The effects of elective total knee arthroplasty on the activation of markers of inflammation, coagulation and endothelial dysfunction

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I would like to dedicate this thesis to my lovely wife, Jacqueline for all her support through the good times and the bad, without whom I would have never have got my research done! And to our two beautiful 'wee monsters Eilidh and Angus.

Abstract

Total knee arthroplasty is a common elective orthopaedic procedure. The surgery itself causes soft tissue and bony trauma leading to a systemic response which includes endocrinological, immunological and haematological events. This thesis aims to investigate the potential association between total knee arthroplasty and such markers of inflammation, endothelium and coagulation.

The study consisted of 4 groups; group 1 underwent an uncemented total knee arthroplasty; group 2 underwent a cemented total knee arthroplasty; group 3 underwent an uncemented total knee arthroplasty but received an intra-operative infiltration of local anaesthetic; group 4 underwent an uncemented total knee arthroplasty but had a post-operative drain for 24hours. Blood sampling was undertaken pre-operatively and at day 1 and day 7 post-operatively for the white cell count, platelets, neutrophils, C-reactive protein, interleukin 6, e-selectin, soluble CD40L, tissue plasminogen activator, von Willebrand factor, CD40 and CD1442a. Statistical analysis was undertaken in the form of pair sampled t-tests between group 1 and each of the other three groups.

Although there some significant changes in one or two of the variables between the groups the only variable which demonstrated a significant difference in all comparisons was the CD1442a count. The exact role of CD1442a is unclear but there evidence to suggest that it may reflect the inflammatory and thrombotic process or contribute directly to the ongoing atherothrombogenesis.

During the statistical analysis it was noted that the majority of the variables showed no clear statistical difference between the groups. In chapter 7 an ANOVA / Freidman analysis demonstrated that all but one of the variables, the CD1442a count, showed no statistical difference between all four groups. This allowed all the variables to be collated and presented as the single largest cohort study to date demonstrating the effects of total knee arthroplasty on the markers of inflammation, endothelium and coagulation. All the variables assessed showed a statistically significant change from pre-operative levels to day 7 post operation.

In summary our studies demonstrate that total knee arthroplasty results in activation of common markers of inflammation, endothelium and coagulation. These changes may explain the increased incidence of venous thrombosis and thrombo-embolism post-operatively as well as a potential risk of venous thrombo-embolism.

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Acknowledgments

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Declarations of own work

Author's Declaration

I declare that, except where explicit reference is made to the contribution of others, that this dissertation is the result of my own work and has not been submitted for any other degree at the University of Glasgow or any other institution.

Signature _____

Printed Name _____

Definitions / Abbreviations

ACCP/SCCM	American College of Chest Physicians / Society of Critical Care Medicine
ACTH	adrenocorticotrophic hormone
ADH	antidiuretic hormone
CRP	C - reactive protein
CD154	CD40 ligand
DAG	diacylglycerol
DMPT	N,N-dimethyl-p-toluidine
EC	endothelial cells
ECAT-DVT	European Concerted Action on Thrombosis deep vein thrombosis
EGF	endothelial growth factor
ELAM	endothelial leukocyte adhesion molecule
FACS	fluorescence-activated cell sorting analysis
FN III	fibronectin type III
FSC	forward scatter channel
FSH	follicle stimulating hormone
GM-CSF	granulocyte/macrophage colony stimulating factor
GPI	glycosyl-phosphatidylinositol
GP-130	130 kDA signal transducing glycoprotein
IFN-γ	interferon-γ
IGF	insulin-like growth factor

IL-1	interleukin 1
IL-1α	interleukin 1 alpha
IL-1ß	interleukin 1 beta
IL-2	interleukin 2
IL7	interleukin 7
IL-6	interleukin 6
IL-8	interleukin-8
IP3	inositol triphosphate
LBP	lipopolysaccharide binding protein
LH	luteinizing hormone
LITE	Longitudinal Investigation of Thromboembolism Etiology Study
LPS	lipopolysaccharide
MCP-1	monocyte chemotactic protein-1
M-CSF	macrophage colony-stimulating factor
MI	myocardial infarction
MIP-1α	macrophage inflammatory protein 1 alpha
NO	nitric oxide
PAI	plasminogen activator inhibitor
PAF	platelet activating factor
PDGF	platelet derived growth factor
PF-4	platelet factor 4
PGE_2	prostaglandin E_2
PMMA	polymethylmethacrylate
PMNL	polymorphonuclear leucocytes

PSGL-1	p-selectin glycoprotein ligand-1
rT3	reverse tri-iodothyronine
SAP	serum amyloid P
sIL-6R	soluble IL-6 receptor
SIRS	systemic inflammatory response syndrome
sCD40L	soluble CD40L
SSC	side scatter channel
T_4	thyroxine
T_3	tri-iodothyronine
TF	tissue factor
TBG	thyroid-binding globulin
TFPI	tissue factor pathway inhibitor
TGF-α	transforming growth factor alpha
TGF-ß	transforming growth factor beta
TNF-α	tumour necrosis factor alpha
TNF-ß	tumour necrosis factor beta
TNFR-1	tumour necrosis factor receptor one
TNFR-2	tumour necrosis factor receptor two
t-PA	tissue plasminogen activator
TSH	thyroid stimulating hormone
VCAM-1	vascular cell adhesion molecule-1
VTE	venous thromboembolism
vWF	von Willebrand factor

List of relevant presentations

‘The effect of cement in total knee arthroplasty on the markers of inflammation and of endothelial function: The ‘PMMA effect’’
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‘The effect of uncemented total knee arthroplasty on markers of inflammation and endothelial function’.
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CHAPTER 1

INTRODUCTION

Introduction

Total knee arthroplasty is a common elective orthopaedic procedure performed in the United Kingdom. The number performed has been steadily rising for the last few decades with over 12 000 in 2007 (Scottish arthroplasty project). There is a multitude of knee prostheses offered by the medical companies but the essential principles of total knee arthroplasty remains the same.

The majority of procedures are performed with a tourniquet to minimise intra-operative bleeding and to aid the surgeon. A standard midline incision is used with the medial parapatellar approach to gain access to the knee joint itself and a degree of soft tissue balancing is carried out before a sequence of jigs are used to make the bone cuts on the tibia and femur. This then allows the knee prosthesis to be implanted with or without polymethylmethacrylate (PMMA) cement, which acts not as the as the name suggests but rather as a space filling device much akin to that of grout.

The surgery itself by its very nature causes a degree of soft tissue and bony trauma which induces hormonal and metabolic changes. This forms part of a wider systemic response to surgical trauma which includes endocrinological, immunological and haematological events (Table 1.1).

Table 1.1: Systemic response to surgery

Sympathetic Nervous System Activation
Endocrine 'stress response' pituitary hormone secretion insulin resistance
Immunological & Haematological Response cytokine production acute phase reaction neutrophil leucocytosis lymphocyte proliferation

This thesis assesses the effect of uncemented total knee arthroplasty on the activation of the immune system and on markers of inflammation. I will also assess any additional response evoked from the added use of polymethylmethacrylate (PMMA) bone cement. My third study will look at the use of local anaesthetic infiltration intra-operatively in uncemented total knee arthroplasty, comparing the effects that this has on the markers of inflammation, endothelium and coagulation. My final study will look at the effect of the use of drains post-operatively on these markers. The quantification of the inflammatory response following elective orthopaedic surgery is important in the light of emerging evidence supporting its association between both venous and arterial thrombosis as well that of atherosclerosis.

In this introduction I will review the systemic response to surgery, then the immune and endothelial response to surgery. I will then summarise the existing literature on these responses to total knee arthroplasty, and the effects of bone cement, local anaesthetic infiltration and the use of post-operative drains.

1.1 The Systemic Response to Surgery

The systemic response to surgery was first described by Cuthbertson in Glasgow Royal Infirmary, in 1932 (1) who described in detail the time course of the metabolic responses of lower limb injuries in four patients as well as quantifying the magnitude of the response. It was here the terms ‘ebb’ and ‘flow’ were first used to portray the time course of events which was one of an initial decrease in activity followed by an increase

in metabolic activity. The initial work on the ebb phase was partly carried out on animals and the flow phase was exaggerated, this has since been redefined (2).

Following surgical trauma there is the characteristic stress response consisting of the hypothalamic activation of the sympathetic nervous system as well as an increased secretion of pituitary hormones as well as secretion from the pancreas and thyroid. The overall effect of these hormonal changes is to mobilise the energy resources to provide energy through catabolism and maintain the intravascular fluid volume through salt and water retention. Stimulation of the autonomic sympathetic nervous system also causes an increased secretion from the adrenal medulla of catecholamines and norepinephrine. This produces the well known 'fight or flight' response with tachycardia and hypertension.

1.1.1 Pituitary hormone secretion

In response to the appropriate stimuli the pituitary gland secretes specific hormones either from its anterior or posterior part. The anterior pituitary stimulated by hypothalamic releasing factors synthesises adrenocorticotrophic hormone (ACTH), also known as corticotrophin. ACTH itself is not secreted but the larger precursor molecule pro-opiomelanocortin is initially secreted instead. This is then metabolised into ACTH, beta-endorphin (an opioid peptide of 31 amino acids, its circulatory level merely reflects the increased secretion from the pituitary gland as itself has no major metabolic activity) and an N-terminal precursor. Adrenocorticotrophic hormone is a 39 amino acid peptide which stimulates adrenal cortical secretion of glucocorticoids. Levels of both ACTH and cortisol rise within minutes of surgery.

The cortisol levels reach a maximum at around 4-6 hours later and may increase above 1500 $nmol^{-1}$ from a baseline value of around 400 $nmol^{-1}$ depending on the degree of surgery or trauma (3). The usual feedback mechanisms whereby the cortisol concentrations have a negative impact on the ACTH secretion are ineffective following surgery. Therefore levels of both the cortisol and ACTH remain high during this period. Cortisol has a variety of metabolic effects mainly by promoting the breakdown of protein and gluconeogenesis in the liver. It also promotes lipolysis producing gluconeogenic precursors from breaking down triglycerides to glycerol and fatty acids. The glucose produced results in elevated blood levels, the use of glucose by cells is also inhibited. As well as this, cortisol has anti-inflammatory activity. It inhibits macrophage and neutrophil buildup in areas of inflammation and interferes with the synthesis of inflammatory mediators namely prostaglandins.

Other hormones secreted are growth hormone and prolactin. Growth hormone, which also goes by the name of somatotrophin, consists of 191 amino acids, it is released in response to growth hormone releasing factor from the hypothalamus. The majority of its actions are mediated through insulin-like growth factors (IGFs), mainly IGF-1, which are small protein hormomes released from liver, muscle and other tissues. As well as regulating growth it also has other effects. It produces a positive protein balance by preventing protein breakdown at the same time as stimulating protein synthesis. As well as this it causes lipolysis (triglycerides into fatty acids and glycerol) and has an anti-insulin effect. Its secretion is related to the severity of the trauma or surgery. The hormone prolactin has 199 amino acids with a chemical structure not too dissimilar from that of the growth hormone. Although released in response to surgery or trauma, and in exercise, it does not affect the metabolic activity.

The levels of the other hormones secreted from the anterior pituitary such as follicle stimulating hormone (FSH) and luteinizing hormone (LH) are not significantly altered by surgery.

Arginine vasopressin, a potent antidiuretic hormone (ADH) is released from the posterior pituitary. ADH also acts along with corticotrophin-releasing factor and causes the release of pro-opiomelanocortin.

1.1.2 Pancreatic hormone secretion

Insulin is a polypeptide of two chains of 21 and 30 amino acids, bound together by two disulphide bridges. It is the main anabolic hormone (3). After food intake it is released from the pancreas specifically from the beta cells. It promoted the uptake of glucose into muscle to form glycogen and into fat cells for conversion into triglycerides. The conversion of glucose to glycogen in the liver is also in response to insulin. As well as its anabolic effects it inhibits catabolic events such as protein catabolism and lipolysis. However there appears to be a failure of insulin secretion that one would expect to accompany the catabolic, hyperglycaemic response seen intra-operatively. This may in part be due to the α-adrenergic inhibition of ß cell secretion. As well as this there is also the lack of the 'insulin resistance' response in the perioperative period.

The pancreas is also responsible for the secretion of glucagon from α cells. Its main effects are in the promotion of hepatic glycogenolysis and in increasing glucose

production in the liver from amino acids. It also promotes lipolysis. However after major surgery despite a rise in the concentrations of glucagon it does not significantly add to the increased glucose levels see in the stress reponse.

1.1.3 Thyroid hormone secretion

Thyroid stimulating hormone (TSH) results in the secretion of thyroxine (T_4) and tri-iodothyronine (T_3) as well as small amounts of the inactive reverse T_3 (r T_3). T_3 is produced peripherally in the tissue where the T_4 undergoes mondeiodination. Metabolically T_3 is in the order of three to five times more active than that of T_4. A siginifcant proportion of these hormones are protein bound, mainly to thyroid-binding globulins (TBG) and thyroxine binding pre-albumins. It is the free unbound percentage that is metabolically active but these low concentrations remain in balance with the concentrations bound to protein in tissue and plasma (4).

The thyroid hormone activity affects both metabolism and heat production. It also stimulates the central and peripheral nervous systems and causes an increased amunt of carbohydrate absorption from the gastro-intestinal tract. The activity of the thyroid hormones is closely linked to catecholamines in that it increases the affinity as well as the number of the ß-adrenoceptors in the cardiac muscle and thereby increasing its response to the catecholamines. Following surgery the TSH levels decrease in the first few hours post-operatively but soon return to their pre-operative levels. The cause of which, is yet unknown.

The net effect of this following surgery is to provide energy from the breakdown of resources such as from carbohydrate, protein and fat. Soon after surgery starts the blood glucose concentrations increase from increased liver glycogenolysis and gluconeogenesis in response to cortisol and catecholamine levels. The uptake of glucose peripherally is reduced. The level in the blood is proportional to the degree of the surgical insult closely following the rise in catecholamines. Due to the apparent lack of glucose control during this period there is a period of hyperglycaemia due to the increased production of glucose, the reduced levels of insulin and the insulin resistance peripherally. The protein catabolism that occurs mainly involves skeletal muscle but visceral muscle is also broken down to its amino acids. These may then be further used to provide energy or alternatively be used to form acute-phase proteins in the liver. With regard to fat the net result is that of increased mobilisation of triglycerides however this does not appear to significantly affect the concentrations of fatty acids and glycerol in the plasma.

The intravascular volume is maintained by the release of arginine vasopressin which causes the retention of water. This increased secretion may continue for several days following surgical trauma. The kidneys release rennin which in turn causes the release of angiotensin II which stimulates the release from the adrenal cortex of aldosterone. The aldosterone then stimulates the distal convoluted tubules of the kidney to absorb both sodium and water (5).

The endocrine response to surgical trauma is first activated by impulses travelling along sensory nerve roots to the spinal cord and medulla with activation of the hypothalamus. It was hypothesised in the 1950s that certain ‘wound hormones’ may be present at the site of the injury which may be in part be responsible for this response. In the now

classical studies by Egdahl (6) it was proved from measuring the adreno-coritcal response in canine lower limb trauma in those with either an intact sciatic nerve, or one that was transected, that there was no elevated levels adrenal hormone in those with a transected sciatic nerve. However this general idea that the presence of these 'local substances' may contribute to the wider stress response seem following surgical trauma was again brought to the forefront with the evolving research into cytokines.

1.2 Cytokines

From 1950 to 1970 the early studies on cytokines described a number of protein factors which were shown to mediate certain functions and were produced by different cell types. They were termed lymphokines as evidence at that time pointed to the T lymphocytes from the thymus as the cells involved in the secretion of these protein factors. As cytokine research evolved many individual cytokines were identified. It came to light that cytokines were synthesised mainly from leucocytes and it was shown that the majority of its actions were directed to other leucocytes, it was at this time they were renamed as interleukins. Often the same cytokine mediated a diverse array of effects. The exact identification and distinction between the various cytokines was due to impure preparations and non specific antibodies for the cytokines. But over the past decade with the use of molecular cloning and the availability of more specific antibodies, the properties and structure of these cytokines has been made possible.

In the presence of tissue trauma these low molecular weight proteins (<80 kDa) can be released from leucocytes (particularly monocytes), endothelial cell as well as

fibroblasts. Following binding to a specific cell receptor they can alter the cellular RNA and affect protein synthesis and it is through this process that they are able to change and regulate cell growth as well as cell development and repair. They alter the cell behaviour through signaling pathways which are predominately intracellular to affect gene transcription (7). Unlike hormones which are secreted into the circulation and affect target cells some distance away cytokines are released to act on either nearby cells (paracrine) or on the same cell (autocrine). They have been termed pleiotropic as they act on many different cell types and usually have more than one effect on the same cell as well as influencing the synthesis and action of other cytokines. They also have a significant role to play in inflammation and immunity and are very powerful at very low concentrations (8). They are extremely potent and generally act at picomolar concentrations. The effect that a specific cytokine will have on a cell will depend on a number of variables such as the levels of the cytokine as well as other regulators which may be present (5).

The majority of cytokines are synthesised and secreted when needed with exceptions such as transforming growth factor-beta (TGF-ß) stored in platelet alpha granules (9) and tumour necrosis factor-α (TNF-α) in mast cells (10). Once synthesized cytokines such as TNF-α and interleukin 1ß (IL-1ß) are stored as membrane proteins (11). They may also bind to an extracellular matrix or a cell surface protein to form a complex such as with interleukin-8 (IL-8) (12;13).

Cytokines have a local and systemic role in response to surgical trauma. The main cytokines involved are interleukin-1 (IL-1), tumour necrosis factor α (TNF-α) and IL-6. Following surgical trauma it is the IL-1 and TNF-α which is released first from the macrophages and monocytes locally, followed by IL-6 and other cytokines are released

(figure 1). But it is the IL-6 that is mainly responsible for inducing the acute phase response (5).

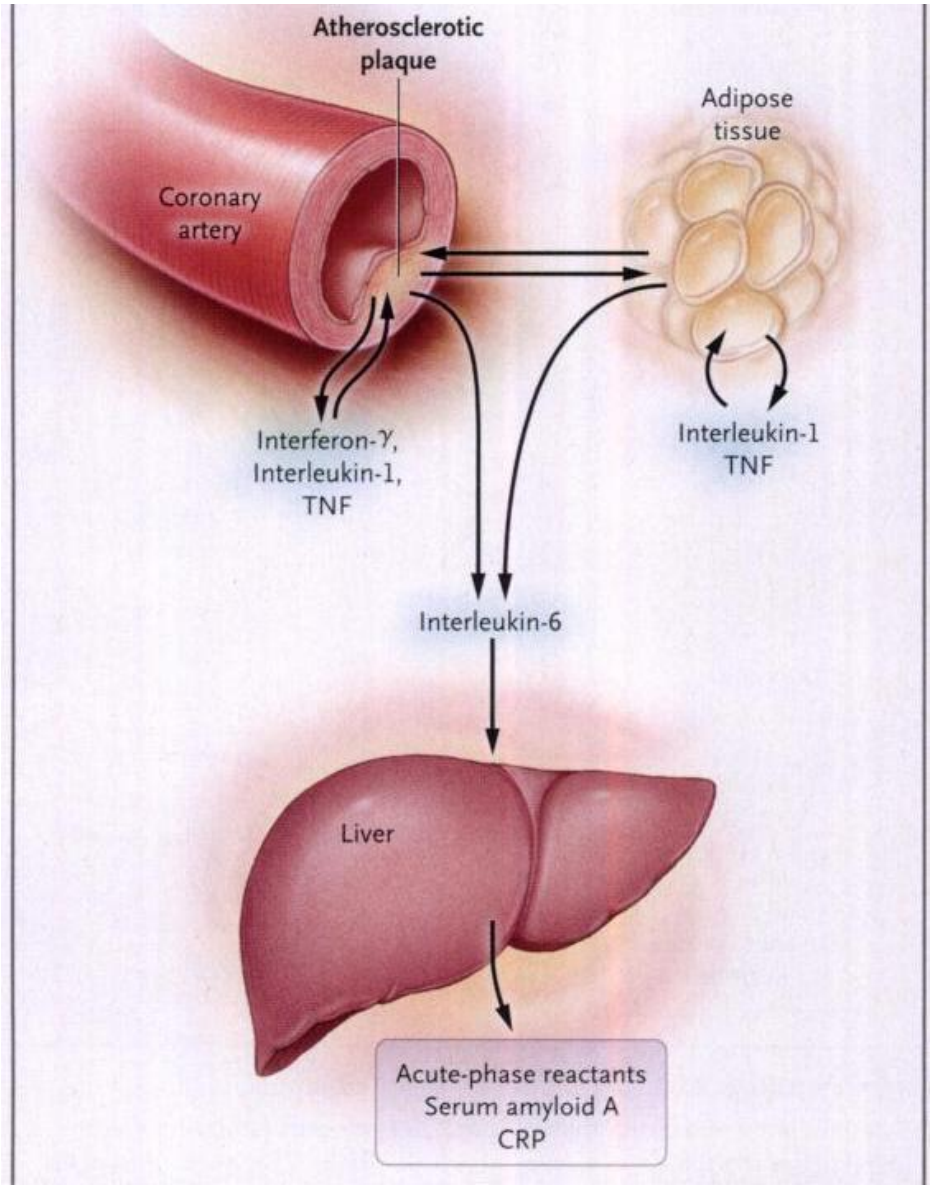


Figure 1: Cytokine cascade – Inflammatory cytokines are released from activated immune cells in the plaque i.e. interferon γ, interleukin-1 and tumour necrosis factor which induces the production of IL-6. IL-6 in turn stimulates the production of acute-phase reactants such as C-reactive protein, serum amyloid A and fibrinogen. (Image taken from Hansson, NEJM 2005 [14], reproduced with kind permission of NEJM)

1.2.1. Cytokine Receptors

The current knowledge on cytokine receptors is that they are transmembrane proteins. The cytokine binds to the extracellular domain and the intracellular domain can have an enzymatic activity, bind with other molecules or use a second messenger system. There are also proteins that exist which are structurally similar to cytokine receptors which are not membrane bound.There are seven families of cytokine receptors grouped either according to the structure of the cytokineor intracellular receptor, or by the type of signal messaging. But the majority of cytokines have different components of their extracellular cytokine receptors which can belong to more than one of the family of receptors.

- Group1

This group usually has more than one extracellular immunoglobulin domain. Although it mainly involves the receptors for IL-1α and ß there are also sequences for IL-6R, M-CSF and PDGF. The IL-1α has a higher affinity for the type I receptor with the IL-1ß binding more to the type II receptor (15). There is also a soluble IL-1 present and there is evidence suggesting that the type II receptor may play a role in binding with this soluble form and in a way affect the IL-1 activity (16). To support this, there is an IL-1 binding protein which matches the type II receptor binding site (17).

- Group 2

This group formerly known as the haematopoietic receptor family is the biggest group. These bind to cytokines with a four α-helical strand structure such as IL-2 to IL-7, G-CSF and GM-CSF.

- Group 3

These are the type I interferon receptors which binds to IFN-α and IFN-ß and the type II receptor binds to IFN-γ.

- Group 4

These are the nerve growth factor receptors which include the two TNF receptors for TNF-α and ß. Either receptor has equal affinity for both TNF-α and ß (18) but they appear to transmit different signals as the intracellular components differ.

Also present are two soluble TNF binding proteins which are present in the healthly population not only in the serum but also in the urine. Although the concentrations normally around 1-2 ng ml $^{-1}$ have been shown to vary from individual to individual they seem to be stable in individuals over time (19). Increased concentrations exist in various conditions like endotoxinaemia (20), systemic lupus erythematous (21), and in malignancies and infections (22;23).

1.2.2 Cytokine Signal Pathways

These pathways are inititated when the cytokine binds to its receptor. The most common pathway involves protein phosphorylation. Some receptors will have intra-cellular enzyme messenging through tyrosine kinase activity), whereas other will use G (GTP-binding) proteins.

The initial cytokine signal can be further amplified by second messenger systems such as by cyclic-AMP-phosphokinase A, regulated by phosphokinase C (24). Phospholipase make up the other second messengers. Phospholipase C produces inositol triphosphate (IP3) and diacylglycerol (DAG) which help regulate calcium within cells (25) and in activating protein kinase C (26).

Following stimulation by the cytokines there can be a rapid or delayed response over hours or days. Ultimately resulting in altering protein synthesis through gene transcription.

Signal transduction and gene transcription is currently being further researched as it presents the possibility of intervention and modulation.

1.2.3 Tumour necrosis factor

Tumour necrosis factor alpha (TNFα) is produced as a pro-hormone, this is then cleaved to give a 157 amino acid (27). Interaction with a specific receptor results in its biological response It is extremely potent requiring only 5% of its receptors to be occupied in order to produce a biochemical response (28). Tumour necrosis factor consists of two different polypeptides, TNF-α and TNF-β are different antigenically. It has a key role along with IL-1 in the initiation of inflammation and the immune response. But over production of TNF is potentially lethal to the host as seen in cachexia (29), autoimmune disorders (30) and meningococcal septicaemia (31). The TNF proteins are involved in a multitude of processes through its ability initiate many different signal transduction paths as well coding for cytokines and acute phase proteins (32).

As with the TNF there are also two TNF receptors, TNFR-I and TNFR-II. Either receptor shows the samw affinity for binding on to either TNF-α or TNF-β. Also present in the serum, are soluble TNF binding proteins acting as receptors which compete with the bound receptors (33).

Tumour necrosis factor is chemotactic for polymorphonuclear leucocytes (PMNL) and causes these cells to release oxygen-derived free radicals.

1.2.4 Interleukin-1

When activated either endothelial cells or macrophages can release interleukin-1 which has a short half-life of around 6 minutes. Similar to TNF IL-1 can be present in two forms, IL-1α and IL-1ß. Both with an equal affinity for their receptors. IL-1α produces its signal through cellular contact. In comparison IL-1ß is present in the circulation and produces similar physiological and metabolic effects to that of TNFα (15). Compared to TNF it has more inflammatory and immunoenhancing effects. It can stimulate myelopoesis directly or indirectly through myelopoetic growth factors such as GM-CSF. It is an endogenous pyrogen and is involved in the anorexic process (34).

As with TNF the main stimulus for its release is lipopolysaccharide (LPS) endotoxin antigen. Both TNF and IL-1 seem to enhance each other's effects when present together (34). Together they cause the release of prostaglandins and IL-6 from monocytes and endothelial cells. It also causes the release of tissue factor from monocytes and endothelium as well as down-regulating the expression of thrombomodulin and so the inflammatory process shifts the haemostatic balance from anticoagulation to coagulation (35). But unlike TNF it does not stimulates the degranulation of PMNLs to release oxygen free radicals.

In general IL-1α and IL-1ß binds to IL-1R type I and IL-1R type II respectively. Recent evidence shows that all the effect of IL-1 is due to the transducing signal from IL-R type I receptor. The function of IL-1R type remains unknown, but similar to TNF it may act to antagonise and regulate the activity of IL-1 (16;36).

1.2.5 Interleukin-6

The acute phase response involves TNF, IL-1 and IL-6 is a mediator of the acute phase. The chances of a subsequent MI following a previous episode increases with increasing baseline IL-6 concentrations (37). Interleukin-6 works by binding to a receptor complex which is made up of IL-6R (80 kDA protein) and gp-130 (130 kDA signal transducing glycoprotein) (38). Interleukin-6 binds to the receptor IL-6R with only low affinity but binds with high affinity in the presence of gp-130. Without IL-6R, IL-6 will not bind to gp-130 unless soluble IL-6 receptor (sIL-6R) is present. This is in contrast to the situation with TNF where the binding of TNF to soluble TNFR renders TNF inactive. The significance and regulation of soluble IL-6R is currently poorly understood but what is known is that increased serum concentration is seen in certain pathological conditions such as multiple myeloma, HIV infection and adult T cell leukaemia. The soluble IL-6 will enhance the actions of IL-6 (39) possibly due to soluble IL-6 binding with IL-6 and gp 130 without the need for a cytoplasmic region (40). This in contrast to most other receptors which require their own cytoplasmic regions for signals.

As well as its inflammatory role it also has a role to play in host defence, immune response and haematopoiesis. Chronic activation is seen in rheumatoid arthritis.

Following surgical trauma IL-6 levels begin to rise and reach significant levels at around 2-4 hours. The cytokine production levels reflect the degree of surgical trauma and so the largest responses are seen in joint replacement surgery, colorectal and major vascular surgery (41). In terms of total knee arthroplasty bilateral simultaneous replacements exhibit a significantly larger IL6 response (42). The levels will reach their

maximal point at around 24 hours, remaining elevated for up to 48 – 72 hours (43), so therefore secretion is a brief and self-limiting event.

There may be some variability between individuals in their response to an inflammatory stimulus, as the increases in IL-6 and CRP seen in those following coronary angioplasty or uncomplicated cardiac catheterisation correlate linearly with baseline and CRP and IL-6 levels (44). This variability in the degree of response may also have a genetic basis (45). In terms of the response to surgical trauma there has been some correlation found between the duration of surgery and the magnitude of the IL-6 response (41).

Current evidence now suggests that elevated IL-6 and CRP levels carry not only more risk of subsequent development of atherosclerosis but also the risk of developing type II diabetes, even in those with no current evidence of insulin resistance (46).

1.2.6 Interferon-γ

Interferon-γ (IFN-γ) is produced from human T helper lymphocytes as well as from natural killer (NK) cells. It levels become detectable at around six hours and remain so for eight days. Tissues which have undergone surgical trauma such as operative wounds also result in IFN- γ production up to 5 to 7 day afterwards (47).

1.2.7 Macrophage / Granulocyte Colony Stimulating Factor

M/G CSF is involved in stimulating leucocytes in the inflammatory response and can also be effective in the wound healing process. Patients undergoing oncologic procedures who have been given macrophage colony stimulating factor (M-CSF) peri-operatively and in those with major burns have shown increased neutrophil activity.

1.3 Acute Phase Response

Following surgical trauma there is an 'acute phase response' which results in the release of certain cytokines. As part of this response the liver produces and releases acute phase proteins (Table 3) which have a role in the mediation of the inflammatory response and in tissue repair. The increased levels of C-reactive protein (CRP) follows that of IL-6. C reactive protein allows for the phagocytosis of bacteria, $alpha_2$ macroglobulin and anti-proteinases. There are many proteins involved. Some are useful markers of the size of an inflammatory process.

Following surgical trauma the release of cytokines (proportional to the degree of surgical trauma) may further increase the release of ACTH from the pituitary and so further increase the cortisol levels. This does not occur as the initial release of cortisol following surgical trauma is enough to depress the IL-6 levles (8).

As the response seen in the cytokines is in direct relation to the severity of the local tissue trauma the type of anaesthetic will have no effect on this response. However, the use of regional anaesthesia can reduce the response to surgery. As seen in pelvic and lower limb surgery, an epidural block along with local anaesthetics will stop the metabolic and endocrine response usually seen following surgery. It has been demonstrated that a T4 to S5 blockade will prevent the increase in cortisol and therefore the increase in glucose following hysterectomies (48).

Table 1.2: Features of the acute phase response

Features of the acute phase response
Fever
Granulocytosis
Production of acute phase proteins in liver CRP Fibrinogen α_2-macroglobulin
Concentration changes in transport proteins Increased ceruloplasmin Decreased transferring, albumin and α_2-macroglobulin
Concentration changes of divalent cations Copper increases Zinc and iron decreases

This is due to the blocking of the signals from the site of trauma to the central nervous system as well as to the hypothalamus as well as the efferent signals to the liver and adrenal medulla. Therefore abolishing the signals to the adrenocortex and the glycaemic responses to surgery. But in the presence of a less extensive blockade this will not occur. A recent animal study has shown that even the use of local lignocaine can attenuate the local acute phase response (49)

In cases of severe polytrauma a more severe response may occur. This systemic inflammatory response syndrome was defined by the American College of Chest Physicians/Society of Critical Care Medicine (ACCP/SCCM) as the 'Systemic Inflammatory Response Syndrome' (SIRS) in 1991 (50). For the diagnosis of SIRS to be made it was agreed that at least two out of the four clinical markers must be present (Table 1.3).

In orthopaedic surgery the surgical stress response to an orthopaedic procedure has been sub-divided into either a primary or a secondary stress response (51). The primary stress response is that which is seen following any elective orthopaedic operation. On the other hand if the patient has already undergone a traumatic event such as a hip fracture following a fall then an emergency operation to deal with the hip fracture would induce a secondary stress response.

Table 1.3: Clinical parameters in SIRS

Clinical Parameters	Values
Heart rate	>90 / min
Breathing rate	>20 / min
Temperature	>38°C or <36°C
Number of leucocytes	>12,000 / mm^3 or <4000 / mm^3

This has been termed the ‘second hit phenomenon’ (52), the ‘first hit phenomenon’ (53;54) being the initial response induced from the hip fracture. The second hit phenomenon has been demonstrated in a paper by Giannoudis et al which reported significant increases in the IL-6 and elastase levels following intramedullary nailing of femoral fractures (55). As mentioned previously the degree of surgical trauma is related to the size of the stress response, but there has also been evidence to suggest that there are potential gender differences in the response as reported by Ono et al. (56) who reported higher levels of TNF-α and suppressive interferon production in men following gastrointestinal surgery. It was concluded that men were at a higher risk of developing SIRS and post-operative infectious complications. These gender variations in stress response have also been supported by other authors (57;58).

The inflammatory mediators have an important role to play in the acute phase response but research over the last few decades has shown a role that inflammation plays in the initial formation and progression of atherosclerosis and in venous thromboembolism.

1.4 Circulating markers of Atherosclerosis, Thrombosis and Inflammation

In the 1970’s atherosclerosis was seen as a lipid storage disease with the lipid deposited in the arteries which eventually builds up eventually causing a blockage of the artery resulting in a myocardial infarction (MI) or a stroke. But recent research has shone some light on the process and development of atherosclerosis. Inflammation appears to be key here and the development of these plaques occur within and not on the arterial walls as previously thought.

Atherosclerosis has been shown to develop slowly over decades starting as the ‘initial fatty streak’ and slowly becoming the complex plaques causing potentially cardiovascular events.

The innermost surfaces of arterial walls are lined by endothelial cells (EC), normally these will resist the adhesion of leucocytes. But this all changes when the endothelial cells express adhesion molecules in response to certain factors such as high saturated fat diet, smoking, high blood pressure, hyperglycaemia and insulin resistnace or obesity. The vascular cell adhesion molecule-1 (VCAM-1) is an example of an adhesion molecule which allows the adhesion of both monocytes and T-lymphocytes, both of which are present in the early developping plaque (Figure 1.2).

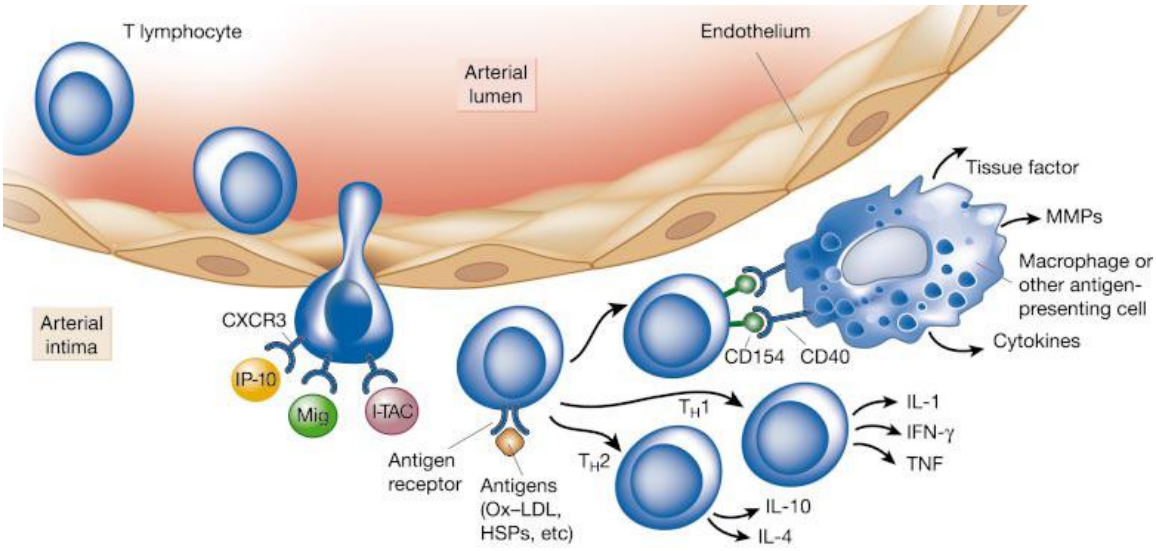


Figure 1.2: Role of T-lymphocytes in atherogenesis (from Libby, Inflammation in atherosclerosis, Nature 2002 [70], produced with kind permission from Nature)

Under normal conditions the central lipid core of these plaques are protected from the bloo contact by a fibrous cap but when this cap fractures it causes the majority of the acute coronary syndromes. This usually results in the formation of a thrombus which will cause an increase in the size of the plaque. The biomechanical strength and resilience of the cap is due to the interstitial collagen and what has been shown is that in those plaques that have ruptured the collagen cap has been thinner than expected (59-61). The smooth muscle cells of the arterial wall produce collagen in response to stimulatory factors such as IL-1, platelet-derived growth factor and transforming growth factor-β . But the T lymphocytes which are present in the plaque has been shown to produce interferon-y which not only inhibits the basal collagen formation but also the inhibits the stimulatory effect on the smooth muscle cells to produce collagen (62). As well as this CD40 ligand and IL-1 is also released from the T-lymphocytes causing the release of enzymes responsible for collagen degradation (63).

1.4.1 Vascular Cell Adhesion Molecule-1 and MCP-1

Mice studies have shown that there is a reduction of lesion formation in those with a poorly functioning VCAM-1 compared with normal VCAM-1 expression (64). The expression of VCAM-1 is in response to the presence of oxidized lipids mediated by cytokines such as nuclear factor-kβ, interleukin-1β and tumour necrosis factor-α.

Laminar blood offers protection against the lesion formation due to the shear stresses generated which causes the release of nitric oxide which reduces the expression of VCAM-1 through the inhibition of nuclear factor-α and platelet clumping (65) (66).

