VENOUS THROMBOEMBOLISM AND PROCOAGULANTS: THE EFFECT OF CHEMOTHERAPY

A thesis submitted to The University of Manchester for the degree of

Doctor of Philosophy

in the faculty of Medical and Human Sciences

2005

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(ENNET)

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Abbreviations

ABC	**Advanced breast cancer**
APTT	**Activated partial thromboplastin time**
CI	**Confidence interval (95%)**
CP	**Cancer Procoagulant**
CRP	**C-reactive protein**
CT	**Computerised tomography**
CV	**Coefficient of variance**
DUI	**Duplex ultrasound imaging**
DVT	**Deep vein thrombosis**
EBC	**Early breast cancer**
EC	**Elastic compression**
ESR	**Erythrocyte sedimentation rate**
FPA	**Fibrinopeptide A**
FDP	**Fibrin degradation product**
HRT	**Hormone replacement therapy**
INR	**International normalised ratio**
IPC	**Intermittent pneumatic compression**
IPG	**Impedance plethysmography**
IQR	**Inter-quartile range**
LDUH	**Low-dose unfractionated heparin**
LMWH	**Low molecular weight heparin**
NBC	**Neoadjuvant breast cancer**

OCP	**Oral contraceptive pill**
PAI-1	**Plasminogen Activator Inhibitor-1**
PBS	**Phosphate buffered saline**
PE	**Pulmonary embolism**
PF1+2	**Prothrombin Fragment 1 + 2**
PT	**Prothrombin time**
r^2	**Spearman correlation coefficient**
RR	**Relative risk**
SD	**Standard deviation**
TAT	**Thrombin-Antithrombin**
TF	**Tissue Factor**
TFPI	**Tissue factor pathway inhibitor**
TSP-1	**Thrombospondin-1**
tPA	**Tissue-type Plasminogen Activator**
TNF-α	**Tumour Necrosis Factor-α**
uPA	**urokinase Plasminogen Activator**
UFH	**Unfractionated heparin**
VCAM-1	**Vascular Cell Adhesion Molecule – 1**
VEGF	**Vascular Endothelial Growth Factor**
VQ	**Ventilation/perfusion**
VTE	**Venous thromboembolism**
vWF	**von Willebrand factor**

THE UNIVERSITY OF MANCHESTER
ABSTRACT OF THESIS submitted by Cliona Clare Kirwan for the degree of PhD and entitled VENOUS THROMBOEMBOLISM AND PROCOAGULANTS: THE EFFECT OF CHEMOTHERAPY July 2005

Introduction
Venous thromboembolism(VTE) is a frequent cause of death in cancer, particularly with advanced cancer. Chemotherapy is associated with a further increase in risk of VTE in both early and advanced cancer. The pathophysiology of this chemotherapy induced hypercoagulability is unknown. Cancer patients who develop VTE may have a worse prognosis than patients without VTE. A tendency to hypercoagulability in cancer may represent a more aggressive form of cancer.

Aims
1. To establish current thromboprophylaxis in oncology patients.
2. To investigate the role of coagulation in cancer and during chemotherapy.

Methods
1. A postal survey of breast surgeons and oncologists in the UK.
2. Serum levels of procoagulant molecules were measured before chemotherapy, and on days one, four and eight, and three months and six months following chemotherapy in early (n=87) and advanced (n=36) breast cancer patients. These levels were compared to serum markers of coagulation and fibrinolysis, VTE formation, response to chemotherapy and survival.

Results
Although thromboprophylaxis was routine (95%) in breast cancer surgery, thromboprophylaxis was rarely used in cancer patients undergoing chemotherapy (16%). Chemotherapy-induced VTE occured in 17% of advanced and 8% of early breast cancer patients. VTE within three months of commencing chemotherapy suggested a poorer outcome. Markers of the haemostatic system (APTT, fibrinogen, D-dimer, tPA, platelet VEGF release, $p \leq 0.05$) and procoagulants (TF, sVEGF, $p \leq 0.05$) were increased in advanced breast cancer patients. Haemostatic markers (fibrinogen, D-dimer, and PF1+2, $p \leq 0.05$) and procoagulants (TF and VEGF, $p \leq 0.05$) were increased, prior to chemotherapy, in patients subsequently developing VTE, and predicted for VTE. Combining D-dimer >700ng/ml and fibrinogen >3g/ml, at baseline, predicted for chemotherapy-induced VTE (sensitivity 67%, specificity 69%). Alterations in haemostasis were apparent within 24 hours of commencing chemotherapy. Neither tumour procoagulant release nor endothelial cell activation, in response to chemotherapy, stimulated VTE. An altered haemostatic response to chemotherapy is identified in patients with poorer prognosis.

Conclusion
VTE during breast cancer chemotherapy is common but thromboprophylaxis in such patients is rarely used. A profile of haemostatic markers may allow the identification of cancer patients at increased risk of VTE. Thromboprophylaxis administered concurrently with chemotherapy may abolish the rapid induction of hypercoagulability.

Declaration

No part of this thesis has been submitted in support of an application for any degree or qualification of the University of Manchester or any other University or Institute of Learning.

Copyright Statement

Ownership Statement

"That swift as Quick-siluer, it courses through

The naturall Gates and Allies of the body;

And with a sodaine vigour it doth posset

And curd, like Aygre droppings into Milke,

The thin and wholsome blood"

Act 1 scene iii, Hamlet

Dedication

I would like to dedicate this thesis to my mother, Una Kirwan and father, Michael Kirwan

Acknowledgements

I am indebted to:

The Royal College of Surgeons of England for awarding me a one year Research Fellowship

Cancer Research UK for awarding me a Project Grant

Pharmacia Upjohn for funding my pilot study

I would like to thank Mr Gerard Byrne for all his support and enthusiasm, Professor Charles McCollum for his guidance and Dr Garry McDowell for all his supervision; Drs Andrew Wardley and Gregory Wilson for making me welcome at the Christie, educating me in oncology and allowing me to study their patients; the Vascular Studies Laboratory for performing duplex ultrasound imaging; the Thrombosis Reference Centre, particularly Dr David Taberner, Jan, Dave Leeming and Steven Craig and Wythenshawe coagulation laboratories, particularly Anne Fisher, for all their help and advice with assays; Mrs Julie Morris for endless advice and patience with the hugh volume of statistics; Miss Janet Walls and Mr John Howat for their faith in me and allowing me the time to complete this thesis and Sanjay Sastry, Kevin Daly, Francis Dix, Francesco Torella, Arnab Bhomick, Angela Cooper, Barbara Milne, Sally Boyle, Sarah Haynes and Carol Devine for making it fun.

Most importantly I would like to thank Mum, Dad and Pam, Jim, Maeve and Michael for their never-ending faith, support and encouragement, and Dave for always, always being there.

PREFACE

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Qualifications

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The Role of Procoagulants in Cancer and Chemotherapy Induced Venous Thromboembolism – a Pilot Study. Pharmacia and Upjohn, £18, 000	2000-01
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Royal College of Surgeons of England Research Fellowship, £35,697	2000-01
Charing Cross and Westminster Medical School Ophthalmology Prize	1994

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SECTION 1

INTRODUCTION

Chapter 1

Cancer and chemotherapy related venous thromboembolism – a summary

Venous thromboembolism (VTE) in cancer is common, and associated with significant mortality and morbidity. VTE occurs in 3-11% (Ambrus *et al.* 1975; Byrd, Divertie, & Spittell, Jr. 1967; Harrison & Breslin 1977; Jose *et al.* 1982; Lieberman *et al.* 1961; Miller *et al.* 1967; Pinzon *et al.* 1986; Rassam & Anderson 1975) of cancer patients, which compares with a population incidence for VTE of 0.1% (Anderson, Jr. *et al.* 1991). Treatments for cancer such as chemotherapy appear to add to this risk, with the incidence rising nearly 200 fold in patients receiving chemotherapy for metastatic breast disease (Goodnough *et al.* 1984). In a study of 14,000 patients, 74% of all VTEs diagnosed in cancer patients at the Christie Hospital occurred during or shortly after completing chemotherapy, 20% in the first cycle and 66% in the first five cycles. Patients who developed VTE had a worse prognosis than those who remained free of VTE (Seward *et al.* 1999).

VTE is a common and well described clinical condition. VTE in the presence of cancer, however, frequently behaves differently to classical VTE, with a presentation varying from Trousseau Syndrome of migratory superficial thrombophlebitis (Sack, Jr., Levin, & Bell 1977) to rapidly propagating thrombus unresponsive to treatment (Harrison & Breslin 1977). Many risk factors are present both as a result of the cancer and its treatment, from immobility to surgery and systemic therapy such as hormone or chemotherapy.

Diagnosis presents its own specific problems in cancer from obstructive pelvic and lung pathology affecting the efficacy of diagnostic imaging, to cancer related biochemical changes invalidating screening tests.

Irrespective of the cause of VTE, mortality from pulmonary embolism (PE) is between five and 17% (Punukollu *et al.* 2005). One autopsy study showed that 60% of all patients who died of PE had localized cancer or limited metastatic disease, which would have allowed for reasonable survival in the absence of fatal PE (Shen & Pollak 1980). Deep vein thrombosis (DVT) of the lower extremity is the commonest presentation of VTE (Anderson *et al.*1991). The clinical course of DVT can be complicated by PE, recurrent DVT and the development of serious post-thrombotic sequelae, such as debilitating pain, intractable oedema, subcutaneous fibrosis, hyperpigmentation, and ultimately venous ulceration. DVT has a risk of recurrence of 25% at five years. This risk is further increased in the presence of cancer (hazard ratio 1.7) (Prandoni *et al.* 1996). The incidence of post-thrombotic syndrome is almost 30% at 5 years (Prandoni *et al.* 1996). Treatment related haemorrhage occurs in approximately 13%, and is considered major in 5% (Prandoni *et al.* 1996). In 2001 the cost of treatment of VTE including initial therapy (the main component), follow-up care, the expected costs of major haemorrhage (due to anticoagulation), recurrent VTE, and post-thrombotic syndrome, was approximately $11,600 (Edelsberg, Ollendorf, & Oster 2001). This represents a national health economic burden of nearly $500 million dollars/ year in the United States (Hawkins 2004). Equivalent costs would apply to the United Kingdom.

Survival of patients from the time of cancer diagnosis with venous thrombosis has been found to be significantly worse than a matched control group without VTE ($p<0.001$), with a two-fold greater risk of dying in gynaecological patients (Morgan *et al.* 2002).

VTE prophylaxis in the surgical setting has been shown to reduce mortality and be cost effective (Salzman & Davies 1980). In orthopaedic surgery, VTE prophylaxis is associated with a reduction in VTE rate of up to 30% (Colwell, Jr. & Spiro 1995). In cancer patients VTE prevention is an even greater priority because the diagnosis of DVT and PE are often more difficult (Gomes & Deitcher 2003; Keefe, Roistacher, & Pierri 1994), the treatment of overt VTE is less successful (Hutten *et al.* 2000; Prandoni *et al.* 2002) and is associated with more bleeding complications (Hutten *et al.* 2000; Prandoni *et al.* 2002).

Coagulation and cancer exist in a symbiotic relationship, with each promoting the existence of the other. At a tissue level tumour cell growth, extravasation and intravasation interrelates with both the molecular and cellular elements of the haemostatic system. At a systemic level, a hypercoagulable state coexists with cancer, and such a state is associated with a poorer cancer outcome.

A greater understanding of the hypercoagulable state of cancer, and how this is altered by cancer therapy, in particular chemotherapy, could guide clinicians to achieve not only a reduction in VTE through prophylaxis, as is being achieved in surgery, but may also provide insight into anticoagulation as an anti-cancer therapy.

VTE occurring in cancer cannot be understood until VTE in the non-cancer setting is reviewed. In the first chapter of the introduction classical VTE will be described in terms of morbidity and mortality, diagnosis, risk factors, pathophysiology, treatment and

prophylaxis. The second chapter of the introduction will explain how these factors transfer into the cancer population. Problems specific to the cancer population will be discussed, for example how presentation of VTE, methods of diagnosis and response to treatment may all differ in this subset. Current understanding of the pathophysiology of VTE in cancer will be reviewed. Finally, in chapter 4, current knowledge about the specific issues related to VTE in the presence of cancer chemotherapy will be presented.

Chapter 2

Venous thromboembolism(VTE)

2.1 Definition

Venous thrombosis is the intravenous formation of thrombus from the constituents of the blood. The detachment of a thrombus, allowing movement of the thrombus through the vascular tree, to impact in a distant vessel is known as thromboembolism. The most frequent forms of VTE are Deep Vein Thrombosis (DVT) and Pulmonary Embolism (PE).

2.2 Mortality and morbidity

VTE, particularly DVT and PE represent a significant source of morbidity and mortality worldwide. Mortality rate from PE is 5% however this increases to 17% in the elderly population (Calder *et al.* 2005). Long-term complications include recurrence, intractable pain and permanent lung damage with loss of function (Lopez, Kearon, & Lee 2004; Pengo *et al.* 2004; Poulsen *et al.* 2001).

Significant complications of acute DVT are pain, swelling, loss of function, and propagation of thrombus with possible development of PE. In the long-term complications include recurrence, with a mortality rate from delayed PE of 2% (Prandoni *et al.* 1997). Moreover chronic pain, swelling and skin ulceration are features of postphlebitic syndrome (Kolbach *et al.* 2004; Walker *et al.* 2003). A recent prospective

longitudinal study in the United States calculated the age-standardized incidence of first time VTE at 1.92 per 1000 person-years, with a 28-day fatality rate of 11% (Cushman *et al.* 2004).

2.3 Diagnosis of venous thromboembolism

2.3.1 Clinical symptoms and signs

Deep vein thrombosis:

DVT of the lower extremity is a serious disorder, with an estimated incidence in the normal population of 1 per 1000 per year (Prandoni *et al.* 1997). The classical presentation of a deep vein thrombosis is pain in the calf, associated with swelling, redness and engorged superficial veins. The affected calf is often warmer and ankle oedema can be present. Calf pain on foot dorsiflexion (Homans' sign) is a common, but not diagnostic, sign (Kumar & Clark 1992). Clinical diagnosis however is inaccurate because many potentially dangerous venous thrombi are nonobstructive and are not associated with inflammation of the vessel wall or of the perivascular tissues. These thrombi may be asymptomatic despite extensive DVTs, or may first present as a PE (Colman *et al.* 1994).

Pulmonary embolism:

A small embolus may present with minor symptoms such as tiredness, effort dyspnoea or syncope, larger emboli causing pulmonary infarction may present with pleuritic chest pain, haemoptysis, cough or dyspnoea. A massive PE is a medical emergency presenting

with shock, severe chest pain, tachypnoea and tachycardia. There may be minimal signs or arrhythmias, pleural rub and the presence of a DVT (Kumar & Clark 1992).

2.3.2 Investigations

Venous thromboembolism:

- *D-Dimer*

In the presence of thrombin, fibrinogen is cleaved to form fibrin monomers. Covalent cross-linkages in the d-domain region of fibrin produce an insoluble fibrin clot. The presence of the fibrin clot triggers plasmin to lyse the clot as well as lysing fibrinogen. Whereas fibrinogenolysis leads to fibrin-degradation products (FDPs), lysis of the fibrin clot generates cross-linked FDPs containing D-dimer (Colman *et al.* 1994). This D-dimer is released into the plasma and can be recognized by commercially available monoclonal antibodies. Qualitative bedside D-dimer testing is poor, with a sensitivity of only 86% and a specificity of 34% (Wilson & Gard 2003). This can be improved by advanced D-dimer quantitative analysis with automated, latex-enhanced turbimetric tests. This produces a sensitivity of 100%, but a still a specificity of just 48% (Wilson & Gard 2003). Even in an elderly population (age over 70), where D-dimer is more commonly elevated, quantitative analysis maintains a sensitivity of 100%, however specificity is reduced to just 14% (Tardy *et al.* 1998).

Deep vein thrombosis:

- *Impedance plethysmography (IPG)*

This is a non-invasive technique that detects volume changes in the leg, measured as changes in electrical resistance (impedance) following inflation and deflation of a pneumatic thigh cuff. These changes are reduced in the presence of obstruction (for example by thrombosis) of the popliteal or more proximal veins. This test has limited use for calf vein thromboses or non occlusive proximal vein thrombi. This test does not distinguish between thrombotic or nonthrombotic obstruction to venous outflow. False positive results may be obtained if a patient is incorrectly positioned or inadequately relaxed, if the vein is compressed by an extravascular mass, or if venous outflow is impaired by raised central venous pressure. Severe obstructive arterial disease, causing reduced arterial inflow can also produce reduced outflow and hence a false positive (Colman *et al.* 1994).

- *Venography*

For many years this has been the reference standard for the diagnosis of DVT, only recently being superceded by duplex ultrasound imaging (DUI) (Bresolette *et al.* 2001; Elias *et al.* 2004; Kassai *et al.* 2004; Schellong 2004). This technique outlines the deep venous system of the leg by injecting radiopaque contrast medium into a distal dorsal foot vein. With good technique, ascending venography outlines the entire deep system of the lower extremities, including the external and common iliac veins in most patients. However, common femoral or iliac venography may be needed if the external and common iliac veins are not properly visualised by the ascending technique, or if the

inferior vena cava must be outlined. The diagnosis of acute DVT is confirmed by the presence of an intraluminal filling defect that is constant in all films and is seen in at least two different projections. Inadequate filling of the venous system with contrast is the main cause of inaccurate interpretation of scans, with this being reported to occur in four to 12% of scans (Colman *et al.* 1994).

- *^{125}Fibrinogen leg scanning*

This technique is dependent on the incorporation of circulating radio-labelled fibrinogen as fibrin into the thrombus. This is detected by measuring the increase in overlying surface radioactivity with an isotope detector. This test has limitations, for example in examining near the bladder, which may contain radioactive urine, or near other sources of fibrin accumulation such as inflammatory reactions, arthritis, haematomas and oedema (Colman *et al.* 1994). This technique is now limited to being a research tool having been superseded by DUI.

- *Duplex ultrasound imaging*

DUI is a non-invasive investigation that combines real-time B-mode ultrasound with Doppler flow analysis. It has a sensitivity of 92-95% and specificity of 97-100% for detecting proximal thrombi (White *et al.* 1989). Its interpretation is subjective, and requires considerable skill and experience to perform reliably, however it is the investigation of choice of suspected DVT for 69% of vascular surgeons (Turton *et al.* 2001). It also has an important diagnostic role in PE as 38% of patients with proven PE will have a positive duplex scan (Paterson & Schwartzman 2001).

Pulmonary embolism:

- *Perfusion lung scan*

Perfusion lung scanning is currently the first-line investigation for investigating PE as normal results virtually exclude PE, and a "high-probability" scan is strongly associated with angiographically proven PE. However, 57% of scans in patients with PE and 78% of scans in patients without PE are non-diagnostic (1990).

- *Pulmonary angiography*

This invasive technique is recognised as the "gold-standard" largely because of its sensitivity in identifying small emboli in the distal pulmonary vasculature, however its invasive nature and associated complications limits its use.

- *Spiral computerised tomography*

Spiral computerised tomography (CT) is replacing pulmonary angiography as the "gold-standard" for diagnosis of PE, particularly in patients with a nondiagnostic perfusion scan and negative leg duplex imaging (Paterson & Schwartzman 2001). It also has the added advantage of diagnosis of other pathologies.

- *Magnetic resonance angiography*

This non-invasive investigation is particularly useful in diagnosing asymptomatic pelvic vein thrombosis in the presence of PE and normal duplex findings, with a positive result being found in 29% of previously undiagnosed patients (Stern *et al.* 2002).

2.4 Management of VTE

The objectives of treatment are

- to prevent death from PE
- to reduce morbidity from the acute event
- to minimize long term complications such as pulmonary hypertension and postphlebetic symptoms.

2.4.1 Treatment

The established treatments are anticoagulant drugs, thrombolytic agents, interruption of the inferior vena cava, and surgical removal of the thromboembolic obstruction. Anticoagulant drugs prevent extension of established venous thrombosis and recurrent embolisation by inhibiting blood coagulation (Colman *et al.*1994). Thrombolytic agents for example streptokinase, urokinase and tissue plasminogen activator (tPA) produce rapid thrombolysis of recent venous thrombi and pulmonary emboli (Hirsh & Hoak 1996). Interruption of the inferior vena cava intercepts emboli from the distal venous system heading to the pulmonary vasculature. Surgical removal is rarely indicated and is reserved for threatened gangrene of a limb due to extensive venous obstruction, or acute circulatory collapse from massive PE (Hirsh & Hoak 1996).

2.4.2 Prophylaxis

Prophylaxis of at risk medical and surgical patients is both successful in the prevention of VTE, and cost-effective (Geerts *et al.* 2001; Halkin *et al.* 1982). Prophylaxis has been

most successfully introduced in surgical patients. LDUH (low-dose unfractionated heparin) was the first antithrombotic agent investigated in large randomized trials. A beneficial effect on reducing serious endpoints such as proximal DVT and PE was consistently demonstrated. Low molecular weight heparin (LMWH) appears equally efficacious as LDUH in preventing VTE in surgical patients, with relative risk (RR) of haematoma development being dependent on dose (Geerts *et al.* 2001). IPC (Intermittent pneumatic compression) has a similar risk-reduction for DVT as LDUH, however the effect on PE is not proven (Geerts *et al.* 2001). Stockings with graded elastic compression (EC) also reduce the risk of leg DVT, but insufficient data exist on EC effect on proximal DVT or PE (Wells, Lensing, & Hirsh 1994). Warfarin may be effective in preventing extensive DVT however the risk of bleeding and delayed onset of action necessitates close laboratory monitoring. Combining prophylactic measures appears to give better protection than when used alone (Geerts *et al.* 2001).

2.5 Pathophysiology

In 1834, de Blainville noted that the intravenous injection of brain tissue led immediately to lethal, massive intravascular clotting (de Blainville 1834). Buchanan (Buchanan 1845) noted that other animal tissues could also promote coagulation. Although the understanding of the homeostasis between coagulation and fibrinolysis has greatly improved, the precise pathology of the development of venous thromboembolism is still unclear. The formation, propagation and dissolution of venous thrombi and pulmonary

emboli reflects a balance between the thrombogenic stimuli and a variety of protective mechanisms.

Deep venous thrombi are intravascular deposits composed predominantly of fibrin and red blood cells, with a variable platelet and leukocyte component. These thrombi usually form in regions of slow or disturbed flow and often begin as small deposits in large venous sinuses in the calf, in valve cusp pockets either in the deep veins of the calf or thigh, or in venous segments that have been exposed to direct trauma. DVT and PE are part of the same pathological process. In a study of patients diagnosed with DVT, 40% were found to have asymptomatic PE. DVT has been identified on contrast venography in over 80% of confirmed PEs (Girard *et al.* 1999), a likely underestimate of the true numbers of DVT in the presence of PE, as whole clots may dislodge from the distal veins resulting in normal subsequent investigations of the deep veins. Authorities suggest that virtually all PEs originate from DVT (Hirsh & Hoak 1996).

Thrombi extend proximally with propagation, and may dislodge and embolize to the pulmonary arteries. This pulmonary artery obstruction, as well as release of vasoactive agents such as serotonin by platelets, elevate pulmonary vascular resistance. The resultant increase in alveolar dead space causes a redistribution of blood flow and a ventilation-perfusion mismatch, with impaired gas exchange. Reflex bronchoconstriction augments airway resistance, and lung oedema decreases lung compliance (Elliott 1992). As right ventricular afterload increases, dilatation, dysfunction and ischaemia of the right ventricle can occur. In the presence of a patent foramen ovale or atrial septal defect, paradoxical embolism may occur, as well as right-to-left shunting of blood causing arterial hypoxaemia.

2.6 Risk factors for venous thromboembolism

Traditionally, due to the work of Virchow, risk factors are classified in the classic triad of:

- changes in the vessel wall
- stasis of the blood and
- changes in the composition of the blood

This is an over-simplification and most modern authorities now classify risk factors into genetic and acquired.

2.6.1 Inherited thrombophilia

Hereditary thrombophilia, was first reported in 1965 when Egeberg identified a deficiency of antithrombin in a family (Egeberg 1965). In the 1980's protein C and protein S defiencies were described in hereditary thrombophilias. These are the major natural inhibitors of the procoagulant system, so a deficiency leads to excessive thrombin formation and an relative risk for thrombosis (RR=6-10). The more recently identified factor V Leiden (a mutation in the clotting factor V) is known to promote resistance to activated protein C and an increase in procoagulant activity, with a similar relative risk of thrombosis as protein C deficiency. Whereas the prevalence of protein C deficiency is estimated at 1:250-500, and that of protein S as even lower, factor V Leiden is relatively common, with a prevalence of three to 7% (Rosendaal 1997). Factor II 20210 G→A, a mutation in the prothrombin gene, is associated with increased plasma concentrations of

prothrombin, increasing the relative risk of thrombosis (RR=2.8). It has a prevalence of one to 3.6% (Rosendaal 1997; Rosendaal 1999). Moreover high concentrations of factor VIII increase thrombosis risk (Anderson, Jr. & Spencer 2003). Because blood group and von Willebrand factor (vWF) are strong determinants of factor VIII levels, non-O blood groups have double the risk of VTE compared to other ABO groups (Jick *et al.* 1969; Rosendaal 1997; Rosendaal 1999).

2.6.2 Acquired

Age:

There is an almost linear increase in VTE risk with age (Coon & Coller 1959). During the fifth decade risk sharply increases reflecting reduced mobility and associated comorbidity (eg. heart failure, surgery, increased circulating procoagulants and reduced circulating fibrinolytic agents) (Motykie *et al.* 2000).

Immobility:

In the 1950s Gibbs reported 15% of patients on bedrest for less than one week before their death, had VTE at autopsy, but this rose to 80% with prolonged bedrest. In hemiplegic stroke patients, asymptomatic DVT is ten times more common on the paralysed side than the unaffected side (Anderson, Jr. & Spencer 2003). Asymptomatic DVTs are found in 10% of passengers following air flights of over eight hours (Scurr *et al.* 2001).

Trauma:

Trauma resulting in pelvic, hip or femoral fracture predisposes to a 35-65% risk for DVT (Motykie *et al.* 2000), either through compression of pelvic and femoral veins or by direct trauma to the vessel wall with subsequent release of tissue factor. Risk estimates for major head injury and spinal injury are 54% and 62% respectively (Rosendaal 1997).

Surgery:

Thirty to 50% of patients undergoing hip and knee surgery without prophylaxis develop VTE, whilst risks of 30% are seen in abdominal, gynaecological and urological surgery (Geerts *et al.* 2001; Rosendaal 1997). Surgery-associated VTE usually starts in the valve cusps within the deep veins of the calf, beginning intraoperatively, with half resolving within 72 hrs. Approximately 16% extend to involve proximal veins, dramatically increasing the risk of subsequent PE (Kearon 2003). Although many VTE begin intraoperatively, delayed development or presentation of surgical VTE is common. Twenty to 30% of patients venographically-proven to be DVT-free one week following hip arthroplasty develop delayed DVT at up to five weeks (Dahl *et al.* 1997; Planes *et al.* 1996).

Other risk factors:

The frequency of VTE in patients suffering congestive cardiac failure and acute myocardial infarction has been quoted as over 20% (Motykie *et al.* 2000).

Pregnant women and women who are less than one month post-partum have a five-fold risk of VTE over non-pregnant women. The greatest risk is during the third trimester (Motykie *et al.* 2000).

Both hormone replacement therapy (HRT) and the oral contraceptive pill (OCP) increases the risk of VTE between two and six fold, with associated increases in coagulation factors and reduction in fibrinolytic activity. In both cases the changes are reversible on cessation of hormone therapy (Anderson, Jr. & Spencer 2003).

Chapter 3

Venous thromboembolism and cancer

In the following pages the relationship between VTE and malignancy will be reviewed, considering:

- A historical perspective
- Epidemiology
- Risk of cancer in patients with VTE
- Risk of VTE in patients with cancer
- Biochemical evidence for hypercoagulability in cancer
- Outcome of patients developing VTE in cancer

3.1 Historical review

In 1865 at the Hotel-Dieu Paris, Armand Trousseau lectured on the association between *phlegmasia alba dolens* and "internal cancerous tumours" (Trousseau 1872). He died two years later from stomach cancer presenting with thrombophlebitis (Nusbacher 1964). In 1906 Haward reported a series of 2,903 post mortems. Pulmonary embolism was the cause of death in 81 cases, with cancer being listed second only to middle ear infection as an underlying disease (Haward 1906). In 1936, Barker reported a series of 166 clinical cases of thrombophlebitis at the Mayo Clinic. Twenty-seven of the 58 thromboses occurring in patients with non-infectious systemic diseases were associated with cancer. Twenty-one of these 27 resulted in fatal pulmonary embolism. "Marantic thrombosis",

first described in the latter half of the nineteenth century as a thrombosis associated with a cachetic state, is described in Barker's paper as commonly resulting in fatal pulmonary embolism. Barker is also the first to mention the presence of cancer "as one factor in setting the stage for possible postoperative thrombosis" (Barker 1936).

In 1938 Sproul reviewed 4258 consecutive post mortems at the Presbyterian Hospital, New York. He found evidence of venous thrombosis in 332 of which 150 or 45% were associated with cancer. Of 598 patients with the commoner cancers, 125 (21%) had evidence of venous thrombosis. In patients with cancer and thrombosis, the cancer originated in the stomach (26%), colon (12%), pancreas (11%) and lung (10%). Of 12 patients with evidence of multiple thromboses, eight or 67% had pancreatic carcinoma (Sproul 1938). Halpert reported, in 1965, evidence of venous thromboembolic disease in 25 of 120 pancreatic carcinoma patients at post mortem. Only five of these patients had VTE diagnosed pre mortem (Halpert, Laszlo, & Jordan 1965). Coon and co-workers, in a series of postmortems in 1976, reported a three-fold increase risk of pulmonary embolism in breast, genitourinary, stomach, colon and lung cancer and at least a six fold increased risk with carcinoma of the pancreas (Coon 1976), confirming similar findings in his 1959 study (Coon & Coller 1959). Historically, tumours arising in the body and/or tail of the pancreas are far more frequently associated with thrombophlebitis than tumours in the head of the organ (Sack, Jr., Levin, & Bell 1977). Reporting bias may have influenced the emphasis on pancreatic cancer in many studies, as early studies in this field specifically concentrated on pancreatic carcinoma-associated VTE (Hoerr & Harper 1957; Leach 1950).

The relationship between venous thromboembolism and cancer is reciprocal. Venous thromboembolism may be the presenting feature of an occult cancer and patients with clinically overt cancer have an increased risk of developing venous thromboembolism at any stage of their disease (Prandoni, Piccioli, & Girolami 1999; Rickles & Levine 2001).

3.2 Epidemiology of venous thromboembolism in cancer

The true incidence of VTE in cancer remains unclear as all studies to date have been retrospective, and relying on clinical presentation or post mortem evidence. Table 1 summarises these studies.

A Swedish study of 21,530 postmortems performed over a 24-year period showed high prevalence of pulmonary embolism for patients with ovarian carcinoma (34.6%), cancer of the extrahepatic bile duct system (31.7%) or stomach (15.2) (Svendsen & Karwinski 1989). However post-mortem studies may overestimate the incidence of VTE, due to post-mortem fibrin deposition. Dhami demonstrated a 23.5% risk of clinical DVT in high grade glioma (Dhami *et al.* 1993). In a recent study of 1041 patients with solid tumours, 81 (7.8%) developed VTE (excluding vascular access-induced thrombosis, superficial thrombophlebitis, thrombosis related to direct extension or compression of tumour and thrombosis in the setting of disseminated intravascular coagulation). Increased tumour stage was an independent risk factor (RR3.2). Renal, pancreatic, gastric and brain tumours were all independent risk factors. However the occurrence of thrombotic events in this study did not adversely affect survival (Sallah, Wan, & Nguyen 2002).

Table 1 Rate of venous thromboembolic events in patients with cancer. A summary of published studies

Year	*Author*	*Site of primary cancer*	*Method of diagnosing VTE*	*Number in study*	*Rate of VTE*
1938	Sproul (Sproul 1938)	Solid tumours	Post mortem	598	21%
1965	Halpert (Halpert, Laszlo, & Jordan 1965)	Pancreas	Post mortem-cause of death	120	21%
1974	Inagaki (Inagaki, Rodriguez, & Bodey 1974)	Solid tumours	Post mortem-cause of death	816	6.6%
1975	Rassam (Rassam & Anderson 1975)	Lung	Clinical	280	3.2%
1994	Quevedo (Quevedo *et al.* 1994)	Glioma	Duplex/venography Perfusion scan/angiography	68	28%
1993	Dhami (Dhami, Bona, Calogero, & Hellman 1993)	Glioma	Diagnosis documented in case notes	68	19%
1999	Levitan (Levitan *et al.* 1999)	All malignancies	Diagnosis documented in case notes	1,211,944	0.6%
2002	Sallah (Sallah, Wan, & Nguyen 2002)	Solid tumours	Duplex/venography Perfusion scan/angiography	1041	7.8%
2004	Blom (Blom, Osanto, & Rosendaal 2004)	Lung	Medical records	537	4.4%

A retrospective study using the MEDPAR discharge coding database calculated relative risk of VTE by cancer site, as compared to other medical conditions (Levitan *et al.* *1*999). Malignancies of the brain, ovary and pancreas are among the most prone to cause VTE, as others have previously published (Baron *et al.* 1998; Sorensen *et al.* 1998). Cancer of the breast and bladder, both with a relative risk for VTE of 0.4, seem here to be associated with a VTE risk lower than other non-cancer hospitalised patients (Thodiyil & Kakkar 2002b). Recently, the risk of VTE in lung carcinoma has been established at 20 fold higher than the general population. Interestingly adenocarcinoma of the lung has a 3.1 hazard ratio as compared to squamous cell carcinoma of the lung (Blom, Osanto, & Rosendaal 2004).

3.3 Epidemiology of cancer with venous thromboembolism

Several early studies have described phlebitis as the presenting sign in malignancy; other symptoms of the underlying neoplasia developing only later (Ackerman & Estes 1951; Barker 1936; Byrd, Divertie, & Spittell, Jr. 1967; Cliffton 1956; Haimovici 1950; Jennings 1954; Lieberman *et al.* 1961). This *thrombophlebitis migrans* classically involves superficial veins in sites that are not typically subject to deep vein thrombosis, such as veins of the upper extremities. It tends to be migratory and resistant to anticoagulation. In 1949 Edwards reported six cases of multiple, migrating and recurrent thrombophlebitis associated with visceral carcinoma and suggested that, in the presence of such thrombophlebitis of unknown cause, exploratory laparotomy should be performed to identify the presence of an underlying carcinoma (Edwards 1949). Byrd in

1967 suggested supraclavicular exploration in such circumstances after reporting on 37 patients with thromboembolism occurring as an early complication of primary bronchogenic carcinoma. Fourteen of these patients presented with thrombophlebitis as the initial presenting sign (Byrd, Divertie, & Spittell, Jr. 1967). Initially authors supported the recommendation of searching for a tumour following a single episode of phlebitis, particularly in individuals over 40 (Byrd, Divertie, & Spittell, Jr. 1967; Cliffton 1956; Durham 1955; Edwards 1949; Jennings 1954; Lieberman *et al.* 1961; Nusbacher 1967; Wooley, Baba, & Ryan 1970).

Other authors have studied the relationship between commoner presentations of VTE, such as DVT and PE with cancer. In a series of 128 patients diagnosed with PE by angiography, 15 (12%) were found to have cancer prior to development of PE, with a further 13 (14.7%) of the remainder developing cancer in the subsequent two years, and six developing cancer after two years. This was compared to an age and sex matched control group, investigated by angiography for suspected PE and found to be negative. Cancer was present in 13 (10%) prior to negative angiography, but did not develop in any in the two years following angiography, and only two patients subsequently. The authors conclude that occult cancer may cause a hypercoagulable state in its very early stages, long before the cancer becomes clinically evident (Gore *et al.* 1982).

In contrast O'Connor, in a retrospective study of 17 cases of idiopathic VTE, found no evidence of malignancy after a mean follow-up of 23 months (O'Connor *et al.* 1984). Griffin demonstrated a relative risk of two for cancer at the time of diagnosis of VTE, but found no increased incidence of subsequent diagnosis of cancer (Griffin *et al.* 1987).

More recently population-based retrospective cohort analyses from large registries, retrospective analyses of large numbers of unselected patients and prospective studies have quantified the risk for a new cancer diagnosis within six to 12 months of the diagnosis of idiopathic VTE. The increased risk in these studies range from between four to seven fold (Rickles & Levine 2001). Patients with recurrent VTE exhibit the greatest risk for subsequent cancer (Monreal & Prandoni 1999).

The need to screen for occult malignancies in patients with idiopathic VTE is still controversial. A recent prospective randomized trial has demonstrated that extensive screening allows the identification of malignancies at an earlier stage. Although this early detection is associated with improved treatment possibilities, it is as yet uncertain whether this improves overall prognosis (Piccioli *et al.* 2004).

3.4 Morbidity and mortality of venous thromboembolism in cancer

Haemostatic problems have been reported to be the second most common cause of death in cancer patients (Donati 1995). A recent prospective longitudinal study shows a 25% mortality rate for VTE occurring in cancer patients compared to 11% in those with no associated cancer (Cushman *et al.* 2004). Sallah demonstrated a VTE incidence of nearly 8% in solid tumours, with a trend for reduced survival in those developing VTE compared to those that remained VTE-free, however this did not reach statistical significance ($p=0.08$) (Sallah, Wan, & Nguyen 2002).

Sorensen and colleagues found a higher mortality in patients whose cancer was diagnosed in the first year after a thrombotic episode (36%) compared to matched cancer

patients without thrombosis (12%). The groups were not matched for stage thus the authors concluded that cancer diagnosed simultaneously with or shortly after an episode of VTE was usually at an advanced stage and so associated with a poor prognosis (Sorensen *et al.* 2000). However Morgan compared gynaecological cancer (ovarian, uterine and cervical) mortality in patients with VTE to site, stage, age and histology-matched cancer controls and was still able to demonstrate a two fold greater mortality in the VTE group (Morgan *et al.* 2002). Similar increased mortality is seen in breast cancer patients who develop VTE whilst receiving chemotherapy as compared to non-VTE patients (Weiss *et al.* 1981b). In a retrospective study of approximately ten million patient episodes from the medicare system, the probability of death amongst patients with cancer and concurrent VTE was more than two fold greater than that observed among patients with cancer but no VTE (Levitan *et al.*1999).

3.5 Clinical symptoms and signs of cancer related venous thromboembolism

3.5.1 Trousseau syndrome

'Trousseau syndome', as described by Armand Trousseau, is a recurrent and migratory superficial thrombophlebitis (Trousseau 1872). Although a relatively rare presentation of VTE, numerous case reports of the association of this form of VTE occurring in association with cancer exist in the literature (Edwards 1949; Hubay & Holden 1954; Oelbaum 1953; Stern 1933). Byrd reports 35 of 37 patients with thromboembolism associated with lung carcinoma, having such a classical picture, sometimes involving

unusual sites like jugular veins and the venous system of the penis (Byrd, Divertie, & Spittell, Jr. 1967).

3.5.2 Other manifestations

There are many clinical manifestations of thrombosis in cancer patients, including lower and upper limb VTE, and subsequent PE; disseminated intravascular coagulation (classically in haematological malignancies and widespread metastatic cancer) (Luzzatto & Schafer 1990); hepatic vein thrombosis, associated with hepatomas and renal cell carcinomas (Rayner, Hoag, & Khan 1987); cerebral venous thrombosis (Hickey *et al.* 1982); digital ischaemia (Albin *et al.* 1986; Taylor, Jr. *et al.* 1987) and arterial embolism, in association with chemotherapy or from non-bacterial thrombotic endocarditis (an insidious complication of a few mucin-secreting adenocarcinomas) (Min, Gyorkey, & Sato 1980; Ondrias, Slugen, & Valach 1985).

3.5.3 Increased severity

In a large prospective study randomising major surgical patients to LMWH or LDUH, post-mortem was performed on the majority of patients who died within 14 days of the discontinuation of anticoagulation. Death due to PE occurred in 0.31% of cancer patients who received prophylaxis compared with 0.09% of non cancer patients ($p=0.001$) (Thodiyil & Kakkar 2002a). Such data suggests that thrombosis may be a more aggressive clinical entity in the cancer patient. The CORTES (Clivarin: Assessment of Regression of Thrombosis. Efficacy and Safety) study, comparing unfractionated heparin (UFH) with LMWH as treatment for DVT, showed a greater thrombus burden in cancer

patients than patients without cancer. Response to treatment was also reduced with 50% without cancer but only 37% with cancer demonstrating a reduction in the Venographic Marder score after three weeks of therapy (Breddin *et al.* 2001; Thodiyil & Kakkar 2002a).

3.5.4 Recurrent venous thromboembolism

One of the more characteristic features of VTE in cancer is the high risk of recurrence, either with or without anticoagulation. The frequency of recurrent VTE in malignancy has been extensively investigated. Once anticoagulation treatment has been interrupted, the recurrence rate in malignancy is greatly increased. In the first few months patients with malignancy exhibit a recurrence rate almost double that of patients free from malignancy (RR=1.72). The cumulative incidence is 30% after eight years (Prandoni *et al.* 1996). Piovella identifies cancer as the only significant predictor of DVT recurrence within three months of the index DVT (Piovella *et al.* 2002).

The cumulative incidence of recurrent VTE during treatment with anticoagulation is approximately 3.5 times higher in patients with malignancy than in cancer-free patients. The recurrence rate appears to be higher in patients with more severe disease, a finding that is not explained by inadequate anticoagulation (Prandoni *et al.* 2002). In the most recent study, overall recurrence rate of DVT in the presence of cancer is 17% (Elting *et al.* 2004).

3.5.5 Bleeding complications

A small proportion of patients with deep vein thrombosis develop bleeding complications during anticoagulant treatment. This risk is increased in the presence of cancer, with a hazard ratio of 2.2. Risk of bleeding is proportional to the cancer stage (Prandoni *et al.* 2002).

3.6 Diagnostic problems of cancer related venous thromboembolism

The diagnostic principles of VTE in cancer are largely similar to that of any VTE, however in many circumstances the accuracy of tests is compromised because of underlying disease. The cancer-related problems of specific investigations are discussed below.

3.6.1 Impedance plethysmography

Patients with pelvic tumours may present with the clinical features of DVT without intraluminal thrombosis; the tumour bulk impeding the flow by direct pressure. IPG is unable to differentiate between obstruction due to thrombosis or external compression. In such patients, IPG has been found to have a sensitivity of only 71%, and specificity of 80% and so DUI is recommended (Keefe, Roistacher, & Pierri 1994).

3.6.2 Venography

Venography may also be difficult to interpret in the presence of pelvic tumours. An intraluminal filling defect is diagnostic of thrombosis, whereas smooth encroachment on the lumen without an obvious intraluminal defect suggests extrinsic compression. Absence of filling is non diagnostic as it could be due to either cause. CT scan is often more helpful in these circumstances.

3.6.3 D-dimer

It is well recognised that circulating D-dimer is elevated in cancer with cancer (Blackwell *et al.* 2000; den Ouden *et al.* 1998; Dirix *et al.* 2002; Oya *et al.* 1998; van Wersch & Tjwa 1991; Vukovich *et al.* 1997). Thus although sensitivity can be maintained, specificity is greatly reduced. In one study sensitivity for identifying VTE in cancer patients with D-dimer was 100%, however specificity was only 9% (Schutgens *et al.* 2002). A retrospective analysis of 1068 consecutive outpatients with suspected VTE assessed D-dimer accuracy in cancer as compared to non cancer patients. Although sensitivity is high in both patients with and without cancer, specificity in patients with cancer (48%) was dramatically lower than patients without cancer (82%) (Lee *et al.* 1999). D-dimer measurement may be further complicated if the patient has had recent surgery, with a reduction in sensitivity and specificity to 93% and 23% respectively in one trial (Bongard *et al.* 1994).

3.6.4 Radiological imaging for pulmonary embolism

Ventilation/ perfusion (VQ) scintigraphy has many limitations in the presence of cancer as pulmonary or pleural disease can interfere with the scan performance. Rarely, the pulmonary artery (or a branch) may be compressed by cancer and so produce the appearance of a high-probability ventilation / perfusion scan. Tumour emboli can also present with symptoms of pulmonary embolism and cause a high-probability scan and filling defects on pulmonary angiography (Gomes & Deitcher 2003).

