-8.32 (-17.18 to 0.53)	-4.1 (-14.28 to 6.08)	

Table 5-35: Pro and anti-coagulant markers in the women with normal Hb (2)

Laboratory marker (reference values)	No HC (n=9) Mean (SD)	POC (n=8) Mean (SD)	COC (n=10) Mean (SD)	P
Factor IX (77.7-130.0 iu/dL)	96.22 (16.70)	102.00 (14.11)	127 90 (44.03)	0.003
Difference (95% CI)	Reference	2.1 (-11.6 to 15.81)	3.13 (2.62 to 18.89)	
Factor X (71.8-130.0 iu/dL)	93.66 (17.72)	92.37 (16.30)	105.30 (21.45)	0.733
Difference (95% CI)	Reference	-1.95 (-9.84 to 5.95)	8.17 (-0.91 to 17.24)	
Factor XI (68-144 iu/dL)	94.44 (9.55)	103.37 (22.28)	99.80 (14.05)	0.081
Difference (95% CI)	Reference	-0.83 (-8.39 to 6.72)	4.04 (-4.65 to 12.72)	
Factor XII (46.7-181.0 iu/dL)	83.77 (24.05)	120.25 (40.14)	116.80 (49.13)	0.158
Difference (95% CI)	Reference	-1.07 (-14.44 to 12.31)	9.76 (-5.62 to 25.14)	
Factor XIIIa (97.4-179.0 iu/dL)	97.77 (20.18)	87.12 (29.14)	68.70 (19.79)	0.482
Difference (95% CI)	Reference	-4.41 (-14.7 to 5.88)	-5.95 (-17.71 to 5.82)	

Table 5-36: Pro and anti-coagulant markers in the women with normal Hb (3)

Laboratory marker (reference values)	No HC (n=9) Mean (SD)	POC (n=8) Mean (SD)	COC (n=10) Mean (SD)	P
Thrombin:anti-thrombin III complex (2 - 4.2 mcg/l)	4.90 (1.91)	4.21 (1.80)	3.53 (0.70)	0.020
Difference (95% CI)	Reference	-9.18 (-18.37 to 0.02)	-7.03 (-17.59 to -3.52)	
Prothrombin fragment 1 + 2 (69 – 229 pmol/L)	144.11 (78.38)	120.12 (46.96)	156.80 (60.94)	0.407
Difference (95% CI)	Reference	-81.84 (-200.39 to 36.7)	-168.36 (-304.47 to -32.25)	
D dimer (ng/ml) (<130)	107.00 (48.24)	137.12 (103.39)	107.00 (54.82)	0.096
Difference (95% CI)	Reference	-148.52 (-456.1 to 159.05)	-407.9 (-759.6 to -56.2)	

Table 5-37 : Coagulation activation markers in the women with normal Hb

Laboratory marker (reference values)	No HC (n=9) Mean (SD)	POC (n=8) Mean (SD)	COC (n=10) Mean (SD)	P
Soluble Platelet selectin(92-212 ng/ml)	53.55 (12.99)	53.25 (11.56)	52.30 (17.45)	0.491
Difference (95% CI)	Reference	-3.03 (-14.78 to 8.72)	-11.41 (-24.74 to 1.92)	
Soluble Cell Differentiation 40 ligand (0.03-3.98 ng/ml)	0.59 (0.18)	2.27 (2.36)	5.49 (0.83)	<0.001
Difference (95% CI)	Reference	0.57 (0.42 to 1.96)	1.71 (0.13 to 3.28)	
Platelet factor 4 (<10 I.U./ml)	82.33 (19.29)	105.00 (16.30)	79.50 (19.09)	0.992
Difference (95% CI)	Reference	14.75 (-19.47 to 48.97)	9.8 (-35.79 to 55.39)	

Table 5-38: Platelet activation markers in the women with normal Hb

Laboratory marker (reference values)	No HC (n=9) Mean (SD)	POC (n=8) Mean (SD)	COC (n=10) Mean (SD)	P
Soluble vascular cell adhesion molecule (131-1223 ng/ml))	507.66 (260.05)	396.00 (154.43)	608.90 (298.83)	<0.001
Difference (95% CI)	Reference	40.59 (-251.27 to 332.44)	-198.64 (-529.78 to -132.5)	
Soluble inter-cellular adhesion molecule-1 (41-669 ng/ml)	354.33 (102.25)	270.31 (42.35)	279.97 (56.34)	0.051
Difference (95% CI)	Reference	-36.11 (-104.36 to 32.15)	-136.86 (-214.3 to --59.42)	
Soluble endothelial-leucocyte adhesion molecule-1 (1.5 – 88.1ng/ml))	66.55 (13.15)	69.87 (31.30)	72.90 (27.85)	0.073
Difference (95% CI)	Reference	-4.98 (-26.03 to 16.07)	31.61 (7.73 to 55.5)	

Table 5-39 Endothelial activation markers in the women with normal Hb

Laboratory marker (reference values)	No HC (n=9) Mean (SD)	POC (n=8) Mean (SD)	COC (n=10) Mean (SD)	P
Micro particles (nMol/ml) (< 10)	8.95 (4.03)	6.70 (2.27)	9.58 (1.11)	<0.001
Difference (95% CI)	Reference	2.66 (-6.51 to 11.83)	-10.64 (-21.16 to -0.11)	
Tissue Factor(<2 pMol/ml)	5.60 (5.90)	6.12 (6.10)	6.12 (6.37)	0.926
Difference (95% CI)	Reference	-2.63 (5.57 to 0.32)	-1.18 (-4.45 to 2.09)	

Table 5-40: Markers of tissue damage in the women with normal Hb

Laboratory marker (reference values)	No HC (n=9) Mean (SD)	POC (n=8) Mean (SD)	COC (n=10) Mean (SD)	P
Lag Time (initiated with 5pMol Tissue Factor) (2.0 - 3.2 minutes)	1.87 (0.40)	2.42 (0.90)	2.22 (0.40)	0.032
Difference (95% CI)	Reference	0.04 (-0.25 to 0.34)	-0.01 (-0.35 to 0.34)	
Endogenous Thrombin Potential (initiated with 5pMol Tissue Factor) (1159 – 2168 nM)	1637.88 (284.27)	1636.87 (364.16)	1837.60 (337.35)	0.798
Difference (95% CI)	Reference	56.97 (-71.08 to 185.01)	229.19 (81.08 to 377.3)	
Peak Height (initiated with 5pMol Tissue Factor) (147 – 359 nM)	322.00 (37.80)	320.75 (84.72)	310.30 (72.63)	0.106
Difference (95% CI)	Reference	-0.02 (-26.45 to 26.41)	5.8 (-24.77 to 36.37)	
Time to Peak (initiated with 5pMol Tissue Factor) (4.6 – 7.4 min)	4.27 (0.47)	4.40 (0.69)	4.60 (0.81)	0.354
Difference (95% CI)	Reference	-0.09 (-0.48 to 0.3)	0.01 (-0.44 to 0.47)	
Slope (33 – 142 nM/min)	134.62 (17.37)	187.55 (101.15)	138.00 (53.60)	<0.001
Difference (95% CI)	Reference	12.83 (-7.84 to 33.5)	5.35 (1.85 to 29.26)	
Start Time (initiated with 5pMol Tissue Factor) (19 28.3 min)	20.22 (1.71)	21.37 (2.86)	24.45 (3.37)	0.187
Difference (95% CI)	Reference	1.47 (-0.01 to 2.95)	3.15 (1.43 to 4.86)	

Table 5-41: Markers of thrombin generation in the women with normal Hb

Laboratory marker (reference values)	No HC (n=9) Mean (SD)	POC (n=8) Mean (SD)	COC (n=10) Mean (SD)	P
Free Hb (0.0 – 0.5.mg/l)	0.05 (0.08)	0.06 (0.05)	0.06 (0.06)	0.520
Difference (95% CI)	Reference	-0.01 (-0.05 to 0.03)	-0.03 (-0.07 to 0.02)	
Haptoglobin (< 1.22 g/l)	1.28 (0.69)	1.19 (0.54)	1.13 (0.44)	0.497
Difference (95% CI)	Reference	-0.01 (-0.26 to 0.24)	-0.21 (-0.49 to 0.08)	
Lactate dehydrogenase (240-480 units/l)	379.14 (70.03)	371.00 (134.91)	432.75 (134.38)	0.262
Difference (95% CI)	Reference	-68.91 (-178.81 to 40.99)	-162.29 (-291.32 to -33.26)	
Bilirubin (< 21 µMol/l)	6.37 (2.13	8.12 (6.24	6.50 (2.07)	0.004
Difference (95% CI)	Reference	-0.43 (-11.95 to 11.09)	-10.96 (-24.3 to -2.37)	

Table 5-42: Markers of haemolysis in the women with normal Hb

Laboratory marker (reference values)	No HC (n=9) Mean (SD)	POC (n=8) Mean (SD)	COC (n=10) Mean (SD)	P
Alanine amino transferase (<33 units/l)	11.37 (2.61)	25.00 (20.88)	15.44 (4.69)	<0.001
Difference (95% CI)	Reference	0.28 (-4.44 to 4.99)	0.54 (0.16 to 4.93)	
Aspartate amino transferase (<31 units/l)	20.87 (4.58)	22.31 (6.39)	25.62 (6.20)	0.661
Difference (95% CI)	Reference	-3.13 (-8.98 to 2.73)	-3.68 (-10.49 to 3.13)	
Alkaline phosphatase (< 129 units/l)	69.62 (17.48)	96.00 (77.63)	69.66 (27.69)	0.001
Difference (95% CI)	Reference	5.48 (-12.91 to 23.87)	-6.85 (-28.19 to -1.48)	
Serum albumin (35-50g/l)	45.37 (1.84)	42.61 (5.60)	43.88 (2.26)	0.007
Difference (95% CI)	Reference	-0.32 (-1.92 to 1.28)	-0.67 (-2.53 to -0.12)	

Table 5-43: Biochemical markers of liver function in the women with normal Hb

Laboratory marker (reference values)	No HC (n=9) Mean (SD)	POC (n=8) Mean (SD)	COC (n=10) Mean (SD)	P
Haemoglobin (11.5-15-5 g/dl)	12.18 (0.49)	11.95 (1.07)	12.04 (0.97)	0.110
Difference (95% CI)	Reference	0.21 (-0.55 to 0.98)	1.36 (0.47 to 2.25)	
Platelet count (140-400 10^9/l)	261.44 (54.42)	228.00 (46.01)	233.50 (70.88)	0.486
Difference (95% CI)	Reference	-12.89 (-62.51 to 36.74)	-34.44 (-91.77 to 22.88)	
Haematocrit (0.35-0.47 l/l)	0.37 (0.02)	0.37 (0.04)	0.37 (0.02)	0.183
Difference (95% CI)	Reference	0 (-0.02 to 0.03)	0.04 (0.01 to 0.07)	

Table 5-44: Blood count values in the women with normal Hb

Laboratory marker (reference values)	No HC (n=9) Mean (SD)	POC (n=8) Mean (SD)	COC (n=10) Mean (SD)	P
Complement reactive protein (0.2-10.0 mcg/ml)	17.66 (20.63)	13.00 (9.98)	16.22 (9.70)	0.139
Difference (95% CI)	Reference	-1.25 (-10.89 to 8.39)	-4.98 (-15.82 to 5.86)	
White blood cells (3.5-11.0 10^9/l))	5.33 (0.93)	6.41 (2.84)	6.99 (2.02)	0.030
Difference (95% CI)	Reference	0.54 (-0.77 to 1.85)	0.89 (-0.64 to 2.43)	
Neutrophils (1.7-8.0 10^9/l))	2.49 (0.44)	2.95 (0.82)	3.32 (1.20)	0.054
Difference (95% CI)	Reference	0.36 (-0.54 to 1.26)	0.73 (-0.3 to 1.76)	
Lymphocytes (1.0-3.510^9/l))	2.23 (0.48)	1.80 (0.78)	2.35 (0.98)	0.203
Difference (95% CI)	Reference	0.18 (-0.26 to 0.61)	0.1 (-0.4 to 0.6)	
Monocytes (0.1-1.010^9/l)	0.45 (0.10)	0.45 (0.09)	0.45 (0.21)	0.101
Difference (95% CI)	Reference	0.13 (-0.04 to 0.31)	0.05 (-0.15 to 0.25)	
Eosinophils (0.0-0.46 10^9/l))	0.13 (0.79)	0.14 (0.10)	0.21 (0.26)	0.007
Difference (95% CI)	Reference	0 (-0.1 to 0.09)	0.05 (0.01 to 0.16)	
Basophils (0.0-0.20 10^9/l))	0.02 (0.008)	0.01 (0.01)	0.02 (0.01)	0.518
Difference (95% CI)	Reference	0 (-0.01 to 0.02)	0 (-0.02 to 0.01)	

Table 5-45: Inflammatory markers in the women with normal Hb

5.10 SUMMARY OF RESULTS FOR WOMEN WITH NORMAL HB

There is even distribution of the duration of contraception use between the three groups. As expected, serious clinical events were minimal during the study period.

Contraceptive side effects are relatively uncommon and evenly distributed between all the women with normal haemoglobin.

All the pro and anti-coagulant markers in women with normal Hb lie within the reference range however, there is a tendency for the APCR mean value to be statistically significantly higher in the group using COC. Factor VIIIC levels were high in women using POC and COC compared with levels in women using no HC. Though DRVVT values remained within the reference range however, there is a statistically significant difference between the groups, with women using COC and POC having the higher levels. Factor IX levels werestatistically significantly higher in women using COC but the values were within the reference range.

Rather strangely, TAT levels were higher than the reference range in women not receiving any hormonal contraception.

D dimer levels were high in women using POC, while PF4 is worse in those women who use POC. MPs levels fell within the reference range in all three groups however COC women showed statistically significantly raised levels compared to other women.

SD40 ligand levels varied significantly between the three groups, being higher in women using COC.

While TF levels were 2-3 times higher than the reference range in all three groups, there was no statistically significant difference between the women using COC and other two groups.

TG results were within the range in all the three groups except the results for the Slope which showed a significant increase in women using POC.

Full blood counts were within the reference range in all the three groups although some values of liver function tests were raised abnormally.

CRP levels were high in the three groups of women with normal Hb.

CHAPTER 6

RESULTS OF THE COMPARISONS BY TYPE OF CONTRACEPTION

6.1 INTRODUCTION

The study results for women using combined oral contraceptive pills (COC) will be presented first in the form of tables and the most important findings will be summarised at the end of these tables. This will be followed by tables and a summary of the results for the women using Progestogen only contraception (POC). Finally, the findings in the women who are not using any hormonal contraception will be presented, again in the forms of tables and the key findings will be summarized. In all the tables, the reference group for comparisons is that of the women with normal Hb.

6.2 CLINICAL EVENTS IN WOMEN USING COC

Clinical variables	SCD (n =9) Mean (SD)	SCT (n =8) Mean (SD)	Hb AA (n=10) Mean (SD)	P
Number of previous pregnancies	0.77 (0.83)	1.125 (0.64)	1.1 (0.87)	0.691
Difference (95% CI)	-0.35(-2.0 to .19)	.01 (-0.53 to 0.51]	Reference	
Duration of use of contraceptive method prior to enrolment (months)	14.77 (13.65)	11.5 (6.48)	15.2 (10.07)	0.168
Difference (95% CI)	3.27(0.91 to .62)	-3.7(-6.44 to -0.97)	Reference	

Table 6-1: The obstetric history and duration of use of their contraceptive method prior to the enrolment interview in women using COC

Clinical events	SCD (n =9)	SCT (n = 8)	Hb AA (n=10)
Severe sickle crises (total)	2	0	0
Number of women with severe sickle crises	1	0	0
Hospital admissions (total)	3	0	2
Number of women with hospital admission	2	0	2
Blood transfusions (total number of sessions)	0	0	0
Number of women receiving transfusion	0	0	0
VTE (DVT & PE)	0	0	0
Strokes & heart attacks	0	0	0

Table 6-2: Clinical events in women using COC

Contraceptive problems	SCD (n =9)	SCT (n = 8)	Hb AA (n=10)
Side effects (i.e. headaches, nausea, mood swings, bloatedness, breast tenderness)	1	2	1
Discontinuation	0	0	0
Unintended interruptions	1	0	2
Planned pregnancies	0	0	0
Unplanned pregnancies	0	0	0
Amenorrhoea	0	0	0

Table 6-3: Contraceptive side effects and problems during the study in women using COC

6.3 LABORATORY RESULTS FOR WOMEN USING COC

Laboratory marker (reference values)	SCD (n= 9) Mean (SD)	SCT (n= 8) Mean (SD)	A+A (n=10) Mean (SD)	P
Prothrombin time (12 – 15 seconds)	14.83 (1.65)	11.73 (1.28)	11.16 (0.97)	0.337
Difference (95% CI)	0.68(0.06 to 1.30)	0.31(-0.31 to 0.93)	Reference	
International normalised ratio (INR)(0.8-1.2)	1.16 (0.11)	1.06 (0.05)	1.04 (0.08)	0.086
Difference (95% CI)	0.03 (-0.05 to 0.08)	-0.03 (-0.08 to 0.02)	Reference	
Activated partial thromboplastin time (25-35 seconds)	29.41 (2.68)	29.54 (1.78)	30.00(2.63)	0.519
Difference (95% CI)	0.05 (-1.21 to 1.31)	-0.89 (-2.15 to 0.37)	Reference	
Thrombin Time (12-14 seconds)	12.86 (0.67)	14.03 (1.23)	14.19(1.08)	0.266
Difference (95% CI)	-0.41 (-0.95 to 0.13)	0.15 (-0,02 to 0.08)	Reference	
Fibrinogen (2.14-3.51 g/dL)	3.47 (0.80)	2.95 (0.66)	3.12 (0.52)	0.488
Difference (95% CI)	0.28 (-0.21 to 0.77)	0.14 (-0.35 to 0.63)	Reference	
Factor VIII Coagulant (I.U./ml)	149.11(38.17)	121.00(34.05)	155.10 (40.00)	0.908
Difference (95% CI)	-1.83 (-22.88 to 19.22)	-5.95 (-27 to -125.25)	Reference	
von Willebrand Factor antigen (46 – 153 iu/dL)	164.22(66.28)	105.87 (33.40)	109.77(35.18)	0.095
Difference (95% CI)	31.1 (-5.70 to 56.50)	-1.78 (-27.18 to 23.62)	Reference	
Dilute Viper Venom Time (ratio) (<1.16)	0.91(0.07)	0.99(0.16)	1.16 (0.19)	0.039
Difference (95% CI)	-0.09 (-0.01 to -0.17)	0.03 (0.01 to 0.11)	Reference	

Table 6-4: Pro and anti-coagulant markers in the women using COC (1)

Laboratory marker (reference values)	SCD (n= 9) Mean (SD)	SCT (n= 8) Mean (SD)	A+A (n=10) Mean (SD)	P
Anti-thrombin : Activity (85-113 iu/dL)	92.03 (13.40)	100.46(9.40)	102.33 (7.64)	0.270
Difference (95% CI)	5.76 (1.43 to 10.09)	1.76 (-2.57 to 6.09)	Reference	
Protein C : Activity (70-135 iu/dL)	75.53(20.24)	102.80(28.04)	98.10(23.53)	0.685
Difference (95% CI)	-3.29 (-11.35 to 4.77)	4.51 (3.55 to 12.57)	Reference	
Free Protein S (75-125 iu/dL)	59.20(16.88)	87.13 (38.14)	78.60(25.61)	0.103
Difference (95% CI)	-8.73 (-0.65 to -16.81)	12.53 (2.45 to 20.61)	Reference	
Activated Protein C Resistance (Factor V Leiden) (>2.0)	2.80(0.14)	2.73(0.25)	2.78(0.08)	0.009
Difference (95% CI)	0.06 (-0.04 to 0.16)	0.17 (0.07 to 0.27)	Reference	
Factor II (84.3-120.0 iu/dL)	91.00(11.94)	107.13(8.32)	117.80(18.75)	0.094
Difference (95% CI)	-6.81(-0.11 to -13.51)	-10.43(-17.13 to -3.73)	Reference	
Factor V (63.85-120.5 iu/dL)	98.89(12.46)	98.00(28.33)	97.70(17.08)	0.083
Difference (95% CI)	-4.62 (-12.58 to 3.34)	11.25 (3.29 to 19.21)	Reference	
Factor VII (77.5-150.5 iu/dL)	83.11(21.31)	76.75(12.19)	95.50(20.30)	0.322
Difference (95% CI)	1.01(-8.39 to 10.42)	-8.11(-11.63 to 1.29)	Reference	

Table 6-5: Pro and anti-coagulant markers in the women using COC (2)

Laboratory marker (reference values)	SCD (n= 9) Mean (SD)	SCT (n= 8) Mean (SD)	A+A (n=10) Mean (SD)	P
Factor IX (77.7-130.0 iu/dL)	121.89(22.28)	95.75(23.11)	127.90(44.04)	0.087
Difference (95% CI)	-21.76(-35.95 to -7.56)	-20.93(-29.49 to -12.36)	Reference	
Factor X (71.8-130.0 iu/dL)	102.56(19.63)	98.25(14.20)	105.30(21.45)	0.542
Difference (95% CI)	-1.82(-9.64 to 6)	-7.25(-17.48 to 2.96)	Reference	
Factor XI (68-144 iu/dL)	89.44(14.59)	100.88(18.31)	99.80(14.05)	0.734
Difference (95% CI)	0.54 (-7.28 to 8.36)	4.26 (-3.56 to 12.08)	Reference	
Factor XII (46.7-181.0 iu/dL)	97.78(32.82)	95.88(20.38)	116.80(49.13)	0.076
Difference (95% CI)	-16.31 (-30.46 to -2.15)to	-28.75(42.90 to -14.60)	Reference	
Factor XIIIa (97.4-179.0 iu/dL)	67.56(26.06)	78.38(27.81)	68.70(19.80)	0.617
Difference (95% CI)	6.26(-3.95 to 16.47)	8.01(-2.20 to 18.22)	Reference	