Therfore areas which lack this laminar blood flow are susceptible to lesion formation. This has been supported by studies which showed that endothelial cells which lack this laminar blood flow have increased expression of nuclear factor-κß (67).

Once adhered, the monocytes enter between the endothelial cells through diapedesis (figure 1.3). The main chemotaxis for this comes from the monocyte chemotactic protein-1 (MCP-1). Without the expression of MCP-1 there is up to 83% less lipid deposit in the vasculature of mice and less leson development compared to MCP-1 producing mice on the same high fat diet (68) (69). Once within intima, macrophages are formed from matured monocytes. These macropages engulf the lipoproteins and become foam cells and as there numbers increase they release further cytokines and growth factors perpetuating the cycle. The macrophage colony-stimulating factor (M-CSF) is the main mediator of this process confirmed by its over expression in experimental and human atherosclerotic plaques (70). Although other cytokines and growth factors are involved in the initiation of this atherosclerotic lesion it is the VCAM-1, MCP-1 and M-CSF that are the key mediators (figure 1.3).

1.4.2 CD40 ligand (CD154)

CD40 ligand (CD154) is another proinflammatory cytokine which contributes to atherogenesis, expressed by leucocytic as well as non-leucocytic cells such as platelets. CD154 is stored pre-synthesised in platelets and released in vitro in seconds after activation and in the formation of thrombus (71). CD154 interacts with CD40.

Interrupting the CD154 signalling pathway will slow the initiation of atherosclerosis (72). Following this further research was carried out to see if interruption of this pathway affected the atherosclerotic progression after the lesion had already been established. It seems that in preventing CD154 signalling the formation as well as the evolution of established plaques is prevented (73).

Also a recent study by Ray et al (74) pointed towards an association of CD40 ligand and the thrombotic cascade but did go as far as to state there was a relationship indicating possible further studies to investigate this.

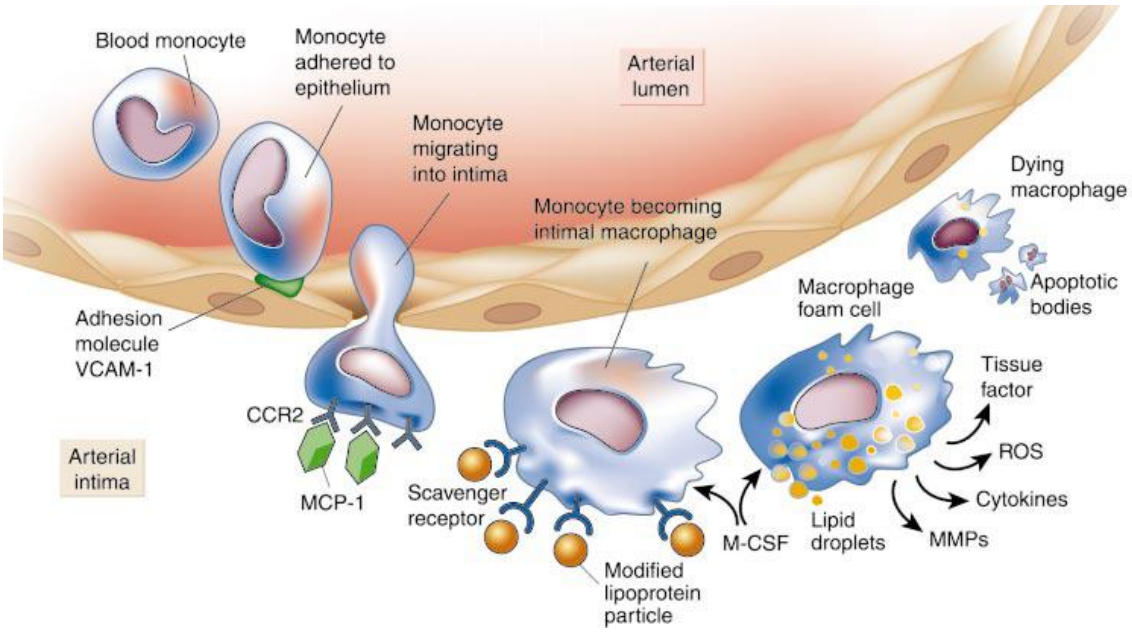


Figure 1.3: Steps in the recruitment of mononuclear phagocytes to the nascent atherosclerotic plaque and some of the functions of these cells in the mature atheroma (from Libby Nature 2002 [75], produced with kind permission from Nature).

1.4.3 Soluble CD40 Ligand

In contrast to the membrane bound CD40L, soluble CD40L (sCD40L) is released mainly from platelets (76). The levels of sCD40L correlate with platelet activation and can identify those at risk of having recurrent ischaemic event (77;78) in patients with known ischaemic heart disease but is not a useful tool in low risk subjects. (79)

The current value of sCD40L as a marker is unknown and further research is needed to evaluate whether sCD40L is of value in the prediction of future cardiac events in a healthy population. It may be more useful as an indication of plaque stability and some pilot studies have shown that sCD40L is more closely associated with plaque composition than volume (80).

There is conflicting evidence as to whether soluble CD40 ligand correlates to CD40L with some suggesting a positive correlation (81) and others a negative (82). Varo et al. (83) highlighted variables that may significantly affect this correlation, these included the time between collection and analysis, centrifugation and the storage temperatures. He stated that this could be avoided through a standardised approach.

1.4.4 CD40

CD40 regulates a wide variety of biological functions, extending from cellular immunity to inflammation, two processes intimately involved in atherogenesis. As such, the endothelium in vessels within inflamed tissue show an increased CD40 expression (84). CD40 signalling can promote the expression of proatherogenic mediators such as certain MMPs, caspase-1, and procoagulant activity.

1.4.5 CD14

In the First Leukocyte Typing Conference held in 1982 a set of monoclonal antinbodies were identified and collectively named unde the title of CD14. These bound only to monocytes and macrophages and so could be used to highlight cells which were of monocytes or macrophage lineage.

CD14 is a 55kDa membrane glycoprotein, consisting of 356 amino acids and a 19 amino acid long N-terminal leader peptide. It is as a receptor for the endotoxin lipopolysaccharide (LPS) (85) which is found in gram-negative bacteria with only small amounts resulting in a rapid immune response. LPS in plasma is bound to LPS binding protein (LBP) which mediates the binding of CD14 to LPS. This results in the production of inflammatory cytokines such as TGFß. CD14 also interacts with other bacteria and fungi, demonstrating its role in innate immunity (86).

The synthesis and expression of CD14 in monocytes has several mediators such as IL-4 and IL-3 which will reduce the expression of CD14 at the transcriptional level in 24-48hrs (87-89) whereas IFNα, IFNγ and TGFß (90) increases CD14 expression.

Recent in vitro experiments suggests that CD14 has other biological functions such as its monocyte-endothelial cell interactions (91). The more mature subpopulation of monocytes, the CD14+/CD16+, have increased numbers in infectious diseases such as sepsis, tuberculosis, the critically ill and in uraemic patients without infections (92-94). It has also been suggested that they have a role in the induction of the inflammatory process involved in atherosclerosis (95). Increased levels are also seen in patients with with coronary artery disease (96). Schlitt et al demonstrated in their study that patients with the highest percentages (upper quartile) of CD14+/CD16+ monocytes had an odds ratio of 4.7 for coronary artery disease after confounding factors such as diabetes, hypertension and lipid profile were considered. Highlighting it as an independent risk factor for coronary artery disease.

1.4.6 CD42a

CD42a belongs to the mucin family and is a small membrane glycoprotein found on the surface of platelets. It combines with CD42b, CD42c and CD42d to form the CD42 complex which acts as a receptor for von Willebrand factor and as a vWF-dependant adhesion receptor and so mediates the adhesion of platelets at high shear rates to vWF in the subendothelial matrices which are exposed when the endothelium is damaged. It also amplifies the response of platelets to thrombin during platelet activation.

1.4.7 CD14/CD42a dyad (platelet-monocyte aggregate)

Current evidence suggests that patients who have undergone an acute coronary syndrome have both increased interactions between platelets as well between platelets and leucocytes, with the later forming in inflammatory states (97). Platelets adhere to circulating leucocytes following activation and degranulation. The P-selectin on the surface of the platelets binds to the P-selectin glycoprotein ligand-1 (PSGL-1) on leucocytes (98). This in turn causes a further expression of CD11b/CD18 on leucocytes enhancing the interaction with platelets (99). A recent study has supported the importance of platelet-leucocyte interaction in vascular disease were human PSGL-1 reduced the degree of reperfusion myocardial damage and endothelial dysfunction in an animal model (100).

There is growing evidence demonstrating the importance of platelet-leucocyte aggregation in atherothrombosis and acute coronary syndromes. Sarma et al (101) demonstrated that higher levels of platelet-monocyte were found those with an acute coronary syndrome and that this was drastically reduced when the PSGL-1 and P-selectin receptors were blocked. Therefore adding further evidence that platelet monocyte binding occurred through PSGL-1 and P-selectin (101). This has been supported by other studies (102). The exact role of platelet-monocyte aggregates in acute coronary syndrome is unknown, whether they are a reflection of the inflammatory and thrombotic process or a directly contribute to the ongoing atherothrombogenesis.

1.4.8 C-reactive protein

Out of the acute phase proteins C-reactive protein was the first one described and is very sensitive in reflecting the ongoing inflammation and tissue damage (103). Plasma CRP is released from liver hepatocytes in response to IL-6produced predominantly from hepatocytes mainly under transcriptional control by IL-6. The CRP levels show no diurnal variation and are unaffected by eating.

The median concentration of CRP in young healthy adults is 0.8 mg/l ranging from 3.0 mg/l (90^{th} centile) to 10 mg/l (99^{th} centile) (104). However, following an acute stimulus the concentration levels may increase by 10,000 fold. Following stimulation CRP synthesis and secretion starts rapidly with levels reaching greater than 5 mg/l by 6 hours and eventually peaking at 48 hours. It's half-life (around 19 hours) remains constant under all conditions and so its rate of synthesis will determine its circulating concentration (105) and is a direct measure of the intensity of the pathological stimulus. When the stimulus is removed the levels of CRP fall almost at the rate of plasma CRP clearance.

C-reactive protein belongs to the pentraxin family of calcium-dependant ligand-binding plasma proteins along with serum amyloid P component (SAP). The pentraxin family was named as such due to it electron micrographic appearance from the Greek *penta ragos* (five berries). It is made up of 5 identical nonglycosylated polypeptide subunits, each subunit with 206 amino acids arranged in a cylic pentameric symmetry (figure 1.4) (106).

C-reactive protein (CRP) has been shown to have a strong association with with the risk cardiovascular disease in healthy people. Although the main source is from the liver, complements proteins and arteries can produce CRP. The presence of C-reactive protein on its own may stimulate the releas of IL-1ß, IL-6 and TNF-α from monocytes (107) and causes the expression of ICAM-1 and VCAM-1. Ridker et al in their study demonstrated a lower risk of recurrent myocardial infarction (MI) and death from cardiovascular cause in those on statin therapy with a lower CRP (less than 2mg/L) (108). High-sensitivity assays have allowed studies to demonstrate a relationship between increased CRP levels and risk of stroke and progression of peripheral arterial disease (109-112). A meta-analysis of all published studies in 2000, totalling 1,953 coronary events demonstrated that those with base values of CRP in the upper third had a relative risk of 2.0 for a future coronary event (112).

However a more recent meta-analysis by Danesh et al in 2004 (113) gave a slightly different result. This included 22 prospective studies with a total of 7068 patient with a weighted mean follow-up 12 years. But they found that studies published before 2000 tended to have more extreme conclusion and made the decision to limit their analysis to four studies with over 500 patients in each (a total of 4107 patients). This gave an overall odds ratio of 1.49 (93% CI of 1.37- 1.62) compared to that of 1.58 (95% CI 1.48 – 1.68) when all 22 studies were included. The authors concluded that the levels of C-reactive protein was only a moderate predictor of the future risk of coronary heart disease in the presence of already well established risk factors.

The Lancet published its results of a meta-analysis from the Emerging Risk Factors Collaboration in 2010 with over 160 000 individuals with no past medical history of vascular disease (114). They concluded that the CRP concentration had a continuous

association with the risk of heart disease, stroke and vascular mortality because it was similarly associated with non-vascular mortality but its relevance in pathogenesis was unclear. They also noted that the association with ischaemic vascular disease depended significantly on the conventional risk factors as well as other markers of inflammation.

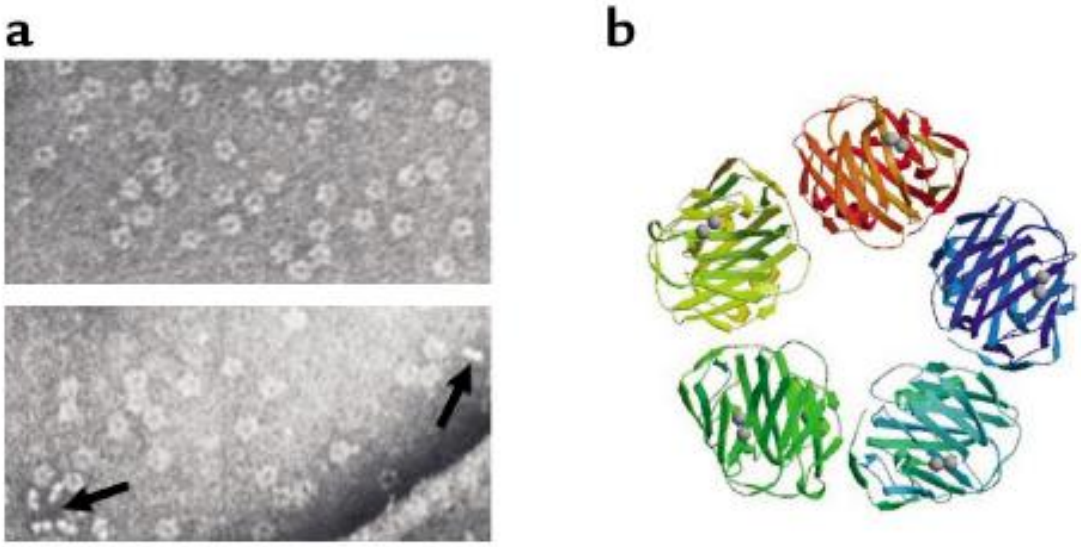


Figure 1.4: A – Electron micrograph showing pentameric disc-like structure face-on and side-on (arrows). B – Ribbon diagram showing the lectin fold and the 2 calcium atoms (spheres) in the ligand-binding site of each protomer (from Pepys et al, J Clin Invest 2003 [103], reproduced with kind permission from J Clin Invest).

1.4.9 Tumour necrosis factor alpha

Tumour necrosis factor alpha (TNFα) is a trimeric protein it was first discovered as a 17kd secreted form but later a noncleaved 27kd precursor form was found in a transmembrane form (115). The 27kd TNFα form is secreted from activated macrophages but it can also be produced from other cell types it then binds to tumour necrosis factor receptors 55 and 75 through cellular contact or bind following cleavage to its soluble form. TNFα has to ability to transform from a hydrophobic to hydrophilic form allowing it cross cell membranes as well as forming ion channels. It has been speculated that this is due to its structural resemblance to that of viral coat proteins (116).

Tumour necrosis factor alpha is a pleiotropic cytokine. It appears to influence the majority of the organs in the body and has a wide spectrum of functions many of which are not understood. It can both stimulate and inhibit growth as well as self regulatory properties. An example of this is that TNFα can cause neutrophil proliferation as well as apoptosis during inflammation and binding to the TNF-R55 receptor respectively (117).

It has beneficial functions such as its role in the necrosis of certain tumours as well as the role it plays in the immune response to infection from either bacterium, viruses or parasitic causes. Tumour necrosis factor has a key role to play in the inflammatory response locally as well as being one of the acute phase proteins. Without TNFα, mice i with gram negative bacteria undergo septicaemic shock (118).

1.4.10 E-selectin

This was originally known as the endothelial leukocyte adhesion molecule (ELAM) because of its localisation to the endothelium and its role in binding leucocytes to the endothelium. However when two other similar adhesive glyoproteins were identified, (the homing receptor and granule membrane protein of molecular weight 140kD) they were renamed under the nomenclature of selectins, with ELAM being renamed as E-selectin (119), the homing receptor named L selectin and the granule membrane protein of molecular weight 140kD was named the P selectin.

All three selectins are either expressed on the surface of platelets (P selectin), vascular endothelial cells (E and P selectin) and leucocytes (L selectin) and function in binding to ligands to promote intercellular adhesion. E-selectin is thought to be expressed only on activated endothelial cells and its expression is induced by cytokines (120).

In addition to mediating leucocyte rolling it is also involved in the conversion from rolling to adhesion. This has been shown by a reduction in adherent leucocytes in mice that are E-selectin deficient (121) (122). Under baseline conditions, E-selectin is expressed by the microvessls of the skin (123) and evidence suggests that it plays an important role in skin inflammation through its role in the recruitment of T lymphocytes specific to the skin (124). The three-dimensional structure of E-selectin (Figure 1.5) was determined using x-ray crystallographic methods (125).

1.4.11 Tissue plasminogen activator

In normal healthy endothelium, thrombus formation is inhibited by several mechanisms. There are the endothelium-derived inhibitors of coagulation such as thrombomodulin, heparin sulphate, protein S, proteoglycans and tissue factor inhibitor, as well as prostacyclin and nitric oxide (NO) which prevent the aggregration of platelets. But following injury or inflammation there is a downregulation of these mechanisms and endothelium becomes procoagulant with tissue factor expression and the realease of fibronectin, vWF and platelet activating factor (126).

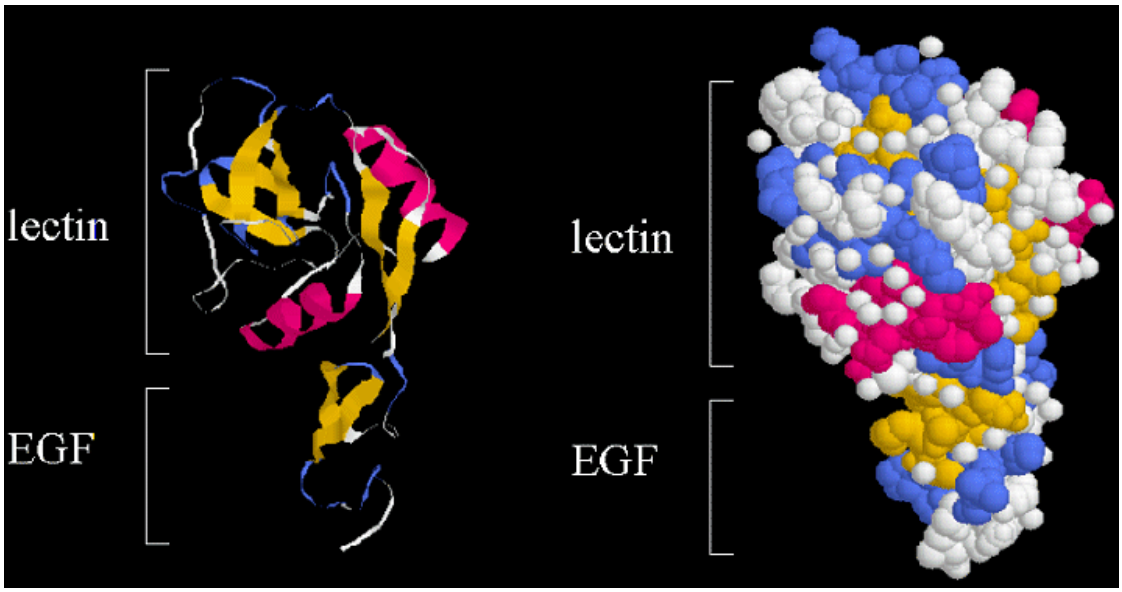


Figure 1.5: 3D image of E-selectin (reproduced with kind permission from the University of Virginia Biomedical engineering website).

After thrombus formation has begun tissue plasminogen activator (t-PA) is released mainly in response to factor Xa and to thrombin (127). T-PA causes the breakdown of the thrombus to soluble fibrin byproducts through converting plasminogen to plasmin which is accelerated by the presence of fibrin at the endothelial cell surface, (128;129) producing localised activation. It is the t-PA release which determines the onset and efficacy of fibrinolysis as plasminogen is present in vast quantities compared to t-PA.

In human plasma the concentration of t-PA is around 3 to 10 ng/ml. But serine protease inhibitors (serpins); mainly plasminogen activator inhibitor (PAI) type 1(PAI-1) as well as PAI-2, PAI-3, ensure that only a small amount is activeproportion is functionally active (130). The half-life of t-PA is around 5 minutes and is cleared by the liver (131). The percentage of active t-PA varies from 2% to 33% (132).

Tissue plasminogen activator is a 68 kDa serine protease consisting of 530 amino acids. Endothelial cells in culture synthesise and secrete t-PA (133). The presence of histamine and thrombin will stimulate its synthesis (134) whereas plasmin will inhibit it (135). Protein kinase C may have a role (136) but the exact mechanism is still unclear.

It is believed by some authors that t-PA and vWF is stored together in Weibel-Palade bodies (137), but strong evidence suggests that t-PA is not stored in the Weibel-Palade bodies (138;139), supported by the observation that the stimulation of the release of t-PA does not cause a simultaneous release of vWF (140;141). The exact signalling mechanism is unclear but G proteins and elevated intra-cellular calcium concentrations seem to have a role (139;142).

The release of t-PA also varies with the anatomic region,with more being released from the upper limbs than the lower limbs (143). The endothelium has a substantial capacity to store and release t-PA and continuous release is possible for several hours without significant tachyphylaxis (144;145).

The main cause of death in acute coronary syndromes is due to the thrombosis that occurs following the rupture of an atheromatous plaque (146). Although comoonly present they are not usually of clinical significance (147). But if there is a disturbance in the equilibrium of the fibrinolytic system then the thrombus formation may continue leading to arterial occlusion (126). A reduction in the release or activity of t-PA in those with stable or unstable angina, has been linked to an increased risk of cardiac events (148) (149).

In 2004, Lowe et al conducted a meta-analysis of twelve relevant prospective studies on t-Pa including their own study cohort, totalling 2119 cases of either a fatal or a non-fatal myocardial infarction and 8832 controls (150). Their combined analysis of the seven studies on the general population (1669 coronary heart disease cases and 5635 controls) yielded an odds ratio 2.18 (1.77-2.69) in those in the top third against those in the bottom third baseline t-Pa values. But this dropped to 1.47 (1.19-1.81) when adjusted for age, sex and vascular risk factors. A similar process carried out on the remaining six studies (based on previous vascular disease), with 450 coronary heart disease cases and 3197 controls revealed an adjusted odds ratio of 1.32 (0.70-2.50). However the authors state that despite the association shown further research was needed to evaluate how independent this was from established risk factors.

1.4.12 Von Willebrand factor

Von Willebrand factor (vWF) is present in the plasma, platelets and endothelium and is synthesized from endiothelial cells as well as from megakaryocytes. vWF is assembled as high molecular weight multimers which are then cleaved by ADAMTS13, a metalloproteinase at specific sites in the vWF molecule to smaller units. It initiates both platelets adhesion and aggregation and so has an important role to play in the formation of thrombus (151). Following damage to the vascular endothelium vWF binds to the subendothelium exposed through injury, mainly collagen types I and III, triggering platelets adhesion to the vessel wall (151).

In a meta-analysis by Whincup et al which included their own study (152) they reviewed a total of six studies on von Willebrand factor. This gave a total of 1524 coronary heart disease cases and 19,830 controls giving a combined odds ratio of 1.5 (95% CI 1.1-2.0). They concluded that an association existed between vWF levels and the risk of coronary heart disease in the future but further research was needed to assess whether this was causal or not.

1.5 The acute phase response, venous thromboembolism and arterial thrombosis

From its first inception venous thromboembolism (VTE) was based upon Virchow's triad of stasis, changes in the vessel wall as well as thrombogenic changes. But since the

1970's, there has been increasing evidence to suggest a link between VTE and inflammation.

Under normal conditions the endothelium resists platelet adhesion, coagulation as well as inflammation and leucocyte activation. It manages this through:

- the thrombomodulin production from the endothelium and protein C activation, which prevents excessive thrombin formation.

- the expression of heparin and dermatan sulphate stimulating anti thrombin and heparin cofactor activity, again preventing excess thrombin formation.

- tissue factor pathway inhibitor expression (TFPI) also prevents excess thrombin formation.

- tissue plasminogen activator (tPA) and urokinase-type plasminogen activator production locally, which stimulates fibrinolysis.

- secretion of nitrous oxide, prostacyclin and interleukin 10 which inhibit the adhesion of leukocytes and platelets as well as producing vasodilatation (153).

This is in contrast to when there is an endothelial disturbance, either physical from vascular trauma or functional as in sepsis. Here the endothelium supports a prothrombotic and proinflammatory state of vasoconstriction (153). This results in vessel vasoconstriction from the release of platelet activating factor (PAF) and endothelium-1 (154). Von Willebrand factor (vWF), tissue factor (TF), plasminogen activator inhibitor 1 (PAI-1) and coagulation factor V is also released promoting thrombosis (153). The endothelial cells are also stimulated to express P-selectin and E-selectin.

There is emerging evidence from epidemiological studies linking VTE, arterial thromboembolism and atherosclerosis. The largest one of these to date by Sorensen et al (2007) (155) demonstrated that patients with a deep vein thrombosis also had an increased risk of suffering a MI (1.6) and stroke (2.6) compared to that of the control group population. Those with a pulmonary embolism had a higher (2.6) risk of MI and for stroke (2.93). The activation of inflammation and haemostasis also has a role to play in atherosclerosis progression, plaque rupture and arterial thrombosis (156-158).

Following lower limb arthroplasty in orthopaedics, myocardial infarction is the most common cause of death post-operatively (159), Parvizi et al examined the records of over 30 000 patients who had undergone a total hip arthroplasty at their institution over a 30 year time period looking specifically at those patients who had died within 30 days of their index operation. Of the 90 who had died 21 (23%) of these were due to a myocardial infarction, 14 (15%) due to a fatal pulmonary embolism and only 1 (0.01%) patient died from a stroke. The highest incidence of deep vein thrombosis has been shown to occur in those following elective total hip arthroplasty (160;161). But in Mantilla's paper (162) the results are subdivided into the incidence of myocardial infarction, pulmonary emboli and deep vein thrombosis following total hip and knee replacement and also bilateral knee replacement. His results show that the incidence of myocardial infarction is comparable between the total hip replacement and knee replacement group (05% and 0.3% respectively) but is highest in the bilateral knee group (0.9%). Winemaker et al also confirmed that simultaneous bilateral total knee replacements carried significantly higher risks post-operatively (42). A similar picture is seen in the incidence of deep vein thrombosis (hip 1.3%, knee 1.5%, bilateral knee 2.2%) and the pulmonary emboli rates (hip 0.6%, knee 0.5%, bilateral knee 1.6%).

Liel et al demonstrated from the Norwegian hip registry with over 67000 total hip arthroplasties that the 60 day post-operative mortality was 0.79%, with vascular diseases being the main cause of death in the early post-operative period (163). Several years later Lie et al performed a similar study but looked at the risks of mortality after both total hip and knee arthroplasty (164) but this time he combined the numbers from the National Joint Replacement Registry of the Australian Orthopaedic Association as well as the Norwegian Arthroplasty Register. The study included 81 856 patients with a total knee replacement and 106 254 patients with a total hip replacement. He demonstrated that in the first 26 days the mortality was increased at 0.187% (95% CI, 0.168% to 0.206%), and that mortality was significantly higher in men and for those between their age group band of 71 to 81. They showed no significant difference in mortality between hip and knee arthroplasties.

The Mayo Clinic (162;165) published their own results after looking at their own database of over 10 000 patients over a 10 year period. Their numbers included 5 233 total hip replacements, 3 601 total knee replacements and 1 410 patients who underwent simultaneous bilateral knee replacements. From this, 224 patients were identified as having an adverse event within 30 days their surgery, which accounted for 2.2%. Of this 0.4% had had a myocardial infarction, 0.7% a pulmonary embolism, 1.5% a deep venous thrombosis and 0.5% died. They also concurred with the Lie et al paper (164) showing that myocardial infarction was more significant in the older age group and in males.

The possible link between venous thrombo-embolism and atherosclerosis was first highlighted in 2003 (158). Schulman and his associates followed up a large cohort of patients for up to 10 years following an acute VTE and found that a significant

proportion had died from either a myocardial infarction or stroke, compared to that of the general population (166). Hong et al (167) investigated the possible link between venous thrombo-embolism and coronary atherosclerotic disease. This was done by measuring the degree of calcification within the coronary arteries on computerised tomography angiographic images. They demonstrated that the degree of calcium was associated with VTE with and odds ratio of 4.3 (95% CI, 1.9-10.1). On a cohort of 23,796 autopsies performed in an urban Swedish population, Eliasson et al (168) demonstrated an increased incidence of venous thrombo-embolism in patients with arterial thrombosis (odds ratio of 1.4 adjusted for age and sex; 95% CI 1.3-1.5). Therefore the evidence presented suggests a possible link between VTE and atherosclerosis but the exact nature of this association is not clear.

Atherosclerosis can potentially promote the progression of thrombotic disorders (figure 1.6). The atherosclerotic process involves platelet activation and blood coagulation. There is also an increased turnover of fibrin which could lead to thrombotic complications. They may share similar mechanisms and or risk factors (figure 1.6). There are several medical conditions which can give rise to both arterial and venous thromboembolic disorders such as side effects of chemotherapy (169) and infection from Chlamydia pneumoniae (170) and HIV infection (171).

Studies have shown an elevation of circulatory markers of activation of inflammation, endothelium and coagulation in those who have or who are at risk of developing coronary artery disease or stroke. Fibrinogen, vWF antigen, t-PA, D-dimer and factor VII are all elevated in those who develop ischaemic coronary artery disease (172-177).

Lowe in 2006 (178) published a comprehensive review article looking at the above associations and stated that these associations should not imply causation and further work was needed to ascertain whether these associations were in his words 'cause, consequence or coincidence'. From prospective case-control studies such as the European Concerted Action on Thrombosis deep vein thrombosis (ECAT-DVT) (179) showed no association with fibrinogen or factor VII, whereas the Longitudinal Investigation of Thromboembolism Etiology Study (LITE) (180) of clinical VTE showed associations with factor VII (above 95th centile), vWF but not fibrinogen. It is possible that the associations with fibrinogen and vWF are due to the consequences of previous thrombosis but this does not explain the associations with fibrinogen. On the other hand D-dimer has been linked with the risk of VTE both in case-control (181;182) and prospective studies (179;183). Whereas t-PA has no association with VTE either in case-control (184) or prospective studies (179;185).

Currently the exact role of fibrinogen in arterial thrombosis is uncertain. Although the meta-analysis (Fibrinogen Studies Collaboration 2005) (175) showed an association with coronary heart disease and stroke this was reduced once adjustment was made for potential confounding factors such as obesity and alcohol. This association would more than likely be further reduced if adjusted for socio-economic status as has been shown in other studies (186). The role of factor VII is not certain as studies have produced inconsistent results (172;187-191).

D-dimer levels have an association with coronary heart disease shown both by meta-analysis (192) and from epidemiology studies (189;191). Whereas t-PA has only a moderate association with coronary heart disease risk from meta-analysis (182).

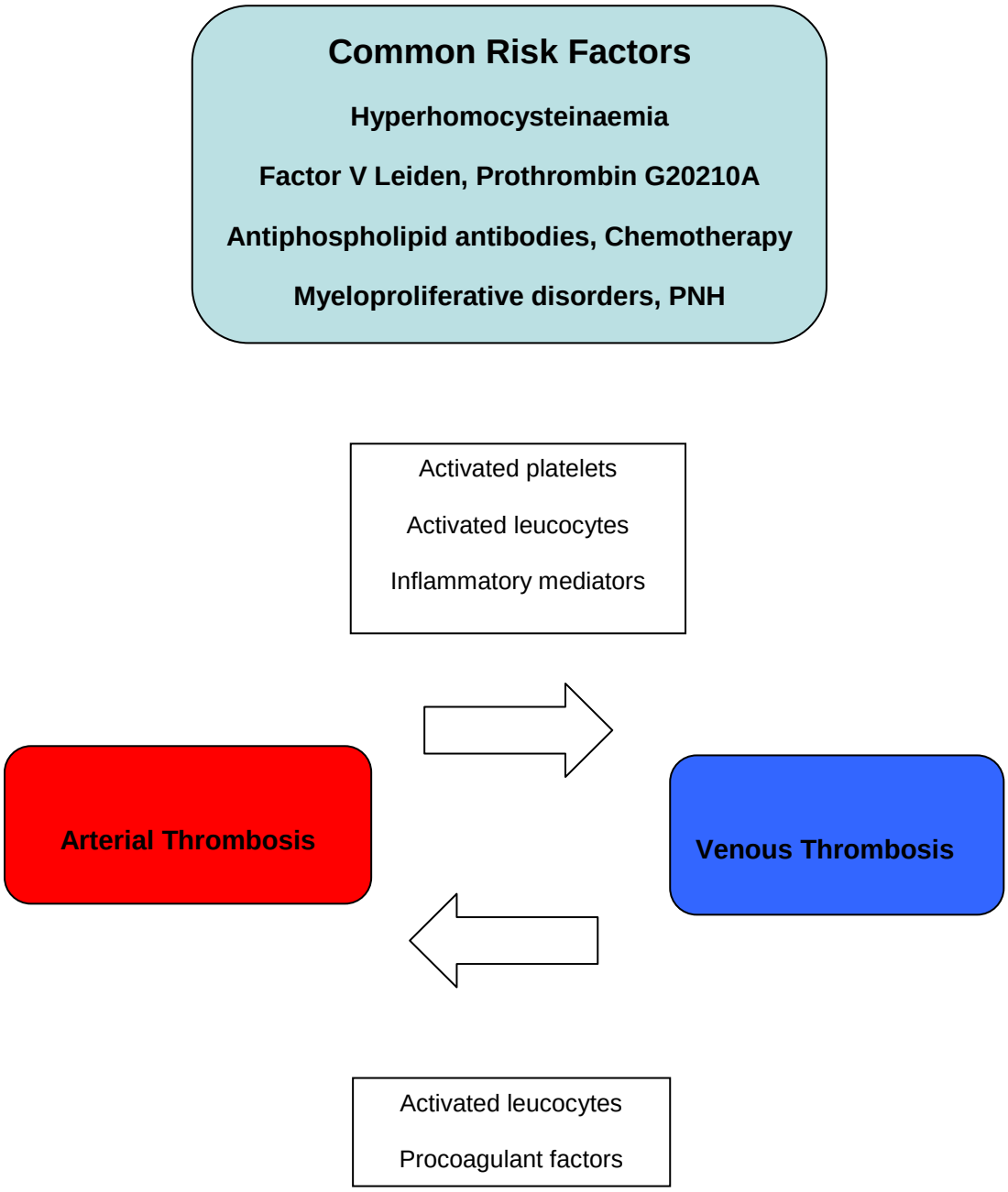


Figure 1.6: Arterial and venous thrombosis pathophysiological links

1.6 Activation of blood cells studied by flow cytometry

- Fluidics system

In addition to the measurement of circulating levels of activation markers, systemic activation of blood platelets and leucocytes can be measured by flow cytometry.

Flow cytometry allows the particular properties of individual particles to be measured. When the sample is first injected into the flow cytometer the particles for analysis are randomly dispersed three-dimensionally. Then through a process known as hydrodynamic focusing these particles are ordered into a single file. This is done by the drag effect caused by the faster flowing fluid that is present in an outer sheath which surrounds the central core into which the sample is injected. Theoretically under ideal conditions the central core fluid will not mix with the outer sheath fluid (figure 1.7). This then allows single particles to be analysed one at a time.

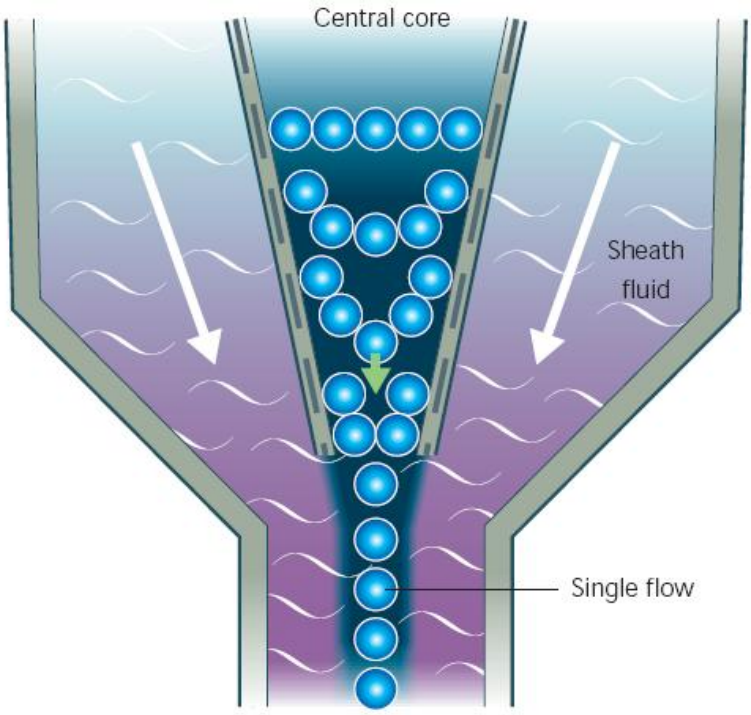


Figure 1.7: Hydrodynamic focusing producing a single stream of particles (reproduced with kind permission from Introduction to Flow Cytometry by Rahman, AbD Serotec).

- Optics and detection

This single stream of particles then is passed through one or more beams of light emitted from a laser or arc lamp. The light scattering as well as the fluorescence emission (if labelled with a fluorochrome) gives specific information about the properties of the particles studied. The light can be scattered in one of two ways, either in a forward or at a 90^{o} angle direction. This is then picked up by one of two lens known as the forward scatter channel (FSC) and the side scatter channel (SSC) respectively. The FSC processes information on the size of the particle but can also distinguish between debris and living cells and the SSC give information regarding the granular content. This information allows for differentiation between cells types.