3.6.5 Duplex ultrasound imaging

DUI has been shown to be useful in the diagnosis of clinically suspected DVT, but fares less well as a screening tool. In post-operative patients following radical prostatectomy, only two of nine DVTs were detected by post-operative screening duplex (Leibovitch *et al.* 1995).

3.7 Management Issues in cancer related venous thromboembolism

Many factors may complicate the initiation of anticoagulant therapy in patients with cancer. Anticoagulant-induced bleeding and recurrent VTE are more common in cancer patients than non-cancer patients (hazard ratio of 3.2 and 2.2 respectively) (Hutten *et al.* 2002). Practical issues include:

- how to manage anticoagulant therapy around the time of invasive procedures
- whether to initiate therapy in a patient with a very short life expectancy
- how to maximise quality of life – ideally at home

- when to use aggressive second-line antithrombotic treatments such as thrombolysis or insertion of an inferior vena cava filter

3.7.1 First line treatment

Current practice dictates that the same treatment regimen is used for patients with or without cancer. Either intravenous UFH, or now more usually LMWH, followed by long-term oral anticoagulation (vitamin K antagonists) are the usual approach (Colman *et al.* 1994).

3.7.2 Long-term management: Heparin versus warfarin

One of the distinguishing features of the hypercoagulability of cancer is its resistance to anticoagulant therapy. Byrd reports over 50% of bronchogenic carcinoma patients with thrombophlebitis have further VTE despite therapeutic prothrombin times (Byrd, Divertie, & Spittell, Jr. 1967). One large prospective trial compared the efficacy of a LMWH to a coumarin derivative, to assess risk of VTE recurrence. The LMWH was twice as effective in reducing the risk of recurrent thromboembolism without increasing the risk of bleeding (Lee *et al.* 2003). The investigators reported that therapeutic International Normalise Ratio (INR) levels were achieved at only 46% of INR monitoring tests. INR was below range 30% of the time and above range 24% of the time. Such poor control may be influenced by factors such as drug interactions, malnutrition, vomiting and liver dysfunction.

A further complication of anticoagulation in patients with cancer is the high risk of bleeding. A cohort study by Bona and co-workers compared bleeding and recurrent

thrombosis in patients with (n=104) and without cancer (n=208) receiving low dose warfarin anticoagulation. Major haemorrhage occurred in 0.4 and 0.3% per treatment month in cancer and non-cancer groups respectively. Recurrent thrombosis occurred in 1.2% and 0.2% per treatment month in the patients with cancer compared with those without cancer, respectively (Bona, Hickey, & Wallace 2000). In contrast, a population-based study of patients with VTE treated with warfarin reported a significantly increased risk of major bleeding in patients with malignancy. The presence of malignant disease was associated with a relative hazard ratio of 4.07 as compared to non-cancer patients (Gitter *et al.* 1995).

In a study of cancer patients with VTE, 146 patients were randomized to either LMWH (enoxaparin) or warfarin for three months following VTE development. The primary endpoint were a major bleeding episode, recurrent DVT, or PE within three months. At three months, fewer patients in the LMWH group than in the warfarin group had experienced a primary outcome event (10.5% versus 21.1%, p=0.09). There were six deaths owing to haemorrhage in the warfarin group compared with none in the LMWH group (Meyer *et al.* 2002). In a similar study of 187 patients with symptomatic VTE, recurrence was documented in 4% of patients on warfarin and 6% on LMWH (p=0.5). Bleeding complications were significantly more frequent in the warfarin group (13% versus 4%, p=0.04) (Pini *et al.* 1994). Thus, current evidence favours the use of LMWH compared to oral anticoagulation both in terms of reduced bleeding and risk of recurrence (Zacharski, Prandoni, & Monreal 2005). Heparin has several antithrombotic mechanisms that warfarin lacks. Heparin releases tPA and tissue factor pathway inhibitor (TFPI) from endothelial binding sites and increases their circulating levels. TFPI is a tri-

domain protein that binds to the complex formed by tissue factor (TF), factor VIIa and factor X and suppresses the generation of Xa by tissue factor. Heparin (both unfractionated and low molecular weight), but not warfarin, can increase the circulating amount of this protein and also increase its activity. Since TF appears to be an important stimulus to coagulation in cancer patients, activation of TFPI by heparin may contribute greatly to the overall antithrombotic effect of heparin.

Large clinical trials have demonstrated safety and efficacy profiles for LMWH in both in-patient and out-patient settings (Gould *et al.* 1999; Quinlan, McQuillan, & Eikelboom 2004). There is no difference in recurrence rate of VTE between patients treated with UFH or LMWH (Levine 2002), however the latter enables home treatment with less monitoring and thus improved quality of life.

3.7.3 Duration of treatment

As yet there are no trials that have examined the optimum duration of anticoagulant therapy for secondary prevention of VTE in cancer patients (Zacharski, Prandoni, & Monreal 2005). The recommended practice is to continue anticoagulation when there is evidence of active cancer and while the patient is receiving anti-neoplastic therapy. In patients without any evidence of residual cancer and who are off treatment, oral anticoagulation is usually stopped after a period of at least three to six months. For patients with metastatic disease this usually means continuing treatment until a contraindication to anticoagulation develops.

3.7.4 Recurrent VTE

When VTE recurs despite adequate anticoagulation the accepted options are either to increase the oral anticoagulation to achieve a higher target INR (3-3.5 from two) or switch to UFH or LMWH (von Depka *et al.* 2000; Zacharski, Prandoni, & Monreal 2005). In patients with recurrent or anti-coagulation resistant VTE a vena cava filter may be required (Yap & McCready 2004). A trial of patients with DVT, randomized to a vena cava filter or no filter, showed that although the filter group had a lower incidence of PE at two years (3.4% versus 6.3%, p=0.16), this was offset by a significantly higher incidence of recurrent DVT (20.8% versus 11.6%, p=0.02) (Decousus *et al.* 1998). The use of vena cava filters is not without risk and is usually restricted to patients with specific contraindications to anticoagulation.

3.7.5 Prophylaxis

VTE prophylaxis in cancer patients has been shown to be effective. In one large study (n=1358) medical patients were randomized to receive LDUH or no prophylaxis, with in-hospital death as the primary outcome. Among the subgroup of patients with cancer, mortality was 32% in the control group and 19% in the LDUH treatment group (Halkin *et al.*1982). Thromboprophylaxis in the cancer patient will be discussed in further detail in chaper 3.12.

3.8 Pathophysiology issues of VTE in the presence of cancer

Although the true mechanism for VTE in cancer is not fully understood, many pathological mechanisms have been suggested, that may even co-exist. Despite the considerable overlap between the three divisions it is useful to consider these mechanisms using Virchows original triad:

3.8.1 Vessel wall damage

The vessel wall may be damaged by invasion of the endothelium by the tumour itself or by the action of proteins secreted by the tumour, for example Vascular Endothelial Growth Factor (VEGF). Many of these cytokines cause increased permeability of endothelial cells, and a shift to a more procoagulant endothelium. Tumour-specific antigens or cytokines may also stimulate vascular endothelial cells to produce procoagulants, such as TF (Semeraro & Colucci 1997), downregulating vascular endothelial cell natural anticoagulant activity. The angiogenesis induced by many tumours causes the creation of complexes of blood vessels that are aberrant in appearance and have very disordered flow, both in magnitude and direction (Folkman 1985). Moreover, chemotherapy may predispose a patient to a coagulation disorder by causing either direct injury to the vascular endothelium or release of endothelium-derived mediators that can activate coagulation. In addition, surgery results in exposure of procoagulant basement membrane with subsequent promotion of a hypercoagulable state.

3.8.2 Venous stasis

This may occur as a result of direct compression of the vessel walls by the tumour or during prolonged periods of bed rest. Long operative procedures (eg malignant gliomas) requiring surgery of greater than four hours has been identified as a risk factor for VTE (Marras, Geerts, & Perry 2000). Blood viscosity measured preoperatively has been correlated with the incidence of postoperative VTE (Humphreys, Walker, & Charlesworth 1976), and is significantly elevated in breast and gynaecological cancer patients compared to patients with the corresponding benign tumour disease (von Tempelhoff *et al.* 2000).

3.8.3 Blood abnormalities

Multiple abnormalities of blood constituents have been described in patients with cancer, resulting in disruption of the homeostatic balance of pro- and anti-coagulation (Falanga *et al.* 1993a). Some are non specific, such as the generation of acute phase reactants and necrosis, associated with the host inflammatory response (Nowacki, Janik, & Nowacki 1996). However, tumour specific clot-promoting mechanisms are most commonly implicated in the hypercoagulability of cancer (Dvorak 1987). Tumour cells interact directly with the haemostatic system through release of molecular mediators, or tumour cells induce procoagulant properties and inhibit anticoagulant properties of other cell types such as platelets and vascular endothelial cells. Both mechanisms may occur either at the site of the tumour, or with entry of tumour cells into the circulation (Piccioli *et al.* 1996). Molecular mediators released by tumour cells that influence the haemostatic system may be directly procoagulant (TF, Cancer Procoagulant (CP), Thrombospondin-1

(TSP-1) (Gordon & Mielicki 1997; Varani *et al.* 1989; Zacharski *et al.* 1993), fibrinolytic (urokinase Plasminogen Activator (uPA), tPA, Plasminogen Activator Inhibitor (PAI)) (Duffy *et al.* 1988; Duffy 2002; Kwaan 1992) or cytokines that alter the activity of other cells for example vascular endothelial cells (Tumour Necrosis Factor–α (TNF-α), VEGF) (Balkwill *et al.* 1987; Dvorak *et al.* 1992). Tumour cell-haemostatic cell interactions may occur through alteration in expression and function of adhesion molecules. In platelets, this is mediated in part through VEGF (Wynendaele *et al.* 1999) and p-selectin (McCarty *et al.* 2000). Endothelial cell mediators include E-selectin (Narita *et al.* 1996) and Vascular Cell Adhesion Molecule-1 (VCAM-1) (Langley *et al.* 2001). Macrophage mediators may include tumour-specific antigens or cytokines such as TNF-α (Balkwill *et al.* 1987).

The following pages will present:

- Evidence for the activation of the haemostatic system at the tumour site
- tumour cell–haemostatic system interactions at the extravascular site of tumour growth
- the circulating sequelae of tumour cell–haemostatic system interactions

3.9 Tumour cell-haemostatic system interactions

3.9.1 Interaction and activation of the haemostatic system at the tumour site

Fibrin deposition at the tumour site:

As early as 1878, Bilroth observed thrombi associated with microscopic intravascular tumour deposits (Bilroth 1878). Moreover, fibrin deposition around tumour cells has

been recognised for over fifty years (Day, Planinsek, & Pressman 1959; Dewey *et al.* 1963). O'Meara in 1958 suggested the existence of a "cancer-coagulative factor" promoting fibrin deposition around tumour tissue, providing a frame for cancer cell growth (Boggust *et al.* 1963; O'Meara 1958a; O'Meara 1958b). Numerous techniques including the use of antisera, monoclonal antibodies, immunohistochemistry and electron microscopy have established that fibrin is a constitutive component of the stroma of many tumours (Costantini & Zacharski 1993; Dvorak *et al.* 1981a). It appears to embrace the surfaces of tumour nodules and individual tumour cells (Zacharski, *et al.* 1993) and is particularly abundant around tumour periphery and at the tumour-host interface (Dvorak *et al.* 1981a; Wojtukiewicz *et al.* 1990). Fibrinogen, the precursor of fibrin, is not synthesised by tumours other than those derived from mature hepatocytes, so it is unlikely that the fibrin found in the majority of malignancies arises from local tumour synthesis (Dvorak 1987). The fibrin deposited around tumours results from extravasation through abnormally permeable microvasculature, and tumour associated clotting mechanisms (Dvorak 1987). Fibrin is thought to provide an extracellular network to support growth of new cells and prevent access of the host's immune system to the tumour.

Coagulation factors at the tumour site:

Many coagulation factors can be identified in the tumour stroma and on cancer cells (Gordon 1992). For example, factors VII, IX, X, and XII can be seen on gastric cancer cells, with prothrombin and Prothrombin Fragment 1 and 2 (PF1+2) demonstrated in the tumour stroma and on cancer cells (Wojtukiewicz *et al.* 2003b). TF is present on cancer

cells and tumour-associated macrophages, but staining for TFPI is minimal. Staining for uPA and tPA in gastric cancer is weak, implying increased coagulation activation as compared to fibrinolysis (Wojtukiewicz *et al.*2003b). Pancreatic cancer displays a similar picture (Wojtukiewicz *et al.* 2001). However in colorectal cancer, an intact coagulation pathway is not easily identified, and laryngeal cancer exhibits stronger staining of fibrinolytic factors and TFPI (Wojtukiewicz *et al.* 2003a). TF has been localized to vascular endothelial and tumour cells, within invasive breast cancer tumours, but not in the vascular endothelial or tumour cells of benign breast disease, following a similar pattern to the distribution of fibrin. This TF expression correlates with the initiation of angiogenesis, supporting the concept that tumour cells can activate nearby vascular endothelial cells and regulate blood vessel growth (Contrino *et al.* 1996). These divergent staining patterns across different tumour types demonstrate that a detailed mechanism for the coagulation activation and widespread fibrin formation at the tissue level is not easily determinable from tissue studies.

In addition antithrombin III has reduced immunoreaction in the vascular endothelium of glioblastomas as compared to normal brain tissue (Isaka *et al.* 1994), implying a reduced role of anticoagulants at the tumour site.

Fibrinolysis at the tumour site:

Tumour progression involves the disruption of anatomical barriers and penetration of tumour cells into normal adjacent host tissues, as well as the infiltration of normal host cells into the tumour (Anderson 1985). The association of fibrinolysis with tumour tissue was first reported in 1911 when it was demonstrated that the *in vitro* cultivation of a

variety of malignant tumours dissolved or liquefied the plasma growth medium (Carroll & Binder 1999). UPA is specifically implicated in this process (Carroll & Binder 1999). It is widely believed that uPA at the tumour cell surface initiates a proteinase cascade, which in turn leads to breakdown of the extracellular matrix and thereby promotes cellular migration. This conclusion is supported by the fact that uPA and the uPA receptor are highly expressed by tumour cells or by surrounding stromal cells, and that they are both independent prognostic indicators in cancer (Reuning *et al.* 1998)

The interaction of coagulation and fibrinolytic factors at the tumour surface is complex and varies between different cancer types. There is an ongoing fluctuating equilibrium that appears to support the growth and spread of cancer cells, through as yet undefined mechanisms. The deposition of fibrin in the connective tissue surrounding viable tumour may interpose between tumour cells and host inflammatory cells that might otherwise invade and destroy the tumour (Rickles & Falanga 2001). Coagulation and fibrinolytic factors may promote angiogenesis, cell adhesion or vascular permability to allow tumour cell intra and extravasation (Rickles & Falanga 2001).

Tumour emboli:

Tumour emboli also demonstrate this interaction with the coagulation and fibrinolytic systems. Warren demonstrated tumour emboli to be covered in a fibrin mesh, which may assist with the adhesion of these emboli to the vessel wall (Warren & Vales 1972). Platelet microemboli have also been associated with growing tumour cells (Gasic *et al.* 1973; Marcum *et al.* 1980). Platelet-tumour cell adhesion appears to be mediated through the thrombin receptor (Akarasereenont *et al.* 2001; Nierodzik *et al.* 1996). This adhesion

is thought to enhance metastatic spread through increased adhesion to vasculature, via fibronectin and von Willebrand Factor (vWF) ligands (Nierodzik, Klepfish, & Karpatkin 1995), and protection of tumour cells from the host immune system.

3.9.2 Molecular mediators expressed by tumour cells

For the purpose of simplicity molecular mediators are divided into procoagulant molecules expressed by tumour cells, fibrinolytic molecules expressed by tumour cells and cytokines that are released by tumour cells and have an impact on haemostasis.

Tumour cell procoagulants

The most well-recognised and researched procoagulant molecules expressed by tumour cells are tissue factor (TF), cancer procoagulant (CP) and thrombospondin-1 (TSP-1).

Tissue Factor

TF is an integral membrane glycoprotein who's extracellular domain initiates coagulation by binding and promoting activation of factor VII (Ott *et al.* 1998). TF is found in normal epithelial tissues including skin, gut mucosa, and the genitor-urinary system (Martin, Wiiger, & Prydz 1998). The expression of TF in normal cells is tightly controlled and is not normally expressed on resting endothelium and monocytes (Rickles & Falanga 2001). Proinflammatory cytokines such as TNF-α induce expression of TF procoagulant activity in these cells (Archipoff *et al.* 1991; Bevilacqua *et al.* 1986). In contrast, constitutive expression of TF is observed both in malignant transformed cells

and on vesicles shed spontaneously from tumour cells both in culture and *in vivo* growth models (Dvorak *et al.* 1983). TF expression has also been found in many malignant tumours; colon, small cell lung carcinoma, transitional cell carcinoma, cervical, pancreatic and gastric carcinoma (Callander *et al.* 1989; Wojtukiewicz *et al.* 1989; Wojtukiewicz *et al.* 1990; Zacharski, Schned, & Sorenson 1983). In the breast, TF has been found to be localised to the vascular endothelial and tumour cells of invasive breast cancer but not benign fibrocystic disease (Contrino *et al.* 1996). This expression of TF is associated with local deposition of coagulation pathway intermediates and thrombin generation as evidenced by the conversion of fibrinogen to fibrin that is found to tightly associate with the surfaces of tumour nodules and individual tumour cells (Zacharski *et al.* 1993). Considerable experimental data exists showing that tumour cells spontaneously shed plasma membrane vesicles during cell turnover that is associated with procoagulant activity interacting at several steps in the clotting cascade (Dvorak *et al.* 1981b; Silberberg, Gordon, & Zucker 1989). Plasma levels of TF are higher in solid tumour cancer patients than normal controls (Kakkar *et al.* 1995).

Cancer Procoagulant

CP is a 68kDA cysteine endopeptidase. The only known physiological substrate for CP is coagulation factor X, thereby activating coagulation independently from both the intrinsic and extrinsic pathways (Donati *et al.* 1986a). CP is synthesised by malignant cells and it appears to be active in many tumours, being found in extracts of colorectal, squamous, melanoma, endometrial, epitheliomas and adeno, squamous and small cell lung cancer (Donati *et al.* 1986a; Gordon, Franks, & Lewis 1975; Gordon & Mielicki

1997; Rucinska *et al.* 1997b). The presence of CP antigen and activity has been detected in sera from cancer patients, and has been shown to have potential as a sensitive and specific early-stage tumour marker (Gordon & Benson 1989; Gordon & Cross 1990; Naschitz *et al.* 1996; Rucinska *et al.* 1997a). CP appears to have vitamin K dependent activity, since in both murine and human tumours it is depressed by treatment with warfarin or vitamin K-deficient diet (Donati *et al.* 1986b; Roncaglioni *et al.* 1986).

Thrombospondin-1

TSP-1 is a high molecular weight adhesive glycoprotein involved in cell-cell and cell-matrix interactions, which is located in the Weibel-Palade bodies of endothelial cells and α-granules of platelets (Lawler 1986). It is also secreted by numerous tumour cell lines including melanoma, fibrosarcoma, and carcinomas (Asch *et al.* 1987; Boukerche *et al.* 1995; Grossfeld *et al.* 1997; Varani *et al.* 1986; Varani *et al.* 1989). TSP-1 has been implicated in the metastatic spread of certain tumours including melanoma, where it promotes tumour cell adhesion and cell motility *in vitro* (Boukerche , *et al.* 1995; Taraboletti *et al.* 1990). It has numerous roles in promoting haemostasis including enhancing cross-linking of platelet-fibrinogen aggregates (Bale & Mosher 1986; Leung 1984), stabilising fibrin clot formation (Bale, Westrick, & Mosher 1985), and modulating fibrinolysis, by slow inhibition of plasmin and uPA (Mosher *et al.* 1992; Silverstein, Harpel, & Nachman 1986; Silverstein & Nachman 1987).

Levels of circulating TSP-1 are increased in breast cancer, and correlate with tumour grade and nodal status (Hayden *et al.* 2000).

Tumour cell fibrinolytic proteins

Elevated levels of plasma markers of fibrinolysis are commonly found in patients with solid tumours (Di Micco *et al.* 2001; Kirchheimer *et al.* 1987; Rocha *et al.* 1989).

Tumour cells can express on their surface all the factors that are required for the regulation of the fibrinolytic pathway. They can express uPA, tPA and can produce PAI 1 and 2 (Kwaan 1992). Among activators, uPA is the most widely expressed within malignant lesions (Stephens *et al.* 1988). Specific receptors (eg uPA receptor) on tumour cells favour the binding of all the fibrinolytic components thus facilitating the activation of the fibrinolytic cascade (Hajjar 1995). However, defects in the generation of normal plasma fibrinolytic activity has been found in patients with solid tumours and may represent another mechanism for the propensity of these patients to develop VTE (Kwaan & Keer 1990).

Fibrinolytic proteins in angiogenesis and tumour growth

Recent evidence supports the role of tPA, uPA, uPA receptor, PAI 1 and 2 in tumour invasion, tumour cell proliferation and metastases. PAI-1 levels are recognized as potentially an important predictor of disease free interval and long term survival in malignant disease (Kwaan 1992). There appears to be a correlation between the uPA expression and both the aggressiveness of some tumours and the histological grade, as well as clinical progression of different carcinomas (Kwaan 1992). This expression of uPA seems to influence the capacity of cancer cells to invade tissues via plasmin-induced effects (Markus 1984).

Tumour cell cytokines

Tumour Necrosis Factor-α

Tumour cells produce cytokines such as Tumour Necrosis factor-α (TNF-α) (Gianni *et al.* 1995; Niiya *et al.* 2003; Rube *et al.* 2003; Vilcek & Lee 1991). TNF-α can elicit important procoagulant effects on the vascular endothelium (Bauer *et al.* 1989). TNF-α induces the expression of TF by vascular endothelial cells (Andree & Nemerson 1995; Archipoff *et al.* 1991; Bevilacqua *et al.* 1986; Spillert *et al.* 1995). TNF-α down-regulates the expression of vascular endothelial cell thrombomodulin, a surface receptor for thrombin that activates the potent anticoagulant protein C (Archipoff *et al.* 1991; Moore, Esmon, & Esmon 1989; von der *et al.* 1993). This up-regulation of TF and down-regulation of thrombomodulin converts the normal anticoagulant endothelium to a prothrombotic endothelium (Moore, Esmon, & Esmon 1989). TNF-α also increases the synthesis of PAI-1 and 2 by vascular endothelial cells in a dose dependent manner further contributing to a prothrombotic endothelium (Zoellner *et al.* 1993). TNF-α also stimulates monocytes to express TF, thus further leading to hypercoagulability (Bottles & Morrissey 1993; Conkling, Greenberg, & Weinberg 1988). Administration of TNF-α to healthy volunteers has been shown to rapidly raise tPA levels, however this increase is followed by a much larger increase in levels of PAI-1, and evidence of increased thrombin and fibrin generation (van Hinsbergh *et al.* 1990).

Significantly increased levels of serum TNF-α have been found in patients with gastric cancer and childhood malignancies including leukaemias and solid tumours (Barber, Fearon, & Ross 1999; McCall, Tuckey, & Parry 1992; Saarinen *et al.* 1990).

Vascular Endothelial Growth Factor

VEGF is a multifunctional cytokine which promotes increased microvascular permeability to plasma proteins, modifying the tumour extracellular matrix and promoting angiogenesis. It is a selective endothelial cell mitogen involved in non-malignant processes such as wound healing (Senger *et al.* 1994). VEGF is expressed by colon, stomach, small bowel, pancreas and breast carcinoma more than by normal tissue, polyps or adenomas (Brown *et al.* 1993; Brown *et al.* 1995). The secretion of VEGF by tumour cells may account for the heightened microvascular permeability found in a wide variety of tumours, and is considered to play a key role in tumour angiogenesis (Dvorak *et al.* 1992).

VEGF stimulates the expression of TF, induces TF mRNA expression and gene promoter activation and TF procoagulant activity in both endothelial cells and macrophages (Armesilla *et al.* 1999; Clauss *et al.* 1990). VEGF is also chemotactic for macrophages (Clauss *et al.* 1990). VEGF co-localises with tissue factor in breast tissue (Shoji *et al.* 1998), and is associated with TF in non-small cell carcinoma of the lung and malignant melanoma (Abe *et al.* 1999; Koomagi & Volm 1998). Serum levels of the soluble form of VEGF are increased with advancing stage of colorectal cancer (Kumar *et al.* 1998), and in breast cancer, particularly oestrogen receptor positive tumours (Heer *et al.* 2001).

3.9.3 Tumour cell to haemostatic cell interactions

The interaction of tumour cells with host cells represents another mechanism by which tumours can manipulate haemostasis and induce VTE. These interactions may occur by

direct cell-cell interaction, usually mediated via adhesive integrins expressed by both tumour cells and host cells, or indirect methods by the release of cytokines.

Platelets:

Platelets appear to be closely associated with the metastatic process. Platelet aggregates surrounding human tumour cells were first described in 1903 (Nand & Messmore 1990). Elevated plasma levels of the platelet specific α-granule proteins α-thromboglobulin and platelet factor 4 have been detected in patients with active malignant disease compared to those in remission (Al Mondhiry 1983). Platelets can be activated by intact human tumour cells and shed membrane vesicles (Bastida & Ordinas 1988; Schwartz & Simantov 1998). Platelet activation encompasses platelet shape change, granule secretion, alterations in platelet glycoprotein receptor function, expression of a procoagulant surface and secretion of bioactive substances from the platelet. Following this initial activation of platelets, platelet aggregation occurs through binding of fibrinogen to newly exposed fibrinogen binding sites on the activated platelets (Sims *et al.* 1991), allowing the formation of fibrinogen bridges between platelets (Ofosu & Nyarko 2000). Activated platelets rapidly express p-selectin when the internal surface of the α-granule is exteriorized, which promotes localization of neutrophils and monocytes to the site of thrombus formation or inflammation (Hamburger & McEver 1990; Larsen *et al.* 1989). TSP-1 and VEGF are also released from the α-granules of platelets. All three molecules are detectable in plasma, with increased levels being indicative of platelet activation (Blann & Lip 1997; Kamath, Blann, & Lip 2001).

Endothelial cells:

Tumour cells can interact with the vascular endothelium by direct and indirect mechanisms. Indirect mechanisms, as described above, include the synthesis and release of inflammatory cytokines by tumour cells, which reduce the anti-thrombotic and enhance the prothrombotic properties of vascular endothelial cells. However direct interaction may take place between tumour cells adherent to vascular endothelial cells or endothelial cell matrix through membrane adhesion molecules. Such molecules include VCAM-1, the ligand for β1 integrins, and E-selectin, ligand for sialyl lewis antigen. TNF-α and interleukin-1 increase the expression of these endothelial cellular adhesion molecules, as well as platelet activating factor. This is accompanied by a loss of anticoagulant molecules such as thrombomodulin (Schwartz & Simantov 1998). By attaching to vascular endothelial cells, tumour cells may play a key role in promoting localised formation of thrombus. Tumour cell release of cytokines induces the adhesive potential of the endothelium, and promotes the adhesion and arrest of other cells such as platelets (McEver 1997). This adhesion of tumour cells either to vascular endothelial cells or other tumour cells, may also facilitate tumour cell migration and extravasation.

Monocyte/Macrophages:

Substantial experimental evidence supports the presence of increased numbers of activated monocytes in the circulation of cancer patients and in proximity to growing tumours (Shoji *et al.* 1998). They may be activated by tumour-specific antigens, immune complexes involving tumour antigens or more directly by cytokines such as TNF-α (Rambaldi *et al.* 1986). Monocytes do not constitutively express TF. On activation they

expose this procoagulant on their surface, and subsequently form cross-linked fibrin in apposition to tumours, perhaps as a primitive effort by the host to limit tumour spread (Falanga & Rickles 1999). Tumour associated macrophages express significantly more TF than control cells. Circulating monocytes from patients with different types of cancer also express increased TF activity (Edwards, Rickles, & Cronlund 1981; Lorenzet *et al.* 1983).

3.10 The hypercoagulable state of cancer

Clinically overt VTE represents only a small degree of the systemic clotting activation, or hypercoagulable state, that occurs in malignancy. Hypercoagulability has been defined as a "condition of a procoagulant imbalance due to heightened enzymatic activation of coagulation zymogens but with no laboratory evidence of deposition of cross-linked fibrin nor clinical signs of thrombosis" (Bauer & Rosenberg 1987).

Seale and Rapaport independently reported cases of hypercoagulability and hypofibrinogenemia in patients with prostatic cancer (Rapaport & Chapman 1959; Seale, Jampolis, & Bargen 1951). Others have also supported a particular propensity to hypercoagulability and intravascular fibrin deposition in prostatic cancer (Charytan & Purtilo 1969; Frick 1956; Goodnight, Jr. 1974; Naeye 1962).

3.10.1 Elevated markers of coagulation

Approximately half of all cancer patients and about 90% of those with metastases exhibit abnormalities of one or more "routine" coagulation parameters. The most common

include elevation of clotting factor levels (fibrinogen, factors V, VIII, IX and XI), the fibrinogen /FDPs levels and thrombocytosis (Falanga *et al.* 1993a). Several of these parameters become more abnormal with disease progression (Edwards *et al.* 1987). Although these markers are indicative of hypercoagulability, none have yet been shown to correlate with development of clinical VTE. However routine functional coagulation screens such as prothrombin time (PT) and activated partial thromboplastin time (APTT) do not appear to show significant difference between cancer and non-cancer groups (Di Micco *et al.* 2001)

More sensitive markers have been developed and applied to the study of hypercoagulability in cancer. Fibrinopeptide A (FPA), a peptide released after cleavage of fibrinogen by thrombin, is a marker of thrombin activity and fibrin formation. This is elevated in the plasma in approximately two-thirds of cancer patients at presentation, and appears to correlate with disease progression. However the assay is limited by *in vitro* artefacts and a short half-life of FPA (Gouin-Thibault, Achkar, & Samama 2001; Rocha *et al.* 1989).

Less transient markers include PF1+2 (released after activation of prothrombin to thrombin and reflecting prothrombinase activity and thrombin generation), thrombin-antithrombin (TAT) (reflecting thrombin generation and inhibition), and FDPs such as D-dimer (generated after activation of both coagulation and fibrinolysis). Several studies have shown that all of these markers are commonly elevated in cancer patients irrespective of the presence of clinical VTE (Gouin-Thibault, Achkar, & Samama 2001), with levels being related to tumour burden (Donati & Falanga 2001; Iversen & Thorlacius-Ussing 2002; Seitz *et al.* 1997). These markers are significantly elevated in

pulmonary venous blood as compared to simultaneously sampled superior vena cava blood in lung cancer patients undergoing surgery (Kalweit *et al.* 2000). In a similar study comparing samples from the tumoural draining vein from colon carcinoma at surgery, markers of hypercoagulability were elevated compared to levels in peripheral veins sampled simultaneously (Garcia-Avello *et al.* 2001), implying a direct contribution of the tumour to hypercoagulability.

The frequency of abnormalities of these markers varies with the extent of hypercoagulation. For example, Tripodi demonstrates approximately 25% of cancer patients studied have elevated FPA, PF1+2 and TAT, but only 10% show concomitant presence of elevated plasma D-dimer levels, indicating the coagulation cascade has proceeded to the point of deposition of cross-linked fibrin (Tripodi *et al.* 1993).

3.10.2 Alteration in fibrinolysis

In the 1950s, both Seale and Rapaport independently reported cases of hypercoagulability and hypofibrinogenemia in patients with prostatic cancer (Rapaport & Chapman 1959; Seale, Jampolis, & Bargen 1951). Functional tests of the fibrinolytic system have demonstrated a shortened fibrinogen have-life in patients with active malignancy compared to those in remission, implying rapid conversion to fibrin (Lyman *et al.* 1978). However fibrinolytic activity is reduced (Rennie & Ogston 1975). Despite this, plasma levels of plasmin-α2-plasmin inhibitor complex, which represents *in vivo* plasmin formation, are significantly raised in lung cancer patients and can act as an independent predictor of survival (Taguchi *et al.* 1996). The measurements of proteins of the fibrinolytic system, for example plasminogen activators and plasminogen activator

inhibitors, also indicate a hypercoagulable state. Elevated circulating levels of uPA, tPA and PAI are well described in association with cancer (Carroll & Binder 1999; Casslen *et al.* 1994; Ho *et al.* 1998). High levels of Plasminogen Activator Inhibitor have been found in the tumoral draining vein of colon carcinoma at surgery, as compared to levels in peripheral veins sampled simultaneously, implying the tumour itself is the origin of some of the fibrinolytic system inhibition (Garcia-Avello *et al.* 2001). Increased circulating levels of tPA seen on the first post-operative day following maxillofacial surgery for malignancy compared to surgery for benign disease suggest a hyper-responsive fibrinolytic response (Wendel *et al.* 1999).

3.10.3 Alteration in anticoagulant activity

Evidence seems to suggest that consumption or compromise of the anticoagulant system may also play a role in hypercoagulation of cancer, however the evidence here is fairly limited (Gordon 1992).

3.10.4 Markers of intravascular fibrin deposition

Early investigation into markers of the intravscualar fibrin deposition in 100 metastatic cancer patients showed marked elevation of fibrinogen in almost all, with highest levels seen in lung and breast carcinoma (Soong & Miller 1970). Fibrinogen correlates significantly with FIGO stage in ovarian malignancy, and is a significant but not independent, risk factor for reduced overall survival (von Tempelhoff *et al.* 1997).

Astedt, in the early 1970s, reported elevated FDPs in 72% of untreated cancer patients compared to 5% in benign disease (Astedt, Svanberg, & Nilsson 1971; Astedt, Svanberg, & Nilsson 1972). FDP levels initially rose during treatment, but fell to normal if treatment was successful. Levels increased again with disease recurrence, implying a potential role as a surrogate marker of recurrence and disease response to treatment (Astedt, Svanberg, & Nilsson 1971; Astedt, Svanberg, & Nilsson 1972).

Circulating D-dimer levels are 20-fold higher in patients with non-metastatic gastric cancer as compared to normal controls (Di Micco *et al.* 2001). Elevated plasma D-dimer levels in patients with colorectal cancer are associated with relatively advanced tumour stage and short postoperative survival after curative resection. In Oya's study of pre-operative circulating levels, D-dimer was found to be the third most powerful prognostic marker, behind lymph node status and preoperative carcinoembryonic antigen levels, in a series of 93 colorectal patients undergoing curative resection (Oya *et al.* 2001).

In a further study, plasma D-dimer levels, in 102 invasive breast carcinoma patients, were shown to correlate with lymphovascular invasion, clinical stage, and lymph node involvement. It has thus been suggested that detectable fibrin degradation, as measured by plasma D-dimer, is a clinically important marker for lymphovascular invasion and early tumour metastases in operable breast cancer (Blackwell *et al.* 2000).

3.10.5 Platelet changes in hypercoagulability of cancer

Paradoxically thrombocytosis and thrombocytopenia are commonly seen in cancer. Thirty to 60% of cancer patients are reported to have thrombocytosis (Edwards *et al.* 1987; Sun *et al.* 1979). As early as 1968 it was shown that artificial induction of

thrombocytopenia in animals protected against the development of metastases (Gasic *et al.* 1973; Gasic, Gasic, & Stewart 1968). Tumour cells can cause platelet aggregation *in vitro*, and in mice, intravenous injection of tumour cells causes thrombocytopenia (Gasic *et al.* 1973). This thrombocytopenia usually occurs after one hour and lasts up to three days. The tumour cells that demonstrated the above characteristics had the greatest potential for metastatic spread. This platelet aggregating activity of tumour cells, both human and animal, was also identified in the plasma membrane vesicles shed by the tumour cells (Karpatkin & Pearlstein 1981; Pearlstein et al. 1980)

3.11 Cancer related risk factors

3.11.1 Risk Factors for VTE that coexist with cancer

Many of the factors that increase the risk of VTE commonly occur in the cancer patient. Immobility, for example, is a common problem in cancer patients for a variety of reasons (e.g. pain, fatigue and neurological compromise). Hospital in-patients with cancer are at a higher risk of developing fatal pulmonary embolism than non-cancer inpatients (14% versus 8%, $p<0.05$) (Shen & Pollak 1980). The presence of paresis is an independent risk factor for the development of VTE in high grade gliomas, with thrombosis more likely to occur in the paretic limb (Dhami *et al.* 1993). The risk of cancer is age dependent. As the cancer population is relatively old, this increase the risk of VTE. Immunity is frequently compromised in cancer, resulting in infection; which is a further risk factor for VTE. Dehydration resulting from poor nutrition, reduced appetite and excessive third space losses may also be a contributory factors.

3.11.2 Cancer therapies as a risk factor for venous thromboembolism

Surgery:

Cancer patients undergoing surgical procedures have at least twice the risk of postoperative DVT and more than three times the risk of fatal PE than noncancer patients undergoing similar procedures (Chan *et al.* 1999; Kakkar & Williamson 1999). Kakkar prospectively studied cancer patients undergoing surgical procedures, using fibrinogen leg scans. He showed 41% of cancer patients developed post-operative DVT compared to 26% of patients of similar age without cancer (Kakkar *et al. 1*970). In the 1975 International Multicentre Trial of Heparin Prophylaxis, patients in the no prophylaxis arm, (eight of 491 (1.6%)) who had operations for cancer developed fatal PE compared with 8 of 1,585 who had operations for non-malignant disease (0.4%) (Lancet 1975). The incidence of DVT in patients undergoing surgery for cancer is 40% (Bick 1978; Pineo *et al.* 1974), compared to 12% in patients without cancer who undergo comparable surgical procedures (Pineo *et al.* 1974).

Clahsen reports a VTE incidence of 0.8% following breast cancer surgery, however use of prophylaxis is not specified. Increased rates of VTE were found in patients who were postmenopausal and those who had mastectomy as compared to wide local excision (Clahsen *et al.* 1994). This is supported by Wedgwood who cites an almost 5% VTE rate following modified radical mastectomy (Wedgwood & Benson 1992). Similar studies however find no increase in VTE incidence.

Increased hypercoagulability following cancer surgery

Markers of both the coagulation and fibrinolytic system have an altered response to surgery in cancer as compared to benign disease (Modrau, Iversen, & Thorlacius-Ussing 2001). Markers of thrombin formation (PF1+2 and TAT) increase following colorectal and maxillofacial surgery, however this increase is significantly greater in surgery for malignant as compared to benign disease (Iversen & Thorlacius-Ussing 2002; Wendel *et al.* 1999).

The increased fibrinolysis seen with IPC in non-malignant patients does not occur with patients with malignant disease (Allenby *et al.* 1973). Elevations in post-operative plasma tPA and D-dimer levels are a systemic, and not local response with no significant difference in levels sampled from peripheral veins or the tumoral draining vein (Garcia-Avello *et al.* 2001).

In breast surgery a significantly greater haemostatic response is seen in patients following surgery for cancer as compared to patients undergoing similar surgery for benign breast disease. This is demonstrated by marked increase in plasma markers of coagulation activity (fibrinogen, TAT), fibrinolytic inhibition (PAI), and fibrinolysis (D-dimer and tPA) (Oberhoff *et al.* 2000).

Chemotherapy:

Sallah demonstrates an overall incidence of VTE of 17% (40 of 242) in solid tumour patients receiving chemotherapy (Sallah, Wan, & Nguyen 2002). VTE occurs in 17.6% of advanced breast cancer patients receiving chemotherapy (Goodnough *et al.* 1984), and 5% of patients receiving chemotherapy for early breast carcinoma (Weiss *et al.* 1981).

Administration of chemotherapy in high grade gliomas was found to be an independent risk factor for the development of VTE (Dhami *et al.* 1993).

Many chemotherapeutic agents affect haemostatic function, including the hepatic synthesis of coagulation factors and their inhibitors, whilst other agents can cause endothelial damage (Gordon & Kwaan 1997; Lazo 1986).

Many vascular abnormalities or toxicities have recognised associations with particular chemotherapies, for example pulmonary veno-occlusive disease (bleomycin), hepatic veno-occlusive disease (conditioning regimens for bone marrow transplantation and cyclophosphamide), the Budd-Chiari syndrome (methotrexate), myocardial infarction (vinca alkaloids), thrombotic thrombocytopenic purpura (mitomycin), and Raynaud phenomenon (vinblastine and bleomycin).

This subject is covered in greater detail in chapter 4.

Indwelling central lines:

Patients with cancer often have central venous catheters placed for the administration of chemotherapy, blood products, fluids, medicines and also for drawing blood. Thrombosis has been venographically documented in 38-62% (Bern *et al.* 1990; Monreal *et al.* 1996) of central venous access devices, although the number of symptomatic thrombi is lower. The overall risk of PE is 13% (Monreal *et al.* 1994). Several factors have been identified that increase the risk of VTE even further. Left subclavian lines are at a higher risk than the right (De Cicco *et al.* 1997). Polyvinyl chloride or polyethylene are associated with a higher PE rate than polyurethane or siliconized lines (Monreal *et al.* 1994). A triple

lumen catheter may be more thrombogenic than a double lumen ($p<0.05$) (Eastridge & Lefor 1995), whilst a line requiring more than one puncture for insertion is more thrombogenic. Total parental nutrition has been found to be more thrombogenic than crystalloid (Koksoy *et al.* 1995). Various mechanisms have been suggested including vessel-wall trauma at the time of insertion, and endothelial abrasion due to catheter movement within the vessel (Masci *et al.* 2003).

The presence of a foreign body is a risk factor for infection. This is well recognised with central venous lines as they provide a direct route of access for bacteria, either along the line tract or within the line itself. The latter case is a particular issue when frequent, recurrent access to the line is required for administering treatments or taking blood. Such line sepsis is associated with an increased risk of thrombosis (Raad *et al.* 1994). Trials of warfarin (Masci *et al.* 2003) and LMWH (Monreal *et al.* 1996) thromboprophylaxis in patients with central lines support anticoagulant usage.

Hormone Therapy:

Hormonal therapy, used in carcinoma of the breast and prostate, is known to increase the incidence of thromboembolism (Deitcher & Gomes 2004; Fisher *et al.* 1989; Lee & Levine 1999; Maenpaa & Ala-Fossi 1997; Rutqvist & Mattsson 1993; Saphner, Tormey, & Gray 1991).

For many years opinion had been divided on the thromboembolic effect of hormone therapy. In 2644 node-negative breast cancer patients randomised to tamoxifen or placebo, Fisher reports two of 1326 (0.15%) thromboembolic events in the placebo group but 12 of 1318 (0.9%) in the tamoxifen arm (Fisher *et al.* 1989). The Stockholm Breast

Cancer Study Group report a relative risk of 1.22 in patients receiving adjuvant tamoxifen alone, as compared to no treatment, following surgery, but no increased risk from tamoxifen if combined with chemotherapy or radiotherapy (Rutqvist & Mattsson 1993). However the NSABP (National Surgical Adjuvant Breast and Bowel Project) Breast Cancer Prevention Trial has unequivocally demonstrated an increased risk of VTE with tamoxifen therapy, independent of the presence of cancer and chemotherapeutic agents (Lee & Levine 1999). When used alone as adjuvant therapy in early breast cancer, the risk of VTE is one to 2%, but greatest in older or postmenopausal women (Lee & Levine 1999). In early breast cancer, anastrazole is also associated with an increased risk of VTE, though to a lesser extent than tamoxifen (Deitcher & Gomes 2004).

Radiotherapy:

In gynaecological malignancies radiotherapy especially during intrauterine / vaginal radium application for 12 to 24 hours, has a reported VTE rate of 2.5% to 6.8%. These rates are unaffected by the administration of LMWH or oral anticoagulation (von Tempelhoff *et al.* 1999). Sohn describes ultrasonographically visible thickening of pelvic vein walls, with deterioration of venous function following radiotherapy in women with gynaecological maliganacies (Sohn *et al.* 1993), impling a local inflammatory effect. In the treatment of Ewing's sarcoma, VTE is seen in two of 40 patients receiving preoperative radiotherapy but none of 28 without preoperative radiotherapy (Rube *et al.* 2003). Radiotherapy has also been shown to increase the risk of VTE in lung cancer (Blom, Osanto, & Rosendaal 2004).

In contrast, however, apart from axillary and subclavian thrombosis reported in the early literature, there is little evidence of increased VTE in patients undergoing modern radiotherapy after breast-conserving surgery (Thodiyil, Walsh, & Kakkar 2001).