Table 6-6: Pro and anti-coagulant markers in the women using COC (3)

Laboratory marker (reference values)	SCD (n= 9) Mean (SD)	SCT (n= 8) Mean (SD)	A+A (n=10) Mean (SD)	P
Thrombin:anti-thrombin III complex (2 - 4.2 mcg/l)	8.15 (4.29)	10.61(19.98)	3.53(0.70)	<0.001
Difference (95% CI)	3.59 (-6.17 to 13.35)	19.28 (9.52 to 29.04)	Reference	
Prothrombin fragment 1 + 2 (69 – 229 pmol/L)	244.63(114.91)	210.00(219.45)	156.80(60.95)	0.003
Difference (95% CI)	53.96 (-60.76 to 168.68)	158.5 (-141.44 to 458.44)	Reference	
D dimer (ng/ml) (<130)	614.44 (283.46)	184.38 (200.72)	107.00(54.82)	< 0.001
Difference (95% CI)	228.64 (217.78 to 239.49)	145.9 (135.04 to 156.76)	Reference	

Table 6-7: Coagulation activation markers in women using COC

Laboratory marker (reference values)	SCD (n= 9) Mean (SD)	SCT (n= 8) Mean (SD)	A+A (n=10) Mean (SD)	P
Soluble Platelet selectin (92-212 ng/ml)	72.00(20.48)	63.50(16.22)	52.30(17.46)	0.811
Difference (95% CI)	3.02 (-7.84 to 13.88)	-1.24 (-12.09 to 9.62)	Reference	
Soluble Cell Differentiation 40 ligand (0.03-3.98 ng/ml)	1.07(0.92)	1.24(1.05)	5.49(8.33)	<0.001
Difference (95% CI)	-7.41(-8.86 to -5.96)	-7.28 (-8.73 to -5.83)	Reference	
Platelet factor 4 (<10 I.U./ml)	150.39(66.91)	137.00(97.58)	79.50(19.09)	0.481
Difference (95% CI)	47.82 (-6.51 to 102.15)	78.49 (24.16 to 132.82)	Reference	

Table 6-8: Platelet activation markers in women using COC

Laboratory marker (reference values)	SCD (n= 9) Mean (SD)	SCT (n= 8) Mean (SD)	A+A (n=10) Mean (SD)	P
Soluble vascular cell adhesion molecule (ng/ml) (131-1223)	1003.22(604.21)	419.75(178.05)	608.90(829.84)	0.002
Difference (95% CI)	-225.63(-282.35 to 54.16)	-651.79 (-931.52 to -372)	Reference	
Soluble inter-cellular adhesion molecule-1(ng/ml) (41-669)	445.07(119.85)	281.63(69.56)	279.97(56.34)	0.083
Difference (95% CI)	63.51 (9.57 to 117.45)	13.22 (-40.22 to 67.16)	Reference	
Soluble endothelial-leucocyte adhesion molecule-1 (ng/ml) (1.5 – 88.1)	89.78(52.84)	136.13 (138.42)	72.90(27.85)	<0.001
Difference (95% CI)	24.99(2.27 to 47.71)	110.57 (87.89 to 133.29)	Reference	

Table 6-9: Endothelial activation markers in women using COC

Laboratory marker (reference values)	SCD (n= 9) Mean (SD)	SCT (n= 8) Mean (SD)	A+A (n=10) Mean (SD)	P
Micro particles (<10 nMol/ml)	25.41(13.46)	9.96(5.36)	9.58(11.10)	0.005
Difference (95% CI)	2.36(-10.39 to 10.39)	-5.74 (-13.78 to -2.29)	Reference	
Tissue Factor(<2pMol/ml)	13.72(5.40)	5.67(5.43)	6.12(6.74)	0.770
Difference (95% CI)	-1.34 (-4.12 to 1.44)	-1.31 (-4.09 to 1.47)	Reference	

Table 6-10: Markers of tissue damage in women using COC

Laboratory marker (reference values)	SCD (n= 9) Mean (SD)	SCT (n= 8) Mean (SD)	A+A (n=10) Mean (SD)	P
Lag Time (initiated with 5pMol Tissue Factor) (2.0 - 3.2 minutes)	1.37(0.34)	1.76(0.27)	2.23(0.41)	0.568
Difference (95% CI)	-0.07(-0.34 to 0.20)	-0.14(-0.41 to 0.13)	Reference	
Endogenous Thrombin Potential (initiated with 5pMol Tissue Factor) (1159 – 2168 nM)	1482.89(348.20)	1608.14(282.05)	1837.60(337.36)	0.859
Difference (95% CI)	10.84 (-120.15 to 141.83)	-55.31(-186.29 to 75.68)	Reference	
Peak Height (initiated with 5pMol Tissue Factor)(147 – 359 nM)	342.67(51.52)	272.52(23.39)	310.30(72.62)	0.035
Difference (95% CI)	-21.1 (-47.62 to -5.42)	49.23 (22.71 to 172.24)	Reference	
Time to Peak (initiated with 5pMol Tissue Factor)(4.6 – 7.4 min)	3.43(0.39)	4.41(0.95)	4.61(0.81)	0.069
Difference (95% CI)	-0.81 (-1.20 to -0.42)	0.14 (-0.25 to 0.53)	Reference	
Slope (33 – 142 nM/min)	168.51(35.64)	114.57(39.87)	138.00(53.60)	0.486
	-17.96 (-39.52 to 3.6)	-13.73 (-35.29 to 7.83)	Reference	
Start Time (initiated with 5pMol Tissue Factor)(19 28.3 min)	17.50(2.29)	23.14(3.91)	24.45(3.37)	0.380
Difference (95% CI)	-1.08 (-2.41 to 0.25)	0.54 (-0.79 to 1.87)	Reference	

Table 6-11: Markers of thrombin generation in women using COC

Laboratory marker (reference values)	SCD (n= 9) Mean (SD)	SCT (n= 8) Mean (SD)	A+A (n=10) Mean (SD)	P
Free Hb (11-15.5mg/l)	0.1 (0.05)	0.05(0.05)	0.06(0.07)	0.604
Difference (95% CI)	-0.02(-0.95 to 0.02)	-0.02(-0.059 to 0.02)	Reference	
Haptoglobin (< 1.22 g/l)	1.59(0.70)	0.88(0.34)	1.14(0.45)	0.151
Difference (95% CI)	0.25(0.015 to 0.74)	-0.11(-0.35 to 0.13)	Reference	
Lactate dehydrogenase (240-480 units/l)	555.50(210.23)	406.71(62.45)	432.75(134.39)	0.027
Difference (95% CI)	75.84(22.80 to 43.04)	-71.94(-398.36 to -36.70)	Reference	
Bilirubin (< 21 µMol/l)	32.00(20.81)	9.63(5.63)	6.50(2.07)	<0.001
Difference (95% CI)	18.74(8.35 to 29.13)	3.56(1.83 to 13.95)	Reference	

Table 6-12: Markers of haemolysis in women using COC

Laboratory marker (reference ranges)	SCD (n= 9) Mean (SD)	SCT (n= 8) Mean (SD)	Hb AA (n=10) Mean (SD)	P
Alanine amino transferase (<33 units/l)	45.13(6.08)	19.13(5.62)	15.44(4.69)	0.015
Difference (95% CI)	1.39(-3.65 to 6.53)	0.93(0.11 to 5.97)	Reference	
Aspartate amino transferase (<31 units/l)	34.00(14.27)	19.75(2.49)	25.63(6.21)	<0.001
Difference (95% CI)	8.06(2.12 to 13.99)	-3.72(-9.66 to -1.50)	Reference	
Alkaline phosphatase (< 129 units/l)	89.67(65.29)	56.63(18.90)	69.67(27.69)	0.003
Difference (95% CI)	37.6(18.19 to 57.00)	-8.79(-28.19 to -10.61)	Reference	
Serum albumin (35-50g/l)	44.67(5.83)	45.13(6.08)	43.89(2.26)	0.031
Difference (95% CI)	3.57(1.88 to 5.26)	3.82(2.13 to 5.50)	Reference	

Table 6-13: Biochemical markers of liver function in women using COC

Laboratory marker (reference values)	SCD (n= 9) Mean (SD)	SCT (n= 8) Mean (SD)	A+A (n=10) Mean (SD)	P
Haemoglobin (11.5-15-5 g/dl)	10.38(1.74)	11.76(1.17)	12.04(0.98)	0.249
Difference (95% CI)	0.76(0.17 to 1.35)	0.19(-0.77 to 0.77)	Reference	
Platelet count (140-400 10^9/l)	310.78(109.49)	278.71(81.43)	233.50(70.88)	0.451
Difference (95% CI)	38.61(-11.37 to 88.59)	10.55(-39.43 to 60.53)	Reference	
Haematocrit (0.35-0.47 l/l)	0.28(0.05)	0.36(0.04)	0.37(0.231)	0.081
Difference (95% CI)	-0.18(-0.18 to -0.34)	-0.191(-0.21 to -0.37)	Reference	

Table 6-14: Blood count values in women using COC

Laboratory marker (reference values)	SCD (n= 9) Mean (SD)	SCT (n= 8) Mean (SD)	A+A (n=10) Mean (SD)	P
Complement reactive protein (0.2-10.0 mcg/ml)	42.11(31.31)	11.13(8.56)	15.22(9.71)	0.001
Difference (95% CI)	21.6(12.78 to 30.42)	1.15(0.97 to 85.9)	Reference	
White blood cells (3.5-11.0 10^9/l))	9.90(2.05)	6.91(2.00)	6.99(2.03)	0.998
Difference (95% CI)	0.02(-1.27 to 1.31)	-0.03(-1.32 to 1.23)	Reference	
Neutrophils (1.7-8.0 10^9/l))	5.55(2.07)	4.00(1.79)	3.33(1.20)	0.426
Difference (95% CI)	0.87(-0.01 to 1.75)	1.59(0.71 to 2.47)	Reference	
Lymphocytes (1.0-3.510^9/l))	2.75(0.86)	2.30(0.37)	2.35(0.98)	0.185
Difference (95% CI)	-0.12(-0.55 to 0.31)	-0.61(-1.04 to -0.18)	Reference	
Monocytes (0.1-1.010^9/l)	0.92(0.55)	0.43(0.13)	0.46(0.21)	0.008
Difference (95% CI)	0.34(0.18 to 0.49)	-0.08(-0.24 to -0.00)	Reference	
Eosinophils (0.0-0.46 10^9/l))	0.34(0.31)	0.18(0.11)	0.21(0.26)	0.131
Difference (95% CI)	0.05(-0.04 to 0.15)	-0.15(-0.25 to -0.05)	Reference	
Basophils (0.0-0.20 10^9/l))	0.04(0.02)	0.03(0.02)	0.02(0.01)	0.426
Difference (95% CI)	0.01(-0.00 to 0.02)	0.01(-0.00 to 0.02)	Reference	

Table 6-15: Inflammatory markers in women using COC

6.4 SUMMARY OF RESULTS FOR THE WOMEN USING COC

All women using COC are well established on their method, and there were no major clinical events among them. There were also no major contraceptive side effects in any of the three groups.

Regarding the pro and anti-coagulant markers in women using COC: VWF antigen were higher than the reference range in women with SCD, however the difference between the three groups did not reach statistical significance. APCR was lower than the reference range in SCD women (an adverse result) but there was no statistically significant difference between the groups. The DRVVT mean level was statistically significantly lower in SCD women, but the actual values were within the reference range. Free Protein S was lower than the reference range (i.e. adverse) in SCD women, and in women with normal Hb, while the SCT women showed normal values, however the differences did not reach statistical significance. PC activity levels were within the reference range in all three groups and the difference between them did not reach statistical significance. Factor II levels were highest in normal Hb women, followed by the women with SCT, and were lowest in the SCD women group, but the differences were not statistically significant. Factor VII and Factor IX levels were also highest in women with normal Hb followed by levels in women with SCD and lowest in SCT, without any statistically significant difference between the groups. Factor XIIIa is low in all COC users and there was no statistically significant difference between the groups.

Coagulation activation markers behaved independently. While, TAT complex and D Dimer mean values were markedly increased in SCD and SCT COC users, Prothrombin fragments 1+2 levels remained within the reference range for all the 3 groups. However, the differences in all of these markers between the groups reached statistical significance

Platelet activation markers i.e. soluble platelet selectin and Soluble CD 40 ligand and platelet factor 4 also varied in trend. SP selectin levels were lower than the reference range in all the three groups, but with a trend of highest in SCD, next in SCT and lowest in normal Hb, but the difference did not reach statistical significance. Soluble CD 40 ligand was raised above the reference range in women with normal Hb, while being normal in women with SCD and SCT (although the SD is wide) and this difference was statistically significant. Platelet factor 4 was markedly elevated in all COC users with a gradation from normal Hb to SCT to SCD, although differences between the Hb types did not reach statistical significance.

Endothelial activation markers differed in trend as well. SVCAM levels were within the reference range (which itself is wide) for all women using COC but showing a counter-intuitive trend lowest in SCT, with normal Hb in the middle and highest in SCD. SICAM-1 levels were also in the reference range but highest in SCD women.

SELAM-1 was significantly highest in SCT women, with mean levels above the reference range (although there is a wide SD).

Markers of tissue damage: microparticle levels were doubled in SCD women using COC .Tissue factor levels were elevated in all COC users, with a particularly marked effect in SCD women.

Parameters of thrombin generation: The lag time was reduced below the reference range in SCD and SCT women, more so in the SCD women. Time to peak and start time were also shortest in SCD women.

Markers of haemolysis were only notably abnormal in the women with SCD and the same applies to the markers of liver function, the blood cell count figures and the general inflammatory markers CRP and WBC count.

6.5 CLINICAL EVENTS IN WOMEN USING POC

Clinical variables	SCD (n =29) Mean (SD)	SCT(n = 7) Mean (SD)	Hb AA(n = 8) Mean (SD)	P
Number of previous pregnancies (mean+/- SD)	1.79 (1.4)	1.71 (0.95)	1.75 (1.16)	0.405
Difference (95% CI)	0.24(-1.21 to 1.69)	-0.21(-1.66 to 1.24)	Reference	
Duration of use of contraceptive method prior to enrolment (months) (mean+/-SD)	33.44 (27.53)	12 (6.80)	14 (16.36)	0.003
Difference (95% CI)	20.73(18.9 to 22.55)	-9.56 (-14.06 to -5.05)	Reference	

Table 6-16: The obstetric history and duration of use of their contraceptive method prior to the enrolment interview in women using POC

Clinical events	SCD (n =29)	SCT (n = 7)	Hb AA (n = 8)
Severe sickle crises (total)	12	0	0
Number of women with severe sickle crises	5	1	0
Hospital admissions (total)	13	0	1
Number of women with hospital admission	7	0	1
Blood transfusions (total number of sessions)	3	0	0
Number of women receiving transfusion	3	0	0
VTE (DVT & PE)	0	0	0
Strokes & heart attacks	0	0	0
Arterial thrombosis	0	0	0

Table 6-17: Clinical events during the study in women using POC

Contraceptive problems	SCD (n =29)	SCT (n = 7)	Hb AA (n = 8)
Side effects (i.e. headaches, nausea, mood swings, bloatedness, breast tenderness)	4	1	3
Discontinuation	0	0	1
Unintended interruptions	1	0	2
Planned pregnancies	0	0	1
Unplanned pregnancies	0	0	0
Amenorrhoea	11	1	2
Menstrual irregularities	8	1	3

Table 6-18: Contraceptive side effects and problems during the study in the women using POC

6.6 LABORATORY RESULTS FOR WOMEN USING POC

Laboratory marker (reference values)	SCD (n= 29) Mean (SD)	SCT (n= 7) Mean (SD)	Hb A+A (n=8) Mean (SD)	P
Prothrombin time (12 – 15 seconds)	15.36 (1.77)	11.51 (0.95)	11.24 (0.57)	0.005
Difference (95% CI)	4.12 (2.89 to 5.35)	0.28 (0.13 to 1.87)	Reference	
International normalised ratio (INR)(0.8-1.2)	1.19 (0.15)	1.03 (0.03)	1.05 (0.05)	<0.001
Difference (95% CI)	0.14 (0.04 to 0.24)	-0.02 (-0.19 to -0.01)	Reference	
Activated partial thromboplastin time (25-35 seconds)	29.24 (3.56)	30.27 (1.30)	30.15 (2.13)	0.020
Difference (95% CI)	-0.91 (-3.41 to 1.6)	0.12 (0.01 to 3.37)	Reference	
Thrombin Time (12-14 seconds	13.13 (1.28)	14.23 (0.83)	14.05 (1.29)	0.471
Difference (95% CI)	-0.92 (-1.92 to .07)	0.18 (-1.11 to 1.46)	Reference	
Fibrinogen (2.14-3.51 g/dL)	3.33 (0.56)	3.26 (0.90)	3.14 (0.64)	0.280
Difference (95% CI)	0.19 (-0.32 to 0.7)	0.12 (-0.55 to 0.78)	Reference	
Factor VIII:C (50 – 150 iu/dL)	154.14 (41.75)	154.57 (80.07)	152.38 (62.82)	0.063
Difference (95% CI)	1.77 (-41.32 to 44.86)	2.2 (-53.43 to 57.83)	Reference	
von willebrand Factor antigen (46 – 153 (iu/dL)	171.33 (64.39)	86.93 (32.00)	129.66 (53.54)	0.174
Difference (95% CI)	41.67 (-5.85 to 89.18)	-42.73 (-104.31 to 18.84)	Reference	
Dilute Viper Venom Time (ratio) (<1.16)	0.92 (0.13)	0.14 (0.27)	1.10 (0.25)	0.010
Difference (95% CI)	-0.18 (-0.32 to -0.03)	0.04 (0.01 to 0.23)	Reference	

Table 6-19: Pro and anti-coagulant markers in the women using POC (1)

Laboratory marker (reference values)	SCD (n= 29) Mean (SD)	SCT (n= 7) Mean (SD)	Hb A+A (n=8) Mean (SD)	P
Anti-thrombin : Activity (85-113 iu/dL)	95.58 (9.18)	108.26 (6.43)	109.07 (9.35)	0.588
Difference (95% CI)	-13.49 (-20.64 to -6.34)	-0.81 (-10.08 to 8.45)	Reference	
Protein C : Antivity (70-135 iu/dL)	82.00 (12.53)	99.80 (15.47)	105.20 (19.57)	0.280
Difference (95% CI)	-23.2 (-34.82 to -11.58)	-5.4 (-20.46 to 9.66)	Reference	
Free Protein S (75-125 iu/dL)	59.07 (14.96)	74.94 (11.78)	66.88 (20.15)	0.390
Difference (95% CI)	-7.81 (-20.37 to 4.75)	8.07 (-8.21 to 24.34)	Reference	
Activated Protein C Resistance (Factor V Leiden) (>2.0)	2.88 (0.32)	2.86 (0.11)	2.71 (0.10)	0.001
Difference (95% CI)	0.17 (-0.05 to 0.38)	0.14 (0.11 to 0.42)	Reference	
Factor II (84.3-120.0 iu/dL)	91.28 (10.00)	104.71 (7.34)	107.75 (16.85)	0.076
Difference (95% CI)	-16.47 (-25.48 to -7.47)	-3.04 (-14.71 to 8.63)	Reference	
Factor V (63.85-120.5 iu/dL)	95.93 (16.34)	97.00 (17.22)	94.88 (10.78)	0.432
Difference (95% CI)	1.06 (-11.58 to 13.69)	2.13 (-14.25 to 18.5)	Reference	
Factor VII (77.5-150.5 iu/dL)	78.17 (22.40)	93.43 (16.68)	83.63 (11.82)	0.155
Difference (95% CI)	-5.45 (-21.73 to 10.82)	9.8 (-11.29 to 30.9)	Reference	

Table 6-20: Pro and anti-coagulant markers in the women using POC (2)

Laboratory marker (reference values)	SCD (n= 29) Mean (SD)	SCT (n= 7) Mean (SD)	Hb A+A (n=8) Mean (SD)	P
Factor IX (77.7-130.0 iu/dL)	89.62 (16.63)	92.38 (16.31)	102.57 (21.43)	0.694
Difference (95% CI)	18.66 (-10.87 to 48.18)	6.57 (-31.69 to 44.83)	Reference	
Factor X (71.8-130.0 iu/dL)	120.66 (41.71)	108.57 (28.41)	102.00 (14.11)	0.013
Difference (95% CI)	-2.75 (-16.76 to 11.25)	10.2 (-7.95 to 28.34)	Reference	
Factor XI (68-144 iu/dL)	86.03 (15.96)	102.43 (13.95)	103.38 (22.29)	0.412
Difference (95% CI)	-17.34 (-31.01 to -3.67)	-0.95 (-18.66 to 16.77)	Reference	
Factor XII (46.7-181.0 iu/dL)	83.69(30.20)	103.14(23.52)	120.25(40.15)	0.402
Difference (95% CI)	-36.56 (-61.8 to -11.33)	-17.11 (-49.81 to 15.6)	Reference	
Factor XIIIa (97.4-179.0 iu/dL)	66.39(18.26)	81.57(30.51)	87.13(29.14)	0.115
Difference (95% CI)	-20.73 (-39.09 to -2.38)	-5.55 (-29.25 to 18.14)	Reference	

Table 6-21: Pro and anti-coagulant markers in the women using POC (3)