If labelled with a fluorochrome then fluorescence measurements can be taken at different wavelengths. This can give specific information on surface receptors, DNA and cytokines. Three main filter are used to allow the detection of specific wavelenghts: (Figure 1.8)

- 'long pass' filters allow light above a cut-off wavelength through
- 'short pass' ones allow light below a cut-off wavelength through
- 'band pass' only allows light within a specified narrow range of wavelength through

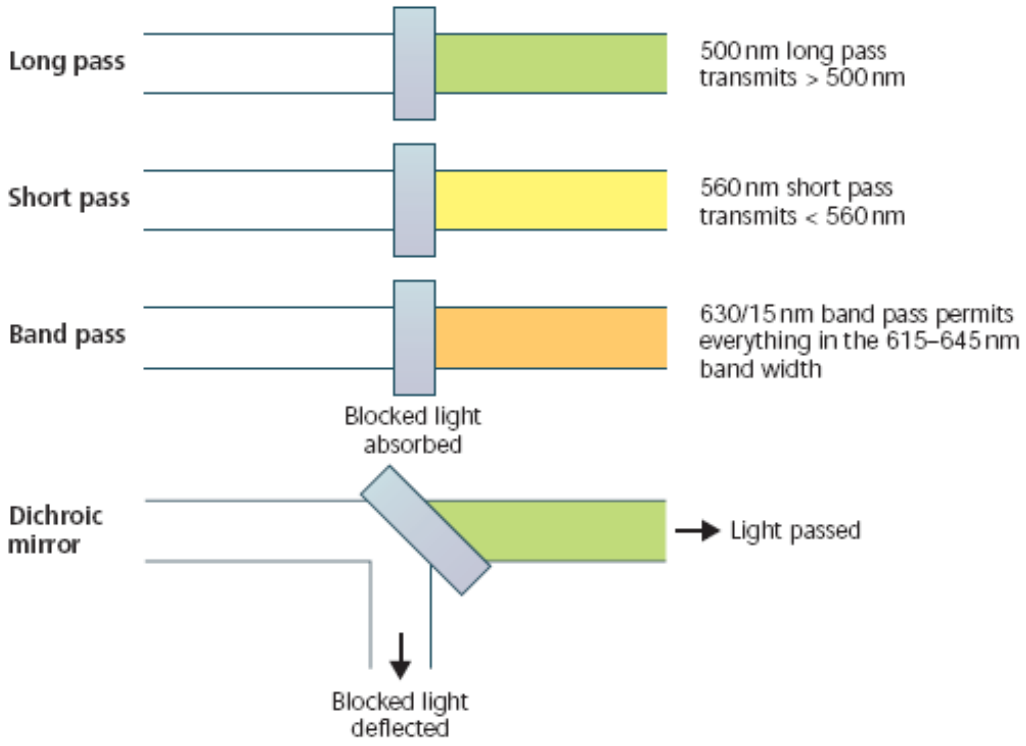


Figure 1.8: Different types of optical filters (reproduced with kind permission from Introduction to Flow Cytometry by Rahman, AbD Serotec).

These filters block light by absorption. Also as demonstrated above if the filter is placed at a 45^{o} angle it becomes a dichroic filter / mirror. This means that the filters perform two functions, firstly they allow light of a specific wavelength to pass through in a forward direction and secondly they deflect the blocked light to a 90^{o} angle.

- Processing of the signals

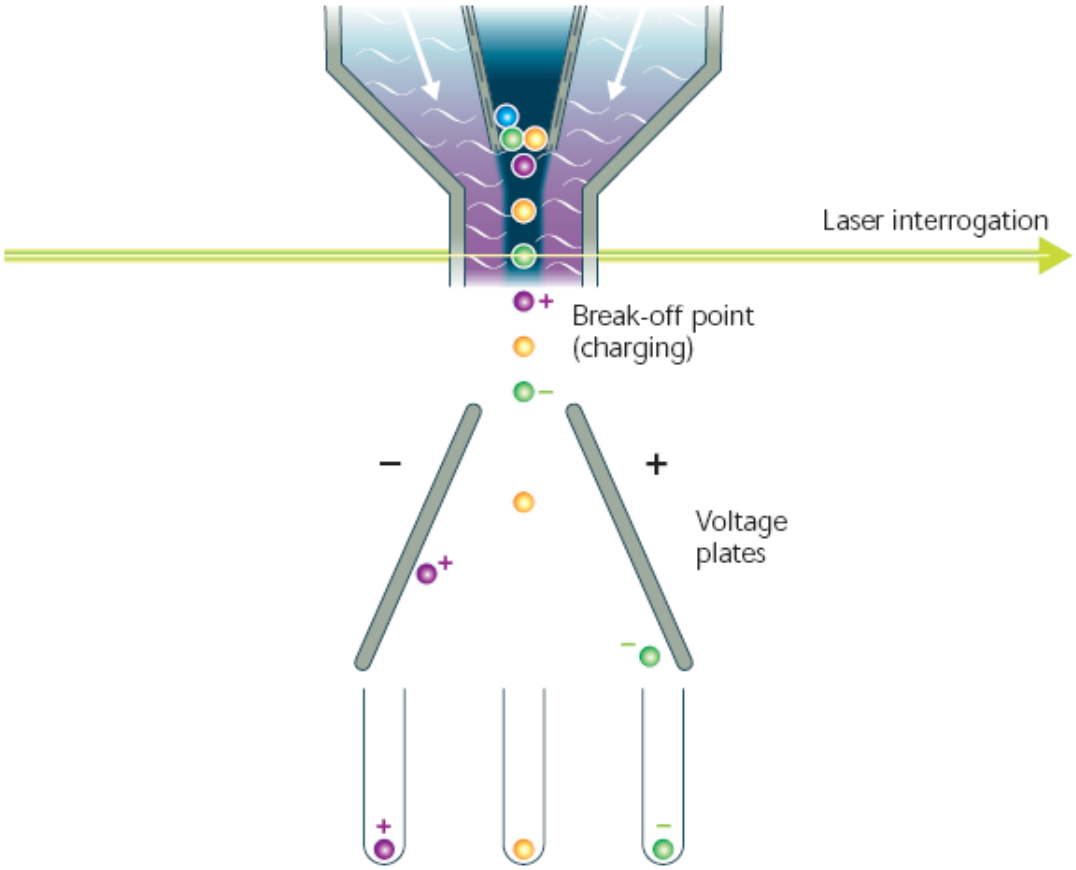
A small current proportional to the number of light photons is generated when they strike the photodetector.This is then amplified and converted to an electrical signal which can then be plotted. The measurement from each detector is called a 'parameter' and the data in each parameter are known as 'events' corresponding to the number of cell detected.

- Electrostatic cell sorting

The main application of flow cytometry is its ability to identify and separate cells depending on their subtype or epitope expression. This is done through a process known as cell sorting or fluorescence-activated cell sorting (FACS^{TM}) analysis. As the sample is orderd into single file it is passed through a beam of light. The information generated from the scatter and fluorescence is then compared against a set a predetermined criteria. If it matches then the fluid is electrically charged and passed though an electromagnetic field allowing the particle to be sorted (figure 1.9).

- Fluorochromes and Stokes shift

As mentioned previously, fluorochromes can be used to identify specific cells. Fluorochromes are dyes which will absorb light at a specific wavelength and releases it at a longer wavelength through a process known as fluorescence. As the light is absorbed by the fluorochrome, the electrons absorb the energy and move from its normal resting state to a more excited state. The amount of energy for this to happen will differ for each fluorochrome (shown as $E_{excitation}$ in figure 1.10). The excited states lasts only for 1-10 nanoseconds before the energy is released as heat and the electrons fall back down to a less excited state known as the 'relaxed electronic singlet state' (shown by 3 in figure 1.10) then as the electron move back to its original state the remaining energy is released as fluorescence (shown by $E_{emission}$ in figure 1.10).



As this $E_{emission}$ contains less energy it appears as a different colour of light to the $E_{excitation}$. This difference is called the Stokes shift.

Figure 1.9: Electrostatic flow sorting (reproduced with kind permission from Introduction to Flow Cytometry by Rahman, AbD Serotec).

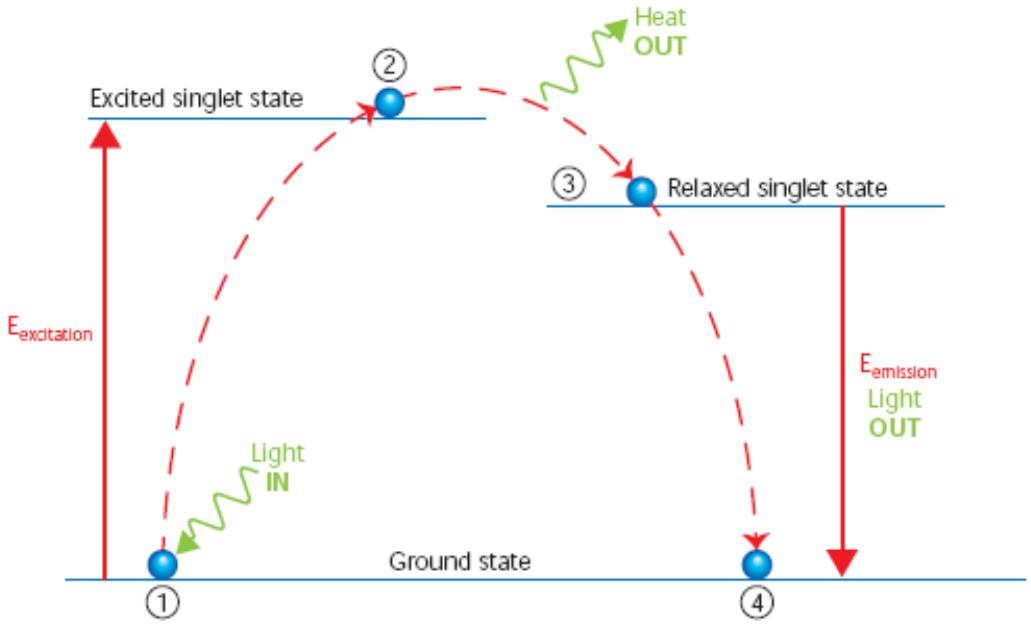


Figure 1.10: Stokes shift (reproduced with kind permission from Introduction to Flow Cytometry by Rahman, AbD Serotec).

- Data analysis

One major advantages of flow cytometry is that it can selectively visualise specific cells of interest through a process known as gating. Traditionally cells are gated according to their physical characteristics such for monocytes, granulocytes and lymphocytes allowing them to be distinguished from each other.

On the density plot (figure 1.11), individual cell are represented by each dot or point. The yellow or green dots show large number of events in the population of cells. The colours give a three-dimensional appearance. Another way of displaying the same data if through the use of contour diagrams, (figure 1.11) here the joined lines represent similar numbers of cells.

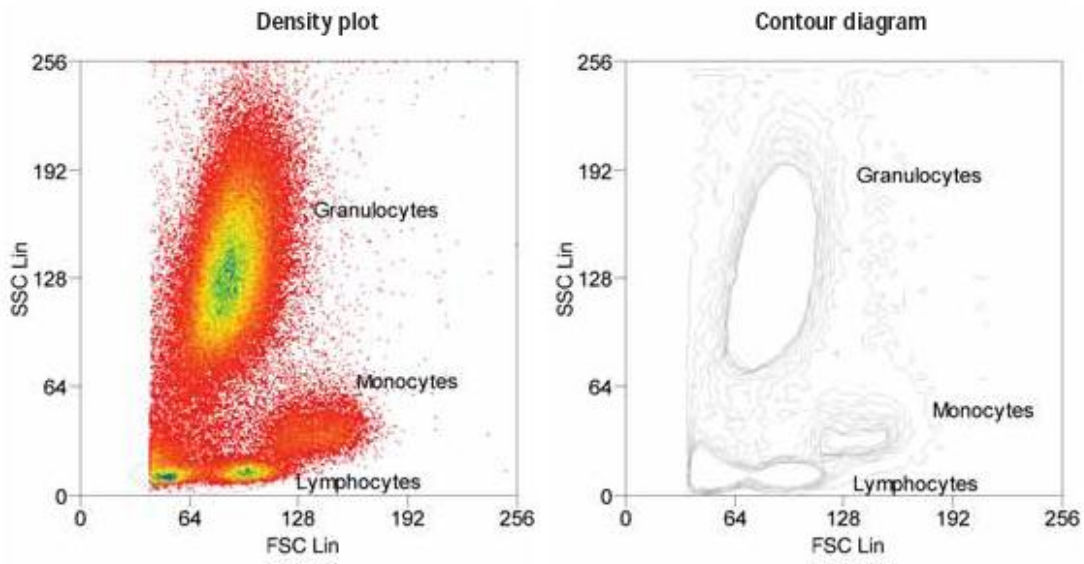


Figure 1.11: Analysis of lysed whole blood using forward and side scatter (reproduced with kind permission from Introduction to Flow Cytometry by Rahman, AbD Serotec).

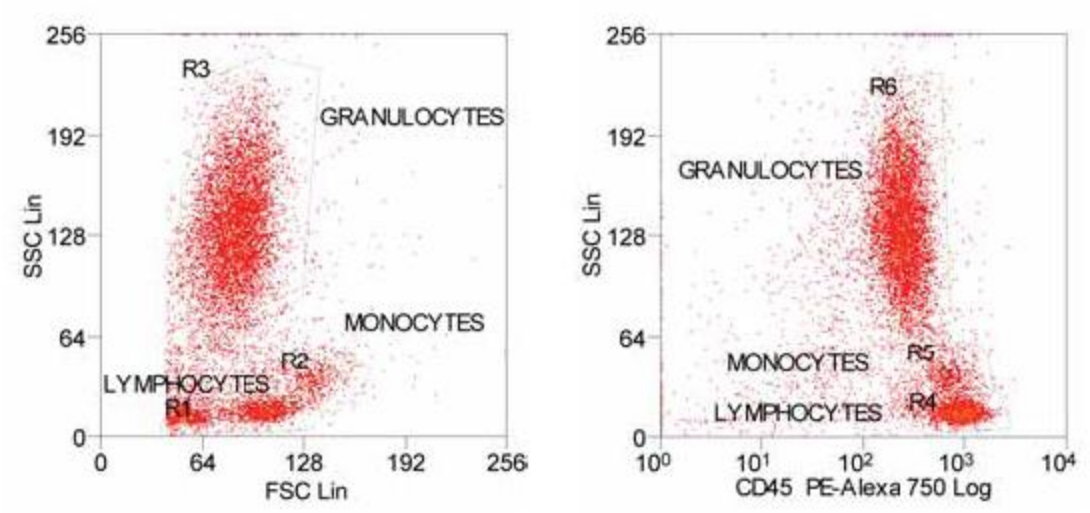


Figure 1.12: Lysed whole blood analysis using scatter and fluorescene scatter (reproduced with kind permission from Introduction to Flow Cytometry by Rahman, AbD Serotec).

In figure 1.12 on the left hand side is a forward and side scatter plot of lysed whole human blood. The lymphocytes are represented in region 1 (R1), the monocytes in region 2 (R2) and the granulocytes in region 3 (R3). The term 'region' is used to refer to the region in the plot which shows the data from the flow cytometer. On the graph on the right the same sample of lysed human whole blood has been plotted but this time CD45 has been added. CD45 is expressed to different degrees by all white blood cells but not by red blood cells. In this plot we see region 4 (R4) representing the lymphocytes (low SSC and high CD45 count), region 6 (R6) showing the granulocytes (high SSC and low CD5 count) and the monocytes in between in region 5 (R5). The main difference between the lymphocytes gated in region 1 and region 4 is the absence of red blood cells in R4.

The data can also be displayed as single or double parameter histograms. A single-parameter histogram is one that displays a single measurement such fluorescence or light scatter intensity on the one axis and the number of events (cell count) on the other (figure 1.13).

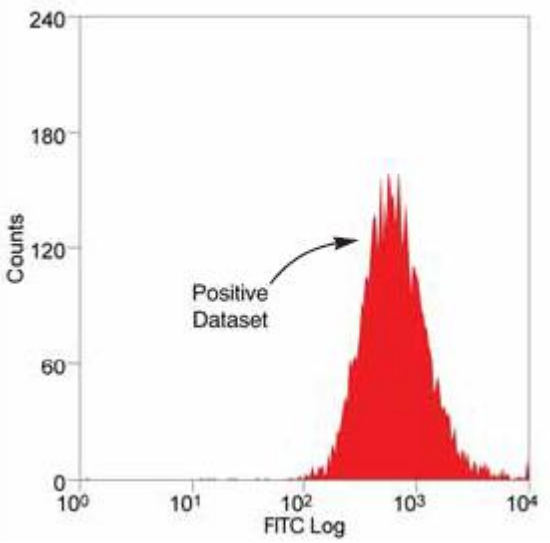


Figure 1.13: Single parameter histogram (reproduced with kind permission from Introduction to Flow Cytometry by Rahman, AbD Serotec).

This type of histogram is useful for quickly assessing the total number of events of inetrest. These selected cells are known as the positive dataset.

The two-parameter histogram displays two measurement parameters, one on the x-axis and y-axis with the cell count as a density (dot) plot or contour map. The parameters could be SSC, FSC or fluorescence (figure 1.14).

In the figure above, the lymphocytes have been stained with anti-CD3 in FITC channel (x-axis), and anti-HLA-DR in the PE channel (y-axis) which are markers for T cells and B cells respectively. Here the R2 reflects the PE-labelled B cells and R5 contains the FITC-labelled T cells. The cells that stains for both markers are reflected in region 3 (R3) and finally R4 contains cells negative for both markers.

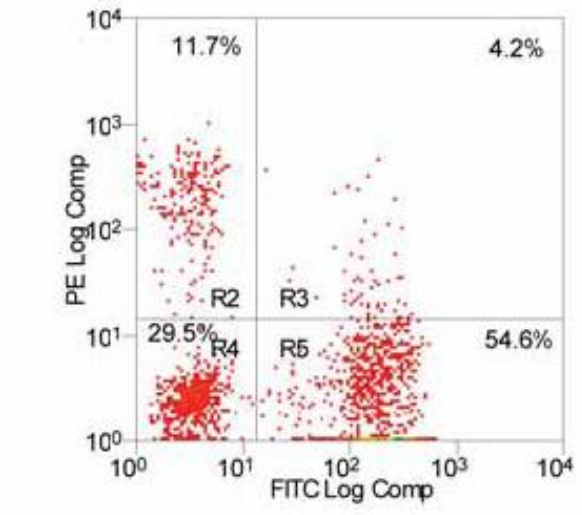


Figure 1.14: Two-parameter (dual-colour fluorescence) histogram (reproduced with kind permission from Introduction to Flow Cytometry by Rahman, AbD Serotec).

1.7 Polymethylmethacrylate Bone Cement

Polymethylmethacrylate (PMMA) has been used in orthopaedic surgery now for over 60 years. The word cement is often used along with PMMA but strictly speaking it is a grout rather than cement. As the orthopaedic speciality has advanced so has our understanding of its properties and it has indirectly helped enhance implant design, tribology and biomechanics.

PMMA was first introduced in 1843 and initially known as '*acide acrylique*' due to the acrid smell of the monomer (193). In 1936 it was discovered that the mixture of monomr and polymer gave a substance that for period a time could be moulded and manipulated. Its early use was in dentistry but Scales and Herschell in 1945 (194) and Judet and Judet in 1950 (195) used it to mould a femoral head prostheses of in the treatment of arthritis. It current use as a grout was first pioneered in 1953 by Haboush (196). The major breakthrough was when it was adapted for orthopaedic use by Sir John Charnley (197;198) and even now remains an integral part of modern orthopaedic practice.

The methylmethacrylate monomer polymerises at room temperature to produce the solid PMMA (199). In its commercial states it consists of packs of the polymer, including additives (table 1.4). The molecular weight of PMMA will vary depending on the brand and the method of sterilisation. For instance gamma radiation for sterilisation will shorten the polymer chains which does not occur with ethylene oxide sterilisation (200).

Table 1.4: Constituents of bone cement

Constituent	**Role**
Powder components	
Polymer	Polymethylmethacrylate
Co-polymers e.g. MM-MMA	Alters physical properties of cement
Barium sulphate or zirconium dioxide	Radio-opacifiers
Antibiotics	Antimicrobial prophylaxis
Dye e.g. chlorophyll	Distinguishes cement from bone
Liquid components	
Monomer	Methylmethacrylate monomer
N,N-dimethyl-p-toluidine (DMPT)	Initiates cold curing of polymer
Benzoyl peroxide	Reacts with DMPT to catalyse polymerisation
Hydroquinone	Stabiliser to prevent premature polymerisation
Dye e.g. chlorophyll	Distinguishes cement from bone

MA-MMA = methacrylate-methylmethacrylate

The polymerisation process is an exothermic one (201). The catalysts form free radicals allowing the breaking of the carbon to carbon bonds of the monomer. This can bind to the polymer increasing its length. This process produces heat in the order of 1.4 to 1.7 $x10^8$ J/m^3 of cement (201). This heat production has been studied both in vitro and in vivo (202). In vitro, the heat produced increases with higher room temperatures, an increased monomer to polymer ratio and when a thicker cement mantle is used (203). The temperatures recorded range from 70°C to 120°C and as collagen denaturing at temperatures above 56ºC, several authors have raised the potential risk of thermal damage to bone (204;205).

However, lower temperatures have been sown in vivo studies (201). Reckling and Dillon (206) in 1977 measured the bone cement interface temperature in 20 total hip replacements. The maximum temperature they found was only 48°C but they attributed this to the possible cooling effects of the blood supply, the large surface are of a metallic stem and the poor thermalconduction of the cement. Others authors have recorded temperatures above 56°C but only for two to three minutes (207).

This exothermic process already been shown to activate the complement system (208). Ritter et al demonstrated that the addition of polymethylmethacrylate contributes to the overall mortality following total hip and knee arthroplasty (209) through measuring levels of gamma-glutamyl-transpeptidase which was significantly elevated compared to a control group of hip fracture fixation using no cement. The methylmethacrylate monomer is an organic solvent which has been shown to be cytotoxic (210). It may act on the pulmonary endothelium to enhancing the procoagulant factors already present adding to its hypercoagulability possibly resulting in the development of DVT (211).

An animal study by Dahl et al (212) compared the frequency of postoperative deep vein thrombosis in eight pigs who underwent a hemiarthroplasty with and without cement (n=16). The levels of thrombin-antithrombin complexes rose substantially during bone preparation followed by a rise in tissue plasminogen activator levels and a gradual fall in plasminogen activator inhibitor activity, with the higher rises seen in those undergoing a cemented prosthesis. Post-operative electron microscope scanning showed femoral vein thrombi in 62% of the animals in the cemented group compared to 25% in the uncemented group. They concluded that bone preparation during surgery induced a significant stimulation of the coagulation pathway and resulted in higher rates of deep vein thrombosis in the proximal veins, more so if cement was used for prosthesis fixation.

1.8 Effect of elective general and orthopaedic surgery on inflammation and endothelial markers

An early study by Cruickshank et al in 1990 investigated the IL-6 response to a variety of elective surgical procedures such as cholecystectomy, cemented total hip arthroplasty and major vascular surgery. They demonstrated a relationship between the duration of surgery and the IL-6 response ($r = 0.8$, $p < 0.001$) and concluded that IL-6 was a sensitive marker of tissue damage and that this response increased with greater surgical trauma (41).

In 2000, Pape et al (172) in a prospective non randomised study investigated 105 patients admitted to their unit for a variety of lower limb orthopaedic procedures.

These were then categorised into four groups. Group 1 represented a group of polytrauma patients with an Injury Severity Score of 18, group 2 were those with a isolated femoral diaphyseal fractures treated with unreamed femoral nailing, group 3 were those with a diagnosis of osteoarthritis admitted for uncemented total hip arthroplasty and the final group 4 were those who had sustained an isolated ankle fracture for open reduction and internal fixation with a plate.

The baseline values of IL-6 and TNFα were sampled from the venous circulation on skin incision and then at insertion of prosthesis i.e. the femoral component of a total hip arthroplasty, insertion of a femoral nail and the application of a plate. They found that the TNFα levels increased the most significantly in the group of polytrauma patients at all time points and although the levels increased in the total hip arthroplasty group, this did not reach statistical significance. The IL-6 levels showed a statistically significant rise at insertion of the femoral component in the total hip arthroplasty group and these rises were of similar magnitude to that of the femoral nailing group, showing a correlation between the groups ($r = 5.83$; $p = 0.0004$).

The authors conceded that the study did not focus solely on skeletal operations. It also did not address the possibility of activation of inflammatory mediators by the methylmethacrylate from the bone cement used (173).

Similar significant rises in IL-6 levels were also reported by Krohn et al (174) but they only looked at 8 adolescents (12-19 yrs) undergoing corrective surgery for thoracic scoliosis. The IL-6 levels were sampled from both the arterial blood as well as blood obtained from drains, the levels in the drained blood reaching much higher levels. Again

as in the previous study, several samples were taken at different time points, pre-operatively and at 1, 2, 4 and 6 hours post-operatively. IL-6 showed a continual rise throughout all the sample points.

They did not demonstrate a significant change in the TNFα apart from a rise during wound closure. They explained their results as possibly due to its short biological half-life. Others have also shown no significant rises in the TNFα levels in the case of total hip arthroplasties (213;214) but Andres et al demonstrated significant levels of local TNFα in surgical drains post-operatively (215).

Other studies have looked into the effects of total knee arthroplasty on monocytes-platelet expression as well as the expression of CD40L on platelets, using flow cytometry. Kageyama et al (175) recruited 24 patients into their study who were then randomised into two groups, one with and the other without the use of a tourniquet intra-operatively. Two patients (one from each group) were excluded due to anaesthetic difficulties resulting in the operation being cancelled. The remaining 22 subjects had venous and arterial blood sampled before and at the end of surgery. Samples were also taken 6 hours then again at 24 hours following surgery. They found that monocytes-platelet expression levels peaked at around 2.5 hours following skin incision and then returned to baseline levels at 24 hours. A similar picture was also seen in CD40 expression with a maximal change of around 25% from baseline levels. They found that circulating levels of sCD40L increased in proportion with levels obtained by flow cytometry ($p<0.05$). These results were increased in the group with tourniquet inflation, however the authors failed to mention if this was statistically significant or not. The authors concluded that their results after total knee arthroplasty may highlight an important role for blood cell activation in the pathogenesis of hypercoagulability and

venous thrombosis. But the numbers of this study are small (n=22) and although an attempt was made to look at the difference with the use of an inflated tourniquet no mention is made as to whether their differences seen were statistically significant but he went on to state that his results support the hypothesis that the use of a tourniquet causes the retention of blood flow, increases the coagulation activity as well as disrupting the vascular endothelium contributing to venous thrombosis. This hypothesis is supported by several smaller studies (216-218).

There is more evidence for elective cemented total hip arthroplasty as previous studies have demonstrated an increase in the thromboplastin activity of monocytes (219). Dahl et al (220) showed that the thromboplastin activity was confined to the cell surface of the monocytes where fibrin formation occurred. This in turn may contribute to the increased coagulability that is induced when blood passes the lung (221). These pericellular fibrin deposits may allow the monocytes to attach to the vessel walls promoting thrombus formation. This may occur more so in an environment where the endothelial surface is already stimulated due to local mechanical, chemical (bone cement) or biochemical factors. As well as this the activated monocytes may have an increased expression of cell surface adhesion molecules e.g. selectins. Therefore this may contribute to the higher frequency of DVT observed in patients receiving cemented hip prostheses (160).

Another study also looked at the levels of CD40L and expression of monocytes-platelets but in subjects undergoing cemented total hip arthroplasty. Ray et al (74) recruited 45 subjects. Their aim was to look at a possible link between CD40L expression and cardiovascular complications 30 days following surgery. Sampling was taken through an arterial line before and then one hour following completion of surgery for flow

cytometry analysis. The recorded adverse cardiovascular events were two patients with non-ST elevation myocardial infarction (NSTEMI), two with atrial fibrillation and another two patients with both and NSTEMI and atrial fibrillation. They found that these 6 patients compared to those with no complications (n=39) were more likely to be diabetic (2/6 versus 1/39, p=0.04) and had a past medical history of cardiovascular disease (3/6 versus 4/39, p=0.04). Flow cytometry revealed that the CD40L expression on platelets before surgery was a median of 11% (IQR 7.9-12.8) in patients with a history of cardiovascular disease compared to 4.7% (IQR 1.9-6.6) for those with none (p=0.001). The increase in CD40L expression in both groups at one hour post procedure was statistically significant.

Bagry et al looked at the effect on the inflammatory response of a continuous peripheral nerve block following a primary total knee arthroplasty in 12 patients (222). All underwent a spinal anaesthetic and were then randomized to either patient-controlled analgesia using morphine (n =6) or lumbar plexus and sciatic nerve blocks with ropivacaine (0.2%) for 48 hours. Venous blood was sampled at specific time points up to 48 hours post-operatively. They found that both leucocyte and CRP levels were significantly lower in those with the continuous nerve block, which therefore attenuated the inflammatory response. The reduction in the inflammatory response can improve the functional recovery by reducing the injury-induced immunosuppression (223) with prolonged elevation of CRP and IL-6 following hip arthroplasty being associated with poor mobilisation and rehabilitation. Other have carried out similar studies with epidural anaesthesia but have not found a significant reduction in the inflammatory response (224) which Bagry et al stated was due to the blocking of both the afferent and efferent fibres innervating the surgical area, preventing primary and secondary hyperalgesia which is not possible with epidural anaesthesia. Bagry et al did however concede that the systemic effect of the local anaesthetic could not be

excluded as research has demonstrated that the use of local anaesthetics either given locally or systemically will affect the inflammatory response in animals (225). However the local attenuation of the inflammatory response has been shown in vitro by Su et al (49).

From this review of the current literature it appears that elective orthopaedic surgery including total knee arthroplasty does activate inflammation, endothelium and blood coagulation, as measured both by circulating levels of activation markers and by flow cytometry. These effects may be influenced by the use of bone cement, by local anaesthetic infiltration intra-operatively or the use of post-operative drains and may also be related to the increased post-operative risks of arterial and venous thrombosis.

To date no systematic study has been reported comparing the effects of bone cement, local anaesthetic infiltration and post-operative drains in elective total knee arthroplasty.

AIM

The aim of the studies reported in this thesis is to examine and compare the effects of 4 different aspects of elective orthopaedic total knee arthroplasty on the activation of selected circulating markers of inflammation, endothelium, coagulation and fibrinolysis:

- IL-6 inflammatory cytokine
- TNFα inflammatory cytokine
- CRP sensitive inflammatory marker
- e-selectin endothelial marker
- sCD40L general marker of inflammation
- tPA endothelial marker
- vWF endothelial marker

As well as this I will study the effects of the 4 aspects of elective orthopaedic total knee arthroplasty on blood cell activation of selected cytokines:

- expression of CD40 on monocytes
- expression of the CD14 / CD42a dyad on monocytes

Four study groups will be compared:

1) Patients undergoing elective uncemented total knee arthroplasty

2) Patients undergoing elective cemented total knee arthroplasty, to investigate any additional response to the exothermic reaction of the PMMA cement.

3) Patients undergoing uncemented total knee arthroplasty, with local infiltration of local anaesthetic intra-operatively.

4) Patients undergoing uncemented total knee arthroplasty, with the use of a drain post-operatively

In order to quantify the effect of the use of cement, intra-operative local anaesthetic infiltration or a drain I will be comparing the following groups:

1) Cemented group versus uncemented group to assess any additional effect the cement may have on the inflammatory or endothelial response.

2) Uncemented group versus the group with local anaesthetic infiltration to assess whether the local anaesthetic would dampen the inflammatory and endothelial response.

3) Uncemented group versus the group with a surgical drain to assess any effect this would have on the inflammatory and endothelial response.

CHAPTER 2

SUBJECTS AND METHODS

2.1 Subjects

All patients attending Monklands Hospital assessed for an elective cemented or uncemented total knee arthroplasty were identified. They were then approached at the pre-assessment clinic (which routinely took place a week before their intended operation date) by the author where the study was explained to them, and they were invited to take part in the study. After informed consent was obtained a patient history was taken which included the history of the presenting complaint, past medical history, drug and social history. The patient's case notes were then reviewed and if they were eligible for the study they were recruited at that point. (Table 2.1) Following recruitment into the study, patients were excluded if they developed a post-operative infection confirmed by bacteriological sampling.

As part of the consent, subjects were told that the average length of hospital stay following a total knee arthroplasty was around 5 to 7 days. This meant that usually by the time the last blood sample was due to be taken, there would a high likelihood that they would have been discharged home. So subjects were only enrolled into the study if they agreed to a home visit by the author at day 7 post operation in order to obtain the third and final sample.

Table 2.1: Inclusion criteria for the study

Subjects were recruited into the study if they met all the following requirements:
- no history of uncontrolled hypertension
- no history of ischaemic heart disease
- were able to give informed consent in the opinion of the researcher

An information sheet (Annex 1) outlining the study along with contact details was given to the subject and a copy of the information sheet was attached to the patient's casenotes to indicate their participation. A cover letter (Annex 2) and information sheet was sent to their general practitioner. Ethical approval (Annex 3) was obtained from the Local Research Ethics Committee.

The subjects were admitted into the elective orthopaedic ward at Monklands Hospital the day before their surgery. They were approached by the author and any questions that had arisen from the previous consultation were addressed. As well as this, a pre-operative electrocardiogram was taken.

The day after their total knee replacement (day 1 post-operation) the second set of blood samples were taken as pre-operatively. A record was also taken of the anaesthetic sheet to note the blood pressure readings during the time of surgery and a post-operative electrocardiogram was done.

At day 7 post total knee arthroplasty if the subject was still in the ward the final set of blood samples were taken as pre-operatively. If they had been discharged home the subject was contacted at home and a suitable time was agreed for the author to visit and take the final samples.

2.2 Blood sampling, handling and laboratory assays

Following enrolment into the study the first of three sets of blood sample was taken. These pre-operative blood samples were collected in the BD Vacutainer system. (Table 2.2) Two BD Vacutainers, SST II Advance (yellow top bottles) were taken and sent to the biochemistry laboratory for routine urea and electrolytes, liver function tests, cholesterol and C-reactive protein. One BD Vacutainer, K3E 7.2mg EDTA (purple top bottle) was sent to the haematology laboratory for a full blood count as well as for a differential white cell count, and one BD Vacutainer, 9NC 0.109M trisodium citrate (light blue top bottle) for coagulation profile. A further two EDTA bottles were taken for flow cytometry analysis and one citrate and a SST bottle for the endothelial markers.

Table 2.2: Overview of BD Vacutainer systems

Colour of bottle top	**Volume (ml)**	**System**	**Additives**
Yellow	5	BD Vacutainer, SST II Advance	Spray coated silica and polymer gel for serum separation
Purple	4	BD Vacutainer, K_3E 7.2mg EDTA	Spray coated K_3 EDTA – anticoagulant
Light Blue	2.7	BD Vacutainer, 9NC 0.109M	3.2% buffered trisodium citrate anticoagulant

The samples for flow cytometry were taken directly to the Haematology laboratory. The peripheral blood was labelled with combinations of FITC-conjugated anti CD14; PE-conjugated anti CD42a and PE-conjugated and anti CD40 (AbD Serotec, Kidlington, UK). After incubation red cells were lysed using EasyLyse ammonium chloride RBC lysing solution (Dako,Denmark). Data acquisition and analysis was performed on a FACS Canto II flow cytometer (Becton Dickinson, San Jose, CA). Monocytes were identified from light scatter properties and a gate applied. 10000 events within the monocyte gate were collected. Number of gated events, mean fluorescence intensity and percentage positive events were recorded. Isotype FITC and PE controls were analysed on each sample to determine background fluorescence. All samples were processed within 24-48hrs.

The citrate and SST bottles were also taken to the Haematology laboratory where they were centrifuged at 1500g for 10 minutes. The plasma was then separated as follows:

- plasma from the citrate bottle was decanted into two 1 ml aliquots

- serum was decanted into two 1 ml aliquots

The samples were then stored in a -40°C degree freezer. All samples were centrifuged and stored within three hours of venepuncture. All samples were stored in the freezer until such time that they could be transferred by the author to the University Department of Medicine laboratory at Glasgow Royal Infirmary for analysis.

Soluble CD40 ligand (sCD40L), e-selectin and interleukin 6 (IL6) were assayed by high-sensitivity enzyme-linked immunoasorbent assay (ELISA) kits from R&D systems, Oxford, United Kingdom.

Plasma von Willebrand factor (vWF) antigen levels were measured with an in-house enzyme linked immunoasorbent assay (ELISA), using rabbit anti-human polyclonal antibodies (DAKO plc, High Wycombe, UK). Tissue plasminogen activator levels were measured with a commercially available enzyme-linked immunoasorbent assay (ELISA) (Trinity Biotech, Sulhampstead, UK).

In the laboratory the intra- and inter-assay coefficient of variations (CV) for IL6 was 7.5% and 8.9% respectively, 4.2% and 3.3% for vWF, 6.6% and 6.5 % for tPA, 5.9% and 8.6% for e-selectin, ~12.6% for TNFα and ~6.0% for sCD40L.

2.3 Statistical Analysis

A Shapiro-Wilk test was done to test for normaility. All variables except for the CD40, CD1442a and e-selectin count were normally distributed. For the parametric data results are presented as mean, SD and SEM. Differences between the three time points (pre-operative, day 1 and day 7) were analysed using pair sampled t-tests. This was done for all the four groups. Independent sample t-tests were used to look for differences between groups. The non-parametric data results are presented as median and IQR. The differences between the three time points were analysed using Wilcoxon

signed rank tests. Mann-Whitney U tests were used to look for differences between the groups.

Advice on statistical analyses was sought from the Robertson Centre for Biostatistics, The University of Glasgow.

Chapter 3

The effect of elective uncemented total knee arthroplasty on the activation of markers of inflammation, endothelium and coagulation

3.1 Subject Characteristics

There were 20 patients recruited into this study, one patient developed a post-operative deep wound infection with positive wound cultures. This required a second procedure in the form of a wound washout and exchange of the polyethylene liner and was therefore removed from the study, leaving 19 patients. The mean body mass index (BMI) was 30.3kg/m^2, standard deviation 5.1. There were 10 males (mean age 65.3 years, standard deviation 7.36) and 9 females (mean age 67.8 years, standard deviation 8.97). A total of 6 total knee arthroplasties were carried out on the left and 13 on the right.