Combined treatments:

When different cancer treatment modalities are combined, the incidence of VTE increases. Saphner, in a study of 2,673 breast cancer patients receiving adjuvant therapy found that premenopausal women receiving chemotherapy and tamoxifen had a 2.8% VTE risk as compared to 0.8% with chemotherapy alone ($p=0.03$). This risk was increased further in postmenopausal women, with a risk of 8.0% with chemotherapy and tamoxifen, 2.3% with just tamoxifen and 0.4% with no treatment ($p<0.001$) (Saphner, Tormey, & Gray 1991). This finding is confirmed by Pritchard (Pritchard *et al.* 1996).

In one randomised study, women with early breast cancer who were treated with one short intensive course of perioperative chemotherapy had three times the risk for developing VTE compared to women who receive surgery alone (Clahsen *et al.* 1994).

In a study of 1027 patients randomised to pre-operative radiotherapy versus surgery alone for rectal carcinoma 7.5% and 3.6% respectively developed VTE ($p=0.01$) (Holm *et al.* 1996). Similar VTE risks are reported by Goldberg, but the effect is confined to the first 30 postoperative days (Goldberg *et al.* 1994).

3.12 Anticoagulation in cancer

3.12.1 The treatment of venous thromboembolism

The treatment of VTE provides particular challenges in the cancer patient, especially with regard to increased risk of bleeding and recurrent thrombosis. Issues such as nausea and vomiting, drug interactions, liver malfunction, venous access and monitoring all have to be considered. This is discussed in more detail in chapter 3.7.

3.12.2 Venous thromboembolism prophylaxis in cancer

Cancer patients not undergoing treatment, and without a history of VTE, generally do not receive thromboembolism prophylaxis. The incidence of VTE is of a sufficient magnitude in patients undergoing cancer treatment that Thodiyil and other authors recommend the routine use of thromboprophylaxis for the duration of treatment (Thodiyil, Walsh, & Kakkar 2001; von Depka *et al.* 2000; Yap & McCready 2004).

Surgery:

Thromboprophylaxis with LDUH is effective in reducing VTE in cancer patients undergoing surgery (Clagett & Reisch 1988). LDUH is also effective in the prevention of fatal PE including those whose operation is undertaken for malignant disease (Kakkar *et al.* 1977). LMWH, introduced in 1982, possesses a more predictable pharmacological profile with a 90 to 95% bioavailability after a single, subcutaneous, once daily dose without the same risks for the development of thrombocytopenia and osteoporosis (Kakkar & Williamson 1999). LMWH offers significant benefits over UFH, including

having a better safety profile and being easier and more convenient to use (Kakkar *et al.* 1993).

Bergqvist and co-workers performed a prospective double-blind randomized multicentre trial of a LMWH, enoxaparin, once daily compared to unfractionated low-dose heparin, three times daily. Patients undergoing elective curative abdominal or pelvic surgery for cancer were recruited. Eighteen and 15% respectively developed venous thromboembolic complications. This study showed that the LMWH enoxaparin was at least as effective and safe as UFH (ENOXACAN Study Group 1997). A follow-up randomized controlled trial of one week prophylaxis versus prolonged prophylaxis for four weeks following abdominal cancer surgery showed a reduction in VTE from 14% to 5.5% at three months (Bergqvist *et al.* 2002). In a study of high versus low dose dalteparin prophylaxis (2500 and 5000 anti FXa IU), in elective general surgery, the higher dose was significantly more effective (DVT rate 14.9% and 8.5% respectively, p=0.001). Cancer patients had no increased bleeding complications with the higher dose (Bergqvist *et al.* 1995).

Hills and colleagues found that intermittent calf compression, during and for 48 hours after surgery, despite greatly reducing the incidence of deep vein thrombosis in patients with non-malignant disease, failed to provide a benefit in patients with malignant disease (Hills *et al.* 1972). However Clagett, in a meta-analysis, demonstrates a reduction in DVT from 21% (control) to 12.8% with IPC (Clagett & Reisch 1988). Other authors support the use of IPC in gynaecological oncology surgery (Ailawadi & Del Priore 2001; Clarke-Pearson *et al.* 1993; Maxwell *et al.* 2001), particularly focusing on cost-effectiveness (Maxwell, Myers, & Clarke-Pearson 2000). Studies have yet to confirm that calf compression reduces the risk of fatal PE.

Indwelling central lines:

One milligram warfarin has been found to be safe and effective prophylaxis against VTE in cancer patients with central venous lines, reducing venographically proven VTE rate from 37.5% to 9.5% (Bern *et al.* 1990) however other studies have suggested an interaction with fluorouracil that promotes an unstable INR (Masci *et al.* 2003). One study assessing the long-term administration of a LMWH compared to no anticoagulation significantly reduced the incidence of VTE in patients with central lines. As the incidence of VTE reached 62% (8/13) in the group without prophylaxis compared to 6% during LMWH treatment, this study was terminated earlier than planned (Monreal *et al.* 1996). In addition, anticoagulant administration reduces catheter-related central venous thrombosis, line malfunction and catheter-related sepsis (Randolph *et al.* 1998).

Radiotherapy:

There are no randomised studies of thromboprophylaxis during radiotherapy treatment alone, however the increased risk of VTE continues for between four and 12 months after cessation of treatment (Thodiyil, Walsh, & Kakkar 2001). Graf and colleagues reviewed 132 patients receiving primary or postoperative radiotherapy, with warfarin therapy. Supra-inguinal thrombosis occurred in 7.5%, with PE in 3.8%, however there was no VTE-related mortality. Bleeding occurred in 5.3%, with 1.5% considered major (Graf *et al.* 1998).

3.12.3 Anticoagulants as an anti-cancer therapy

Anticoagulants have been shown to have an inhibitory effect on tumour growth and metastasis both *in vitro* and *in vivo* (Hejna, Raderer, & Zielinski 1999; Zacharski 1986; Zacharski & Loynes 2002; Zacharski & Ornstein 1998). Warfarin was reported to double the survival of patients with small-cell lung cancer in a prospective clinical trial (Zacharski *et al.* 1981) but such a convincing finding has never been confirmed. However, other studies have shown tumour regression and an increased disease-free interval in small cell lung cancer when warfarin has been added to conventional therapy (Aisner *et al.* 1992; Chahinian *et al.* 1989). However in another trial of warfarin in small cell lung cancer, patients who received chemotherapy and radiotherapy did not have an improved outcome when warfarin was added to their treatment (Maurer *et al.* 1997). In a trial of patients with recurrent VTE, the incidence of cancer, over a mean follow-up of 8.1 years, was significantly lower among subjects randomly assigned to six months of anticoagulation with warfarin than among those randomly assigned to only six weeks of anticoagulation (Schulman & Lindmarker 2000).

LMWH, compared to UFH, has been shown to significantly reduce cancer-related mortality when used in treating DVT (Green *et al.* 1992; Hull *et al.* 1992; Lensing *et al.* 1995; Prandoni *et al.* 1992). Meta-analysis of cancer patients who receive treatment with LMWH or UFH, largely for the treatment of VTE, has shown a better survival outcome in terms of cancer (Cosgrove *et al.* 2002). In retrospective analysis this improved life expectancy appears independent of complications from VTE (Cosgrove *et al.* 2002). One prospective study designed to evaluate the influence of heparin on long term survival was performed in patients with small cell lung cancer. This study showed an increase from

24% to 37% in response to chemotherapy in patients who received five weeks of UFH therapy and an increase in survival from 261 to 317 days (Lebeau *et al.* 1994). The potential antitumour effects of LMWHs are thought to have a greater impact on early cancer compared with more advanced, disseminated malignancy (Schulman & Lindmarker 2000). In the CLOT trial (Comparison of Low-molecular-weight heparin versus Oral anticoagulation Therapy for the prevention of recurrent VTE in patients with cancer), cancer patients with symptomatic DVT were randomised to warfarin or LMWH (dalteparin) for six months. At 12 months, in the metastatic subgroup, there was no difference in mortality between the two treatment groups. In contrast, among those with non-metastatic disease at entry to the study, the 12-month cumulative mortality was 20% for those in the LMWH group compared with 35% in the oral anticoagulation group (hazard ratio 0.5, p=0.03) (Lee *et al.* 2003). These results are consistent with the recently reported FAMOUS (Fragmin Advanced Malignancy Outcome Study) trial. FAMOUS is the first randomized, placebo-controlled trial to assess the survival impact of LMWH therapy in patients with advanced solid tumours, without evidence of underlying thrombosis. A total of 385 patients were randomized to either 5,000 anti-Xa units of dalteparin or matched placebo injection, daily for one year. Among the subgroup of patients with good prognosis, Kaplan-Meier survival estimates for two and three years after randomization were significantly higher in the dalteparin group than in the placebo group (78% and 55% respectively, for dalteparin and 60% and 36%, respectively, for placebo; p=0.03) (Kakkar *et al.* 2004). Preliminary results from a similar, but not placebo-controlled, trial support these findings (Klerk *et al.* 2005).

Possible anticancer mechanisms of action of heparin include the inhibition of microthrombi formation, inhibition of angiogenesis, reduction in tumour cell adhesion and inhibition of several proteinases (Engelberg 1999). Further studies are required to investigate if this reduction in cancer-related mortality persists with the use of LMWH in DVT prophylaxis.

Chapter 4

Venous thromboembolism during chemotherapy

Although it is well established that chemotherapy is a risk factor for cancer-related VTE, data on the frequency of VTE is limited, except in breast cancer. The mechanism of action is only hypothesised. There are no predictive markers for development of VTE that would allow targeted prophylaxis in a group that is at high risk not only of VTE, but also the complications associated with thromboprophylaxis.

In the subsequent chapter current knowledge on the epidemiology morbidity and mortality of VTE during chemotherapy is presented. Additional risk factors for VTE that occur during chemotherapy are discussed. Current evidence for a chemotherapy-induced hypercoagulable state is presented and possible mechanisms for development of hypercoagulability and VTE during chemotherapy are outlined.

4.1 Epidemiology

Chemotherapy is associated with a hypercoagulable state and an increased incidence of VTE, over and above that seen in cancer *per se*, in the majority of cancers studied. Cases of VTE have been reported in association with a variety of chemotherapy regimens and different types of malignancies (Shlebak & Smith 1997). VTE occurs in over 10% of all ovarian cancers receiving chemotherapy, particularly early in therapy (von Tempelhoff *et al.* 1997), 7% of all germ cell cancers during chemotherapy (Weijl *et al.* 2000) and up to

20% of clear cell ovarian cancers receiving platinum-based chemotherapy (Recio *et al.* 1996).

The most extensively investigated cancer in terms of VTE risk during chemotherapy is breast cancer, providing evidence for the additional risk of chemotherapy in an already hypercoagulable patient. In the absence of adjuvant chemotherapy, the risk of VTE in early breast cancer is relatively low, between 0.2% and 0.8%, as compared to other cancers (Lee & Levine 1999; Saphner, Tormey, & Gray 1991). However early breast cancer is associated with a two to 10% risk of VTE during chemotherapy, and patients with advanced breast cancer receiving chemotherapy have a risk of up to 17.6% (Clahsen *et al.* 1994; Weiss *et al.* 1981a). Several studies have revealed that almost all of the VTE occur whilst patients are receiving chemotherapy, particularly early in treatment, within the first three cycles (Goodnough *et al.* 1984; Pritchard *et al.* 1996;Seward *et al.* 1999; von Tempelhoff *et al.* 1996; Weiss *et al.* 1981c). Moreover Levine demonstrates a prolonged risk of VTE in patients receiving long-course chemotherapy. In a randomized trial of 12 versus 36 weeks of chemotherapy in stage II breast cancer, no VTE occurred in the former group after 12 weeks but five events (0.5%, p=0.03) occurred in the 36-week group (Levine *et al.* 1988), implying a causal role for chemotherapy in VTE development.

Arterial thrombotic events are commonly referred to in the literature, in relation to chemotherapy. Thirty five separate cases of arterial thrombosis during chemotherapy for germ cell tumours are reported in the literature, with 1.7% of germ cell cancer patients receiving chemotherapy developing an arterial thrombosis (Weijl *et al.* 2000). Whether these arterial events are due to a primary arterial pathology, or a subclinical venous

thrombosis passing through a patent foramen ovale (present in 5-20% of the population) can only be speculated. Despite a literature review revealing a risk of arterial thrombosis during chemotherapy of 1%, Saphner failed to find any correlation between chemotherapy and arterial thrombosis in breast cancer patients (Saphner, Tormey, & Gray 1991).

4.2 Morbidity and mortality of chemotherapy related venous thromboembolism

VTE mortality in patients undergoing chemotherapy for germ cell tumours has been reported at 0.5% (Weijl *et al.* 2000). In patients receiving chemotherapy for early breast cancer, VTE-related mortality is 0.23%-0.46% (Clahsen *et al.* 1994; Weiss *et al.* 1981c). Pritchard and colleagues reported VTE to be the biggest cause of death in a randomized trial of over 700 postmenopausal node-positive women randomized to tamoxifen alone or in combination with chemotherapy (Pritchard *et al.* 1996). This is a substantial risk in patients who are potentially curable. In advanced breast cancer patients receiving chemotherapy Goodnough reports a VTE-associated death rate of 7% (Goodnough *et al.* 1984).

4.3 Chemotherapy related risk factors

Many of the risk factors that contribute to VTE in chemotherapy patients are applicable to cancer patients in general. Immobility and dehydration secondary to vomiting are frequent complications of toxic chemotherapy.

Placement of an indwelling long-term central venous catheter may be required for administration of specific chemotherapies for example administration via pumps, for ease of access in the presence of difficult peripheral access, or simply patient choice. Without prophylaxis this increases the risk of VTE to up to 62% (Monreal *et al.* 1996).

Infectious complications of chemotherapy are common, and increase thr risk of VTE. Fever occurs in up to 70% of small-cell lung cancer patients receiving chemotherapy (Crawford *et al.* 1991; Trillet-Lenoir *et al.* 1993). In solid-organ cancer patients the incidence of documented infections has approached 5%, with a 2% infectious mortality (Viscoli 1998). Most episodes of neutropenia lasting more than a week are complicated by fever, with documented infection occurring in 50% (Viscoli 1998). Central venous catheter sepsis is common and often associated with thrombosis. Electron microscopy reveals universal colonization by cocci of the fibrin catheter sleeve (Raad *et al.* 1994). At post-mortem 10% of indwelling central line cancer patients are found to have catheter-related septicaemia. All are related to mural thrombosis, with thrombosis occurring in 43% (Raad *et al.* 1994).

Infection-associated fever, dehydration and immobility promote VTE.

There is some evidence to suggest that concurrent administration of steroids with chemotherapy, commonly used as an antiemetic, is associated with an increased risk of

VTE. During chemotherapy for germ cell cancers, administration of greater than 80mg dexamethasone is an independent risk factor for the development of VTE (Weijl *et al.* 2000). This is supported by several reports about the hypercoagulable state of patients with Cushing's syndrome and the occurrence of VTE in patients receiving high dose steroids for non-malignant conditions (Jorgensen, Sorensen, & Freund 1982).

In cancer patients, surgery commonly preceeds chemotherapy. In patients with malignancy, haemostatic markers of coagulation are raised for several weeks after surgery (Rasmussen 2003). Prolonged post-operative anticoagulation is associated with a 60% reduction in relative risk of VTE. Reducing the interval between surgery and adjuvant chemotherapy has been shown to prolong disease-free survival in node-negative breast cancer patients (Ludwig 1989), however the impact on hypercoagulability of a further treatment in already recovering, inactive and hypercoagulable patients has yet to be established.

4.4 Hypercoagulability

4.4.1 Coagulation system

Breast cancer patients receiving chemotherapy have a significantly shortened PT compared to those under observation (Goodnough *et al.* 1984; Pectasides *et al.* 1999). Canobbio also found a shortened thrombin time and APTT, but Pectasides found a statistical prolongation of APTT in early breast cancer patients receiving chemotherapy (Canobbio *et al.* 1986; Pectasides *et al.* 1999). Others have found no such alteration (Rella *et al.* 1996). Several studies show an increase in FPA following chemotherapy

(Kuzel *et al.* 1990; Ruiz *et al.* 1989; Zurborn *et al.* 1991). Edwards has demonstrated a rapid increase in FPA within 45 minutes of administration of intravenous chemotherapy, an effect that is abolished by the administration of heparin prior to chemotherapy (Edwards *et al.* 1990). However no clear effect of chemotherapy has been established on other markers of coagulation such as PF1+2 and TAT (Zurborn *et al.* 1991).

Pectasides also observed a significant decrease in plasma levels of the anticoagulants ATIII, and protein C and S during chemotherapy, particularly mid therapy, in stage II breast cancer patients. Levels return to normal after cessation of therapy (Pectasides *et al.* 1999). This confirms Rella's previous findings of chemotherapy-induced decrease in protein C and S (Rella *et al.* 1996). Similar decreases have also been seen in metastatic breast cancer patients (Feffer, Carmosino, & Fox 1989; Rogers *et al.* 1988).

4.4.2 Fibrinolytic system

Several studies demonstrate an increase in circulating PAI following chemotherapy (Rella *et al.* 1996; Zurborn *et al.* 1991), with Ruiz also demonstrating a reciprocal decrease in plasma tPA levels in advanced lung cancer patients receiving chemotherapy (Ruiz *et al.* 1989). Pectasides noticed no significant change in plasma levels of D-dimer during chemotherapy in stage II breast cancer patients (Pectasides *et al.* 1999). Gabazza shows a significant decrease in D-dimer and fibrinogen in the first week after commencement of chemotherapy in lung cancer. This decrease continued to the second cycle of chemotherapy (Gabazza *et al.* 1994). He suggests that although the decreased levels of these plasma markers of the fibrinolytic system during chemotherapy may be accounted for by a drug-induced normalisation of the fibrinolytic system, the increased

activation of the coagulation system suggests that a transient imbalance of the coagulation- fibrinolytic system is the most probable mechanism of the reduced fibrinolytic activity during administration of systemic cytotoxic therapy.

4.4.3 Platelets

Pectasides further demonstrated a significant decrease in platelet count, to below normal, during chemotherapy in stage II breast cancer patients, with levels returning to normal after cessation of therapy (Pectasides *et al.* 1999), however other investigators have not found such a change (Canobbio *et al.* 1986). Moreover, vincristine has been reported to affect platelet aggregation by inducing a higher threshold for adrenaline-induced second phase aggregation, a delay in the onset of collagen-induced aggragation, and abnormal platelet adhesiveness in patients with leukaemia (Steinherz *et al.* 1976). Cyclophosphamide appears to have an inhibitory effect on platelet aggregation in children with solid tumours (Komp *et al.* 1974).

4.4.4 L-asparaginase

This drug, used almost exclusively in the treatment of acute lymphoblastic leukaemia, is particularly associated with clinical and laboratory coagulation abnormalities. L-asparaginase effects the depletion of L-asparagine, which in turn inhibits production of many plasma proteins, including fibrinogen, plasminogen, antithrombin III, protein C and protein S. However not all chemotherapies have such a straight-forward mechanism for disrupting coagulation (Lee & Levine 1999).

4.5 Pathophysiology of chemotherapy related venous thromboembolism

There are several potential mechanisms by which chemotherapy may upregulate the hypercoagulability of cancer, either by action on host tissue or tumour cells, resulting in a systemic effect. Experimental evidence supports many of these mechanisms, which may even co-exist.

4.5.1 Upregulation of tumour cell procoagulant expression and release

Chemotherapy, through targeted cell damage, may induce increased tumour cell expression or release of procoagulants. These may act directly on the coagulation cascade or indirectly on cells such as platelets, vascular endothelial cells or monocytes (von Tempelhoff *et al.* 1999).

4.5.2 Upregulation of tumour cell cytokine expression and release

In a similar way, chemotherapy may alter the expression and release of tumour cell cytokines, directly activating coagulation or stimulating cells such as platelets, vascular endothelial cells or monocytes as intermediaries in the establishment of hypercoagulability. Bertomeu has demonstrated an increase in endothelial cell reactivity when endothelial cells are incubated with post-chemotherapy plasma from breast cancer patients. This is thought to be caused by inducing the release of interleukin-1 thus facilitating adhesion molecule expression on the endothelial cell surface (Bertomeu *et al.* 1990).

4.5.3 Increased circulating tumour cells / tumour cell fragments

Malignant cells were initially discovered in the venous drainage of tumours in the 1950s, but with no correlation being identified between occurrence and distribution of metastases (Engell 1959; Roberts *et al.* 1967). However, a recent study of number of circulating breast cancer cells has found this to be an independent predictor of survival (Cristofanilli *et al.* 2004). Hardaway (Hardaway 1966) and Wood (Wood, Holyoke, & Yardley 1961) have observed circulating tumour cells in patients and animals with various neoplasms and have suggested that these may be possible sources of TF. Chemotherapy may increase the procoagulant activity of these circulating cells through alteration in procoagulant and cytokine expression.

Cell division and death is a continual process in tumour growth, with spontaneous shedding of tumour cell vesicles occurring with cell turnover (Dvorak *et al.* 1983; Milas, Stephens, & Meyn 1994). Chemotherapy induced cell death, either through apoptosis (programmed cell death) or disordered cell destruction (e.g. necrosis), may lead to an increase in circulating tumour cell fragments or microvesicles with associated procoagulant activity (Dvorak *et al.* 1983). Massive tumour cell death associated with coagulopathy is best exemplified by the tumour lysis syndrome, in which acute disseminated intravascular coagulation is almost always present (Barton 1989). Moreover, cell membrane changes occur with apoptosis that can result in the exteriorixation of the inner cell membrane, with exposure of coagulation activating molecules (Wang *et al.* 2001).

In vitro, chemotherapy induces apoptosis within hours of administration (Milas, Stephens, & Meyn 1994). Circulating neurone specific enolase, released from

neuroendocrine tumours, shows a significant increase at 24 hours, but not 3 hours after intra-hepatic chemotherapy (Cunningham *et al.* 1990). This is thought to be due to chemotherapy-induced cell lysis, and gives an indication of the time period over which chemotherapy may induce cell death.

A significant correlation between chemotherapy-induced apoptosis and thrombin generation has been demonstrated in various tumour cell lines. This thrombin generation is inhibited by anti-TF antibodies. It appears that this apoptosis results in increased exteriorisation and expression of TF by tumour cells (Morel et al. 2004; Wang *et al.* 2001).

4.5.4 Vascular endothelial cell activation

Chemotherapy may have a toxic effect on vascular endothelial cells resulting in reduction of their anticoagulant or an increase in procoagulant function. *In vitro* experiments on the effects of chemotherapy on endothelium has shown that several chemotherapies including bleomycin, vincristine and adriamycin induce endothelial cell retraction (occurring rapidly with the former two chemotherapies) and subsequent binding of platelets and tumour cells to the exposed subendothelial matrix (Nicolson & Custead 1985). This may occur as a systemic effect or be localised to angiogenic tumour vessels. Direct toxic effects on the endothelium by a number of different chemotherapies have been reported (Lazo 1986). *In vivo*, chemotherapy has been found to cause arteritis (Bodensteiner 1981; Schaeppi *et al.* 1973) with associated increases in markers of endothelial injury (Licciardello *et al.* 1985).

Most solid tumours undergo neovascularization (angiogenesis) in order to grow. The potentially abnormal endothelial lining of the new vessels might be responsible for activation of the contact system thus triggering blood clotting through the intrinsic pathway (Bick 1978).

Plasma levels of vWF, a marker of endothelial cell perturbation, increase during chemotherapy (Bazarbachi *et al.* 1993) and there is anecdotal evidence that levels increase further in patients who subsequently develop arterial occlusive complications (Licciardello *et al.* 1985). Both PAI and tPA are secreted products from endothelial cells. An abnormal chemotherapy-induced fibrinolytic response may result from decreased functional levels of tPA as well as higher systemic concentrations of PAI during chemotherapy (Ruiz *et al.* 1989). The levels of these two substances have also been found to be altered in other hypofibrinolytic states (Juhan-Vague *et al.* 1987).

4.5.5 Acute phase response

Several studies demonstrate activation of the acute phase response following chemotherapy (Zurborn *et al.* 1991). C-reactive protein (CRP) is synthesised in the liver and is normally present as a trace constituent of serum or plasma. In various disease states resulting in tissue injury, infection or inflammation, CRP values may rise above normal to 20 to 500mg/L within four to eight hours after an acute event (Kushner & Rzewnicki 1994). Serum or plasma CRP levels rise and fall more rapidly than the Erythrocyte Sedimentation Rate (ESR), returning to normal often days before the ESR normalises. Zurborn demonstrates an increase in CRP following aggressive chemotherapy in NHL and leukaemia patients (Zurborn *et al.* 1991).

4.5.6 Upregulation of platelet reactivity

Current published evidence is inconclusive about the effects of chemotherapy on platelet count (Canobbio *et al.* 1986; Pectasides *et al.* 1999). Vincristine and other chemotherapies, including chemotherapy for breast cancer, have been shown to inhibit aspects of platelet aggregation *in vitro* and *in vivo* (Klener, Kubisz, & Suranova 1977; Kumar, Chaturvedi, & Gupta 1996; Panella *et al.* 1990; Steinherz *et al.* 1976). *In vitro*, anthracyclins have been shown to increase the expression of activation-associated membrane molecules (Foss *et al.* 2002).

4.5.7 Upregulation of monocyte procoagulant activity

Mononuclear cells form an integral part of the lymphoreticular infiltrate of tumours, and may undergo functional changes upon contact with cancer cells, stimulating production of procoagulant activity such as TF (Grignani & Maiolo 2000; Lwaleed, Francis, & Chisholm 2000).

In vitro studies have demonstrated the capacity of some chemotherapies, for example cisplatin and adriamycin, to directly stimulate the expression of TF procoagulant activity by macrophages and monocytes. This effect appears independent of cell lysis as TF activity does not increase with increasing chemotherapy doses and hence reduced cell viability (Walsh, Wheeler, & Geczy 1992).

4.6 Thromboprophylaxis during chemotherapy

Studies of VTE prophylaxis during chemotherapy are limited, concentrating on oral anticoagulation. Levine and co-workers demonstrated, in a double-blind randomized-controlled trial, that low-dose warfarin is safe and effective in the prevention of VTE in patients with metastatic breast cancer receiving chemotherapy. Seven of 159 patients receiving placebo developed VTE, compared to one of 152 patients receiving low-dose warfarin (p=0.03). Major bleeding occurred in two placebo-treated patients and in one patient receiving warfarin (Levine *et al.* 1994). Rajan and colleagues performed a cost/ benefit analysis using the results of this trial and showed that very low dose warfarin can be provided to women with metastatic breast cancer receiving chemotherapy without an increase in health-care costs (Rajan *et al.* 1995). In a similar study of metastatic breast cancer patients receiving chemotherapy, Falanga demonstrated a VTE rate of 12% in a placebo group (n=16) and 0% in a low dose warfarin group (n=16), with concurrent significant reduction in markers of coagulation in the latter group (Falanga *et al.* 1998).

The need for monitoring INR is a drawback of such a prophylactic programme. For agents to be suitable for prophylaxis they must be safe in chronic administration, practical and acceptable to patients. For these reasons UFH and mechanical methods have limitations. LMWH may be suitable but are yet to be evaluated for these indications. However, it has been shown that heparin can diminish laboratory evidence of chemotherapy-related coagulopathy (Edwards *et al.* 1990).

Several papers suggest that the extent of pre-existing coagulation activation may signal an increased risk for VTE development following either surgery or chemotherapy. A role for pre-treatment measurement of, for example, TAT, PF1+2 and D-dimer has been

suggested to identify a risk profile for VTE prophylaxis (Falanga *et al.* 1993b; Iversen, Okholm, & Thorlacius-Ussing 1996; von Tempelhoff *et al.* 1996).

Chapter 5

Conclusions of Introduction

VTE in cancer is common, occurring in three to 11% of all cancer patients. The incidence of VTE rises to 17.6% in patients on chemotherapy for metastatic breast cancer. This complication of cancer, and particularly chemotherapy, results in potentially avoidable morbidity, with associated healthcare costs, and premature death.

Despite the common occurrence of coagulation abnormalities in patients with cancer, the utility of coagulation markers in determining either the extent of malignant disease or their value in predicting those who will develop thrombosis during therapy remains obscure (Kakkar *et al.* 1995). No studies to date have provided conclusive evidence that any of the plasma markers of coagulation activation are capable of identifying those at risk for the development of thrombotic complications during chemotherapy (Kakkar & Williamson 1999). The nature, extent and chronology of changes in the coagulation and fibrinolytic systems in response to chemotherapy remain unclear.

Alterations in haemostatic function in response to chemotherapy is evident. It remains unclear whether this is primarily a response to intravenous toxin (chemotherapy) or whether it is a result of the action of chemotherapy on tumour cells, initiating a tumour procoagulant response. It is reasonable to hypothesise that chemotherapy resulting in greater cell death, may release greater quantities of tumour procoagulants into the circulation, resulting in hypercoagulability and ultimately VTE. Numerous circulating tumour cell procoagulants have been identified, but their response to chemotherapy and

subsequent relationship to markers of hypercoagulability and development of VTE require clarification.

There is evidence to suggest that development of VTE is a poor prognostic sign for cancer survival, so markers of hypercoagulability may have some predictive value for cancer outcome, and changes in these markers may predict response to chemotherapy. Tumour cell procoagulants, as the potential source of this hypercoagulability, may also have some predictive value for cancer outcome and response to chemotherapy.

Emerging evidence supports the role of anticoagulant therapy as having a potential anticancer role, however even the role of anticoagulants as thromboprophylactic agents remains controversial, and in need of clarification.

Chapter 6 Thesis aims

1. To determine current practice for VTE prophylaxis in breast cancer patients undergoing surgery and breast cancer patients undergoing chemotherapy.
2. To determine the incidence of hypercoagulability and VTE in advanced and early breast cancer patients receiving chemotherapy.
3. To determine whether formation of VTE or hypercoagulability predicts for poor cancer outcome.
4. To determine the response of markers of hypercoagulability, fibrinolysis, platelet function and acute phase response to chemotherapy in the presence of high tumour load (advanced breast cancer) and low tumour load (early breast cancer).
5. To determine the response of circulating levels of tumour cell procoagulants and cytokines to chemotherapy in the presence of high tumour load (advanced breast cancer) and low tumour load (early breast cancer).
6. To assess whether changes in markers of hypercoagulability, fibrinolysis, platelet function and acute phase response, in response to chemotherapy, can predict for VTE formation.
7. To assess whether changes in markers of hypercoagulability, fibrinolysis, platelet function and acute phase response, in response to chemotherapy, can predict for tumour response and survival.
8. To assess whether changes in circulating levels of tumour cell procoagulants and cytokines, in response to chemotherapy, can predict for VTE formation.

9. To assess whether changes in circulating levels of tumour cell procoagulants and cytokines, in response to chemotherapy, can predict for tumour response and survival.

SECTION 2

SURVEY OF CURRENT THROMBOPROPHYLAXIS PRACTICE

Chapter 1

Venous thromboembolism prophylaxis in breast cancer surgery

1.1 Introduction

A large body of literature has developed to support the use of low-molecular weight heparin as a thromboprophylactic agent in general surgery. As early as 1988, the Lancet published a meta-analysis of thirty-one trials of patients undergoing general surgery in which there was a control group that received either placebo or no prophylaxis; these trials gave a combined incidence of fibrinogen uptake test-diagnosed DVT of 27.0% (Colditz, Tuden, & Oster 1986). For studies using a clinical diagnosis of DVT, the rate was estimated at only 3.1%. More recently a meta-analysis of all available randomised trials in general surgery was performed comparing low molecular-weight heparin (LMWH) or ultra-fractionated heparin (UFH) with placebo or no treatment (Mismetti *et al.* 2001). Eight of the studies analysed, containing 5520 patients, compared LMWH administration with placebo or no treatment (Balas 1992; Bergqvist *et al.* 1996; Ho *et al.* 1999; Le Gagneux & Le Guillou 1987; Marassi *et al.* 1993; Mismetti *et al.* 2001; Ockelford, Patterson, & Johns 1989; Pezzuoli *et al.* 1989; Pezzuoli *et al.* 1990; Valle, Sola, & Origone 1988). The adjusted incidences of events (SD) were 14.5 (2.2) % for DVT detected at the end of the treatment period ten days after surgery, 0.5 (0.1)% for clinical PE, 0.9 (0.2)% for clinical VTE and 0.9 (0.2)% for death. A statistically significant 72% reduction in risk of asymptomatic DVT was obtained with LMWH (n=513; RR 0.28 (95% confidence interval 0.14-0.54); $p<0.001$) and was associated with a 75% reduction for

clinical PE (RR 0.25 (0.08-0.79); p=0.018) and 71% for clinical VTE (RR 0.29 (0.11-0.73); p=0.009).

No randomised trial to date has compared the benefits of VTE prophylaxis or its omission in patients undergoing surgery for breast disease specifically. The effects of prolonged anaesthesia on VTE risk and breast cancer induced hypercoagulability are well recognised and anecdotally many surgeons recount experience of patients undergoing surgery for breast disease who suffer fatal pulmonary embolism or severe lower limb DVT following surgery. As a result many use heparin, graduated elastic compression stockings or a combination of these routinely in patients undergoing breast surgery. In contrast, many surgeons avoid anticoagulants in these patients because of the perceived risk of wound haematoma and its associated morbidity.

The aim of this study was to explore current VTE prophylaxis practice policy amongst surgeons operating on women with breast disease in the UK.

1.2 Methods

A postal questionnaire was sent to all breast surgeons (n=412) in England, Scotland and Wales identified by internet search, the medical directory and recent published league tables (Appendix 1). Close-ended questions with tick boxes and scoring systems were used to establish speciality; main surgical practice, current practice in different clinical scenarios for VTE prophylaxis and influences of current practice.

1.3 Results

Two hundred and seventy eight of 412 (68%) replies were received, of which 240 (86%) were suitable for inclusion. Twenty-seven (10%) of respondents were plastic surgeons engaged in only reconstructive work and thus felt the questionnaire was not applicable to their practice. From this point only the relevant 240 responders will be analysed.

Eighty surgeons (33%) replied anonymously. Seventy-six (32%) worked in teaching hospitals and 146 (61%) worked in district general hospitals. Mean time in specialist breast practice was 11 years, (range 1-30 years). One hundred and twenty (50%) of respondents were pure breast surgeons, 80 (33%) were general surgeons and 9 (4%) were plastic surgeons. The median number of breast patients operated upon annually was 150 (IQR 100-240).

We presented three clinical scenarios of different VTE risk and asked surgeons what their protocol or usual practice for VTE prophylaxis was. Options presented were either no prophylaxis; subcutaneous heparin; elastic compression (EC), aspirin, intermittent pneumatic compression (IPC) or "other". For simplicity results are combined into no prophylaxis, physical measures (EC or IPC), anticoagulant measures (heparin, aspirin etc) or both. The results are summarised in Table 2.

The first clinical scenario was: A 35 year old female, previously fit and well, undergoing excision of a breast lump that is a fibroadenoma on triple assessment. One hundred and seven (45%) of respondents would use no prophylaxis, 109 (45%) would use physical measures alone, 7 (3%) would use anticoagulants alone and 14 (6%) would use a combination of compression and anticoagulants. Six surgeons (3%) would not operate in such a case.

The second clinical scenario was: A 55 year old female, previously fit and well, undergoing wide local excision of a breast lump that is a TII, NI carcinoma on triple assessment. For this case, fourteen surgeons (6%) would use no prophylaxis, 55 (23%) would use compression methods alone, 13 (5%) would use anticoagulants and 158 (66%) would use a combination.

The final scenario was: A 70 year old female, with known ischaemic heart disease, undergoing mastectomy and axillary node clearance following a partial response to neoadjuvant chemotherapy for an inflammatory carcinoma. Ten surgeons (4%) stated they would use no prophylaxis, 28 (12%) would use compression methods, 10 (4%) would use anticoagulants alone and 191 (80%) would use a combination.

Table 2: Surgeons preferred selection of prophylaxis for different clinical scenarios in breast surgery. (Number out of 240 respondents, and percentage)

Method of prophylaxis	*Clinical scenario*		
	Fibroadenoma	Wide local excision	Mastectomy
None	107 (45%)	14 (6%)	10 (4%)
Compression	109 (45%)	55 (23%)	28 (12%)
Anticoagulation	7 (3%)	13 (5%)	10 4%)
Compression and anticoagulation	14 (6%)	158 (66%)	191 (80%)

Eleven surgeons (5%) did not use any VTE prophylaxis and 41 (17%) used compression rather than anticoagulants as prophylaxis specifically to reduce the risk

of post-operative haematoma. One hundred and forty one respondents (59%) would not stop pre-operative hormone therapy if patients required further surgery.

We investigated which risk factors altered prophylaxis practice (Table 3).

Table 3: Risk factors reported by surgeons to influence thromboprophylaxis prescribing practice in breast surgery

Risk factor	Not a significant risk	Minor risk for VTE	Moderate risk for VTE	Major risk for VTE
Previous VTE	40 (17%)	3 (1%)	59 (25%)	138 (58%)
Thrombophilia	68 (28%)	9 (4%)	64 (27%)	99 (41%)
Immobility	67 (28%)	23 (10%)	86 (36%)	64 (27%)
Obesity	69 (29%)	23 (10%)	86 (36%)	64 (27%)
HRT	85 (35%)	41 (17%)	81 (34%)	33 (14%)
Age	96 (40%)	41 (17%)	82 (34%)	21 (9%)
Family history of VTE	127 (53%)	47 (20%)	50 (21%)	16 (7%)

(HRT= Hormone replacement therapy)

The pre-existing risk factor with the most influence on practice was previous VTE, followed in descending priority by thrombophilia, immobility, obesity, hormone therapy, age and family history. Twenty-seven (11%) specifically mentioned cancer as a risk factor that would alter prophylaxis practice, and two (1%) mentioned prolonged operative time. Only three surgeons (1%) cited a hospital-wide protocol governed their practice.

Twenty surgeons (8%) would not use VTE prophylaxis on day case patients but only five (2%) said that they would never use VTE prophylaxis in any circumstances.

When asked to estimate the number of VTE events occurring in their patients in one year, 68 (28%) estimated zero, 40 (17%) estimated less than one, and 77 (32%) estimated between one to two. Five (2%) of respondents acknowledged the possibility of under-reporting.

When asked to estimate the number of patients on prophylaxis, 14 (6%) estimated less than 5%, 22 (10%) estimated between 10-60%, 91 (38%) said 61-90% and 86 (36%) said 91-100%.

1.4 Discussion

This study demonstrates a lack of consensus in prescribing policy for all aspects of VTE prophylaxis amongst surgeons operating on women with breast disease within the UK. The higher rates of combined prophylaxis usage in the higher risk patients suggest that most surgeons employ some risk stratification analysis prior to the prescription of prophylaxis. Amongst those surgeons who do not use heparin-based prophylaxis regimes, only a small number avoided heparin on the basis of haematoma risk. This data demonstrates an understanding by most UK surgeons of the common predisposing factors which may precipitate venous thrombosis, however most surgeons perceive the incidence of VTE to be low in the patients on which they operate.

This study is a single questionnaire-based, cross-sectional survey and the results represent a snapshot of current UK prescribing practice. It does not demonstrate changes in practice that may have occurred in recent years. Moreover, the three scenarios that were presented to the respondents are stylised cases and as such do not

necessarily reflect the actual prescribing practices of the responding surgeons. Although the response rate of 68% is high for such a postal survey, the reasons for non-response have not been explored.

The rationale for thromboprophylaxis is based on the high prevalence of VTE among surgical patients, the clinically silent nature of the disease in many patients and the morbidity, costs and potential mortality associated with un-prevented thrombi.

Consultant surgeons in this survey report a high use of VTE prophylaxis, however 6% and 4% fail to use any prophylaxis in moderate and high risk breast cancer surgery cases respectively. 14% of surgeons have 60% or less of patients on prophylaxis. IPC and EC are both inexpensive and associated with no risk factors, particularly haematoma.

Twenty-two percent of surgeons were concerned about bleeding complications from anticoagulants. Many meta-analysis and placebo-controlled, double-blind, randomized trials of anticoagulants in general surgery demonstrate no or minimal increase in major bleeding (Geerts *et al.* 2004). Little data exists on the risk of anticoagulation in breast cancer surgery, although in one recent study risk of wound haematoma, a specific concern in breast surgery, had an odds ratio of three when LMWH was used as compared to EC (Friis *et al.* 2004). However mechanical methods of prophylaxis carry no risk of bleeding and have been shown to be effective (Clagett & Reisch 1988).

A major reason for poor use of VTE prophylaxis is likely to be due to subjective perceptions of the magnitude of the problem of VTE and effects of prophylaxis in clinical practice. VTE is often clinically silent, or has a delayed presentation to physicians without the surgeon's knowledge. Overt VTE in an individual surgeons

practice is perceived as rare (Laverick, Croal, & Mollan 1991). In our study 45% of surgeons report less than 1% VTE rate in their patients. A reduction in rate of VTE would be difficult to appreciate in an individual clinicians practice however a bleeding complication is less likely to occur unnoticed.

Although respondents believed a high percentage of their patients received prophylaxis, this is dependent on effective prescription of medical prophylaxis by junior doctors, or mechanical prophylaxis by nurses, theatre staff and compliant patients. With only 1% of respondents referring to a hospital policy, this impression of effective prophylaxis may be spurious. A prophylaxis policy would also allow consideration of difficult issues such as management of added VTE risk factors such as concurrent hormone therapy.

Table 4: Recommendations for surgical thromboprophylaxis (Geerts *et al.* 2004)

Group	*Risk factors*	*Prophylaxis*
Low risk	Minor/short operation Age <40 No additional risk factors	Early ambulation
Moderate risk	Age 40-60 + no risk factors or minor operation + additional risk factors	LDUH 12 hourly or LMWH daily (<3,400u) or Properly used EC or IPC
High risk	Age >60 or age 40-60 + additional risk factors	LDUH 8 hourly or LMWH daily (>3,400u) or IPC (especially if risk of bleeding)
Highest risk	Multiple risk factors (age >40, cancer, prior VTE)	LMWH daily (>3,400u) or Oral vitamin K antagonist (INR 2-3) or IPC/EC + LDUH/LMWH

Geerts and colleagues suggest a preventive strategy in general surgery, taking into account the risk of VTE, the effectiveness of various agents, and the expense and possible complications incurred by their use (Table 4) (Geerts *et al.* 2004). Riber and co-workers document a near zero risk for the development of VTE after minor procedures in low-risk patients (Riber *et al.* 1996).

VTE as a post-operative complication is still poorly documented, with no formal method of reporting. A national database for recording confirmed VTE would allow identification of risk factors and thus stratification of at risk patients into a prophylaxis protocol specifically for breast surgery.

Chapter 2

Venous thromboembolism prophylaxis in cancer therapy by oncologists

2.1 Aims

Venous thromboembolism in cancer is common, and this risk is exacerbated by the majority of treatment modalities. We assessed current thromboprophylaxis practice during cancer treatment in the north of England.

2.2 Methods

A postal questionnaire was sent to all oncologists in the north of England identified by internet search and the medical directory (Appendix 2). Close-ended questions with tick boxes and scoring systems were used to establish: speciality; main cancer type treated; main modality used (of chemotherapy, hormone therapy and radiotherapy); estimate of VTE risk of treatments used; and current prophylaxis practice.

2.3 Results

From 166 questionnaires, we received 123 responses, of which 106 (64%) were suitable for inclusion. The specialities were: 56 (53%) clinical oncology; 31(30%) medical oncology; 7(7%) surgery; five (5%) gynaecological oncology; five (5%) paediatrics; one (1%) urology; and one (1%) radiology. Chemotherapy was the most frequently used treatment by 41 (39%), and least or no answer by 17 and seven respectively (total 23%); hormone therapy was most frequently used by 10 (9%), and least or no answer by 29 and

34 (total 59%); radiotherapy was most frequently used by 44 (42%), and least or no answer by 13 and 24 (total 35%). Range and frequency of types of tumour treated is illustrated in figure 1.

When asked the question "Do you consider VTE to be a significant risk in your patients during treatment", 29 (27.3%) described **no** significant risk in their patients. Figure 1 separates the response to this question by tumour type treated. When asked about the increase in VTE risk with the different treatment modalities 71 (67%) believed there to be little or no increased risk associated with hormone therapy, 83 (79%) described little or no increased risk associated with chemotherapy and 96 (91%) felt the same about radiotherapy.