Laboratory marker (reference values)	SCD (n= 29) Mean (SD)	SCT (n= 7) Mean (SD)	Hb A+A (n=8) Mean (SD)	P
Thrombin:anti-thrombin III complex (2 - 4.2 mcg/L)	5.46 (3.06)	4.04 (1.49)	4.21(1.81)	0.070
Difference (95% CI)	1.25 (-0.94 to 3.43)	-0.17 [-2.98 to 2.64)	Reference	
Prothrombin fragment 1 + 2 (69 – 229 pmol/L)	374.37 (277.51)	139.00 (51.36)	120.13 (46.96)	<0.001
Difference (95% CI)	254.25 (68.32 to 440.17)	18.88 (12.17 to 257.92)	Reference	
D dimer (<130 ng/ml)	762.17 (728.77)	196.00 (106.70)	137.13 (103.39)	<0.001
Difference (95% CI)	625.05 (137 to 1113.1)	58.88 (45.62 to 691.37)	Reference	

Table 6-22: Coagulation activation markers in women using POC

Laboratory marker (reference values)	SCD (n=29) Mean (SD)	SCT (n=7) Mean (SD)	Hb A+A (n=8) Mean (SD)	P
Soluble Platelet selectin (92-212 ng/ml)	81.78 (26.72)	47.14 (20.60)	53.25 (11.56)	0.066
Difference (95% CI)	28.53 (9.17 to 47.88)	-6.11 (-30.99 to 18.78)	Reference	
Soluble Cell Differentiation 40 ligand (0.03-3.98 ng/ml)	1.52 (1.69)	1.25 (1.37)	2.28 (2.37)	0.351
Difference (95% CI)	-0.76 (-2.21 to 0.7)	-1.03 (-2.9 to 0.85)	Reference	
Platelet factor 4 (I.U./ml) (< 10)	147.52 (67.63)	78.50 (17.68)	105.00 (10.23)	0.254
Difference (95% CI)	42.52 (-96.59 to 181.63]	-26.5 (-193.69 to 140.69)	Reference	

Table 6-23: Platelet activation markers in women using POC

Laboratory marker (reference values)	SCD (n= 29) Mean (SD)	SCT (n= 7) Mean (SD)	Hb A+A (n=8) Mean (SD)	P
Soluble vascular cell adhesion molecule (ng/ml) (131-1223)	1162.04 (917.68)	606.43 (505.56)	396.00 (154.44)	<0.001
Difference (95% CI)	766.04 [132.71 to 1399.36]	210.43 [60.85 to 1024.7]	Reference	
Soluble inter-cellular adhesion molecule-1(ng/ml) (41-669)	528.03 (128.45)	271.87 (41.29)	270.31 (42.36)	0.001
Difference (95% CI)	257.72 [170.09 to 345.35]	1.56 [1.11 to 14.23]	Reference	
Soluble endothelial-leucocyte adhesion molecule-1 (ng/ml) (1.5 – 88.1)	52.67 (29.59)	81.00 (35.19)	69.88 (31.30)	0.860
Difference (95% CI)	-17.21 [-42.3 to -7.89]	11.13 [2.14 to 43.39]	Reference	

Table 6-24: Endothelial activation markers in women using POC

Laboratory marker (reference values)	SCD (n= 29) Mean (SD)	SCT (n= 7) Mean (SD)	Hb A+A (n=8) Mean (SD)	P
Micro particles (<10nMol/ml)	39.03 (27.11)	8.33 (4.02)	6.70 (2.28)	<0.001
Difference (95% CI)	32.33 (14.21 to 50.46)	1.63 (0.21 to 25.12)	Reference	
Tissue Factor(<2pMol/ml)	8.84 (6.95)	2.13 (1.89)	6.13 (6.11)	0.011
Difference (95% CI)	2.71 (2.44 to 7.86)	-4 (-10.53 to -2.53)	Reference	

Table 6-25: Markers of tissue damage in women using POC

Laboratory marker (reference values)	SCD (n= 29) Mean (SD)	SCT (n= 7) Mean (SD)	Hb A+A (n=8) Mean (SD)	P
Lag Time (initiated with 5pMol Tissue Factor) (2.0 - 3.2 minutes)	1.60 (0.53)	0.160	2.42 (0.91)	0.002
Difference (95% CI)	-0.82 (-1.33 to -0.32)	0.18 (0.083 to 0.47)	Reference	
Endogenous Thrombin Potential (initiated with 5pMol Tissue Factor) (1159 – 2168 nMol)	1480.68 (269.80)	0.463	1636.88(364.17)	0.176
Difference (95% CI)	-156.2 (-385.82 to 73.42)	-333.87 (-630.31 to -37.44)	Reference	
Peak Height (initiated with 5pMol Tissue Factor)(147 – 359 nM)	319.07 (52.31)	0.111	320.75 (84.73)	0.949
Difference (95% CI)	-1.68 (-54.41 to 51.06)	-86.89 (-154.97 to -18.81)	Reference	
Time to Peak (initiated with 5pMol Tissue Factor) (4.6 – 7.4 min)	3.92 (0.83)	0.868	4.40 (0.70)	0.141
Difference (95% CI)	-0.48 (-1.13 to 0.17)	-0.28 (-1.12 to 0.56)	Reference	
Slope (33 – 142 nM/min)	143.25 (39.33)	0.002	187.55 (101.15)	0.067
Difference (95% CI)	-44.3 (-91.87 to 3.28)	-55.14 (-116.56 to 6.28)	Reference	
Start Time (initiated with 5pMol Tissue Factor) (19 28.3 min)	18.57 (2.75)	0.048	21.38 (2.86)	0.042
Difference (95% CI)	-2.8 (-5.5 to -0.11)	2.84 (-0.64 to 6.32)	Reference	

Table 6-26: Markers of thrombin generation in women using POC

Laboratory marker (reference values)	SCD (n= 29) Mean (SD)	SCT (n= 7) Mean (SD)	Hb A+A (n=8) Mean (SD)	P
Free Hb (11-15.5mg/l)	0.09(0.10	0.12(0.10	0.06(0.05	0.177
Difference (95% CI)	0.02 (-0.05 to 0.1)	0.06 (-0.04 to 0.16)	Reference	
Haptoglobin (< 1.22 g/l)	1.56 (0.66)	1.15 (0.45)	1.19 (0.55)	0.510
Difference (95% CI)	0.37 (-0.13 to 0.88)	-0.04 (-0.68 to 0.6)	Reference	
Lactate dehydrogenase (240-480 units/l)	637.23 (227.29)	467.29 (57.30)	371.00 (134.91)	0.004
Difference (95% CI)	266.23 (97.18 to 435.28)	96.29 (-115.92 to 308.49)	Reference	
Bilirubin (< 21 µMol/l)	38.11 (32.64)	7.00 (3.46)	8.13 (6.24)	<0.001
Difference (95% CI)	29.99 (8.15 to 51.82)	-1.12 (-29.2 to -0.26)	Reference	

Table 6-27: Markers of haemolysis in women using POC

Laboratory marker (reference values)	SCD (n= 29) Mean (SD)	SCT (n= 7) Mean (SD)	Hb A+A (n=8) Mean (SD)	P
Alanine amino transferase (<33 units/l)	19.97(11.89)	14.71(3.90)	25.00(20.89)	0.001
Difference (95% CI)	-5.03 (-15.65 to 5.58)	-10.29 (-24.05 to 3.47)	Reference	
Aspartate amino transferase (<31 units/l)	29.79(11.91)	23.14(4.88)	22.31(6.40)	0.023
Difference (95% CI)	7.47 (-0.89 to 15.84)	0.83 (-9.97 to 11.63)	Reference	
Alkaline phosphatase (< 129 units/l)	86.86(48.12)	64.14(17.78)	96.00(77.64)	0.023
Difference (95% CI)	-9.14 (-50.71 to 32.43)	-31.86 (-85.73 to -22.0)	Reference	
Serum albumin (35-50g/l)	45.79(3.57)	43.71(2.14)	42.61(5.60)	0.064
Difference (95% CI)	3.18 (0.09 to 6.28)	1.1 (-2.91 to 5.11)	Reference	

Table 6-28: Biochemical markers of liver function in women using POC

Laboratory marker (reference values)	SCD (n= 29) Mean (SD)	SCT (n= 7) Mean (SD)	Hb A+A (n=8) Mean (SD)	P
Haemoglobin (11.5-15-5 g/dl)	9.23 (1.56)	12.47(1.03)	11.95 (1.07)	0.310
Difference (95% CI)	-2.72 (-3.87 to -1.58)	0.52 (-0.96 to 2)	Reference	
Platelet count (140-400 10^9/l)	323.39 (134.13)	251.71 (70.91)	228.00 (46.02)	0.007
Difference (95% CI)	95.39 (2.06 to 188.72)	23.71 (9.67 to 144.2)	Reference	
Haematocrit (0.35-0.47 l/l)	0.27(0.05)	0.38 (0.03)	0.37 (0.04)	0.779
Difference (95% CI)	-0.1 (-0.14 to -0.07)	0.01 -0.05 to 0.07)	Reference	

Table 6-29: Blood count values in women using POC

Laboratory marker (reference values)	SCD (n= 29) Mean (SD)	SCT (n= 7) Mean (SD)		
Complement reactive protein (0.2-10.0 mcg/ml)	35.58 (19.20)	13.57 (16.30)	13.00 (15.98)	0.793
Difference (95% CI)	22.58 (7.55 to 37.61)	0.57 (-18.48 to 19.63)	Reference	
White blood cells (3.5-11.0 10^9/l))	8.46 (3.43)	4.17 (1.27)	6.42 (2.85)	0.573
Difference (95% CI)	2.04 (-0.99 to 5.07)	-2.25 (-7.73 to 3.23)	Reference	
Neutrophils (1.7-8.0 10^9/l))	4.50 (2.39)	1.90 (0.94)	2.95 (0.83)	0.061
Difference (95% CI)	1.55 (-0.46 to 3.56)	-1.05 (-4.66 to 2.57)	Reference	
Lymphocytes (1.0-3.510^9/l))	2.84 (0.90)	1.75 (0.08)	1.80 (0.79)	0.191
Difference (95% CI)	1.03 (0.23 to 1.84)	-0.05 (-1.49 to 1.4)	Reference	
Monocytes (0.1-1.010^9/l)	0.83 (0.36)	0.34 (0.08)	0.45 (0.10)	0.018
Difference (95% CI)	0.38 (0.07 to 0.68)	-0.11 (-0.65 to 0.43)	Reference	
Eosinophils (0.0-0.46 10^9/l))	0.23 (0.19)	0.15 (0.16)	0.14 (0.11)	0.396
Difference (95% CI)	0.08 (-0.08 to 0.25)	0 (-0.29 to 0.3)	Reference	
Basophils (0.0-0.20 10^9/l))	0.05 (0.05)	0.01 (0.01)	0.02 (0.01)	0.396
Difference (95% CI)	0.03 (-0.01 to 0.07)	0 (-0.07 to 0.07)	Reference	

Table 6-30: Inflammatory markers in women using POC

6.7 SUMMARY OF RESULTS FOR THE WOMEN USING POC

As already observed in Chapter 5, the SCD women using POC had been using that method significantly longer than the SCT and normal Hb women. Clinically significant events were much higher in the SCD women on POC, but seemed to be related to their SCD itself, and no POC users had clinically recognised episodes of VTE or arterial thrombosis.

The occurrence of contraceptive side effects with women using POCs was similar in all the three groups. These were mainly amenorrhoea and menstrual irregularities, and it should be noted that the SCD women were the largest group on POC, as has been previously highlighted (Chapter 5).

Looking at the pro and anti-coagulant markers, PT and INR levels were at the upper limit of the reference range in the SCD women and the difference reached statistical significance. The DRVVT, AT:activity and PC: activity levels all fell within the reference ranges, with values lowest in SCD women. However only the difference in DRVVT reached statistical significance. Although activated protein C resistance levels lie within the reference range, the difference between the three groups is statistically significant, with levels being lower in women with normal Hb. Protein S levels were lower than the reference range (i.e. adverse) in all women using POC, but most markedly so with SCD women.

Factors II, VII, XI, and XII mean values lie within the reference range, being lowest in SCD women. Factor X was highest in SCD women but again all the mean values were within the reference range.

Factor XIIIa levels were lower than the reference range in all POC users but lowest in those with SCD.

With regard to coagulation activation markers, TAT complex and Prothrombin F1+2 were normal in women using POC, except in SCD women, where the levels were markedly raised.

D dimer levels were higher than the reference range in all women using POC; however, they were most markedly elevated in SCD, with an upward gradation from normal Hb to SCT to SCD.

6.8 CLINICAL EVENTS IN WOMEN NOT USING HC

Clinical variables	SCD (n=30) Mean (SD)	SCT (n=7) Mean (SD)	Hb AA (n=9) Mean (SD)	P
Number of previous pregnancies (mean+/-SD)	1.2 (1.09)	1.42 (1.13)	1.44 (0.88)	0.752
Difference (95% CI)	0.21(-6.06 to 6.48)	0.25(-6.02 to 6.52)	Reference	
Duration of use of contraceptive method prior to enrolment (months) (mean+/-SD)	18(4.72)	16(5.12)	20(7.68)	0.271
Difference (95% CI)	-0.4 (-7.26 to 5.87)	-2.56(-9.36 to 4.3)	Reference	

Table 6-31: The obstetric history and duration of use of their contraceptive method prior to the enrolment interview in women not using HC

Clinical events	SCD (n=30)	SCT (n=7)	Hb AA (n =9)
Severe sickle crises (total)	14	0	0
Number of women with severe sickle crises	10	2	0
Hospital admissions (total)	16	0	3
Number of women with hospital admission	11	0	3
Blood transfusions (total number of sessions)	5	0	0
Number of women receiving transfusion	3	0	0
VTE (DVT & PE)	1	0	0
Strokes & heart attacks	0	0	0
Arterial thrombosis	0	0	0

Table 6-32: Clinical events during the study in women not using HC

Contraceptive problems	SCD (n=30)	SCT (n=7)	Hb AA (n=9)
Side effects (i.e. headaches, nausea, mood swings, bloatedness, breast tenderness)	0	0	0
Discontinuation	0	0	0
Unintended interruptions	0	0	0
Planned pregnancies	1	0	0
Unplanned pregnancies	1	0	1
Amenorrhoea	0	1	0
Menstrual irregularities	4	2	2

Table 6-33: Contraceptive side effects and problems during the study in women not using HC

6.9 LABORATORY RESULTS FOR WOMEN NOT USING HC

Laboratory marker (reference values)	SCD (n=30) Mean (SD)	SCT (n=7) Mean (SD)	Hb AA (n=9) Mean (SD)	P
Prothrombin time (12 – 15 seconds)	14.94 (1.57)	11.03 (0.78)	10.77 (0.72)	0.019
Difference (95% CI)	4.16 (3.12 to 5.2)	0.25 (-1.13 to 1.63)	Reference	
International normalised ratio (INR)(0.8-1.2)	1.00 (0.06)	1.04 (0.07)	1.16 (0.13)	0.024
Difference (95% CI)	0.16 (0.07 to 0.25)	0.04 (-0.08 to 0.15)	Reference	
Activated partial thromboplastin time (25-35 seconds)	27.98 (2.35)	32.06 (4.44)	31.09 (2.19)	0.060
Difference (95% CI)	-3.11 (-5.19 to -1.03)	0.97 (-1.79 to 3.73)	Reference	
Thrombin Time (12-14 seconds)	13.80(1.00)	15.38(1.80)	15.011(0.76)	0.033
Difference (95% CI)	-1.2 (-2.07 to -0.34)	0.37 (-0.78 to 1.53)	Reference	
Fibrinogen (2.14-3.51 g/dL)	3.10 (0.62)	4.94 (3.60)	2.82 (0.51)	<0.001
Difference (95% CI)	0.27 (-0.84 to 1.39)	2.11 (0.64 to 3.59)	Reference	
Factor VIII:C (50 – 150 iu/dL)	168.93 (51.59)	148.00 (19.00)	143.56 (42.87)	0.045
Difference (95% CI)	25.38 (-10.47 to 61.22)	4.44 (-43.09 to 51.98)	Reference	
von willebrand factor antigen (46 – 153 iu/dL)	181.96 (68.43)	124.32 (36.75)	143.13 (45.89)	0.138
Difference (95% CI)	38.83 (-8.03 to 85.7)	-18.8 (-80.95 to 43.34)	Reference	
Dilute Viper Venom Time (ratio) (<1.16)	0.92 (0.17)	0.94(0.21)	0.91(0.04)	0.001
Difference (95% CI)	0.01 (-0.11 to 0.14)	0.03 (0.01 to 0.2)	Reference	

Table 6-34: Pro and anti-coagulant markers in the women not using HC (1)

Laboratory marker (reference values)	SCD (n=30) Mean (SD)	SCT (n=7) Mean (SD)	Hb AA (n=9) Mean (SD)	P
Anti-thrombin : Antivity (85-113 iu/dL)	98.57 (10.90)	108.09 (8.74)	105.88 (5.82)	0.149
Difference (95% CI)	-7.31 (-14.86 to .24)	2.21 (-7.81 to 12.22)	Reference	
Protein C : Activity (70-135 iu/dL)	85.03 (15.50)	100.29(21.62)	100.39 (23.49)	0.241
Difference (95% CI)	-15.36 (-29.28 to -1.44)	-0.1 (-18.56 to 18.35)	Reference	
Free Protein S (75-125 iu/dL)	56.30 (13.30)	70.28 (14.63)	73.22 (10.60)	0.685
Difference (95% CI)	-16.92 (-26.92 to -6.92)	-2.94 (-16.19 to 10.32)	Reference	
Activated Protein C Resistance (Factor V Leiden) (>2.0)	2.84 (0.35)	2.71 (0.12)	2.71 (0.03)	<0.001
Difference (95% CI)	0.13 (0.1 to 0.36)	0 (0.29 to 3)	Reference	
Factor II (84.3-120.0 iu/dL)	90.33 (14.28)	95.71 (20.88)	101.67 (26.41)	0.056
Difference (95% CI)	-11.33 (-25.22 to 2.55)	-5.95 (-24.36 to 12.46)	Reference	
Factor V (63.85-120.5 iu/dL/ml)	100.30 (18.63)	81.71 (23.03)	80.56 (8.89)	0.053
Difference (95% CI)	19.74 (5.98 to 33.51)	1.16 (0.17 to 19.41)	Reference	
Factor VII (77.5-150.5 iu/dL)	92.40 (22.70)	86.89 (24.80)	83.14 (21.11)	0.915
Difference (95% CI)	5.51 (-12.04 to 23.06)	-3.75 (-27.02 to 19.52)	Reference	

Table 6-35: Pro and anti- coagulant markers in women not using HC (2)

Laboratory marker (reference values)	SCD (n=30) Mean (SD)	SCT (n=7) Mean (SD)	Hb AA (n=9) Mean (SD)	P
Factor IX (77.7-130.0 iu/dL)	121.17 (29.70)	101.14 (16.76)	96.22 (16.70)	0.082
Difference (95% CI)	24.94 (4.87 to 45.02)	4.92 (-21.69 to 31.54)	Reference	
Factor X (71.8-130.0 iu/dL)	94.83 (22.96)	91.71 (10.64)	93.67 (17.72)	0.113
Difference (95% CI)	1.17 (-14.72 to 17.06)	-1.95 (-23.02 to 19.12)	Reference	
Factor XI (68-144 iu/dL)	92.37 (20.84)	91.43 (21.46)	94.44 (9.55)	0.066
Difference (95% CI)	-2.08 (-16.9 to 12.75)	-3.02 (-22.68 to 16.64)	Reference	
Factor XII (46.7-181.0 iu/dL)	99.77 (24.17)	85.71 (36.11)	83.78 (24.06)	0.383
Difference (95% CI)	15.99 (-4.05 to 36.03)	1.94 (-24.64 to 28.51)	Reference	
Factor XIIIa (97.4-179.0 iu/dL)	66.10 (21.90)	97.86 (14.62)	97.78 (20.19)	0.522
Difference (95% CI)	-31.68 (-47.56 to -15.8)	0.08 (-20.98 to 21.14)	Reference	

Table 6-36: Pro and anti- coagulant markers in women not using HC (3)

Laboratory marker (reference values)	SCD (n=30) Mean (SD)	SCT (n=7) Mean (SD)	Hb AA (n=9) Mean (SD)	P
Thrombin:anti-thrombin III complex (2 - 4.2 mcg/l)	19.70 (40.28)	3.13 (0.58)	4.90 (1.91)	<0.001
Difference (95% CI)	-14.8 (-15.30 to -4.13)	1.77 (0.35 to 31.69)	Reference	
Prothrombin fragment1 + 2 (69 – 229 pmol/L)	492.72 (380.72)	142.71 (109.00)	144.11 (78.38)	< 0.001
Difference (95% CI)	348.61 (105.72 to 591.51)	-1.4 (-322.2 to 319.41)	Reference	
D dimer (<130 ng/ml)	943.77 (319.40)	126.20 (70.59)	107.00 (48.24)	<0.001
Difference (95% CI)	836.77 (90.01 to 1583.52)	19.2 (-1022.5 to 1060.9)	Reference	

Table 6-37: Coagulation activation markers in women not using HC (3)

Laboratory marker (reference values)	SCD (n=30) Mean (SD)	SCT (n=7) Mean (SD)	Hb AA (n=9) Mean (SD)	P
Soluble Platelet selectin(92-212 ng/ml)	84.89 (32.91)	52.57 1(4.05)	53.55 (12.99)	0.004
Difference (95% CI)	31.34 (9.8 to 52.89)	-0.98 (-1.44 to -0.27)	Reference	
Soluble Cell Differentiation 40 ligand(0.03-3.98 ng/ml)	1.33 (0.20)	0.48 (0.32)	0.59(0.18)	<0.001
Difference (95% CI)	0.73 (0.12 to 2.74)	-0.11 (-2.77 to -0.05)	Reference	
Platelet factor 4 (<10I.U./ml)	140.63 (74.10)	77.00 (6.28)	82.33 (19.29)	<0.001
Difference (95% CI)	58.29 (24.77 to 141.35)	-5.33 (-10.5 to -0.94)	Reference	