3.2 Blood cell count and CRP

The mean values, standard deviations and standard error of means of blood cell counts and CRP are summarised in table 3.1.

Each of these variables were compared over three time periods, from pre-operative values to day 1 post-operation (P1-D1), from day 1 to day 7 post-operation (D1-D7) and from pre-operative to day 7 post-operation (P1-D7).

There were significant changes at all three time points for the platelets (Plat) (Figure 3.1), white cell count (WCC) (Figure 3.2) and neutrophils (Neutro) (Figure 3.3), with an

initial rise then fall except for the platelets which had an initial fall in their levels. The monocytes (Mono) (Figure 3.4) also had a significant rise but just failed to reach significance when their levels dropped at day 7. C-reactive protein (CRP) showed a significant rise in the first 24 hours and remained significantly elevated at day 7 (Figure 3.5).

Table 3.1: Mean, standard deviations and standard error of means of platelets (Plat), white cell count (WCC), neutrophils (Neutro), monocytes (Mono) (all 10^9/L) and C-reactive protein (CRP) (mg/L) pre-operatively (P1) and at day 1 (D1) and day 7 (D7) post-operatively in the uncemented total knee arthroplasty group (n=19)

	Mean	Standard Deviation	Standard Error of Mean
Plat P1	260	71	16
Plat D1	204	57	13
Plat D7	333	79	18
WCC P1	8.1	1.5	0.3
WCC D1	9.9	2.2	0.5
WCC D7	8.9	2.1	0.5
Neutro P1	4.7	1.1	0.2
Neutro D1	7.3	1.9	0.4
Neutro D7	5.8	2.0	0.4
Mono P1	0.6	0.2	0.1
Mono D1	1.1	0.5	0.1
Mono D7	0.8	0.3	0.1
CRP P1	2	3	1
CRP D1	68	38	9
CRP D7	67	31	7

Table 3.2: Paired sample t-test of platelets, WCC, neutrophils, monocytes and CRP in the uncemented total knee arthroplasty group (n=19)

		Mean	Standard Deviation	P value
Plat	P1-D1	55	33	<0.01
	D1-D7	-129	51	<0.01
	P1-D7	-73	53	<0.01
WCC	P1-D1	-1.8	1.9	<0.01
	D1-D7	1	2.0	0.05
	P1-D7	-0.8	1.5	0.02
Neutro	P1-D1	-2.6	1.8	<0.01
	D1-D7	1.4	2.2	0.01
	P1-D7	-1.2	1.6	<0.01
Mono	P1-D1	-0.5	0.4	<0.01
	D1-D7	0.4	0.4	<0.01
	P1-D7	-0.1	0.3	0.07
CRP	P1-D1	-66	38	<0.01
	D1-D7	0.5	52	0.97
	P1-D7	-65	30	<0.01

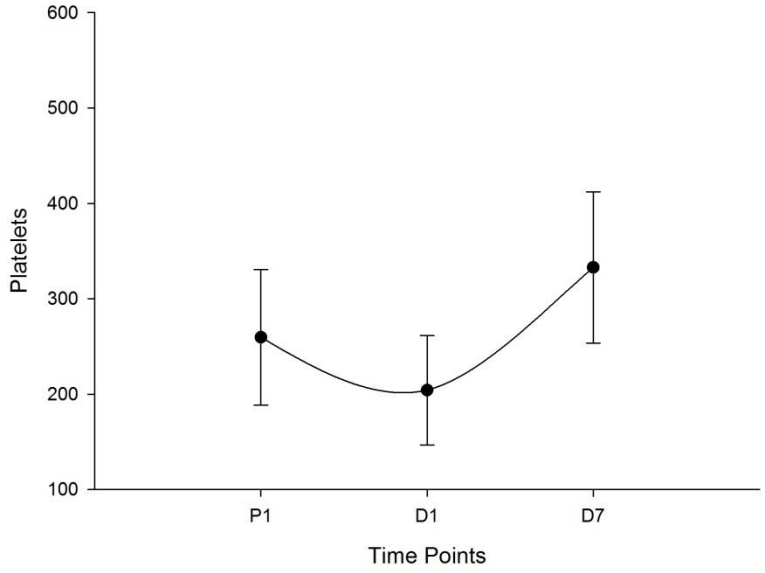


Figure 3.1: Graph of changes in platelet levels (x10^9/L) over the three time points (mean and standard deviation) in the uncemented total knee arthroplasty group (n=19)

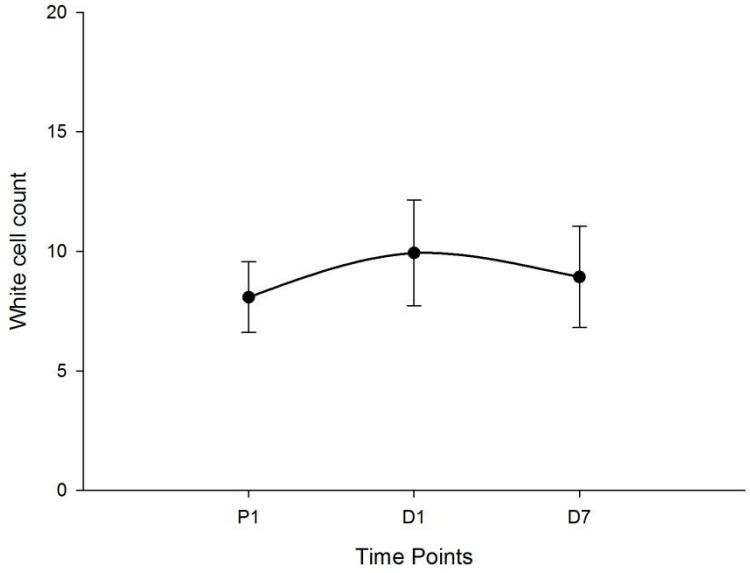


Figure 3.2: Graph of changes in white cell count levels (x10^9/L) over the three time points (mean and standard deviation) in the uncemented total knee arthroplasty group (n=19)

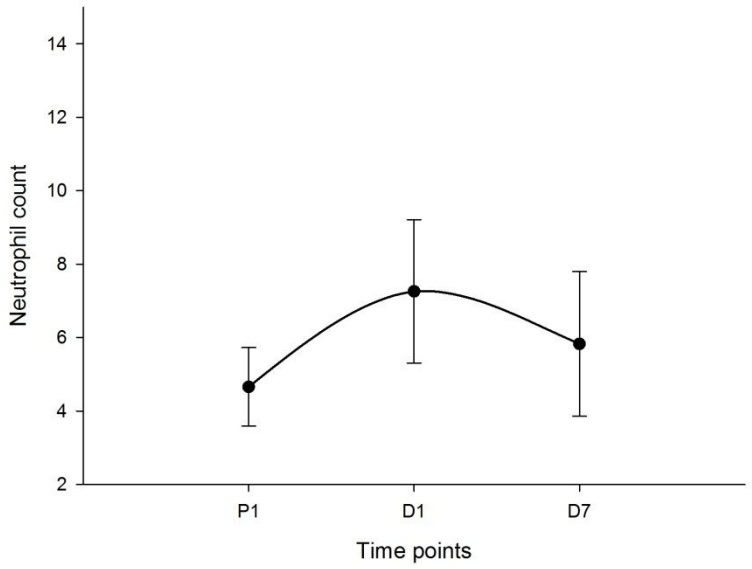


Figure 3.3: Graph of changes in neutrophil levels (x10^9/L) over the three time points (mean and standard deviation) in the uncemented total knee arthroplasty group (n=19)

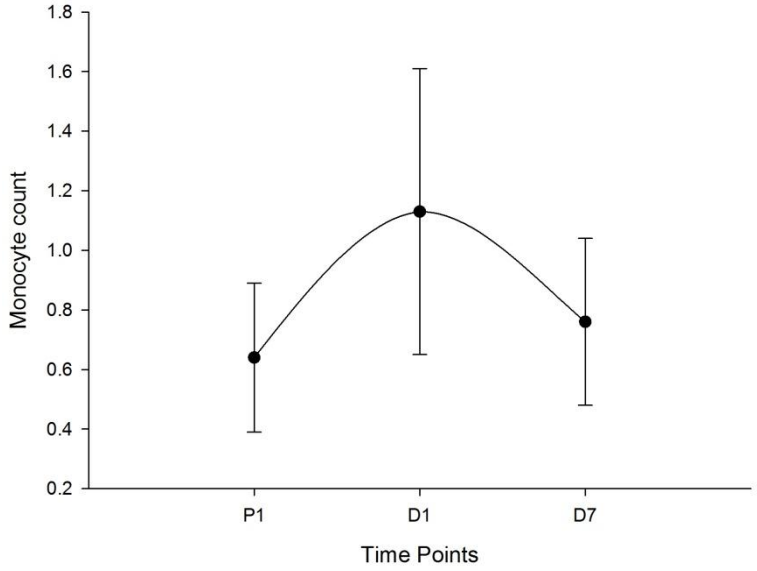


Figure 3.4: Graph of changes in monocyte levels (x10^9/L) over the three time points (mean and standard deviation) in the uncemented total knee arthroplasty group (n=19)

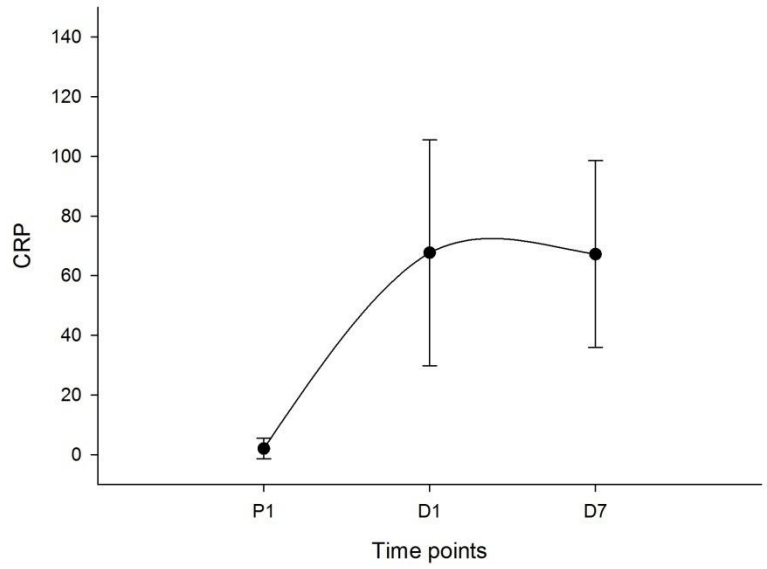


Figure 3.5: Graph of changes in C-reactive protein levels (mg/L) over the three time points (mean and standard deviation) in the uncemented total knee arthroplasty group (n=19)

3.3 Circulating activation markers

The mean, standard deviations and standard error of means of tissue plasminogen activator (t-PA), Von Willebrand factor (vWF), soluble CD40 ligand (sCD40L), interleukin 6 (IL6), tumour necrosis factor (TNFα) and e-selectin are summarised in table 3.3.

Each variable was then compared over the three time points from pre-operative values to day 1 post-operation (P1-D1), from day 1 to day 7 post-operation (D1-D7) and from pre-operative to day 7 post-operation (P1-D7) (Table 3.4).

The t-PA levels showed a non-significant rise in the first 24 hours ($p=0.06$) but by day 7 there was a significant rise from the pre-operative levels ($p=0.01$) (Figure 3.6). The vWF levels rose significantly in the first 24 hours ($p=0.00$) and remained higher at day 7 (Figure 3.7). The sCD40L showed a significant fall from pre-operative levels and rose from day 1 levels to day 7 ($p=<0.01$) (Figure 3.8). IL 6 rose and fell significantly (Figure 3.9). TNFα rose significantly at day 7 (Figure 3.10). The e-selectin levels did not show any significant changes at any of the time points (Figure 3.11).

Table 3.3a: Mean, standard deviations and standard error of means of tissue plasminogen activator (t-PA) (ng/ml), von Willebrand factor (vWF) (IU/dl), soluble CD40 ligand (sCD40L) (pg/ml), interleukin 6 (IL6) (pg/ml) and tumour necrosis factor (TNFα) (pg/ml) in the uncemented total knee arthroplasty group (n=19)

	Mean	Standard Deviation	Standard Error Mean
t-PA P1	11.5	4.5	1.1
t-PA D1	14.4	6.0	1.4
t-PA D7	15.1	7.1	1.7
vWF P1	119	38	9.
vWF D1	197	73	18
vWF D7	206	45	11
sCD40L P1	3300	2020	476
sCD40L D1	2803	2227	525
sCD40L D7	4282	2417	570
IL6 P1	4.3	2.6	0.6
IL6 D1	173.3	113.9	26.8
IL6 D7	31.9	47.9	12.0
TNFα P1	1.8	0.8	0.3
TNFα D1	2.2	1.0	0.3
TNFα D7	2.3	1.0	0.3

Table 3.3b: Median and Inter-quartile ranges (IQR) of e-selectin (ng/ml) in the uncemented total knee arthroplasty group (n=19)

		Percentiles		
		25th	**50th (Median)**	**75th**
E-selectin	P1	15.5	24.5	35.1
	D1	15.0	26.2	29.1
	P1	13.7	23.9	27.1

Table 3.4a: Paired sample t-test of tissue plasminogen activator (t-PA) (ng/ml), von Willebrand factor (vWF) (IU/dl), soluble CD40 ligand (sCD40L) (pg/ml), interleukin 6 (IL6) (pg/ml) and tumour necrosis factor (TNFα) (pg/ml) in the uncemented total knee arthroplasty group (n=19)

		Mean	Standard Deviation	P value
t-PA	P1-D1	-2.8	6.0	0.06
	D1-D7	-0.8	7.1	0.65
	P1-D7	-3.61	5.31	0.01
vWF	P1-D1	-77	71	<0.01
	D1-D7	-9	69	0.60
	P1-D7	-82	38	<0.01
sCD40L	P1-D1	497	2047	0.32
	D1-D7	-1480	1973	<0.01
	P1-D7	-983	1735	0.03
IL6	P1-D1	-169.0	113.4	<0.01
	D1-D7	146.2	139.2	<0.01
	P1-D7	-27.8	48.0	0.03
TNFα	P1-D1	-0.4	0.8	0.13
	D1-D7	-0.1	0.9	0.69
	P1-D7	-0.5	0.3	<0.01

Table 3.4b: Wilcoxon signed rank test for e-selectin (ng/ml) in the uncemented total knee arthroplasty group (n=19)

		P-value
E-selectin	P1-D1	0.20
	D1-D7	0.94
	P1-D7	0.09

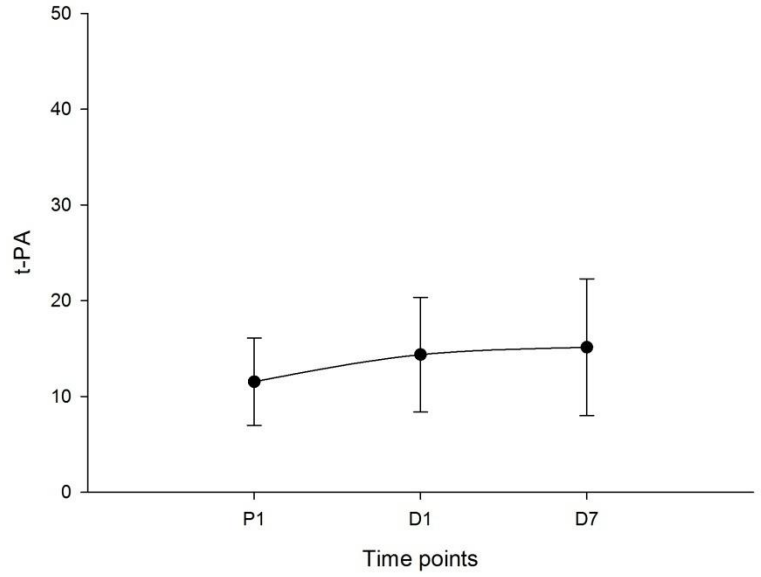


Figure 3.6: Graph of changes in tissue plasminogen activator levels (ng/ml) over the three time points (mean and standard deviation) in the uncemented total knee arthroplasty group (n=19)

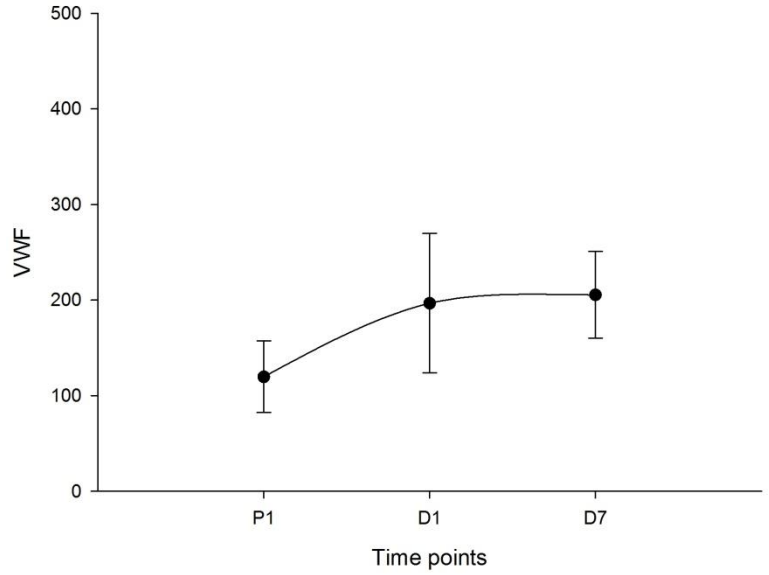


Figure 3.7: Graph of changes in von Willebrand factor levels (IU/dL) over the three time points (mean and standard deviation) in the uncemented total knee arthroplasty group (n=19)

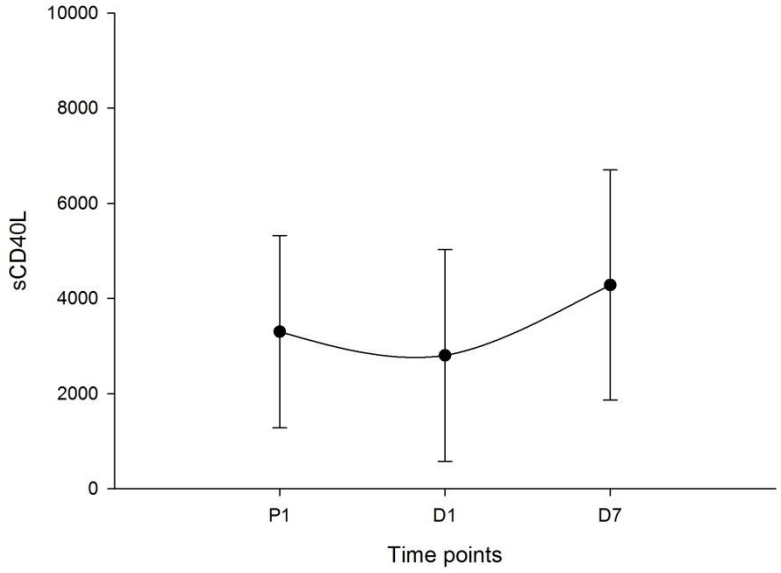


Figure 3.8: Graph of changes in soluble CD40 ligand levels (pg/ml) over the three time points (mean and standard deviation) in the uncemented total knee arthroplasty group (n=19)

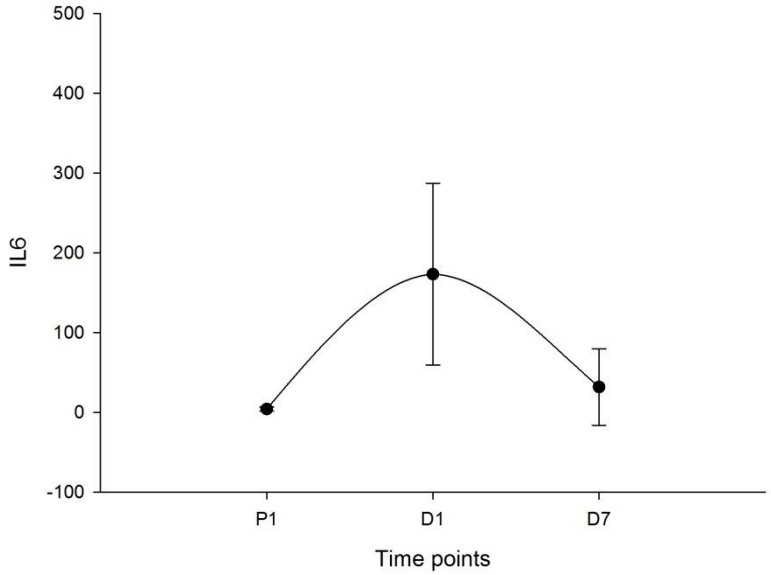


Figure 3.9: Graph of changes in soluble interleukin 6 levels (pg/ml) over the three time points (mean and standard deviation) in the uncemented total knee arthroplasty group (n=19)

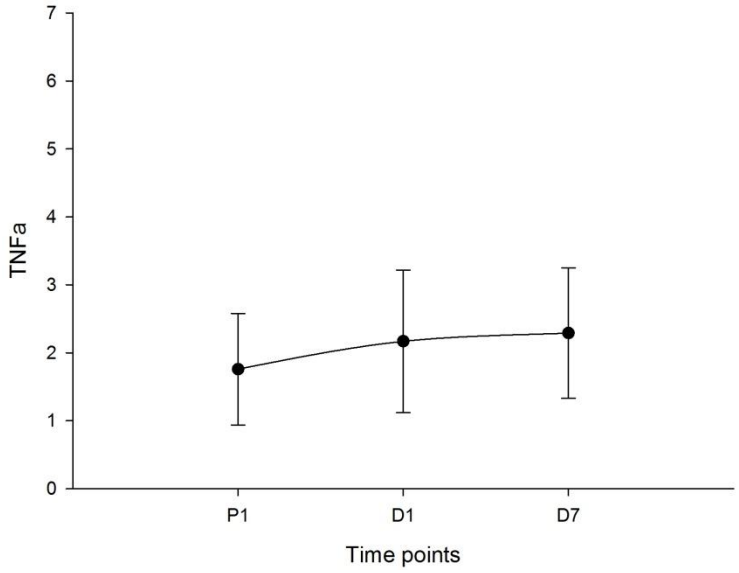


Figure 3.10: Graph of changes in tumour necrosis factor alpha levels (pg/ml) over the three time points (mean and standard deviation) in the uncemented total knee arthroplasty group (n=19)

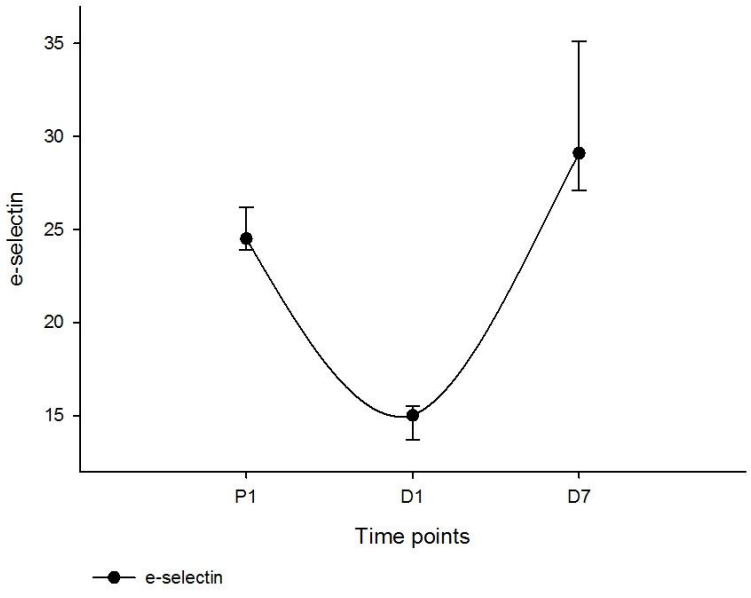


Figure 3.11: Graph of changes in soluble e-selectin levels (ng/ml) over the three time points (median and interquartile ranges) in the uncemented total knee arthroplasty group (n=19)

3.4 Flow cytometry assays of cell cytokine expression

The mean, standard deviations and standard error of means of the CD1442a and CD40 flow cytometry values are summarised in table 3.5. The values are described both in absolute values (number of cell counts) and in percentages.

The flow cytometry readings were also analysed at the three times points using paired sampled t-tests (table 3.6). The analysis was carried using both the absolute values and chi squared tests for percentage values. The only statistically significant change was seen in the CD40 from the pre-operative levels (P1) to the day 1 (D1) post-operative levels when looking at both the absolute numbers and percentage changes ($p= 0.01$ and 0.004 respectively), (Figure 3.12). No significant changes were seen with the CD1442a levels (Figure 3.13).

Table 3.5a: The mean, standard deviation and standard error of means of CD1442a and CD40, expressed as percentage values in the uncemented total knee arthroplasty group (n=19)

	Mean	Standard Deviation	Standard Error Mean
CD1442a percent P1	6.4	6.3	1.4
CD1442a percent D1	6.7	6.1	1.4
CD1442a percent D7	7.6	7.5	1.7
CD40 percent P1	0.8	0.7	0.2
CD40 percent D1	0.3	0.3	0.1
CD40 percent D7	1.2	0.8	0.2

Table 3.5b: Median and Inter-quartile ranges (IQR) of CD1442a and CD40, expressed as absolute values in the uncemented total knee arthroplasty group (n=19)

		Percentiles		
		25th	**50th (Median)**	**75th**
CD1442a	P1	254	456	1362
	D1	185	492	1006
	P7	158	352	1250
CD40	P1	20	50	146
	D1	10	32	54
	P7	62	89	200

Table 3.6a: Mean, standard deviation and p values of CD1442a andCD40 (mean and SD) in the uncemented total knee arthroplasty group (n=19)

		Mean	Standard deviation	P value
CD1442a percentage	P1-D1	-0.4	3.9	0.67
	D1-D7	-0.9	5.6	0.50
	P1-D7	-1.2	5.4	0.33
CD40 percentage	P1-D1	0.4	0.5	0.04
	D1-D7	-0.8	0.6	<0.01
	P1-D7	-0.4	0.7	0.21

Table 3.6b: Wilcoxon signed rank test for CD1442a andCD40 in the uncemented total knee arthroplasty group (n=19)

		P-value
CD1442a	P1-D1	0.33
	D1-D7	0.86
	P1-D7	0.81
CD40	P1-D1	0.01
	D1-D7	0.01
	P1-D7	0.16

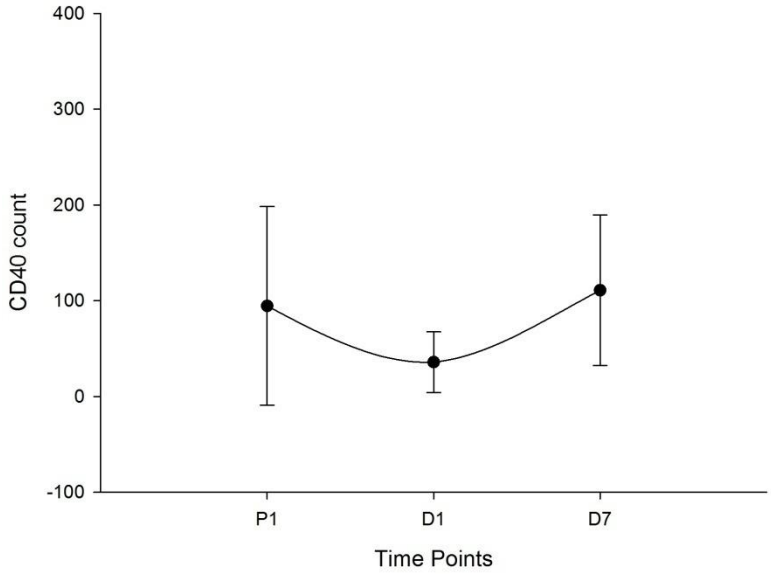


Figure 3.12: Graph of changes in CD40 counts over the three time points (median and interquartile ranges) in the uncemented total knee arthroplasty group (n=19)

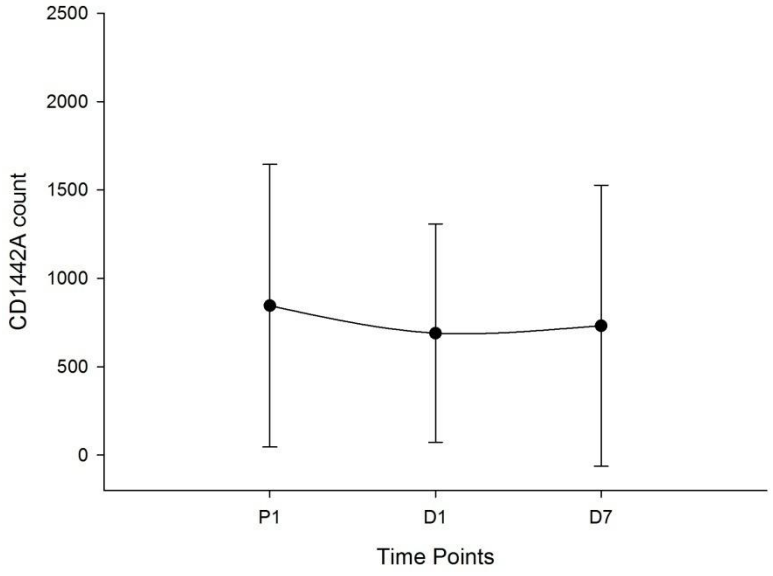


Figure 3.13: Graph of changes in CD1442a counts over the three time points (median and interquartile ranges) in the uncemented total knee arthroplasty group (n=19)

3.5 Discussion

The transient rise in the white cell count, neutrophils, monocytes and platelets supports the existing literature on the acute phase response following surgery (226).

The CRP levels responded mainly in the first 24 hours and even at 7 days remained significantly risen reflecting the ongoing surgical stimulus. This may be predominantly due to the continued high levels of IL6 which remained significantly elevated throughout the 7 days reflecting the ongoing inflammatory process. The IL6 levels reflect the degree of surgical trauma (40) and in most instances the secretion of IL6 is brief and self-limiting lasting from 48-72 hours (41) but this demonstrates that the stimulus of the surgical trauma continues at least up to 7 days post-operatively.

Following the surgical trauma to the tissues and vessels the vascular endothelium becomes procoagulant with the release of several factors including vWF. In response to the thrombus formation t-PA is released from the endothelium (127) and our results reflect this with significant levels of both vWF and t-PA in the first 24 hours with levels remaining significantly elevated to at least 7 days.

The TNFα levels are significant at day 7 possibly reflecting the ongoing inflammatory response and its role in initiating the cytokine cascade, increasing vascular permeability and in recruiting macrophages and neutrophils. The e-selectin levels did not demonstrate a significant change in this study.

The CD40 expression showed a significant change from the pre-operative levels up to day 7 post-operatively. This reflects the release from the endothelium in the vessels within the inflamed tissue in the operative field (84).But the CD1442a changes remained insignificant.

It is not possible to compare these results to the current literature as there has not been a study to date to allow a direct comparison. There have been several studies that have looked at the CRP and IL6 response following trauma (41;227) but these have been in heterogeneous groups comprising of general surgical as well as orthopaedic cases and the orthopaedics cases included trauma as well other elective cases such as ankle fixation and total hip replacements. This study does show a significant rise in the TNFα levels at day 7, the current literature has demonstrated that no significant changes occur but these studies were only carried out on those undergoing a total hip replacement (213;214).

No comparison is possible in terms of the CD40 and CD1442a changes as no study has specifically looked at these following either total hip or total knee replacement.

CHAPTER 4

The effect of elective cemented total knee arthroplasty on the activation of markers of inflammation, endothelium and coagulation

4.1 Subject characteristics

There were 20 patients recruited into this study, one patient was subsequently excluded due to a deep vein thrombosis confirmed on ultrasound leaving 19 patients in this cohort. The patient was excluded as the deep vein thrombosis would have contributed to the inflammatory response. The mean body mass index (BMI) was 30.5kg/m^2, standard deviation 5.2. There were 6 males (mean age 60.3 years, standard deviation 12.74) and 13 females (mean age 70.7 years, standard deviation 8.57). A total of 9 total knee arthroplasties were carried out on the left and 10 on the right. None of these characteristics was significantly different from the uncemented group in Chapter 3.

4.2 Blood cell counts and CRP

The mean values and standard deviations and standard error of mean of white cell count (WCC), neutrophils (Neutro), monocytes (Mono) and C-reactive protein (CRP) are summarised in table 4.1.

Each of these variables were compared over three time periods, from pre-operative values to day 1 post-operation (P1-D1), from day 1 to day 7 post-operation (D1-D7) and from pre-operative to day 7 post-operation (P1-D7), the results of which are summarised in table 4.2. The paired sample t-tests revealed a significant decrease then increase for the platelet count (Figure 4.1) and a significant increase then decrease for the white cell count (Figure 4.2). Neutrophils (Figure 4.3), monocytes (Figure 4.4) and

C-reactive protein (CRP) levels (Figure 4.5) showed a significant rise in the first 24 hour period and remained significantly elevated at day 7.