In response to questioning about routine use of prophylaxis, of those that responded 84 (84%), 79 (88%) and 86 (96%) did not use routine prophylaxis in chemotherapy, hormone therapy and radiotherapy respectively. Nine of the 10 that routinely or occasionally used prophylaxis in chemotherapy specifically mentioned long lines as a risk factor, and six of the 11 that routinely or occasionally used prophylaxis in hormone therapy specifically referred to stilboestrel. The choice of prophylaxis was aspirin by 13; warfarin 12, subcutaneous heparin 10; stockings 9 and the WARP study (randomising patients with long lines to different warfarin regimens) by three. Indications that prompted the **routine** use of heparin were: previous VTE (45%); immobility (44%); thrombophilia (24%); long line (17%); obesity (15%); surgery (8%); concurrent hormone therapy (8%); family history (6%); pelvic mass (3%); superior vena caval obstruction (2%); age (2%); brachytherapy (1%); multiple myeloma (1%); hyperviscosity (1%) and travel (1%). 19 (18%) never used VTE prophylaxis.

When asked for an estimation of the percentage of patients currently on VTE prophylaxis 37% of those answering said less than 1%, and 62% said less than 5%. 16% did not answer this question.

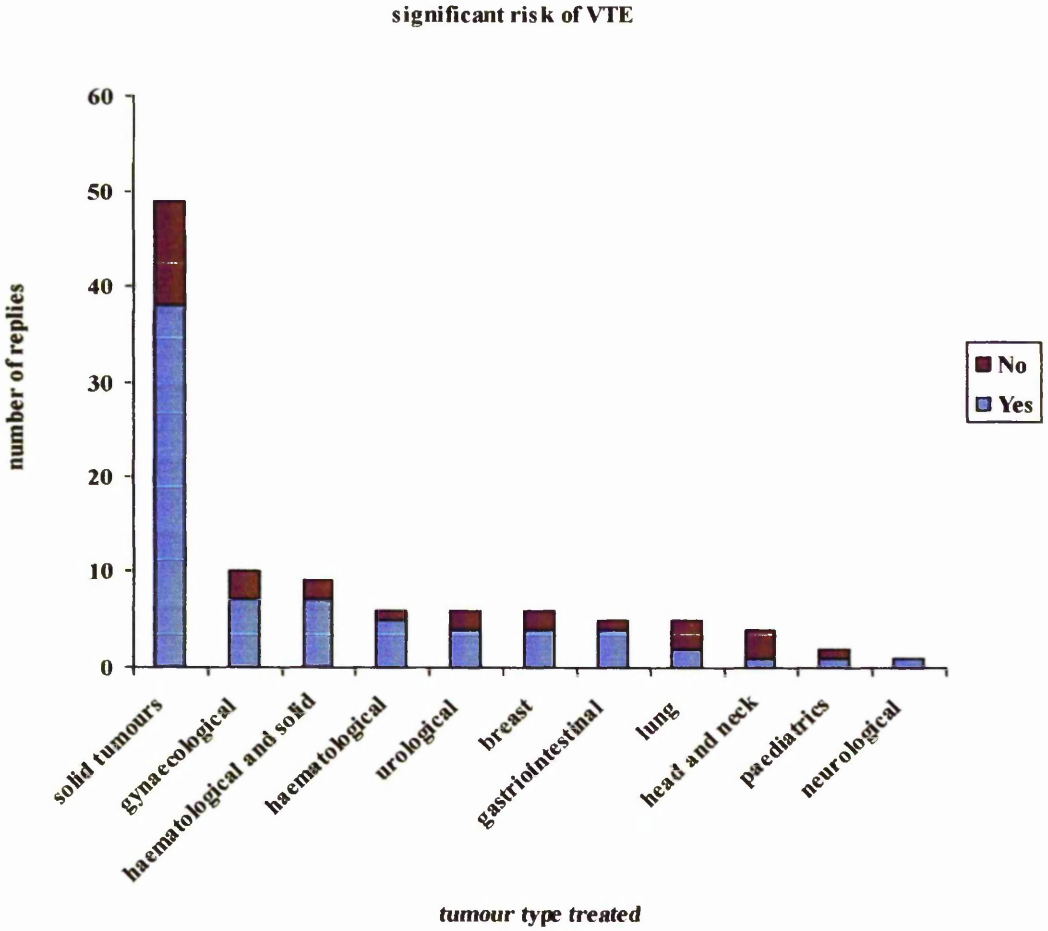

Figure 1: Number of respondents identifying patients at risk (yes) or not at risk (no) from VTE. Results presented by separating oncological specialities

2.4 Comments

Over one quarter of oncologists surveyed do not recognise the significant thrombogenic effects of cancer treatments. The high (27.3%) response of no significant risk of VTE is not biased by lower risk specialities such as paediatrics. The good (74%) response rate to this questionnaire demonstrates a reliable representation of current practice in the north of England. Thromboprophylaxis is rarely used in cancer patients undergoing treatment. The estimated percentage of patients on prophylaxis is surprisingly low bearing in mind that almost half of respondents quoted previous VTE and immobility as an indication for routine prophylaxis. There may be a need for national guidelines on VTE prophylaxis during cancer treatment.

(Published: Kirwan, C. C., Nath, E., Byrne, G. J., & McCollum, C. N. 2003, "Prophylaxis for venous thromboembolism during treatment for cancer: questionnaire survey", *BMJ*, vol. 327, no. 7415, pp. 597-598.)

SECTION 3

THE ROLE OF PROCOAGULANTS IN CANCER AND CHEMOTHERAPY INDUCED VENOUS THROMBOEMBOLISM

Chapter 1

Introduction

Chemotherapy is a risk factor for cancer-related VTE. VTE rates of 2-10% are reported in early breast cancer (Clahsen *et al.* 1994; Weiss *et al.* 1981) and up to 17.6% (Goodnough *et al.* 1984) in advanced breast cancer patients receiving chemotherapy. The pathophysiology of VTE during chemotherapy is poorly understood. Some data suggests a poorer cancer outcome in those chemotherapy patients developing VTE (Seward *et al.* 1999), however this issue is not resolved in the literature. However VTE in cancer appears to be a cause of premature mortality (Shen & Pollak 1980). Predictive criteria for development of VTE, and hence uses of prophylaxis, during chemotherapy remain limited to such factors as indwelling central lines (Kakkar *et al.* 1995; Kakkar & Williamson 1999; Monreal *et al.* 1996).

This study aims to establish the prevalence of VTE in advanced and early breast cancer patients undergoing chemotherapy. Whether development of VTE is associated with a worse cancer outcome will also be assessed.

Advanced breast cancer patients receiving chemotherapy will form the study patient group, enabling the effects of chemotherapy on cancer patients to be studied. Early breast cancer patients will act as a control group, representing patients who are receiving chemotherapy, but have minimal or no cancer. A non-cancer control group will establish baseline "normal" levels of studied molecules.

Table 5 describes the circulating markers of clotting activation that will be measured.

Table 5: Circulating haemostatic markers used in this study to assess hypercoagulability, fibrinolysis, and acute phase response

Group	*Marker*	*Function*
Hyper-coagulability	APTT	Measures intrinsic clotting pathway
	PT	Measures extrinsic clotting system and common pathway
	PF1+2	Released during conversion of prothrombin to thrombin
	TAT	Generated during inhibition of thrombin by the natural anticoagulant antithrombin
Intravascular fibrin formation	Fibrinogen	Substrate for thrombin Represents final step in coagulation cascade
	D-dimer	Fibrin degradation product produced as sequelae of fibrin formation and reflecting active fibrinolytic system
Fibrinolysis	tPA	Converts plasminogen to plasmin Principally synthesised by endothelium
	uPA	Serine protease involved in breakdown of extracellular matrix Converts plasminogen to plasmin
	PAI-1	The primary physiological inhibitor of uPA and tPA
Platelets	Platelet count	
	Platelet VEGF release	$\frac{\text{(Serum VEGF)} - \text{(plasma VEGF)}}{\text{platelet count}}$
Acute phase reactant	CRP	Synthesised in liver. Released within hours in response to injury/infection/inflammation

Table 6 lists the circulating procoagulant molecules and endothelial adhesion molecules expressed in cancer, that will be measure in this study.

Table 6: Procoagulants and endothelial adhesion molecules acting as possible initiators of hypercoagulability in cancer and chemotherapy

Group	*Circulating marker expressed in cancer*
Procoagulant	Tissue Factor
	Cancer Procoagulant
	Thrombospondin-1
	Tumour Necrosis Factor - α
	Vascular Endothelial Growth Factor (plasma and serum)
Endothelial Adhesion Molecule	Vascular Cell Adhesion Molecule-1 (soluble)
	E-selectin (soluble)

Early and advanced breast cancer patients will be compared to controls at baseline. Patients prior to neoadjuvant chemotherapy for large or inflammatory breast cancers will also be compared to the two subject groups and controls at baseline. The two subject groups will be compared to assess a response to chemotherapy, and how this may differ between the two groups. In this way we hope to identify a "chemotherapy effect" (as demonstrated in the early breast cancer group) or a "chemotherapy on cancer (or tissue death) effect" as demonstrated by the advanced breast cancer group.

The above circulating markers (at baseline and changes in response to chemotherapy) will be assessed for predictive value for:

- VTE formation
- tumour response
- overall outcome

Chapter 2

Aims and Objectives

2.1 Objective

To investigate the role of coagulation in cancer and during chemotherapy.

2.2 Aims

1. To determine the incidence of venous thromboembolism formation and hypercoagulability in advanced and early breast cancer patients receiving chemotherapy
2. To determine whether formation of VTE or hypercoagulability predicts for poor cancer outcome
3. To determine the response of markers of hypercoagulability, fibrinolysis, platelet function and acute phase response to chemotherapy in the presence of high tumour load (advanced breast cancer) and low tumour load (early breast cancer)
4. To determine the response of circulating levels of tumour cell procoagulants, cytokines and endothelial adhesion molecules to chemotherapy in the presence of high tumour load (advanced breast cancer) and low tumour load (early breast cancer)
5. To assess whether changes in markers of hypercoagulability, fibrinolysis, platelet function and acute phase response, in response to chemotherapy, can predict for VTE formation.

6. To assess whether changes in markers of hypercoagulability, fibrinolysis, platelet function and acute phase response, in response to chemotherapy, can predict for tumour response and survival.
7. To assess whether changes in circulating levels of tumour cell procoagulants, cytokines and endothelial adhesion molecules, in response to chemotherapy, can predict for VTE formation.
8. To assess whether changes in circulating levels of tumour cell procoagulants, cytokines and endothelial adhesion molecules, in response to chemotherapy, can predict for tumour response and survival.

Chapter 3 Summary of study plan

This was a prospective cohort study of advanced (high tumour load), neoadjuvant (moderate, localised tumour load) and adjuvant (low tumour load) breast cancer patients receiving chemotherapy. Circulating tumour procoagulant molecules (TF, TSP-1 and CP), tumour cell procoagulant cytokines (TNF-α and VEGF), and endothelial adhesion molecules (VCAM-1and E-selectin) were measured at baseline (pre-chemotherapy), at one day, four days, eight days, three and six months following the commencement of chemotherapy. Markers of the coagulation system (PT, APTT, TAT, PF1+2), markers of the fibrinolytic system (PAI-1, tPA, and uPA), markers of intravascular fibrin deposition (Fibrinogen and D-dimer), platelet count, acute phase response (C-reactive protein) and a clinical assessment for VTE were measured at the same timepoints. DUI for DVT was performed three weeks following the start of chemotherapy. Levels of procoagulant were related to hypercoagulability, intravascular fibrin formation, DUI confirmed VTE, response to chemotherapy and survival.

3.4.1 Subjects

Three groups of patients were studied:

i) advanced breast cancer patients receiving chemotherapy (n=120),

ii) age matched early breast cancer patients following curative surgery, but receiving adjuvant chemotherapy (n=120), and

iii) patients with localised large or inflammatory breast tumours receiving chemotherapy prior to curative surgery (n=120).

The three patient groups were compared to normal controls prior to chemotherapy, to compare the effect of high, moderate and low/ no tumour load. The advanced and early breast cancer groups were compared to assess the difference in response to chemotherapy in the presence of high and low/no tumour load. The early breast cancer group form a control group, receiving chemotherapy, but theoretically cancer-free. The advanced breast cancer group provide the main subject group, with a large, systemic cancer load, allowing us to study the procoagulant effect of chemotherapy on breast cancer. A group of age-matched, cancer-free women acted as a normal control for baseline comparison.

Inclusion criteria

i) Advanced breast cancer patients:

Women over the age of 18, with confirmed metastatic breast cancer prior to commencement of chemotherapy (CMF (cyclophosphamide, methotrexate and 5-fluorouracil); FEC (5-fluorouracil, etoposide and cyclophosphamide) or a taxane and/ or epirubicin).

ii) Early cancer patients:

Women over the age of 18 with newly diagnosed breast carcinoma who have undergone apparently curative surgery and have no clinical or radiological evidence of advanced disease but who will undergo adjuvant chemotherapy (CMF and FEC).

iii) Neoadjuvant breast cancer patients:

Women over the age of 18 with newly diagnosed breast carcinoma, with no clinical or radiological evidence of advanced disease. These patients will receive neoadjuvant chemotherapy prior to curative surgery (AC (Cyclophosphamide and Adriamycin) or Epirubicin and Navelbine), because of large primary tumour size or significant markers of tumour aggression.

iv) Non-cancer controls:

Women over the age of 18 with no previous or current diagnosis of cancer.

Exclusion criteria

1. Patients who were less than 18 days following surgery (to minimise surgery-related hypercoagulability as a confounding variable)
2. Patients who were less than two months from previous chemotherapy (advanced group, to exclude previous chemotherapy-related hypercoagulability as a confounding variable)
3. Patients with biochemical evidence of liver or renal failure (impaired liver function is associated with altered coagulation, and renal failure is associated with an altered angiogenic response and coagulopathy (Ambuhl *et al.* 1997; Choi *et al.* 2004))
4. Patients with haemophilia, other clotting disorders; or anticoagulant drug therapy (altered haemostasis)
5. Patients receiving hormonal treatment containing oestrogen or anti-oestrogens (recognised as thrombogenic (Deitcher & Gomes 2004; Kemmeren, Algra, & Grobbee 2001))
6. Patients with a past history of VTE or on anticoagulant therapy
7. Patients receiving chemotherapy via a long line (infrequent in breast cancer) (recognised as thrombogenic (Monreal *et al.* 1996))
8. Patients with pelvic disease or para-aortic nodes (to avoid venous compression)
9. Patients with impaired mobility defined as WHO performance status of 3-4 (compounding risk factor for VTE) (Appendix 3)
10. Patients who live more than one hour from the Christie Hospital (to standardise blood handling and storage technique)

4.2 Assessment and follow-up

Peripheral blood was analysed, using the methods described in chapter 4.4:

i) immediately pre-chemotherapy (baseline);

ii) one day post chemotherapy (response to maximum intravascular drug concentration);

iii) four days post chemotherapy (maximum chemotherapy-induced cell death);

iv) eight days post chemotherapy (at-risk period for VTE);

v) three months after first chemotherapy treatment (pre- fifth or sixth cycle of chemotherapy);

vi) six months after first chemotherapy treatment (approximately six weeks following completion of chemotherapy).

Patients were assessed in their own homes when they were not due to attend the outpatient clinic to reduce potential compliance difficulties. DUI was performed three weeks after the start of chemotherapy in the Vascular Studies Unit at South Manchester University Hospital. Suspected pulmonary embolism was confirmed by ventilation-perfusion isotope imaging. Patients were reviewed at three, six, 12 and 24 months for clinical VTE, response to treatment and survival. Blood biochemistry was monitored throughout chemotherapy as part of routine management.

4.3 Outcome measures

4.3.1 Primary outcome measures

1. Levels of tumour procoagulant molecules (TF, TSP-1 and CP), tumour cytokines (TNF-α and VEGF) and endothelial adhesion molecules (VCAM-1and E-selectin) at baseline

2. Alterations in levels of these molecules in response to chemotherapy
3. VTE on DUI
4. Overall survival and cause of death

4.3.2 Secondary outcome measures

1. Evidence of systemic hypercoagulability (PT, APTT, TAT, PF1+2)
2. Circulating evidence of intravascular fibrin formation (D-dimer, Fibrinogen)
3. Circulating evidence of fibrinolysis (tPA, uPA and PAI-1)
4. Altered platelet release
5. Acute phase response
6. Response to treatment (Appendix 4)

4.4 Details of storage and analyses

Atraumatic venous blood sampling was performed at the antecubital fossa and all specimens separated and stored within two hours. Citrate, EDTA (Ethylene Diamine Tetra-acetic Acid) and CTAD (Citrate Theophylline Adenosine Dipyridamole) (Ahnadi, Chapman, & Hoang 2000) samples were immediately taken onto ice, serum samples were allowed to clot at room temperature. All samples (except full blood count and clotting screen) were centrifuged for 20 minutes at 4°C, 2500g and the plasma or serum removed from the cells. Serum and citrated plasma samples were then divided into 0.3ml aliquots. CTAD, EDTA and further citrate plasma samples were re-spun and platelet-poor plasma aliquoted as above. All samples were stored at -80°C until analysis.

4.4.1 Measures of hypercoagulability

Prothrombin time and activated partial thromboplastin time

PT and APTT were performed by the Thrombosis Reference Centre (TRC), South Manchester University Hospital. PT and APTT were performed on an Instrumentation Laboratory ACL 3000, using TRC reagents. Normal ranges were 9-13.5 seconds for PT, and 16.5-24.5 seconds for APTT.

Thrombin-Antithrombin complex

Serum samples were analysed using the Enzygnost® TAT micro ELISA by Dade Behring, Marburg, Germany, using the following method:

Sample buffer (50µl, Tris buffer solution (100mmol/l), Tween (10ml/l), EDTA (37g/l)) and samples (50µl) were added to wells coated with rabbit antibodies against human thrombin incubated at 37°C for 15 minutes. Any unbound substances were removed by washing three times with Washing Solution (Phosphate Buffered Saline (PBS) (90mmol/l), Tween (18g/l)). Conjugate (100µl, rabbit anti-human ATIII, peroxidase conjugated) was added and the assay further incubated at 37°C for 15 minutes. After three further washes with Wash Solution, substrate solution (100µl, hydrogen peroxide (0.3g/l) in citrate buffer mixed with o-phenylenediamine dihydrochloride) was then added, so that colour developed in proportion to the amount of bound TAT. The assay was incubated at 20°C and colour development stopped using sulphuric acid (100µl of 0.5M H_2SO_4) after 30 minutes. The optical density of each well was determined using a microplate reader set to 492nm.

The TAT ELISA has a minimum sensitivity of 1μg/ml. TAT levels were determined in duplicate. In the range between 2 and 6μg/l the intra-assay coefficient of variance (CV) fell between 4 and 6%, and the inter-assay CV fell between 6 and 9%.

Prothrombin Fragments 1+2

PF 1+2 was performed by the Thrombosis Reference Centre (TRC), South Manchester University Hospital, using the Enzygnost® F1+2 micro ELISA by Dade Behring, Marburg, Germany. This was performed as part of a pilot study with a more heterogeneous group of patients including males and females with solid tumours (breast, colorectal and stomach).

4.4.2 Markers of intravascular fibrin formation

Fibrinogen

Derived fibrinogen was measured by the Thrombosis Reference Centre (TRC), South Manchester University Hospital. Analysis was performed on an Instrumentation Laboratory ACL 3000, using TRC reagents. Normal ranges were 1.5-5.0g/l for derived fibrinogen.

D-dimer

D-dimer was performed by the Thrombosis Reference Centre (TRC), South Manchester University Hospital.

Plasma samples were analysed using the VIDAS® D-Dimer (bioMérieux, Marcy l'Etoile, France) system. This is a quantitative fully automated ELISA assay with fluorescence detection performed on the VIDAS immunoanalyzer. A solid phase receptacle is coated with an anti-D-Dimer monoclonal antibody and serves both as a solid phase and pipetting device. The conjugate is an alkaline phosphatase labelled anti-D-Dimer monoclonal antibody. The VIDAS has a minimum sensitivity of less than 45ng/ml. The TRC used an upper limit of normal of 500ng/ml.

4.4.3 Markers of fibrinolysis

Tissue-type Plasminogen Activator

Plasma samples were analysed using an enzyme-linked immunosorbent assay (ELISA) by Bender MedSystems, Vienna, Austria using the following method:

After an initial two washes with Wash Buffer (PBS, 1% Tween 20), sample diluent (90µl) and citrate samples (10µl) were added to wells coated with polyclonal sheep antibody to human tPA. Anti-tPA monoclonal murine antibody conjugate (50µl) was added and the assay incubated for 120 minutes at room temperature. Any unbound substances were then removed by washing three times with Wash Buffer. Tetramethylbenzidine substrate (100µl) solution was then added. Colour developed in proportion to the amount of t-PA bound in the initial step. Colour development was stopped using phosphoric acid (100µl of 1M H_3PO_4) after 15 minutes. The optical density of each well was determined using a microplate reader set to 450nm with a correction wavelength set to 620nm, within 30 minutes.

The t-PA ELISA has a minimum sensitivity of 8pg/ml. tPA levels were determined in duplicate. Intra-assay CVs were assessed at three levels as follows: at 0.37g/ml CV is 5.9%; at 3.2ng/ml CV is 7.8%; and at 8.7ng/ml CV is 1.8%. Inter-assay CVs were assessed at three levels as follows: at 0.38ng/ml CV is 7.8%; at 3.0ng/ml CV is 9.2%; and at 8.9ng/ml CV is 6.9%.

Urokinase Plasminogen Activator

Plasma samples were analysed using an enzyme-linked immunosorbent assay (ELISA) by Imubund®, American Diagnostica, Connecticut, USA using the following method:

Plasma samples (100µl), diluted 1:20 in sample buffer (1 gm BSA/100ml wash buffer) were added to wells coated with murine monoclonal antibody against human uPA and incubated overnight at 4°C. After washing four times with Wash Buffer (PBS with 0.1% Triton X-100, pH7.4), detection antibody (100µl, biotinylated anti-human uPA) was added and incubated at room temperature for 60 minutes. After a further four washes with Wash Buffer, diluted streptavidin-horseradish peroxidase conjugate (100µl) was added and the assay further incubated for 60 minutes at room temperature. Any unbound substances were then removed by four washes, and tetramethylbenzidine substrate solution (100µl) added. Colour development was stopped using sulphuric acid (100µl of 0.5M H_2SO_4) after 20 minutes. The optical density of each well was determined using a microplate reader set to 450nm, within 30 minutes.

uPA levels were determined in duplicate.

Plasminogen Activator Inhibitor-1

PAI was performed by the Thrombosis Reference Centre (TRC), South Manchester University Hospital, using an immulyse® ELISA by Biopool, Brey, Ireland. This assay was also part of the initial pilot study and so performed on the same patients as described above.

4.4.4 Platelet count and function

Platelet Count

This was performed by the haematology department, South Manchester University Hospital, using the Advia 120 Haematology System by Bayer.

Platelet Function

Platelet function was inferred by calculating release of VEGF per platelet following degranulation (Banks *et al.* 1999):

$$\frac{\text{(Serum VEGF – plasma VEGF)}}{\text{platelet count}}$$

Measurement of VEGF is described in chapter 4.4.6 below.

4.4.5 Acute phase response

C-reactive protein (CRP)

A highly sensitive C-reactive protein (CRP) assay was performed, by the biochemistry department, South Manchester University Hospital, with a BNII automated system from

BN® Systems, Germany. The assay uses particle-enhanced immunonephelometry to quantitate CRP in serum samples. Polystyrene particles coated with monoclonal antibodies against CRP become agglutinated when mixed with samples containing CRP. The intensity of light scattering due to the agglutination reaction is measured by the nephelometer and is directly related to the CRP concentration. Samples are automatically diluted 20-fold by the instrument prior to analysis.

4.4.6 Procoagulants

Tissue Factor

Samples were analysed using an enzyme-linked immunosorbent assay (ELISA) by American Diagnostica inc., Greenwich, Connecticut, USA using the following method:

Plasma samples (100µl), diluted 1:4 in sample buffer (1 gm Bovine Serum Albumin/ 100ml wash buffer), were added to wells coated with murine monoclonal antibody against TF and incubated overnight at 4°C. Any unbound substances were then removed by washing the wells four times with Wash Buffer (PBS with 0.1% Triton X-100, pH7.4). Detection antibody (100µl, biotinylated anti-human TF antibody fragment $F(ab')_2$) was added and incubated at room temperature for 60 minutes. After four washes with Wash Buffer, conjugate (100µl, streptavidin conjugated horseradish peroxidase) was added to the wells and incubated for a further 60 minutes at room temperature. Following a further four washes with Wash Buffer to remove any unbound antibody-enzyme reagent, tetramethylbenzidine substrate solution (100µl) was added. Colour develops in proportion to the amount of bound TF bound. Colour development was stopped with sulphuric acid

(50µl of 0.5M H_2SO_4) after 20 minutes. The optical density of each well was determined using a microplate reader set to 450nm, within 30 minutes.

The TF ELISA has a sensitivity of 10µg/ml. TF levels were determined in duplicate.

Cancer Procoagulant

CP was measured indirectly using a three-stage chromogenic assay to assess cancer procoagulant activity as described by Mielicki and co-workers (Mielicki *et al.* 1999). Two modifications to the methods were made. The chromogenic substrate concentration was increased to 1.25mg/ml, and the final plate read was performed at 3 minutes. Assays were performed by the Thrombosis Reference Centre, South Manchester University Hospital, with advice from W.P. Mielicki, Medical University of Lodz, Poland.

Thrombospondin-1

TSP-1 was measured using the radioimmunoassay technique described as previously described (Hayden *et al.* 2000), based on the competitive binding of rabbit anti-TSP-1 antibody with either the TSP contained within the sample or added radiolabelled TSP-1.

An initial dilution of 1:20 was done on plasma samples, with repeat analysis being performed at 1:10 and 1:120 where samples were below and above the working range of the assay respectively (assay buffer: 0.05 mol/L phosphate buffer, pH 7.4, containing 2% horse serum and 1% Tween 20 (Polyoxyethylene sorbital monolaurate (Sigma Chemical Co., Poole, UK).

Standard solutions of known concentrations of TSP-1 were prepared using commercial TSP-1 diluted in horse serum. Samples (50µl) were added to a mixture of 125I-labelled TSP-1 (200µl, 0.5ng/tube) and rabbit anti-TSP-1 antiserum(200µl), and incubated overnight at 4°C.

Anti-rabbit anti-serum (300µl Sac-cell AA-SAC1) was added to each sample and the samples incubated for a further 30 min at room temperature. Wash buffer (3ml, 0.9% sodium chloride and 0.05% Tween 20) was added, the samples centrifuged for 20 min at 1500g at 8°C, and the supernatant discarded.

The precipitate was then counted for 120s on an LKB 1260 multiwell counter (Wallac, Milton Keynes, UK), and sample levels calculated against the generated standard curve. TSP-1 levels were determined in duplicate.

Tumour Necrosis Factor -α

TNF-α samples were analysed using an enzyme-linked immunosorbent assay (ELISA) by R&D Systems®, Oxon, UK using the following method:

Assay diluent (50µl, buffered protein base) and serum samples (200µl) were added to wells coated with murine monoclonal antibody against TNF-α and incubated for 120 minutes at room temperature. Any unbound substances were then removed by washing three times with Wash Buffer (25-fold concentrated solution of buffered surfactant). Conjugate (200µl, polyclonal antibody against TNF-α conjugated to horseradish peroxidase) was added to the wells and incubated for a further 120 minutes at room temperature. Following a three further washes with Wash Buffer to remove any unbound

antibody-enzyme reagent, tetramethylbenzidine substrate solution (200µl) was then added. Colour develops in proportion to the amount of TNF-α bound in the initial step. Colour development was stopped after 20 minutes, using sulphuric acid (50µl of 0.5M H_2SO_4). The optical density of each well was determined using a microplate reader set to 450nm, with a correction wavelength set to 540nm, within 30 minutes.

The TNF-α ELISA has a sensitivity of 4.4µg/ml. TNF-α levels were determined in duplicate. Intra-assay CVs were assessed at three levels as follows: at 48.1µg/ml CV is 5.2%; at 317µg/ml CV is 4.2%; and at 587µg/ml CV is 4.6%. Inter-assay CVs were assessed at three levels as follows: at 45.8µg/ml CV is 7.4%; at 301µg/ml CV is 4.6%; and at 587µg/ml CV is 5.4%.

Vascular Endothelial Growth Factor (VEGF)

Both plasma and serum VEGF were analysed to establish circulating (pVEGF) and platelet released (sVEGF) levels.

Samples were analysed using an enzyme-linked immunosorbent assay (ELISA) by R&D Systems®, Oxon, UK using the following method:

Assay diluent (100µl, protein buffered base) and samples (100µl) were added to wells coated with murine monoclonal antibody against VEGF and incubated for 120 minutes at room temperature. Any unbound substances were then removed by washing three times with Wash Buffer (25-fold concentrated solution of buffered surfactant). Conjugate (200µl, polyclonal antibody against VEGF conjugated to horseradish peroxidase) was added to the wells and incubated for a further 120 minutes at room temperature.

Following a three further washes with Wash Buffer to remove any unbound antibody-enzyme reagent, substrate solution (200µl, tetramethylbenzidine and hydrogen peroxide mixture) was added. Colour develops in proportion to the amount of VEGF bound in the initial step. Colour development was stopped with sulphuric acid (50µl of 0.5M H_2SO_4) after 25 minutes. The optical density of each well determined using a microplate reader set to 540nm, with a correction wavelength set to 540nm, within 30 minutes.

The VEGF ELISA has a sensitivity of 9µg/ml. VEGF levels were determined in duplicate. Intra-assay CVs were assessed at three levels as follows: at 53.7µg/ml CV is 6.7%; at 235µg/ml CV is 4.5%; and at 910µg/ml CV is 5.1%. Inter-assay CVs were assessed at three levels as follows: at 64.5µg/ml CV is 8.8%; at 250µg/ml CV is 7.0%; and at 1003µg/ml CV is 6.2%.

4.4.7 Markers of endothelial cell activation

Vascular Cell Adhesion Molecule-1 (VCAM-1)

Serum samples were analysed using an enzyme-linked immunosorbent assay (ELISA) by R&D Systems® Oxon, UK using the following method:

Conjugate (100µl, antibody to recombinant human soluble VCAM-1 (sVCAM-1) conjugated to horseradish peroxidase) was added to wells coated with murine monoclonal antibody to human sVCAM-1. Serum samples (100µl, diluted 50-fold with protein buffered base) were then added and incubated for 90 minutes at room temperature. Any unbound substances and/or antibody-enzyme reagent were removed by washing six times with Wash Buffer (25-fold concentrated solution of buffered surfactant). Tetramethylbenzidine substrate solution (100µl) was then added, so that colour develops

in proportion to the amount of bound sVCAM-1. Colour development was stopped using sulphuric acid (50µl of 0.5M H_2SO_4) after 20 minutes. The optical density of each well was determined using a microplate reader set to 450nm, with a correction wavelength set to 620nm, within 30 minutes.

The VCAM-1 ELISA has a minimum sensitivity of 2ng/ml. VCAM-1 levels were determined in duplicate. Intra-assay CVs were assessed at three levels as follows: at 11.7ng/ml CV is 5.9%; at 28.6ng/ml CV is 4.3%; and at 55.1ng/ml CV is 4.9%. Inter-assay CVs were assessed at three levels as follows: at 9.8ng/ml CV is 10.2%; at 24.9ng/ml CV is 8.5%; and at 49.6ng/ml CV is 8.9%.

E-selectin

Serum samples were analysed using an enzyme-linked immunosorbent assay (ELISA) by R&D® Systems Oxon, UK using the following method:

Conjugate (100µl, antibody to human soluble E-Selectin (sE-Selectin) conjugated to horseradish peroxidase in buffer) was added to wells coated with murine monoclonal antibody to human sE-Selectin. Serum samples (100µl, diluted 20-fold with protein buffered base) were then added and incubated for 90 minutes. Any unbound substances and/or antibody-enzyme reagent were removed by washing six times with Wash Buffer (25-fold concentrated solution of buffered surfactant). Tetramethylbenzidine substrate solution (100µl) was then added, so that colour developed in proportion to the amount of bound sE-selectin. Colour development was stopped using sulphuric acid (100µl of 0.5M H_2SO_4) after 30 minutes. The optical density of each well was determined using a microplate reader set to 450nm, with correction wavelength of 620nm, within 30 minutes.

The E-selectin ELISA has a minimum sensitivity of less than 0.1ng/ml. E-selectin levels were determined in duplicate. Intra-assay CVs were assessed at three levels as follows: at 21.9ng/ml CV is 5.0%; at 56.6ng/ml CV is 4.8%; and at 115ng/ml CV is 4.7%. Inter-assay CVs were assessed at three levels as follows: at 20.4ng/ml CV is 8.8%; at 54.7ng/ml CV is 5.7%; and at 115ng/ml CV is 7.4%.

4.5 Data management, analysis and statistical methods

All data were recorded by hand on purpose-designed recording forms and then transferred to a statistics computer package (SPSS 11.5 for Windows) for analysis. All manually recorded data was cleaned after being transferred from paper to database. The majority of the data were normally distributed, however several molecules required log transformation to produce an adequate approximation to a normal distribution prior to parametric analysis. One way ANOVA and Independent T-test were used to compare independent samples (for example advanced versus early versus control, and VTE positive versus negative). Correlation analysis used Spearman's correlation coefficient. Univariate analysis and paired T-tests were used to assess change from baseline. Greenhouse Geiser correction was used with repeated measures analysis to assess change over time. Binary logistic regression to identify, for example, predictors of VTE or progression and Cox regression survival analysis was also performed. Analysis was performed on baseline data, and change from baseline. Appropriate corrections were made for cancer stage and age.

4.6 Study power

The sample size calculation was based on the X^2 test with Yates' correction. A sample size of 120 per group gives an 80% power to detect the difference between a Group 1 proportion, π^1, of 0.170 and a Group 2 proportion, π^2, 0f 0.050. (Group 1 = ABC receiving palliative chemotherapy, group 2 = EBC receiving adjuvant chemotherapy).

Chapter 5

Incidence of VTE during chemotherapy, and impact on survival

5.1 Introduction

Published data of the frequency of VTE during chemotherapy is limited. The majority of published studies focus on chemotherapy during breast cancer. In the absence of chemotherapy, the risk of VTE in early breast cancer is between 0.2% and 0.8% (Lee & Levine 1999; Saphner, Tormey, & Gray 1991), rising to two to 10% if the patient receives chemotherapy (Levine *et al.* 1988; von Tempelhoff *et al.* 1996;Weiss *et al.* 1981). In advanced breast cancer patients receiving chemotherapy, as many as 17.6% of patients develop VTE (Goodnough *et al.* 1984). Most reported VTEs occur early in treatment (within the first three cycles) (Goodnough *et al.*1984; Pritchard *et al.* 1996; Seward *et al.* 1999; von Tempelhoff *et al.* 1996; Weiss *et al.* 1981). The rate of VTE during neoadjuvant chemotherapy has not previously been studied.

There is some evidence to suggest that cancer patients who develop VTE have a poorer *cancer* outcome than those who do not (Morgan *et al.* 2002; Seward *et al.* 1999); suggesting VTE may be a surrogate marker for more aggressive disease.

In the first part of this study we will address the following:

- Incidence of VTE in early breast cancer patients undergoing chemotherapy
- Incidence of VTE in advanced breast cancer patients undergoing chemotherapy
- Incidence of VTE in neoadjuvant breast cancer patients undergoing chemotherapy
- Risk factors for development of VTE during chemotherapy

- Effect of development of VTE on cancer outcome, in terms of progression and survival

5.2 Results

5.2.1 Demographics of study groups

One hundred and thirty four breast cancer patients were recruited. Of these, 36 had advanced breast cancer, 87 had early breast cancer following curative surgery and 11 were neoadjuvant or inflammatory breast cancer patients undergoing chemotherapy prior to curative surgery. 68 control women without cancer were recruited. There was a trend for the advanced group to be older than the neoadjuvant and control group, however this failed to reach significance (p=0.09 and 0.07 respectively) (Table 7).

Table 7: Age of patients recruited (mean and range for each patient group)

Group (n)	*Age (Range)*
Advanced breast cancer (**36**)	55.8 (34-78)
Early breast cancer (**87**)	51.3 (31-74)
Neoadjuvant breast cancer (**11**)	46.1 (35-58)
Control (**68**)	49.8 (31-78)

There was no difference in frequency of oestrogen, progesterone or Her 2 neu receptor positivity between the patient groups (Table 8).

Table 8: Receptor positivity (+) and negativity (-) for each patient group

	ABC +/-	*EBC* +/-	*NBC* +/-	*Total* +/-	*Difference between groups*
Oestrogen receptor	20/16	60/27	5/6	85/49	p=0.16
Progesterone receptor	16/19	47/40	4/7	67/66	p=0.45
Her 2 neu receptor	10/18	16/32	4/5	30/55	p=0.82

More women with advanced breast cancer were post-menopausal compared to early, neoadjuvant and control groups (Table 9).

Table 9: Numbers of pre and postmenopausal women in each patient group.

The ratio of premenopausal and post menopausal women in advanced breast cancer is compared to the ratio of premenopausal and post menopausal women in other groups (Chi squared).

	Premenopausal	*Postmenopausal*	*Ratio of pre/post menopausal women compared to advanced breast cancer (p)*
Advanced breast cancer (**36**)	9	27	
Early breast cancer (**87**)	39	48	0.05
Neoadjuvant breast cancer (**11**)	8	3	0.009
Control (**68**)	42	26	0.001

The groups were not matched for type of chemotherapy administered. Chemotherapy regimens used for advanced breast cancer were docetaxol (n=15); cyclophosphamide, methotrexate, 5-fluorouracil (n=8); epirubicin, docetaxol (n=6); vinorelbine, mitomycin (n=3); E7070 (sulphonamide antimetabolite) (n=1); epirubicin (n=1); 5-fluorouracil, epirubicin and cyclophosphamide (n=1); and vinorelbine, 5-fluorouracil (n=1).

Chemotherapy regimens used for early breast cancer were 5-fluorouracil, epirubicin and cyclophosphamide (n=65); cyclophosphamide, methotrexate, 5-fluorouracil (n=15); epirubicin (n=3); and epirubicin and cyclophosphamide (n=4).

Chemotherapy regimens used for neoadjuvant breast cancer were doxorubicin, cyclophosphamide (n=9) and epirubicin, vinorelbine (n=2).

Table 10: Different chemotherapy classes administered for advanced, early and neoadjuvant breast cancer patients (see Appendix 4 for chemotherapy class)

Chemotherapy class	*ABC yes(%) /no(%)*	*EBC yes(%) /no(%)*	*NBC yes(%)/no(%)*
Alkylating	9(25) / 27(75)	84(97) / 3(3)	9(82) / 2(18)
Anthracycline	11(31) / 25(69)	72(83) / 15(17)	11(100) / 0(0)
Antimetabolite	11(31) / 25(69)	80(92) / 7(8)	0(0) / 11(100)
Vinca alkaloid	4(11) / 32(89)	0(0) / 87(100)	2(18) / 9(82)
Taxane	21(58) / 15(42)	0(0) / 87(100)	0(0) / 11(100)

Advanced breast cancer (ABC), early breast cancer (EBC), neoadjuvant breast cancer (NBC)

5.2.2 Prevalence of VTE in advanced versus early breast cancer patients undergoing chemotherapy

Thirteen (10%) patients developed VTE, of which nine (7%) were symptomatic. Six of 36 (17%) advanced breast cancer patients and seven of 87 (8%) early breast cancer patients developed VTE. None of 11 neoadjuvant breast cancer patients developed VTE. The increased rate of VTE in advanced breast cancer compared to early breast cancer approached significance (p=0.06). Of patients with symptomatic VTE, four of 36 (11%) were in the advanced breast cancer group and five of 87 (6%) were in the early breast cancer group (p=0.1) (table 11).

Three (2.2%) patients died from VTE; all had advanced breast cancer (8.3%). All three developed VTE within the first three months of commencement of chemotherapy. Survival from entry into the study for these three patients was 55, 66 and 879 days. The former two patients died within one and seven days, respectively, of development of VTE. The later patient was found to have calf vein thrombosis at screening, but died over two years later of PE. Of patients with VTE (symptomatic and screen-detected), the 28 day mortality rate was 15%, but of patients with symptomatic VTE, the 28 day mortality was 22%.

Nine of the 13 (69%) patients that developed VTE did so within the first three months of commencing chemotherapy. All six advanced breast cancer patients who developed VTE did so within three months, but only three of the seven early breast cancer patients developed VTE within three months of starting adjuvant chemotherapy.

Ten of the 13 (77%) VTE events occurred in postmenopausal women, however neither age nor menopausal status was associated with an increased risk of VTE (p=0.2 and 0.2).

Table 11: Demographics and outcome of patients developing VTE

Age	*Stage*	*Days from commencing chemo to VTE*	*Site of VTE*	*Symptomatic or asymptomatic*	*Outcome (cause of death)*
41	ABC	27	tibio-peroneal	symptomatic	Died day 169 (not recorded)
77	ABC	59	PE	symptomatic	Died day 66 (PE)
58	EBC	181	common femoral	symptomatic	remission
49	ABC	35	soleal veins	asymptomatic	died day 879 (PE)
49	EBC	35	posterior tibial	asymptomatic	died day 834 (widespread metastatic breast cancer)
54	EBC	122	peroneal vein	symptomatic	remission
52	ABC	4	internal jugular (PICC line at 2nd cycle)	symptomatic	died day 377 (liver failure)
70	EBC	87	femoral vein	symptomatic	remission
61	EBC	21	upper femoral vein	symptomatic	remission
46	EBC	721	internal iliac	symptomatic	died day 906 (not recorded)
40	ABC	55	PE	symptomatic	died day 55 (PE)
67	ABC	19	femoral vein	asymptomatic	died day 339 (not recorded)
61	EBC	36	posterior tibial	asymptomatic	remission

Development of VTE in the early breast cancer group did not correlate with the number of days from surgery to commencement of chemotherapy (p=0.7).

5.2.3 Does development of VTE predict for a worse cancer outcome?

Cancer outcome was categorized into 'response', 'stabilisation', 'progression' and 'death' at three, six, 12 and 24 months (Tables 12 to 15).

Table 12 reports the number of patients that have radiological or clinical cancer progression or response within three months of commencing chemotherapy. The number of patients dying (either from VTE or cancer) is reported in the last column.

The number of patients developing VTE in each group is reported in brackets.

Table 12: Response to treatment at three months (development of VTE)

	Response	*Stable*	*Progression*	*Death*
Advanced breast cancer	15 (2)	6	9 (2)	6 (2)
Early breast cancer	0	87 (3)	0	0
Neoadjuvant breast cancer	9	2	0	0
Total	24 (2)	95 (3)	9 (2)	6 (2)

Table 13 reports the number of patients that have radiological or clinical cancer progression or response within six months of commencing chemotherapy. The same format is used as described for Table 12.

Table 13: Response to treatment at six months (development of VTE)

	Response	*Stable*	*Progression*	*Death*
Advanced breast cancer	4	10	11 (3)	11 (3)
Early breast cancer	0	87 (5)	0	0
Neoadjuvant breast cancer	0	11	0	0
Total	4	108 (5)	11 (3)	11 (3)

Table 14 reports the number of patients that have radiological or clinical cancer progression or response within twelve months of commencing chemotherapy. The same format is used as described for Table 12.

Table 14: Response to treatment at 12 months (development of VTE)

	Response	*Stable*	*Progression*	*Death*
Advanced breast cancer	1	3	10 (3)	22 (3)
Early breast cancer	0	85 (6)	1	1
Neoadjuvant breast cancer	0	10	0	1
Total	1	98 (6)	11 (3)	24 (3)

Table 15 reports the number of patients that have radiological or clinical cancer progression or response within 24 months of commencing chemotherapy. The same format is used as described for Table 12.

Table 15: Response to treatment at 24 months (development of VTE)

	Response	*Stable*	*Progression*	*Death*
Advanced breast cancer	1	3	2 (2)	30 (4)
Early breast cancer	0	81 (5)	5 (2)	1
Neoadjuvant breast cancer	0	7	2	2
Total	1	91 (5)	9 (4)	33 (4)

Development of VTE did not predict for progression in advanced breast cancer patients by three and six months (p=0.17 and 0.15 respectively). VTE demonstrated a trend for predicting for progression by two years (p=0.08 for early breast cancer patients and p=0.07 for all cancer patients, with correction for cancer stage on logistic regression). Using Cox regression survival analysis, there was no survival advantage in those with or without VTE (p=0.4).