Table 6-38: Platelet activation markers in women not using HC

Laboratory marker (reference values)	SCD (n=30) Mean (SD)	SCT (n=7) Mean (SD)	Hb AA (n=9) Mean (SD)	P
Soluble vascular cell adhesion molecule (ng/ml) (131-1223)	1095.11 (563.90)	486.43 (207.00)	507.67 (260.05)	0.005
Difference (95% CI)	587.44 (217.35 to 957.53)	-21.24 (-510.03 to -4.65)	Reference	
Soluble inter-cellular adhesion molecule-1(ng/ml) (41-669)	555.07 (162.63)	281.44 (70.20)	354.33 (102.26)	0.046
Difference (95% CI)	200.73 (90.94 to 310.52)	-72.89 (-217.9 to 72.12)	Reference	
Soluble endothelial-leucocyte adhesion molecule-1 (ng/ml) (1.5 – 88.1)	68.86 (38.49)	51.14 (22.98)	66.55 (13.15)	0.006
Difference (95% CI)	2.31 (-23.19 to 27.81)	-15.41 (-49.09 to 18.27)	Reference	

Table 6-39: Endothelial activation markers in women not using HC

Laboratory marker (reference values)	SCD (n=30) Mean (SD)	SCT (n=7) Mean (SD)	Hb AA (n=9) Mean (SD)	P
Micro particles (<10nMol/ml)	34.94 (20.37)	8.34 (5.15)	8.96 (4.04)	<0.001
Difference (95% CI)	25.99 (13.03 to 38.95)	-0.61 (-17.73 to 0.02)	Reference	
Tissue Factor(<2pMol/ml)	12.08 (5.96)	5.49 (7.55)	5.60 (5.90)	0.734
Difference (95% CI)	6.47 (1.67 to 11.28)	-0.11 (-6.43 to 6.21)	Reference	

Table 6-40: Markers of tissue damage in women not using HC

Laboratory marker (reference values)	SCD (n=30) Mean (SD)	SCT (n=7) Mean (SD)	Hb AA (n=9) Mean (SD)	P
Lag Time (initiated with 5pMol Tissue Factor) (2.0 - 3.2 minutes)	1.66 (0.67)	2.38 (1.39)	1.87 (0.41)	0.003
Difference (95% CI)	-0.21 (-0.8 to 0.03)	0.51 (0.27 to 1.3)	Reference	
Endogenous Thrombin Potential (initiated with 5pMol Tissue Factor)(1159 – 2168 nM)	1345.43 (245.32)	1484.71 (332.93)	1637.89 (284.27)	0.581
Difference (95% CI)	-292.46 (-496.81 to -88.1)	-153.17 (-424.15 to 117.8)	Reference	
Peak Height (initiated with 5pMol Tissue Factor)(147 – 359 nM)	305.65 (58.93)	283.86 (59.42)	322.00 (37.81)	0.363
Difference (95% CI)	-16.35 (-59.03 to 26.33)	-38.14 (-94.73 to 18.45)	Reference	
Time to Peak (initiated with 5pMol Tissue Factor)(4.6 – 7.4 min)	3.90 (0.96)	4.93 (1.64)	4.28 (0.48)	0.010
Difference (95% CI)	-0.38 (-1.16 to -0.04)	0.65 (0.39 to 1.69)	Reference	
Slope (33 – 142 nM/min)	142.72 (40.36)	114.90 (33.83)	134.62 (17.37)	0.048
Difference (95% CI)	8.1 (-19.69 to 35.89)	-19.72 (-56.57 to 17.12)	Reference	
Start Time (initiated with 5pMol Tissue Factor)(19 -28.3 min)	17.48 (2.01)	20.93 (2.23)	20.22 (1.72)	0.793
Difference (95% CI)	-2.74 (-4.27 to -1.21)	0.71 (-1.32 to 2.73)	Reference	

Table 6-41: Markers of thrombin generation in women not using HC

Laboratory marker (reference values)	SCD (n=30) Mean (SD)	SCT (n=7) Mean (SD)	Hb AA (n=9) Mean (SD)	P
Free Hb (11-15.5mg/l)	0.10 (0.09)	0.14 (0.20)	0.06 (0.08)	0.006
Difference (95% CI)	0.04 (0.01 to 0.12)	0.08 (-0.3 to -0.01)	Reference	
Haptoglobin (< 1.22 g/l)	1.54 (0.47)	1.12 (0.43)	1.28 (0.69)	0.299
Difference (95% CI)	0.26 (-0.13 to 0.66)	-0.16 (-0.69 to 0.36)	Reference	
Lactate dehydrogenase (240-480 units/l)	734.17 (321.21)	417.83 (76.16)	379.14 (70.04)	<0.001
Difference (95% CI)	355.03 (120.87 to 589.19)	38.69 (-270.67 to 348.05)	Reference	
Bilirubin (< 21 µMol/l)	39.74 (27.72)	5.29 (1.70)	6.38 (2.13)	<0.001
Difference (95% CI)	33.37 (14.91 to 51.82)	-1.09 (-24.81 to -0.22)	Reference	

Table 6-42: Markers of haemolysis in women not using HC

Laboratory marker (reference values)	SCD (n=30) Mean (SD)	SCT (n=7) Mean (SD)	Hb AA (n=9) Mean (SD)	P
Alanine amino transferase (<33 units/l)	22.07 (9.25)	20.14 (13.36)	11.38 (2.62)	0.141
Difference (95% CI)	10.7 (3.2 to 18.2)	8.77 (-0.91 to 18.45)	Reference	
Aspartate amino transferase (<31 units/l)	35.19 (19.56)	23.71 (9.18)	20.88 (4.58)	0.002
Difference (95% CI)	14.32 (0.9 to 27.74)	2.84 (1.43 to 20.02)	Reference	
Alkaline phosphatase (< 129 units/l)	88.13 (36.43)	54.57 (22.99)	69.62 (17.48)	0.001
Difference (95% CI)	18.51 (7.45 to 44.47)	-15.05 (-48.7 to -1.85)	Reference	
Serum albumin (35-50g/l)	45.83 (2.92)	42.43 (1.62)	45.38 (1.85)	0.069
Difference (95% CI)	0.45 (-1.65 to 2.55)	-2.95 (-5.67 to -0.23)	Reference	

Table 6-43: Biochemical markers of liver function in women not using HC

Laboratory marker (reference values)	SCD (n=30) Mean (SD)	SCT (n=7) Mean (SD)	Hb AA (n=9) Mean (SD)	P
Haemoglobin (11.5-15-5 g/dl)	9.07 (1.44)	11.55 (0.55)	12.19 (0.49)	0.002
Difference (95% CI)	-3.12 (-4.06 to -0.21)	-0.64 (-1.95 to -0.07)	Reference	
Platelet count (140-400 10^9/l)	331.40 (144.40)	252.33 (84.91)	261.44 (54.42)	0.012
Difference (95% CI)	69.96 (-26.51 to 166.42)	-9.11 (-142.89 to 124.66)	Reference	
Haematocrit (0.35-0.47 l/l)	0.26(0.03)	0.35 (0.02)	0.37 (0.02)	0.222
Difference (95% CI)	-0.11 (-0.14 to -0.09)	-0.02 (-0.06 to 0.01)	Reference	

Table 6-44: Blood count values in women not using HC

Laboratory marker (reference values)	SCD (n=30) Mean (SD)	SCT (n=7) Mean (SD)	Hb AA (n=9) Mean (SD)	P
Complement reactive protein (0.2-10.0 mcg/ml)	35.39 (22.07)	13.29 (10.80)	17.67 (20.64)	0.04 (0.03)
Difference (95% CI)	17.73 (1.85 to 33.6)	-4.38 (-25.26 to 16.5)	Reference	
White blood cells (3.5-11.0 10^9/l))	8.23 (2.69)	5.78 (2.59)	5.33 (0.93)	0.013
Difference (95% CI)	2.9 (1.02 to 4.77)	0.45 (-2.15 to 3.05)	Reference	
Neutrophils (1.7-8.0 10^9/l))	4.22 (1.71)	3.21 (1.62)	2.50 (0.45)	0.002
Difference (95% CI)	1.73 (0.54 to 2.91)	0.71 (-0.91 to 2.34)	Reference	
Lymphocytes (1.0-3.510^9/l))	2.57 (1.04)	3.21 (1.62)	2.23 (0.48)	0.050
Difference (95% CI)	0.34 (-0.44 to 1.11)	-0.29 (-1.35 to 0.77)	Reference	
Monocytes (0.1-1.010^9/l)	0.71 (0.38)	0.31 (0.15)	0.45 (0.10)	0.001
Difference (95% CI)	0.26 (0.01 to 0.51)	-0.14 (-0.48 to 0.2)	Reference	
Eosinophils (0.0-0.46 10^9/l))	0.231 (0.22)	0.22 (0.17)	0.13 (0.07)	0.015
Difference (95% CI)	0.1 (-0.06 to 0.25)	0.09 (-0.12 to 0.3)	Reference	
Basophils (0.0-0.20 10^9/l))	0.04 (0.03)	0.03 (0.02)	0.02 (0.01)	0.001
Difference (95% CI)	0.02 (0.01 to 0.04)	0 (0.03 to 3.2)	Reference	

Table 6-45: Inflammatory markers in women not using HC

6.10 SUMMARY OF RESULTS FOR THE WOMEN NOT USING HORMONAL CONTRACEPTION

There are no statistically significant differences between the three groups in terms of parity or duration of non-use of HC. Women with SCD had more complications and one woman suffered an episode of VTE.

Pro and anti-coagulant markers showed varying trends. Free Protein S levels were lower than the reference range in all three groups. Fibrinogen levels were statistically significantly higher in women with SCT. Women with SCD had higher levels of Factor VIII and VWF antigen. Activated Protein C resistance levels were again statistically significantly different between the three groups, the lowest mean value being in women with normal Hb. Factor XIII was lowest in women with SCD (ranging from 66.10 to 97.78.).

Coagulation activation markers (TAT complexes, D Dimers and Prothrombin F1+2) were all statistically significantly elevated in women with SCD.

Although, all the platelet activation markers studied, except PF4, were normal in the three groups, the difference between the groups is statistically significant (with levels being higher in women with SCD).

Endothelial activation markers were normal in all women not using HC. E elevated levels of TF were displayed by all the three groups but the difference between them did not reach statistical significance. Microparticle levels were statistically significantly higher in the SCD women.

Thrombin generation markers were normal in all three groups.

The free Haemoglobin levels were significantly higher in women with SCT, while LDH and Bilirubin were elevated in women with SCD.

CRP levels were high in all women not using HC, however the differences between the groups did not reach statistical significance.

Although it is to be expected that women with SCD will show, as they did, increased pro-coagulant markers, increased coagulation activation markers and platelet activation markers, some of the findings in the SCT and normal Hb women have some unexpected features. All the women, regardless of Hb type, had low Protein S (a natural anticoagulant), raised endothelial activation markers and

raised CRP, compared with general population reference ranges. All of these being adverse indicators of VTE risk. In addition, the SCT women, rather unexpectedly, had raised fibrinogen, and raised free Hb levels, a marker of haemolysis which predisposes to thrombosis. The implications of these findings being that some degree of VTE risk may be relevant to all women of black ethnicity, and additionally to women with SCT. Bearing in mind that the women described in Tables 6.31 to 6.40 are using no hormonal contraception, these features deserve further exploration.

CHAPTER 7

DISCUSSION AND CONCLUSIONS

7.1 AIMS

This research aims to answer the question whether hormonal contraception poses any additional clinical or haematological risks to women with SCD and SCT over and above the risks inherent in the disease itself. Although the UK Medical Eligibility Criteria (MEC) for contraceptive use, in 2009, specified that the use of combined hormonal contraception in SCD is MEC "category 2" (i.e. the benefits of use outweigh the risks) and the use of progestogen-only contraception is "category 1" (i.e. no restriction on their use), this was followed by the US MEC recommendation, in 2010, that women with sickle cell disease should not be discouraged from the use of any particular contraceptive method, stating that combined hormonal contraceptive methods and copper IUDs are a US MEC "Category 2", while progestogen-only methods are US MEC "Category 1" (U S. Medical Eligibility Criteria for Contraceptive Use, 2010). However, the first recommendation is based more on the fact that pregnancy carries many hazards to women with SCD and not on any evidence to support their complete safety, while the second recommendation, albeit very positive, is based also on limited evidence, as has been shown in Chapter 1.

7.2 IMPORTANCE OF THE STUDY

This study is important because it addresses an area, contraception in women with SCD, which at present is poorly understood. Advances in understanding in this area

may help to reduce the high rate of unplanned pregnancies in this cohort of women and may assist medical practitioners and their patients in finding the right balance between the risks of VTE associated with the use of some types of hormonal contraception in women with SCD, and alternatively, the major complications of pregnancy. It is also the first study to combine clinical and laboratory markers in studying contraception in women with SCD using hormonal contraception. This study has also pioneered the assessment of contraception in women with SCT.

The number of unplanned pregnancies in SCD women is still unacceptably high. In a survey conducted by the Author in 2010, 53% of the pregnancies in SCD women were unplanned. This showed some improvement compared with a similar survey in 1993 (64.2%). These findings emphasise the continuing unmet need for effective contraceptive advice for SCD women (Appendix 2).

7.3 PRACTICALITIES AND DIFFICULTIES WITH THE RESEARCH

Clinically, this study covered vascular complications, such as VTE incidences, arterial thrombosis and strokes, sickle cell crises, the need for blood transfusion and hospital admissions as well as looking at side effects and failures associated with any particular method of contraception. In order for this information to be reliable I have only counted those events occurring prospectively during the study periods. However, at the interview, some women told me retrospectively that they had experienced complications and hence had changed their method of

contraception. This inevitably raises the possibility of bias in these women at the time I was observing them.

I delayed taking blood samples until participants had been enrolled in the study for three months, to make sure that the participants have a plateau from exposure to their chosen contraception method because the risk of VTE is highest in the four months following initiation of combined hormonal contraception (WHO, 1995). This three-month period also helped me to organise the timings for blood collection in SCD women with regard to their crises, blood transfusions and their menstrual periods, where necessary.

The laboratory tests in this study were selected to test all possible SCD patho-physiological manifestations as well as some known haemostatic effects of hormonal contraception, as discussed in chapters 1 and 3. I have grouped all types of SCD together and deliberately avoided subdividing them by specific type. I am aware that some people may criticise this. My justification for this approach is that data on pregnancy-related complications shows that specific types of SCD and preceding haematological clinical history do not correlate with complications during pregnancy and the puerperum (Howard *et al*., 1995). This knowledge could be extrapolated to hormonal contraception use, since pregnancy physiological changes are E2 and progesterone related.

I have studied all the effects of the different COC preparations together as one group, as well as studied the POC preparations as a single group. While this helps to observe and identify any possible interactions of these hormones with SCD and SCT, it would not help in stratifying the risks with the different preparations.

The small number of participants in some of the study arms arises partly from the major time delays with the ethics and R&D approval process and the failure to include some hospitals with SCD populations as discussed in chapter 3. It is also due to fact that there are extremely few women with SCD who are using COC among the potential study population. This small number of COC users in the community reflects the fact that these women are not prescribed the COC as readily as POC.

The study did involve looking at many facets of laboratory markers and clinical parameters, in the participants, in order to ensure good coverage to help answering the study question. However, I appreciate that studying many parameters could also be a source of bias, as the chance of identifying an adverse linkage with the use of hormonal contraception could increase. To deal with this potential source of bias additional analysis was done using logistic regression testing beside the main statiscal analysis by Linear regression (LR)in order to identify any true effects of contraception used by these women. As previously

discussed (para 3.9), however, unfortunately this did not prove useful, possibly because of the relatively small numbers involved.

Of those women on any hormonal contraception, the duration of POC use is almost three times the COC use, and that probably results from the tendency of contraception prescribers to be influenced by anecdotal suggestions that POC is the better choice for women with SCD. Data obtained from the women's interviews highlighted that sometimes SCD women accept POC use as their best choice even if they experience problems taking it.

7.4 SIGINFICANCE OF SOME OF THE HAEMOSTATIC AND HAEMATOLOGICAL TESTS USED

7.4.1 INTRODUCTION

Some of the haemostatic tests used in this study and their relevance to SCD pathogenesis or hormonal contraception were already discussed in chapters 1 and 3. A further review of some of the other tests and their clinical value will be given in this section.

7.4.2 PRO AND ANTI-COAGULANT MARKERS

In this paragraph I will explain further the relevance of activated Protein C resistance (APCR), Free Protein S (FPS) and the Dilute Russell's Viper Venom Time

(DRVVT) to this study. APCR is proven to occur in patients with SCD (Wright *et al*., 1997a) and in women using COC (Raps *et al*., 2013) and it can predict the risk of VTE (De Visser *et al.*, 1999).

Protein S is one of the naturally occurring anticoagulants which together with PC and AT regulate c stimulates the Tissue Factor Inhibitor pathway, leading to the inhibition of coagulation (Castoldi *et al*., 2010). It is also known to be adversely affected by SCD, with low levels usually noted even in the steady state. However, there is a suggestion that low FPS levels in SCD patients may result from hepatic dysfunction rather than coagulation activation (Wright *et al.*, 1997b) and may also be due to reduced endothelial production of PS, secondary to SCD-related endothelial damage *(Schnog et al*., 2004). Acquired Protein S deficiency is also reported to be caused by COC use, as mentioned in chapter 1

7.4.3 COAGULATION ACTIVATION MARKERS

Thrombin anti-thrombin complexes (TAT) and Prothrombin Fragment 1+ 2 levels are well known to be raised in sickle cell disease (Peters *et al.*, 1994), however there is evidence that these complexes could be also raised in other conditions such as inflammatory bowel disease (Souto *et al*., 1995). Elevated levels are also found in patients with VTE (Ota *et al.*, 2008), malignancy (Tripodi *et al*., 1993) and in patients with thrombophilia (Simioni *et al*., 1996). Levels may be abnormally low in patients who are on anticoagulant therapy.

7.4.4 PLATELET ACTIVATION MARKERS

The platelet activation markers studied in this project were: sPselectin, Platelet Factor 4 (PF4) and SCD40 ligand. All these markers were shown to be relevant to thrombosis (Davis *et al*., 2003). **s**Pselectin is an adhesion molecule that supports the binding of WBCs to activated platelets and the endothelium. It is normally present in granules in the membranes of unstimulated platelets and it is mobilised and migrates to the surface of platelets during activation. SP selectins are also present in endothelial cells and megakaryocyte membranes. The appearance of p-selectin on the cell surface is usually very rapid. Excessive accumulation of neutrophilis, accompanied by high exposure of P-selectin, has been implicated in a number of inflammatory disorders (Fijnheer *et al*., 1997). PF4 is released from activated platelet α granules. It has a very short half life of less than five minutes, as it is quickly bound to endothelial cell glycos-aminoglycans, where it is stored. PF4 determination of platelet reactivity should be taken in conjunction with other markers of a hypercoagulable state including Fibrinopeptide A (FPA) and D-dimer, because raised levels can be found in cardio-vascular disease, diabetes, and malignancy (Kaplan and Owen, 1986; Mosnier, 2011). SCD40 ligand is a platelet-activation marker which could also signal T cell activation. Hence its levels can increase in pathological immune conditions such as Chronic Myeloid Leukemia (CLL), hyper IgM syndrome, B-cell hybridisms and auto-immune diseases. In conditions of pathological thrombosis the presence of high peripheral blood levels of sCD40 ligand positively correlates with the amount of platelet content in the thrombus, giving an indication of the extent of thrombosis (Silvain *et al*., 2011). It

has also been suggested that COC preparations containing spironolactione increase SCD40 ligand release (Kebapcilar *et al*.,2010).

7.4.5 ENDOTHELIAL CELL ACTIVATION

Selectins are adhesion molecules that guide non-activated inflammatory cells to areas of inflammation and create loose contacts with endothelial cells (EC). The ones included in this study are sE-selectin, sVCAM-1, VWF: Ag and white cell activation slCAM-1.

SE selectin is a non-specific disease marker expressed on many cells in the body. It is expressed when cytokines activate ECs and contributes to the adhesion of leukocytes to the endothelium. E-selectin is maximally expressed 2-4 hours after EC activation by exerting chemotactic signals on WBCs. Hence it is usually raised in many inflammatory conditions, septic shock, vascular infection and inflammation and inflammatory bowel disease (Azimi-Nezhad *et al*., 2013).

Soluble Intercellular adhesion molecule -1 (sICAM-1) is produced by various cells including vascular endothelial cells (ECs), fibroblasts, epithelial cells and tissue macrophages. It is part of the immunoglobulin super-gene family and functions as a ligand for the lymphocyte function-associated antigen -1 (LFA-1), an initial

marker of the inflammatory reaction. Its role in predicting thrombosis is limited by the fact that it is raised in many medical and immunological conditions such as allergic airways inflammation, allergic contact dermatitis, cancers, acute transplant rejection, in insulin-dependent diabetes mellitus, following acute myocardial infarction, glomerulonephritis, and asthma.

Soluble Vascular cell adhesion molecule-1 (sVCAM-1) supports adhesion of leukocytes to the endothelium. V-CAM-1 is not usually expressed on endothelium, but can be upregulated by EC. It is present on tissue macrophages, and other immune system cells. Soluble VCAM-1 levels can be found in healthy individuals, however levels are elevated in malignancies, auto-immune diseases, infections, inflammation, cirrhosis and impaired renal function (Jin *et al*., 2010).

7.4.6 THROMBIN GENERATION

Thrombin generation (TG) has previously been shown to be increased in patients with high levels of FVIII C, FIX , FXI and in women using COC or hormone replacement therapy (Chantarangkul *et al*., 2004; Dargaud *et al*., 2003; Eilertsen *et al*., 2007; Mackie *et al*., 2001; Regnault *et al*., 2003; Siegemund *et al*., 2004; Tchaikovski and Rosing, 2010; Tchaikovski *et al*., 2007; van Hylckama Vlieg *et al*., 2007).