Table 4.1 : Mean, standard deviations and standard error of means of platelets, white cell count (WCC), neutrophils (Neutro), monocytes (Mono) (all 10^9/L) and C-reactive Protein (CRP) (mg/L) pre-operatively (P1) and on days1 (D1) and day 7 (D7) post-operatively in the cemented total knee arthroplasty group (n=19)

	Mean	Standard Deviation	Standard Error Mean
Plat P1	241	32	8
Plat D1	210	61	14
Plat D7	299	109	26
WCC P1	7.5	1.4	0.3
WCC D1	8.8	2.3	0.5
WCC D7	7.6	1.9	0.4
Neutro P1	5.0	1.7	0.4
Neutro D1	6.3	2.2	0.5
Neutro D7	5.3	1.6	0.4
Mono P1	0.5	0.1	0.03
Mono D1	0.9	0.4	0.1
Mono D7	0.6	0.2	0.05
CRP P1	1.5	1.8	0.4
CRP D1	71	36	9
CRP D7	86	66	15

Table 4.2: Paired sample t-tests of WCC, neutrophils, monocytes and CRP in the cemented total knee arthroplasty group (n=19)

		Mean	Standard Deviation	P value
Platelets	P1-D1	31	54	0.02
	D1-D7	-89	70	<0.01
	P1-D7	-58	97	0.02
WCC	P1-D1	-1.3	2.0	0.01
	D1-D7	1.1	2.4	0.06
	P1-D7	-0.2	1.5	0.66
Neutro	P1-D1	-1.3	2.0	0.01
	D1-D7	1.0	2.4	0.08
	P1-D7	-0.3	1.2	0.36
Mono	P1-D1	-0.4	0.4	<0.01
	D1-D7	0.3	0.4	<0.01
	P1-D7	-0.1	0.2	0.08
CRP	P1-D1	-70	36	<0.01
	D1-D7	-14	71	0.41
	P1-D7	-84.	66	<0.01

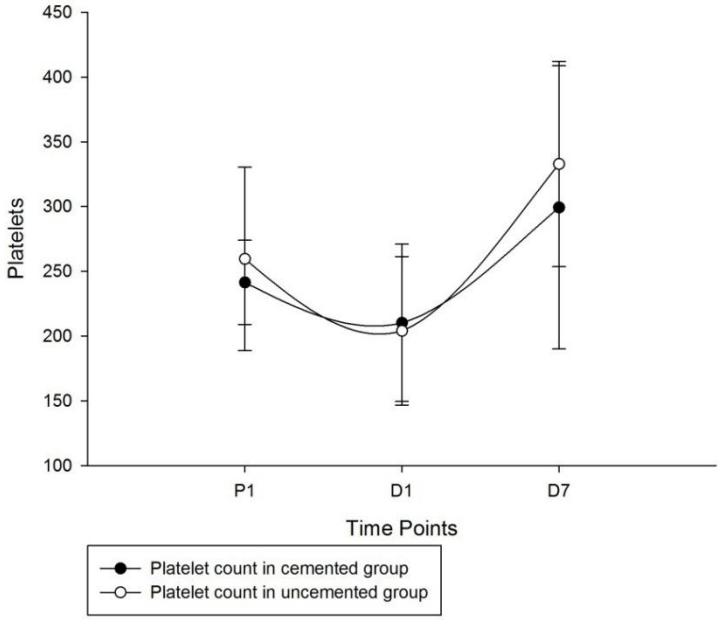


Figure 4.1: Graph of changes in platelet count (x10^9/L) over the three time points (mean and standard deviation) in the cemented total knee arthroplasty group (n=19) against the uncemented total knee arthroplasty group (n=19)

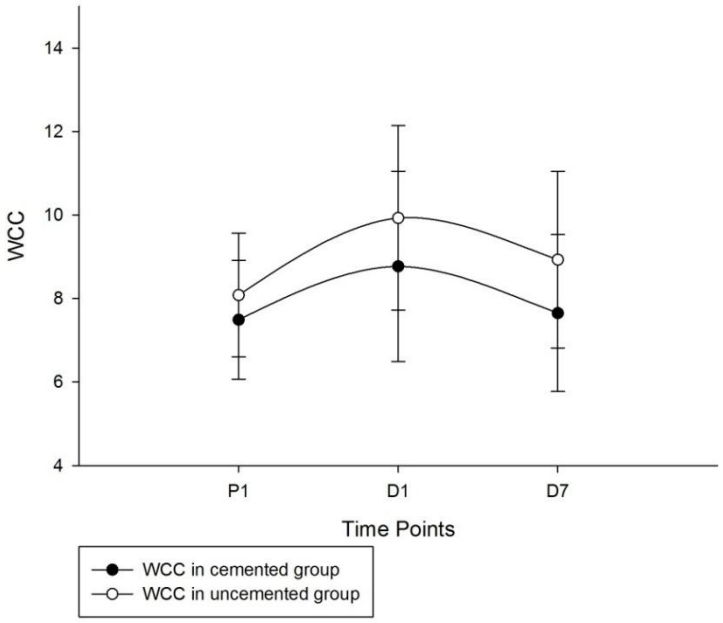


Figure 4.2: Graph of changes in white cell count (x10^9/L) over the three time points (mean and standard deviation) in the cemented total knee arthroplasty group (n=19) against the uncemented total knee arthroplasty group (n=19)

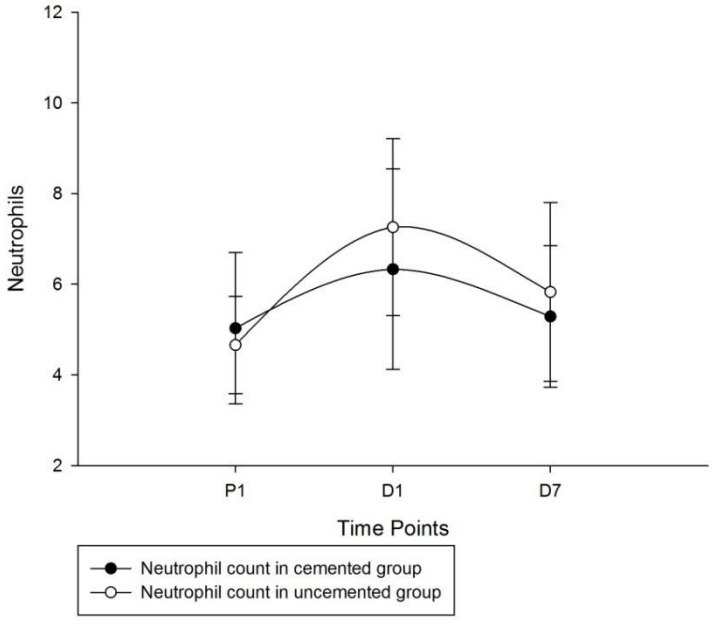


Figure 4.3: Graph of changes in neutrophil count (x10^9/L) over the three time points (mean and standard deviation) in the cemented total knee arthroplasty group (n=19) against the uncemented total knee arthroplasty group (n=19)

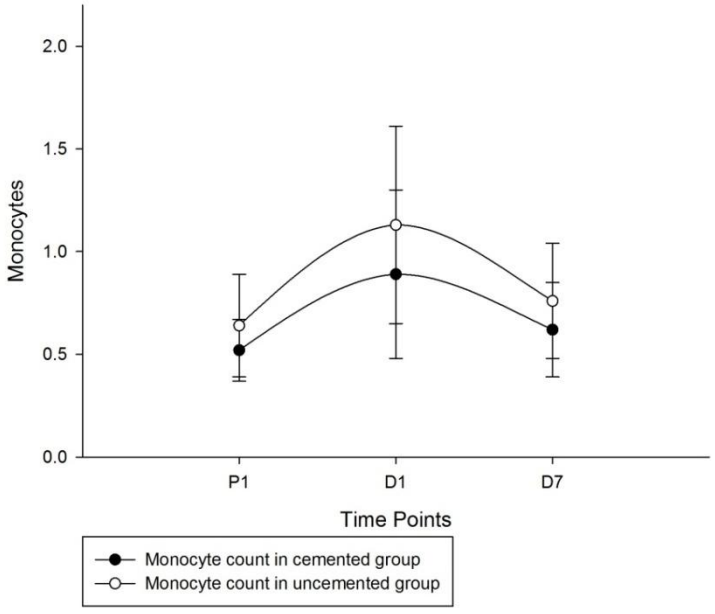


Figure 4.4: Graph of changes in monocyte count (x10^9/L) over the three time points (mean and standard deviation) in the cemented total knee arthroplasty group (n=19) against the uncemented total knee arthroplasty group (n=19)

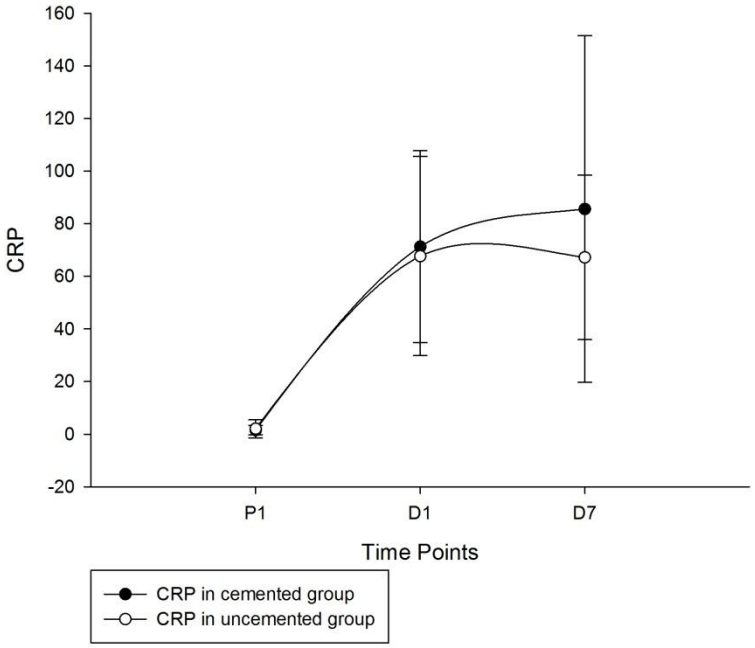


Figure 4.5: Graph of changes in C-reactive protein (mg/L) over the three time points (mean and standard deviation) in the cemented total knee arthroplasty group (n=19) against the uncemented total knee arthroplasty group (n=19)

4.3 Circulating activation markers

The mean, standard deviations and standard error of means of tissue plasminogen activator (t-PA), von Willebrand factor (vWF), soluble CD40 ligand (sCD40L), interleukin 6 (IL6), tumour necrosis factor (TNFα) and e-selectin are summarised in table 4.3.

Each variable was then compared over the three time points (Table 4.4). The t-PA levels showed a non-significant rise initially but did become significant at day 7 post-operatively (p=0.04) (Figure 4.6). The vWF levels showed similar changes becoming significant after 24 hours and remaining so at day 7 (<0.01) (Figure 4.7). The sCD40L levels were significantly lower by 24 hours and remained so at day 7 (Figure 4.8). The IL- 6 levels rose and fell significantly (Figure 4.9). TNFα levels did not initially show a significant rise but by day 7 their levels had significantly risen compared to the pre-operative (Figure 4.10). E-selectin levels fell significantly by day 7 (Figure 4.11).

Table 4.3a: Mean, standard deviations and standard error of means of tissue plasminogen activator (t-PA) (ng/ml), von Willebrand factor (vWF) (IU/dl), soluble CD40 ligand (sCD40L) (pg/ml), interleukin 6 (IL6) (pg/ml) and tumour necrosis factor (TNFα) in the cemented total knee arthroplasty group (n=19)

	Mean	Standard Deviation	Standard Error Mean
t-PA P1	12.3	2.4	0.7
t-PA D1	14.8	5.9	1.9
t-PA D7	17.4	6.9	2.1
vWF P1	153	72	19
vWF D1	187	68	18
vWF D7	233	60	15
sCD40L P1	4243	2594	693
sCD40L D1	3052	2366	632
sCD40L D7	4638	2076	536
IL6 P1	4.5	2.3	0.6
IL6 D1	189.2	160.1	41.3
IL6 D7	61.3	80.0	20.0
TNFα P1	2.7	0.7	0.3
TNFα D1	3.5	1.3	0.6
TNFα D7	3.7	1.0	0.5

Table 4.3b: Median and Inter-quartile ranges (IQR) of e-selectin (ng/ml) in the cemented total knee arthroplasty group (n=19)

		Percentiles		
		25th	**50th (Median)**	**75th**
E-selectin	P1	23.8	35.8	46.5
	D1	21.3	30.3	35.3
	P1	23.88	30.3	34.3

Table 4.4a: Paired sample t-test of tissue plasminogen activator (t-PA) (ng/ml), von Willebrand factor (vWF) (IU/dl), soluble CD40 ligand (sCD40L) (pg/ml), interleukin 6 (IL6) (pg/ml) and tumour necrosis factor (TNFα) (pg/ml) in the cemented total knee arthroplasty group (n=19)

		Mean	Standard Deviation	P value
t-PA	P1-D1	-2.5	5.6	0.20
	D1-D7	-2.8	7.0	0.22
	P1-D7	-4.0	5.4	0.04
vWF	P1-D1	-35	64	0.56
	D1-D7	-47	43	<0.01
	P1-D7	-80	67	<0.01
sCD40L	P1-D1	1191	1939	0.04
	D1-D7	-1543	2031	0.01
	P1-D7	-318	1556	0.46
IL 6	P1-D1	-184.6	159.5	<0.01
	D1-D7	141.8	203.8	0.01
	P1-D7	-56.6	82.9	0.02
TNFα	P1-D1	-0.8	1.1	0.17
	D1-D7	-0.2	1.4	0.72
	P1-D7	-1.0	0.8	0.05

Table 4.4b: Wilcoxon signed rank test for e-selectin (ng/ml) in the cemented total knee arthroplasty group (n=19)

		P-value
E-selectin	P1-D1	0.42
	D1-D7	0.80
	P1-D7	0.18

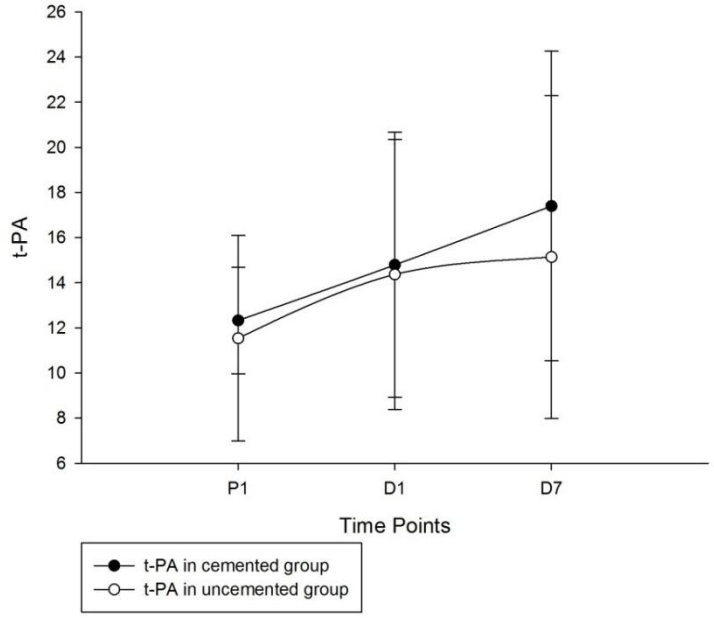


Figure 4.6: Graph of changes in tissue plasminogen activator levels (ng/ml) over the three time points (mean and standard deviation) in the cemented total knee arthroplasty group (n=19) against the uncemented total knee arthroplasty group (n=19)

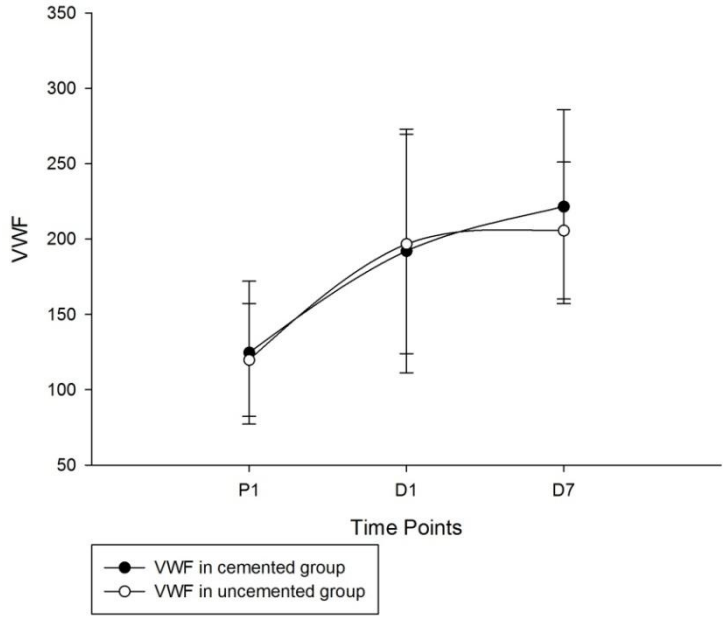


Figure 4.7: Graph of changes in von Willebrand Factor (vWF) (IU/dl) over the three time points (mean and standard deviation) in the cemented total knee arthroplasty group (n=19) against the uncemented total knee arthroplasty group (n=19)

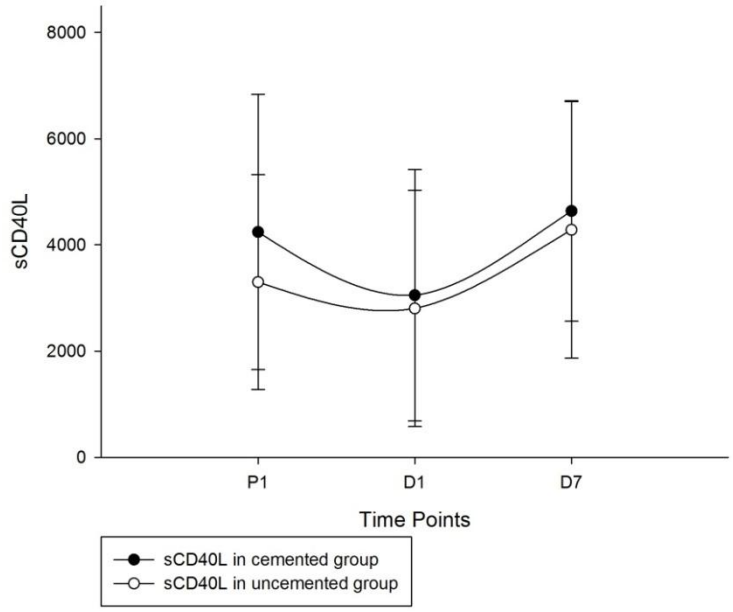


Figure 4.8: Graph of changes in soluble CD40 ligand levels (pg/ml) over the three time points (mean and standard deviation) in the cemented total knee arthroplasty group (n=19) against the uncemented total knee arthroplasty group (n=19)

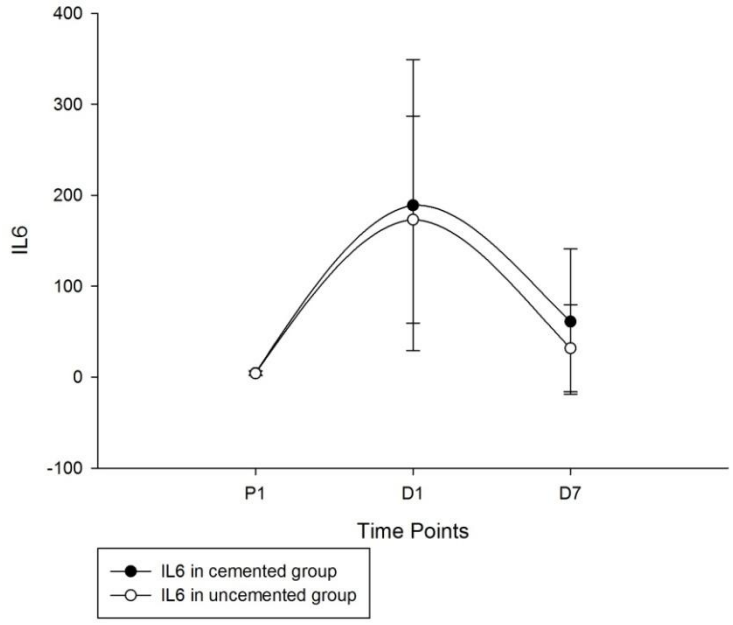


Figure 4.9: Graph of changes in interleukin 6 levels (pg/ml) over the three time points (mean and standard deviation) in the cemented total knee arthroplasty group (n=19) against the uncemented total knee arthroplasty group (n=19)

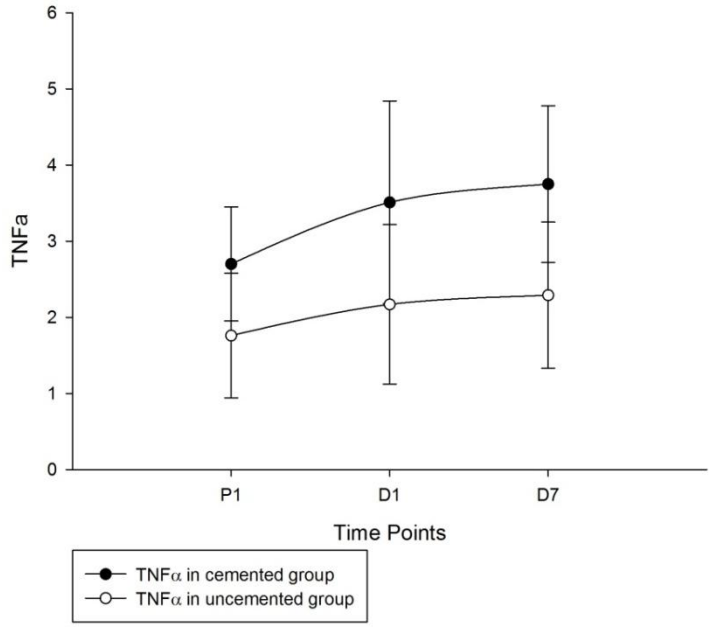


Figure 4.10: Graph of changes in tumour necrosis factor alpha levels (pg/ml) over the three time points (mean and standard deviation) in the cemented total knee arthroplasty group (n=19) against the uncemented total knee arthroplasty group (n=19)

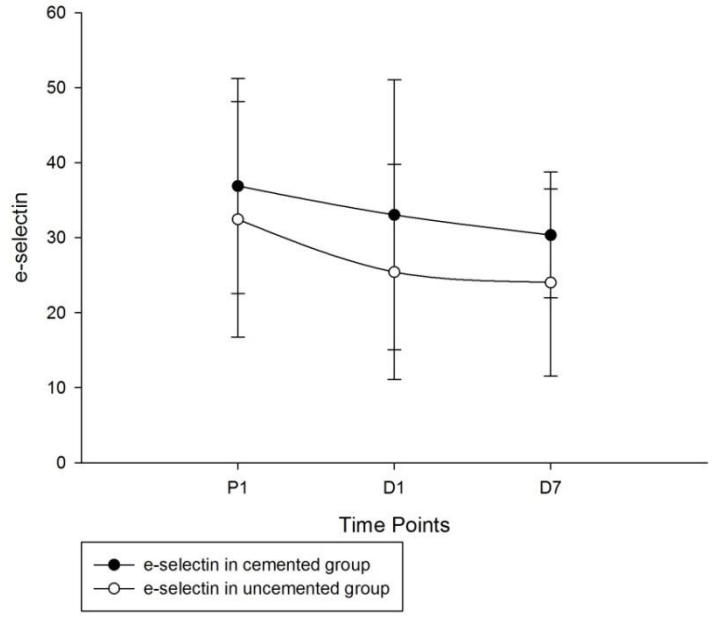


Figure 4.11: Graph of changes in e-selectin levels (ng/ml) over the three time points (median and interquartile ranges) in the cemented total knee arthroplasty group (n=19) against the uncemented total knee arthroplasty group (n=19)

4.4 Flow cytometry assays of cell cytokine expression

The mean, standard deviations and standard error of means of the CD1442a and CD40 flow cytometry values are summarised in table 4.5. The values are described both in absolute values (cell count) and in percentages. The flow results were then analysed to look at changes between the three time points, the results of which are summarised in table 4.6.

The CD1442a counts showed a statistically significant fall in the first 24 hours (p=0.022), then rose by day 7 which is also statistically significant at day 7 and also when compared to the pre-operative levels (p=0.006 and 0.025 respectively) (Figure 4.12). These changes are also mirrored when we look at the percentage changes. The CD40 counts on the other hand showed a significant change from pre-operative to day 1 and from day 1 to day 7, for the absolute values (p=0.01 and 0.04) and the percentage values showed a significant change from day 1 to day 7 only (p=0.027) (Figure 4.13).

Table 4.5a: The mean, standard deviation and standard error means of CD1442a and CD40, expressed as percentage values in the cemented total knee arthroplasty group (n=19)

	Mean	Standard Deviation	Standard Error Mean
CD1442a percent P1	11.0	6.0	1.5
CD1442a percent D1	5.7	5.4	1.4
CD1442a percent D7	16.5	12.0	3.0
CD40 percent P1	1.7	2.4	0.6
CD40 percent D1	0.6	0.5	0.1
CD40 percent D7	1.9	3.2	0.8

Table 4.5b: Median and Inter-quartile ranges (IQR) of CD1442a and CD40, expressed as absolute values in the cemented total knee arthroplasty group (n=19)

		Percentiles		
		25th	**50th (Median)**	**75th**
CD1442a	P1	285.5	1006.5	1455.5
	D1	124.0	296.0	1100.0
	D7	419.2	1547.0	2378.5
CD40	P1	34.2	110.0	183.5
	D1	12.0	52.0	78.0
	D7	20.7	72.0	184.0

Table 4.6a: Mean, standard deviation and p values of CD1442a and CD40 expressed as percentage values in the cemented total knee arthroplasty group (n=19)

		Mean	Standard deviation	P value
CD1442a percentage	P1-D1	5.3	6.0	<0.01
	D1-D7	-11.1	13.0	<0.01
	P1-D7	-5.6	10.3	0.04
CD40 percentage	P1-D1	1.1	2.4	0.09
	D1-D7	-1.3	3.1	0.10
	P1-D7	-0.8	2.9	0.26

Table 4.6b: Wilcoxon signed rank test for CD1442a andCD40 absolute values in the cemented total knee arthroplasty group (n=19)

		P-value
CD1442a	P1-D1	0.02
	D1-D7	0.01
	P1-D7	0.01
CD40	P1-D1	0.01
	D1-D7	0.04
	P1-D7	0.74

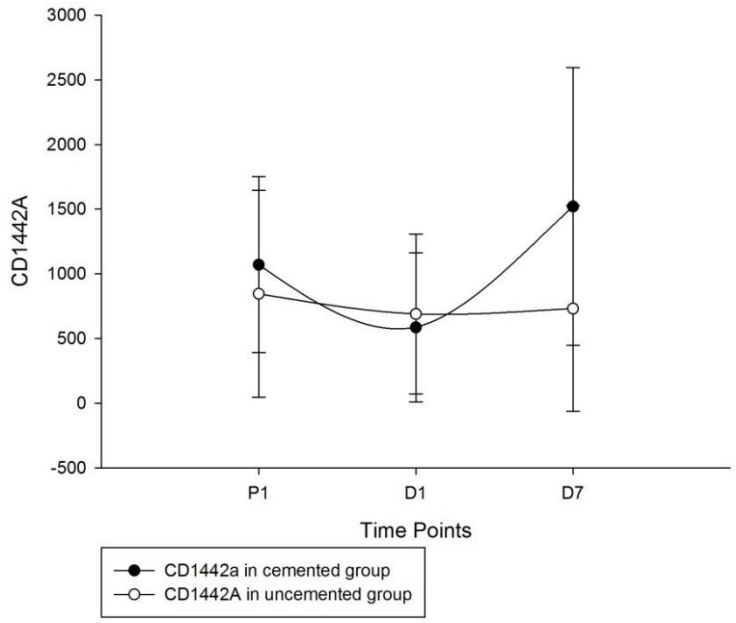


Figure 4.12: Graph of changes in CD1442a counts over the three time points (median and interquartile ranges) in the cemented total knee arthroplasty group (n=19) against the uncemented total knee arthroplasty group (n=19)

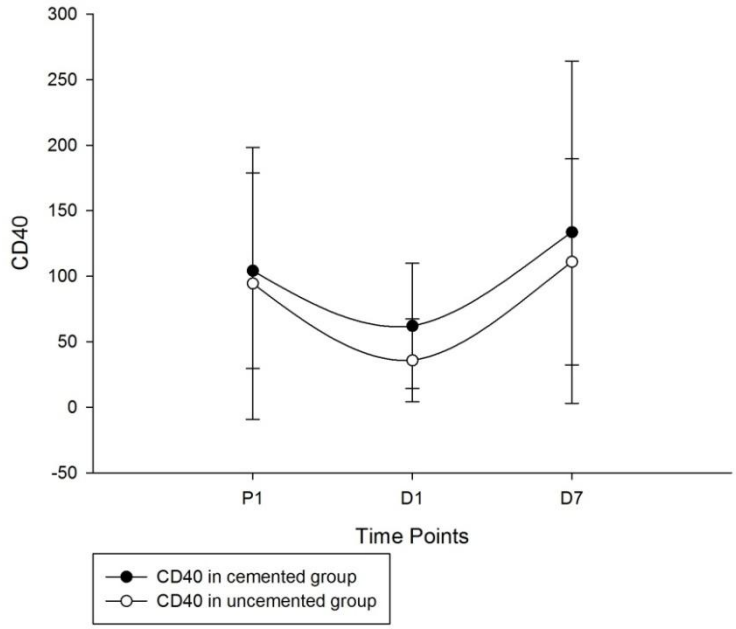


Figure 4.13: Graph of changes in CD40 counts over the three time points (median and interquartile ranges) in the cemented total knee arthroplasty group (n=19) against the uncemented total knee arthroplasty group (n=19)

4.5 Comparison of cemented versus uncemented groups

The groups were compared using independent sample t-test to look for significant differences in the inflammatory and endothelial response with the additional use of cement (polymethylmethacrylate). Table 4.7 below summarises the results.

There was a statistically significant difference in the platelets response between the cemented and uncemented groups with respect to the time period between day 1 and day 7 (D1-D7) (p=0.05). From the table 4.7 we see that for the period from day 1 to day 7 there is less of a rise in the platelet levels in the cemented group. There is also a similar difference in the neutrophil levels which rose less in the cemented group (Figure 4.3). There was also no statistical difference between the two groups with respect to the changes in the white cell count. There was no difference with regards to the monocytes and C-reactive protein changes between the two groups.

Table 4.8 (below) is a summary of the results when we compare the tissue plasminogen activator (t-PA), von Willebrand factor (vWF), soluble CD40 ligand (sCD40L), interleukin 6 (IL6), tumour necrosis factor alpha (TNFα) and e-selectin levels.

None of the variables demonstrated a statistically significant difference between the cemented and uncemented groups.

Comparing the flow cytometry counts between the two groups (Table 4.9) showed a significant difference in the CD1442a levels both for the absolute values and percentage

changes from day 1 to day 7 (D1-D7) (p=0.03 and 0.02 respectively) (Figure 4.12). There were also significant differences in the first 24 hours (P1-D1) when we look at the percentage changes (<0.01). There was no significant difference between the two groups for the CD40 levels.

The only parameter which demonstrated a more significant change in the cemented group compared to that of the uncemented group was that of the CD1442a levels. This showed a more statistically significant change not only in the first 24 hours but also in the period between day 1 to day 7 (<0.01 and p=0.02 respectively).

We can therefore conclude from these results that the use of bone cement does cause a statistically significant rise in CD1442a levels in this group of patients which occurs in the first 24 hours and continues to at least day 7 post-operatively. The exact relevance is not clear as the actual role played by CD1442a is not well understood and is thought to reflect the inflammatory and thrombotic processes or in fact contribute directly to the ongoing atherothrombogenesis (101).

Table 4.7: Mean difference, standard error difference and significance of blood parameters between the cemented total knee arthroplasty group (n=19) and uncemented total knee arthroplasty group (n=19)

		Mean difference	Standard error difference	P value
Platelets	P1-D1	-24	15	0.11
	D1-D7	40	20	0.05
	P1-D7	15	25	0.55
White Cell Count	P1-D1	0.6	0.6	0.39
	D1-D7	0.6	0.7	0.93
	P1-D7	1.1	0.6	0.09
Neutrophils	P1-D1	1.3	0.6	0.48
	D1-D7	-0.4	0.7	0.54
	P1-D7	1.1	0.5	0.03
Monocytes	P1-D1	0.1	0.1	0.40
	D1-D7	-0.8	0.1	0.55
	P1-D7	0.1	0.1	0.76
C-reactive protein	P1-D1	-4	12.	0.71
	D1-D7	-15	20	0.47
	P1-D7	-19	17	0.25

Table 4.8a: Mean difference, standard error difference and significance of tissue plasminogen activator (t-PA), von Willebrand factor (vWF), soluble CD40 ligand (sCD40L0, interleukin 6 (IL6) and tumour necrosis factor alpha (TNFα) levels between the cemented total knee arthroplasty group (n=19) and uncemented total knee arthroplasty group (n=19)

		Mean difference	Standard error difference	P value
t-PA	P1-D1	0.4	2.3	0.87
	D1-D7	-2.0	2.7	0.46
	P1-D7	-0.4	2.1	0.86
vWF	P1-D1	42	24.	0.09
	D1-D7	-37	21	0.09
	P1-D7	2	18	0.91
sCD40L	P1-D1	694	713	0.34
	D1-D7	-63	699	0.93
	P1-D7	664	591	0.27
IL6	P1-D1	-23.9	47.6	0.62
	D1-D7	-4.4	61.7	0.94
	P1-D7	-28.9	24.1	0.24
TNFα	P1-D1	-0.4	0.5	0.42
	D1-D7	-0.1	0.6	0.85
	P1-D7	-0.5	0.3	0.09

Table 4.8b: Mann-Whitney U test for e-selectin (ng/ml) between the cemented total knee arthroplasty group (n=19) and uncemented total knee arthroplasty group (n=19)

		Percentiles			P-value
		25th	**50th (Median)**	**75th**	
E-selectin	P1-D1	-6.3	-4.3	1.5	0.84
	D1-D7	-3.4	0.1	3.6	0.82
	P1-D7	-9.9	-2.7	1.9	0.84

Table 4.9a: Mean difference, standard error difference and significance of CD1442a and CD40 percentage values between the cemented total knee arthroplasty group (n=19) and uncemented total knee arthroplasty group (n=19)

		Mean difference	Standard error difference	P value
CD1442a	P1-D1	275	235	0.25
	D1-D7	-736	322	0.03
	P1-D7	-460	278	0.11
CD1442a percentage	P1-D1	5.1	1.7	<0.01
	D1-D7	-8.3	3.4	0.02
	P1-D7	-3.1	2.8	0.26
CD40	P1-D1	43	59	0.46
	D1-D7	24	33	0.47
	P1-D7	67	72	0.35
CD40 percentage	P1-D1	0.7	0.5	0.23
	D1-D7	-0.2	0.7	0.73
	P1-D7	0.4	0.9	0.64

Table 4.9b: Mann-Whitney U test for CD1442a and CD40 absolute values between the cemented total knee arthroplasty group (n=19) and uncemented total knee arthroplasty group (n=19)

		Percentiles			P-value
		25th	50th (Median)	75th	
CD1442a	P1-D1	-719	-177	41	0.15
	D1-D7	-112	358	1206	0.04
	P1-D7	-286	171	720	0.07
CD40	P1-D1	-114	-54	-4	0.93
	D1-D7	16	64	169	0.17
	P1-D7	-32	19	130	0.35

4.6 Discussion

This study differs from the previous one in that bone cement (PMMA) was used to 'cement' in the knee prosthesis. We see a rise in the CRP levels which is significant in the first 24 hours as well as at day 7 post-operatively. This is accompanied by a significant rise in IL-6 levels at all three time points which mirrors the response seen in the previous study. When we look at the changes in the t-PA and vWF levels one might have expected a significant rise in the first 24 hours from the surgical trauma and assumed exothermic trauma from the cement to the surrounding tissues and vasculature. But there was not a significant rise in the first 24 hours and the vWF levels only became significant after 24 hours from day 1 to day 7 and remained significantly elevated at day 7 compared to the pre-operative levels. The t-PA levels again did not show a significant rise in the first 24 hours and only became significant at day 7 compared to the pre-operative levels. None of these changes were statistically significant between the uncemented and cemented groups.

The TNFα levels were similar to the previous study in that the levels were not significantly elevated until at day 7. In contrast the e-selectin levels were significant at day 7 which may be attributed to the exothermic reaction of the bone cement and relevant due to e-selectin's role in mediating leucocyte rolling and its role in conversion of rolling to firm adhesion but this was not statistically different to that of the uncemented group.

The sCD40L levels reduced significantly in the first 24 hours occurring earlier than the group without cement which only became significantly reduced after the initial 24

hours. The levels in the cemented group continued to rise significantly from day 1 to day 7 reflecting a more acute rise in its levels possibly due to the cement used. This may also reflect a more acute rise in the CD154 levels.

The release of CD40 from the endothelium rose significantly from day 1 to day 7 as opposed to only the first 24 hours in the uncemented group, reflecting a prolonged and gradual rise in its levels from the exothermic reaction of the bone cement. In contrast to the uncemented group where there was no significant rise in the CD1442a levels at any of the time points, the levels in the cemented group demonstrated a significant rise at all time points. This was the only variable which was statistically different between the uncemented and cemented groups.

Our current understanding of the exact role of CD1442a is not clearly understood. Whether it reflects the inflammatory and thrombotic process or in fact directly contributes to the ongoing atherothrombogenesis (101). Either way it appears that the used of bone cement here has a significant effect on its levels albeit the relevance at this current time is unknown.

A review of the current literature highlights that this is the only study so far to specifically look at the potential effect of polymethylmethacrylate on markers of inflammation, endothelium and coagulation.

CHAPTER 5

Effect of local anaesthetic infiltration intra-operatively on the activation of markers of inflammation, endothelium and coagulation after uncemented total knee arthroplasty

5.1 Subject characteristics

There were 20 patients recruited into this study, 2 patients were excluded one due to wound dehiscence which required to be taken back to theatre for a wound lavage and secondary closure and the other patient fell during rehabilitation sustaining a humeral fracture. Both these patients were excluded due to the potential effects of further tissue trauma and the 'second hit' phenomenom of the humeral fracture on the stress response. The mean body mass index (BMI) was 30.35kg/m^2, standard deviation 6.1. There were 7 males (mean age 62.3 years, standard deviation 12.08) and 11 females (mean age 67.5 years, standard deviation 8.74). A total of 10 total knee arthroplasties were carried out on the left and 8 on the right. None of these characteristics were significantly different from the patients in the uncemented total knee arthroplasty group in Chapter 3.

5.2 Blood cell counts and CRP

The mean values, standard deviations and standard error of means of the white cell count (WCC), neutrophils (Neutro), monocytes (Mono) and C-reactive protein (CRP) are summarised below in table 5.1.

Each of these variables where compared over three time periods, from pre-operative values to day 1 post-operation (P1-D1), from day 1 to day 7 post-operation (D1-D7) and

from pre-operative to day 7 post-operation (P1-D7), the results of which are summarised in table 5.2.

Table 5.1: Mean, standard deviations and standard error of means of white cell count (WCC), neutrophils (Neutro), monocytes (Mono) (all x10^9/L) and C-reactive protein (CRP) (mg/L) pre-operatively (P1) and at day 1 (D1) and day 7 (D7) post-operatively in the uncemented total knee arthroplasty group with local anaesthetic infiltration (n=18)

	Mean	Standard Deviation	Standard Error Mean
Plat P1	276	72	17
Plat D1	218	73	18
Plat D7	314	115	29
WCC P1	7.6	1.7	0.4
WCC D1	8.5	2.2	0.5
WCC D7	8.0	2.4	0.6
Neutro P1	4.7	1.6	0.4
Neutro D1	6.1	2.1	0.5
Neutro D7	5.6	2.1	0.5
Mono P1	0.5	0.2	0.1
Mono D1	0.8	0.3	0.1
Mono D7	0.6	0.2	0.1
CRP P1	3	11.	3
CRP D1	74	62	15
CRP D7	73	30	7

Table 5.2: Paired sample t-test of WCC, Neutrophils, monocytes and CRP in the uncemented total knee arthroplasty group with local anaesthetic infiltration (n=18)

		Mean	Standard Deviation	P value
Platelets	P1-D1	59	58	<0.01
	D1-D7	-94	63	<0.01
	P1-D7	-37	75	0.02
WCC	P1-D1	-0.8	1.7	0.06
	D1-D7	0.4	2.3	0.46
	P1-D7	-0.4	1.6	0.34
Neutro	P1-D1	-1.4	1.9	<0.01
	D1-D7	0.4	2.2	0.51
	P1-D7	-1.0	1.4	0.01
Mono	P1-D1	-0.3	0.2	<0.01
	D1-D7	0.2	0.3	<0.01
	P1-D7	-0.1	-0.2	0.88
CRP	P1-D1	-71	51	<0.01
	D1-D7	3	67	0.88
	P1-D7	-70	30	<0.01

There were significant changes in the platelets at all three time points (Figure 5.1). There was no statistically significant change in the white cell count in any of the three time points, although the time point between P1 and D1 the rise just failed to reach significance (p=0.061) (Figure 5.2). The neutrophils had a significant rise in the first 24 hours (p=0.008) and remained significant elevated at day 7 compared to the pre-operative levels (Figure 5.3). The monocytes had a significant rise in the first 24, then fell at day 7 (p=0.006) (Figure 5.4). The majority of the rise in C-reactive protein was in the first 24 hours (<0.01) and was still significant when compared to the pre-operative levels (Figure 5.5).

There was no significant difference between these patients and those in the uncemented group. The only parameter which differed was the platelet levels between day 1 and day 7 (Table 5.3 below).