5.3 Discussion

5.3.1 Prevalence of VTE in advanced versus early breast cancer patients undergoing chemotherapy

The rate of VTE during chemotherapy in both early breast cancer and advanced breast cancer patients in this study concurs with much of the current literature (Goodnough *et al.* 1984; Levine *et al.* 1988; von Tempelhoff *et al.* 1996; Weiss *et al.* 1981) (Table 16).

Table 16: Rate of VTE during chemotherapy for breast cancer. Summary of the literature

Author, year	*Patient group*	*Study Design*	*VTE diagnosis*	*Design faults*	*VTE rate (mortality)*
Goodnough, 1984 (Goodnough *et al.* 1984)	ABC	Observational Patients commencing new course of chemotherapy	Clinical presentation, imaging, post mortem	No VTE screening Possible concurrent hormone therapy	28(14)/ 159 during study period 24(11)/ 159 during chemotherapy
Weiss, 1981 (Weiss *et al.* 1981)	Node positive EBC	Observational Commencing 2 years chemotherapy following surgery	Not stated	No VTE screening Largely clinical diagnosis	22(2)/ 433 all during chemotherapy
Saphner, 1991 (Saphner, Tormey, & Gray 1991)	EBC	Retrospective 7 RCTs of hormone, chemotherapy or both	Clinical presentation, imaging, post mortem	No VTE screening Retrospective	4(?)/471 premenopausal 5(?)/132 postmenopausal all during chemotherapy
Levine, 1988 (Levine *et al.* 1988)	Node positive EBC	Prospective RCT: 12 weeks chemotherapy + tamoxifen or 36 weeks chemotherapy	IPG or Duplex screening at 2, 4, 8, 12, 16, 24, 36 weeks	Includes 3 superficial thrombophlebitis	4(0)/102 in first 12 weeks (chemotherapy only group) 9(0)/102 during 36 weeks of chemotherapy

Author, year	*Patient group*	*Study Design*	*VTE diagnosis*	*Design faults*	*VTE rate (mortality)*
Clahsen, 1994 (Clahsen *et al.* 1994)	EBC	Retrospective RCT surgery +/- peri-operative chemotherapy	Clinical presentation, imaging, post mortem	No VTE screening Retrospective Surgery as confounding risk factor	27(3)/1292 with chemotherapy 10(0)/1332 without chemotherapy
von Tempelhoff, 1996 (von Tempelhoff *et al.* 1996)	Node positive EBC	Observational Commencing chemotherapy following surgery	IPG screening before each cycle, then 3 monthly	IPG poor screening tool for non-occlusive thrombi(Colman et al. 1994)	5(0)/50 all during chemotherapy
Levine, 1994 (Levine *et al.* 1994)	ABC	Prospective RCT: warfarin or placebo during chemotherapy	Clinical presentation, imaging	No VTE screening Some patients commenced on chemotherapy prior to entry into study	7(0)/152 during chemotherapy
Kirwan, 2005	ABC, EBC and NBC	Prospective cohort	Duplex screening at 4 weeks. Clinical presentation, imaging	One screening assessment only Insufficient NBC for assessment	6(3)/36 ABC 7(0)/87 EBC 0(0)/11 NBC

Despite difficulties with recruitment within the limitations of the study time, we were still able to confirm the previously quoted literature on rates of VTE, and the increased

rate that occurs in ABC compared to EBC. Although many studies with VTE as an endpoint use venography to screen for DVT, we used DUI. The quality of imaging in our specialist unit is extremely high, with a high rate for identifying even asymptomatic VTE. This non-invasive test allowed increased patient compliance, and is well recognised as a screening tool for VTE (Bresolette *et al.* 2001; Elias *et al.* 2004; Kassai *et al.* 2004; Schellong 2004).

Saphner reports a 0.8% (4 of 471) VTE rate in pre-menopausal women receiving adjuvant chemotherapy in a retrospective study (Saphner, Tormey, & Gray 1991). Weiss prospectively reports a 5% (22 of 433) VTE rate in node-positive breast cancer patients receiving adjuvant chemotherapy (Weiss *et al.* 1981). Both studies rely on clinical presentation (either as an emergency by the patient or by examination at follow-up by the doctor), rather than screen detection. A 9% (nine of 102) and 10% (five of 50) VTE rate in node positive, early breast cancer patients receiving chemotherapy, as detected by IPG and DUI screening was found by Levine (Levine *et al.* 1988) and von Tempelhoff (von Tempelhoff *et al.* 1996) respectively. Levine's study of advanced breast cancer patients receiving chemotherapy, and randomised to warfarin or no treatment reports a VTE rate of only 4.5%. In this study, however, VTE screening was by clinical methods only. Patients recruited to the study had already commenced chemotherapy, and so those patients developing VTE early in response to chemotherapy would have been excluded. As this appears to be the majority of patients (Seward *et al.* 1999; von Tempelhoff *et al.* 1996), this biases the recruited population to a low-risk group (Levine *et al.* 1994).

This present study is not powered to confirm the previously quoted VTE mortality in early breast cancer of 0.2-0.5% (Clahsen *et al.*1994; Weiss *et al.* 1981), however with a

mortality rate of 8% it does confirm Goodnough's finding of a 7% VTE mortality rate during chemotherapy for advanced breast cancer (Goodnough *et al.* 1984).

This study also confirms the previously reported finding of approximately two thirds of all VTEs occurring within three months of commencing chemotherapy (Seward *et al.* 1999; von Tempelhoff *et al.* 1996). In this study 43% of VTEs in early breast cancer patients, occurred within the first three months. Levine, in a study of adjuvant chemotherapy administered over a 36 week period found 44% (four of nine) of VTEs occurring in the same three month time period. The remaining 56% (five of nine) occurred in the following 24 weeks, with no VTEs occurring after treatment was completed (Levine *et al.* 1988). It is interesting that all the VTEs in advanced cancer patients, in this current study, occurred within three months. This may be because these patients are nearer a VTE threshold, which they rapidly cross with the added procoagulant stimulus of chemotherapy (Rosendaal 1997). Goodnough supports this finding with 86% of advanced breast cancer patients developing VTE during chemotherapy (24 events during 1236 months of treatment), but only 14% whilst under observation (four events during 8852 months of treatment), however he fails to comment whether any patients were receiving concurrent hormone therapy (Goodnough *et al.* 1984).

The 28 day mortality rate for symptomatic VTE of 22% is similar to that reported by Cushmans cohort study, where a 28 day fatality rate after first time VTE of 11% was found, but this increased to 25% for cancer associated thrombosis (Cushman *et al.* 2004).

Due to limited availability of eligible patients and hence low recruitment, this study has insufficient numbers to assess risk of VTE in patients receiving neoadjuvant chemotherapy.

Some authors report an increased risk of thrombosis amongst postmenopausal women (Clahsen *et al.* 1994; Saphner, Tormey, & Gray 1991; Weiss *et al.* 1981) however an association was not demonstrated in this study. Saphner (Saphner, Tormey, & Gray 1991) demonstrated oestrogen receptor positivity as a risk factor for chemotherapy-induced VTE, however this is not supported by Levine (Levine *et al.* 1988), Clahsen (Clahsen *et al.* 1994) or indeed this present study. This implies that Saphner's finding may have been due to chance.

In malignancy, haemostatic markers of coagulation are raised for several weeks after surgery (Rasmussen 2003). A recent multicenter, double-blind, randomized, placebo-controlled study of cancer patients undergoing abdominal surgery, compared prolonged (four week) VTE prophylaxis with the standard one-week regimen. There was a 60% reduction in the relative risk of VTE in patients receiving prolonged prophylaxis (4.8% versus 12.0%, p=0.02) (Bergqvist *et al.* 2002). These results have been supported by further meta-analysis (Rasmussen 2003). Reducing the interval between surgery and adjuvant chemotherapy by administering chemotherapy within 36 hours of mastectomy has been shown to prolong disease-free survival in node-negative breast cancer patients (Ludwig Group 1989), however the impact on hypercoagulability of a further treatment in already recovering and inactive patients has yet to be established. In this study there is no demonstrable added risk for VTE by commencing chemotherapy shortly after surgery.

One potential weakness of this current study is that no screening for VTE was performed prior to commencement of chemotherapy. Identified thrombi could have been pre-existing and therefore as a result of the cancer, or in the case of EBC patients, as a result of surgery. It was unfortunately not possible, for logistical reasons to screen patients for VTE prior to chemotherapy. If anything, however, this increases the significance of the greater VTE rate in patients with a high cancer load (ABC).

It is recognised that in this group, the two study groups, EBC and ABC, are heterogeneous for chemotherapy type. It is also recognised that the ABC patients are heterogeneous for site of metastatic disease. It was felt by the author that it was not appropriate to further subgroup ABC patients into tumour load as the micrometastatic disease cannot be adequately quantified.

5.3.2 Does development of VTE predict for a worse cancer outcome?

Previous research has shown that patients that develop VTE have a worse cancer outcome than those that remain free of VTE complications (Levitan *et al.* 1999; Morgan *et al.* 2002;Seward *et al.* 1999; Sorensen *et al.* 2000). However to date all studies have been retrospective. Both Seward and Morgan studied medical records of cancer patients presenting with VTE at major medical centres. Seward found that cancer patients who developed VTE had a worse prognosis than those who remained free of VTE, but failed to account for missed, more minor VTE that may have presented to smaller district hospitals (Seward *et al.* 1999). Morgan reported a significantly reduced survival time if cancer presented concurrently with VTE compared to a matched control group without VTE ($p<0.001$) (Morgan *et al.* 2002). Sorensens retrospective study of the National

Registry acknowledges that VTE occur more commonly in patients with more advanced disease, compared to early cancer. This may contribute to the reduced survival of patients with VTE in cancer, seen in his study, and hence bias survival analysis (Sorensen, Mellemkjaer, Olsen, & Baron 2000) . The increased VTE rate in advanced cancer is supported by this present study. Levitan's study of Medicare records, although finding a significantly reduced survival in patients presenting with concurrent VTE, compared to cancer alone, has a similar problem (Levitan *et al.* 1999). Interestingly, in Weiss' observational study of early breast cancer patients commencing adjuvant chemotherapy, 35% (seven of 20) of those with VTE developed recurrence of their breast cancer within two years, compared to 15% for the entire group of patients in the study (65 of 433). Statistical significance for this is not reported (Weiss *et al.* 1981).

This current study provides further evidence that development of VTE predicts for cancer progression. VTE within 3 months following cancer chemotherapy suggests a poorer outcome ($p=0.07$). However with cancer progression occurring in 61% of advanced breast cancer patients and no early breast cancer patients at three months, and 89% of advanced breast cancer patients but only 7% of early breast cancer patients by two years, analysis is complicated by strong covariates. Sub-group analysis is limited by small numbers.

Although the body of evidence supports a worse outcome when VTE and cancer coexist as compared to either diagnosis alone, a large prospective study is required to confirm this, and clarify whether any premature death is due primarily to VTE or to a more aggressive cancer.

3.5.4 Conclusion

The current study confirms the rates of chemotherapy induced VTE (8% in early breast cancer patients and 17% in advanced breast cancer patients) (Goodnough *et al.* 1984; Levine *et al.* 1988; von Tempelhoff *et al.* 1996; Weiss *et al.* 1981). The difference between the two study groups, may reflect pre-existing factors within the advanced cancer group, for example age, immobility or veno-occlusion from nodal disease, however in this study we have attempted to exclude or correct for all such factors. All early breast cancer patients underwent surgery shortly before commencement of chemotherapy, however with this study size, no identifiable surgery-induced hypercoagulability, in terms of VTE development, is observed. The most important difference between the two groups is the presence of cancer. Early breast cancer patients receive chemotherapy in the presence of minimal or no cancer load, whereas advanced breast cancer patients receive chemotherapy in the presence of extensive, radiologically identifiable cancer. The 8% VTE rate in the former group demonstrates that chemotherapy *per se* induces thrombus formation. However the increased rate seen in the presence of cancer suggests a role for *chemotherapy acting on cancer* as a stimulus for thrombosis. This theory would be further supported if patients demonstrating a large response to chemotherapy (ie disease regression), had an increased thrombotic response. With only six VTE events in the advanced cancer group, we fail to demonstrate such a link in this study. More subtle haemostatic changes will be investigated in the following chapter to identify those patients that develop a hypercoagulable state.

It is evident that early and advanced breast cancer patients demonstrate a different thrombotic response to chemotherapy. By assessing haemostatic, vascular endothelial

and tumour procoagulant response during chemotherapy in these groups this study hopes to clarify whether advanced breast cancer patients demonstrate an upregulated response to chemotherapy, or whether the *chemotherapy acting on cancer* is the source of the procoagulant response.

The high rates of VTE presented in this study have important implications on both patient quality of life and health economics. This study demonstrates that although VTE may be associated with cancer progression, early breast cancer patients with good prognosis are also at risk of VTE, making VTE prevention more important. In this study we identify chemotherapy for advanced breast cancer as an increased risk factor for VTE, however no other routinely measured demographics (eg age, menopausal status, nodal involvement, receptor status) are identified as risk factors. In the subsequent chapters markers of the haemostatic system, and circulating procoagulant markers from the cancer itself will be assessed for predictive value of VTE. Identification of a high VTE risk subset would allow targeted thromboprophylaxis.

Chapter 6

Effect of cancer and chemotherapy on haemostatic markers, cancer-related procoagulants and endothelial activation

In this chapter, the effect of cancer on markers of coagulation, procoagulants and endothelial adhesion molecules is presented as:

baseline levels of these markers in i) advanced breast cancer patients (high tumour load), ii) neoadjuvant breast cancer patients (moderate tumour load), iii) early breast cancer patients (low/no tumour load) and iv) controls.

The effect of chemotherapy on coagulation, procoagulants and endothelial adhesion molecules, and the differing responses in advanced and early breast cancer patients is reported.

6.1 Results: Haemostatic markers in breast cancer patients and controls

Aim

To measure markers of hypercoagulability or fibrinolysis, including platelets and acute phase response, in i) advanced breast cancer, ii) neoadjuvant breast cancer and iii) early breast cancer patients prior to chemotherapy and non-cancer controls.

Table 17: Baseline haemostatic markers prior to chemotherapy

Analysis of the difference between groups (ABC/ EBC/ NBC and controls) used Analysis of Variance (ANOVA) or Kruskal Wallis. Where differences were found, further analysis (between pairs of groups) was performed with Scheffe or Mann-Whitney U tests, with Bonferroni correction. (Standard deviation (SD), 95% Confidence interval (CI)).

Coagulation marker	*Advanced breast cancer*	*Early breast cancer*	*Neoadjuvant breast cancer*	*Control*	*p ANOVA / Kruskal Wallis (Scheffe/Mann-Whitney+Bonferroni)*
PT secs, mean (SD) ***(n)***	11.7 (0.8) ***(26)***	11.7 (0.7) ***(77)***	11.6 (0.6) ***(9)***	11.4 (0.6) ***(45)***	*0.08*
APTT secs, mean (SD) ***(n)***	22.6 (2.8)* ***(26)***	23.2 (2.4)† ***(77)***	22.0 (2.1) ***(9)***	20.8 (2.9)*† ***(45)***	*<0.001* (* 0.02, †<0.001)
PF1+2 nmol/l, median (range) ***(n)***	0.7 (0.5-1.6) ***(16)***	0.9 (0.7-3.3) ***(14)***	***(0)***	***(0)***	*0.2*
TAT µg/ml, median (range) ***(n)***	7.0 (2.8-280)*† ***(14)***	3.8 (2.5-22.2)* ***(11)***	3.6 (2.2-24.9) ***(3)***	2.9 (2.0-18.6)† ***(13)***	*0.05* *(*0.08, †0.1)*
Fibrinogen g/L, mean (SD) ***(n)***	4.8 (1.8)*† ***(21)***	3.1 (0.9)*‡ ***(73)***	3.4 (0.8) ***(9)***	2.7 (0.6)†‡ ***(45)***	*<0.001* *(*<0.001, †<0.001, ‡0.06)*
D-dimer ng/ml, geometric mean (CI) ***(n)***	1335 (970-1838)*†‡ ***(35)***	667 (585-764)*§ ***(85)***	374 (200-700)† ***(10)***	288 (253-338)‡§ ***(61)***	*<0.001* *(*<0.001, †<0.001, ‡<0.001,* §<0.001 *)*
tPA ng/ml, median (range) ***(n)***	11442 (3082-30845)* ***(16)***	8147 (4943-22296)† ***(20)***	8863 (4636-15540) ***(4)***	5798 (3727-12129)*† ***(27)***	*<0.001* *(*<0.001, †0.08)*

Coagulation marker	*Advanced breast cancer*	*Early breast cancer*	*Neoadjuvant breast cancer*	*Control*	*p ANOVA / Kruskal Wallis (Scheffe/Mann-Whitney+Bonferroni)*
uPA ng/ml, median (range) *(n)*	0.56 (0.01-3.81)* ***(16)***	0.75 (0.01-19.41)† ***(19)***	0.50 (0.47-2.04) ***(4)***	0.34 (0.01-0.82)*† ***(20)***	*<0.001 (*0.01, †0.001)*
PAI-1 ng/ml, geometric mean (CI) *(n)*	22.2 (15.6-31.5) ***(16)***	15.4 (11.3-21.0) ***(14)***	***(0)***	***(0)***	*0.1*
Platelet count $x10^9$/l, mean (SD) *(n)*	327* (118) ***(36)***	310 (78) ***(87)***	336 (76) ***(11)***	279 (65)* ***(53)***	*0.03 (*0.08)*
Platelet function VEGF µg/ml per platelet $x10^9$, median (range) *(n)*	1.02 (0.17-3.53)*†‡ ***(35)***	0.53 (0.05-2.89)* ***(83)***	0.43 (0.08-1.05)†***(10)***	0.52 (0.007-2.48)‡ ***(48)***	*0.05 (*<0.001, †0.008, ‡0.002)*
CRP mg/l, median (range) *(n)*	8.9 (1.8-226) ***(13)***	1.1 (0.5-39) ***(13)***	***(0)***	***(0)***	*0.04*

6.1.1 Markers of hypercoagulability

Slower clotting times were observed in advanced and early breast cancer patients at baseline compared to controls. This was significant when assessing activated partial thromboplastin time (APTT) (Table 17).

There was a trend towards elevated TAT in advanced compared to early breast cancer patients and controls. As expected, TAT correlated with PT (Spearman correlation coefficient (r^2) 0.69, $p<0.001$, $n=31$).

PF1+2, PAI-1 and CRP were assessed as part of a pilot study only. The patient groups were more heterogeneous, in terms of site of primary cancer and sex. There was no control.

Table 18: Site of primary tumour in pilot study group (for measurement of PF1+2, PAI-1 and CRP)

Tumour site	*Advanced*	*Early*	*Total*
Breast	7	7	14
Colorectal	7	7	14
Stomach	2	0	2
Total	16	14	30

There were 9 males and 21 females, with equal distribution between the advanced and early cancer groups (advanced: five male, 11 female; early: four male, ten female). There was no significant difference in age between the advanced and early cancer groups (mean age 58 and 54 respectively, p=0.4).

There was no significant difference between advanced and early breast cancer pre-chemotherapy levels of PF1+2 (median (range) 0.7 (0.5-1.6) and 0.9 (0.7-3.3) nmol/l respectively, $p=0.2$), although levels were significantly decreased in advanced colorectal cancer compared to early colorectal cancer (median (range) 1.1 (0.8-1.5) nmol/l and 1.23 (1.0-4.2) nmol/l respectively, p=0.04).

6.1.2 Markers of intravascular fibrin formation

Circulating levels of fibrinogen and D-dimer were increased in advanced compared to early breast cancer patients, and early breast cancer patients compared to controls.

As expected, markers of intravascular fibrin formation correlated with many markers of hypercoagulability and fibrinolysis (Tables 19 and 20).

Table 19: Molecular markers of hypercoagulability and fibrinolysis correlating with D-dimer

Spearman correlation used for all correlation analysis.

Molecule correlating with D-dimer	*Spearman correlation coefficient (p)(n)*
Thrombin-antithrombin	0.68 (p<0.001) (**39**)
Fibrinogen	0.41 (p<0.001) (**144**)
Tissue plasminogen activator	0.50 (p<0.001) (**64**)
Urokinase plasminogen activator	0.39 (p=0.003) (**56**)
Platelet count	0.24 (p=0.001) (**182**)

Table 20: Molecular markers of hypercoagulability and fibrinolysis correlating with Fibrinogen

Molecule correlating with Fibrinogen	*Spearman correlation coefficient (p)(n)*
D-dimer	0.41 (p<0.001) (**144**)
Tissue plasminogen activator	0.47 (p=0.001) (**48**)
Platelet count	0.23 (p=0.006) (**145**)

6.1.3 Markers of fibrinolysis

Pre-chemotherapy levels of both tPA and uPA tended to be higher in breast cancer patients (Table 17).

In the pilot study, PAI-1 tended to be higher in advanced compared to early cancer patients (geometric mean 22.2 and 15.4ng/ml respectively, p=0.1). This trend was evident when breast cancer alone was analysed (geometric mean 27.9 and 13.3ng/ml, p=0.1).

6.1.4 Platelets

There was a trend for increased platelet count in advanced breast cancer patients, compared to both early breast cancer patients and controls (mean (SD) platelet count 326.7(117.5) x 10^9/l, 309.6(77.7) x 10^9/l and 278.9(65.4) x 10^9/l respectively, n=36, 87 and 53 respectively, p=0.03).

Twelve (33%) advanced breast cancer patients, 13 (15%) early breast cancer patients, one (9%) neoadjuvant breast cancer patients and two (4%) controls had thrombocytosis (platelet count >400x10^9/l). Three (8%) advanced breast cancer patients and one (2%) control had thrombocytopenia (platelet count <150x10^9/l).

Calculated VEGF release per platelet from advanced cancer patients was elevated compared to early, and neoadjuvant breast cancer patients and the control group (see table 17).

Calculated platelet function correlated with TAT (r^2=0.42, p=0.01, n=34), D-dimer (r^2=0.17, p=0.02, n=175) (Figure 2) and fibrinogen (r^2=0.22, p=0.01, n=139).

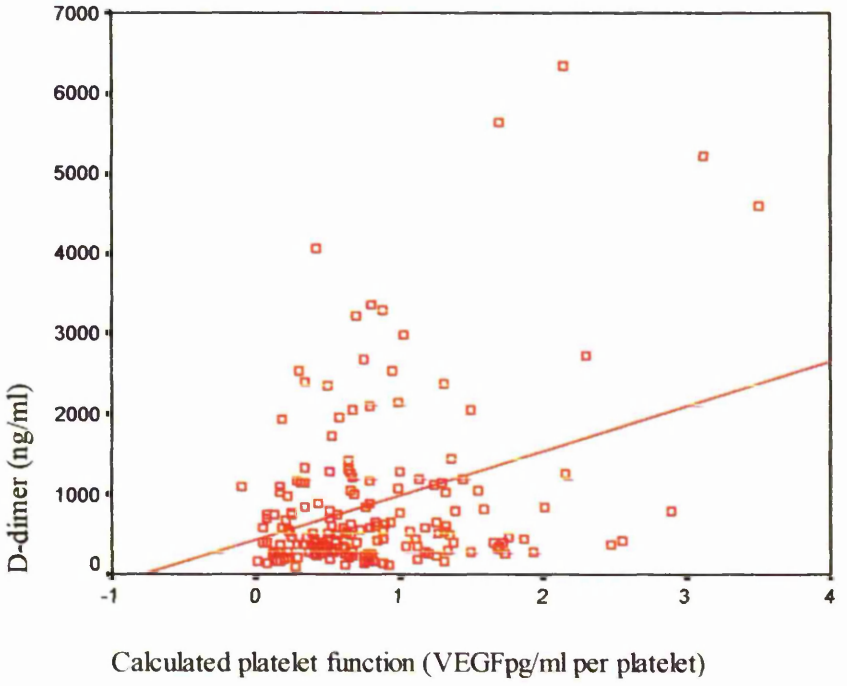

Figure 2: Correlation of platelet function with D-dimer, prior to chemotherapy

Platelet function was calculated as VEGF release per platelet (described in chapter 4.4.4).

6.1.5 Acute phase response

In the pilot study, CRP was increased in advanced compared to early cancer patients (median (range) 8.9(1.8-226)mg/l and 1.1(0.5-39)mg/l respectively, *p=0.04*).

CRP correlated with fibrinogen (r^2=0.77, p<0.001, n=20), another marker of the acute phase response.

6.1.6 Influence of time from surgery on markers of coagulation

In the early breast cancer group, there was a negative correlation between both baseline D-dimer and platelet count and the number of days following surgery, despite the exclusion of patients commencing chemotherapy within 18 days of surgery (Table 21 and Figure 3).

Table 21: Correlation of markers of coagulation with number of days since surgery (early breast cancer patients only)

Coagulation marker	*Spearman correlation coefficient (p)(n)*
PT	-0.01 (0.9) (**59**)
APTT	-0.07 (0.6) (**59**)
PF1+2	0.25 (1.0) (**14**)
TAT	-0.35 (0.7) (**9**)
Fibrinogen	0.04 (0.8) (**55**)
D-dimer	-0.40 (0.001) (**68**)
tPA	0.06 (0.8) (**16**)
uPA	-0.43 (0.1) (**15**)
PAI-1	0.35 (0.4) (**14**)
Platelet count	-0.38 (0.001) (**68**)
Platelet function	-0.08 (0.6) (**65**)
CRP	-0.36 (0.5) (**13**)

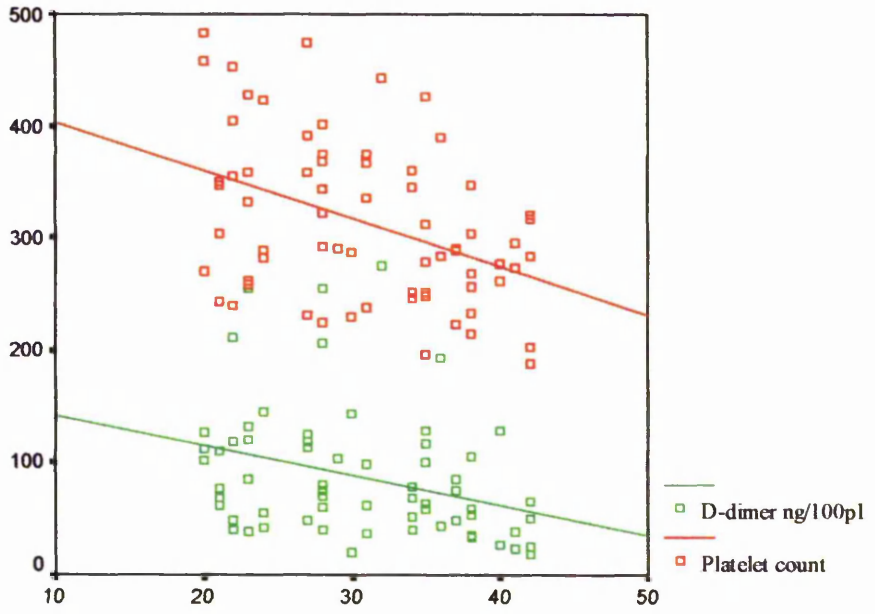

Figure 3: Correlation of D-dimer and platelet count with number of days since curative surgery in early breast cancer patients

D-dimer is shown as nanogram per 100 microlitre. Platelet count is $x10^9$ per litre.

6.1.7 Age, menopause and hypercoagulability

Only D-dimer correlated with age (r^2=0.27, p<0.001, n=200) and so was significantly elevated in post menopausal women compared to premenopausal women (geometric mean 692ng/ml and 446ng/ml, n=65 and 64 respectively, p<0.001). Fibrinogen and TAT, both of which correlated with D-dimer, demonstrated a weak correlation with age (Fibrinogen: p=0.08, n=148, TAT: p=0.15, n=41) (Appendix 7).

6.1.8 Association between tumour hormone receptor status and haemostatic markers

Only fibrinogen was associated with hormone receptor status, levels being higher in Her 2 neu negative compared with Her 2 neu positive patients (mean 3.9 and 3.2g/l, p=0.03). Corrected for multiple tests, a p value cut-off for significance of 0.01 would be more appropriate. (Appendices 8, 9 and 10).

6.1.9 Tumour stage in early breast cancer and markers of hypercoagulability

D-dimer was increased in early breast cancer patients with poorer prognosis as predicted by the Nottingham Prognostic Index (grade 3 compared to grade 1 and 2) (geometric mean 809 and 585ng/ml, n=36 and 51 respectively, p=0.02). Fibrinogen was reduced in early breast cancer patients with poorer prognosis, however this was of questionable significance due to multiple testing (p=0.04). D-dimer correlated with the size of the excised tumour (r^2=0.27, p=0.01, n=85) (Figure 4 and appendix 11).

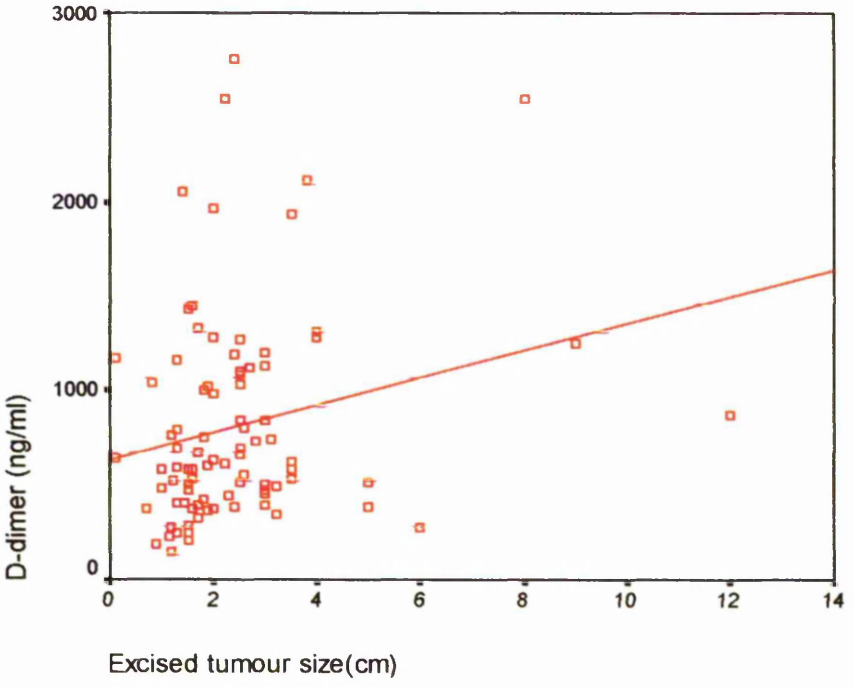

Figure 4: Correlation of D-dimer with excised tumour size in early breast cancer patients

6.2 Results: The effect of chemotherapy on haemostasis, platelet function and the acute phase response

6.2.1 Hypercoagulability

PT increased (or slowed) in the ten days following chemotherapy(p=0.002), but started to decrease at three months(p=0.007). By six months, there was a trend for quickening of PT to faster than pre-chemotherapy (p=0.009).

APTT demonstrated a marked quickening (ie a reduction in APTT) in the first 24 hours following chemotherapy ($p<0.001$). This was maintained up to six months. (Figure 5 and Appendix 10).

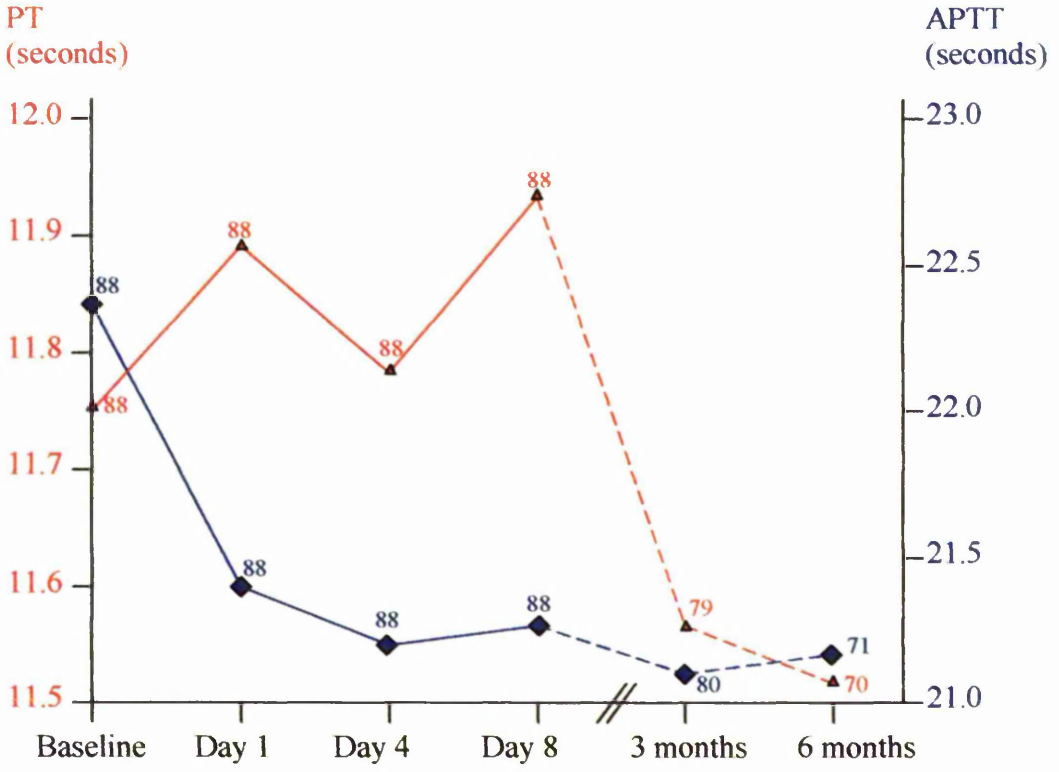

Figure 5: Clotting times: Effect of chemotherapy

Mean values. Patient numbers given at each timepoint in all graphs

TAT showed a borderline significant trend for an increase at 24 hours after chemotherapy, followed by a steady decrease ($p=0.08$). However PF1+2 levels did not alter in response to chemotherapy (Figure 6 and appendix 12).

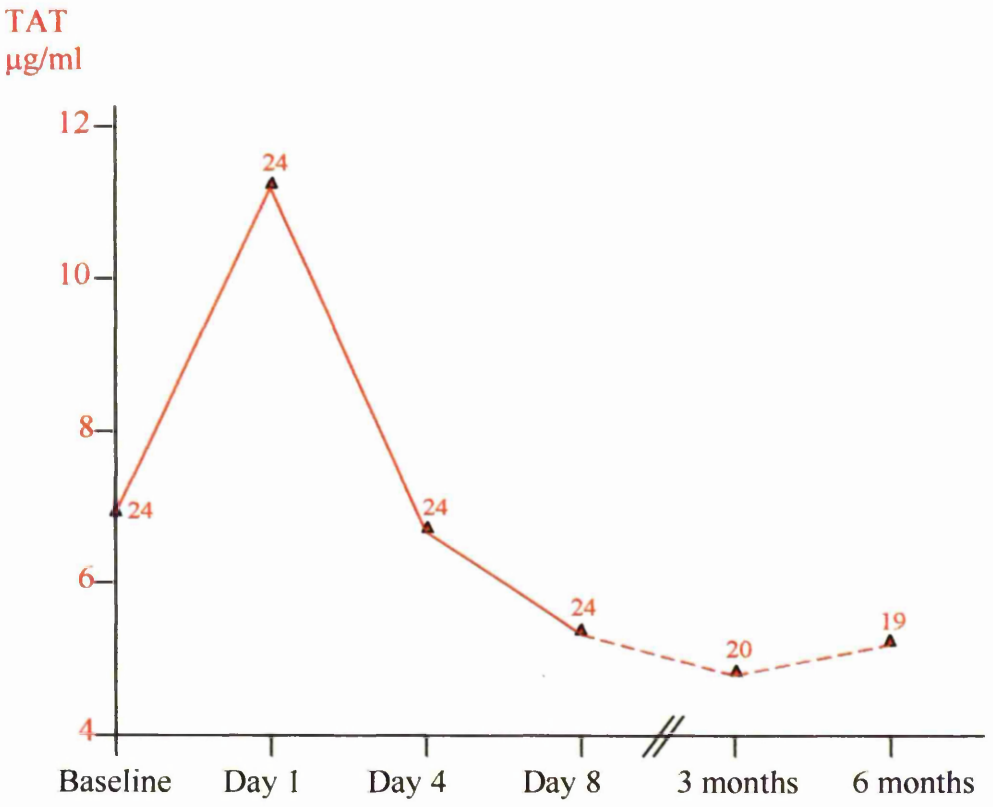

Figure 6: Circulating Thrombin-Antithrombin: Effect of chemotherapy

Geometric mean. Patient numbers given at each timepoint

6.2.2 Intravascular fibrin formation

Fibrinogen decreased in response to chemotherapy, but increased to above pre-chemotherapy levels by three months ($p<0.001$). D-dimer did not show any significant response to chemotherapy. Between the three and six month timepoints, both fibrinogen and D-dimer decreased significantly ($p<0.001$) (Figure 7 and appendix 12).

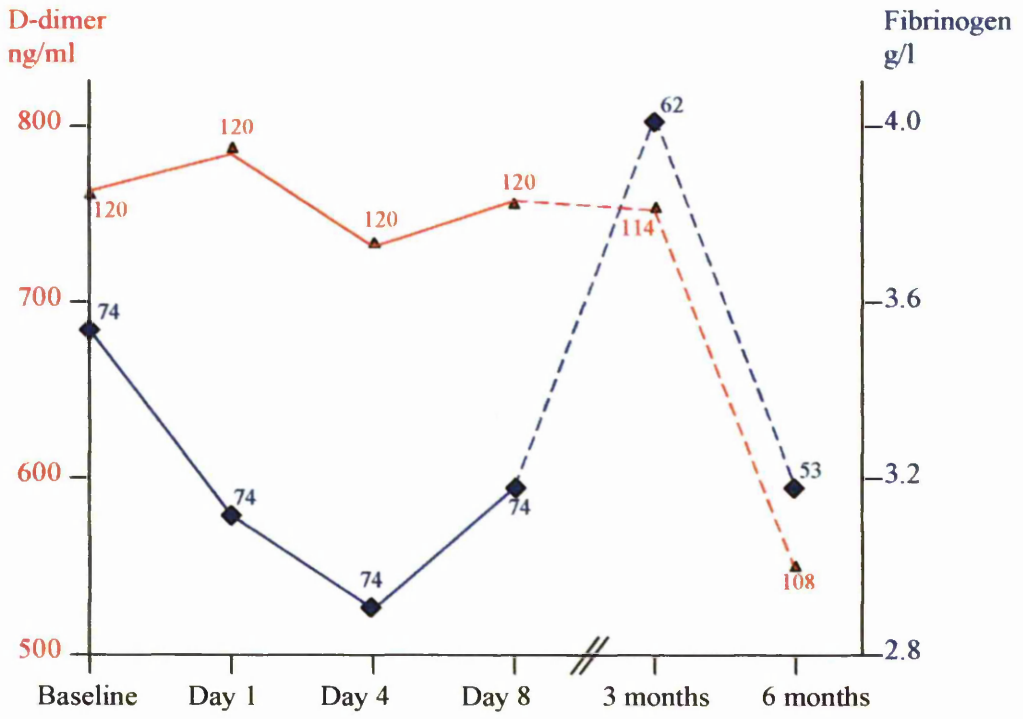

Figure 7: Circulating markers of intravascular fibrin formation: Effect of chemotherapy

Fibrinogen: mean values. D-dimer: geometric mean. Patient numbers given at each timepoint.

6.2.3 Fibrinolysis

PAI showed a tendency for decrease, with the trend approaching significance at six months ($p=0.06$).

tPA showed a significant trend for a slight decrease in the eight days following chemotherapy ($p<0.001$) with a significant trend for increase to above pre-chemotherapy levels by six months ($p=0.03$).

There was no significant trend in uPA levels as a response to chemotherapy within the first 8 days (p=0.15), however by three months uPA levels increased to above baseline (p=0.01). This trend for increasing circulating uPA continued to the six month timepoint (p=0.01) (Figure 8 and appendix 12).

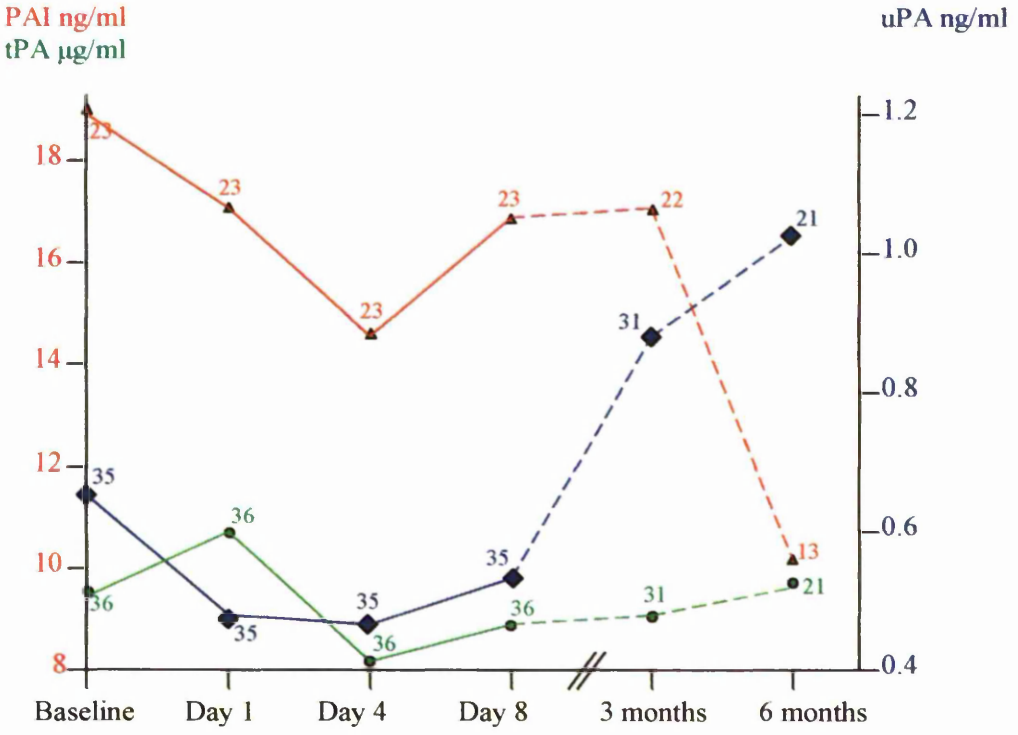

Figure 8: Circulating markers of fibrinolysis: Effect of chemotherapy

Geometric mean. Patient numbers given at each timepoint

6.2.4 Platelets

There was a significant trend for platelet count to decrease from baseline in the first eight days following chemotherapy (p<0.001), recover by three months (p<0.001), and then decrease again by six months (p<0.001).

Platelet release (of VEGF *per platelet*) also showed a significant trend in response to chemotherapy, that mirrored the change in platelet count. There was a decline in platelet function by four and eight days ($p<0.001$), increasing to above pre-chemotherapy activity by three months, and returning to nearer baseline by six months (Figure 9 and appendix 12).

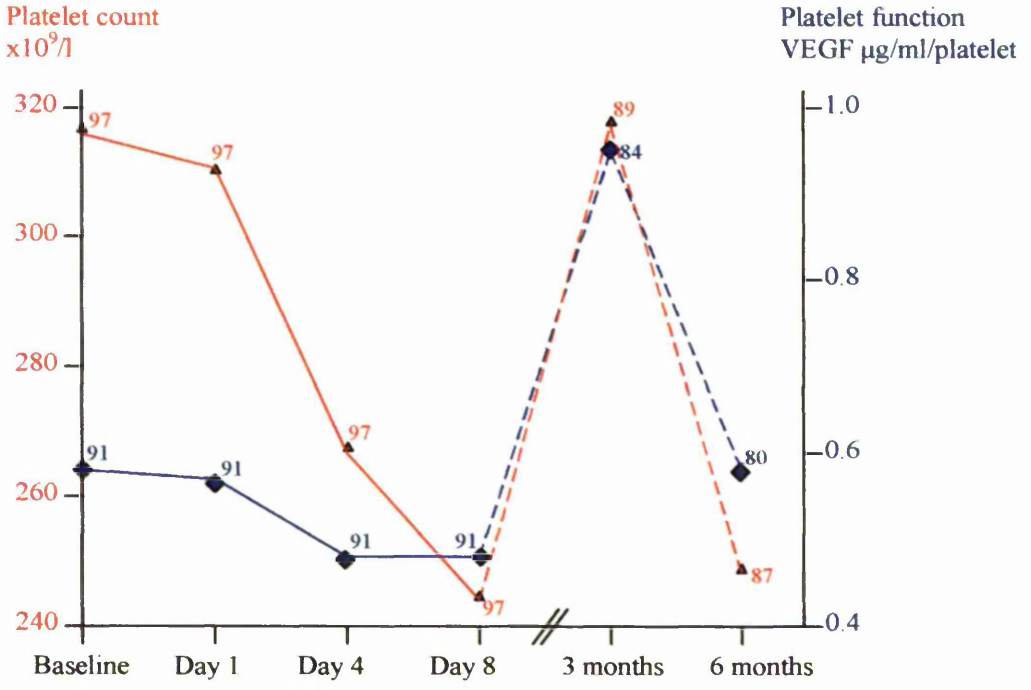

Figure 9: Platelet count and function: Effect of chemotherapy

Platelet count: mean. VEGF release per platelet: geometric mean

6.2.5 Acute phase response

CRP did not demonstrate any significant response to chemotherapy at any time ($p=0.5$) (Appendix 12).