Thrombin-generation (TG) assays are reported to be useful in detecting hypercoaguability in affected patients (Pereira *et al*., 2006).They have also been used to detect differences in clinical severity among patients with bleeding disorders, and to monitor patients on anticoagulants and those who are having anticoagulation reversed (Van Veen *et al*., 2008).However, TG tests measure a physiological process, and the usefulnes of this assay in pathological conditions is not straight forward.This could limit its clinical use at present, as people carrying similar TG defects could show different clinical manifestations depending on the presence of other confounding factors in haemostasis such as comorbidities, immobilisation, pregnancy and dehydration.. Some studies have suggested that a high ETP in patients with thrombosis may indicate a higher risk of recurrence (Besser *et al*.,2008). TGA testing using the CAT analyser has been found to be a reliable and relatively easy way to measure TG (Brandts *et al*., 2007), however, it is still a research tool .

7.5 COMPARISONS BY TYPE OF HAEMOGLOBIN

7.5.1 CLINICAL COMPLICATIONS AND CONTRACEPTION SIDE EFFECTS IN WOMEN WITH SCD

Within the limits of the small numbers of participants, as mentioned already, the data do not support any excess of clinical complications in SCD women using COC, while women with SCD using POC had more complications. The statistically significant difference in age between women with SCD using COC and those using

POC could have been a source of bias in the comparison between them. Generally COC are not prescribed for older women, even if they do not have haemoglobinopathies, as age alone puts women at higher risk of complications. COC should usually only be prescribed until the age of 50 years. Amongst all women using POC there was no age difference between those with SCD, SCT and normal haemoglobin and yet women with SCD fared worse than the other groups. However, this noticeable increase in clinical complications in women with SCD using POC could be attributed to their disease severity and not the contraception use, as there is a tendency for women with severe problems from the disease to be prescribed POC rather than COC.

The increased incidence of clinical complications in SCD women who are not using any hormonal contraception could also result from a selection bias, as there is a chance that SCD women who are not on contraception could have more severe phenotypes of the disease.

The occurrence of one episode of proven VTE in a cohort of 68 SCD women over a period of two years gives an incidence of 0.7%, which is slightly higher than the one quoted in the literature (0.44 risk of PE in Stein *et al*, 2006 study). Nonetheless, the literature about clinical VTEs in SCD patients is limited. The fact that the participants in this study are cared for at major referral centres for SCD could also be a possible source of bias here, as these patients may suffer from more

disease complications than other SCD women who are not referred to these centres.

The side effects from contraception, the occurrence of unplanned pregnancies and the discontinuation of contraception occurred in a very small amount of participants with little difference amongst the three groups.The small number of cases of menstrual irregularities noted in all SCD women using COC is an expected finding as well as the increased rate of menstrual irregularities and amenorrhoea in SCD women on POC, which is the same as observed in the general population, as discussed in chapter 1.

Despite, the study design not making any controlled assignment of the participants to any specific study group, there were, however, no significant differences in BMI, smoking and parity between them, which is a positive point in the study.

7.5.2 CLINICAL COMPLICATIONS AND CONTRACEPTION SIDE EFFECTS IN WOMEN WITH SCT

As expected, there were minimal serious clinical events during the period of the study in women with SCT using COC and POC. Contraceptive side effects are also few in this cohort. In women with SCT the prior contraceptive period use is more

evenly distributed between the different contraceptive methods than in the SCD women. This is believed to be a true reflection of the situation in the SCT population, as there was no contraception method recruitment in the study, which could give such bias. Paradoxically, this also demonstrates that "pill" prescribers fail to recognise recent research evidence, which implies that there are increased risks of thrombosis in SCT women, as discussed in chapter 1.

7.5.3 CLINICAL COMPLICATIONS AND CONTRACEPTION SIDE EFFECTS IN WOMEN WITH NORMAL HAEMOGLOBIN

There are also minimal clinical and contraceptive complications in these women. The different prior contraceptive-use duration is not statistically significant between the women in this group.

7.5.4 HAEMOSTATIC AND HAEMATOLOGICAL RESULTS IN WOMEN WITH SCD

Thelaboratory markers used did not support the theory that COC use increases the thrombotic risks in SCD women further. Activated protein C levels in this group are statistically significantly lower than in the other SCD women. However, the absolute value of APCR itself is within the normal range. Thus one can suggest that COC use has no clinically relevant additive or multiplicative effect on APCR in SCD women, because when SCD women using COC are compared to SCT women and

women with normal Hb using the same methods the women with SCD have better values (see Table 6.5).

It is well known that COC use could decrease Protein S levels. However, the low levels of free Protein S in women with SCD using COC could be attributable to the disease activity, because all SCD women, regardless of their method of contraception, had low free Protein S levels (Table 5.5) and because COC use did not alter the levels of free Protein S in SCT women and in women with normal Hb (see Table 6.5). There is also no indication that this deficiency is made worse by COC use because the lowest levels were seen in women with SCD who are not using any hormonal contraception.

Von willebrand factor antigen is higher than the reference range in women with SCD using COC; however this could also be a manifestation of the disease, as this is noticeable in all SCD women and is more marked in SCD women not using any hormonal contraception (see Table 5.4). Von Willebrand factor is an endothelial biomarker which is a marker of risk for ischemic heart disease or stroke (Constans and Conri, 2006) hence there is a chance that those women who have higher levels may be prone to such events. All the clotting factors studied were normal in women with SCD on COC which is unsurprising, as the multi factorial prothrombotic condition of SCD seems not to involve increased levels of clotting factors (Setty *et al*., 2001)

Two of the coagulation activation markers studied showed levels higher than the reference range (TAT and D Dimer), though the levels of these markers levels were high in all the SCD women regardless of their method of contraception, and being worst in those on no HC (see Table 5.7). TAT is reported to be elevated in thrombotic events and could predict a predisposition to thrombosis; however it can be raised in other medical conditions such as liver dysfunction, septicaemia and malignancy (Fidan *et al*., 2012). A multinomial logistic regression

model found a Relative risk Ratio of 1.02 for D Dimer in SCD women regardless of their contraceptive method with a[95% CI: 1.01 to1.03) for and p value of 0.001. There is evidence that high D Dimer levels signal micro-thrombi which could predispose to the development of pulmonary hypertension, which is a recognised complication of SCD (Shitrit *et al.*, 2002). In this study, women with known pulmonary hypertension were specifically excluded. As mentioned in chapter 3, D dimer levels could also be raised in other inflammatory conditions and in cases of malignancy.

Platelet activation factor 4 (PF4) is raised in all women with SCD (see Table 5.8). As mentioned earlier, this marker is not very sensitive in predicting thrombosis. In the presence of high D dimer levels this could denote an increased risk of thrombosis in these women. However, the uniform rise in all the SCD women

makes it more likely that this is a disease manifestation which is not influenced much by hormonal contraception.

Endothelial activation markers are within the reference range in all women with SCD. This finding was unexpected because of published findings that these markers are elevated in patients with SCD, as mentioned in chapter 1.

The increased levels of microparticles seen in all SCD women is an expected finding (Tantawy *et al*., 2012). However, micorparticle levels are not increased by using COC, as there is a 40-60% reduction in the level in the group of women using COC. TF is also another marker which is expected to be high in women with SCD and yet again it was high in all women regardless of their contraceptive use (see Table 5.10). The increased TF levels are strongly associated with an increased risk of thrombosis.

The tests for TG using CAT showed normal results in all except the slope, which is higher than the reference range in SCD women using COC. Nonetheless, the normal TG test in these women, with a clear trend shift towards thrombosis could be due to the fact that this test fails to pick up ongoing prothrombotic changes. Though, Shah et al (2012) found that CAT measured TG markers were significantly higher in SCD crises compared to the steady state, however, they found that there were no

differences in clinical outcomes studied simultaneously. It has been suggested that TG tests could be beneficial in assessing bleeding disorders (Van Veen *et al*., 2008). These results could indicate that TG tests fits less well with thrombosis.

All markers of haemolysis are uniformly elevated in all women with SCD, which again fits with the pathology of SCD, but the levels of these markers did not indicate that hormonal contraception worsened their rate of haemolysis in the steady state.

SCD women on POC had a number of statistically significantly worse markers than other SCD women such as SCD 40 L, SVCAM and TP although the absolute values emained within the reference ranges.. Again, this could be selection bias, with this group being more ill and hence being prescribed POC. Nonetheless, SCD40L has a stronger correlation with thrombosis, as mentioned in section 7.4.4, and this could reflect an increased risk of thrombosis in these women. On the other hand, SVCAM can be raised in many diseases and it may not in itself indicate an increased risk of thrombosis. Amongst the TG tests done on SCD women on POC, time to peak (TP) is the only marker which is statistically significantly different between SCD women on differing forms of contraception. Women using POC have the highest values, however as mentioned earlier, the actual values themselves are within the reference range.

Women with SCD not using hormonal contraception had the greatest number of statistically significant adverse markers i.e DVVT, TAT, Prothrombin F1+2, D

Dimer and AST. Again, this group of women could have more severe disease states, and the adverse results would be a manifestation of the disease itself which is known to be a prothrombotic condition, as discussed in chapter 1. The fact that the woman who suffered VTE was in this group fits with this picture of adverse laboratory markers. As markers of haemolysis are uniformly elevated in all women with SCD, which again confirms the pathology of SCD, but does not imply that hormonal contraception has added further insult. This speculation (that the decision not to prescribe HC to these individuals was because they were already known to have a more severe SCD phenotype) cannot be explored by the data I have, since no comparison was made between them and the HC using SCD women before the latter started their HC.

CRP levels are high in all women with SCD, and this is in agreement with the evidence that there is a high degree of endothelial activation and damage seen in these patients, even in the steady state.(Kanavaki *et al*., 2012).

7.5.5 HAEMOSTATIC AND HAEMATOLOGICAL RESULTS IN WOMEN WITH SCT

With regard to SCT women, the laboratory markers studied found higher than expected prothrombotic tendencies. SCT women who are using COC, however, had no adverse pro or anticoagulant markers, but there was a statistically significant increase in Factor II levels, compared to the SCT women using POC or those women who were not using any hormonal contraception.

On the other hand SCT women using POC had higher Factor VIIIC levels, which is statistically different from the other SCT women. They had also slightly lower free protein S levels. However, SCT women on no hormonal contraception had even lower levels of free Protein S. This deficiency of Protein S is most likely due to the condition itself, as it occurred in women not using any hormonal contraception. This finding is interesting, because while the association with SCD and protein S deficiency is well known, there is little information about this deficiency in the SCT population. A case report from 2008 described recurrent priapism in a man with SCT and Protein S deficiency, where the Protein S deficiency was found incidentally during the course of investigations (Rehman *et al*., 2008).SCT women not using any hormonal contraception have also significantly higher APTT and Fibrinogen levels compared to other SCT patients.

SCT women on POC and on COC exhibited adversely higher levels of D Dimer, The logistic regression modeling found a Relative risk Ratio of 1.01 for D Dimer in SCT women regardless of their contraceptive method with a [95% CI:0.020 to 1.002) and a p value of 0.020. This raises the question of SCT itself having an increased thrombotic risk and whether COC increases this risk further.SCT women on COC also exhibited adversely higher levels of TAT which were statistically significant. Another explanation is that this prothrombotic tendency is more related to the fact that these women are of black ethnicity. This agrees with a similar finding Lutsey *et al* (2006) when they reported that people of black ethnic origin had the highest

levels of factor VIII, D-Dimer and von Willebrand factor, among all participants from four different ethnic groups

Recent literature suggests that sickle cell trait is not a benign disease when it comes to thrombosis, as discussed in Chapter 1, with a quoted doubling of risk for VTE, and a 4 four fold increase in PE. The levels of Prothrombin 1+2-fragment in women with SCT using COC is significantly higher (almost 30 %) compared with those women who are not using hormonal contraception, although the highest value is still within the reference range. In the published literature, Saleh *et al*, 1993, found no increase in Prothrombin 1+2-fragment with conjugated equine estrogenuse, and Régine Sitruk-Ware, 2007, found no increased levels with E2 use.

Women with SCT using COC had high levels of PF4, more than 10 fold above the reference range, although PF4 was also high in other women with SCT in the study, the difference was statistically significant. As mentioned in section 7.4.4, this marker may not necessarily denote a higher risk of thrombosis. SCD40L, which is a more accurate predictor of platelet activation, was within the reference range in all women with SCT. However, there is still a statistically significant difference between the groups, with levels in those women on COC and those women on POC being 2.5 times the levels in women without hormonal contraception. This increase in SCD40L in COC users supports the current literature by Divani *et al* (2012). However, the increased level in women with SCT using POC is more

puzzling. There is evidence that high levels of SCD40L may be a marker for atherosclerosis and cardiovascular disease, but whether these women are already affected, or will develop such changes in the future, is unknown.

P-selectin is a marker for procoagulant properties and the prothrombotic state, and thus it is expected to be raised in subjects with conditions similar to SCD (Pabinger and Ay, 2009). However, P-selectin results showed levels lower than the reference range for participants of the three haemoglobin types studied. This result was unexpected, and will need further evaluation in future studies. However, whatever the reason behind these low levels of P-selectin, this is a reassuring point in this study as it suggests that the platelets do not undergo activation at the time of blood collection, and thus the results of the haemostatic markers are valid and reliable. Endothelial activation markers in all women with SCT lie within the reference range, apart from the soluble endothelial-leucocyte adhesion molecule-1 (sELAM), which was found to be higher in SCT women using COC. This increase was statistically significant, with levels being greater than twice those in the women not using any hormonal contraception. However sELAM can increase in inflammatory conditions, so the interpretation of this finding is as yet unclear.

Tisssue factor is another haemostatic marker which was increased 2-fold in women with SCT who were using COC. This adverse result was statistically significantly different from the results in women with SCT not using hormonal

contraception. The relevance of TF to thrombosis has already been highlighted in chapter 1. This increase appears to be more related to the sickle carrier status that to the oral contraception use, as this increase was also seen in women with SCT not using any hormonal contraception.

TG tests remained normal in all women with SCT, although the actual levels were statistically significantly different in two of these (LT and PH), with the levels being high in the group not using any hormonal contraception. Again, TG testing here did not suggest any thrombotic tendency, which raises the question about its usefulness in predicting prothrombotic conditions.

All markers of haemolysis were normal in all women with SCT. This finding is to be expected as these women are not known to have un-provoked haemolysis. However, the presence of elevated thrombotic markers in these women leads to the question as to whether the haemolysis contribution to thrombosis in SCD patients (discussed in section 1.6.1.2) is only a small fraction of a very wide and complicated mechanism.

The biochemical markers and full blood counts were normal in all SCT women. CRP was elevated in all those with SCT, although to a lesser extent than women with

SCD, which may indicate that the SCT women were also undergoing a degree of chronic inflammatory changes.

7.5.6 HAEMOSTATIC AND HAEMATOLOGICAL RESULTS IN WOMEN WITH NORMAL HAEMOGLOBIN

With regard to the women with normal haemoglobin, women on COC had statistically significantly higher levels of DVVT, although the mean value was still within the reference range. Secondly, the mean level of APCR in women with normal Hb who were using COC was statistically significantly different from the rest. Again however, the value was still within the normal range. Women with normal Hb and using COC had levels of Factor VIIIC which were higher than the reference range, and this is also noticeable in women using POC. As discussed in section 1.7.1.1.1, COC use may increase Factor VIII and these results support this. However, rather unexpectedly, the women with normal Hb on POC, and those not using any hormonal contraception, had Free Protein S levels which were lower than the reference range. This could be an ethnic characteristic, as low Free Protein S results are reported to be more prevalent in people of black African origin (Jerrard-Dunne *et al*., 2003).

TAT levels were significantly different between the women with normal haemoglobin, with the group not using any hormonal contraception showing

results higher than the reference range, while the other groups had normal results. This again could be a feature of their ethnicity (Hagger *et al*., 1995).

The adversely raised sCD40L levels in the women with normal Hb using COC was significantly different from the other two groups. As mentioned in section 7.4.4, this could signal an increased risk of thrombosis.

PF4 was high in all the women with normal haemoglobin. The significance of this elevation is difficult to interpret, as mentioned in section 7.4.4. Although microparticle levels were normal in all the women with normal haemoglobin, the levels in the group using COC was statistically significantly higher.

Thrombin generation tests were normal in all the women with normal Hb, except the slope which was raised in women using POC.

The normal haemolysis markers seen in all the women with normal Hb is an expected result, as is the normal full blood count parameters.

Some liver function markers (ALT, ALP and albumin) showed statistically significant adverse results in the group of women with normal Hb using POC.

Whether there are some participants in this group with undiagnosed liver problems is unknown.

CRP is elevated in all the women with normal Hb. The association of high CRP with future health risks is reported in the literature. Folsom *et al* (2009) and Divani *et al* (2009). suggested that elevated CRP is independently associated with increased risk of VTE .It has also been suggested that there is an association between minor CRP elevation and future major cardiovascular events (Black *et al*., 2004). (Folsom et al., 2009)

7.6 COMPARISONS BY TYPE OF CONTRACEPTION

7.6.1 CLINICAL COMPLICATIONS

Within the limits of small numbers, the data do not support any excessive clinical problems in women with SCD or SCT, and there was no evidence that these women experienced more side effects with hormonal contraception**.**

7.6.2 HAEMOSTATIC AND HAEMATOLOGICAL RESULTS FOR WOMEN USING COMBINED ORAL CONTRACEPTION (COC)

With regard to the findings on pro- and anti-coagulant markers, the results do not support the assumption that women with SCD and SCT using COC have an excessively high risk of VTE. Women with SCD using COC had two "adverse"

results ie relatively high levels of von willebrand antigen and low levels of free Protein S. However, these parameters were not statistically significantly different from the results of COC women with SCT or normal Hb (see Tables 6.4 and 6.5). While women with SCT showed no adverse values, women with normal Hb using COC had high Factor VIIIC levels, although the difference is not statistically significant. The only statistically significant difference among women using COC were the lower APCR values in SCT women, but the actual values remained within the reference range (Table 6.5).

All coagulation activation markers studied were statistically significantly different between the three Hb groups, with TAT and D Dimer levels elevated in the SCD and SCT groups, while PF 1+2 was raised in the SCD group (Table 6.7). In women with SCD, all three values were outside the reference range, women with SCT had two values outside the reference range, while women with normal haemoglobin had all three in the normal range. This is in keeping with the known fact that SCD is a prothrombotic disease, and that SCT is also not completely benign when it comes to the risk of thrombosis. At first glance, these data (Table 6.7). might raise concerns about the VTE risk of combining SCD with COC use. However, the data comparisons of Table 5.7 demonstrate that none of these coagulation activation markers is increased in SCD women by COC use, and in fact my data show the reverse, although that aspect might reflect a selection bias on the part of the prescriptions chosen for these women.

With regard to platelet activation markers, the only statistically significantly different marker was SCD40L (see Table 6.8). The actual levels were high only in women with normal Hb who were using COC .These levels were more than 5 fold higher than the levels in women with SCD. As discussed previously, this supports the known fact that COC use increases thrombotic risks. In addition, the tendency for women of black African ethnicity to have high levels of SCD40 is significant and could be greater than the risk from SCD itself.

Endothelial activation markers (Table 6.9). are another area where the three Hb groups differ significantly in two of the three markers studied. Women in the SCD group had higher levels of sCVAM (but still within the reference range), while women with SCT had higher sE selectin-1, which was outside the reference range. Again, these results indicate that SCD and SCT are conditions with a prothrombotic tendency, but COC use adds little to this risk, as demonstrated in Tables 5.9 and 5.24.

The fact that women with SCD had microparticle levels which were 2.5 times the levels in SCT women and in those women with normal Hb (Table 10.6), is to be expected as there is evidence that microparticle levels are always increased in SCD, as discussed previously in relation to the data of Table 5.10.

Thrombin generation tests were all normal in all women who were taking COC, except for the slope, which was high in women with SCD, but the difference did not reach statistical significance.(Table 6.11).

Markers of haemolysis and liver function tests were raised in women with SCD, as expected. However, full blood count parametersin COC users were not statistically different from those with SCT and those with normal Hb.

While CRP levels were high in all three groups, women with SCD showed levels 3-4 times the levels of those in the other groups, indicating the chronic inflammatory nature of SCD even in when the woman is in steady state.

7.6.3 HAEMOSTATIC AND HAEMATOLOGICAL RESULTS FOR WOMEN USING PROGESTOGEN ONLY CONTRACEPTION (POC)

There are six prothrombotic and anti-coagulant marker levels which are significantly different between the three groups of women who were using POC.(Tables 6.19, 6.20 and 6.21). Three of these markers were elevated in women with SCD (PT, INR and FX). and the other two were raised in women with SCT (APTT and DVVT). The last marker was APCR which showed the lowest levels (i.e adverse) in women with normal Hb. Nevertheless, within each group of these

women there are markers which are outside the reference range, such as PT, FVIII and PS free in women with SCD using POC. However the data of Table 5.4 and 5.5 demonstrate that this derives from their SCD and not from their POC use. PS free levels were low in POC users, regardless of Hb type, with no statistically significant difference between them (Table 6.20)

Factor XIIIa levels were low in all women taking POC, with the lowest results seen in women with SCD. The same finding was noticed with women using COC, when similarly the lowest levels were in women with SCD (Table 5.6). There is reported evidence that Factor XIIIa has ethnic and genetic variation (Saha et al., 2000). However, no studies of factor XIIIa have been done in people of black ethnic origin, and the possibility that the reference range for them is different should be addressed in future studies.

All coagulation activation marker levels were raised in women with SCD; however, D Dimer was also raised in other women. The differences between SCD group and the other women who were using POC are statistically significant for D Dimer and P F1+2. The increase of D Dimer in women with normal Hb is minimal, however as mentioned previously this could signal future health risks.