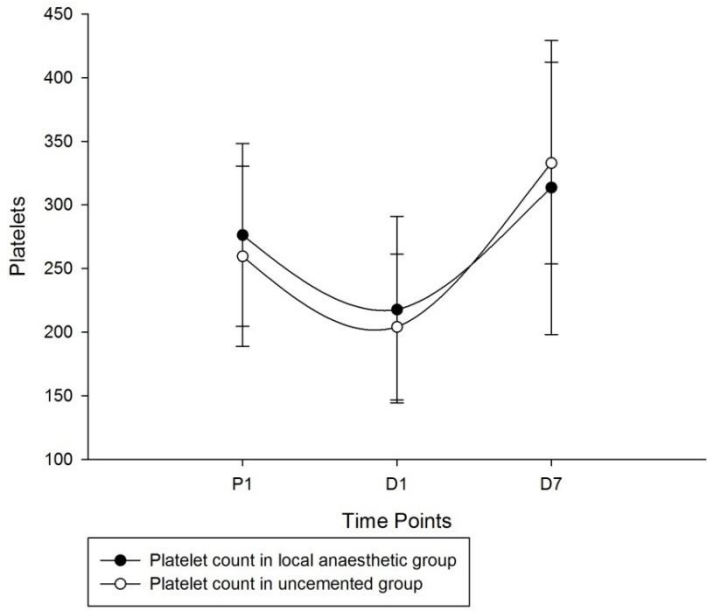


Figure 5.1: Graph of changes in platelet count (x10^9/L) over the three time points (mean and standard deviation) in the uncemented total knee arthroplasty group with local anaesthetic infiltration (n=18) against the uncemented total knee arthroplasty group on its own (n=19)

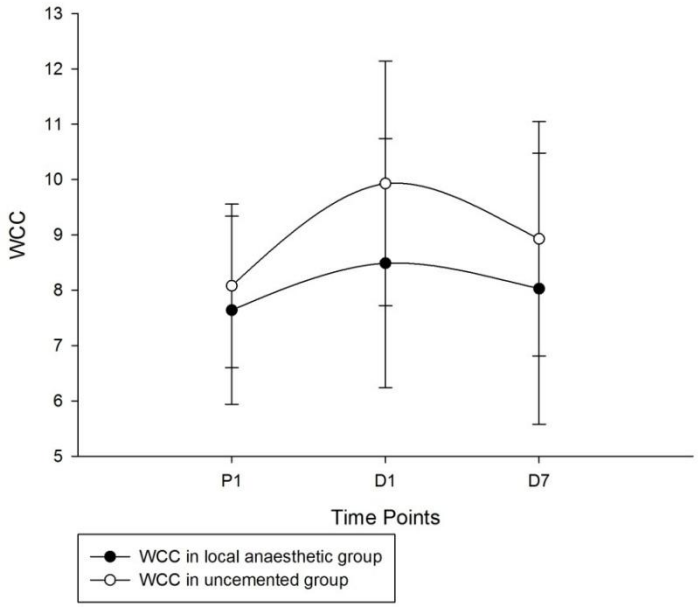


Figure 5.2: Graph of changes in white cell count (x10^9/L) over the three time points (mean and standard deviation) in the uncemented total knee arthroplasty group with local anaesthetic infiltration (n=18) against the uncemented total knee arthroplasty group on its own (n=19)

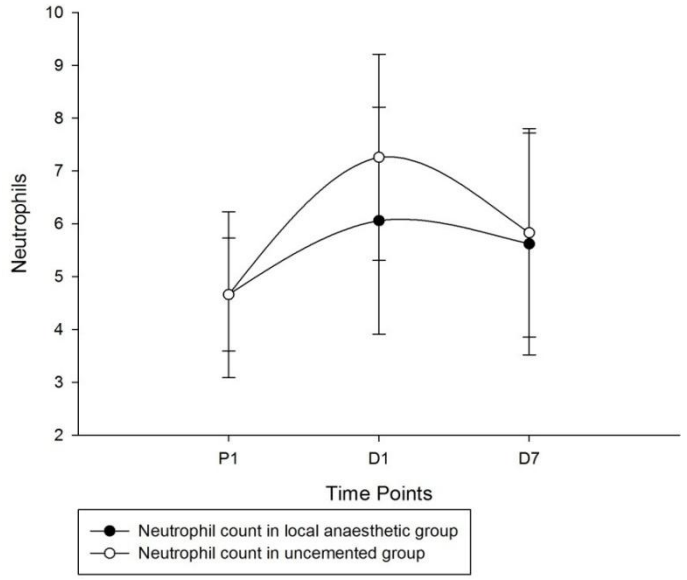


Figure 5.3: Graph of changes in neutrophil count (x10^9/L) over the three time points (mean and standard deviation) in the uncemented total knee arthroplasty group with local anaesthetic infiltration (n=18) against the uncemented total knee arthroplasty group on its own (n=19)

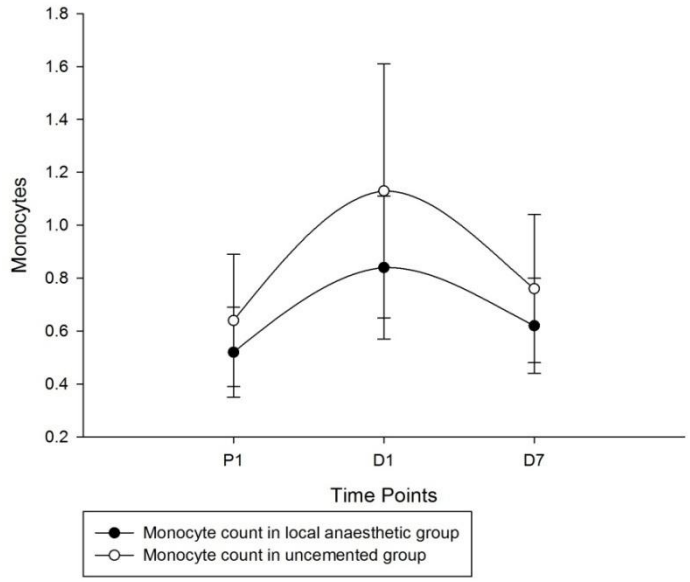


Figure 5.4: Graph of changes in monocyte count (x10^9/L) over the three time points (mean and standard deviation) in the uncemented total knee arthroplasty group with local anaesthetic infiltration (n=18) against the uncemented total knee arthroplasty group on its own (n=19)

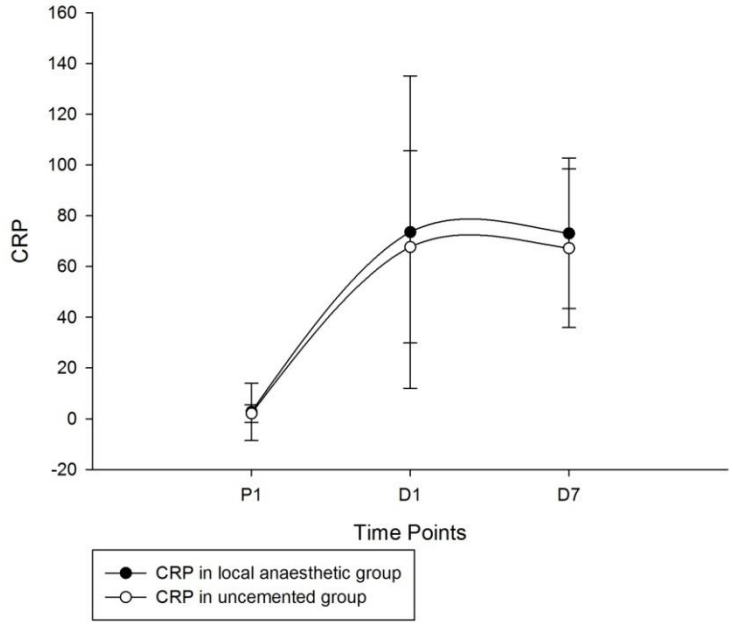


Figure 5.5: Graph of changes in C-reactive protein count (mg/L) over the three time points (mean and standard deviation) in the uncemented total knee arthroplasty group with local anaesthetic infiltration (n=18) against the uncemented total knee arthroplasty group on its own (n=19)

Table 5.3: Mean difference, standard error difference and significance of blood parameters between cemented and uncemented group in the uncemented total knee arthroplasty group with local anaesthetic infiltration (n=18)

		Mean difference	Standard error difference	P value
Platelets	P1-D1	3	16	0.84
	D1-D7	51	24	0.04
	P1-D7	54	32	0.09
White Cell Count	P1-D1	1	0.6	0.11
	D1-D7	0.1	0.8	0.9
	P1-D7	0.9	0.7	0.19
Neutrophils	P1-D1	1.2	0.6	0.06
	D1-D7	-0.7	0.8	0.43
	P1-D7	0.5	0.6	0.38
Monocytes	P1-D1	0.2	0.1	0.12
	D1-D7	-0.1	0.1	0.41
	P1-D7	0.1	0.1	0.45
C-reactive protein	P1-D1	-5	15	0.72
	D1-D7	4	19	0.83
	P1-D7	-1	10	0.91

5.3 Circulating activation markers

The mean, standard deviations and standard error of means of tissue plasminogen activator (t-PA), von Willebrand factor (vWF), soluble CD40 ligand (sCD40L), interleukin 6 (IL6), tumour necrosis factor (TNFα) and e-selectin (median and IQRs) are summarised in table 5.4a & b.

Each variable was then compared over the three time points from pre-operative values to day 1 post-operation (P1-D1), from day 1 to day 7 post-operation (D1-D7) and from pre-operative to day 7 post-operation (P1-D7) (Table 5.5).

The increase in t-PA levels just failed to reach significance in the first 24 hours (p=0.06) but became significant at day 7 (p=0.01) (Figure 5.6). VWF levels were significantly higher at days 1 and 7 (Figure 5.7). The sCD40L levels showed a significant fall in the day 1 (p=0.04) rising by day 7 (p=0.07) (Figure 5.8). IL 6 levels were significantly elevated at day 1 and fell by day 7 (p=0.37) (Figure 5.9). E-selectin was significantly lower at day 1 (p=0.01) (Figure 5.10). TNFα could not be arranged in this study due to exhaustion of laboratory funding. Comparing these results with the uncemented group (Table 5.6) we see that the vWF level from day 1 to day 7 is the only parameter which has shown a statistically significant difference between the two groups.

Table 5.4a: Mean, standard deviations and standard error of means of tissue plasminogen activator (t-PA) (ng/ml), von Willebrand factor (vWF) (IU/dl), soluble CD40 ligand (sCD40L) (pg/ml), interleukin 6 (IL6) (pg/ml) and tumour necrosis factor (TNFα) (pg/ml) in the uncemented total knee arthroplasty group with local anaesthetic infiltration (n=18)

	Mean	Standard Deviation	Standard Error Mean
t-PA P1	11.6	4.1	1.2
t-PA D1	13.5	4.5	1.4
t-PA D7	16.5	6.5	2.0
vWF P1	110	42	10
vWF D1	152	45	11
vWF D7	212	42	10
sCD40L P1	6047	1998	499
sCD40L D1	4583	1926	481
sCD40L D7	6218	2208	570
IL6 P1	6.1	7.0	1.7
IL6 D1	170.8	111.4	27.0
IL6 D7	49.0	77.0	18.7

Table 5.4b: Median and Inter-quartile ranges (IQR) of e-selectin (ng/ml) in the uncemented total knee arthroplasty group with local anaesthetic infiltration (n=18)

		Percentiles		
		25th	**50th (Median)**	**75th**
E-selectin	P1	24.1	33.0	42.0
	D1	23.5	27.2	33.2
	D7	23.3	27.6	32.8

Table 5.5a: Paired sample t-test of tissue plasminogen activator (t-PA), von Willebrand factor (vWF), soluble CD40 ligand (sCD40L), interleukin 6 (IL6) and tumour necrosis factor (TNFα) in the uncemented total knee arthroplasty group with local anaesthetic infiltration (n=18)

		Mean	Standard Deviation	P value
t-PA	P1-D1	-1.9	3.0	0.06
	D1-D7	-3.0	6.2	0.14
	P1-D7	-4.9	5.6	0.01
vWF	P1-D1	-42	27	<0.01
	D1-D7	-60	32	<0.01
	P1-D7	-101	26	<0.01
sCD40L	P1-D1	1464	2644	0.04
	D1-D7	-1614	3195	0.07
	P1-D7	-240	2734	0.74
IL6	P1-D1	-164.6	109.9	<0.01
	D1-D7	121.7	112.9	<0.01
	P1-D7	-42.9	77.9	0.37

Table 5.5b: Wilcoxon signed rank test for e-selectin (ng/ml) in the uncemented total knee arthroplasty group with local anaesthetic infiltration (n=18)

		P-value
E-selectin	P1-D1	0.01
	D1-D7	0.28
	P1-D7	0.29

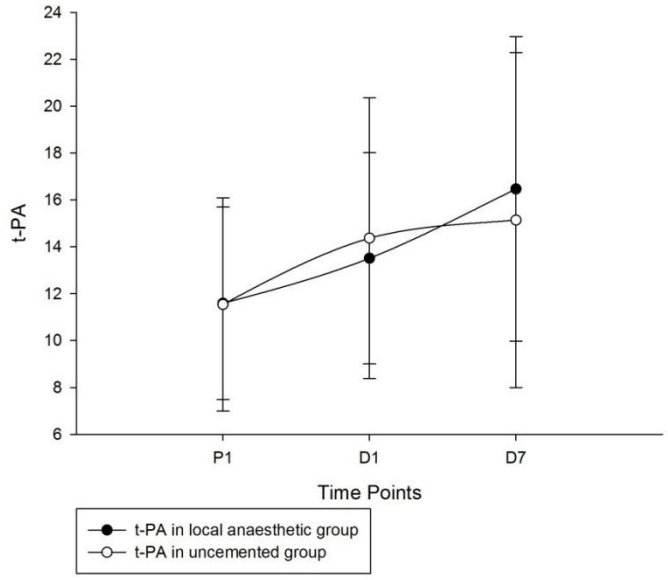


Figure 5.6: Graph of changes in tissue plasminogen activator level (ng/ml) over the three time points (mean and standard deviation) in the uncemented total knee arthroplasty group with local anaesthetic infiltration (n=18) against the uncemented total knee arthroplasty group on its own (n=19)

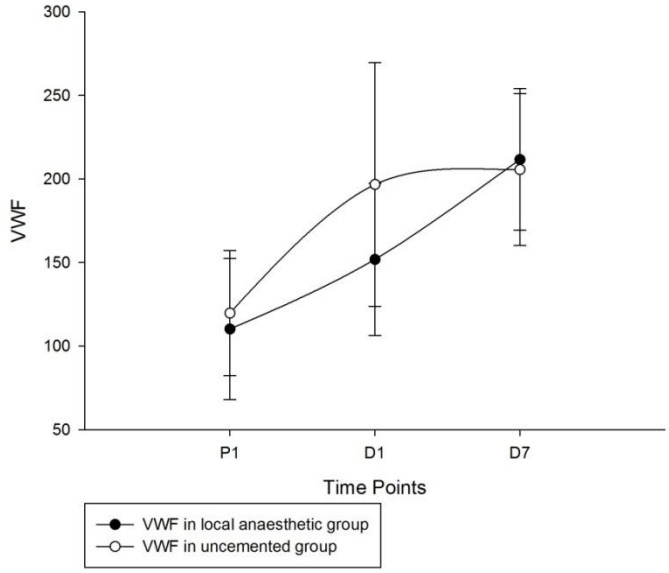


Figure 5.7: Graph of changes in von Willebrand Factor level (IU/dl) over the three time points (mean and standard deviation) in the uncemented total knee arthroplasty group with local anaesthetic infiltration (n=18) against the uncemented total knee arthroplasty group on its own (n=19)

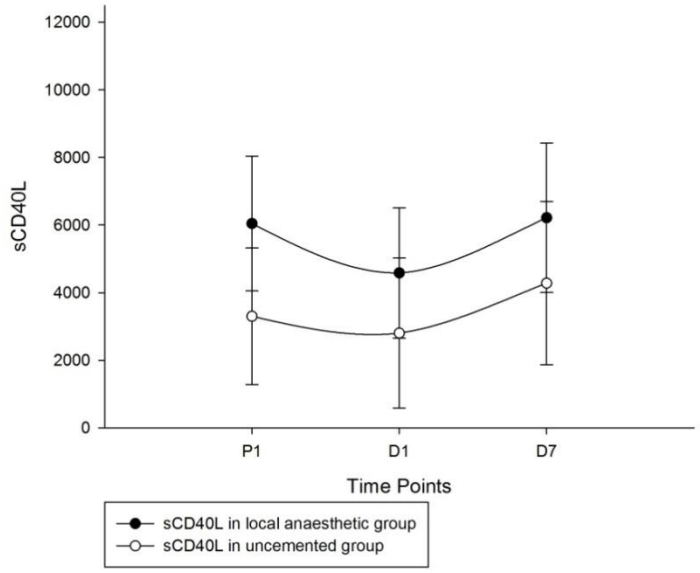


Figure 5.8: Graph of changes in soluble CD40 ligand levels (pg/ml) over the three time points (mean and standard deviation) in the uncemented total knee arthroplasty group with local anaesthetic infiltration (n=18) against the uncemented total knee arthroplasty group on its own (n=19)

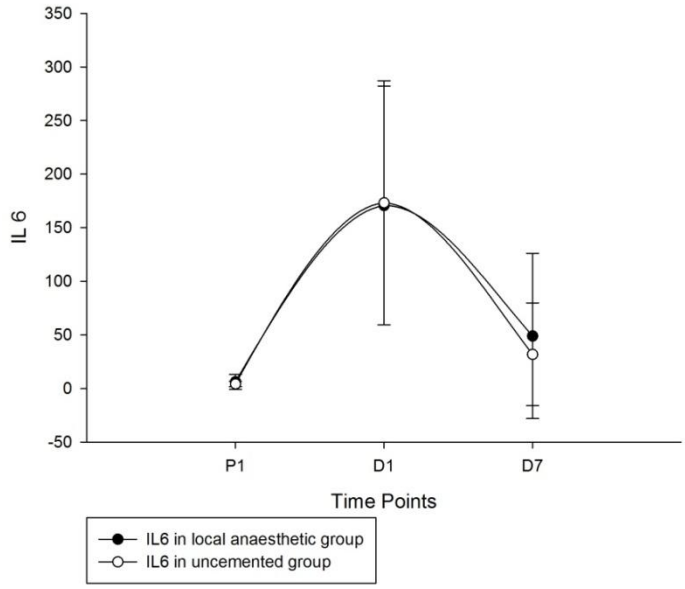


Figure 5.9: Graph of changes in interleukin 6 levels (pg/ml) over the three time points (mean and standard deviation) in the uncemented total knee arthroplasty group with local anaesthetic infiltration (n=18) against the uncemented total knee arthroplasty group on its own (n=19)

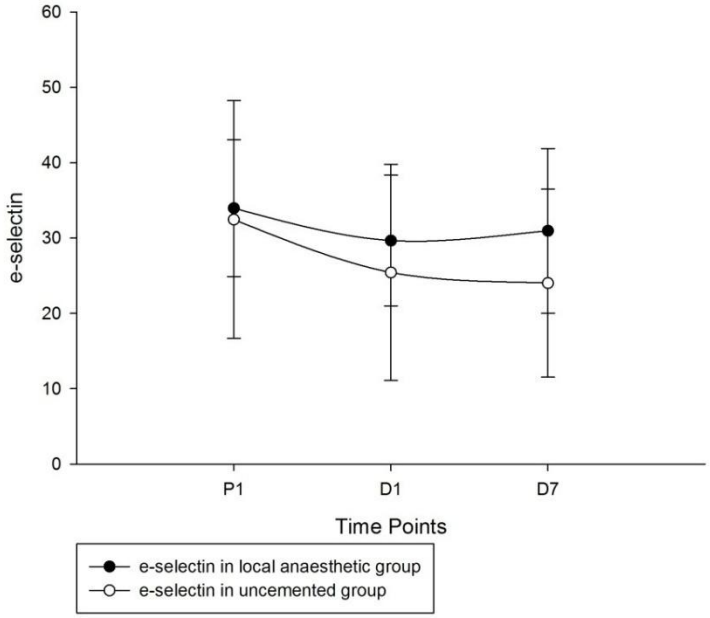


Figure 5.10: Graph of changes in e-selectin levels (ng/ml) over the three time points (median and interquartile ranges) in the uncemented total knee arthroplasty group with local anaesthetic infiltration (n=18) against the uncemented total knee arthroplasty group on its own (n=19)

5.4 Flow cytometry assays of cell cytokine expression

The mean, standard deviations and standard error of means of the CD1442a and CD40 flow cytometry values are summarised in table 5.6 a & b. The values are described both in absolute values (cell counts) and in percentages.

The flow results were then analysed to look at changes between the three time points, the results of which are summarised in table 5.7a & b.

The CD1442a count fell significantly in the first 24 hours and rose significantly in the time period between day 1 and day 7 ($p=0.04$, <0.01) but was not significantly elevated at day 7 compared to the pre-operative levels (Figure 5.11). This is reflected when we look at percentage changes over the same time period ($p=0.01$ and $p=0.05$ respectively). The CD40 counts on the other hand decreased significantly in the first 24 hour period (P1-D1) with respect to both the absolute values and percentage changes ($p=0.00$ and 0.01 respectively) (Figure 5.12) and then rose significantly to day 7 ($p=0.01$)

The CD1442a levels fell significantly and then rose significantly at day 7 compared to the patients in the uncemented group (Table 5.10a &b).

Table 5.6a: The mean, standard deviation and standard error means of CD1442a and CD40, expressed as percentage values in the uncemened total knee arthroplasty group with local anaesthetic infiltration (n=18)

	Mean	Standard Deviation	Standard Error Mean
CD1442a percent P1	12.1	5.7	1.4
CD1442a percent D1	9.1	6.9	1.7
CD1442a percent D7	17.0	8.9	2.2
CD40 percent P1	1.8	0.8	0.2
CD40 percent D1	0.9	0.7	0.2
CD40 percent D7	8.2	14.8	3.8

Table 5.6b: Median and Inter-quartile ranges (IQR) of CD1442a and CD40, expressed as absolute values in the uncemened total knee arthroplasty group with local anaesthetic infiltration (n=18)

		Percentiles		
		25th	**50th (Median)**	**75th**
CD1442a	P1	897	1232	1578
	D1	185	758	1231
	P1	939	1915	2508
CD40	P1	121	168	236
	D1	48	67	116
	D7	132	240	362

Table 5.7a: Mean, standard deviation and p values of CD1442a and CD40 expressed as percentage values in the total knee arthroplasty group with local anaesthetic infiltration (n=18)

		Mean	Standard deviation	P value
CD1442a percentage	P1-D1	3.8	8.0	0.05
	D1-D7	-7.9	11.4	0.01
	P1-D7	-4.0	9.0	0.10
CD40 percentage	P1-D1	0.8	1.0	<0.01
	D1-D7	-7.4	14.4	0.06
	P1-D7	-6.9	14.9	0.10

Table 5.7b: Wilcoxon signed rank test for CD1442a and CD40 absolute values in the total knee arthroplasty group with local anaesthetic infiltration (n=18)

		P-value
CD1442a	P1-D1	0.03
	D1-D7	0.01
	P1-D7	0.07
CD40	P1-D1	0.01
	D1-D7	0.03
	P1-D7	0.01

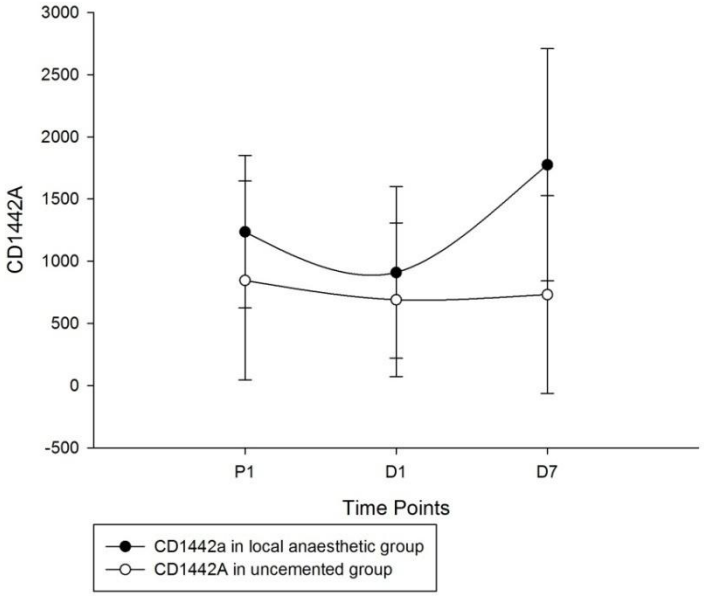


Figure 5.11: Graph of changes in CD1442a counts over the three time points (median and interquartile ranges) in the uncemented total knee arthroplasty group with local anaesthetic infiltration (n=18) against the uncemented total knee arthroplasty group on its own (n=19)

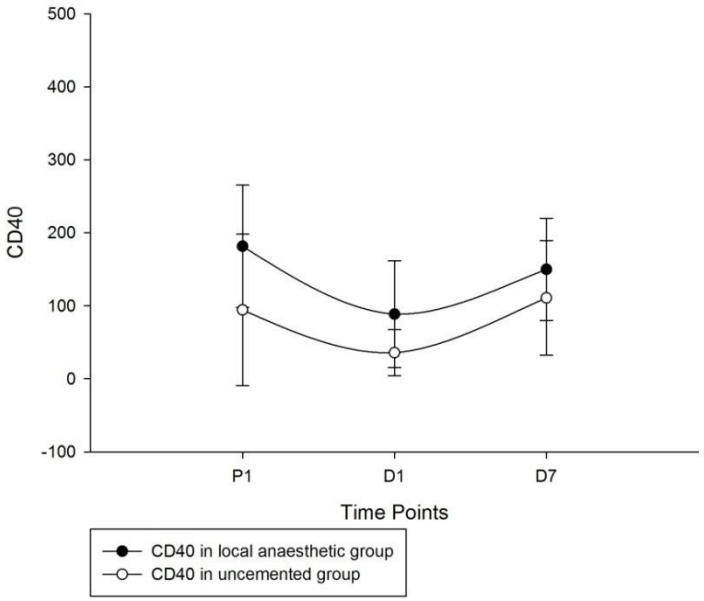


Figure 5.12: Graph of changes in CD40 counts over the three time points (median and interquartile ranges) in the uncemented total knee arthroplasty group with local anaesthetic infiltration (n=18) against the uncemented total knee arthroplasty group on its own (n=19)

5.5 Uncemented versus local anaesthetic infiltration group to assess whether the local anaesthetic would dampen the inflammatory and endothelial response

The groups were compared to look for significant differences in the inflammatory and endothelial response with the additional use of local anaesthetic infiltration intra-operatively into the joint capsule and surrounding soft tissues (40ml 0.25% Chirocaine with 10ml 1:100 000 adrenaline). The table below summarises the results (Table 5.8).

When we compare the blood parameters we see that there is a significant difference between the uncemented group and the group which received the local anaesthetic infiltration with reference to the changes in the platelet counts from day 1 to day 7 ($p=0.04$) (Figure 5.13), here the platelets levels rose less in the local anaesthetic group. The changes in the neutrophil counts in the first 24 hours (P1-D1) just failed to reach significance ($p=0.06$). There was no significant difference with regards to the white cell count and C-reactive protein.

Table 5.8: Mean difference, standard error difference and significance of blood parameters between the uncemented total knee arthroplasty group (n=19) and the uncemented total knee arthroplasty group with local anaethetic infiltration (n=18)

		Mean difference	Standard error difference	Significance
Platelets	P1-D1	3	16	0.84
	D1-D7	51	24	0.04
	P1-D7	54	31	0.09
White Cell Count	P1-D1	1	0.6	0.11
	D1-D7	0.1	0.8	0.9
	P1-D7	0.9	0.7	0.19
Neutrophils	P1-D1	1.2	0.6	0.06
	D1-D7	-0.7	0.8	0.43
	P1-D7	0.5	0.6	0.38
Monocytes	P1-D1	0.2	0.1	0.12
	D1-D7	-0.1	0.1	0.41
	P1-D7	0.1	0.1	0.45
C-reactive protein	P1-D1	-5	15	0.72
	D1-D7	4	19	0.83
	P1-D7	-1	10	0.91

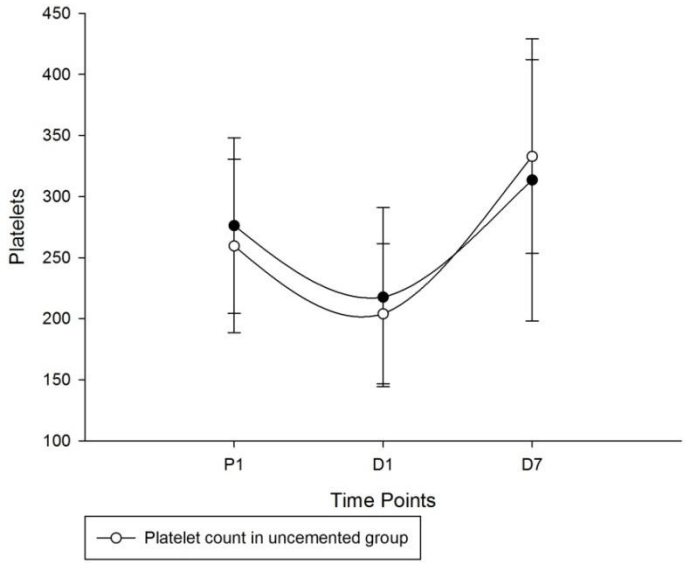


Figure 5.13: Graph of changes in platelet count (x10^9/L) over the three time points (mean and standard deviation) in the uncemented total knee arthroplasty group with local anaesthetic infiltration (n=18) against the uncemented total knee arthroplasty group on its own (n=19)

The table below (table 5.9a & b) is a summary of the results when we compare the tissue plasminogen activator (t-PA), von Willebrand factor (vWF), soluble CD40 ligand (sCD40L0, interleukin 6 (IL-6) and e-selectin levels.

The only variable to show a significant difference was the levels of von Willebrand factor from day 1 to day 7 when comparing between the two groups (p=0.01) here the levels rose significantly less than the uncemented group (Figure 5.7)

The results of the flow cytometry are outlined in table 5.11. There were significant differences between the two groups with regards to the CD1442a counts for both the absolute values and percentage changes up to day 7 (Figure 5.11). From the figure below we see that the CD1442a levels fall and then rise more significantly than the uncemented group.

Table 5.9a: Mean difference, standard error difference and significance of tissue plasminogen activator (t-PA), von Willebrand factor (vWF), soluble CD40 ligand (sCD40L0, interleukin 6 (IL6) and tumour necrosis factor alpha (TNFα) levels between the uncemented total knee arthroplasty group with local anaesthetic infiltration (n=18) and the uncemented total knee arthroplasty group on its own (n=19)

		Mean difference	Standard error difference	P value
t-PA	P1-D1	0.9	2.0	0.65
	D1-D7	-2.2	2.6	0.40
	P1-D7	-1.3	2.1	0.54
vWF	P1-D1	35	18	0.06
	D1-D7	-51	18	0.01
	P1-D7	-19	11	0.09
sCD40L	P1-D1	966	806	0.24
	D1-D7	-135	908	0.88
	P1-D7	742	784	0.35
IL6	P1-D1	41.5	59.8	0.49
	D1-D7	-24.4	44.0	0.58
	P1-D7	30.3	53.4	0.57

Table 5.9b: Mann-Whitney U test for e-selectin (ng/ml) between the uncemented total knee arthroplasty group with local anaesthetic infiltration (n=18) and the uncemented total knee arthroplasty group on its own (n=19)

		Percentiles			P-value
		25th	**50th (Median)**	**75th**	
E-selectin	P1-D1	-6.3	-4.3	1.5	0.27
	D1-D7	-3.4	0.1	3.6	0.27
	P1-D7	-9.9	-2.7	1.9	0.69

Table 5.10a: Mean difference, standard error difference and significance of CD1442a and CD40 percentage values between the uncemented total knee arthroplasty group (n=19) and the uncemented total knee arthroplasty group with local anaethetic infiltration (n=18)

		Mean difference	Standard error difference	P value
CD1442a percentage	P1-D1	4.2	2.0	0.05
	D1-D7	-7.0	3.0	0.02
	P1-D7	-2.7	2.5	0.28
CD40 percentage	P1-D1	0.4	0.3	0.15
	D1-D7	-4.2	4.2	0.32
	P1-D7	-3.9	4.2	0.36

Table 5.10b: Mann-Whitney U test for CD1442a and CD40 absolute values between the uncemented total knee arthroplasty group (n=19) and the uncemented total knee arthroplasty group with local anaethetic infiltration (n=18)

		Percentiles			P-value
		25th	50th (Median)	75th	
CD1442a	P1-D1	-719	-177	41	0.18
	D1-D7	-112	358	1206	0.02
	P1-D7	-286	171	720	0.06
CD40	P1-D1	-114	-54	-4	0.07
	D1-D7	16	64	169	0.22
	P1-D7	-32	19	130	0.33

5.6 Discussion

This is the first study to look specifically at the effects of local anaesthetic infiltration intra-operatively on the activation of inflammatory, endothelium and coagulation markers. The hypothesis of this study was that the local infiltration of local anaesthetic would attenuate the inflammatory response (49).

The only variables which were statistically significant when compared to the uncemented group were the platelets, von Willebrand factor and the CD1442a. The platelets showed a significant difference from day 1 to day 7 whereas the von Willebrand factor was significant from pre-operative levels to day 1 and from day 1 to day 7. This difference may in part be due to the possible further local surgical trauma from the infiltration of the relatively large volume of local anaesthetic into the peri-operative field. But as we will see in Chapter 7 these changes in the platelets and von Willebrand factor are not significant in the context of all the four groups together.

In the anaesthetic group we again see a significant change in the CD1442a levels both over the first 24 hours and from day 1 to day 7 which is not reflected in the uncemented group. This is similar to the result we see when we compared the uncemented and cemented group. The significance as noted before is unclear, whether this reflects the thrombotic and inflammatory process or the process of atherothrombogenesis. It may reflect the thrombotic process as we see a significant difference in the vWF levels in the local anaesthetic group not seen in the uncemented group. One possible reason is that in the first 24 hours the levels may have been reduced by the use of local

anaesthetic to a level so that as their levels rose back up again it became significant. There is no difference as regards the changes in the CD40 level changes.

CHAPTER 6

The effect of using of a drain post-operatively on the activation of markers of inflammation, endothelium and coagulation after uncemented total knee arthroplasty

6. Results

There were 20 patients recruited into this study, 2 patients were excluded from the study due to superficial wound infections, leaving 18 patients. Both of the excluded patients had positive wound cultures. One was successfully treated with antibiotics but the other patient required a secondary procedure in the form of a wound wash-out. These patients were excluded due to possible effects of the wound infection and wound wash-out would have on the inflammatory response. The mean body mass index (BMI) was 28.37kg/m^2 with a standard deviation of 6.1. There were 6 males (mean age 70.3 years, standard deviation 3.98) and 12 females (mean age 64 years, standard deviation 10.32). A total of 6 total knee arthroplasties were carried out on the left and 12 on the right. None of these characteristics was significantly different from the patients in the uncemented group in Chapter 3.

6.1 Blood cell counts and CRP

The mean values, standard deviation and standard error of means of the white cell count (WCC), neutrophils (Neutro), monocytes (Mono) and C-reactive protein (CRP) are summarised below in table 6.1. Each of those variables were compared over three time periods, from pre-operative values to day 1 post-operation (P1-D1), from day 1 to day 7 post-operation (D1-D7) and from pre-operative to day 7 post-operation (P1-D7), the results of which are summarised in table 6.2.

The platelet count fell then rose significantly (Figure 6.1). The white cell count rose then fell significantly (Figure 6.2) but the neutrophils did not show a significant change (Figure 6.3). The monocytes showed a significant rise in the first 24 hours and were still significantly higher than the pre-operative levels at day 7 ($p=0.00$) (Figure 6.4). C-reactive protein levels (Figure 6.5), which were significantly higher at day 1 and 7.