6.3 Results: The influence of cancer on the haemostatic, platelet and acute phase response to chemotherapy

Aim

To assess whether the presence of cancer alters the haemostatic response to chemotherapy.

In this section, the effect of chemotherapy on patients with high tumour load (advanced breast cancer) will be compared to those with low on no tumour load (early breast cancer following 'curative' surgery).

6.3.1 Hypercoagulability

The change in clotting times induced by chemotherapy was significantly different for advanced breast cancer patients compared to early breast cancer patients. Both APTT and PT had a greater prolongation in advanced breast cancer. A trend was evident by eight days (p=0.2 and 0.13 respectively), but became significant by three months (p=0.002 and 0.004). This *difference in trend* between advanced and early breast cancer was maintained at six months, when both clotting times in advanced cancer (APTT and PT) approximated early cancer times (p=0.001 and 0.03). An attrition rate, largely due to death, of 37% and 17% occurred in advanced and early breast cancer respectively from pre-chemotherapy to six months (Figures 10 and 11, and appendices 13 and 14).

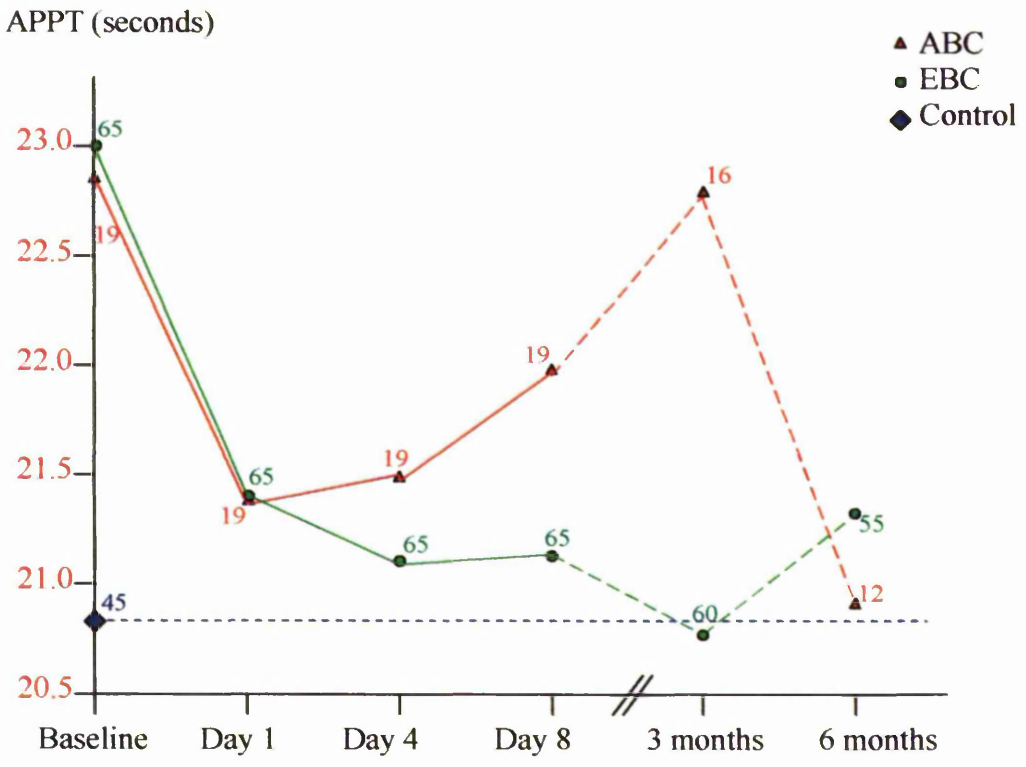

Figure 10: Chemotherapy-induced changes in activated partial thromboplastin time: The effect of cancer

Mean values. Patient numbers given at each timepoint

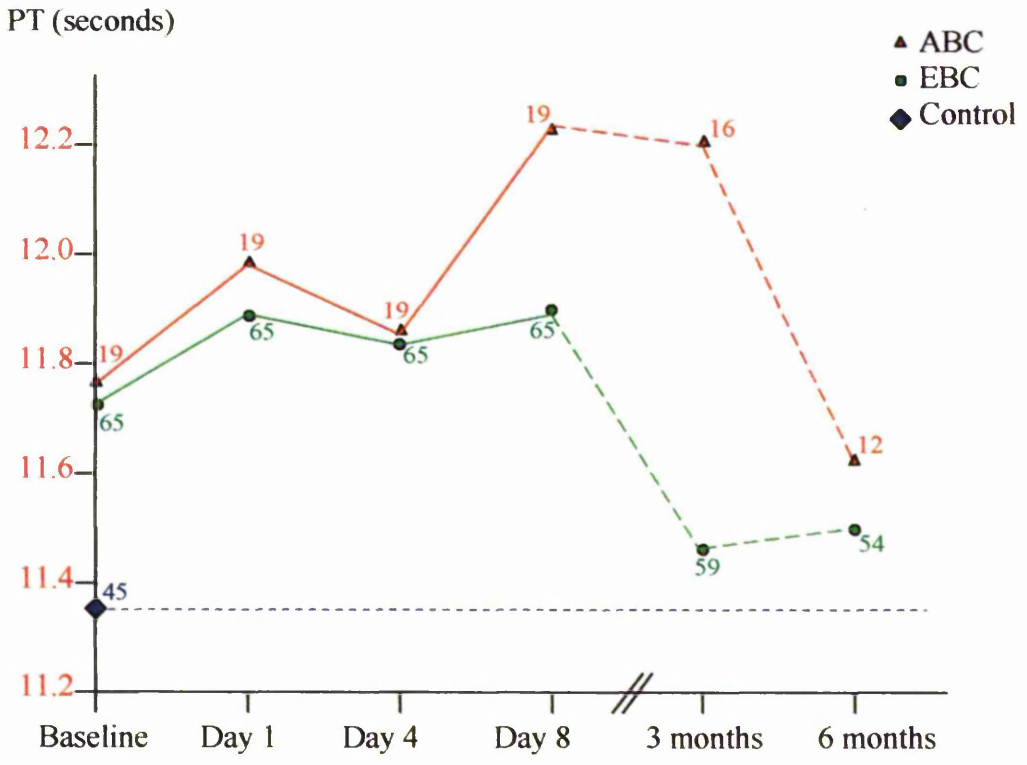

Figure 11: Chemotherapy-induced changes in prothrombin time: Effect of cancer

Mean values. Patient numbers given at each timepoint

The TAT response that occured following chemotherapy (increase at 24 hours followed by decrease, see figure 6) appeared to occur independently of cancer group (p=0.6) (Appendices 13 and 14). There was, however, a significant difference in response between advanced and early cancer demonstrated by PF1+2 levels. A steady increase in circulating PF1+2 was seen in advanced cancer following chemotherapy, that was evident at eight days (p=0.02), but continued with borderline significance at three month (p=0.1) and six months (p=0.1). This was despite an attrition rate of 64% in advanced cancer and 23% in early cancer (Figure 12 and appendices 13 and 14).

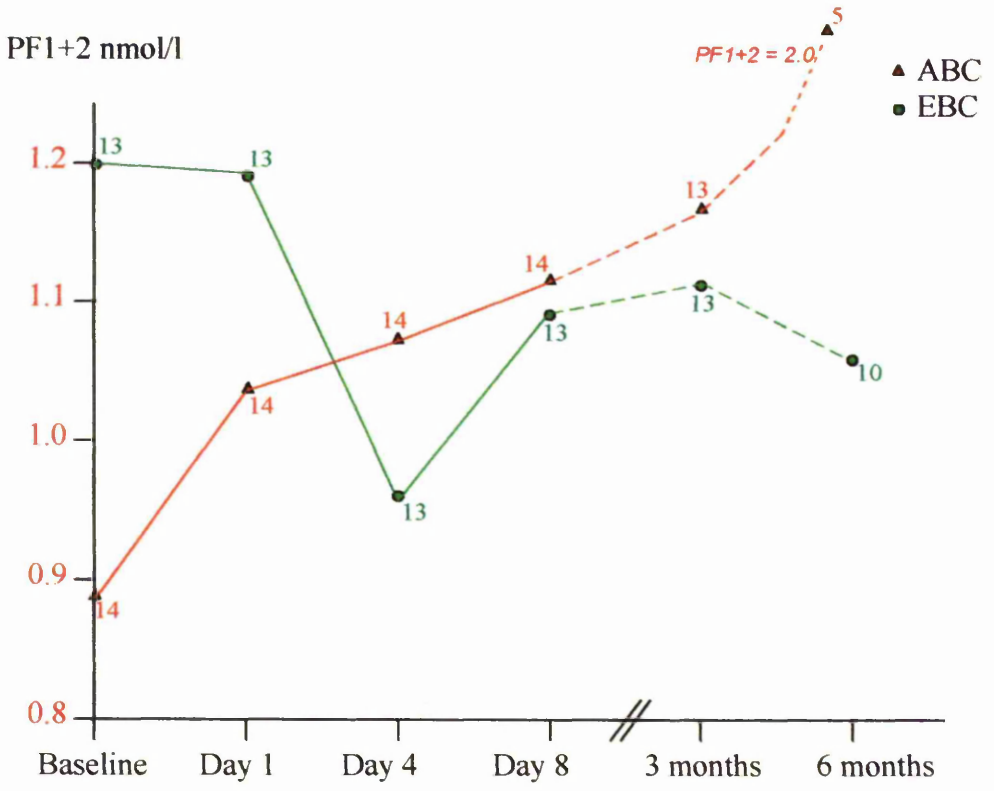

Figure 12: Chemotherapy-induced changes in prothrombin fragments 1 and 2: Effect of cancer

Geometric mean. Patient numbers given at each timepoint

6.3.2 Intravascular fibrin deposition

There was an altered response of circulating fibrinogen levels in advanced compared to early breast cancer patients. The initial decrease in levels was more prolonged in advanced breast cancer, continuing until eight days post-chemotherapy, compared to four days in early breast cancer (p=0.04). The increase demonstrated at three months was significantly more pronounced in early breast cancer patients (p=0.01). By six months the difference in trend was lost (p=0.6). There was, however, an attrition rate of 54% in

advanced breast cancer and 25% in early breast cancer (Figure 13 and appendices 13 and 14).

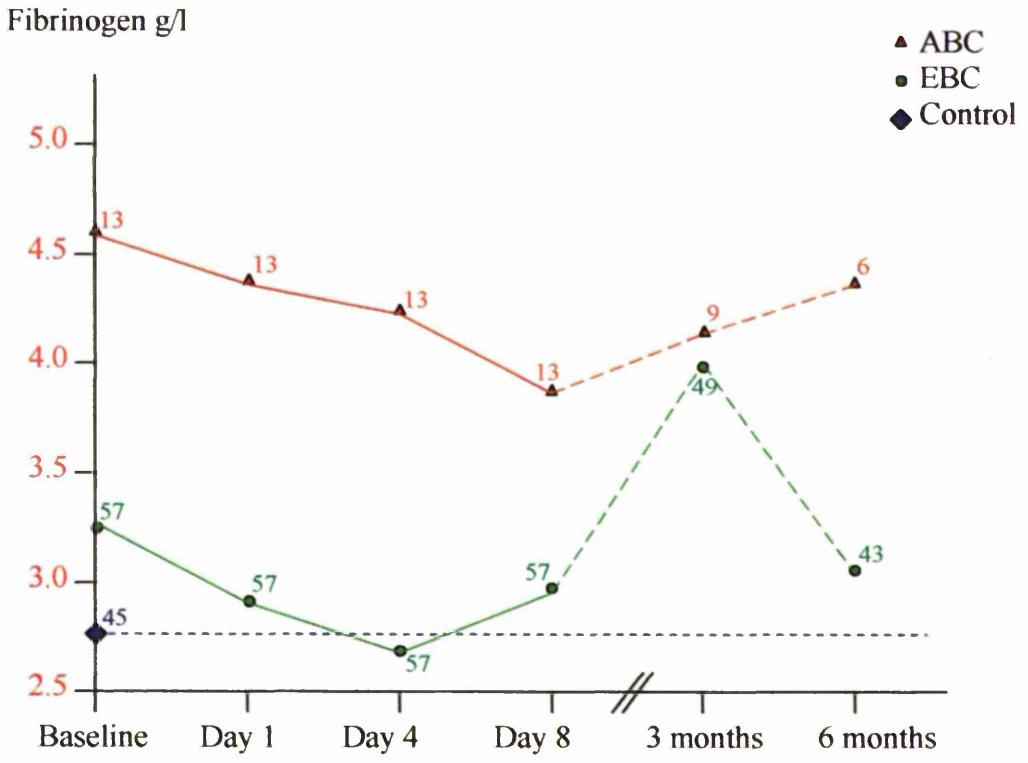

Figure 13: Chemotherapy-induced changes in circulating fibrinogen: Effect of cancer

Mean values. Patient numbers given at each timepoint

There was no difference in the trend of circulating D-dimer levels in advanced compared to early breast cancer patients following chemotherapy (p=0.2) (Appendices 13 and 14).

6.3.3 Fibrinolysis

There was no difference in the alteration of circulating PAI-1 levels in advanced compared to early breast cancer patients following chemotherapy ($p=0.5$).

tPA levels showed a trend for a greater decrease in advanced cancer compared to early breast cancer patients in the eight days following chemotherapy ($p=0.08$), however by three months this difference was no longer apparent. (Appendices 11 and 12)

Circulating uPA levels fell within 24 hours following chemotherapy in early but not advanced breast cancer patients ($p=0.03$). Although uPA appeared to return to a similar level in advanced and early breast cancer patients by six months, the response to chemotherapy at three ($p=0.04$) and six months ($p=0.07$) was different (Figure 14).

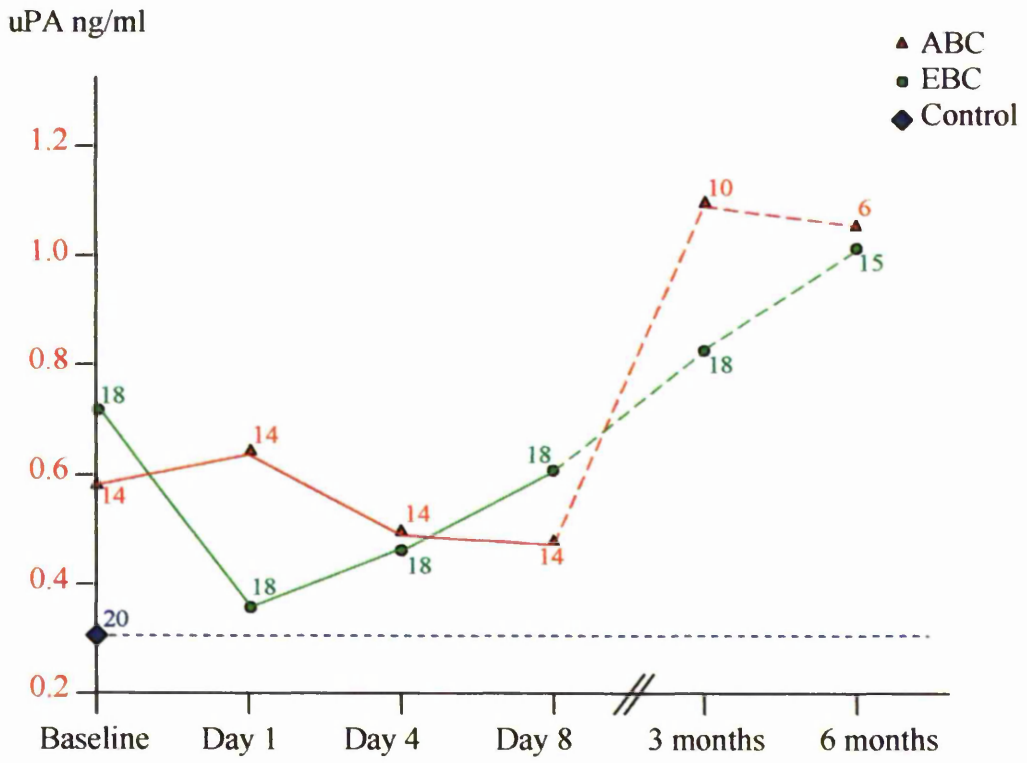

Figure 14: Chemotherapy-induced changes in soluble urokinase (uPA): Effect of cancer.

Geometric mean. Patient numbers given at each timepoint

6.3.4 Platelets

Although platelet count appeared to decrease in both patient groups during the eight days following chemotherapy, this decrease was greater in advanced cancer (p=0.01). The subsequent increase in platelet count seen at three months, and decrease by six months, was more pronounced in advanced cancer (p=0.06 and p=0.007 respectively) (Figure 15, and appendices 13 and 14).

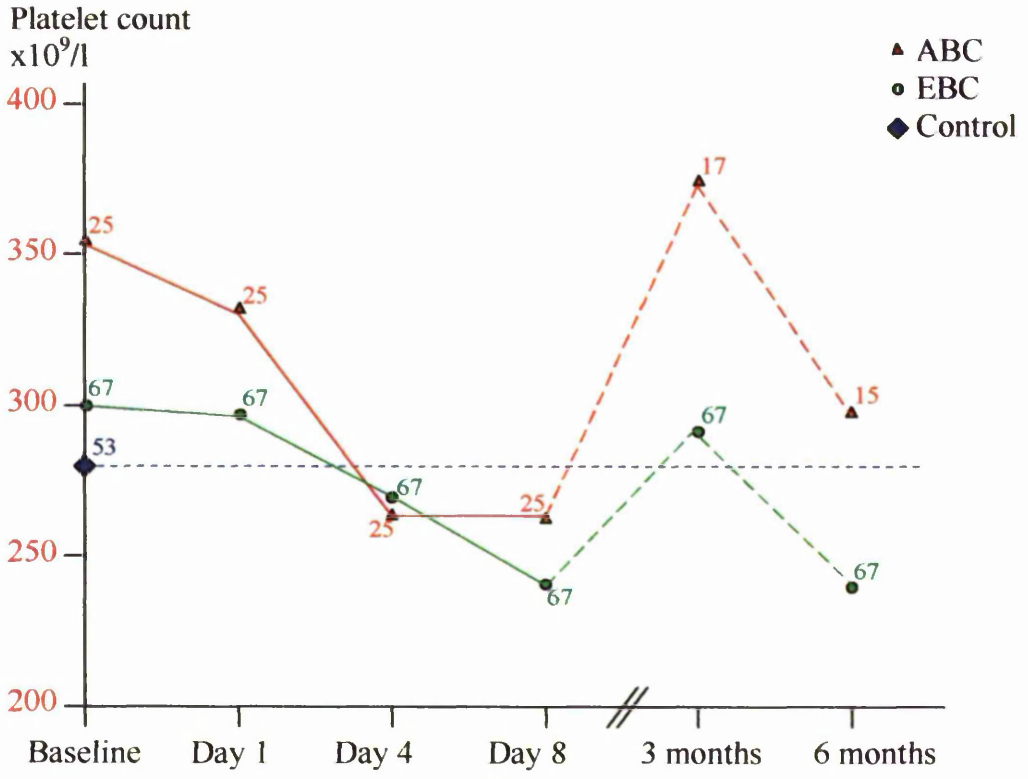

Figure 15: Chemotherapy-induced changes in platelet count: Effect of cancer

Mean values. Patient numbers given at each timepoint

The alteration in platelet release of VEGF following chemotherapy was significantly different in advanced breast cancer compared to early breast cancer patients. Although both groups appeared to show a decline in platelet release of VEGF initially following chemotherapy, this was not significant in advanced cancer even at 24 hours ($p=0.15$), compared to early cancer ($p<0.001$ at day eight). The difference in trend was significant ($p=0.007$). The subsequent apparent increase in platelet VEGF levels in advance cancer up to six months also did not reach significance ($p=0.17$). However the reactive increase in VEGF content of platelet seen at three months in early breast cancer, and the

subsequent return to baseline levels following completion of chemotherapy was significant ($p<0.001$ and $p<0.001$). (Figure 16, and appendices 13 and 14).

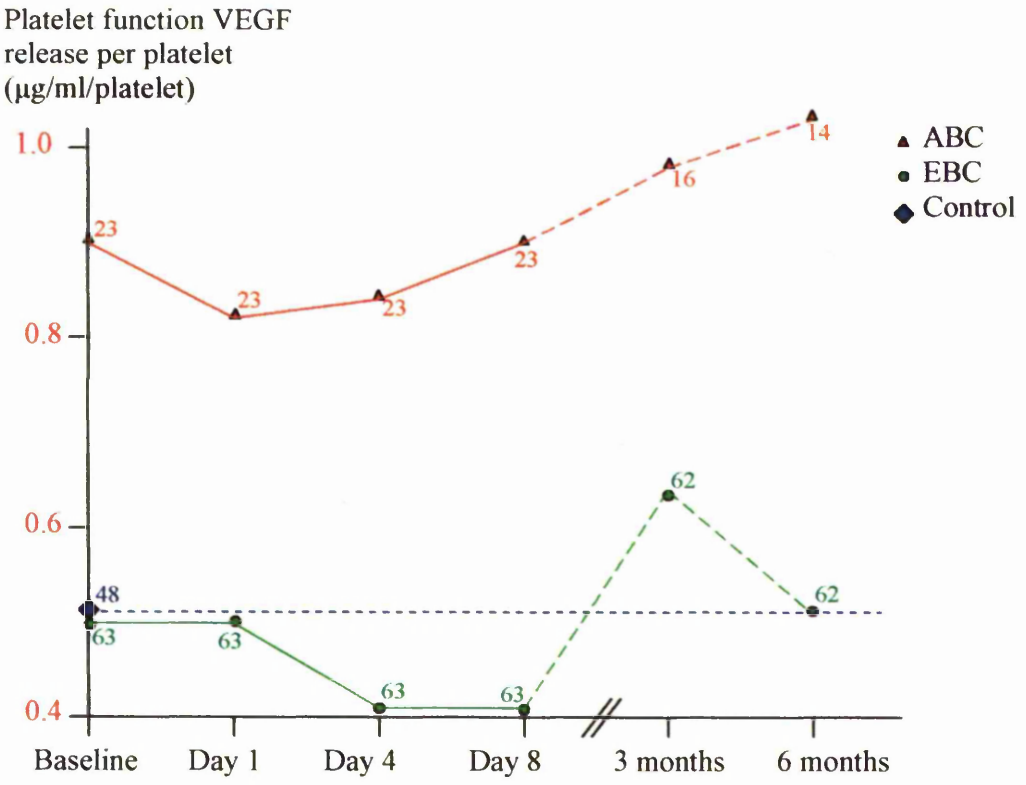

Figure 16: Chemotherapy-induced changes in platelet function: Effect of cancer

Geometric mean. Patient numbers given at each timepoint

6.3.5 Acute phase response

CRP did not demonstrate an altered response to chemotherapy in advanced cancer patients compared to early cancer patients. (Appendices 13 and 14).

6.4 Procoagulants and endothelial adhesion molecules in advanced compared to early breast cancer patients

Aim

To determine whether circulating levels of procoagulants and endothelial adhesion molecules are raised in advanced and early breast cancer patients, prior to chemotherapy, compared to non-cancer controls

Table 22: Procoagulants and endothelial adhesion molecules in advanced, neoadjuvant and early breast cancer patients compared to normal controls

Analysis of the difference between groups (ABC/ EBC/ NBC and controls) used Analysis of Variance (ANOVA) or Kruskal Wallis. Where differences were found, further analysis (between pairs of groups) was performed with Scheffe or Mann-Whitney U tests, with Bonferroni correction.

Procoagulant/ adhesion molecule	*Advanced breast cancer*	*Early breast cancer*	*Neoadjuvant breast cancer*	*Control*	*p ANOVA / Kruskal Wallis (Scheffe/Mann-Whitney+Bonferroni)*
TF µg/ml, median (range) *(n)*	187 (23-644)*† *(36)*	122 (1-1535)* *(85)*	132 (25-634) *(10)*	101 (0-4303)† *(67)*	*0.002 (*0.02, †<0.001)*
CP, mU, geometric mean (CI) *(n)*	28.3 (24.8-32.3)* *(33)*	33.1 (30.7-35.7)*† *(78)*	36.2 (25.2-52.2) *(8)*	28.5 (25.5-31.9)† *(34)*	*0.04 (*0.2, †0.2)*
TSP-1 ng/ml, geometric mean (CI) *(n)*	1095 (831-1442)* *(11)*	610 (408-911) *(32)*	206 (9-4671)* *(3)*	*(0)*	*0.04 (*0.05)*

Procoagulant/ adhesion molecule	*Advanced breast cancer*	*Early breast cancer*	*Neoadjuvant breast cancer*	*Control*	*p ANOVA / Kruskal Wallis (Scheffe/Mann-Whitney+Bonferroni)*
TNF-α µg/ml, geometric mean (CI) *(n)*	3.47 (2.90-4.14) *(22)*	3.19 (2.80-3.64) *(42)*	3.61 (2.36-5.55) *(4)*	3.39 (2.76-4.15) *(19)*	*0.8*
pVEGF µg/ml, geometric mean (CI) *(n)*	22.7 (17.9-29.4)*† *(35)*	14.2 (12.1-16.6)* *(84)*	17.6 (11.1-27.7) *(10)*	15.1 (12.7-17.9)† *(69)*	*0.01 (*0.02, †0.06)*
sVEGF µg/ml, geometric mean (CI) *(n)*	344 (271-437)*†‡ *(36)*	181 (156-210)* *(83)*	160 (92-279)‡ *(10)*	176 (139-222)† *(66)*	*<0.001(*0.001,* †0.001, ‡ 0.07)
VCAM ng/ml, geometric mean (CI) *(n)*	733 (617-870)*† *(36)*	629 (586-674)* *(86)*	547 (462-648)† *(10)*	677 (629-728 *(68)*	*0.05 (*0.2, †0.15)*
E-sel ng/ml, geometric mean (CI) *(n)*	33.0 (26.4-41.2) *(35)*	28.0 (25.3-30.9) *(87)*	31.4 (20.3-48.4) *(10)*	30.1 (26.9-33.7) *(69)*	*0.4*

6.4.1 Procoagulants

Tissue Factor

Prior to chemotherapy, circulating TF was significantly elevated in advanced compared to early breast cancer (p=0.02) and non-cancer controls (p<0.001). Levels in early breast cancer were not significantly elevated compared to non-cancer controls (p=0.2) (Table 22).

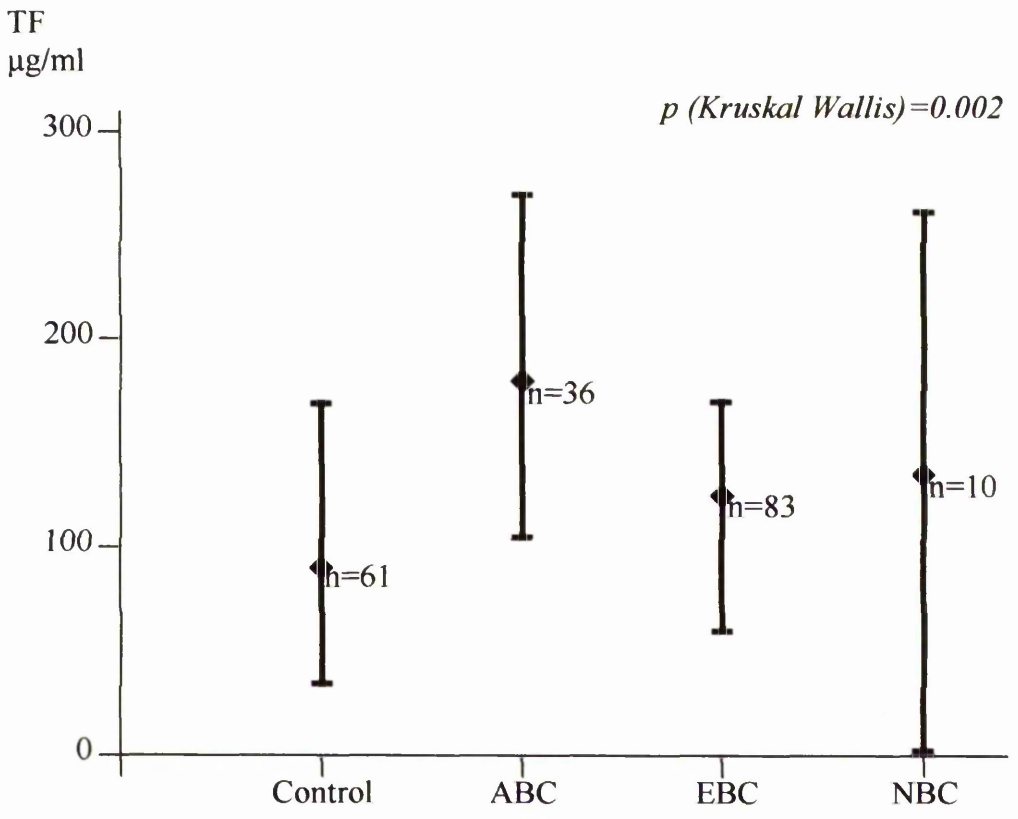

Figure 17: Circulating Tissue Factor levels in advanced, early and neoadjuvant breast cancer, and non-cancer controls.

Median and inter-quartile range shown

Table 23: Molecular markers of intravascular fibrin formation and fibrinolysis correlating with Tissue Factor, prior to chemotherapy

Molecule correlating with tissue factor	*Spearman correlation coefficient (p)(**n**)*
D-dimer	0.23 (p=0.001) (**195**)
Fibrinogen	0.41 (p=0.009) (**143**)
Tissue plasminogen activator	0.36 (p=0.003) (**67**)
Urokinase plasminogen activator	0.42 (p=0.001) (**59**)

TF correlated with intravascular fibrin formation and fibrinolysis (Table 23 and Figure 18).

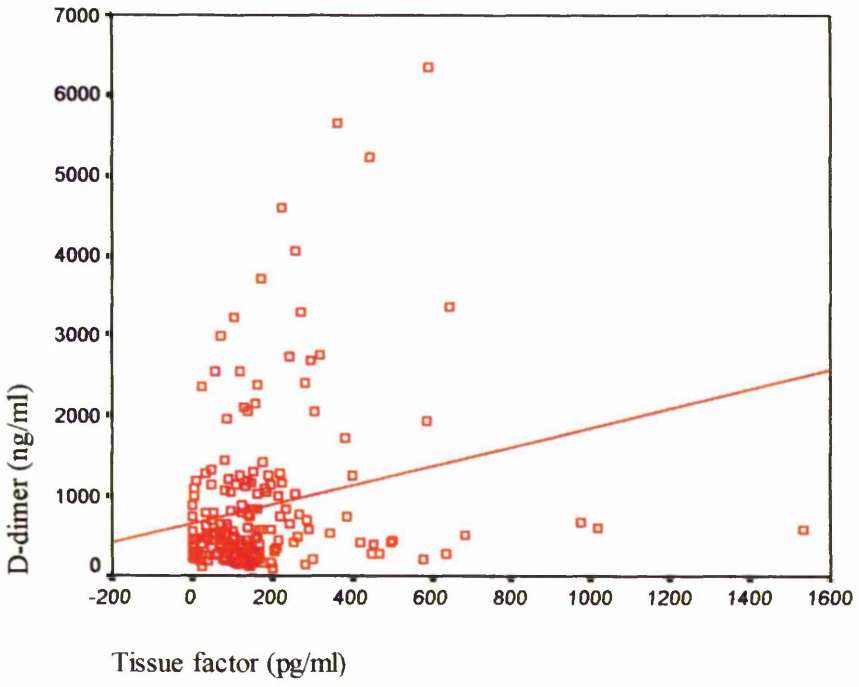

Figure 18: Correlation of Tissue Factor with D-dimer, prior to chemotherapy

Cancer Procoagulant

Circulating CP activity, prior to chemotherapy, was different in the control and cancer groups (p=0.04) (Table 22). When analysed independently, early breast cancer patients demonstrated a trend for increased CP activity compared to both advanced breast cancer patients and non-cancer controls (p=0.2 and 0.2). Although this trend appeared weak, with repeated measures analysis of different timepoints, levels in early breast cancer were significantly increased compared to advanced breast cancer (p<0.001) (Appendices

19 and 20). There was no demonstrable difference in CP activity between advanced breast cancer patients and the non-cancer controls (p=1.0).

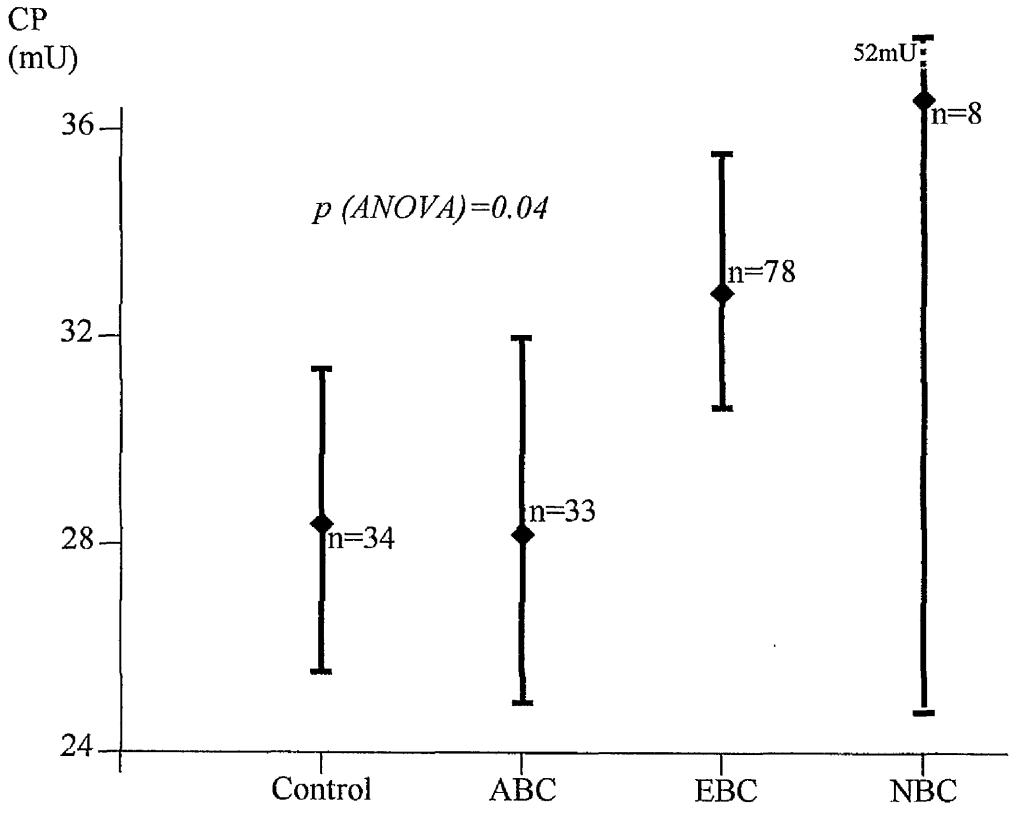

Figure 19: Circulating Cancer Procoagulant levels in advanced, early and neoadjuvant breast cancer, and non-cancer controls.

Geometric mean and 95% confidence intervals (CI) shown.

CP negatively correlated with pVEGF (r^2= -0.25, p=0.002, n=148) (Figure 20).

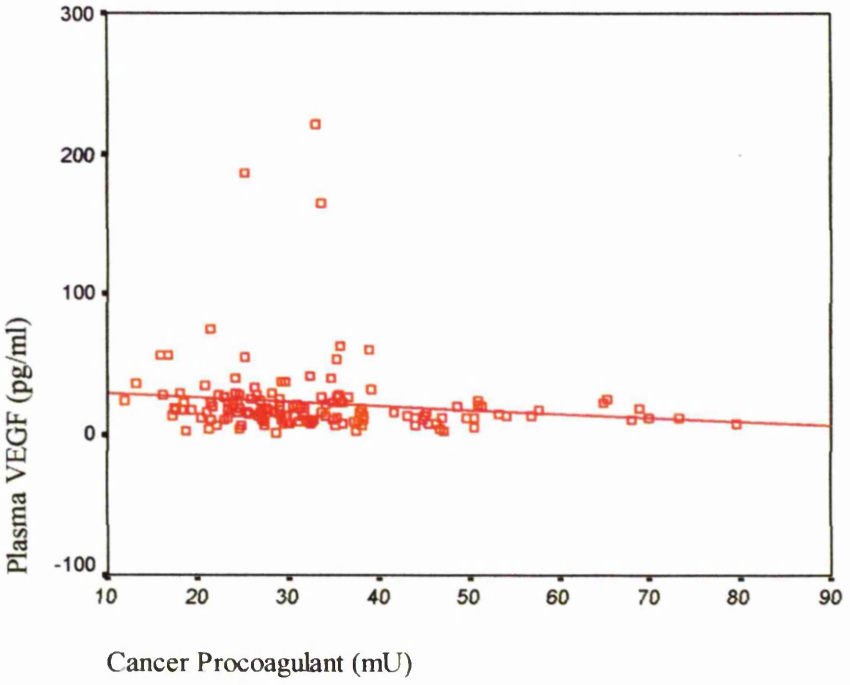

Figure 20: Correlation of Cancer Procoagulant with plasma VEGF prior to chemotherapy

Thrombospondin-1

TSP was initially analysed on EDTA samples. A pilot study of TSP-1 analysis on CTAD samples revealed much lower levels in the later group, implying a more successful inhibition of platelet release of TSP-1 by CTAD as compared to EDTA (geometric mean TSP-EDTA 3464ng/ml (n=34), TSP-CTAD 653ng/ml (n=46)). Subsequent analysis was performed on CTAD samples, the results of which will be presented here.

TSP-1 had a tendency for increased levels in advanced breast cancer compared to neoadjuvant breast cancer patients (p=0.05). Increased levels in advanced breast cancer

patients did not reach significance when compared to early breast cancer patients (p=0.26) (Table 22).

TSP-1 correlated with VCAM-1 (r^2=0.44, p=0.003, n=45) (Figure 21).

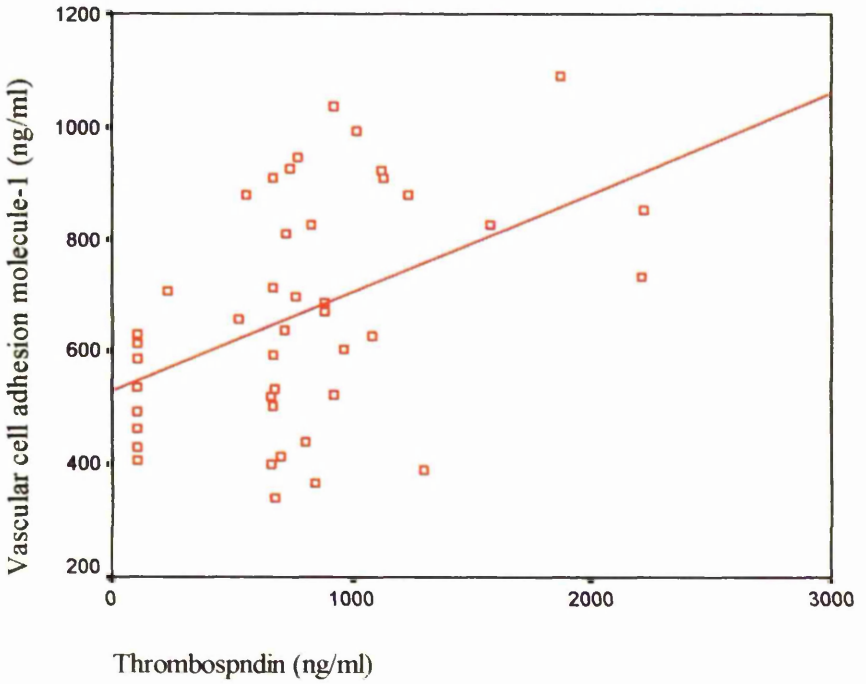

Figure 21: Correlation of circulating Thrombospondin-1 with soluble VCAM-1 prior to chemotherapy

Tumour Necrosis Factor-α

There was no significant difference in baseline TNF-α levels between advanced, early, neoadjuvant breast cancer patients and non-cancer controls (ANOVA p=0.8) (Table 22).

Vascular Endothelial Growth Factor

The different patient groups, and control group showed a significant difference in baseline levels of serum and plasma VEGF (ANOVA, $p<0.001$ and $p=0.01$ respectively). Serum VEGF and pVEGF levels were increased in advanced breast cancer as compared to early breast cancer ($p=0.001$ and $p=0.02$ respectively) and non-cancer controls ($p=0.001$ and $p=0.06$). Serum, but not plasma, VEGF in advanced cancer showed a trend for increased levels compared to neoadjuvant breast cancer patients ($p=0.07$ and $p=0.8$ respectively) (Table 22).

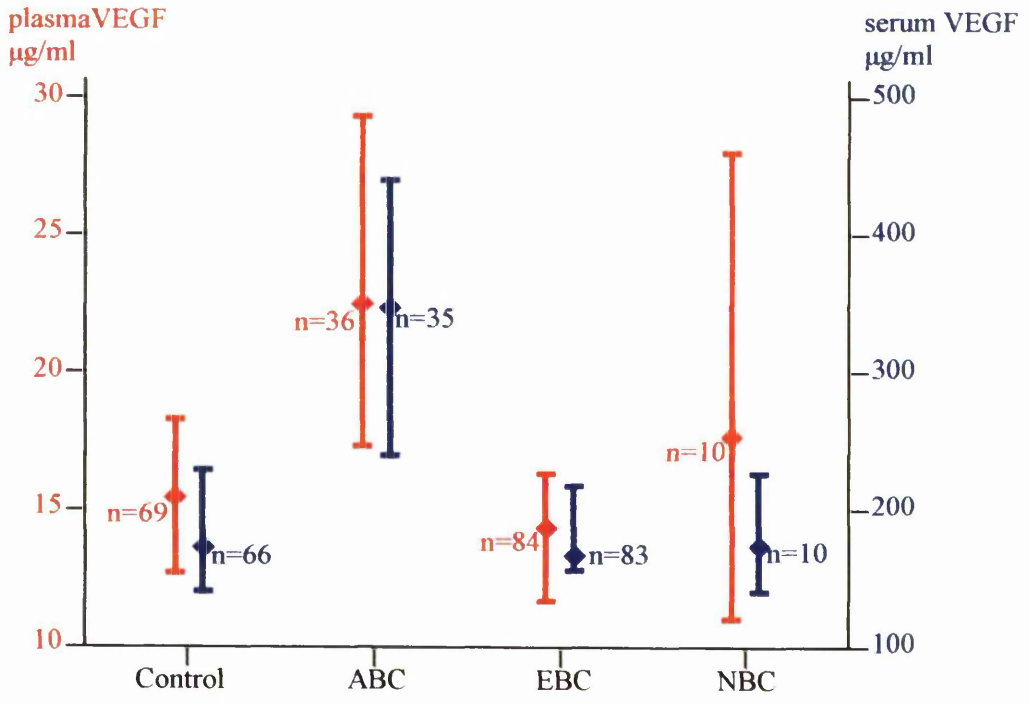

Figure 22: Serum and plasma VEGF levels in advanced, early and neoadjuvant breast cancer, and non-cancer controls

Geometric mean and 95% confidence intervals (CI) shown.

Both pVEGF and sVEGF correlate with platelet count (r^2= 0.15 and 0.30, n=181 and 179, p=0.04 and <0.001 respectively) (Figure 23).