Again, sP Selectin levels are low in all these women, while PF4 levels are high in all. This consistent pattern is puzzling and needs further exploration, and may even represent a technical artifact.

SVCAM and sICAM-1 are statistically significantly higher in women with SCD, but all values were within the reference ranges (Table 6.24). .This finding is expected

as this shows disease activity. This is a similar phenomenon to the increase in microparticles and tissue factor (a 4-fold increase for both).

The Thrombin generation tests persistently failed to pick up any indication of thrombotic tendency in the different comparisons groups, and again showed normal results in all (Table 6.26). However, the Start time and the Lag time were both highest in the women with normal Hb.

Bilirubin and LDH are statistically significantly higher in women with SCD as expected, while free Hb and Haptoglobin show no such differences. ALT, AST, ALP and Platelets are also significantly higher in women with SCD, which is to be expected. However, the surprising result is that the difference in CRP levels between the three groups does not reach statistical significance. This stresses the fact that all the study participants, regardless of their contraception or haemoglobin, exhibit some degree of chronic inflammation which may be a manifestation of their shared ethnicity (Clark *et al*., 2007).

7.6.4 HAEMOSTATIC AND HAEMATOLOGICAL RESULTS FOR WOMEN NOT USING HORMONAL CONTRACEPTION

The comparison here followed most of the patterns already seen, Free Protein S is low in all the three groups, which is likely to reflect their shared ethnicity rather than any differences in Hb type. Women with SCT also had statistically significantly

higher levels of Fibrinogen, while women with SCD had higher levels of Factor VIIIC and von Willebrand Factor.(Table 6.34). At the same time activated Protein C resistance is statistically significantly different between the three groups, the lowest (adverse) mean values being in women with normal Hb and with SCT.

Again, Factor X111a is lowest in women with SCD and coagulation marker levels are significantly raised in the SCD women. Apart from PF4, all platelet activation markers are normal; however, these are statistically significantly higher in women with SCD (Table 6.38).

Other unsurprising results are:-the statistically significantly higher levels of endothelial activation markers and markers of tissue damage. However, all women, regardless of their haemoglobin type, had elevated levels of TF. Thrombin generation again did not reveal any differences between these women apart from in the Lag time. The free Haemoglobin levels are significantly higher in women with SCT, while LDH and Bilirubin are elevated in women with SCD.

CRP was statistically significantly higher in women with SCD; however the levels are also elevated in the other two groups, as already discussed.

In general women with normal haemoglobin and from black African ethnic origin showed evidence of chronic inflammation and some raised prothrombotic markers, albeit being of a lesser degree.

7.7 LIMITATIONS OF THE STUDY AND POSSIBLE SOURCES OF ERROR

Some hospitals with large SCD populations were not included in this study for reasons mentioned in Chapter 4. This gap in recruitment raises the issue of whether the women not included could have been different in some of the studied aspects, and this could have potentially influenced the study outcomes.

The observational design of the study carries some inherent problems eg, self selection. The contraceptive method used could also be a source of bias, especially in the SCD group, as the sicker women are prescribed a more limited range of contraception options and generally tend to be prescribed long acting POCs if anything. This also limits the ability to match comparable groups; in particular women with SCD on the COC were statistically significantly younger than their counterparts.

Women with SCD who were using COC are extremely few in the potential eligible population approached. This also reflects the fact that these women are not prescribed COC as readily as POC, and resulted in this subgroup of women being smaller than I had hoped for.

All POC preparations were studied together (ie DMPA, POP, Contraceptive implants and Mirena IUS) due to the relatively small number of women on different progestogen preparations.

The plan to include the SCT group arose at an advanced stage in the study, thus the participant number is smaller, compared with the SCD group.

Unfortunately, it was not possible to ascertain the cause of death of the woman who died in Nigeria. It is therefore difficult to speculate about any relevant complications in her case.

I used laboratory markers as a surrogate of actual clinical problems. There are limitations to the appropriate interpretationof in-vitro data. A potential danger lies in assuming direct correlation between these laboratory investigation findings and the related clinical events. However, the in-vitro tests had the advantage of producing a larger data resource than the criteria studied in vitro. Also the ascertainment of the laboratory findings was much amenable to consistent control than the in-vivo features. Furthermore, there is good evidence validating the correlation between a number of the markers I have used and clinically evident VTE events e.g. the pro and anti-coagulant markers, coagulation activation markers, and platelet activation markers (see paragraph 7.4).

7.8 CONCLUSION

My hypothesis was that there are no additional clinical or haematological risks to Sickle Cell Disease patients using hormonal contraceptive methods, over and above those inherent in their SCD.

My further hypothesis was that there are no additional clinical or haematological risks to Sickle Cell Trait (SCT) women using hormonal contraceptive methods. With regard to women with SCT, there is no noticeable increase in the incidence of clinical complications, nor any increase in the contraception-related complications in those women who are using COC or POC.

A two-year clinical follow-up of women with sickle cell disease using combined oral contraceptive pills (COC) shows that the occurrence of clinical complications such as sickle crises, the need for blood transfusion, episodes of VTE, and hospital admissions are minimal. It also demonstrates that these complications (other than sickle crises) could be comparable to women with normal haemoglobin, and in some aspects are better than in women with SCD using Progestogen Only Contraception (POC) and in women not using any hormonal contraception.

Menstrual irregularities, contraceptive side effects, the discontinuation of contraception, accidental interruptions and the number of planned and unplanned pregnancies are not increased in women with SCD using COC.

From the results of the laboratory markers researched in this study, I can conclude that the thrombotic tendency of COC has no or minimal additive effect to the disease's known hypercoaguable state and hence COC use does not increase the thrombotic risks in SCD women, nor does it adversely affect their liver function or exacerbate any inflammatory changes.

Progestogen only contraception is associated with an increased incidence of menstrual irregularities, however this is inherent to this type of contraception and these side effects are not significantly different from those noted in women with normal haemoglobin taking POC.

Thus, I hope that my findings will encourage clinicians looking after women with SCD to remember to discuss effective contraception with them on all relevant occasions. They should be aware that the hormonal methods are the most efficacious of the reversible options. My findings should reassure clinicians that the impact of hormonal contraceptives on coagulability is minimal in comparison with the inherent hypercoagulability of the sickle cell condition itself. In other words, any concerns about VTE risk should be sensibly balanced by an appreciation of the

many advantages of effective hormonal contraception, not least, the avoidance of unplanned pregnancies, which are currently so prevalent in women with SCD, and which carry far greater risks for them.

7.9 FUTURE RESEARCH

Future research involving a collaboration of all the Sickle Cell Centres, the Sickle Cell Society and the UK Forum for Haemogolobin Disorders will yield a wider data base and is greatly needed. Women from these sources could be recruited early in their reproductive life. These women could be encouraged to ask their doctors proactively to be put on hormonal contraception. Blood collections could be done before starting the HC, within the first three months of starting it and at 6 months. The clinical follow up could continue as long as the women are using HC.

My study of laboratory haemostatic and haematological markers in women with SCT showed significant increases in indicators of some of the pro and anti-coagulant markers, coagulation activation markers, markers of tissue damage, platelet and endothelial activation markers. This phenomenon appears more marked in women with SCT using COC. However a larger randomised controlled study is needed to further assess this recently-noted phenomenon.

An incidental finding of my studies suggests that women of black African ethnic origin show increased inflammatory and endothelial activation markers and

reduced anti-coagulation factors, regardless of their method of contraception. Ironically, a study which set out to explore hormonal effects on women with SCD, has revealed equally interesting features of women with SCT and women of black African ethnicity who have normal Hb.

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Appendices

Appendix 1	Pregnancy outcome in women with SCD
Appendix 2	Trends in family planning and counselling for women with sickle cell disease in the UK over two decades.
Appendix 3	Ethics approval letter
Appendix 4	Laborotary reference ranges (Haemostatic markers)
Appendix 5	Laborotary reference ranges (Liver function and biochemical markers)
Appendix 6	Patient Information sheet (PIS)
Appendix 7	Katherine Dormandy Haemophilia Centre Plasma Bank PIS
Appendix 8	Katherine Dormandy Haemophilia Centre Plasma consent form
Appendix 9	Study consent form

Appendix 1

Manuscript to be submitted for publication

Pregnancy outcome in women with sickle cell disease in the United Kingdom: a trend analysis of three surveys from 1975 to 2004.

Pregnancy outcome in women with sickle cell disease in the United Kingdom: a trend analysis of three surveys from 1975 to 2004.

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Abstract

We have undertaken three studies of pregnancies in women with sickle cell disease in the United Kingdom, which covered 1975-1982, 1991-1993 and 2000-2004. Differences, similarities and trends in the findings are analysed and discussed, and we compared trends in maternal and perinatal mortality among the cohort of women affected by sickle cell disease with overall national rates. Maternal mortality rates in sickle cell disease appear to have shown no significant change in the United Kingdom during the time covered by these three studies.

Background

Sickle cell disease (SCD) is the most common genetically inherited disorder worldwide (1) its prevalence, albeit low, is increasing in the United Kingdom, with estimated numbers 5,000 in 1986 and 12,000 in 2010 (2–4). Recent advances in the medical management of SCD and the improvement in life expectancy for these patients, result in many more reaching reproductive age and becoming pregnant. The indications are that the fertility of women with SCD is normal (5).

Maternal sickle cell disease is associated with a higher rate of complications, than pregnant women without this disease (6–11).

It is estimated that there are currently between 60 and 100 pregnancies per year in women with SCD in the UK (9).

There have been a variety of changes in pregnancy outcome among women with SCD in the UK over the past few decades. Data from a multi-centre survey carried out in the 1990s suggest SCD pregnancies, in some respects, experienced worsening outcomes compared with a similar survey of pregnancies in the 1970s (10) and 1980s (11).

Similar deteriorations in pregnancy outcomes have been reported from the USA and from Jamaica (8,12–16).These impressions are also supported by the data from the Confidential Enquiries into Maternal Deaths in the UK relating to the 1970's, 1980's and 1990's (20, 21). In the 1970's the maternal mortality rate nationally in women with SCD was approximately 3% (with one death per year and an estimated 30 pregnancies per year). This was 200-times the overall contemporaneous maternal mortality rate in the UK, which then stood at 15 per 100,000. In the 1990s the maternal mortality rate in women with SCD was approximately 2.2% (with one death in an estimated 45 pregnancies per year).

However, by this time the overall maternal mortality rate in the UK had fallen to 9.8 per 100,000 births (21). The maternal mortality rate in SCD had thus risen, in relative terms, to 224 times the overall rate. The perinatal mortality rate (PMR) decreased from 88 per 1000 from 1975 – 1982, to 60 per 1000 during the period 1991 – 93. (The definition of stillbirth in the U.K. changed in 1992, to include pregnancies delivered from 24 weeks gestation, rather than 28 weeks. The survey data have been adjusted accordingly in order to allow valid comparisons to be made).

It is possible that this continuing high mortality reflects the survival to adult life of individuals with more adverse disease states than in earlier decades. However, it is notable that there were no maternal deaths and only one perinatal loss in three more recent single hospital series from London teaching hospitals in 25, 62 and 71 pregnancies, respectively, between 2000 and 2007 (17,18). This suggests that pregnancy outcomes in SCD can be significantly improved by the consistent delivery of care by experienced clinicians.

The current analysis therefore sets out to examine how the national picture may be changing in more recent times. It is inevitably difficult to ascertain whether changing outcomes over time simply reflect the improved survival to reproductive age of women with severe disease, or whether they should be ascribed to other factors such as altered demographics, changes in clinical care and experience, or indeed be an artefact of our improved network of data collection. For all three series, the intention was to contact as many hospitals looking after SCD women as possible, including those with small numbers and less clinical experience, in order to derive a realistic view of the total national picture. The contact list for all three surveys was obtained from the members of the UK Forum on Haemoglobin Disorders (22).

The study received ethical approval from the West Midlands Multi-centre Research Ethics Committee on behalf of the National Multi-centre Research Ethics Committee.

Methods:

This study compares maternal and fetal outcomes in pregnancies complicated by SCD from three retrospective surveys in the UK, covering the periods 1975 to 1981 (survey 1), 1991 to 1993 (survey 2), and 2000 to 2004 (survey 3). The numbers of pregnancies in each survey are 125, 82, and 206, respectively. The same data collection proforma was used for all surveys. Data from the first two surveys were obtained from two MD theses (SMT and RJH). Data for the final survey was collected by the first author.

During each survey, women with SCD who had pregnancies during the relevant time periods were identified by their consultant haematologist from the following sources: SCD databases, laboratory registers, maternity records, and the network of the UK Forum on Haemoglobin Disorders. Eligible women were identified as those with the following haemoglobin types: SS, SC, and S-beta° thalassaemia.

Data were extracted from the patient's general and maternity hospital notes, if she consented to take part in the survey. Patient information was anonymised and handled in accordance with the Data Protection Act 1998.

A total of 23 hospital in the UK with both maternity and haemoglobinopathy departments were invited to take part in the 2000 to 2004 survey. Ethical approval for data collection was obtained in 78% (18/23), and 61% (11/18) hospitals provided data. The remaining seven hospitals did not contribute to the data collection because, either there were no

pregnancies in the relevant time period or there was no means of identifying pregnancies from their records system.

Data

No raw data from the first two surveys were available. Relevant information from these two surveys was extracted from the published tables.

Maternal and demographic characteristics included: maternal age, parity, sickle cell type, past medical history, socio-demographic characteristics, past obstetric history. Pregnancy related information included: antenatal and pregnancy complications, labour and delivery, pregnancy management, and pregnancy outcome (maternal and fetal).

Analysis

Data were summarised using mean and standard deviation or median and range, depending on the distribution. Categorical data were summarised using count and percentages. 95% confidence intervals are provided for all estimates.

A oneway analysis of variance (ANOVA) was performed for surveys 1, 2 and 3 using the ''aovsum'' Stata command, which can generate a statistical model from summary statistics. The "aovsum" generates synthetic data which enables the use of regression technique to assess trends across the three surveys for numerical variables. A non-parametric method (nptrendi) was used to test for trend across categorical variables.

All analyses were carried out in Stata V.11 (23).

Demographic variables		**Survey 1 n=125**	**Survey 2 n=82**	**Survey 3 n=206**
Age in years	mean (SD)	22 (3.9)	28 (5.2)	28 (6.5)
	Difference (95% CI)	base	6 (4.4 to 7.5)	6 (4.7 to 7.2)
	Test for trend			
Smoker		28 (22%)		23 (11%)
	Difference (95% CI)	-11% (20% to -3%)		
	Test for trend	0.006**		
Para 0	Count (%)	64 (51%)	35 (43%)	112 (54%)
	Difference (95% CI)	base	-8% (-22% to 5%)	3% (-7% to 14%)
	Test for trend	0.456		
Para 1	count (%)	39 (31%)	33 (40%)	60 (29%)
	Difference (95% CI)	base	9% (-4% to 22%)	-2% (-12% to 8%)
	Test for trend	0.555		
Para 2	count (%)	14 (11%)	12 (15%)	29 (14%)
	Difference (95% CI)	base	3% (-6% to 13%)	3% (-4% to 10%)
	Test for trend	0.485		
Para 3+	count (%)	8 (6%)	2 (2%)	5 (2%)
	Difference (95% CI)	base	-4% (-9% to 15%)	-4% (-9% to 0.8%)
	Test for trend	0.074		
Haemoglobinopathy type				
SC	Count (%)	59 (47%)	34 (41.5%)	87 (42.2%)
	Difference (95% CI)	base	-6% (-19% to 8%)	-5% (-16% to 6%)
	Test for trend	0.407		
SS	Count (%)	54 (43%)	43 (52.4%)	117 (57%)
	Difference (95% CI)		9% (-5% to 23%)	14% (2% to 25%)
	Test for trend	0.018*		
Sβ° thal	Count (%)	12(9.6%)	5(6.1%)	2(0.97%)
	Difference (95% CI)	base	-3% (-11% to 4%)	-9% (-14% to -3%)
	Test for trend	0.002***		

Comparison of the demographic characteristics of women from the three surveys

*p-value significant at 5%; *** p-value significant at 0.1%

Mode of delivery		**Survey 1 n=125**	**Survey 2 n=82**	**Survey 3 n=206**
Elective caesarean s	Count (%)	23 (18%)	12(15%)	18(9%)
	Difference (95% CI)	Base	-3% (-14% to 6%)	-9% (-17% to -2%)
	Test for trend	0.010**		
Emergency caesarean S	Count (%)	20 (16%)	28 (34%)	69 (33%)
	Difference (95% CI)	Base	18% (6% to 30%)	17% (8% to 26%)
	Test for trend	0.001**		
Operative vaginal delivery	Count (%)	21 (16%)	2 (2%)	11 (5%)
	Difference (95% CI)	base	-14% (-22% to -7%)	-11% (-18% to -4%)
	Test for trend	0.001***		
Spontaneous normal vaginal delivery	Count (%)	56 (45%)	40 (49%)	84 (41%)
	Difference (95% CI)		4% (-10% to 18%)	-4% (-15% to 7%)
	Test for trend	0.407		

Comparison of mode of delivery across the three surveys

Complication		**Survey 1 n=125**	**Survey 2 n=82**	**Survey 3 n=206**
Pre-eclampsia	Count (%)	17(13.6%)	6 (7.3%)	19(9.2%)
	Difference (95% CI)	Base	-6.3%(-6.162 to -6.44)	-4.4%(-4.26 to -4.54)
	Test for trend	0.280		
UTI	Count (%)	35(28%)	12(14.6%)	20(9.7)%
	Difference (95% CI)	Base	-13.4 %(-13.56 to -13.24)	-18.3 (-18.46 to 18.14)
	Test for trend	<0.001		
Sickle crises	Count (%)	48(38.4%)	33(40.2%)	87(42.2%)
	Difference (95% CI)	Base	1.8% (1.623 to 1.976)	3.83%(3.65 to 4)
	Test for trend	0.051		
Chest complications	Count (%)		11(13%)	24(12%)
	Difference (95% CI)		Base	-1% (-4.79 to 1.93)
	Test for trend	0.786		
Blood transfusion	Count (%)	89(71.2%)	43(52.4%)	70(34%)
	Difference (95% CI)	Base	-18.8 % (-18.93 to -18.64)	-37.2 %(-30 35 to -37.04)
	Test for trend	<0.001		

Pregnancy Complications *Severe pre-eclampsia is defined as blood pressure above 140/90 mm Hg, with proteinuria, biochemical disturbance and clinical symptoms.

Complication		Survey 1 n=125	Survey 2 n=82	Survey 3 n=206
ITU admission	Count (%)	7(5.7%)	6(7.3%)	13(7%)
	Difference (95% CI)	Base	1.6%(1.4 to 1.74)	1.3 %(1.14 to 1.48)
	Test for trend	0.897		
Sickle crises	Count (%)	17(13.7%)	4(4.9%)	29(15.5%)
	Difference (95% CI)	Base	-8.8% (-8.92 to 8.68)	1.6% (1.2 to 2)
	Test for trend	0.051		
Infection	Count (%)	77(62%)	1(1.2%)	6(3.2%)
	Difference (95% CI)	Base	-60.8% (-60.62 to -60.98)	-58.8% (-59.9 to -58.21)
	Test for trend	<0.000		

Postnatal complications

Parameter		Survey 1 n=125	Survey 2 n=82	Survey 3 n=206
Fetal Birth weight in grams	Mean (SD)	2883 (822)	2805 (719)	2772 (656)
	Difference (95%CI)	base	-78 (-291.5 to 135.5)	-111 (-286.6 to 59.6)
	Test for trend	0.182		
Male	Count (%)		35 (42%)	79 (38%)
	Difference (95%CI)		-4% (-17% to 8%)	
	Test for trend	0.497		
Female	Count (%)		47 (57%)	91 (44%)
	Difference (95%CI)		13% (-26% to -0.4%)	
	Test for trend	0.044*		
Gestational age at delivery in weeks	Mean (SD)	37.9 (3.19)	37.4 (3.05)	37.1 (3.27)
	Difference (95%CI)	base	-0.5 (-1.4 to 0.40)	-0.8 (-1.5 to -0.08)
	Test for trend	0.029*		
Perinatal death	Count (rate per 1000 births)	11 (88)*	5 (60)	11 (53)
	Difference (95%CI)			
	Test for trend	0.863		
Maternal death	count (rate per 1000 pregnancies)	0	2 (24)	1(4.8)

Comparison of pregnancy outcomes from the three surveys

Discussion:

The experience of any individual clinician or hospital department in the UK with SCD pregnancies will not necessarily reflect the general trend of outcomes for these pregnancies. Some pregnancy complications, such as severe sickle cell crises, pulmonary complications and severe pre-eclampsia have remained the same over the past three decades, whilst urinary tract infections showed a significant reduction over the three decades, and this could be explained by the greater awareness of infection as an important issue.

The finding of a persistently high incidence of severe pre eclampsia across all three surveys is notable. It is possible that there is an inherent association between severe pre eclampsia and SCD, which may be triggered by activation of the endothelium of blood vessels in SCD (19) However, not all researchers in this field have concurred with this observation (12).

The last two decades have witnessed more selective practice in the use of blood transfusion in SCD patients generally, and our finding of a significant decline in the use of blood transfusions in pregnancy over this time is in keeping with this.

The overall caesarean section rate has increased in the UK over this period, and is currently 23.8% (24). However the rate for SCD women is almost double this. The UK perinatal mortality rate has fallen since the 1970s, but that in SCD pregnancies has fallen to a much lesser extent. The rate is still seven times the overall national rate (which currently stands at 7.6 per 1,000). The miscarriage rate in the last survey, conducted between 2000 – 2004, was 9.3%. However, no trend analysis was done, as this information was not included in the previous surveys.