Table 6.1: Mean, standard deviation and standard error of means of white cell count (WCC), neutrophils (Neutro), monocytes (Mono) (all $x10^9$/L) and C-reactive protein (CRP) (mg/L) at pre-operative (P1), day 1 (D1) and day 7 (D7) post-operatively in the uncemented total knee arthroplasty group with a post-operative surgical drain (n=18)

	Mean	Standard Deviation	Standard Error Mean
Plat P1	241	32	8
Plat D1	210	61	14
Plat D7	299	109	26
WCC P1	248.4	70.4	17
WCC D1	196.4	46.4	11.3
WCC D7	309.6	60.3	14.6
Neutro P1	7.2	1.4	0.4
Neutro D1	8.1	2.1	0.5
Neutro D7	7.7	1.8	0.4
Mono P1	0.5	0.1	0.1
Mono D1	0.8	0.3	0.1
Mono D7	0.6	0.2	0.1
CRP P1	1	3	0.8
CRP D1	69	36	9
CRP D7	69	40	10

Table 6.2: Paired sample t-tests of WCC, neutrophils, monocytes and CRP in the uncemented total knee arthroplasty group with a post-operative surgical drain (n=18)

		Mean	Standard Deviation	P value
Plat	P1-D1	31	54	0.02
	D1-D7	89	70	<0.01
	P1-D7	58	97	0.02
WCC	P1-D1	52.0	34.6	<0.01
	D1-D7	-113.2	49.2	<0.01
	P1-D7	-61.2	57.7	<0.01
Neutro	P1-D1	-0.9	2.1	0.09
	D1-D7	0.5	2.3	0.42
	P1-D7	-0.4	1.4	0.23
Mono	P1-D1	-1.6	2.1	0.01
	D1-D7	0.6	1.9	0.24
	P1-D7	-1.0	1.3	0.01
CRP	P1-D1	-68	37	<0.01
	D1-D7	0.1	61	0.99
	P1-D7	-68	40	<0.01

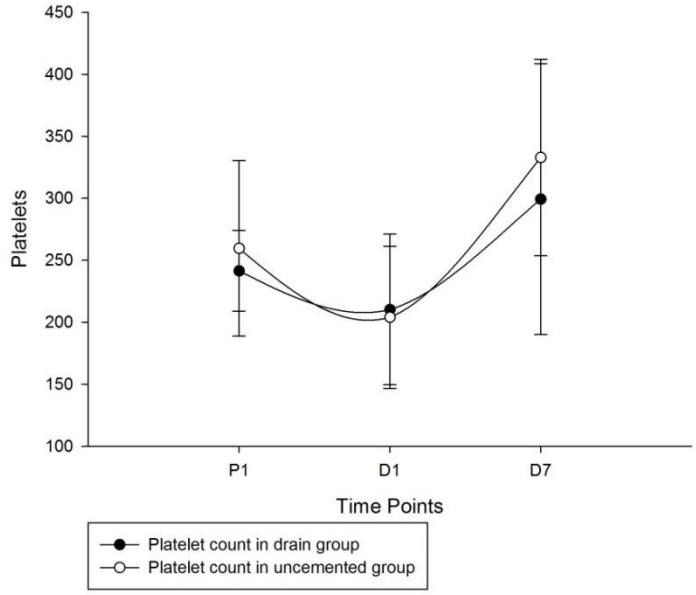


Figure 6.1: Graph of changes in platelet count (x10^9/L) over the three time points (mean and standard deviation) in the uncemented total knee arthroplasty group with a post-operative surgical drain (n=18) against the uncemented total knee arthroplasty group on its own (n=19)

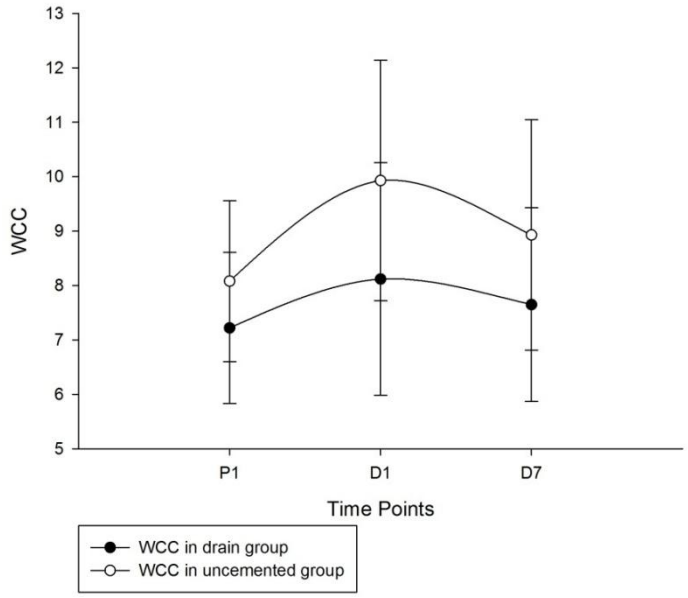


Figure 6.2: Graph of changes in white cell count (x10^9/L) over the three time points (mean and standard deviation) in the uncemented total knee arthroplasty group with a post-operative surgical drain (n=18) against the uncemented total knee arthroplasty group on its own (n=19)

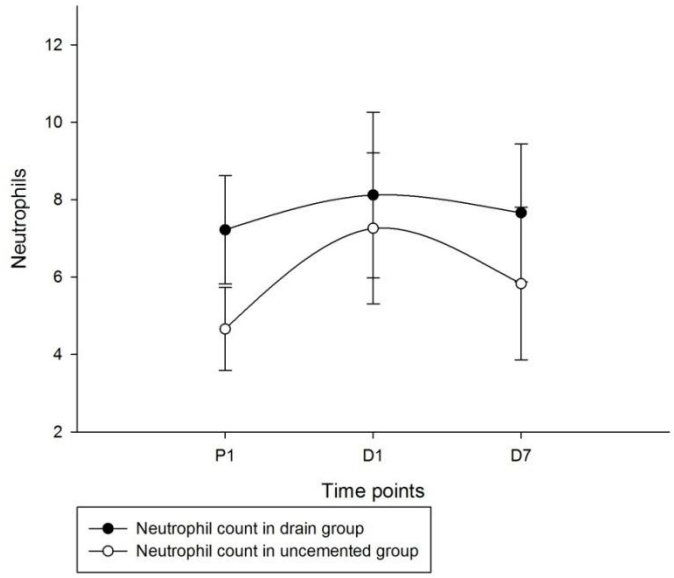


Figure 6.3: Graph of changes in neutrophils count (x10^9/L) over the three time points (mean and standard deviation) in the uncemented total knee arthroplasty group with a post-operative surgical drain (n=18) against the uncemented total knee arthroplasty group on its own (n=19)

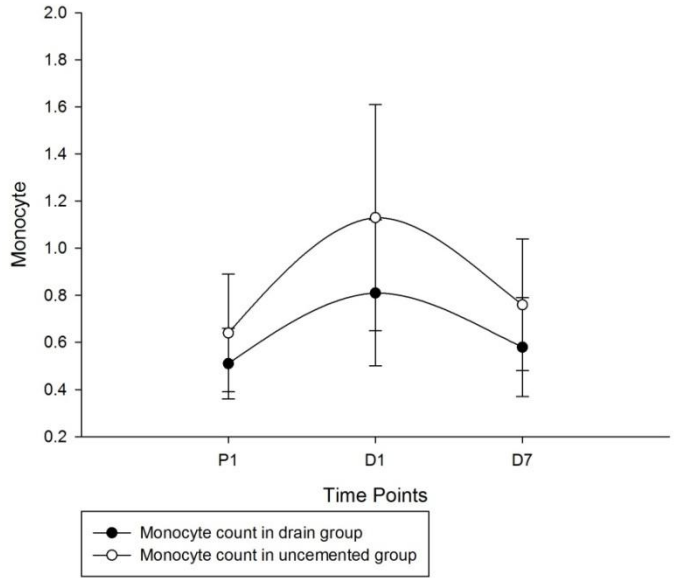


Figure 6.4: Graph of changes in monocyte count (x10^9/L) over the three time points (mean and standard deviation) in the uncemented total knee arthroplasty group with a post-operative surgical drain (n=18) against the uncemented total knee arthroplasty group on its own (n=19)

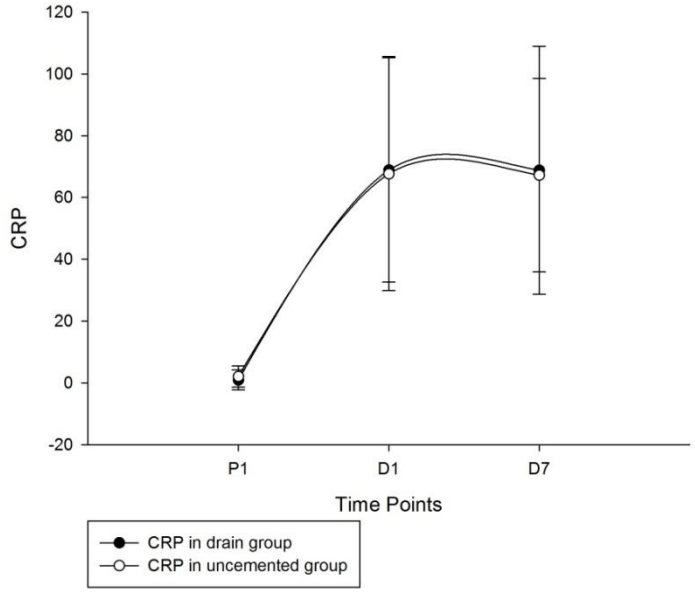


Figure 6.5: Graph of changes in C-reactive protein (mg/l) level over the three time points (mean and standard deviation) in the uncemented total knee arthroplasty group with a post-operative surgical drain (n=18) against the uncemented total knee arthroplasty group on its own (n=19)

6.3 Circulating activation markers

The mean, standard deviations and standard error of means of tissue plasminogen activator (t-PA), von Willebrand factor (vWF), soluble CD40 ligand (sCD40L), interleukin 6 (IL-6), tumour necrosis factor (TNFα) and e-selectin are summarised in table 6.3a & b.

Each variable was then compared over the three time points from pre-operative values to day 1 post-operation (P1-D1), from day 1 to day 7 post-operation (D1-D7) and from pre-operative to day 7 post-operation (P1-D7) (Table 6.4a & b).

Table 6.3a: Mean, standard deviations and standard error of means of tissue plasminogen activator (t-PA) (ng/ml), von Willebrand factor (vWF) (IU/dl), soluble CD40 ligand (sCD40L) (pg/ml), interleukin 6 (IL6) (pg/ml) and tumour necrosis factor (TNFα) (pg/ml) in the uncemented total knee arthroplasty group with a post-operative surgical drain (n=18)

	Mean	Standard Deviation	Standard Error Mean
t-PA P1	13.1	3.6	0.9
t-PA D1	13.5	4.4	1.2
t-PA D7	17.3	7.7	2.0
vWF P1	125	47	12
vWF D1	192	81	21
vWF D7	222	64	17
sCD40L P1	5233	2383	615
sCD40L D1	3605	2106	544
sCD40L D7	6000	2711	699
IL6 P1	3.9	2.0	0.5
IL6 D1	137.8	88.5	22.8
IL6 D7	42.9	84.4	21.8

Table 6.3b: Median and Inter-quartile ranges (IQR) of e-selectin (ng/ml) in the uncemented total knee arthroplasty group with a post-operative surgical drain (n=18)

		Percentiles		
		25th	**50th (Median)**	**75th**
E-selectin	P1	21.3	24.9	39.1
	D1	17.0	25.9	31.2
	D7	20.7	25.8	32.9

Table 6.4a: Paired sample t-test of tissue plasminogen activator (t-PA) (nG/ML), von Willebrand factor (vWF) (IU/dl), soluble CD40 ligand (sCD40L) (pg/ml), interleukin 6 (IL6) (pg/ml)and tumour necrosis factor (TNFα) (pg/ml) in the uncemented total knee arthroplasty group with a post-operative surgical drain (n=18)

		Mean	Standard Deviation	P value
t-PA	P1-D1	-0.4	4.3	0.75
	D1-D7	-3.8	6.3	0.04
	P1-D7	-4.2	7.0	0.04
vWF	P1-D1	-67	67	<0.01
	D1-D7	-29	79	0.17
	P1-D7	-97	38	<0.01
sCD40L	P1-D1	1628	1490	<0.01
	D1-D7	-2395	2518	<0.01
	P1-D7	-766	2272	0.21
IL6	P1-D1	-133.9	88.2	<0.01
	D1-D7	94.8	86.9	0.01
	P1-D7	-39.0	88.1	0.09

Table 6.4b: Wilcoxon signed rank test for e-selectin (ng/ml) in the uncemented total knee arthroplasty group with a post-operative surgical drain (n=18)

		P-value
E-selectin	P1-D1	0.06
	D1-D7	0.30
	P1-D7	0.92

The t-PA levels showed a rise which was maintained to day 7 (p=0.04) (Figure 6.6). The vWF was significant at 24 hours and remained so at day 7 when compared to the pre-operative levels (p=0.00) (Figure 6.7). The levels of sCD40L fell significantly in the first 24 hours and rose from day 1 to day 7 (p=0.00) (Figure 6.8). IL6 levels rose significantly to day 7 (p=0.00) (Figure 6.9). E-selectin did not change significantly (Figure 6.10).

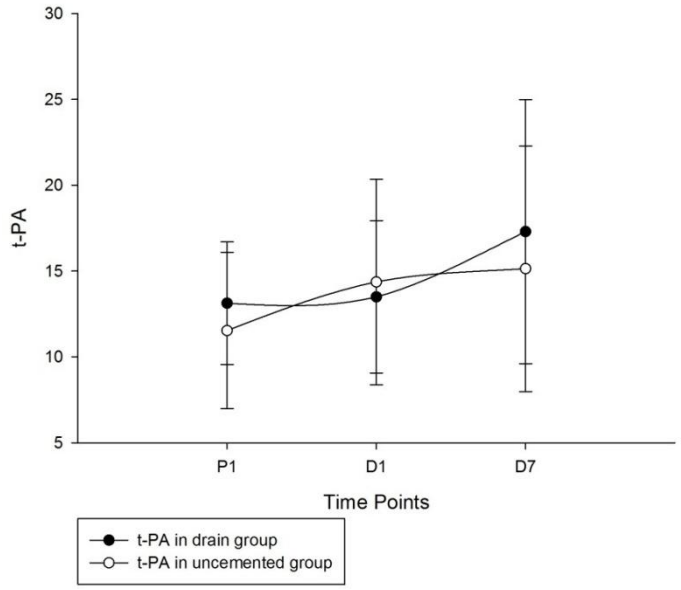


Figure 6.6: Graph of changes in tissue plasminogen activator level (ng/ml) over the three time points (mean and standard deviation) in the uncemented total knee arthroplasty group with a post-operative surgical drain (n=18) against the uncemented total knee arthroplasty group on its own (n=19)

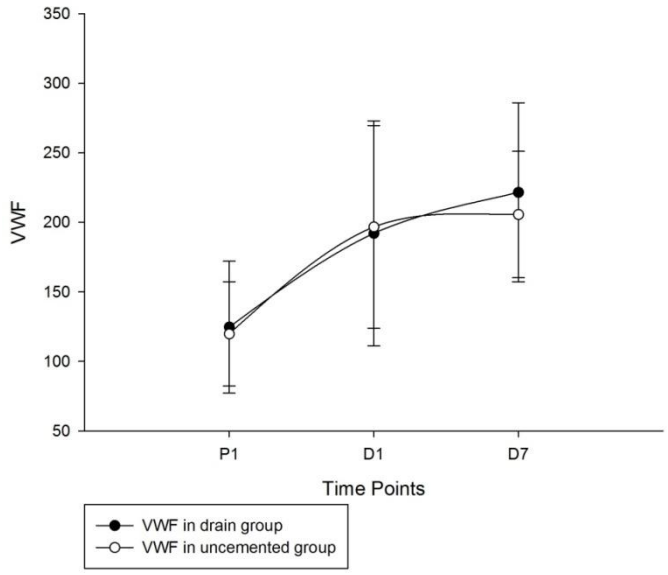


Figure 6.7: Graph of changes in von Willebrand Factor level (IU/dl) over the three time points (mean and standard deviation) in the uncemented total knee arthroplasty group with a post-operative surgical drain (n=18) against the uncemented total knee arthroplasty group on its own (n=19)

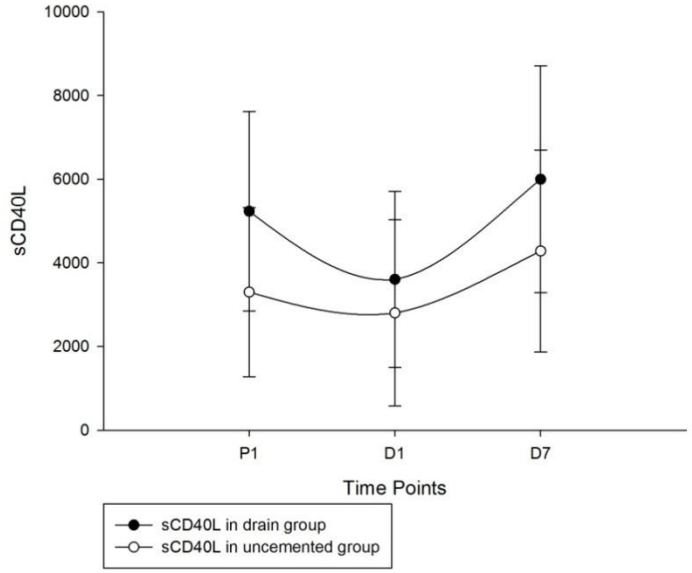


Figure 6.8: Graph of changes in soluble CD40 ligand level (pg/ml) over the three time points (mean and standard deviation) in the uncemented total knee arthroplasty group with a post-operative surgical drain (n=18) against the uncemented total knee arthroplasty group on its own (n=19)

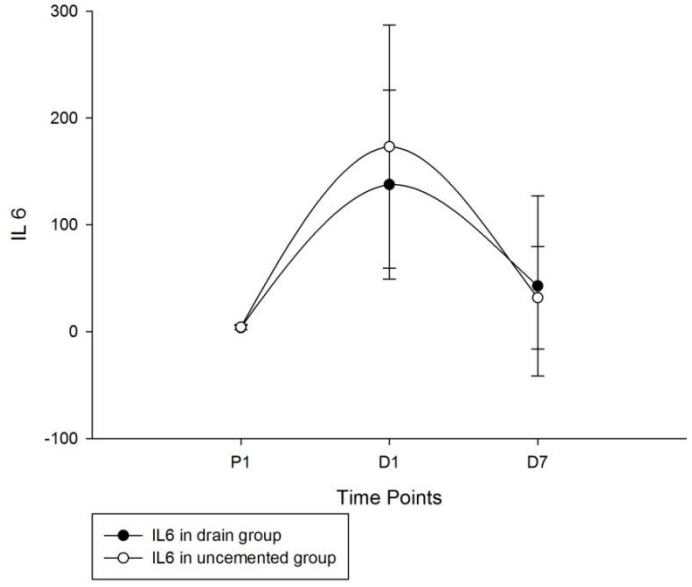


Figure 6.9: Graph of changes in interleukin 6 level (pg/ml) over the three time points (mean and standard deviation) in the uncemented total knee arthroplasty group with a post-operative surgical drain (n=18) against the uncemented total knee arthroplasty group on its own (n=19)

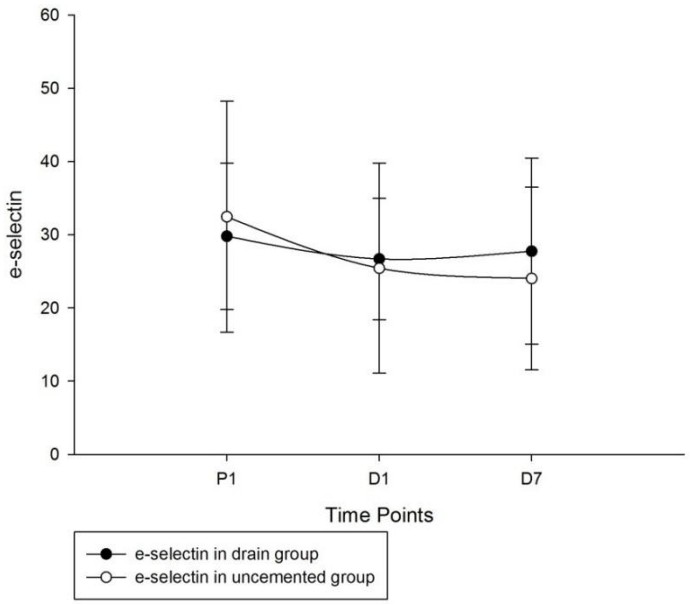


Figure 6.10: Graph of changes in e-selectin level (ng/ml) over the three time points (median and interquartile ranges) in the uncemented total knee arthroplasty group with a post-operative surgical drain (n=18) against the uncemented total knee arthroplasty group on its own (n=19)

6.4 Flow cytometry assays of cell cytokine expression

The mean, standard deviation and standard error of mean of the CD1442a and CD40 levels are summarised in table 6.6a & b for both absolute (cell counts) and percentage values.

The flow results were then analysed to look at changes between the three time points, the results of which are summarised in table 6.7a & b.

There were no significant changes in the CD1442a counts at any of the three time points for either the absolute or percentage levels (Figure 6.11). The CD40 counts fell significantly in the first 24 hours (P1 to D1) only ($p=0.009$) (Figure 6.12).

Table 6.5a: The mean, standard deviation and standard error means of CD1442a and CD40, expressed as percentage values in the uncemented total knee arthroplasty group with surgical drain (n=18)

	Mean	Standard Deviation	Standard Error Mean
CD1442a percent P1	10.00	6.13	1.53
CD1442a percent D1	8.16	5.33	1.33
CD1442a percent D7	11.44	8.86	2.21
CD40 percent P1	1.43	1.20	0.29
CD40 percent D1	0.88	0.73	0.18
CD40 percent D7	1.43	0.83	0.20

Table 6.5b: Median and Inter-quartile ranges (IQR) of CD1442a and CD40, expressed as absolute values in the uncemented total knee arthroplasty group with surgical drain (n=18)

		Percentiles		
		25th	**50th (Median)**	**75th**
CD1442a	P1	527	1262	1533
	D1	181	1022	1188
	D7	399	1118	2154
CD40	P1	62	122	186
	D1	42	58	131
	D7	73	129	303

Table 6.6a: Mean, standard deviation and p values of CD1442a and CD40 expressed as percentage values in the uncemented total knee arthroplasty group with surgical drain (n=18)

		Mean	Standard deviation	P value
CD1442a percentage	P1-D1	1.8	5.2	0.18
	D1-D7	-3.3	7.4	0.09
	P1-D7	-1.4	6.3	0.37
CD40 percentage	P1-D1	0.5	0.8	0.01
	D1-D7	-0.5	0.7	>0.01
	P1-D7	-0.01	0.9	0.99

Table 6.6b: Wilcoxon signed rank test for CD1442a andCD40 absolute values in the uncemented total knee arthroplasty group with surgical drain (n=18)

		P-value
CD1442a	P1-D1	0.05
	D1-D7	0.20
	P1-D7	0.27
CD40	P1-D1	0.01
	D1-D7	<0.01
	P1-D7	0.18

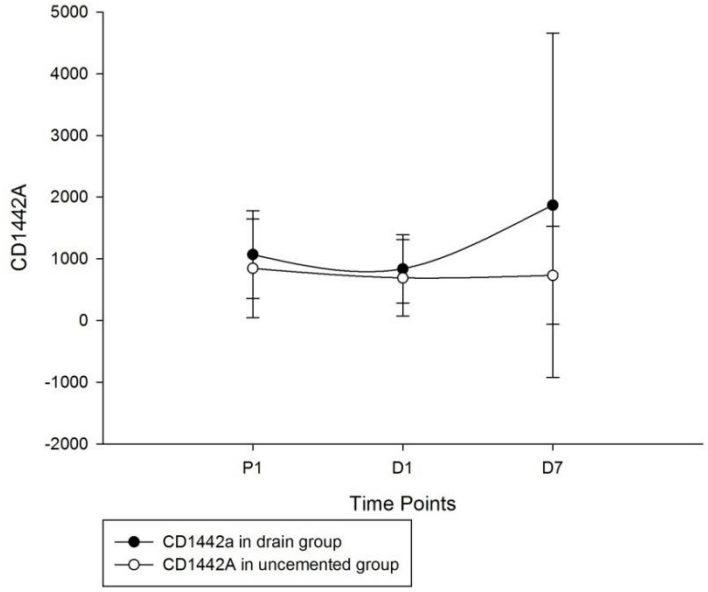


Figure 6.11: Graph of changes in CD1442a counts over the three time points (median and interquartile ranges) in the uncemented total knee arthroplasty group with surgical drain (n=18) against the uncemented total knee arthroplasty group on its own (n=19)

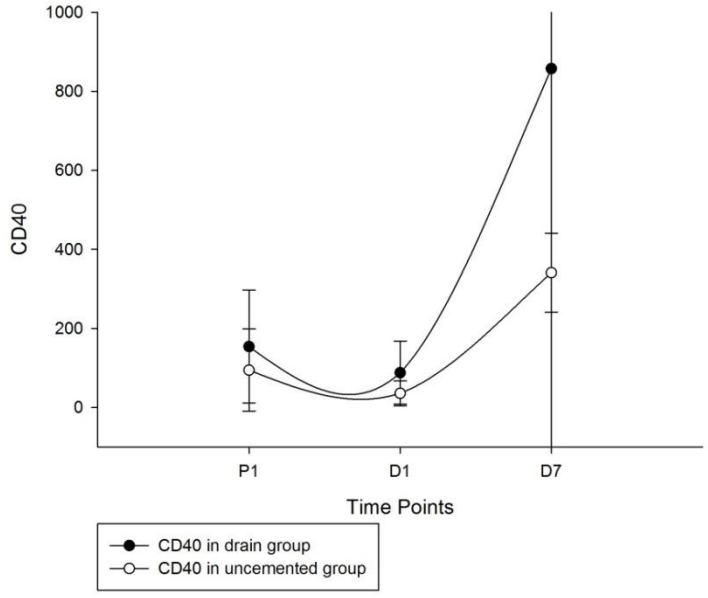


Figure 6.12: Graph of changes in CD40 counts over the three time points (median and interquartile ranges) in the uncemented total knee arthroplasty group with surgical drain (n=18) against the uncemented total knee arthroplasty group on its own (n=19)

6.5 Uncemented group versus the group with a surgical drain to assess any effect this would have on the inflammatory and endothelial response.

The groups were compared using independent sample t-test to look for significant differences in the inflammatory and endothelial response with the additional use of a surgical drain post-operatively for 24 hours.

The table below summarises the results of the independent sample t-tests for the blood parameters (table 6.9).

The only blood parameter which was significantly different between the two groups was the change in monocyte counts in the first 24 hours (p=0.04) (Figure 6.13). Here the monocytes rose less significantly then the uncemented group.

Table 6.7: Mean difference, standard error difference and significance of blood parameters between uncemented total knee arthroplasty group with surgical drain (n=18) and the uncemented total knee arthroplasty group on its own (n=19).

		Mean difference	Standard error difference	P value
Platelets	P1-D1	-4	13	0.76
	D1-D7	16	17	0.36
	P1-D7	34	28	0.23
White Cell Count	P1-D1	1.5	0.9	0.08
	D1-D7	-0.6	0.7	0.43
	P1-D7	1.0	0.7	0.18
Neutrophils	P1-D1	1.4	0.8	0.07
	D1-D7	-0.9	0.7	0.19
	P1-D7	0.5	0.6	0.41
Monocytes	P1-D1	0.2	0.1	0.04
	D1-D7	-0.1	0.1	0.25
	P1-D7	0.1	0.1	0.29
C-reactive protein	P1-D1	1	13	0.91
	D1-D7	-0.4	18	0.98
	P1-D7	1	12	0.93

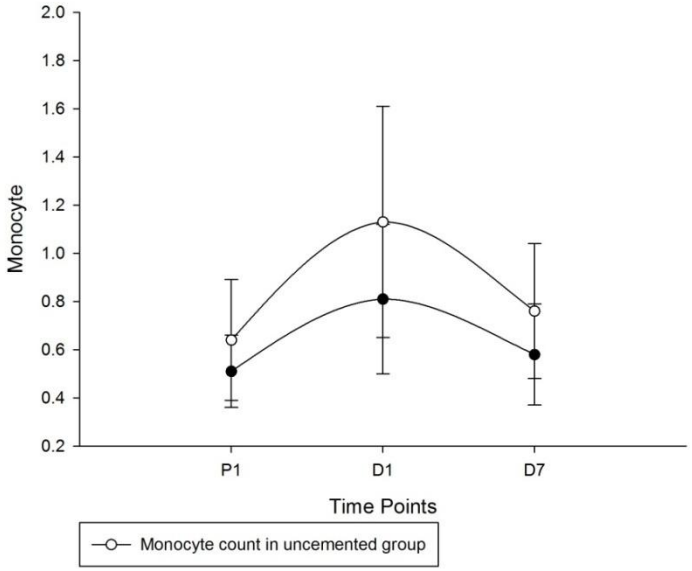


Figure 6.13: Graph of changes in monocyte count (x10^9/L) over the three time points (mean and standard deviation) in the uncemented total knee arthroplasty group with surgical drain (n=18) against the uncemented total knee arthroplasty group on its own (n=19)

The table below (Table 6.8a & b) is a summary of the results when we compare the tissue plasminogen activator (t-PA), von Willebrand factor (vWF), soluble CD40 ligand (sCD40L), interleukin 6 (IL-6), tumour necrosis factor alpha (TNFα) and e-selectin levels between the two groups. There does not appear to have been a statistically significant difference with the additional usage of a post-operative drain.

In terms of the flow cytometry results (Table 6.9a & b) there were no significant difference between the uncemented group and the group with the additional use of a surgical drain. The changes in the drain group did not show a statistically significant difference with regards to the CRP levels, although the IL-6 levels in this group were only significantly elevated from day 1 to day 7 the changes in their levels were not significantly different to that of the uncemented group.

There was no difference in the t-PA, vWF and e-selectin levels. The sCD40L showed a significant change in the first 24 hours and from day1 to day 7, similar to that of the cemented group. But when we look at the difference in the levels between the three time point we se that there is no difference between the drain and uncemented group. There was also no difference in the change in the CD1442a and CD40 levels.

Table 6.8a: Mean difference, standard error difference and significance of tissue plasminogen activator (t-PA), von Willebrand factor (vWF), soluble CD40 ligand (sCD40L0, interleukin 6 (IL-6) and tumour necrosis factor alpha (TNFα) levels between the uncemented total knee arthroplasty group with surgical drain (n=18) and the uncemented total knee arthroplasty group on its own (n=19)

		Mean difference	Standard error difference	P value
t-PA	P1-D1	2.5	1.9	0.21
	D1-D7	-3.0	2.4	0.22
	P1-D7	-0.6	2.2	0.79
vWF	P1-D1	9	25	0.70
	D1-D7	-21	26	0.44
	P1-D7	-15	13	0.27
sCD40L	P1-D1	1131	635	0.08
	D1-D7	-915	782	0.25
	P1-D7	216	698	0.76
IL6	P1-D1	23	36	0.52
	D1-D7	-51	42	0.23
	P1-D7	-13	24	0.59

Table 6.8b: Mann-Whitney U test for e-selectin (ng/ml) between the uncemented total knee arthroplasty group with surgical drain (n=18) and the uncemented total knee arthroplasty group on its own (n=19)

		Percentiles			P-value
		25th	**50th (Median)**	**75th**	
E-selectin	P1-D1	-6.3	-4.3	1.5	0.52
	D1-D7	-3.4	0.1	3.6	0.56
	P1-D7	-9.9	-2.7	1.9	0.29

Table 6.9a: Mean difference, standard error difference and significance of CD1442a and CD40 percentage values between the uncemented total knee arthroplasty group with surgical drain (n=18) and the uncemented total knee arthroplasty group on its own (n=19).

		Mean difference	Standard error difference	P value
CD1442a percentage	P1-D1	2.2	1.5	0.16
	D1-D7	-2.4	2.2	0.28
	P1-D7	-0.2	2.0	0.93
CD40 percentage	P1-D1	0.2	0.2	0.48
	D1-D7	-6.1	3.4	0.07
	P1-D7	-5.9	3.3	0.09

Table 6.9b: Mann-Whitney U test for CD1442a and CD40 absolute values between the uncemented total knee arthroplasty group with surgical drain (n=18) and the uncemented total knee arthroplasty group on its own (n=19).

		Percentiles			P-value
		25th	**50th (Median)**	**75th**	
CD1442a	P1-D1	-719	-177	41	0.45
	D1-D7	-112	358	1206	0.05
	P1-D7	-286	171	720	0.35
CD40	P1-D1	-114	-54	-4	0.80
	D1-D7	16	64	169	0.90
	P1-D7	-32	19	130	0.82

6.6 Discussion

This is the first study to specifically look at the effect of the use of a post-operative drain on the activation of markers of inflammation, endothelium and coagulation in TKA patients. The hypothesis of this study was that the use of a surgical drain would allow for the local inflammatory mediators such as IL-6 and TNFα to drain away from the operative site and so attenuate the local inflammatory response (215)

In this group this use of a drain post-operatively did not affect the CRP response which was similar to that of the uncemented group. However the IL6 response was different in that there was no significant rise in the first 24 hours and rose significantly from day 1 to day 7, even then the levels reached were not significantly higher that those of the pre-operative levels. Therefore there is some suggestion that the use of a drain may somehow dampen the local IL-6 response although this was not statistically different to the uncemented group or all four groups combined (Chapter 7).

Both the CD1442a and CD40 levels show similar changes but was not statistically different when compared to the uncemented group.

CHAPTER 7

Comparison of the four study groups and collaboration of the results

7.1 Introduction

During the data collection and following analysis of the variables in the previous chapters we can see that although there were significant changes following total knee arthroplasty there was no significance when the groups were compared, except for the flow cytometry comparison with the cemented group.

This aim of this chapter was to formally compare the changes in those variables across all four groups. These will then be collated to present the effects of total knee arthroplasty on the activation of markers of endothelium, inflammation and coagulation in 76 patients, which will be the single largest study to date to report these findings.

7.2 Statistical analysis

For the parametric data repeated measures ANOVA was used to look for statistical difference in the variables between groups. A *p* value of less than or equal to 0.05 was considered to be significant. The group was analysed as a whole, utilising repeated measures ANOVA with group (cemented, uncemented, local anaesthetic and drain) as a between subjects factor and time as a within subjects factor. Age, sex and BMI were covariates. A Bonferroni correction for multiple comparisons was performed.

In the case of the non-parametric data (e-selectin, CD1442a and CD40 count) the Freidmans test was used with post hoc Wilcoxon Signed Rank test to look for differences between the groups with a p-value of less then 0.017 (0.05 divived by the 3) as being significant. All analyses were performed using SPSS version 19.0 for Windows (SPSS Inc., Chicago, IL, USA).

7.3 Results

The table below (Table 7.1a & b) shows the results of all the variables with their p-values. The only variable which showed a statistical difference between the groups (cemented versus uncemented) was in the CD1442a percentage change which confirms the conclusions drawn from previous chapters.

Although there was perceived significance for the time period for CD40 from day 1 to day 7 ($p=0.01$), post-hoc Wilcoxon Signed-Rank test was conducted with a Bonferroni correction applied, giving a significance level of $p<0.017$. Analysis of the day 1 to day 7 timepoint for CD40 gave p values of 0.44 (uncemented versus cemented group); 0.06 (uncemented versus local anaesthetic group); 0.39 (uncemented versus local drain group) therefore showing this not to be significant.

Table 7.1a: Results of repeated measures ANOVA with p-values in all four total knee arthroplasty groups except e-selectin and CD40 count (n=74)

Variables	P value
Platelets	0.31
White cell count	0.62
Neutrophils	0.24
Monocytes	0.29
CRP	0.74
TPA	0.93
vWF	0.14
Soluble CD40L	0.75
IL6	0.99
TNF	0.84
CD1442a %	<0.01
CD40 %	0.49

Table 7.1b: Results of Freidman test on CD40 and e-selectin (ng/ml) count for all three time points in four total knee arthroplasty groups (n=74)

		P-value
CD1442a	P1-D1	0.43
	D1-D7	0.10
	P1-D7	0.31
CD40	P1-D1	0.31
	D1-D7	0.01
	P1-D7	0.19
e-selectin	P1-D1	0.98
	D1-D7	0.87
	P1-D7	0.77

The data was then combined (excluding the data on CD1442a) and analysed to report the changes between each of the three time points for the 76 patients (the only exception was in the analysis of TNFα in only 40 patients). The mean, standard deviation and standard error of mean are shown below in table 7.2a and the median and IQRs for CD40 and e-selectin count in table 7.2b.

Table 7.3a & b demonstrates that all variables show a significant changes at all three time point apart from e-selectin and C-reactive protein at the day 1 to day 7 time period and interleukin 6 for the pre-operative to day 7 time period, reflecting and confirming the results of the previous chapters. Graphs 7.1 to 7.12 are graphical representations of the changes in table 7.3.