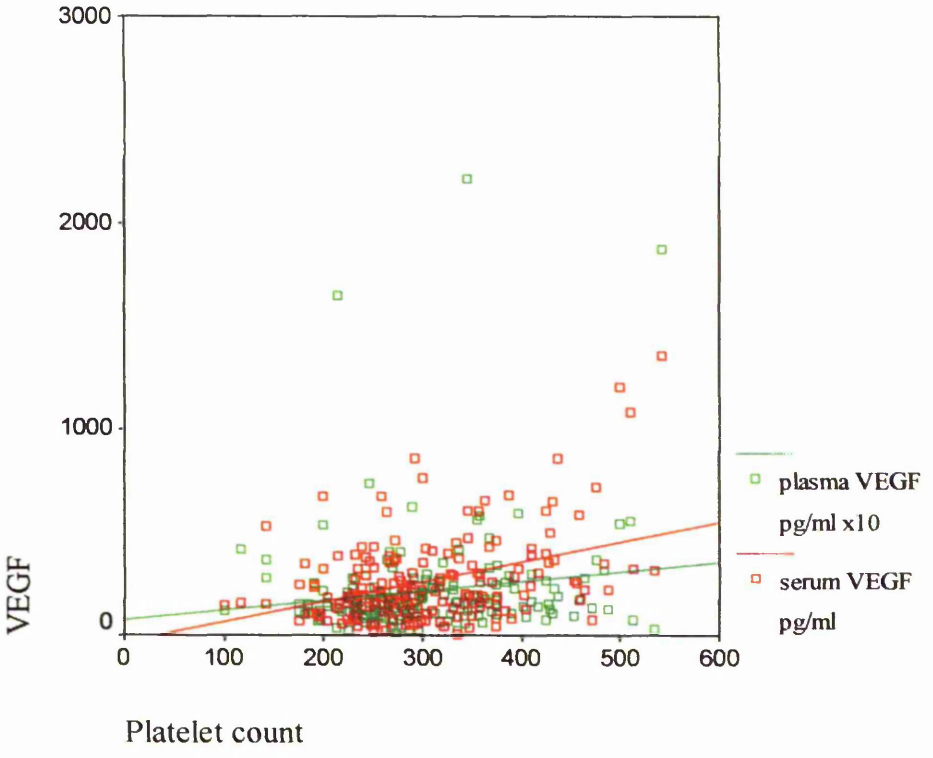

Figure 23: Correlation of serum and plasma VEGF with platelet count prior to chemotherapy

Plasma VEGF concentrations multiplied by 10 (pg/ml x 10)

Table 24: Correlation of serum and plasma VEGF with markers of hypercoagulability and intravascular fibrin formation

VEGF	*Molecule correlating with VEGF*	*Spearman correlation coefficient (p)(**n**)*
sVEGF	Thrombin-antithrombin	0.37 (p=0.02) (**39**)
sVEGF	D-dimer	0.23 (p=0.001) (**196**)
sVEGF	Fibrinogen	0.25 (p=0.003) (**143**)
pVEGF	Thrombin-antithrombin	0.40 (p=0.01) (**41**)
pVEGF	D-dimer	0.24 (p=0.002) (**196**)

Serum and plasma VEGF correlated with markers of hypercoagulability and intravascular fibrin formation (Table 24 and Figure 24).

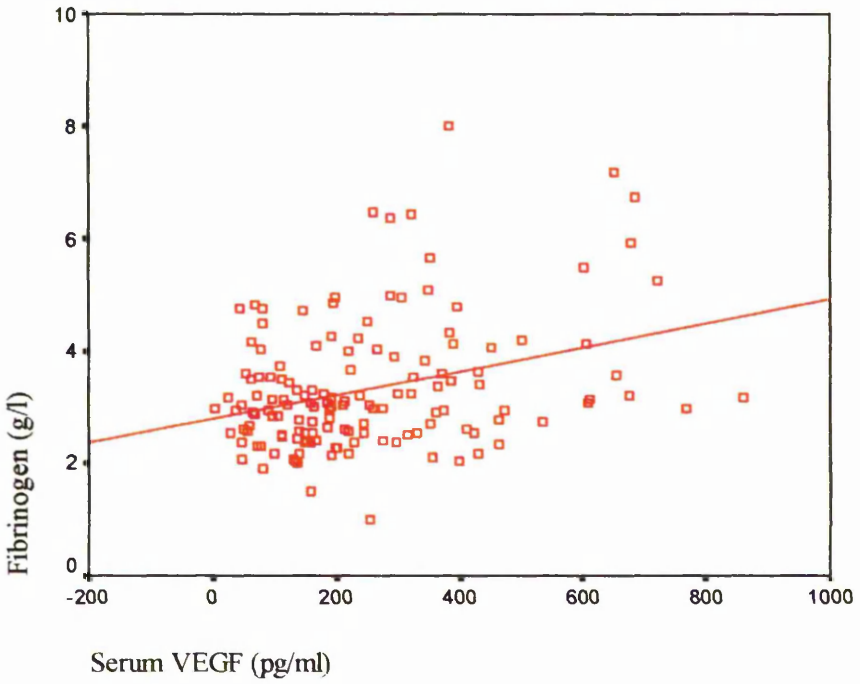

Figure 24: Correlation of serum VEGF with fibrinogen, prior to chemotherapy

6.4.2 Endothelial adhesion molecules

Circulating VCAM-1, but not E-selectin, was altered in the different cancer and the non-cancer groups (p=0.05 and 0.4 respectively). However on paired comparison of the different groups, the difference in VCAM-1 was weak. In advanced breast cancer, VCAM-1 levels prior to chemotherapy showed a possible increase compared to early and neoadjuvant breast cancer but not non-cancer controls (Table 22).

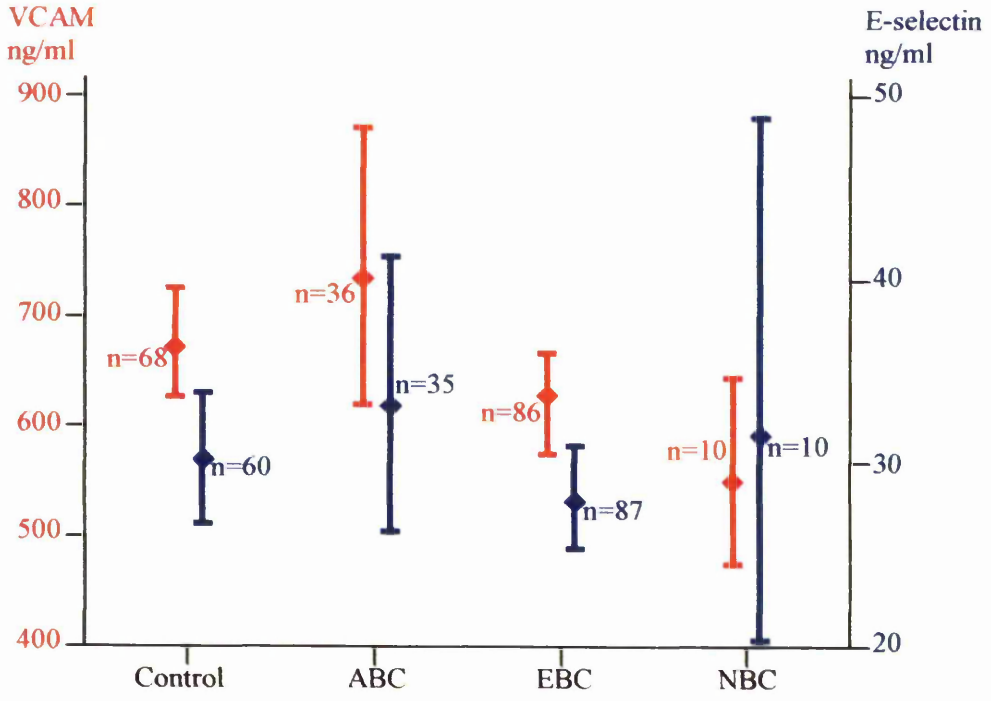

Figure 25: Levels of circulating endothelial adhesion molecules levels in advanced, early and neoadjuvant breast cancer, and non-cancer controls

Geometric mean and 95% confidence intervals (CI) shown.

6.4.3 Influence of time from surgery on procoagulants and markers of endothelial cell activation

Table 25: Correlation of markers of coagulation with number of days since surgery (early breast cancer patients only)

Prcoagulant / endothelial adhesion molecule	*Spearman correlation coefficient (p)(**n**)*
TF	-0.12 (0.3) (**66**)
CP	0.02 (0.8) (**60**)
TSP-1	0.02 (0.9) (**26**)
TNF-α	0.43 (0.01) (**32**)
pVEGF	-0.01 (0.7) (**66**)
sVEGF	-0.23 (0.06) (**67**)
VCAM-1	-0.08 (0.5) (**62**)
E-selectin	0.27 (0.02) (**68**)

Circulating levels of TNF-α and E-selectin correlated with the number of days since recent surgery (within six weeks) in early breast cancer patients (Table 25 and Figure 26).

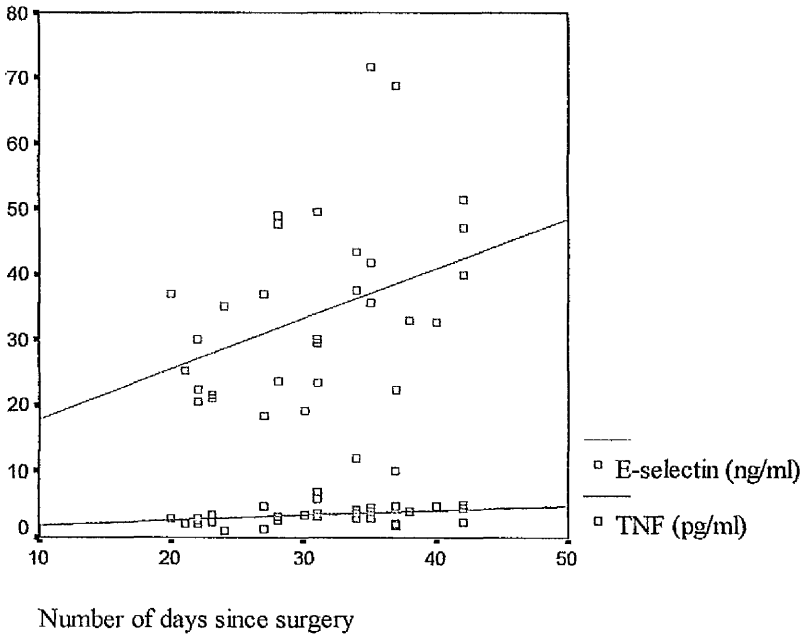

Figure 26: Correlation of circulating E-selectin and TNF-α with number of days since surgery in early breast cancer

6.4.4 Influence of age and menopausal status on procoagulants and markers of endothelial cell activation

TF, CP and VCAM correlate with age, with increased levels of TF and CP being seen in post menopausal women compared to pre-menopausal women (Table 26).

Table 26: Association between age, menopausal status and procoagulants and endothelial adhesion molecules

Levels in pre- and postmenopausal patients compared using an Independent T-test

Procoagulant/ adhesion molecule	*Pre menopause*	*Post menopause*	*p*	*Correlation with age-p (Spearman coefficient) (n)*
TF µg/ml, geometric mean (CI) *(n)*	65.1 (45.1-93.9) *(95)*	118.4 (91.4-153.2) *(103)*	*0.008*	0.005 (0.20) *(198)*
CP mU, geometric mean (CI) *(n)*	29.0 (26.9-31.3) *(69)*	32.9 (30.4-35.6) *(84)*	*0.03*	0.03 (0.18) *(153)*
TSP ng/ml, geometric mean (CI) *(n)*	478 (238-959) *(15)*	760 (541-1070) *(31)*	*0.2*	0.4 (-0.13) *(46)*
TNF-α µg/ml, geometric mean (CI) *(n)*	3.43 (2.95-3.98) *(35)*	3.25 (2.92-3.62 *(52)*	*0.6*	0.4 (-0.09) *(87)*
pVEGF µg/ml, geometric mean (CI) *(n)*	15.0 (12.9-17.5) *(97)*	16.9 (14.7-19.5) *(102)*	*0.2*	0.4 (0.06) *(199)*
sVEGF µg/ml, geometric mean (CI) *(n)*	178 (151-209) *(95)*	223 (188-263) *(102)*	*0.06*	0.1 (0.11) *(197)*
VCAM ng/ml, geometric mean (CI) *(n)*	650 (606-715) *(96)*	665 (619-715) *(104)*	*0.7*	0.04 *(200)* (0.15)
E-sel ng/ml, geometric mean (CI) *(n)*	30.9 (28.0-34.1) *(98)*	28.6 (25.8-31.7) *(103)*	*0.3*	0.5 (-0.05) *(201)*

6.4.5 Association between hormone receptor status and procoagulants and endothelial adhesion molecules

TSP-1 was significantly elevated in oestrogen receptor negative compared to oestrogen receptor positive breast cancer patients (geometric mean 1220ng/ml and 497ng/ml, n=14 and 32 respectively, p=0.007). VCAM-1 was significantly elevated in Her 2 neu receptor positive compared to Her 2 neu receptor negative breast cancer patients (geometric mean 741ng/ml and 617ng/ml, n=30 and 50 respectively, p=0.04). Corrected for multiple tests, a p value cut-off for significance of 0.01 would be more appropriate. No other markers correlated with oestrogen, progesterone or Her 2 neu receptor status (Appendices 15,16 and 17).

6.4.6 Relationship of procoagulants and endothelial adhesion molecules to tumour stage in early breast cancer

In early breast cancer, procoagulant molecules and endothelial adhesion molecules did not correlate with tumour size, or Nottingham Prognostic Index (Appendix 18).

6.5 Results: The effect of chemotherapy on circulating procoagulants and endothelial adhesion molecules

6.5.1 Procoagulants

Tissue Factor

There was no early alteration in circulating TF levels (within eight days), however by six months a trend for increased TF could be seen (p=0.06) (Figure 28 and appendix 19).

Cancer Procoagulant

CP rose rapidly following chemotherapy, but returned towards baseline by eight days. Levels decreased further by three months but appeared to return towards baseline by six months. This trend was significant (p=0.002). (Figure 27 and appendix 19).

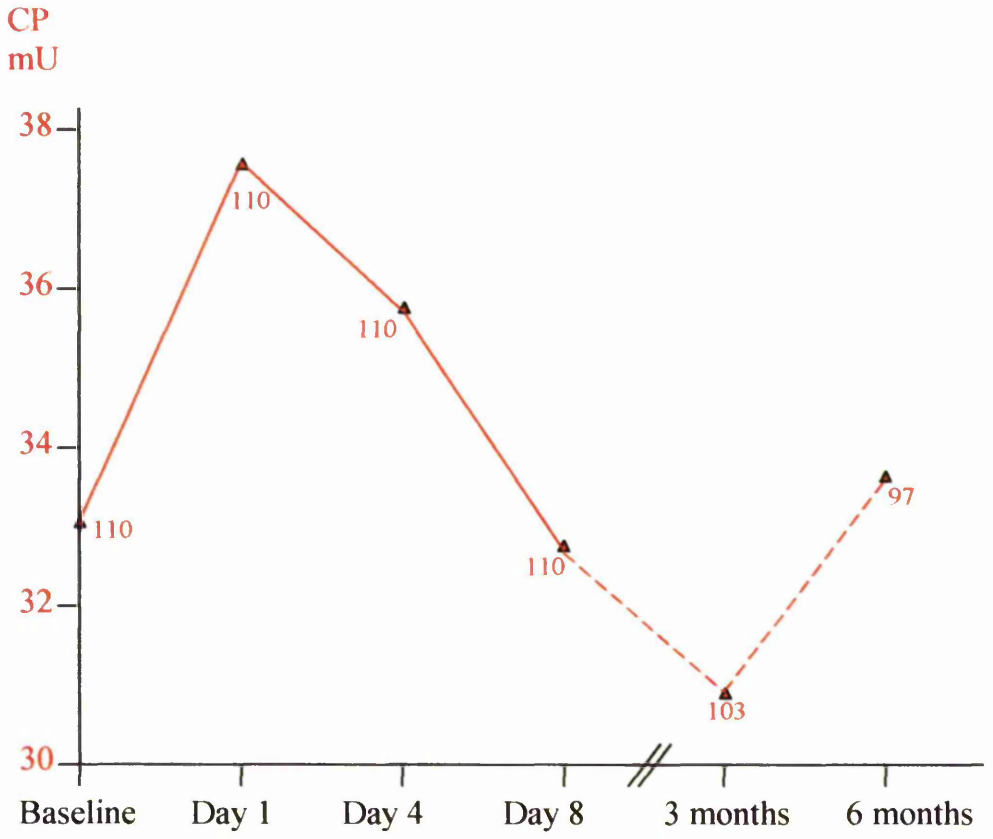

Figure 27: Circulating cancer procoagulant levels: Effect of chemotherapy

Geometric mean. Patient numbers given at each timepoint

Thrombospondin-1

There was no alteration in circulating TSP-1 levels in response to chemotherapy (p=0.5) (Appendix 19).

Tumour Necrosis Factor-α

Chemotherapy caused an initial decrease in circulating TNF-α levels (p=0.02) in the first eight days. By three months, levels showed a reactive increase (p<0.001). By six months the trend was for a return to baseline (p=0.002). (Figure 28 and appendix 19).

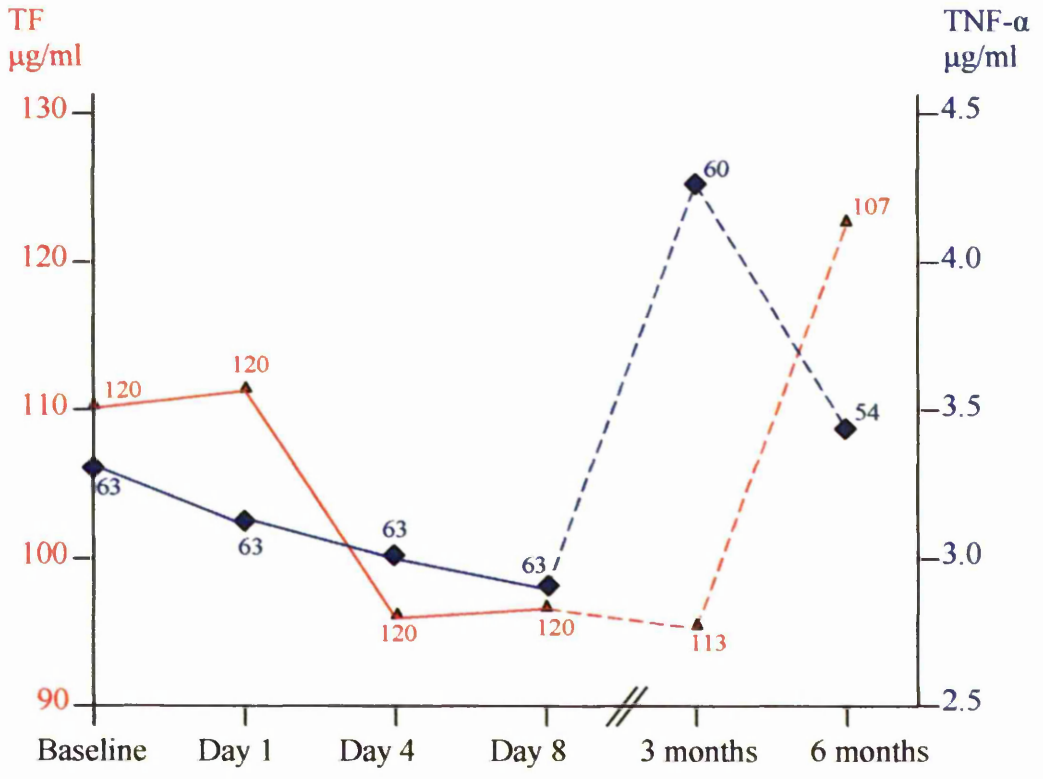

Figure 28: Circulating levels of Tissue Factor and Tumour Necrosis Factor-α: Effect of chemotherapy

Geometric mean. Patient numbers given at each timepoint

Vascular Endothelial Growth Factor

Serum VEGF decreased during the eight days following chemotherapy, however plasma VEGF increased during the same time period. Both trends were significant (p<0.001). By three months, levels of both pVEGF and sVEGF showed a trend for increase (p<0.001

and p<0.001 respectively). By six months the levels of both pVEGF and sVEGF were decreasing (p<0.001). (Figure 29 and appendix 19).

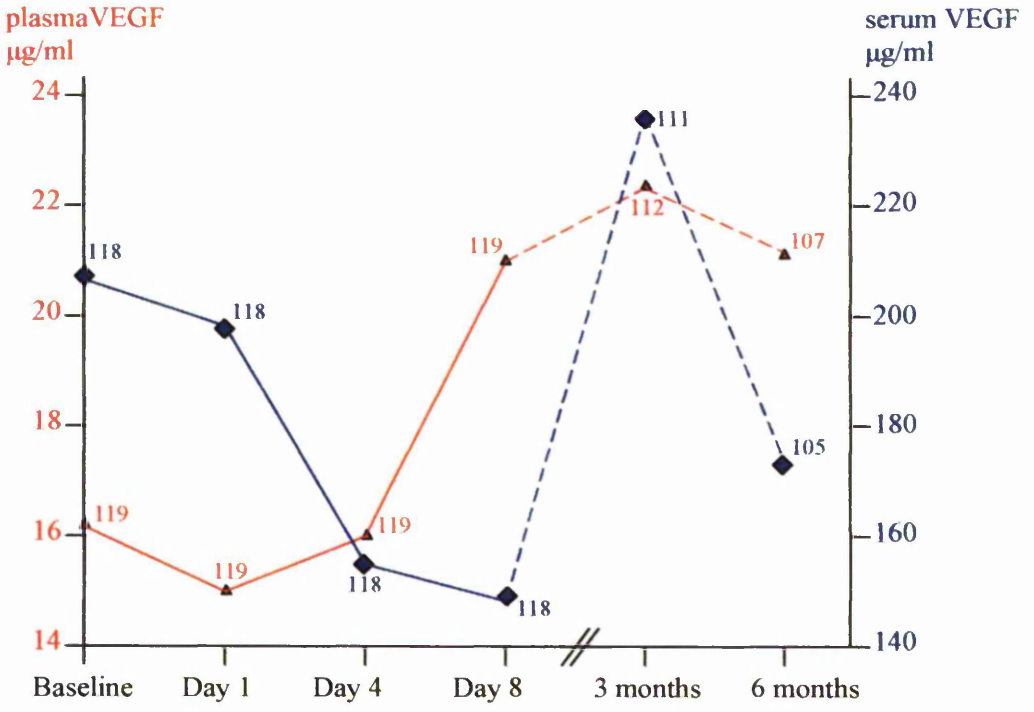

Figure 29: Circulating levels of plasma and serum VEGF: Effect of chemotherapy

Geometric mean. Patient numbers given at each timepoint

6.5.2 Endothelial Adhesion Molecules

Both VCAM-1 and E-selectin showed a trend for decrease in the eight days following chemotherapy (p<0.001). At three and six months following commencement of chemotherapy, levels of both VCAM and E-sel had steadily increased (p<0.001). (Figure 30 and appendix 19).

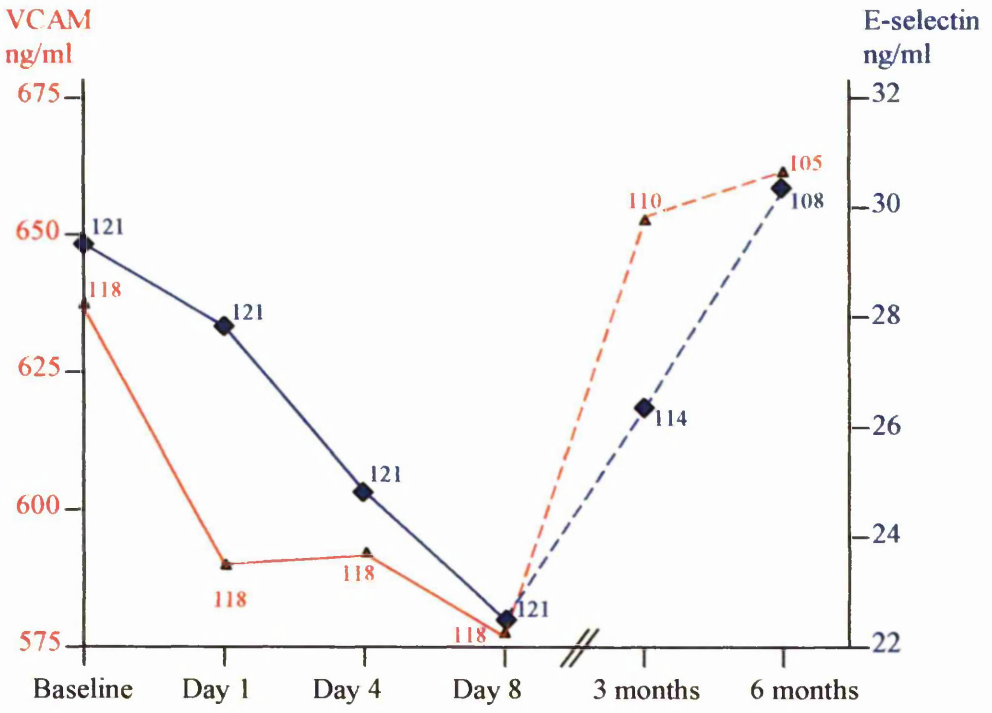

Figure 30: Circulating levels of endothelial adhesion molecules: Effect of chemotherapy

Geometric mean. Patient numbers given at each timepoint

6.6 Results: The influence of cancer on the response of procoagulants and endothelial adhesion molecules to chemotherapy

6.6.1 Procoagulants

Tissue Factor and Thrombospondin-1

There was no difference in the response to chemotherapy of circulating TF or TSP-1 levels in advanced compared to early breast cancer patients (p=0.7 and 0.6 respectively) (Appendices 20 and 21).

Cancer Procoagulant

In the first three months following commencement of chemotherapy, there was no difference in the pattern of change of circulating CP in advanced compared to early breast cancer patients. By six months, a trend for decreasing levels in advanced breast cancer was apparent, which was different from the relatively steady levels seen in early breast cancer (p=0.02). The decrease, from baseline to six months, in circulating CP in advanced compared to early breast cancer was significant (<0.001) (Figure 31 and appendices 20 and 21).

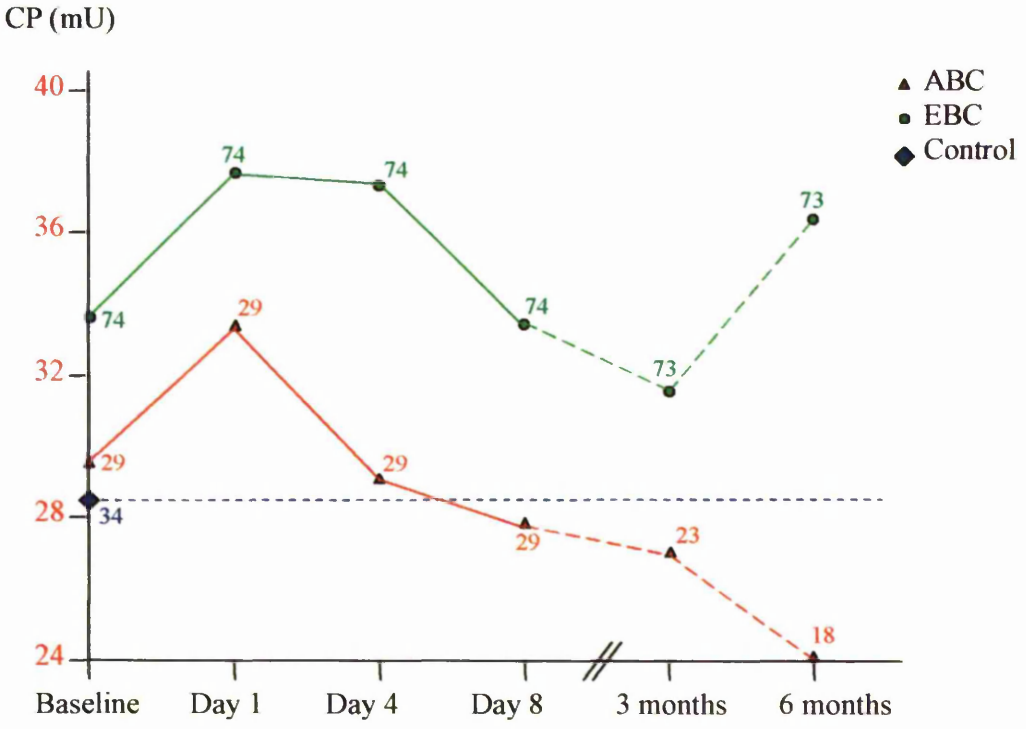

Figure 31: Chemotherapy-induced changes in cancer procoagulant: Effect of cancer

Geometric mean. Patient numbers given at each timepoint

CP in early breast cancer remained significantly elevated, compared to controls, at three and six months following commencement of chemotherapy ($p=0.03$ and $p<0.001$ respectively) (Appendices 20 and 21).

Tumour Necrosis Factor-α

There was a difference in the response to chemotherapy of circulating TNF-α levels in advanced compared to early breast cancer patients. Although both show an overall trend for decreasing levels in the first eight days, in advanced cancer this was preceded by an initial increase in circulating TNF-α in response to chemotherapy at 24 hours ($p=0.02$). This resulted in differing trends between the two cancer groups in the first eight days ($p=0.06$). By three and six months, these differences in response to chemotherapy had resolved ($p=0.2$ and 0.6 respectively) (Figure 32 and appendices 20 and 21).

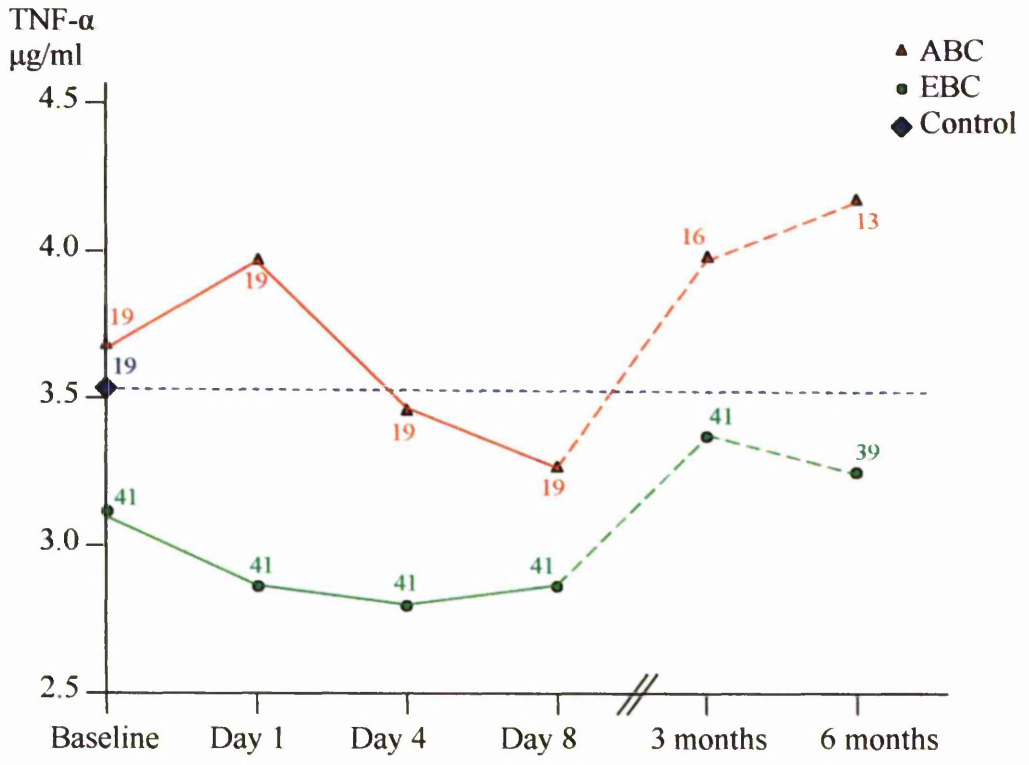

Figure 32: Chemotherapy-induced changes in TNF-α: Effect of cancer

Geometric mean. Patient numbers given at each timepoint

Vascular Endothelial Growth Factor

A differing pattern of change was observed in serum VEGF between advanced and early breast cancer patients which was not reflected in plasma VEGF. The difference was apparent in the first eight days following chemotherapy, where sVEGF levels in advanced cancer showed a greater decrease, from baseline, at 24 hours ($p=0.004$) and four days ($p=0.002$) compared to early breast cancer. The difference between the trends seen in response to chemotherapy in advanced and early breast cancer patients was no longer apparent by three and six months ($p=0.2$ and 0.2 respectively).

The rapid decrease in sVEGF levels, in advanced breast cancer, as an early response to chemotherapy was not mirrored by pVEGF levels. (Figure 33 and appendices 20 and 21).

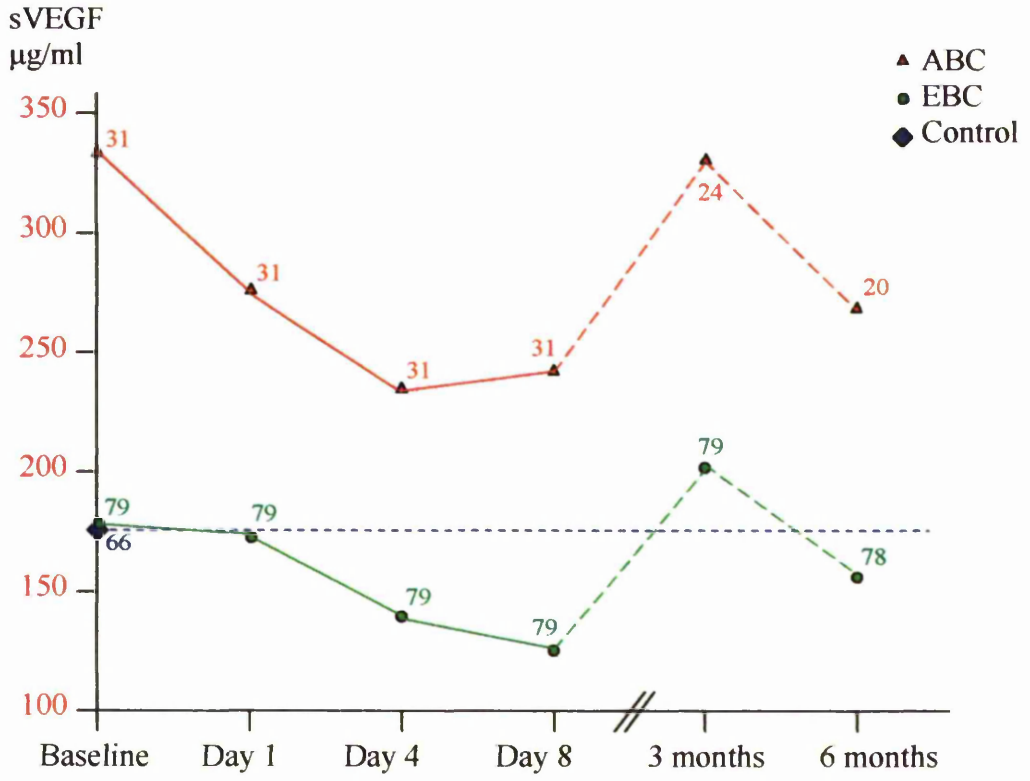

Figure 33: Chemotherapy-induced changes in serum VEGF: Effect of cancer

Geometric mean. Patient numbers given at each timepoint

6.6.2 Endothelial Adhesion Molecules

Both VCAM and E-selectin demonstrated different trends following chemotherapy administration in advanced compared to early breast cancer. Levels of soluble VCAM-1 in both advanced and early breast cancer showed a similar reduction up to day eight ($p=0.2$), and an increase by three months ($p=0.07$). At six months, levels in advanced

breast cancer had continued to increase however the opposite was true for early breast cancer (p=0.01) (Figure 34 and appendices 20 and 21).

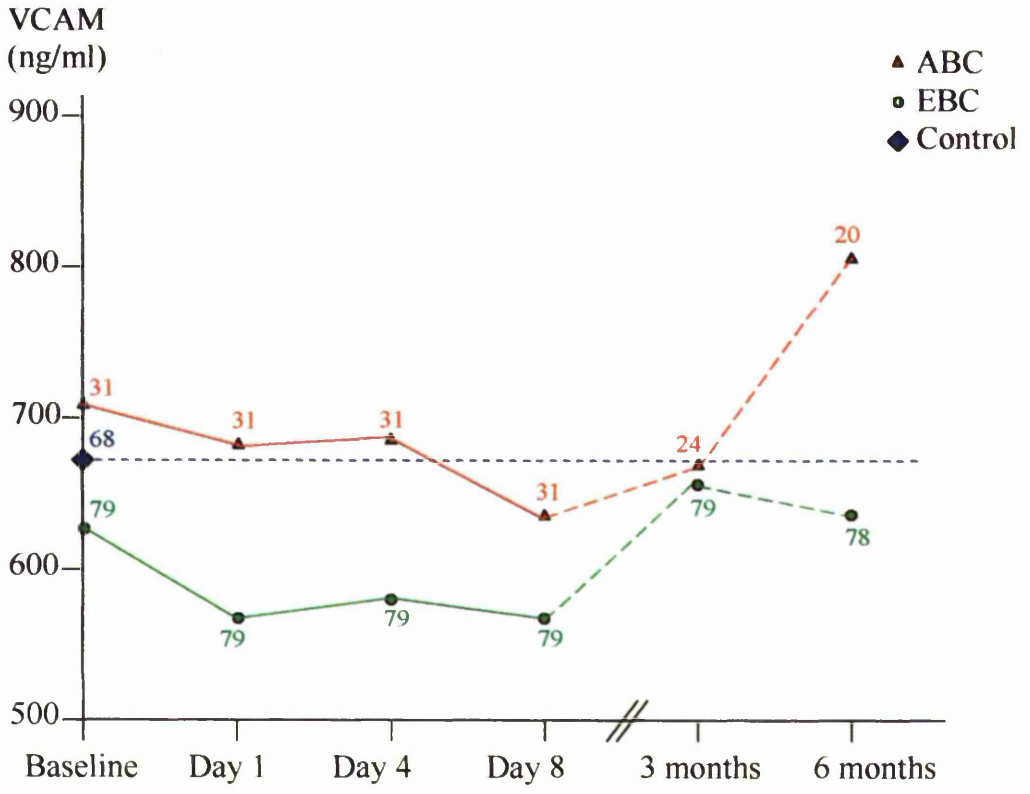

Figure 34: Effect of cancer on chemotherapy-induced changes in soluble Vascular Cell Adhesion Molecule-1

Geometric mean. Patient numbers given at each timepoint

Circulating E-selectin levels also decreased in the eight days following chemotherapy, however this trend was delayed and exaggerated in advanced breast cancer ($p<0.001$). The change in circulating E-selectin levels from baseline to 24 hours and four days was significantly different in advanced and early breast cancer patients ($p=0.01$ and $p<0.001$ respectively). At three and six months, levels in both groups were increased and the

difference in trend was no longer apparent (p=0.09 and 0.2 respectively) (Figure 35 and appendices 20 and 21).

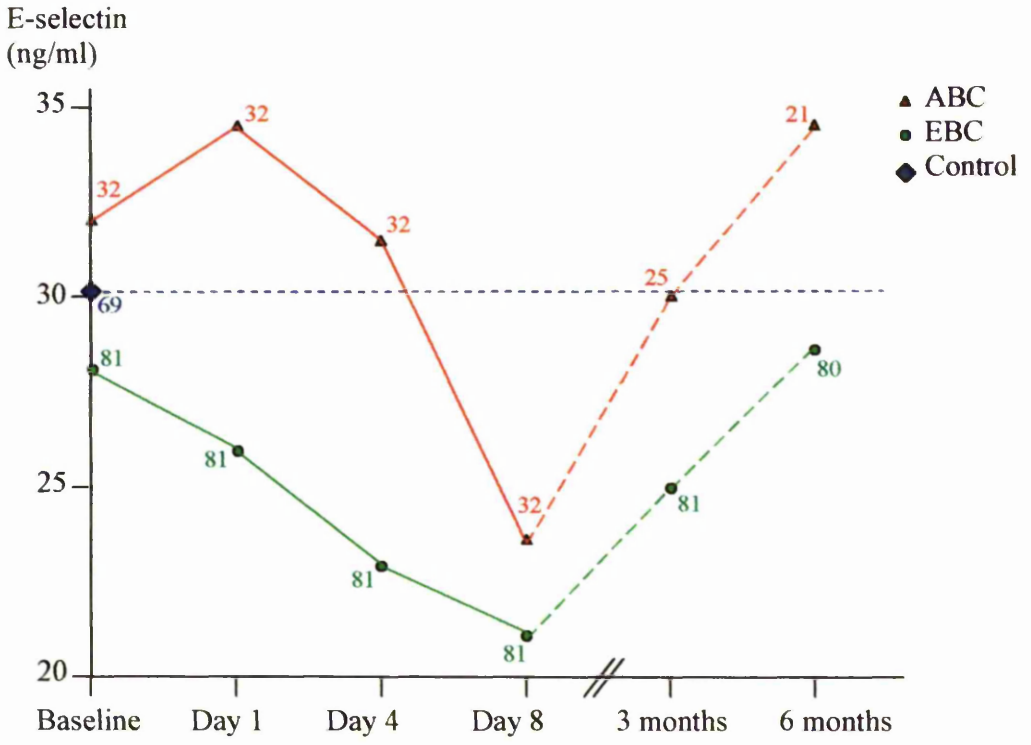

Figure 35: Chemotherapy-induced changes soluble E-selectin: Effect of cancer

Geometric mean. Patient numbers given at each timepoint

6.7 Discussion: The influence of cancer and chemotherapy on haemostatic, platelet and acute phase responses

6.7.1 Haemostatic markers in breast cancer and normal controls

The increase in PT and APTT in breast cancer patients (advanced and early) in this study paradoxically suggests a *prolongation* of clotting times prior to chemotherapy, compared to non-cancer controls. This has been reported previously (Abbasciano *et al.* 1995; Canobbio *et al.* 1986; Di Micco *et al.* 2001; Mielicki *et al.* 1999; Unsal *et al.* 2004).

PF1+2 is widely recognised to be increased in cancer (Di Micco *et al.* 2001;Kakkar *et al.* 1995; Roselli *et al.* 2003), however in this pilot group subset, a trend for decreased circulating levels in advanced cancer compared to early cancer was observed.

The elevated pre-chemotherapy TAT levels in advanced breast cancer patients in this study support previous findings in breast (Donati & Falanga 2001; Falanga *et al.* 1998), lung (Gabazza *et al.* 1992; Seitz *et al.* 1997) and colorectal cancer (Iversen, Okholm, & Thorlacius-Ussing 1996; Iversen & Thorlacius-Ussing 2002). Although TAT was elevated in early breast cancer patients compared to controls in this current study, as in Ozyilkan's study, this increase was not significant (Ozyilkan *et al.* 1998).

The raised fibrinogen and D-dimer levels in patients with breast cancer, particularly advanced breast cancer, observed in this study is supported by others (Blackwell *et al.* 2000; Blann *et al.* 2001; Miller & Heilmann 1988; Oberhoff *et al.* 2000).

The correlation of D-dimer with Nottingham Prognostic Index and tumour size in early breast cancer supports Blackwells conclusion, from a study of 140 patients undergoing breast surgery, that plasma D-dimer correlates with clinical stage and lymph node

involvement (Blackwell *et al.* 2000). The fact that D-dimer still acts as a staging marker after removal of the tumour may reflect the slow correction of haemostatic abnormalities following surgery. This is supported by Oberhoff, who reports mean D-dimer levels ten days after breast cancer surgery at over twice that of pre-surgery levels, with a steady increase in that mean since surgery. Unfortunately his study did not measure D-dimer after ten days, to assess the endurance of this result (Oberhoff *et al.* 2000). The results of this current study that D-dimer levels negatively correlate with number of days since surgery, for up to six weeks, demonstrates that surgical induced haemostatic alterations are more prolonged than is generally recognised.

Although the majority of studies to date have investigated tumour tissue expression of plasminogen activators, several papers support the finding of increased circulating tPA in cancer. Casslen reports increased circulating tPA, uPA and PAI-1 in ovarian cancer compared to healthy controls (Casslen *et al.* 1994). Di Micco found increased tPA in gastric cancer patients compared to controls (Di Micco *et al.* 2001), and Oberhoff found similar results in breast cancer patients (Oberhoff *et al.* 2000). Although no previous relationship between cancer stage and plasma tPA has been identified, there is a trend in this current study for increased tPA in advanced cancer compare to early breast cancer, despite limited numbers. The use of plasma tPA as a screening test for cancer recurrence has already shown potential, with a sensitivity of 79% and specificity of 61% in rectal cancer (Barillari *et al.* 1992).