Though our 2000-to-2004 survey reveals one maternal death, contemporaneous national data from the Centre for Maternal and Child Enquiries (CMACE) reported four pregnancy related deaths in SCD women during this period. CMACE uses different search and reporting methodology and collects deaths up to one year after birth. The unclear trend in maternal mortality shown by our data unfortunately includes an ascertainment bias. The most recent CMACE reports indicate continuing high national rates of maternal mortality for SCD women, with three deaths in the 2000 to 2002 triennium, two deaths in the 2003 to 2005 triennium, and three deaths in the 2006 to 2008 triennium. This would equate to a contemporary national mortality rate in SCD of approximately 1.6% (with an estimated 60 pregnancies per year in the UK), compared with the current overall maternal mortality rate of 11.4 per 100,000, which is 140 times lower than the rate in SCD women.

Limitations of the study

Unfortunately complete case ascertainment was inevitably not achieved in these surveys. The increased demands of research ethics requirements certainly contributed to the failure to follow through on initial agreements to allow access to patients' notes in some hospitals. This data would have enhanced the most recent survey. Early miscarriages not coming to medical attention would also not be known to the researchers. Venous thromboembolic (VTE) event-rates were not included in the trend analysis, since the diagnosis of this condition was often unclear or not fully established.

Clinical data are likely to be unique to the population and health services being surveyed, and international extrapolations should not necessarily be made. The fluctuating natural course of sickle cell disease and the influence of other confounding factors on pregnancy mean that research in this field is challenging. Continuing data collection and shared clinical experience are essential to ensure future progress for the care of these patients.

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Appendix 2

Trends in family planning and counselling for women with sickle cell disease in the UK over two decades

Article submitted for publication

Abstract

Background Pregnancies in women with sickle cell disease (SCD) are known to have high rates of maternal and fetal mortality and morbidity. Given these pregnancy-associated problems for women with SCD, advice both about pregnancy planning and about effective contraception are of paramount importance. This study sought to discover the contraception methods used by women with SCD, what complications women with SCD encounter with contraception, and their experiences of pre-pregnancy counselling and pregnancy planning, and how such issues may have changed over the past two decades.

Method The study was a multicentre, interview-based, cross-sectional study. Interviews were carried out with 102 women with SCD, in north and central London during 2010, concerning their current and previous contraceptive use, their pregnancy history, their menstrual history, and the advice they received concerning pregnancy planning and contraception. Patient information was anonymised and ethical approval was obtained. These data were compared with data from a similar study undertaken in 1993.

Results There were significant differences in a number of key areas: the number of unplanned pregnancies decreased from 64% in 1993 to 53% in 2010. The number of women with SCD who were advised not to become pregnant also fell, from 36% to 15%. The use of combined oral contraceptive pills declined, from 45% of the women in 1993 to 31% in 2010. Conversely the use of depot medroxyprogesterone acetate contraception (DMPA) and the levonorgestrel intrauterine system (LNG-IUS) both increased.

Conclusions Significant changes in the contraceptive methods used by women with SCD are demonstrated in the London population. LNG-IUS use in SCD has not been investigated before. There has been an encouraging decrease in the number of women with SCD who are advised not to become pregnant, perhaps reflecting an improvement in their overall health. Although the number of unplanned pregnancies has fallen, it remains high – emphasising the continuing need for women with SCD to have access to informed advice about pregnancy-associated issues and contraception.

Method: The study is a multi-centre, interview-based, cross sectional study. Interviews were carried out with 102 women with SCD, in north and central London during 2010,

concerning their current and previous contraceptive use, their pregnancy history, their menstrual history, and the advice they received concerning pregnancy planning and contraception. Patient information was anonymised, and ethical approval was obtained. These data were compared with data from a similar study undertaken in 1993.

Results: There were significant differences in a number of key areas: the number of pregnancies which were unplanned fell, from 64.2% in 1993 to 53% in 2010. The number of women with SCD who are advised not to become pregnant has also fallen, from 36% to 15%. The use of Combined Oral Contraceptive Pills (COCPs) declined, from 45% of the women in 1993 to 31% in 2010. The use of Depo-Provera contraception, and the Mirena IUS meanwhile both increased.

Conclusions: Significant changes in the contraceptive methods used by women with SCD are demonstrated in the London population. Mirena IUS use in SCD has not be investigated before. There has been an encouraging decrease in the number of women with SCD who are advised not to become pregnant, perhaps reflecting an improvement in their overall health. Although the number of unplanned pregnancies has fallen, it remains high, emphasising the continuing need for women with SCD to have access to informed advice about pregnancy-associated issues and contraception

Background

Sickle cell disease (SCD) is an inherited, incurable abnormality of haemoglobin, characterised by multiple acute episodes and chronic complications and a shorter-than-average life expectancy. There are an estimated 12,500 people with sickle cell disease in the UK (1). Pregnancies in women with SCD have a high rate of maternal and fetal mortality and morbidity (2–5).

The maternal death rate in women with SCD in the UK is approximately 1.6%, in contrast to the overall national pregnancy-related death rate in the UK which is 0.01%. (6) The perinatal mortality rate (stillbirths and deaths in the first week after birth) in SCD is approximately 60/1000 compared with overall rate in the UK of 7.9/1000. (4) . Serious complications such as sickling crises occur in 40% of pregnancies (5). These significantly increased maternal and fetal risks necessitate effective family planning advice, so that women with SCD, recognising the problems in pregnancy associated

with SCD, and are able to optimise the timing of, and preparation for, their pregnancies. However, this clear need is unfortunately handicapped by the extreme paucity of evidence-based contraceptive advice for women with SCD(7), resulting in the family planning advice given to these women being generally negative and unhelpful.

Contraceptive choices for women with SCD are complicated by three main areas of concern: firstly, the combined oral contraceptive pill (COC) is associated with an increased risk of venous thromboembolism (VTE) (8–10). SCD is known to be a prothrombotic condition. Thrombosis is triggered by deformed red blood cells whose abnormal cell membrane has lost their natural phospholipid asymmetry, hence becoming stickier and adhering to the endothelium, forming a port to which prothrombotic enzymes dock. Thrombosis is also more likely with sickle cell disease due to the presence of free circulating haemoglobin (because of red cell breakdown), the occurrence of chronic inflammatory reactions, and the slow microcirculation (11,12). It has therefore been assumed that these two risks would be additive or indeed multiplicative. This presumed increased thrombosis risk is theoretical and has not been demonstrated in any well designed studies (7) Secondly, perhaps because of this concern over the thrombotic-risk of COCs, there is a tendency to advise SCD women to use progestogen-only forms of contraception, either oral progestogen-only pills, (POPs), or long-acting reversible contraceptives (LARCs), which have a lower VTE risk in the general population (13–16). Due to the need to adhere to a strict schedule for effective use, in practice the failure rate with traditional POPs is believed to be higher than with COC use (17), although, this is not a problem with desogestrel only pills. Despite the high efficacy of depot medroxyprogesterone acetate (DMPA), this commonly results in amenorrhoea, which some women find unacceptable, and also risks the problems of bone density loss (18). Both POPs and LARCs frequently cause irregular vaginal bleeding, which is the commonest cause of discontinuation (19–21). Thirdly, standard copper-containing intrauterine contraceptive devices (IUDs) are often regarded as inappropriate for SCD women because of the increased menstrual blood loss associated with them, which would exacerbate the chronic anaemia of SCD, and because of anecdotal evidence of an increased risk of infection with IUD use (22,23), which could potentially provoke acute

sickling episodes. However, more recent studies have found no association between IUD use and infection (24).

The UK Medical Eligibility Criteria (MEC) for contraceptive use, in 2009, stated that the use of COC in SCD is MEC "category 2" (i.e. the benefits of use outweigh the risks) and the use of progestogen-only contraception (POC) is "category 1" (i.e. no restriction on their use), However this recommendation is based on evidence that pregnancy carries many hazards in women with SCD, and not on evidence specifically supporting their safety (25).

This study aims to answer the following questions: what is the experience of pregnancy planning for women with SCD, what guidance do they receive, and what methods of contraception are they using? The study also aims to ascertain the proportion of their pregnancies which were unplanned. The survey provides some limited further data on what complications women with SCD experience with specific methods of contraception, and also looks at their menstrual histories and the counselling they receive about the reproductive health aspects of their disease.

These data were compared with data obtained from a similar survey conducted in 1993(26) to explore how the knowledge and attitudes of women with SCD and that of their health care professionals might have changed over time.

Study Design:

This is a multi-centre, interview based, cross-sectional study involving four acute hospital Trusts and two Sickle Cell and Thalassaemia Centres in north and central London. The participants in the study were identified from lists of patients attending these services, over a twelve-month period from 2009 to 2010. The inclusion criteria were women with sickle cell disease (Haemoglobin SS, SC, and S-beta thalassaemia), aged 18 years and over, and consenting to be interviewed. The participants were interviewed in confidence by the principal researcher (AE) using a structured validated questionnaire at their local hospital. Each interview lasted about 30 minutes. Patient information was anonymised to preserve confidentiality and to comply with the Data

Protection Act 1978. Data was obtained on demographic and clinical characteristics, contraceptive methods used, and side effects experienced. In addition, information was ascertained about past contraception, pregnancy history, and the health care advice they received regarding pregnancy planning.

The results are summarised using the mean and standard deviation, or median and range. Categorical data were summarised using count and percentages. The 95% confidence intervals for the differences between proportions or means are presented, where appropriate. The contraceptive complications are reported as percentages in woman-years of use (the summation of duration of use by individual women expressed in years). These data are compared with a similar survey conducted in 1993 from the same hospitals and Haemoglobinopathy Centres in north London, using the chi-square test or logistic regression, as appropriate. All analyses were carried out in Stata Version.11 (27). Consent to verify a reported serious illness, such as an episode of thrombosis requiring hospital admission, investigation and treatment, was sought and the researcher viewed the woman's hospital records, in order to obtain clinical details of these complications. The inability to verify such details was a drawback to the 1993 survey.

The study received ethical approval from the Cambridge 4 Research Ethics Committee on behalf of the National Multi-centre Research Ethics Committee. This survey was supported by the multi-professional and multidisciplinary membership of the UK Forum for Haemoglobin Disorders which includes patient representatives from the Sickle Cell Society.

Results

In total 114 women were invited to participate and 102 consented to do so, 75 of them carry haemoglobin SS type, 19 haemoglobin SC, and 8 have S beta thalassaemia. In the 1993 survey 156 consented to participate, of whom 102 had haemoglobin SS, 42 haemoglobin SC, and 12 had S beta thalassaemia.

The mean age at interview was 34.6 (19-65) years in the 2010 survey, while the mean age in the1993 survey was 28.7 (17-53) years. Age at menarche was 14 years in the 2010 survey and 15 years in the 1993 survey.

The 102 women surveyed in 2010 had had 150 pregnancies, and there had been 207 pregnancies in the 156 interviewed in 1993. The unplanned pregnancy rate was 53% in the 2010 survey, compared with 64% in the 1993 survey. Sixteen women (15%) in the 2010 survey said they had received advice against pregnancy, compared with 54 women (36%) in the 1993 survey. The number of women understanding the mode of inheritance of SCD increased from 28% in the 1993 survey to 88 % in the 2010 survey.

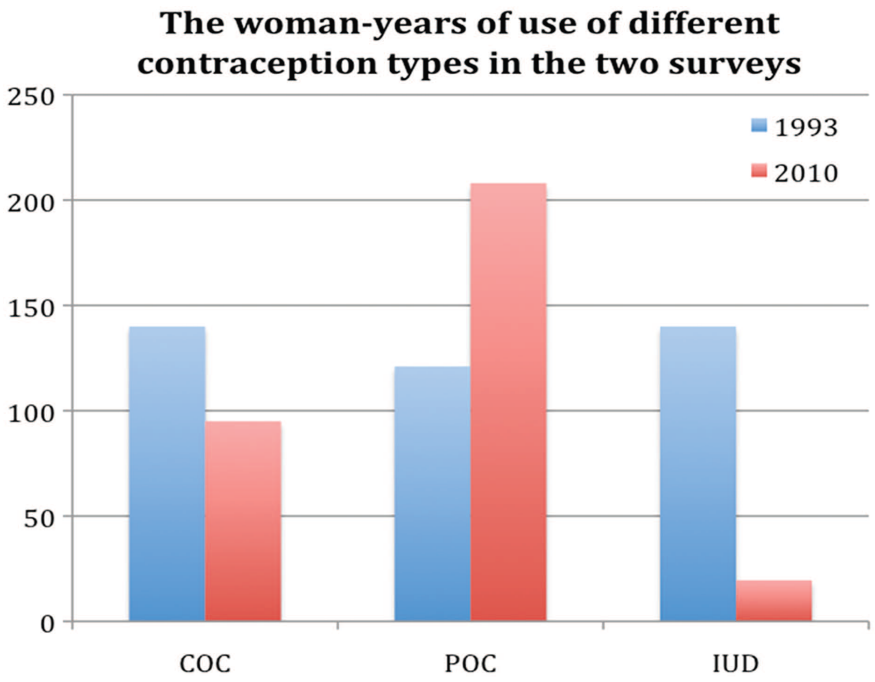
The COCs used by the women in the survey were standard formulations containing 35 micrograms, 30 micrograms or 20 micrograms of ethinylestradiol, with norethisterone acetate, levonorgestrel, noregestimate, or drospirenone progestogens. None of the participants reported use of "third generation" COCs containing desogestrel or gestodene as the progestogen.

The POPs used were levonorgestrel, norethisterone or desogestrel.

Trends in the use of contraceptive methods over a period of 17 years

Contraceptive	**1993 Count (%)**	**2010 Count (%)**	**p**
COC	67 (45%)	29 (31%)	
Difference in proportions (95% Confidence interval)	14% (1% to 26%)		0.035
DMPA	26 (17%)	35 (38%)	
Difference in proportions (95% Confidence interval)	21% (10% to 32%)		<0.001
POP	30 (20%)	14 (15%)	
Difference in proportions (95% Confidence interval)	5% (4 to15%)		0.0325
IUD	28 (19%)	2 (2%)	
Difference in proportions (95% Confidence interval)	17% (10% to 32%)		<0.001

CI, confidence interval; COC, combined oral contraceptive pill; DMPA, depot medroxyprogesterone acetate; IUD, intrauterine device; POP, progestogen only pill



The woman-years of use of different contraception types in the two surveys conducted in 1993 and 2010.COV, combined oral contraceptive pill; IUD, intrauterine device; POC, progesterone-only contraceptive.

Percentages and trends of women reporting side effects with the use of different contraceptive methods in the two surveys conducted in 1993 and 2010

	COC (Number; %)		**p**	**DMPA (Number; %)**		**p**	**POP (Number; %)**		**p**	**Copper IUD (Number; %)**		**p**
	1993 N=67	2010 N= 29		1993 N= 26	2010 N=35		1993 N=30	2010 N= 14		1993 N= 28	2010 N= 2	
Woman years of use	148	98		44	77		77	53		140	7	
Irregular bleeding	3 (4.5)	1 (3.4)	0.817	8 (30.8)	6 (20)	0.009	6 (20)	1 (7.1)	0.278	0	1(50)	
Forgetfulness depression.	7 (10.4)	4 (13.8)	0.637	1 (3.8)	1 (3.3)	0	1 (3.3)	0		0	0	
Amenorrhoea	0	0		5 (19.2)	1 (3.3)	0.634	1 (3.3)	0		0	0	
Menorrhagia	2 (2.9)	0		4 (15.4)	0	0.016	0	0		11 (39.2)	0	0.009
Weight gain	11 (16.4)	0	0.020	0	3 (10)	0.074	3 (10)	2 (14.3)	0.072	0	0	
DVT, PE	2 (2.9)	0		0	0		0	0		0	0	
Stroke	0	0		0	0		0	0		0	0	
Failure	15 (22.4)	1 (3.4)	0.022	0	6 (21.4)		6 (21.4)	0	0.072	6 (21.4)	0	0.464
Discontinuation due to side effects	8 (12)	2 (6.9)	0.458	3(11.6)	2 (6.7)	0.039	2 (6.7)	0	0.323	0	0	
Increased crises	4 (5.9)	1(3.4)	0.610	0	0		0	0		1 (3.5)	0	0.786

COC, combined oral contraceptive pill; POC, progestogen-only contraceptives; Cu-IUD, copper –containing intrauterine device, DMPA, depot medroxyprogesterone acetate; DVT, deep vein thrombosis; PE, pulmonary embolism

The intra uterine contraceptive devices (IUDs) used were all standard copper and plastic devices, but the specific design for each woman is not known.

In the 2010 survey eight women (8.7%) were using the levonorgestrel intrauterine system (LNG-IUS), with a total of 15 years' use. Six women (6.5%) were using etonorgestrel subcutaneous implants with a total of 14 years' use. The side effects were minimal. Amenorrhoea was experienced by one woman in each group (12.5 % and 16.7% respectively). Irregular bleeding was reported by one woman in each group. There were no reports of VTE in either group, but one woman using an implant had a stroke, and there were no contraceptive failures with either of these methods. The LNG-IUS, and the implant were not included in the trend analysis as these were not used by any of the women interviewed in the 1993 study.

Discussion:

The number of unplanned pregnancies in SCD women is still unacceptably high, albeit there is some improvement in 2010 compared with the 1993 survey (53% vs 64.2%). That over fifty-percent of SCD pregnancies are still unplanned in the 2010 survey confirms that there is a continuing unmet need for effective contraceptive advice for this patient group. Further efforts to educate healthcare professionals on this issue are clearly needed, as well as initiatives to include contraceptive advice in the routine medical care of young women with SCD.

There is a statistically significant declining trend in the use of COCs, which decreased by about a third from 1993 to 2010, while the use of DMPA has more than doubled over this period. The authors believe that the studies conducted by de Ceular et al in 1982 and by de Aboud et al in 1997, both of which concluded that DMPA decreases the incidence of painful sickling crises, led to a considerable change in the family planning practice of those looking after women with SCD, with a reversal of the rate of use between COCs and DMPA (28,29). This trend is opposite to that seen in the general population, where COC use is quoted to be ten times higher than the use of long acting reversible contraceptives (30). DMPA use in our 2010 study is reported by 35% of SCD women,

compared with 3% for both DMPA and implants in the general population (30). However, this increased use of DMPA is supported only by the two small studies mentioned (28, 29).

Despite, the statistically significant decline in the use of POP in women with SCD, there were still 15% of them using it compared with 5% in the general population (30). This change occurred despite no evidence for an increased risk of thrombosis from progestogen only contraception in SCD (7) The 2010 survey has included the relatively newer methods, the LNG-IUS and contraceptive implants. These methods together constitute 15% of the methods used in 2010 and are associated with very few reported complications. This contrasts with a marked decrease in the use of copper IUDs.

With regard to serious complications with the use of the different methods, our data probably suggest a similar incidence of VTE with COC use in the two cohorts (2 episodes during 148 women-years in the 1993 survey, and no episodes in 98 women-years in the 2010 survey). Taken at face value, this is higher than with hormonal contraceptive use in the general population, but in keeping with the usual incidence of VTE in women with SCD. Only one woman experienced a stroke, whilst using LARC, in the 2010 survey, and it is clearly not possible to deduce any comparisons from this. Four COC users in the 1993 survey reported an increased frequency of acute sickling crises, and one COC user had the same problem in the 2010 survey. The rate of contraceptive failure with COC use does seem to have reduced considerably (15 failures in the 1993 survey compared with one failure in the 2010 survey). Anecdotally in the 1993 survey, the commonest reason for failure with COCs was an accidental interruption of medication during emergency hospital admissions. None of our data included any serious complications with POP use. However, it was associated with 6 contraceptive failures in the 1993 survey (during 77 woman-years of use), although none in the 2010 survey, which hopefully reflects a better awareness of the potential reasons for failure with this method. The only serious complication in our data with DMPA use was one episode of VTE in the 2010 survey, and there were no contraceptive failures with this method, which is consistent with its performance in the general population.

There appears also to be some progress in the number of women receiving advice against pregnancy (36% vs 15%). This may reflect the improving medical care of this group, and an improvement in their overall health, such that pregnancy is thought to impose less of a risk to their well being. It may also reflect a more general shift away from issuing prescriptive or prohibitive advice.

The interpretation of these data faces the inevitable problems of relatively small numbers. Information derived from interviews with the SCD women themselves has potential flaws in accuracy of recall. In the 1993 survey, the terms of the consent given by women to participate precluded cross-checking with their medical records, but this was permitted by the women participating in the 2010 survey. Another limitation of this study is that we do not have full demographic information for both groups for comparative analysis. However, the studies were conducted at the same centres in both 1993 and 2010. All the women participating in both studies were from black African or Afro-Carribbean ethnic groups.

All the women interviewed in both surveys felt strongly that the issues relating to pregnancy planning and contraceptive use were of high importance to them, and many of them encountered a lack of knowledge and of active discussion by their doctors of these matters. Without exception, the women were enthusiastic in their participation in this work, and took great trouble to provide answers to the structured questionnaire to the best of their ability.

This study highlights that healthcare professionals need to be better educated about the risks these women face in pregnancy, and thus their clear, but often unmet, need for advice about effective and safe contraception. We also note the paucity of well conducted research looking at appropriate contraceptive options for women with SCD. Within the limitations of the data described from our research, however, we have not found any strong suggestion of particular difficulties with any of the standard methods of contraception in current use.