Table 7.2a: Mean, standard deviation and standard error of mean of platelets, white cell count (WCC), neutrophils, monocytes (all 10^9/L), C-reactive protein (mg/L), tissue plasminogen activator (t-PA) (ng/ml), von Willebrand factor (vWF) (IU/dl), soluble CD40L (pg/ml), interleukin 6 (IL6) (pg/ml) and CD40 percentage values excluding CD1442 in all four total knee arthroplasty groups (n=74)

	Mean	Standard deviation	Standard error of mean
Plat P1	256	63	8
Plat D1	207	59	7
Plat D7	314	92	11
WCC P1	7.6	1.5	0.2
WCC D1	8.9	2.3	0.3
WCC D7	8.1	2.1	0.2
Neutro P1	4.7	1.4	0.2
Neutro D1	6.4	2.1	0.2
Neutro D7	5.5	1.7	0.2
Mono P1	0.6	0.2	0.02
Mono D1	0.9	0.4	0.05
Mono D7	0.7	0.2	0.03
CRP P1	2	6	0.7
CRP D1	70	43	5
CRP D7	74	44	5
t-PA P1	12.1	3.8	0.5
t-PA D1	14.0	5.2	0.7
t-PA D7	16.4	7.0	0.9
vWF P1	126	52	7
vWF D1	181	68	8

vWF D7	217	53	7
sCD40L P1	4667	2435	307
sCD40L D1	3501	2219	279
sCD40L D7	5229	2481	315
IL6 P1	28.5	134.9	16.7
IL6 D1	168.1	119.2	14.8
IL6 D7	46.4	73.1	9.2
CD40 P1 percent	1.6	1.5	0.2
CD40 D1 percent	0.8	0.7	0.1
CD40 D7 percent	5.0	11.0	1.4

Table 7.2b: Median and Inter-quartile ranges (IQR) of e-selectin (ng/ml) and CD40 count in all four total knee arthroplasty groups (n=74)

		Percentiles		
		25th	**50th (Median)**	**75th**
E-selectin	P1	-6.3	-4.3	1.5
	D1	-3.4	0.1	3.6
	D7	-9.9	-2.7	1.9
CD40	P1	-112.5	-49.0	-4.0
	D1	16.0	66.0	174.0
	D7	-18.0	24.0	139.0

Table 7.3a: Mean difference, standard error difference and significance of platelets, white cell count, neutrophils, monocytes, C-reactive protein, tissue plasminogen activator (t-PA), von Willebrand Factor (vWF), soluble CD40 ligand and CD40 percentage values in all four total knee arthroplasty groups (n=74)

		Mean	Standard deviation	P value
Platelets	P1-D1	49	46	<0.01
	D1-D7	-107	60	<0.01
	P1-D7	-58	80	<0.01
White Cell Count	P1-D1	-1.2	1.9	<0.01
	D1-D7	0.8	2.2	<0.01
	P1-D7	-0.5	1.5	0.01
Neutrophils	P1-D1	-1.7	2.0	<0.01
	D1-D7	0.9	2.2	<0.01
	P1-D7	-0.9	1.4	<0.01
Monocytes	P1-D1	-0.4	0.3	<0.01
	D1-D7	0.3	0.4	<0.01
	P1-D7	-0.1	0.3	<0.01
C-reactive protein	P1-D1	-68	40	<0.01
	D1-D7	-3	62	0.70
	P1-D7	-72	44	<0.01
t-PA	P1-D1	-1.9	5.0	0.01
	D1-D7	-2.4	6.6	0.01
	P1-D7	-4.1	5.7	<0.01
vWF	P1-D1	-55	61	<0.01
	D1-D7	-69	61	<0.01
	P1-D7	-90	44	<0.01
sCD40L	P1-D1	1166	2082	<0.01

	D1-D7	-1745	2423	<0.01
	P1-D7	-601	2087	0.02
IL6	P1-D1	-139.6	184.0	<0.01
	D1-D7	126.6	140.8	<0.01
	P1-D7	-16.9	158.4	0.40
CD40 percentage	P1-D1	0.8	1.4	<0.01
	D1-D7	-4.3	10.8	<0.01
	P1-D7	-3.7	10.7	0.01

Table 7.4b: Wilcoxon signed rank test for eselectin and CD40 absolute values in all four total knee arthroplasty groups (n=74)

		P-value
e-selectin	P1-D1	0.03
	D1-D7	<0.01
	P1-D7	0.99
CD40	P1-D1	<0.01
	D1-D7	<0.01
	P1-D7	<0.01

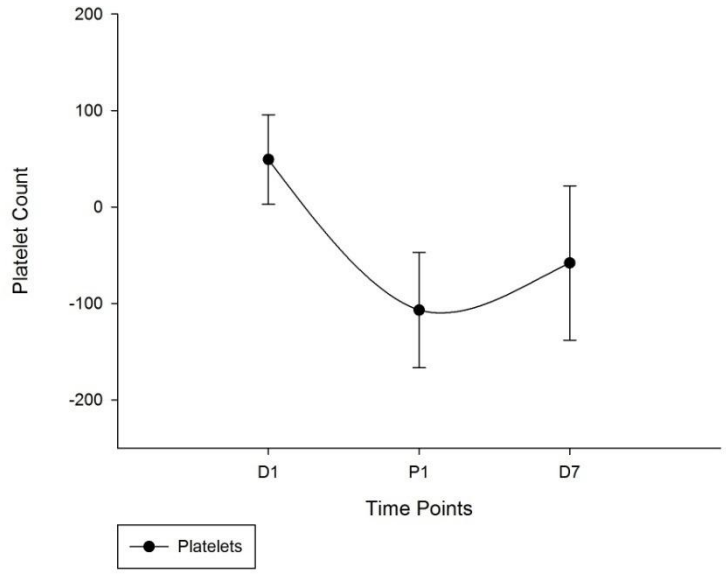


Figure 7.1: Graph of changes in platelet counts over the three time points (mean and standard deviation) in all four total knee arthroplasty groups (n=74)

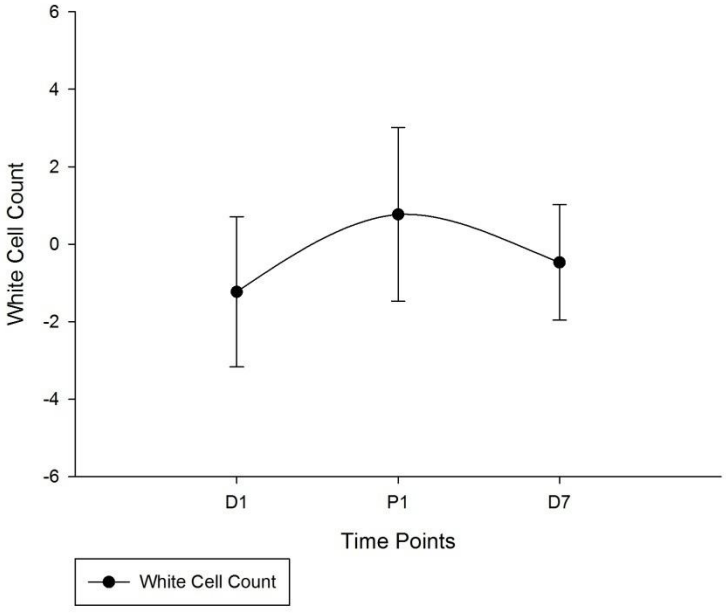


Figure 7.2: Graph of changes in white cell counts over the three time points (mean and standard deviation) in all four total knee arthroplasty groups (n=74)

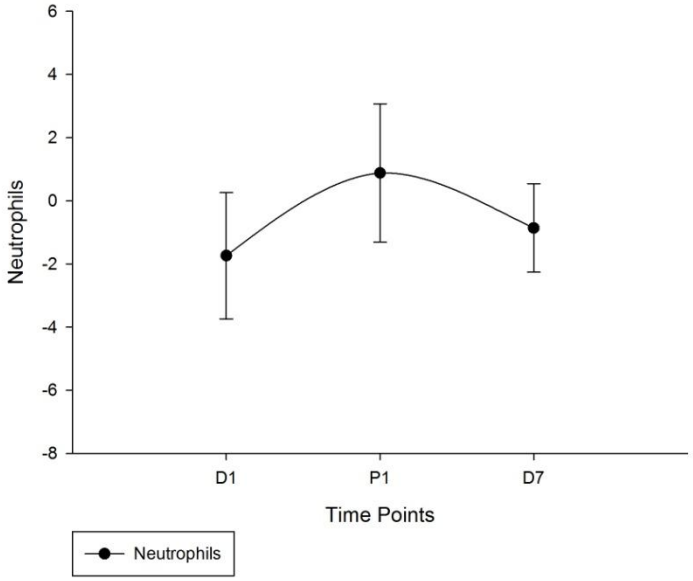


Figure 7.3: Graph of changes in neutrophil counts over the three time points (mean and standard deviation) in all four total knee arthroplasty groups (n=74)

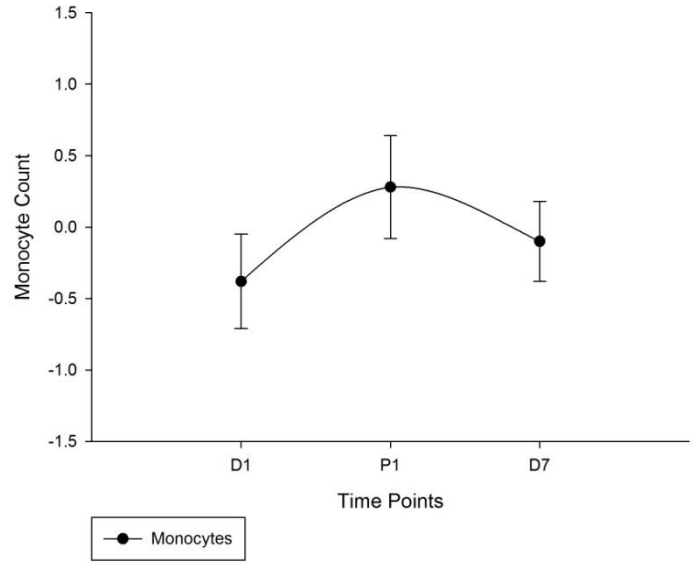


Figure 7.4: Graph of changes in monocyte counts over the three time points (mean and standard deviation) in all four total knee arthroplasty groups (n=74)

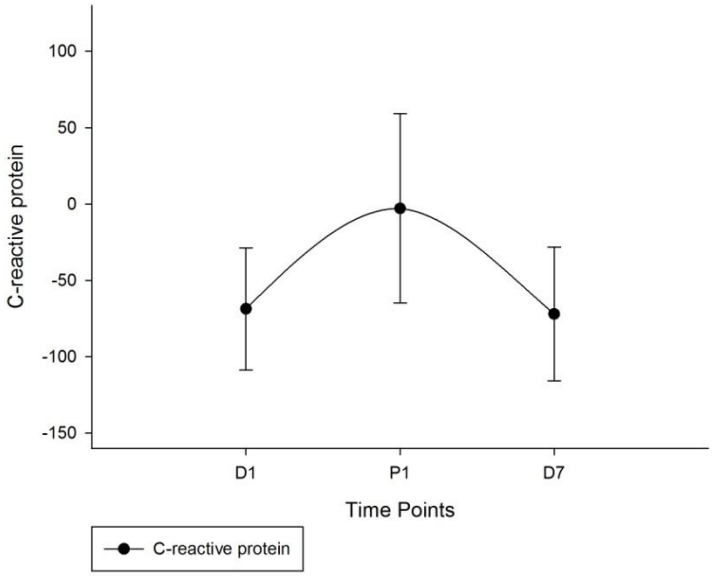


Figure 7.5: Graph of changes in C-reactive protein levels over the three time points (mean and standard deviation) in all four total knee arthroplasty groups (n=74)

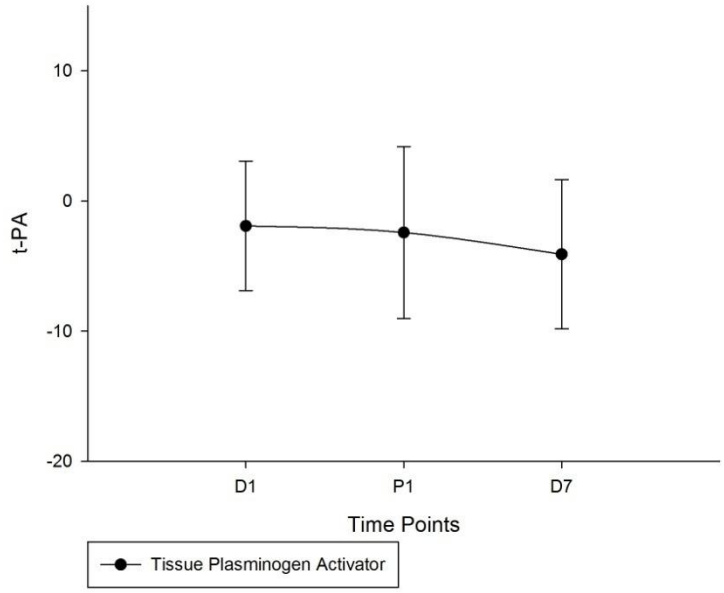


Figure 7.6: Graph of changes in tissue plasminogen activator levels over the three time points (mean and standard deviation) in all four total knee arthroplasty groups (n=74)

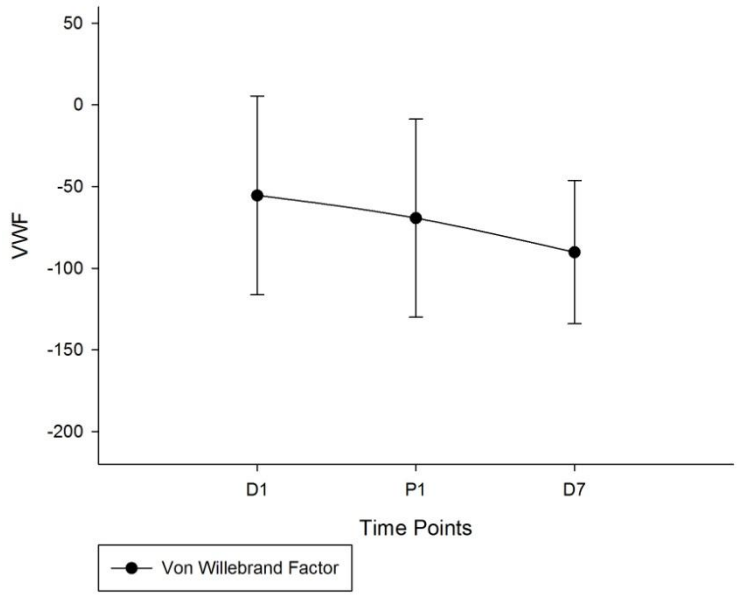


Figure 7.7: Graph of changes in von Willebrand factor levels over the three time points (mean and standard deviation) in all four total knee arthroplasty groups (n=74)

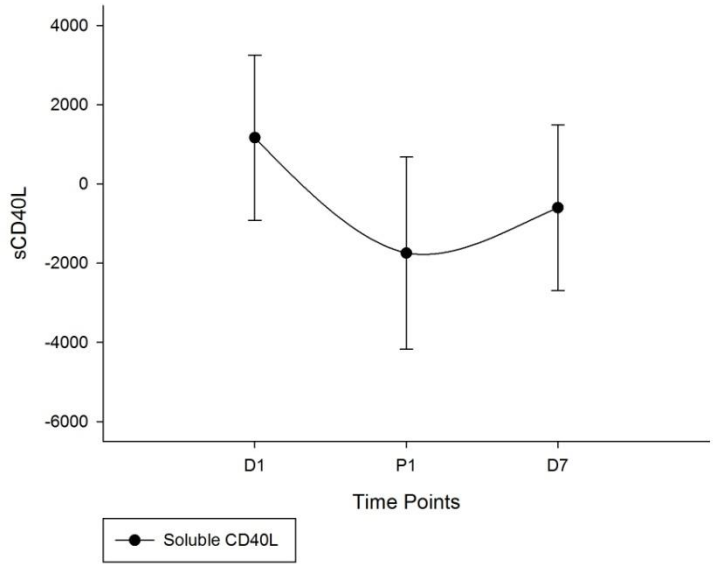


Figure 7.8: Graph of changes in soluble CD40L levels over the three time points (mean and standard deviation) in all four total knee arthroplasty groups (n=74)

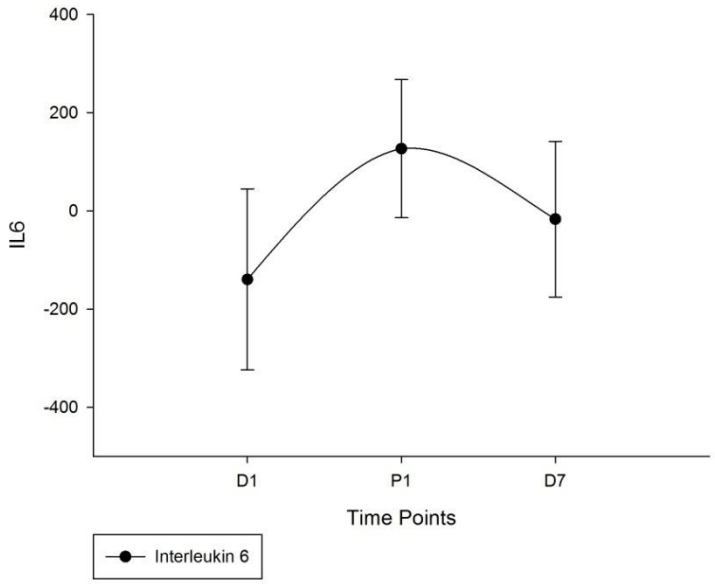


Figure 7.9: Graph of changes in interleukin 6 levels over the three time points (mean and standard deviation) in all four total knee arthroplasty groups (n=74)

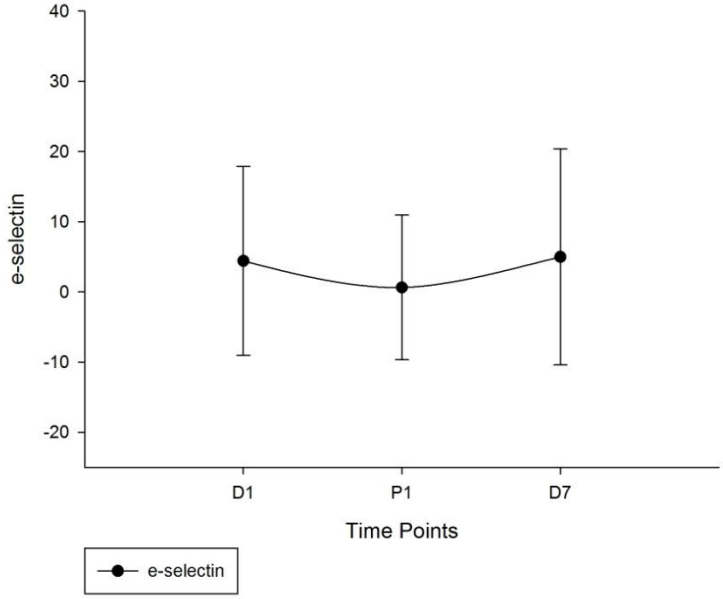


Figure 7.10: Graph of changes in e-selectin levels over the three time points (median and interquartile ranges) in all four total knee arthroplasty groups (n=74)

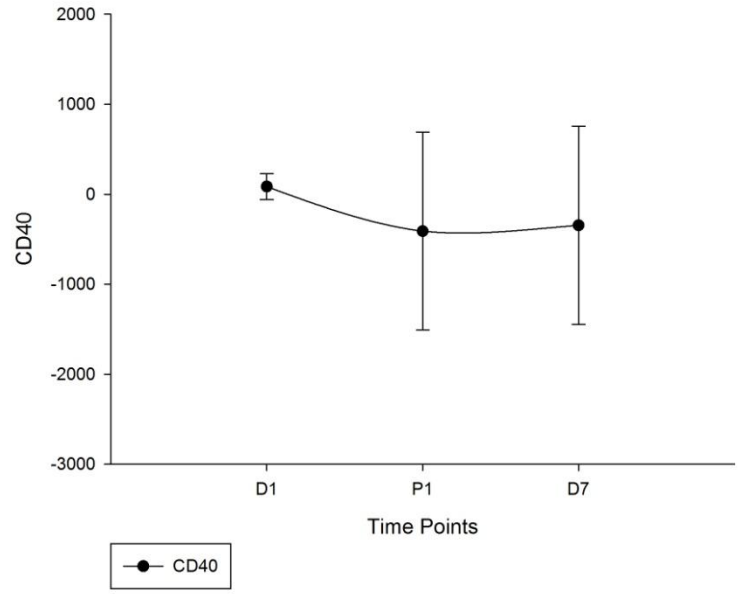


Figure 7.11: Graph of changes in CD40 levels over the three time points (median and interquartile ranges) in all four total knee arthroplasty groups (n=74)

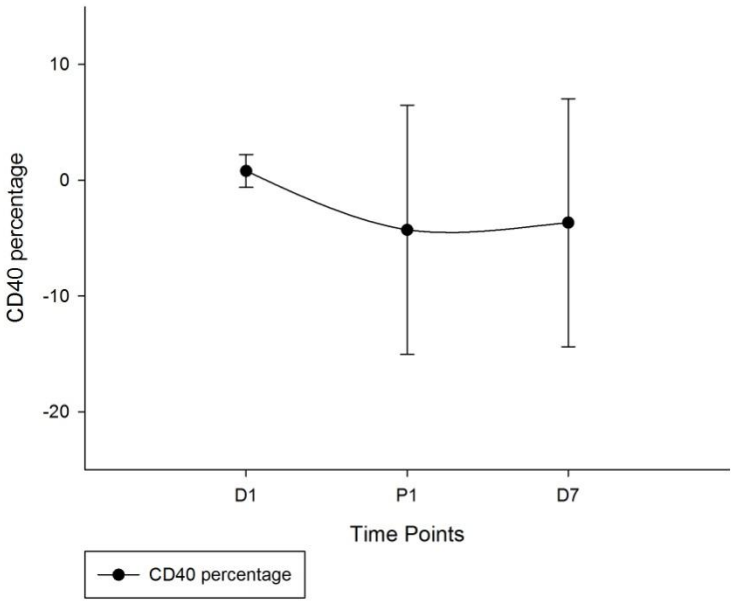


Figure 7.12: Graph of changes in CD40 percentage over the three time points (mean and standard deviation) in all four total knee arthroplasty groups (n=74)

7.4 Discussion

This is the single largest study to date looking at the effects of total knee arthroplasty (n=74) on the markers of inflammation, coagulation and of endothelial function. As part of the 'surgical response' to surgery we see a significant fall then rise with regards to the platelet levels due to the activation of the coagulation pathways ($p<0.01$) (figure 7.1). This is also reflected by the significant falls in the levels of both the vWF and t-PA at all three time points ($p<0.01$) (figure 7.6 & 7.7).

The white cell count, monocyte and neutrophils levels all showed a significant rise from the pre-operative levels to day 1 and fell significantly from day 1 to day 7 ($p<0.01$). Even at day 7 the levels of the white cell count, monocyte and neutrophils still remained significantly elevated compared to the pre-operative levels reflecting the continued surgical stimulus ($p<0.01$). The C-reactive protein levels rose significantly in the first 24 hours following surgery ($p<0.01$) as part of the acute phase response and although the levels continued to rise there was not a significant rise from day 1 to day 7 ($p=0.70$). But the levels at day 7 still remained significantly elevated compared to the pre-operative levels. By comparison the IL6 although were significantly elevated in the first 24 hours and continued to significantly rise to day 7 ($p<0.01$) were not significantly elevated at day 7 when compared to the pre-operative levels ($p=0.40$).

The sCD40L showed a signficant fall in the first 24 hours ($p<0.01$) followed by a significant rise from day 1 to day 7 ($p<0.01$) but remained significantly low at day 7 compared to the pre-operative levels. The levels of sCD40L has been shown to reflect plaque stability and composition (80). The CD40 levels expressed from the endothelium of inflamed tissues (84) was significantly elevated in the first 24 hours and continues to do so to day 7 ($p<0.01$). At day 7 it remains significantly elevated compared to the pre-

operative levels (p=0.01). CD40 has been shown to be involved in a variety of functions from immunity to inflammation (84).

This study demonstrates that elective total knee arthroplasty results in significant changes in the markers of inflammation, coagulation and of endothelial function.

CHAPTER 8

Discussion and Future Research

8.1 Discussion

There is very little in the current literature which investigates the effect of total knee replacement on the markers of inflammation, endothelium and coagulation. The aim of the studies undertaken in this thesis were to investigate such a potential association. Although the instrumentation to undertake the knee replacement is generally standardised there are variations which exits. Traditionally 'bone cement' would be used to 'cement in' the prosthesis but an option now exists to implant these uncemented. Some surgeons regularly use a post-operative surgical drain for 24-48 hours and with the current drive to increase efficiency and workload through the National Health Service, local anaesthetic infiltration has been used intra-operatively to improve post-operative pain control and patient mobilisation to allow for early discharge. The other three studies in this thesis specifically examines the use of bone cement, surgical drain and local anaesthetic and the effect on the markers of inflammation, endothelium and coagulation.

In chapter 4 the cemented and uncemented groups were directly compare to one another. The only parameter which showed a significant difference between the groups was the CD1442a levels. The levels were significantly different in the first 24 hours ($p<0.01$) and from day 1 to day 7 post-operatvely. The exact role of CD1442a is not known, so the difference between these two groups with respect to the CD1442a levels may reflect the impact that the cement has on the inflammatory and thrombotic process or on its contribution to the ongoing atherothrombogenesis. A comparison between the uncemented and local anaesthetic group (Chapter 5) revealed that both the platelet and von Willebrand factor levels rose less significantly from day 1 to day 7. There was no difference in the IL-6 levels between the groups which does not support

the in vitro studies which demonstrate that local anaesthetic results in attenuation of the inflammatory response (49). When a comparison was made to the surgical drain group the monocytes rose less from pre-operative levels to day 1 ($p=0.04$). The IL-6 levels compared to the uncemented group also showed a significant difference. Here there was no significant rise in the first 24 hours as was seen in the uncemented group and although the levels rose thereafter the day 7 levels were not significant compared to the pre-operative levels. This would possibly support the hypothesis that the use of the surgical drain allows the local IL-6 accumulation to drain away (215) and so contribute less to the systemic response. The only variable which showed a statistically significant difference in all three of the comparison groups was the CD1442a count. The exact role of CD1442a is not clearly understood, whether it reflects the inflammatory and thrombotic process or in fact directly contributes to the ongoing atherothrombogenesis (103). This would fit well with the hypothesis that the exothermic reaction of the bone cement in the cemented group would have caused additional inflammatory and thrombotic response. Unfortunately there is no current literature to support or refute this.

It was during the writing up of the results in the previous chapters when it was noted that it seemed that the majority of the variables demonstrated similar changes throughout all the four study groups. In chapter 7 an ANOVA and Friedman analysis was undertaken which showed that in fact all but one of the variables, the CD1442a count, showed no statistical difference between all the groups. These variables were then combined to present the single largest cohort to date investigating the effects of total knee replacement on the markers of inflammation, endothelium and coagulation. This showed that all variables (except CD1442a) showed a significant change at all three time points following total knee replacement. The only exceptions to these were the

changes in e-selectin from day 1 to day 7, C-reactive protein from day 1 to day 7 and IL6 levels from pre-operative to day 7.

No direct comparison to any current literature is possible as although there has been smaller studies looking at some of these changes in heterogeneous cohorts. Cruickshank et al (41) looked at other surgical procedures as well as total hip arthroplasty and found a correlation between the IL-6 response and the duration as well as the severity of the surgical trauma. In our cohort all patients were operated on by one of four consultant surgeons all using the standard approach and the same instrumentation with no significant variation in surgical operating time. Other studies have looked at changes in the IL-6 response following total knee arthroplasty. Lisowska et al in 2009 studied 40 patients all with a history of rheumatoid arthritis undergoing total knee arthropasty (228). Their study comprised of 35 females and 5 males with an average age of 58.9 +/- 8.5 years. All subjects received a spinal anaesthetic and a tourniquet was used. Venous samples were taken pre-operatively and up to 36 hours post-operatively. They showed significant rises in both the IL-6 and CRP responses with CRP peaking at 36 hours but the IL-6 response not being significant at 36 hours. Our study differs in that samples were taken at day 7 post-operatively with the CRP levels remaining significantly raised at day 7 and with a continued significant rise from day 1 to day 7 of the IL-6 levels. The authors did also state in their results that they could find no correlation between the tourniquet time and the IL-6 and CRP response. There was no mention of whether bone cement was used or not. Our results support that of Honsawck et al in 2011 who looked at the IL-6 and CRP response following cemented total knee arthroplasty (229). All patients (n=49) had either a general or spinal anaesthetic with a post-operative drain used for 24 hours afterwards. Venous samples were taken pre and post-operatively as well as at 2, 6, 14 and 26 weeks later. They found that it took up to 2 weeks for the levels to return to their pre-operative levels.

Hughes et al undertook a small study of only 10 patients (mean age 77) in 2010, this comprised of mxture of 5 total knee and 5 total hip arthroplasties (230). All patients underwent a general anaesthetic and had venous blood sampling up to day 5 post-operatively. They demonstrated significant rises in both groups with regards to the white cell count which peaked after 24 hours and returned to baseline at day 5 ($p<0.05$). Whereas in our sample the white cell count levels remained significantly elevated at day 7 ($p=0.01$). This correlates with a study by Defi et al who studied 24 patients (19 females; 5 males) undergoing a total hip arthroplasty with sampling up to 1 week post-operatively (231). They demonstrated significant rises in both the white cell count and CRP levels up to 1 week post-operatively The neutrophil count rose significantly at day 3 in the hip arthroplasty group ($p<0.05$) and at day 1 in the knee arthroplasty group ($p<0.05$) with levels returning to baseline at day 5. The monocyte and VWF levels did not show a significant change in the hip arthroplasty group and peaked at day 1 and day 3 respectively in the knee arthroplasty group, again returning to baseline levels at day 5. Both the neutrophil, monocyte and VWF levels in our study remained significant up to day 7. Hughes et al also conducted a similar albeit shorter study looking at upper limb surgery in 2010 (232) ($n=10$). Samples were taken for both CRP and VWF levels but only up to 15 minutes post-procedure. They demonstrated a significant rise in the CRP levels only ($p<0.05$).

Pape et al in 2000 looked at a heterogeneous group ranging from polytrauma to ankle fixation (227) and found that a significant rise in TNFα following femoral nailing but not in total hip arthroplasty. In his conclusion he stated that he was not able to comment on the effect of the bone cement on the markers measured in his study. Due to a limitation of resources we were unable to carry out the TNFα analysis on the local anaesthetic and surgical drain group but they were carried out in the cemented and uncemented group with the uncemented group demonstrating a significant change at day 7. This correlates

with a study by Reikeraas et al in 2005 who studied 7 patients (4 female; 3 male) undergoing and uncemented total knee arthroplasty. Samples were taked up to day 6 post-operatively. They demonstrated significant changes on the TNFα levels up to day 6.

Krohn et al (1999) also looked at changes to the TNFα levels following thoracic spinal surgery (n=8), 6 females; 2 males with a mean age of 15 (233). He found that TNFα levels significantly increased in the blood sampling but did not show a significant rise in the drained blood. He also showed that the levels of IL-6 in the drained blood were of a greater concentration (by the order of 10) compared to the levels in the venous blood. This has been reflected in other studies such as the one by Arnold et al (1995) who measured the IL-6 levels in the wound drainage blood following total hip arthroplasty (n=10) (234), he also showed significant levels of IL-6 in the wound drainage blood. Bastian et al also found that levels of both IL-6 and TNFα in the wound drainage blood was significantly higher than the levels found systemically (n=7) in those undergoing a total hip replacement (235).

Other studies have demonstrated significant changes in t-PA levels following total hip arthroplasty, this is the first study to confirm that similar changes also occur following total knee arthroplasty (219).Our results correlate with that of Kageyama et al (226) who showed a significant expression of CD40 as well as correlation with sCD40L following total knee arthroplasty. His paper did highlight the possible effect of the use of a tourniquet but the numbers involved were too small. All our subjects were operated on by consultants with an operating time ranging from sixty to ninety minutes with no significant variation in the operating time between all four groups. A pneumatic tourniquet was used for all subjects. There has been some evidence to suggest that the use of a tourniquet induces blood hypercoagulability due to the retention of blood flow

and disorders of the vascular endothelium (216-218). The potential 'effect' of the tourniquet has been negated by the fact that all the subjects in the studies had a tourniquet but the possible effects that its use may have had on the results cannot be assessed or quantified.

Currently there are no published studies to compare our findings as regards the sCD40L changes seen following total knee arthroplasty. There have been studies into sCD40L but not in orthopaedics. Antoniades et al found that in coronary bypass graft surgery the pre-operative levels of sCD40L were predictive of the chances of developing post-operative atrial fibrillation (236) in 144 patients. Desideri et al conducted an observational prospective study of 300 patients over 24 months following haemodialysis and found that higher levels of sCD40L were associated with a significant risk of cardiovascular morbidity and mortality (237)

The strength of our studies are the fact that they were all conducted in a single centre. All the operations were undertaken by one of four surgeons using the same basic approach and instrumentation. There was no significant difference in the tourniquet or operating time and the blood sampling was undertaken as per protocol. The potential weaknesses are the unknown effect that the tourniquet may have had on the study results. As some of the variables had a short half-life significant changes may have been missed as the first sampling following the operation was taken 24 hours later. Also the patients in this study were operated on by one of four surgeons with surgeons operating on patients from each of the 4 study groups. The effect that this may have on the results is difficult to quantify but with all four surgeons using the standard approach with the same instrumentation and same knee prosthesis this would minimize the potential impact of this.

In conclusion these studies demonstrate that total knee arthroplasty results in the activation of common markers of inflammation, endothelium and coagulation. These changes may explain the increased incidence of venous thrombosis and thromboembolism post-operatively as well as a potential increased risk of arterial thrombosis and sequelae from atherosclerotic plaque rupture.

8.2 Future direction

Future research would be needed to further investigate these issues. This would involve a single centred randomized large cohort study involving just one surgeon. To fully assess the potential effect of the tourniquet a large study group would be needed which would be randomized into either a with or without tourniquet group performed by the one surgeon. If this did not show a significant difference then this group as a whole could be compared to a second single surgeon group undergoing a cemented total knee arthrplasty group to assess the effects of the bone cement on the markers of inflammation, coagulation and of endothelial dysfunction. If however a difference is demonstrated by the use of the tourniquet then a single surgeon study would be needed with randomization into either a cemented or uncemented total knee arthroplasty with no tourniquet.

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ANNEX 1

Participant Information Sheet

NON-INVASIVE MARKERS OF ENDOTHELIAL FUNCTION IN ADULTS UNDERGOING TOTAL KNEE REPLACEMENT

You are being invited to take part in a research study. Before you decide, it is important that you should understand why this research is being done, and what it will involve. Please take time to read this information sheet and to discuss it with family or friends if you wish. Please ask about anything you are not clear about or do not understand.

What is the purpose of the study?

The main purpose of the study is to examine the effects of joint replacement on blood vessels. It is already known that a small number of people who present with broken bones or undergo joint replacement are at risk of developing some complications after their operation like heart disease or stroke. Inflammation in the blood vessels may contribute to these problems. This study aims to look at whether joint replacement may cause inflammation in blood vessels.

Why have I been chosen? Do I have to take part?

You have been invited to take part because you are undergoing a total knee replacement. The decision to take part or not is entirely up to you. If you do decide to take part, you are free to withdraw at any time.

What does the study involve?

You will be asked to sign the consent form attached to this Information Sheet. Some of your medical details will be discussed with you to ensure that you are eligible for the study.

At the pre-assessment clinic at the same time as your routine blood samples are being taken some additional blood tubes will be collected so that no additional needles are needed. The total amount of extra blood collected will be about 30ml (six teaspoonfuls) of blood. In addition to this you will also undergo a tracing of your heart (otherwise known as an ECG) and a test of the blood vessels in your arms and legs (otherwise known as ABPI). The ABPI test involves measuring the blood pressure in all your arms and legs.

At days number 1 and 7 after your operation, again whilst you are getting your normal bloods checked we shall also take off another 30 mls of blood.

What are the risks or side effects of taking part?

There are no foreseeable risks. Blood will be taken, which would be taken anyway.

What are the possible benefits of taking part? Will I be paid?

If the tracing of your heart or the test of the blood vessels in your arms/legs reveals any abnormalities we shall refer you onto the appropriate specialists.

You will not be paid for taking part in the study.

Confidentiality

If you decide to take part in this study, we will inform your GP. This is so that all doctors involved in your care are fully informed. Otherwise, no information about you will leave the Department of Orthopaedics. Confidential records will be kept secure.

A scientific paper will be prepared using results from the study, and it is likely that this will be published in a medical journal. You will not be identifiable from any report.

Who is organising and funding this research?

The chief investigator is Mr Alec Campbell of the Department of Orthopaedics at Monklands Hospital, using research funds belonging to the Department

Further information

You can discuss this study with the clinical investigator who will be carrying out the study:

Mr Kenneth Cheng

Department of Orthopaedics,

Monklands Hospital,

Airdrie

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You can speak to someone who is able to give independent advice about the study:

Mr I McLeod, Consultant Orthopaedic Surgeon

Department of Orthopaedics,

Monklands Hospital,

Airdrie

01236-712-294

CONSENT FORM

Title of Project: NON-INVASIVE MARKERS OF ENDOTHELIAL FUNCTION IN ADULTS UNDERGOING TOTAL KNEE REPLACEMENT

Names of Researchers: Mr A Campbell

Mr K Cheng

Please initial each statement

1. I confirm that I have read and understand the information sheet dated 18/06/07 for this study and have had the opportunity to ask questions. ____

2. 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. ____

3. I understand that sections of any of my medical notes may be looked at by responsible individuals 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. ____

4. I understand that my general practitioner will be informed of my participation in this study ____

5. I agree to take part in the above study. ____

Name of Subject	Date	Signature
Name of person taking consent (if different from researcher)	Date	Signature
Name of Researcher	Date	Signature

1 for patient, 1 for researcher, 1 to be kept with hospital notes

ANNEX 2

Department of Orthopaedic Surgery,

Monklands Hospital,

Monkscourt Avenue

Airdrie

ML6 0JS

Dear Dr,

For your information, the above patient has agreed to take part in a research study based here at the Department of Orthopaedics. I enclose a copy of the patient information sheet for your perusal.

If you wish to discuss this further please feel free to contact me.

Yours sincerely,

Mr Kenneth Cheng

Orthopaedic SpR

Phone 07803203888

E-mail: kennethcheng@nhs.net

ANNEX 3

Lanarkshire Local Research Ethics Committee

Lanarkshire NHS Board

14 Beckford St

Hamilton

ML3 OTA

Telephone: 01698 281313

Facsimile:

11 January 2008

Mr A Campbell

Consultant Orthopaedic Surgeon

Monklands Hospital

Monkscourt Avenue

Airdrie

Dear Mr Campbell

Full title of study:	**Non invasive markers of endothelial function in adults undergoing total knee replacement**
REC reference number:	**07/S1001/51**

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

The further information has been considered on behalf of the Committee by the 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 favourable opinion applies to the research sites listed on the attached form.

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	5.4	14 August 2007	
Investigator CV	2	January 2008	
Protocol	1	18 June 2007	
Covering Letter	1	14 August 2007	
Statistician Comments	1	09 August 2007	
GP/Consultant Information Sheets	1	18 June 2007	
Participant Information Sheet: PIS	1	18 June 2007	
Participant Consent Form: PCF	1	18 June 2007	

R&D approval

All researchers and research collaborators who will be participating in the research at NHS sites should apply for R&D approval from the relevant care organisation, if they have not yet done so. R&D approval is required, whether or not the study is exempt from SSA. You should advise researchers and local collaborators accordingly.

Guidance on applying for R&D approval is available from http://www.rdforum.nhs.uk/rdform.htm.

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.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Website > After Review

Here you will find links to the following

a) Providing feedback. You are invited to give your view of the service that you have received from the National Research Ethics Service on the application procedure. If you wish to make your views known please use the feedback form available on the website.

b) Progress Reports. Please refer to the attached Standard conditions of approval by Research Ethics Committees.

c) Safety Reports. Please refer to the attached Standard conditions of approval by Research Ethics Committees.

d) Amendments. Please refer to the attached Standard conditions of approval by Research Ethics Committees.

e) End of Study/Project. Please refer to the attached Standard conditions of approval by Research Ethics Committees.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nationalres.org.uk .

07/S1001/51	**Please quote this number on all correspondence**

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

Yours sincerely

MRS. P. CONWAY

SECRETARY TO THE GROUP

Enclosures: *Standard approval conditions [SL-AC1 for CTIMPs, SL-AC2 for other studies]*

Site approval form

Lanarkshire Local Research Ethics Committee

LIST OF SITES WITH A FAVOURABLE ETHICAL OPINION

For all studies requiring site-specific assessment, this form is issued by the main REC to the Chief Investigator and sponsor with the favourable opinion letter and following subsequent notifications from site assessors. For issue 2 onwards, all sites with a favourable opinion are listed, adding the new sites approved.

REC reference number:	07/S1001/51	**Issue number:**	0	**Date of issue:**	11 January 2008
Chief Investigator:	Mr A Campbell				
Full title of study:	Non invasive markers of endothelial function in adults undergoing total knee replacement				

This study was given a favourable ethical opinion by Lanarkshire Local Research Ethics Committee on 11 January 2008. The favourable opinion is extended to each of the sites listed below. The research may commence at each NHS site when management approval from the relevant NHS care organisation has been confirmed.

Principal Investigator	*Post*	*Research site*	*Site assessor*	*Date of favourable opinion for this site*	*Notes* [(1)]

Mr A Campbell	Consultant Orthopaedic surgeon	Monklands Hospital - NHS Lanarkshire	Lanarkshire Local Research Ethics Committee	11/01/2008	
Approved by the Chair on behalf of the REC: …… (Signature of Chair/Co-ordinator) (delete as applicable) ……. (Name)					

(1) *The notes column may be used by the main REC to record the early closure or withdrawal of a site (where notified by the Chief Investigator or sponsor), the suspension of termination of the favourable opinion for an individual site, or any other relevant development. The date should be recorded*