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Appendix 3

Letter for approval for this study from the Cambridge 4 Ethics Committee

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NW3 2 QG

Dear Dr Elssa

Full title of study: **The clinical and haematological effects of hormonal contraception on women with sickle cell disease(SCD).**

REC reference number: **06/MRE05/59**

Thank you for your letter 26 February 2007, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information was considered at the meeting of the Sub-Committee of the REC held on 06 March 2007 by Dr Sandra Evans, Chair and Dr Mark Wilkinson, Vice Chair.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

Ethical review of research sites

The Committee has not yet been notified of the outcome of any site-specific assessment (SSA) for the research site(s) taking part in this study. The favourable opinion does not therefore apply to any site at present. We will write to you again as soon as one Research Ethics Committee has notified the outcome of a SSA. In the meantime no study procedures should be initiated at sites requiring SSA.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Application	AB/89218/1	29 August 2006
Investigator CV - Dr Asma Elssa		
Supervisor CV – Dr S Tuck		
Protocol	04	04 December 2006
Covering Letter		26 February 2007
Covering Letter		04 December 2006
Covering Letter		01 September 2006
Statistician Comments Email from Richard Morris		17 July 2006
Statistical Analysis		
Compensation Arrangements - Insurance Certificate		01 August 2006 – 01 August 2007
Letter of invitation to participant with Opt in reply slip		
Participant Information Sheet	04	19 February 2007
Participant Consent Form	05	
GP letter	03	19 February 2007
Consultant Letter	06	04 December 2006
List of Laboratory tests per individual participant		
Letter from Royal Free Hospital REC		01 June 2006
Letter from R and D office, Royal Free NHS Trust		22 March 2006
Letter from Sponsor		08 February 2006
Letter from funder		23 February 2006
Proforma for symptom and clinical assessment		
Peer review – Sheila Radhakrishnan		
Email confirming no external review		29 November 2006
Response to Request for Further Information		04 December 2006
Response to Request for Further Information		26 February 2007

R&D approval

The study should not commence at any NHS site until the local Principal Investigator has obtained final approval from the R&D office for the relevant NHS care organisation.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

06/MRE05/59 **Please quote this number on all correspondence**

With the Committee's best wishes for the success of this project

Yours sincerely

Dr Sandra Evans
Chair

Email: emma.clark@eoe.nhs.uk

Enclosures: *Standard approval conditions*

Site approval form

Copy to: Dr Oke Avwenagha
Room G652, Admin corridor
Royal Free & UCL Medical School
Hampstead Campus, Rowland Hill Street
London
NW3 2PF

Miss Susan Tuck
Department of Obstetrics & Gynaecology
Royal Free Hospital
Pond Street
London
NW3 2QG

Appendix 4

Reference range for haemostatic markers

Data derived from 40 normal adults presented as two standard deviations below and above the mean.

Analysis conducted by Dr Anne Riddell, Chief Biomedical Scientist, Haemophilia and Haemostasis Centre, Royal Free Hospital, London

Antithrombin activity (AT AC)	85.000-113.000 IU/ml
Factor II (FII)	84.300 - 120.000 IU/ml
Factor IX (FIX)	77.700 - 130.000 IU/ml
Factor V (FV)	63.850 - 120.500 IU/ml
Factor VII (FVII)	77.500 - 150.500 IU/ml
Factor VIII FVIII	66.000 - 174.000 IU/ml
Factor X FX	71.800 - 130.000 IU/ml
Factor XI FXI	68.000 - 144.000 IU/ml
Factor XII FXII	46.700 - 181.000 IU/ml
Factor XIIa FXIIIa	97.400 - 179.300 IU/ml
Protein C Activity (PC AC)	70.100 - 135.600 IU/ml
FREE Protein S	75.000 - 124.000 IU/ml
Von Willebrand Antigen (VW:AG)	46.000 - 153.000 IU/ml
International normalised ratio (INR)	0.8-1.2 ratio
APTT	25-35 seconds
Thrombin time (TT)	12 - 14 secs
Fibrinogen	1.5 – 4.0 g/dL
D-dimer	<130ng/mL
Dilute viper venom time (DVVT)	<1.16 sec
Activated Protein C resistance (APCR-V)	>2.0
Micro particles (MP)	<10nM
Soluble Platelets selectin (sP selectin)	92--212 ng/mL
Slouable sell differentiation 40 ligand (sCD40 ligand)	0.03-3.98 ng/mL
Haptoglobin	<1.22g/L g/L
CRP	0.2 -10 ug/mL
Thrombin- anti thrombin complexes (TAT)	<2-4.2µg/L
Prothrombin Time (PT)	12 – 15.5 secs
Tissue factor (TF)	< 2 pMol
Factor VIII Coagulant (FVIII C)	50 – 150 iu/dL
Prothrombin F1+2	69 – 229 pmol/L
Lag Time (initiated with 5pM Tissue Factor) (time/min)	2.0 - 3.2 minutes
Endogenous Thrombin Potential (initiated with 5pM Tissue Factor)	1159 – 2168 nM
Peak Height (initiated with 5pM Tissue Factor)	147 – 359 nM
Time to Peak (initiated with 5pM Tissue Factor)	4.6 – 7.4 min
Slope	33 – 142 nM/min
Start Time (initiated with 5pM Tissue Factor)	19 -28.3 min

Appendix 5

Reference ranges for liver function tests and biochemical markers (two standard deviations above and below mean values in healthy adults).

Neutrophils	1.7-8.0 10^9/l
Lymphocytes	1.0-3.510^9/l
Monocytes	0.1-1.010^9/l
Eosinophils	0.0-0.46 10^9/l
Basophils	0.0-0.20 10^9/l
Complement reactive protein	0.2-10.0 mcg/ml
White blood cells	3.5-11.0 10^9/l
Haemoglobin	11.5-15-5 g/dl
Platelet count	140-400 10^9/l
Haematocrit	0.35-0.47 l/l
Alanine amino transferase	<33 units/l
Aspartate amino transferase	<31 units/l
Alkaline phosphatase	< 129 units/l
Serum albumin	35-50g/l
Haptoglobin	< 1.22 g/l
Lactate dehydrogenase	240-480 units/l
Bilirubin	< 21 µMol/l
Free Hb	11-15.5mg/l

Appendix 6

Participants' information sheet

30/11/06

Royal Free Hospital NHS

Royal Free Hospital
Pond Street
London NW3 2QG

Participants information sheet

The clinical and haematological effects of hormonal contraception (e.g. "the pill") on women with Sickle Cell Disease.

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish.

Part 1 tells you the purpose of this study and what will happen to you if you take part.

Part 2 gives you more detailed information about the conduct of the study.

Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

Part 1

1. What is the purpose of the study?

The aim of this research is to test whether hormonal contraceptive methods pose any additional risk of complications for women with Sickle Cell Disease (SCD) compared with women with SCD not taking hormonal contraception. This mainly involves the combined pill and other "pills" like progesterone-only tablets ("the mini-pill") and depot injections, as well as the Mirena (progesterone) coil.

Whilst doing this, I will also look at what women with SCD experience with their menstrual cycles and reproductive health, i.e. use of different contraceptive methods, fertility issues and pregnancy planning. I realise that this information may be sensitive, but it forms an important part of understanding how women with SCD experience issues relating to pregnancy planning.

2. Why have I been chosen?

You have been chosen because you are a woman living in London who has SCD, and you do not feel that you are planning pregnancy in the near future. You are using your own chosen method of contraception or you do not need contraception at present, and you are in the age group 18-54 years.

Your Haematology Consultant has suggested your name to me as someone who might be interested in joining the project.

3. Do I have to take part?

No. It is up to you to decide whether or not to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you receive.

4. What will happen to me if I take part?

I am the Chief researcher, Dr Asma Eissa; I am a postgraduate trainee and member of the Royal College of Obstetricians & Gynaecologists in London; with over eight years of experience in Gynaecology. I am working with Miss Susan Tuck (Consultant Obstetrician & Gynaecologist at the Royal Free Hospital, London).

I will see you throughout this project.

In the first meeting I will check your medical history and spend 30-45 minutes going through a set of questions about your SCD, your menstrual periods, your pregnancy plans and your experience of contraception methods.

I will also check your blood pressure and your weight. Your name will not be written on the forms which I use to write your answers and the examination findings. Only a code number will be used. There will be only one copy of the list which matches women's names and their project code number. I will keep this list securely in my office at the Royal Free Hospital, which is always locked if I am out of the office. This means that I will be the only person who can know which personal details are from which woman.

Then I will take a blood sample to analyse for blood clotting tests and other investigations such as your haemoglobin concentration and the proportion of your red blood cells which have sickled.

Some of the research tests will be to detect if there is any damage to the lining of your blood vessels caused by the hormonal contraception method you are using.

The amount of blood will be around 30 ml (about 3 dessert spoonfuls). I will see you again for the same checks and blood tests every 3-4 months for the 2 years of the project.

5. What will happen to any samples I give?

There will be secure procedures for collecting, using and storing your blood samples The storage duration will be two years (till the end of the study).

There is no intention to use these samples in the future for research that cannot yet be specified.

I will have access to these samples as well as the senior biomedical scientist in the Haemophilia Centre at the Royal Free Hospital (where the tests will be carried out).

These tests are carried out three times a year, during the study. The inconvenience of the numerous venepunctures will be kept to minimum and you are obviously well used to having blood samples taken! All the usual hygiene precautions will be undertaken. However there is a small risk of infection and thrombosis from these procedures. Please report to your GP “as soon as possible” any swelling or unusual pain at the site of the needle insertion.

Your conversations with me, the physical examination and the blood tests will be private and confidential.

The time will be arranged to suit you. The place of the meeting will be in your usual hospital, either in the Haematology Department or in the Gynaecology Clinic, whichever you prefer.

Your blood samples will have your name written on them when they are sent to the laboratory for testing. However the information from this is always treated as being confidential and they will be protected by the Data Protection Act procedures. The final analysis of these test results from all the women who participate will be totally anonymous.

In case the project finds an unexpected test result on you, you will need to let me know how you would like to be informed about this. For example, if a blood sample test shows that you have hepatitis and you need to be prescribed some treatment for this. I could either let you know about this myself, or ask your Haematologist to let you know, or your G.P. It will also be helpful for you to let me know how you would like to be contacted. This could be either by letter or by telephone. If you do need any particular treatment, which would obviously be discussed with you by your usual doctors.

I will be talking to about 110 women with SCD during this project. These women will fall into two groups, one group is those who are using hormonal contraception and the other group is those who are using other forms of contraception (or not using contraception at all). I will then be able to compare the health and blood test results of the two groups of women and see if there are any particular differences between them.

This study is expected to run for two years, because we know that your health with SCD does vary from time to time. By looking over a reasonably long period of time I hope to get the most realistic picture of how these issues are.

6.What are the benefits of taking part?

We cannot promise the study will help you but the information we get might help improve the treatment of women with SCD for the future. However, you will be helping us to improve our understanding of important and effective methods of contraception and family planning for women with SCD. This will help doctors to provide women with SCD with the best possible family planning advice in the future.

Part 2

Your own Haematology Consultant knows all about this project and is helping with the recruitment of appropriate women to participate in it.

This project is intended to be presented for a higher degree in medicine, (Medical Doctorate (MD Res) of University College, London) by the Chief researcher, Dr Asma Eissa.

1. Involvement of the General Practitioner/Family doctor (GP)

We would like to inform your GP about your participation in this study if you permit us. Please tick the relevant box in the consent form if you wish so.

2. What if there is a problem?

If you have a concern about any aspect of this study, you should speak to me, and I will do my best to answer your questions. My mobile phone number is 07717 084 861 and my phone number at the Royal Free Hospital is 020 7 830 2565. If you remain unhappy and wish to complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from your usual hospital or from the Royal Free (www.royalfree.nhs.uk).

University College London has no-fault indemnity (insurance) arrangements in place, in the event that something does go wrong and you are harmed during the research study. If you are harmed and this is due to someone's negligence then you may have grounds for a legal action for compensation, but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you.

3. What will happen to the results of the study?

All the final information will be anonymous. Individual results will be put together for the two groups of women. These will be analysed and categorised without giving any personal details about individuals. I hope that our findings will be published in medical journals so that other doctors can read and learn from it.

If you would like a copy of the report to be sent to you when I have completed the study, please let me know.

4. Who is organising and funding this survey

The study has been organized by Miss Susan M Tuck, Consultant Gynecologist at the Royal Free Hampstead NHS Trust, North London & me, Dr Asma A. Eissa, Clinical Research Fellow in the Department of Obstetrics & Gynaecology. Both of us have experience of conducting various research projects concerning pregnancy and gynaecological issues affecting women with SCD. This study is being funded from in-house funds of the Department of Obstetrics and Gynaecology at University College London. These funds cover all the hospitals involved in the study.

5. Who has reviewed the study project?

The study is reviewed by the researcher supervisor (Miss S M Tuck) & it has been externally reviewed by Miss Radhakrishnan (Consultant Gynaecologist at the Royal Free Hospital who is in charge of the family planning clinics). The ethical basis for this study was assessed independently by the Eastern Multicentre Research Ethics Committee, based in Cambridge.

6.Travel expenses

All your travel expenses will be paid for you. Please let me know what you need for these when you confirm your participation in the survey. If you are willing to take part in this study I will give you a consent form to sign and keep.

Contact for further information:

Dr Asma A. Eissa

Department of Obstetrics and Gynaecology

Royal Free Hampstead NHS Trust

Pond Street

London NW3 2QG

Mobile phone 07717 084 861

Work phone 020 7 830 2565

Fax: 02078302799

E mail: alwan12@hotmail.co.uk

Thank you very much for helping us, if you can, with this survey, and thank you for taking the time to read this information sheet.

Appendix 7

Participants' information for Katherine Dormandy Coagulation Research Plasma Bank

HAEMOPHILIA CENTRE & THROMBOSIS UNIT

Director: Professor Edward Tuddenham

Dr Pratima Chowdary, Dr Anja Drebes, Dr Keith Gomez, Dr Mary Mathias, Dr Thynn Thynn Yee

Telephone: 020 7830 2068 Fax: 020 7472 6759

Participant Information for the KD Coagulation Research Plasma Bank

You are being invited to contribute to research being co-ordinated by the Haemophilia Centre and Thrombosis Unit by giving blood samples. Please take time to read the following information carefully before you decide. Ask us if there is anything that is not clear or if you would like more information.

What is blood coagulation?

Blood coagulation is a complex process by which blood forms clots, necessary to prevent excessive blood loss after injury. The bleeding and thrombotic disorders are due to under or over active coagulation factors and platelets.

What is the purpose of the KD Coagulation Research Plasma Bank?

The purpose of the plasma bank is to enable research into the investigation and management of inherited and acquired coagulation disorders. The plasma bank will store samples from patients with a diagnosed or suspected inherited or acquired bleeding or

thrombotic disorder for future research. Samples will be processed in the laboratory and either tested immediately or kept for long term storage at -80°C.

The three research areas of interest for which the samples and data from plasma bank will be used are;

1. Developing tests to predict the effects of your condition - Previous studies have shown that the severity of a clotting disorder cannot always be predicted by currently available tests. There are newer tests in development which may give a better indication of how a disease will affect patients.
2. Tailoring treatment for individual patients - Effective treatment is available for most coagulation disorders. but the response can vary between different individuals or in the same person over time. A better understanding of how treatment responses vary may lead to better treatment for individual patients
3. Validation of new tests. When new tests are developed they need to be tested on samples from patients who are known to have a particular clotting disorder. This will show whether they are better, or offer further information, when compared with existing tests.

Why have I been chosen?

You have been invited to contribute to this ongoing research programme either because you are attending the Haemophilia and Thrombosis Unit for investigation of either a bleeding disorder or a thrombotic disorder or you have some blood clotting problems in relation to your underlying medical condition and the doctors from your team in discussion with the Doctors in the Haemophilia Centre and Thrombosis Unit have identified you as a potential donor.

Do I have to take part?

It is up to you to decide whether or not to take part. If you decide to take part, you will be given this information sheet to keep and be asked to sign a consent form. Whether you decide to partake or not it will not affect the number of hospital visits you make, the duration of an inpatient stay or the treatment of your medical condition.

What if I change my mind ?

Your participation is entirely voluntary and you are free to withdraw at any time, without giving any reason, without your medical care or legal rights being affected. If you decide to withdraw we will dispose of the blood samples and delete the information collected on you. Where your sample has already been used, that data cannot be destroyed, but no further use will be permitted

What will happen to me if I take part?

10 -20 mls of blood (two- four teaspoonfuls) will be taken at the time of your clinic visits or during inpatient stay. This will be taken at the same time as your routine blood tests so there will be no need for an extra needle stick. At the same time we will collect some information about yourself and your medical condition to link to the sample. This information will be your name, date of birth, hospital number, a list of your medical problems, medical history, medications that you are taking, and results of the laboratory investigations done for your condition. This information will be stored electronically and be accessible only to authorised personnel. There will be no changes in the number of hospital visits, duration of inpatient stay or the management of your medical condition.

What do I have to do?

No lifestyle or dietary changes are required for participation in this trial. Any changes that are made have been done as a part of your routine care.

Is there any Financial Compensation?

No, There is no financial compensation if you decide to donate a sample for this research programme.

What are the possible disadvantages, risks and possible benefits of taking part?

If you agree to take part we do not anticipate any disadvantages to you and we cannot promise that donating blood samples will help you directly. It is entirely possible that investigations done on the samples at a later date might reveal new information, which might be useful in the management of your underlying condition. In such situations you will be written to by one of the doctors taking care of you and offered an appointment for further discussion. We hope that the research co-ordinated by us will help advance the treatment of people, including yourself, with bleeding or thrombotic conditions.

Will my taking part in this study be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential. If you consent to take part in the research, your data will be handled in a manner in accordance with the Data Protection Act 1998 and the rights you have under this Act. For interpreting the information obtained from the plasma sample we will need information on your medical conditions The information that is used will be the information collected at the time of the blood sampling. It is our intention to store the

samples for a maximum of 10 years. The results will be published in various journals and international meetings in a way that you will not be identified.

Where samples are sent outside of the trust, to other researchers in the United Kingdom and European Union accompanied by clinical information, all personal identifiable information such as your name, and date of birth, will be removed so that you will not be recognised from that information.

Representatives of regulatory authorities, research and development team at the Royal Free Hampstead NHS Trust or ethics committee will be allowed to see your medical notes as required to ensure the research is being properly conducted and that the data collected is accurate. Your privacy will be respected at all times.

You have the right to ask and see the data that has been collected about your health and if you think anything is incorrect, to have it corrected. The only person outside the trust able to view any identifiable data will be your GP to help with your ongoing care.

Who has reviewed the study?

This research programme has been reviewed by Sheffield Ethics Committee, and the reference number is **09/H1308/115**

What happens if there is a problem?

We would not expect you to suffer any harm or injury by donating samples for the plasma bank. If you are harmed by taking part there is no special compensation arrangement. If you are harmed due to someone's negligence then you may have grounds for legal action, but you may have to pay your legal cost. Regardless of this, if you wish to complain or have any concerns about any aspect of the way you have been approached or treated during the

course of this consent process, the normal National Health Service Complaints mechanism is available to you. If you have any concerns regarding the care you have received or as an initial point of contact if you have a complaint, please contact the Patient Advice and Liaison Service (PALS) at the address given below;

PALS office: 020 7830 2174

pals@royalfree.nhs.uk

PALS

Royal Free Hospital,

Pond Street,

London,

NW3 2QG

Contact for further information?

If you require any further information please do not hesitate to discuss with any of the nurses or clinicians looking after you.

THANK YOU FOR YOUR PATIENCE IN READING THIS LEAFLET.

Appendix 8

Donor consent form for the Katherine Dormandy Coagulation Research Plasma Bank.

Royal Free Hospital NHS

Royal Free Hospital
Pond Street
London NW3 2QG

30/11/06

HAEMOPHILIA CENTRE & THROMBOSIS UNIT

Director: Professor Edward Tuddenham

Dr Pratima Chowdary, Dr Anja Drebes, Dr Keith Gomez, Dr Mary Mathias, Dr Thynn Thynn Yee

Telephone: 020 7830 2068 Fax: 020 7472 6759

Donor Consent Form for the KD Coagulation Research Plasma Bank

(KDCRPB)

Patient details (or affix pre-printed label)

Patient's Full Name:

Date of Birth:

Hospital Number:

Research Ethics committee Ref: 09/H1308/115

	Patient Initials
I confirm that I have read and understood the information sheet, version 2, dated 17 Nov. 2009 for the above plasma bank and have had the opportunity to ask questions.	
I understand that additional blood may be taken during the investigation and / or treatment of my condition for the purposes of the plasma bank, and there is no need for extra needle sticks	
I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	
Medical information collected about myself at the time of blood samples will be treated as confidential.	
I understand that sections of my medical notes may be looked at by	

authorised individuals from regulatory authorities or by the ethics committee, where it is relevant to my participation in this study. All my medical information will be treated as confidential.

I have the right to ask to see the data collected about me and if anything is incorrect, I can ask for it to be corrected.

If you have any preferences or exclusions for use of the donated plasma samples, or any other comments, please elaborate in the box below.

_____

Name of Patient Date Signature

_____

Name of Person taking consent Date Signature

Appendix 9

Participants' consent form for project: The clinical and haematological effects of hormonal contraception on women with sickle cell disease.

30/11/06

Royal Free Hospital NHS

Royal Free Hospital
Pond Street
London NW3 2QG

CONSENT FORM

The clinical and haematological effects of hormonal contraception on women with sickle cell disease Name of Researcher: Dr Asma A .Eissa **Please initial box**

1. I confirm that I have read and understand the information sheet dated…01/09/2006 for the above study and have had the opportunity to ask questions ☐
2. I understand that Dr Asma A. Eissa will spend around 30-45 minutes to ask me questions relating to my medical history, examine me and take blood samples. ☐
3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
4. I understand that sections of any of my medical notes may be looked at by Dr Asma Eissa With my consent, if that would help the accuracy of the survey . ☐
5. I understand that sections of any of my medical notes may be looked at by responsible individuals from Royal Free Hospital, London or from regulatory authorities where it is relevant to my taking part in research. I give permission for these individuals to have access to my records. ☐
6. I agree to take part in the above study. ☐
7. I agree for my GP being informed about my participation in this study. ☐

Name of Patient

Signature Date

_____

Name of Patient Date Signature

_____

Name of Person taking consent Date Signature

When completed: 1 for patient; 1 for researcher; 1 to be kept with hospital notes