Increased tumour uPA expression is associated with poor prognosis in primary breast cancer. Paradoxically, high tissue concentrations of PAI-1, an endogenous inhibitor of uPA also correlate with poor prognosis in patients with breast cancer. Although elevated

plasma uPA has been reported in pancreatic and colorectal cancer (Kirchheimer *et al.* 1987), other authors disagree. Montemurro failed to observe an increase of uPA in 41 colorectal cancer patients compared to 40 controls (Montemurro *et al.* 1995). In addition, no increase has been found in gastric (Ho *et al.* 1998; Kirchheimer *et al.* 1987), lung (Pavey, Hawson, & Marsh 1999) or biliary cancers (Kirchheimer *et al.* 1987). The finding of significantly increased uPA in the early breast cancer patients, but only a trend for increase in the advanced group does not clarify this issue.

The literature consistently reports elevated PAI-1 levels in cancer, which supports the trends seen in our pilot study (Rocha *et al.* 1989; von Tempelhoff *et al.* 1997).

The trend for a relative thrombocytosis in advanced breast cancer concurs with Pedersens findings of thrombocytosis being more common in the more advanced TNM stages of primary lung cancer. In 1115 lung cancer patients, he found thrombocytosis (>400 $x10^9$) in 32% compared to only 6% in benign lung disease controls (Pedersen & Milman 1996). Paradoxically, thrombocytosis and thrombocytopenia are commonly seen in cancer. Sun and co-workers found 57% of a heterogeneous group of cancer patients had thrombocytosis, and 11% had thrombocytopenia (Sun *et al.* 1979). In this study, data on aspirin usage was incomplete so the effect of this on platelet function in terms of VEGF release was not determined.

Previous research has shown that platelet lysates from breast cancer patients have increased VEGF concentrations compared to controls (Caine, Lip, & Blann 2004). In this study we show that this is limited to advanced cancer patients. A previous study by Salgado and colleagues shows increased VEGF release per platelet in advanced cancer compared to early cancer (prior to surgery), and early cancer compared to control.

However in their study they fail to correct for background circulating VEGF (pVEGF) (Salgado *et al.* 2001). In the present study, the platelets of early cancer patients following tumour removal release VEGF of equivalent quantities to normal controls.

Our data support previous findings of an acute phase response in metastatic cancer (O'Hanlon *et al.* 2002b; Sattar & McMillan 1998; Weinstein *et al.* 1984).

6.7.2 The effect of chemotherapy on haemostatic markers in advanced and early breast cancer

Chemotherapy caused a rapid and marked fall in APTT, and a more subtle increase in PT. There is a marked reduction in both clotting times by three months in early cancer patients, compared to controls. In a similar study performed in early breast cancer patients, Pectasides also demonstrated a significant decrease in PT at three months, however unlike in this study, he found an increase in APTT after three months of adjuvant chemotherapy (Pectasides *et al.* 1999). Although Rella found no alteration in clotting times in 38 early breast cancer patients following chemotherapy (Rella *et al.* 1996), Canobbio found a significant decrease in thrombin time and APTT following adjuvant chemotherapy in 49 breast cancer patients (Canobbio *et al.* 1986). Gabazza reported a reduction in PT and APTT in the two weeks following chemotherapy for lung cancer (Gabazza *et al.* 1994). In advanced breast cancer patients, the marked reduction in APTT at 24 hours is temporary, beginning to revert after only four days, returning to pre-chemotherapy rate by three months. PT has further increased following three months of chemotherapy in advanced breast cancer. At six months both APTT and PT are similar in advanced breast cancer, early breast cancer and the control group, however the attrition

rate due to death in the advanced group biases this timepoint to less aggressive advanced breast cancer patients. The prolonged clotting times, prior to chemotherapy, in early breast cancer may represent a post-surgery effect. Chemotherapy appears to cause a reduction in clotting times, however in advanced cancer this is only temporary.

The marked increase in PF1+2 following chemotherapy in advanced cancer, that is not reflected in early cancer, may represent a hypercoagulable response due to chemotherapy *acting on cancer cells*. These results are supported by Zurborn's findings of increase circulating PF1+2 following aggressive chemotherapy for non-Hodgkins lymphoma, a factor they related to high tumour cell destruction (Zurborn *et al.* 1991). Such an increase in PF1+2 is not seen with less aggressive chemotherapy in lymphoma, or in early breast cancer patients receiving chemotherapy (Rella *et al.* 1996; Zurborn *et al.* 1991).

The rise in TAT within 24 hours of chemotherapy, irrelevant of cancer stage, implies a rapid procoagulant stimulus from chemotherapy. Previous studies in breast cancer have failed to detect this early response as, in both studies, blood samples were only taken prior to each cycle of chemotherapy (JABCSG and JCOG 1993; Rella *et al.* 1996).

Within 24 hours of commencing chemotherapy a reduction in fibrinogen levels is evident in this current study. This is more prolonged in advanced cancer. Pectasides' study looking at the effect of chemotherapy on early breast cancer, refers to reduced fibrinogen levels prior to third and sixth cycle of chemotherapy, however it is unclear whether this decrease is compared to a control group, or the pre-chemotherapy levels. Graphical illustrations do not support the latter (Pectasides *et al.* 1999). Another similar study does not detect any alteration in fibrinogen levels, however the analysis is only preformed before each cycle (Rella *et al.* 1996). This early reduction in circulating fibrinogen levels

may reflect consumption of fibrinogen, induced by chemotherapy. The prolongation of this effect in the presence of cancer supports the more labile haemostatic balance in these patients.

Unlike fibrinogen, D-dimer is not altered by chemotherapy in either patient group. Although decrease in D-dimer has been described within a week of lung cancer patients receiving chemotherapy (Gabazza *et al.* 1994), no such change is seen in early breast cancer patients or in lymphoma patients receiving aggressive chemotherapy (Rella *et al.* 1996; Zurborn *et al.* 1991). The marked decline at six months appears to reflect the high attrition rate in advanced breast cancer due to patient death.

TPA levels measured in advanced lung cancer patients receiving chemotherapy have been shown to decrease within 48 hours of treatment (Ruiz *et al.* 1989). This supports the findings in this present study of a greater decrease in circulating tPA levels at four days. The minimal changes occurring in early breast cancer suggest the lytic action of chemotherapy on tumour cells may have a role in this altered fibrinolytic response.

There is a marked response, in circulating uPA levels, to chemotherapy in early breast cancer only. There is a rapid decrease at 24 hours and subsequent steady increase for six months. This change is absent in advanced breast cancer. The altered response in the presence of extensive cancer may reflect release of uPA from tumour cells masking the initial decrease in levels, and rebound correction.

The decrease in circulating PAI-1 six months after chemotherapy, as with other markers, may simply reflect attrition in the advanced cancer group.

The marked decline in platelet count, particularly in advanced breast cancer, within eight days of chemotherapy has not been previously described. Although Pectasides describes a reduction in platelet count after three months chemotherapy in early breast cancer, this is not supported by the present study. The chemotherapy-induced reduction in platelet count is clearly resisted in advanced breast cancer. Substantial evidence exists to support the interaction between platelets, leucocytes and haematogenously borne tumour cells, creating tumour microemboli (Gasic, Tuszynski, and Gorelik 1986). These microemboli may facilitate evasion of immuno-surveillance, arrest in distant organs and subsequent interaction with endothelial cells (Kim *et al.* 1998). Experimental studies show that under flow conditions, platelets enhance tumour cell adhesion to endothelial cells (Bastida, Almirall, and Ordinas 1989). This relative thrombocytosis in advanced cancer may reflect tumour aggression.

After an initial decrease in platelet VEGF content, possibly due to VEGF release stimulated by chemotherapy, the possible increase in platelet VEGF content in advanced cancer only, suggests an important store of this angiogenic molecule in active cancer. The profound effect of chemotherapy on platelet VEGF content in early breast cancer suggests an initial early release of VEGF by platelets in response to chemotherapy, hence depleting stores. This appears to be followed by a reactive increase in VEGF storage, which is mirrored by the absolute platelet count. Verheul and co-workers, in a study of 27 breast cancer patients receiving chemotherapy, reported an initial thrombocytopenia followed by a strong platelet rebound coinciding closely with a sVEGF peak. However, by not correcting this for background (plasma) levels of VEGF, or calculating VEGF per platelet, they concluded that sVEGF simply reflects platelet count (Verheul *et al.* 1997).

The data from this present study suggests not just a chemotherapy-induced rebound in platelet count (ie decrease, followed by steady increase), but also a similar decrease and then steady increase in individual platelet content of VEGF, irrespective of platelet count. This implies an alteration in platelet function.

The acute phase response described in response to chemotherapy for small cell lung cancer (Milroy *et al.* 1989), non-Hodgkins lymphoma and leukaemia (Zurborn *et al.* 1991) was not demonstrated in this current study.

Data on the use of growth factors for stimulation of bone marrow was not recorded, however usage was felt to be minimal in the patient groups studied. Erythropoeitin, a known added thrombogenic stimulus during chemotherapy, was not used in these patients (Bokemeyer *et al.* 2004).

6.8 Discussion: The influence of cancer and chemotherapy on procoagulants and endothelial adhesion molecules

6.8.1 Procoagulants and endothelial adhesion molecules in advanced breast cancer, early breast cancer and normal controls

Procoagulants

Elevated systemic TF levels in breast cancer patients is extensively documented in the literature (Lwaleed, Chisholm, & Francis 1999; Ueno *et al.* 2000). This study supports these findings with raised levels in the advanced cancer group. The lack of increased TF

at baseline in the early breast cancer group provides evidence for this group acting as an appropriate "non-cancer" control receiving chemotherapy.

The extrinsic pathway of the blood coagulation cascade is initiated when TF binds to factor VII. The correlation of TF with markers of intravascular fibrin formation and fibrinolysis supports the hypothesis that increased circulating TF results in activation of coagulation.

The increased levels of CP in early breast cancer but not advanced breast cancer supports the previous study by Mielicki and colleagues, although the advanced group in the latter study consisted of only locally advanced breast cancer patients and not those with distant metastases (Mielicki *et al.* 1999). An explanation for the normal CP levels in advanced breast cancer is suppression of CP activity by the anti-CP antibody, possibly present in the advanced cancer patients' serum (Mielicki *et al.* 1999). Clearly in this current study, the circulating CP levels in the early cancer group have not returned to control levels despite surgery. Rucinska published a series of 16 lung cancer patients undergoing curative surgery that demonstrated a marked decline in CP within ten days of surgery, however no statistical analysis was published to confirm if the decrease was significant, or if the post-operative levels were comparable to controls (Rucinska *et al.* 1997).

There is a marked increase in CP in both advanced breast cancer patients and early breast cancer patients at 24 hours following chemotherapy, in keeping with a potential hypercoagulable trigger. As with Mielicki's study, there was no correlation between CP and haemostatic markers in the present study, contary to other evidence that CP is an important initiator of cancer-related VTE (Curatolo *et al.* 1979). Interestingly, however, we demonstrate a negative correlation between pVEGF and CP. Previous work by Olas

and co-workers has shown that CP stimulates platelet activation and adhesion (Olas, Wachowicz, & Mielicki 2001). The alteration in platelet function induced by CP may increase platelet scavenging of circulating VEGF, resulting in the negative correlation with pVEGF but not sVEGF.

The trend towards increased TSP-1 levels in advanced breast cancer supports previously published data showing increased circulating levels in breast cancer, particularly with lymph node metastases (Hayden *et al.* 2000; Tuszynski *et al.* 1992), however the small numbers and the lack of a non-cancer control group in this analysis limits the conclusions that can be made. The association between oestrogen receptor-positivity and increased TSP-1 in early breast cancer warrants further investigation.

The correlation between VCAM-1 and TSP is unexpected. A platelet response to endothelial cell activation may explain this, but further work is needed to confirm this finding. Further analysis on CTAD samples may provide interesting data on the effect of chemotherapy.

TNF-α is not increased in the cancer groups in this study compared to normal controls. Limited reports in the literature of increased circulating TNF-α in cancer have focused on advanced pancreatic cancer, where cachexia frequently occurs (Barber, Fearon, & Ross 1999). Serum TNF-α correlates with insulin resistance, an important factor in cancer-cahexia (McCall, Tuckey, & Parry 1992). TNF-α levels are elevated in advanced breast cancer patients in the presence of weight loss compared to stable weight (Knapp *et al.* 1991). As the advanced cancer patients recruited in this study had a WHO status of one or two, cancer-cachexia was minimal.

TNF-α, in this study, did not correlate with VCAM-1 or E-selectin despite TNF-α's ability to enhance their expression (Bevilacqua *et al.* 1987; Laferriere *et al.* 2001; Okahara *et al.* 1994). This may simply be due to different temporal peaks in their circulating levels (Fa, Schalkwijk, & van Hinsbergh 2003).

The higher VEGF seen in serum compared to plasma samples, and the correlation of pVEGF and particularly sVEGF with platelet count, is consistent with previous studies reporting platelet release of VEGF as the main source of serum VEGF (Banks *et al.* 1998; Salgado *et al.* 1999). The much lower levels of pVEGF may represent ongoing platelet release of VEGF in addition to other sources, including tumour cells. Consistent with previously published literature, sVEGF and pVEGF levels in this study are significantly elevated in advanced breast cancer patients(Adams *et al.* 2000). Several studies report elevated serum and plasma VEGF in early cancer patients, prior to curative surgery (Adams *et al.* 2000; Belgore *et al.* 2001). The absence of increased sVEGF in early breast cancer in this current study once again supports the use of this group as a control.

Serum, plasma VEGF, and VEGF release per platelet correlate with markers of hypercoagulability and intravascular fibrin formation. This finding suggests a link between circulating VEGF or alterations in platelet function, and the hypercoagulability of cancer.

Endothelial adhesion molecules

In this study raised levels of circulating VCAM or E-selectin in advanced breast cancer patients was not demonstrated, although a trend was apparent. In a larger study by

O'Hanlon and co-workers, both endothelial adhesion molecules were found to be elevated in advanced breast cancer compared to normal controls (O'Hanlon *et al.* 2002a). Other authors agree with these latter findings (Banks *et al.* 1993; Hebbar *et al.* 1998; Regidor *et al.* 1998).

The positive correlation of E-selectin with number of days since surgery in the early breast cancer group may represents an angiogenic response to surgery. The finding that VCAM-1 does not correlate with number of days following surgery supports Mancuso's report of circulating levels decreasing within 24 hours of surgical resection for breast cancer (Mancuso *et al.* 2003). The association between Her 2 neu receptors and increased VCAM-1 in early breast cancer is unexpected and may be a chance finding. Further research is needed to clarify this. The correlation between E-selectin and VCAM-1 has previously been reported, and reflects the specificity of both of these molecules as endothelial markers (Alexiou *et al.* 2003).

6.8.2 The effect of chemotherapy on procoagulants and endothelial adhesion molecules in advanced and early breast cancer

Chemotherapy does not appear to alter TF levels either in the presence or absence of cancer. Tissue factor has been described as the main determinant of coagulation activation in cancer patients (Costantini *et al.* 1998). Its role in chemotherapy-induced hypercoagulability does not appear significant.

The increase in CP activity within 24 hours of chemotherapy, irrespective of cancer is difficult to explain. The decline in CP in advanced cancer over six months is consistent with reduced circulating CP in advanced cancer compared to early cancer. CP has been

identified in a broad spectrum of malignant tissues, but it has not been found in normally differentiated tissues (Gordon 1992). The ongoing presence of increased CP activity, in early breast cancer patients following tumour removal, which increases further at six months is also difficult to explain. Lichetenbeld reports that cultured tumour cells induce factor-X activating procoagulant activity on endothelial cells, independently of tissue factor. Tumour cells, although present in the culture system, did not require direct contact with endothelial cells (Lichtenbeld *et al.* 1993). Such procoagulant activity may be due to CP expression, and could explain the prolonged CP activity occurring after tumour removal *in vivo*.

The decline in TNF-α levels in the eight days following chemotherapy may reflect a chemotherapy induced down-regulation of monocyte/macrophage production, as this is the predominant cellular source of TNF-α (Vilcek & Lee 1991). However, the initial increase in TNF-α within 24 hours of chemotherapy administration may reflect release from cancer cells, as this is the principal cell population that differs between the advanced and early breast cancer groups. *In vitro* experiments by Niiya and colleagues have demonstrated upregulation of TNF-α expression by doxorubicin in human lung carcinoma (Niiya *et al.* 2003). The increase in circulating levels may be as a result of increased TNF-α from upregulated functioning tumour cells, or may occur as a result of membrane breakdown with cell death. This raises the possibility of TNF-α as a marker of chemotherapy-induced tumour cell death.

In the first eight days following chemotherapy, the increase in pVEGF is matched by a decrease in sVEGF. This may reflect increased platelet release of VEGF as a response to chemotherapy, with a consequent reduction in stored levels of VEGF in the platelet α-

granules (Salgado *et al.* 2001). This is supported by the reduction in calculated VEGF per platelet in the eight days following chemotherapy. The decrease seen in serum VEGF is particularly marked in advanced cancer (both absolute sVEGF, and VEGF released per platelet). This implies the platelet response to chemotherapy is altered in the presence of cancer. The lack of difference in pVEGF levels between advanced and early breast cancer implies that tumour release of VEGF is not noticably affected by chemotherapy. The increase in pVEGF and particularly sVEGF at three months occurs in both advanced and early breast cancer, implying that this is not a tumour response, but a systemic response to chemotherapy. As platelet content of VEGF increases, this must be either due to increased platelet (megakaryocyte) production of VEGF (Mohle *et al.* 1997) or platelet scavenging of circulating VEGF produced from other cellular sources (Vermeulen *et al.* 1999). The lack of difference between advanced and early breast cancer response to chemotherapy provides evidence to refute previous theories that increased platelet content of VEGF in cancer is due to platelet scavenging of tumour VEGF (George *et al.* 2000; Salgado et al. 2001).

The rapid and marked decrease in circulating endothelial adhesion molecules in the eight days following chemotherapy, may represent increased uptake of these circulating molecules by activated endothelial cells. Levels appear to recover prior to the three month administration of chemotherapy. With respect to VCAM-1, this response is similar in advanced and early cancer, implying it is a chemotherapy-induced effect. The further increase in VCAM-1 at six months, seen in advanced cancer patients only, supports the previous findings of O'Hanlon, that circulating levels are increased in advanced breast cancer patients(O'Hanlon *et al.* 2002a).

The difference in E-selectin levels seen in advanced and early breast cancer patients following chemotherapy may reflect a release of E-selectin from tumour vasculature, initially over-riding the chemotherapy-induced decrease in circulating E-selectin levels. As with VCAM, levels further increase above control at six months, supporting previous authors reports of elevated circulating E-selectin in advanced breast cancer (Hebbar *et al.* 1998).

6.9 Conclusion

Increased circulating levels of TAT, fibrinogen, D-dimer, tPA, platelet count and platelet release of VEGF suggest the activation of coagulation in advanced breast cancer patients. In contrast, in early breast cancer patients there is a minimal activation of the haemostatic systems, suggesting that post-surgery early breast cancer patients show the effects of chemotherapy, rather than the effects of chemotherapy-induced tumour cell lysis, on coagulation.

However, despite the much lower level, or normalization, of haemostasis in early breast cancer patients following curative surgery, it is interesting that D-dimer levels still correlate with Nottingham Prognostic Index and tumour size many weeks after surgery, at a time when the pro-thrombotic stimulus of chemotherapy is added.

The prolongation of PT and APTT is maintained in advanced breast cancer, but returns to normal in early breast cancer during chemotherapy. The rapid decrease in fibrinogen and increase in TAT within the first few days of chemotherapy implies that alterations in coagulation occur early after commencement of chemotherapy. An increase in D-dimer may be expected, however it is possible that a peak is missed because the eight day blood

sample may be too early for the D-dimer, a fibrin-degradation product, to appear. The changes in circulating PF1+2, fibrinogen and tPA suggest that the early alteration of coagulation is more pronounced in patients with advanced breast cancer.

Platelet count shows a rapid and profound response (or decrease) to chemotherapy in advanced breast cancer compared to early breast cancer, with a return to baseline in early breast cancer but a marked increase in platelet count in advanced breast cancer by three months. Platelet function, in terms of VEGF release, was also altered by chemotherapy. However unlike in early breast cancer patients, in advanced breast cancer patients platelet release of VEGF is maintained in the eight days following chemotherapy.

Circulating TF, TSP-1, VEGF and possibly VCAM-1 were elevated in advanced breast cancer as compared to early breast cancer controls. The lack of increase of TF and VEGF in the post-surgery early breast cancer patients supports the use of these patients as a control group receiving chemotherapy.

Both TF and VEGF levels correlate with markers of hypercoagulability. It is not possible to conclude whether they act as a trigger of coagulation, or simply markers of activation of the cascade, with for example VEGF reflecting altered platelet function.

Although TNF-α was not increased in advanced breast cancer, it increased following chemotherapy in advanced but not early breast cancer. This increase raises the possibility that TNF-α may be a surrogate marker of chemotherapy induced tumour-cell death.

VEGF release by platelets appears to increase in the eight days following chemotherapy, with a decrease in sVEGF and an increase in pVEGF (ie platelet VEGF decreases, but

background VEGF increases). This is more apparent in advanced breast cancer, where the decrease in sVEGF is significantly greater. If tumour release of VEGF was a major source of circulating VEGF, then an increase in pVEGF following chemotherapy may be expected, however an increase is not demonstrated in this study.

The marked fall in circulating endothelial adhesion molecules following chemotherapy may reflect vascular endothelial cell activation and increased uptake of circulating endothelial adhesion molecules as an early response to chemotherapy.

Chemotherapy causes a marked alteration in numerous markers of the haemostatic system, procoagulants and endothelial adhesion molecules, within 24 hours to eight days of commencing chemotherapy, however this is more marked in patients with advanced breast cancer.

This early hypercoagulable response may be the critical trigger to initiate a VTE. In chapter 7 baseline levels of haemostatic markers, procoagulants and endothelial adhesion molecules are measured in patients who subsequently develop VTE, assessing whether they predict for development of VTE. The response to chemotherapy, in terms of changing levels of haemostatic markers, procoagulants and endothelial adhesion molecules will be compared in patients with, compared to without subsequent VTE.

The more pronounced alterations in circulating procoagulants and markers of haemostasis in advanced breast cancer patients following chemotherapy may reflect the upregulation of the haemostasis, procoagulants and endothelial adhesion molecules at baseline, prior to chemotherapy. The pronounced response may also be a result of the chemotherapy effect on cancer cells, releasing procoagulants into the circulation following tumour cell lysis. If the latter is true, a more marked hypercoagulable response

may be expected in those patients with the greatest cell death as a response to chemotherapy. In chapter 8 we investigate whether those patients with the greatest response to chemotherapy have an altered haemostatic and procoagulant response to chemotherapy.

Chapter 7

Correlation of haemostatic markers, procoagulants and endothelial adhesion molecules with chemotherapy-induced venous thromboembolism

Chemotherapy for the treatment of breast cancer is associated with an increased risk of development of VTE. This is more common in advanced breast cancer compared to early breast cancer patients receiving chemotherapy. In the previous chapter a significant haemostatic, procoagulant and endothelial adhesion molecule response in the presence of advanced breast cancer was established. A more subtle hypercoagulable state in early breast cancer patients following curative surgery was demonstrated. A significant haemostatic and procoagulant response has been shown following the initiation of chemotherapy. This is more marked in advanced breast cancer, the group at particular risk from VTE. The aim of this chapter is to

- identify if haemostatic markers, procoagulants and endothelial adhesion molecules are raised, prior to chemotherapy, in patients who subsequently develop VTE
- identify molecular predictors of VTE
- identify altered haemostatic, procoagulant and endeothelial adhesion molecule response to chemotherapy in those patients developing VTE

7.1 Haemostatic markers in patients developing VTE compared to those remaining free of VTE

Aim

To determine if haemostatic markers, prior to chemotherapy, are raised in breast cancer patients who subsequently develop VTE, and establish if such markers predict for development of VTE

7.1.1 Are haemostatic markers increased in patients who develop VTE?

Table 27: Haemostatic markers, prior to chemotherapy, in patients developing VTE compared to those remaining free of VTE

Analysis compares patients with and without VTE at three months following commencement of chemotherapy, and patients with and without VTE at two years following commencement of chemotherapy. Comparison is made using an independent T-test. Mean and standard deviation or geometric mean and 95% confidence interval.

Coagulation marker	*VTE within 3 months (n)*	*No VTE within 3 months (n)*	*p*	*VTE within study period (n)*	*No VTE in study period (n)*	*p*
PT secs, mean (SD)	11.6 (11.0-12.3) *(7)*	11.7 (11.5-11.8) *(105)*	*0.7*	11.6 (11.1-12.0) *(10)*	11.7 (11.5-11.8) *(102)*	*0.6*
APTT secs, mean (SD)	21.5 (17.2-25.8) *(7)*	23.0 (22.6-23.5) *(105)*	*0.1*	22.5 (19.4-25.7) *(10)*	23.0 (22.5-23.4) *(102)*	*0.6*
PF1+2 nmol/l, geometric mean (CI)	1.9 (0.9-4.1 *(5)*	1.0 (0.8-1.1) *(25)*	*0.004*	1.9 (0.9-4.1 *(5)*	1.0 (0.8-1.1) *(25)*	*0.004*

Coagulation marker	*VTE within 3 months (**n**)*	*No VTE within 3 months (**n**)*	*p*	*VTE within study period (**n**)*	*No VTE in study period (**n**)*	*p*
TAT µg/ml, geometric mean (CI)	13.0 (2.3-72.2) *(**6**)*	5.4 (3.9-7.4) *(**22**)*	*0.1*	8.0 (2.6-25.1) *(**9**)*	5.9 (4.1-8.4) *(**19**)*	*0.5*
Fibrinogen g/L, mean (SD)	5.1 (2.8-7.5) *(**6**)*	3.4 (3.2-3.7) *(**97**)*	*0.004*	4.7 (3.0-6.4) *(**8**)*	3.4 (3.2-3.7) *(**95**)*	*0.013*
D-dimer ng/ml, geometric mean (CI)	1655 (837-3274) *(**9**)*	727 (631-837) *(**123**)*	*0.05*	1514 (936-2448) *(**13**)*	714 (618-824) *(**119**)*	*0.009*
tPA ng/ml, geometric mean (CI)	14412 (8958-23126) *(**4**)*	8948 (7640-10480) *(**36**)*	*0.07*	14412 (8958-23126) *(**4**)*	8948 (7640-10480) *(**36**)*	*0.07*
uPA ng/ml, geometric mean (CI)	2.1 (0.7-6.5) *(**4**)*	0.6 (0.4-0.9) *(**35**)*	*0.05*	2.1 (0.7-6.5) *(**4**)*	0.6 (0.4-0.9) *(**35**)*	*0.05*
PAI-1 ng/ml, geometric mean (CI)	16.3 (8.9-29.8) *(**5**)*	19.2 (14.7-25.2) *(**25**)*	*0.5*	16.3 (8.9-29.8) *(**5**)*	19.2 (14.7-25.2) *(**25**)*	*0.5*
Platelet count $x10^9$/l, mean (SD)	348 (250-446) *(**9**)*	314 (299-329) *(**125**)*	*0.3*	339 (269-410) *(**13**)*	314 (298-329) *(**121**)*	*0.4*
Platelet function-VEGF µg/ml per platelet $x10^9$, geo. mean (CI)	0.9 (0.4-1.9) *(**9**)*	0.6 (0.5-0.7) *(**118**)*	*0.6*	0.7 (0.4-1.3) *(**12**)*	0.6 (0.5-1.3) *(**115**)*	*0.7*
CRP mg/l geometric mean (CI)	5.9 (1.7-20.6) *(**5**)*	4.8 (2.3-10.2) *(**21**)*	*0.8*	5.9 (1.7-20.6) *(**5**)*	4.8 (2.3-10.2) *(**21**)*	*0.8*

7.1.2 Do haemostatic markers, prior to chemotherapy, predict for chemotherapy-induced venous thromboembolism?

In early breast cancer, shortened APTT, prior to chemotherapy, is associated with an increased risk of developing VTE. A one second reduction in APTT is associated with a 2.5 fold increase in risk of VTE (binary logistic regression, p=0.01).

In both early and advanced breast cancer patients, pre-chemotherapy fibrinogen and D-dimer are predictors for the development of VTE. Every one gram increase in fibrinogen doubles the risk of VTE (p=0.005), and every 1000ng/ml increase in D-dimer is associated with a 1.8 fold increased risk of VTE (p=0.005).

In the pilot study of 30 colorectal, stomach and breast cancer patients, five developed VTE, all occurring within three months of commencement of chemotherapy. Four VTE occurred in patients with colorectal cancer (one advanced cancer and three early cancer) and one in a patient with advanced stomach cancer. In this group PF1+2 had a predictive value for VTE, with a 1nMol/l increase in PF1+2 being associated with a four-fold increased risk of VTE (p=0.03).

The trend for increased platelet count in patients developing VTE was significant in the advanced breast cancer group. Of those developing VTE, mean platelet count was not only increased compared to advanced breast cancer patients without VTE, but was above the normal range (mean platelet count 313 and 434 $x10^9$/l, n=30 and 4, p=0.05, normal range 150-400 $x10^9$/l).

7.1.3 Can a clinically useful screen be established to identify hypercoagulable patients at-risk of venous thromboembolism

A clinically useful predictor of VTE requires readily available assays. Potentially useful markers are PT, APTT, D-dimer, fibrinogen and platelet count. Of these, both D-dimer and fibrinogen are elevated at baseline in those patients that subsequently develop VTE and predict for VTE. We established sensitivity and specificity for predicting VTE using D-dimer and fibrinogen and D-dimer combined. A cut off value of 3g was chosen for fibrinogen (median fibrinogen for all patients) and 700ng/ml for D-dimer (higher value than median, to allow for greatly increased D-dimer levels in advanced breast cancer).

Table 28: Sensitivity and specificity for potentially clinically useful markers of patients at-risk of developing VTE

Criteria for predictive test	*Sensitivity*	*Specificity*	*False negative rate*	*False positive rate*
D-dimer >700ng/ml Advanced breast cancer	100%	31%	0%	69%
D-dimer >700ng/ml and fibrinogen >3g/l Advanced breast cancer	100%	53%	0%	47%
D-dimer >700ng/ml All breast cancer	78%	53%	22%	47%
D-dimer >700ng/ml and fibrinogen >3g/l All breast cancer	67%	69%	33%	31%

7.2 Response of haemostatic markers to chemotherapy in patients developing VTE

7.2.1 Is there an altered haemostatic response to chemotherapy in breast cancer patients developing VTE?

Table 29: The haemostatic response to chemotherapy. Is it altered in patients developing VTE, and can it predict for development of VTE? A summary

Coagulation marker	*Demonstrate a different response to chemotherapy in those developing VTE, compared to VTE-free patients?*	*Do early (within 8 days) changes predict for VTE (Binary logistic regression)*
PT	Yes	Yes
APTT	Yes	Yes
PF1+2	No	No
TAT	Yes	No
Fibrinogen	No	No
D-dimer	Yes	No
tPA	No	No
uPA	No	No
PAI-1	No	No
Platelet count	No	No
Platelet function	No	No
CRP	No	No

(Appendices 22 and 23)

In patients who develop VTE, particularly those patients developing a VTE within the first three months of commencing chemotherapy, a marked increase in PT was demonstrated in the first eight days, compared to patients that remained free of VTE, following initiation of chemotherapy (n=6 and 82 respectively, p=0.005) (Figure 36 and appendices 22 and 23).

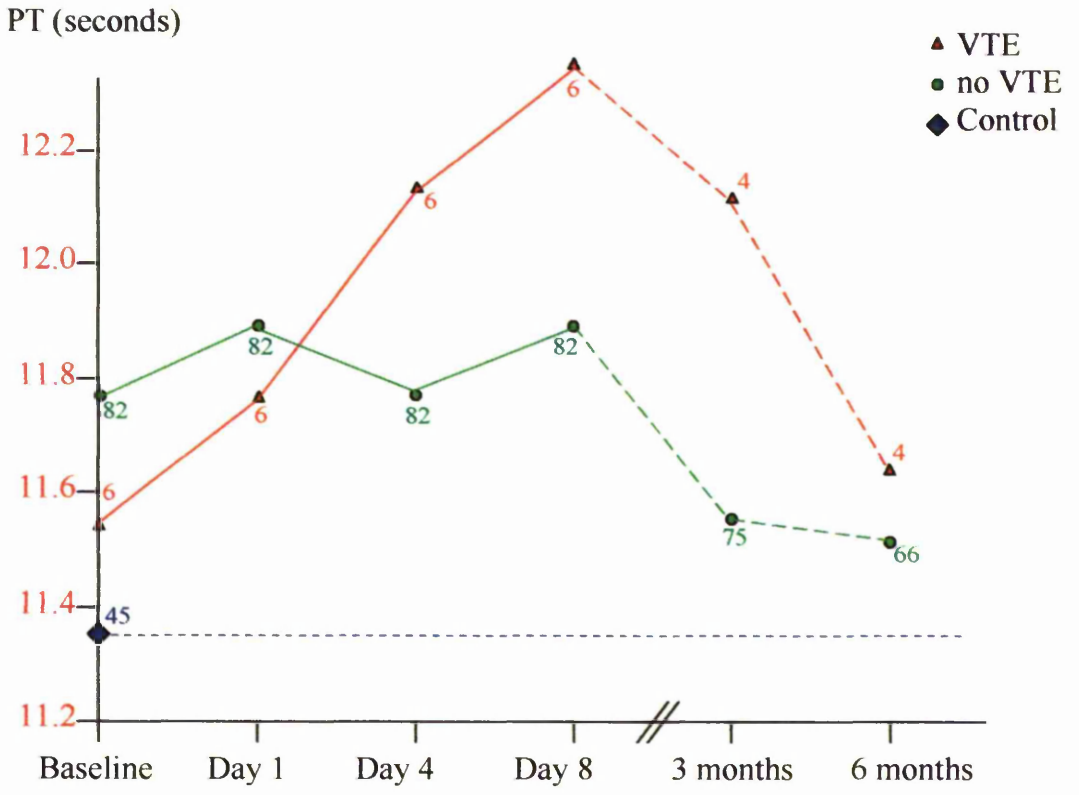

Figure 36: Prothrombin time in patients developing VTE compared to those remaining free of VTE: Effect of chemotherapy

Mean values. Patient numbers given at each timepoint

Prior to chemotherapy, PT demonstrated a predictive value for VTE, with a 1 second increase in PT from baseline to day 4 and day 8 being associated with a three and six fold

increase in risk of development of VTE (p=0.06 and 0.016 respectively, n=7+92 and 6+94 respectively).

APTT demonstrated a marked decrease within 24 hours in patients who developed VTE (either within three months, or at any time in the study period) as compared to patients who remain free of VTE (within three months: n=7 and 94, p=0.006; within two year study period: n=10 and 91, p=0.007) (Figure 37 and appendices 22 and 23)

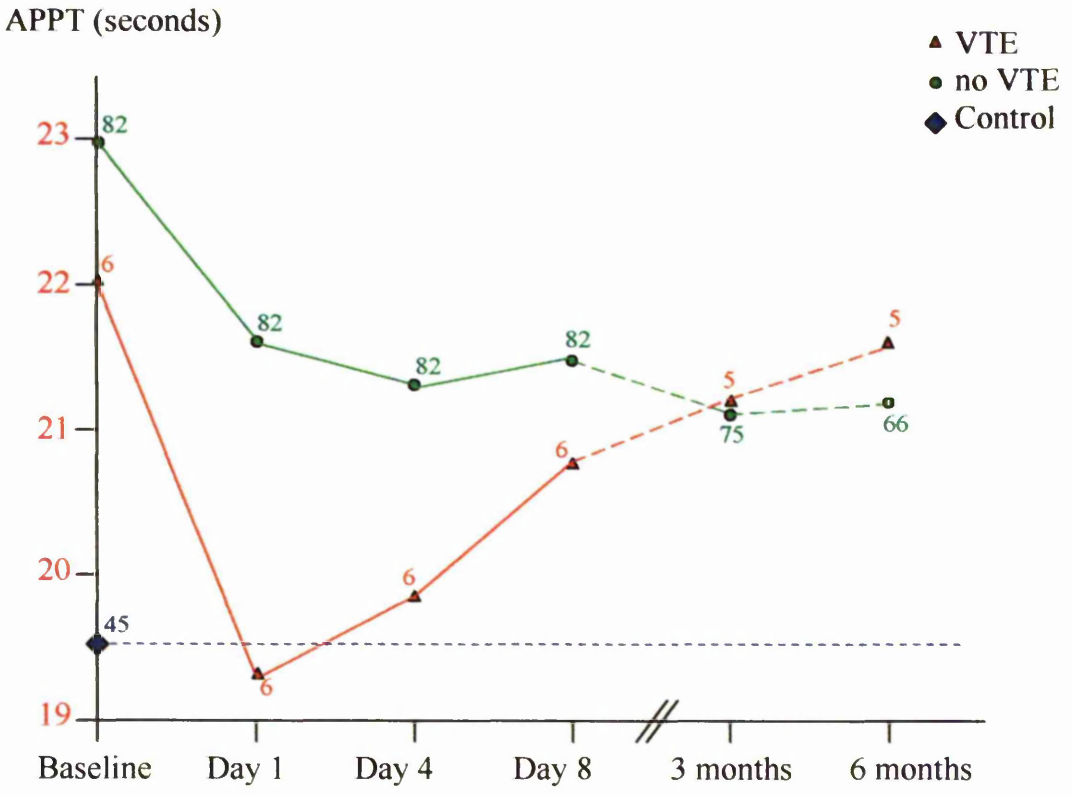

Figure 37: Activated partial thromboplastin time in patients developing VTE compared to those remaining free of VTE: Effect of chemotherapy

Mean values. Patient numbers given at each timepoint

The early APTT response to chemotherapy also demonstrated a predictive value for development of VTE, with a one second decrease in APTT being associated with a 25% increase in risk of development of VTE within 3 months (0.004).

TAT significantly increased within 24 hours in patients that developed VTE as compared to those that remained free of VTE (n=9 and 17, p=0.01) (Figure 38 and appendices 22 and 23).

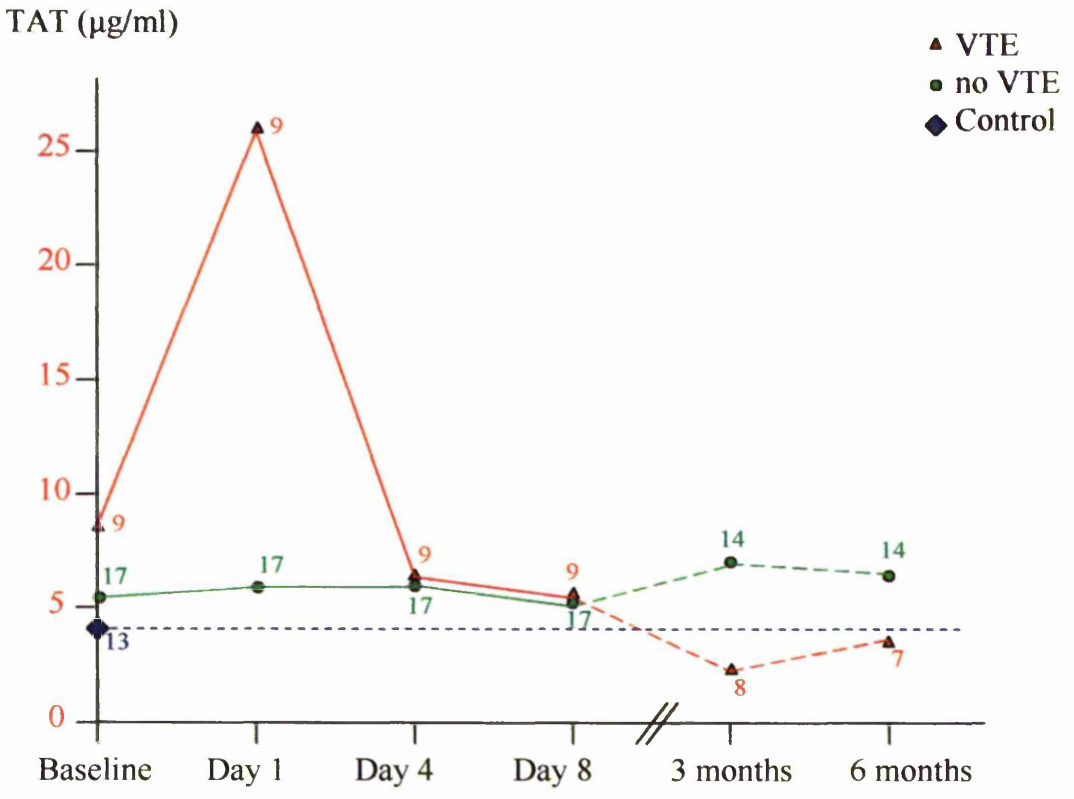

Figure 38: Thrombin-antithrombin in patients developing VTE compared to those remaining free of VTE: Effect of chemotherapy

Mean values. Patient numbers given at each timepoint

D-dimer demonstrated a significant decreasing trend over six months in patients who developed VTE (p=0.02). This trend was not present in those that remained free of VTE. There was, however, no significant difference in trend in D-dimer, in patients with and without VTE, in the first eight days (p=0.9) (Figure 39 and appendices 22 and 23).

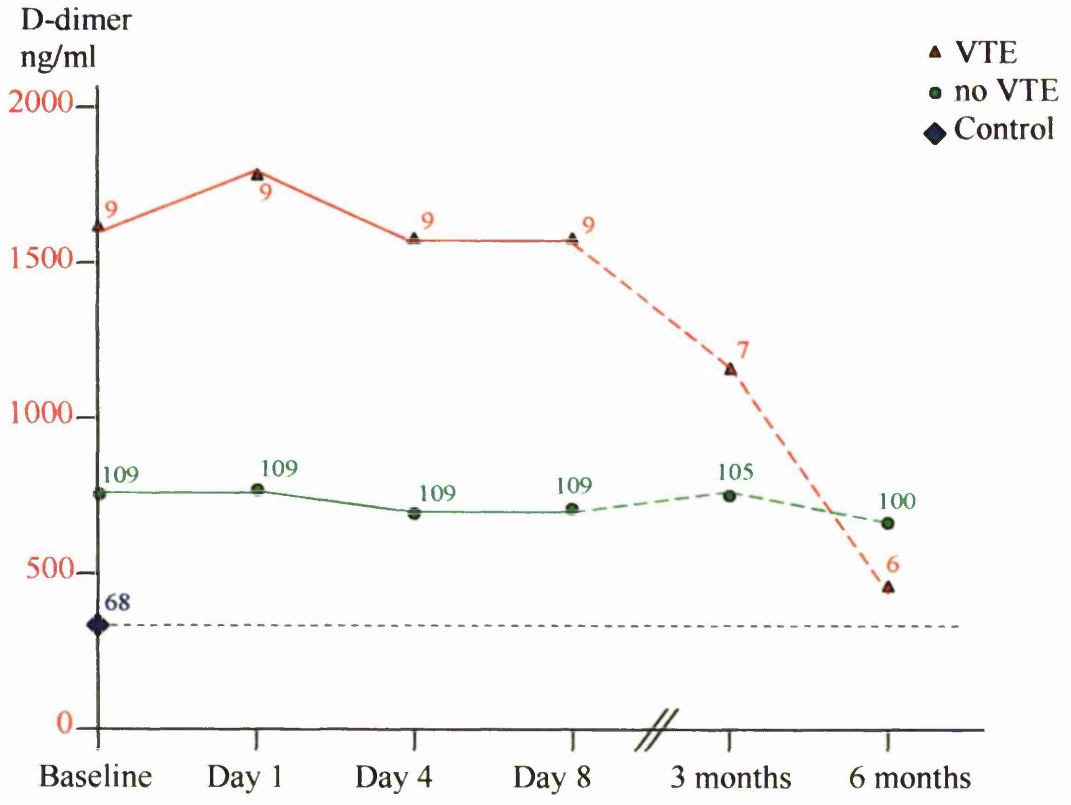

Figure 39: D-dimer in patients developing VTE compared to those remaining free of VTE: Effect of chemotherapy

Geometric means. Patient numbers given at each timepoint

7.3 Procoagulants and endothelial adhesion molecules in patients developing VTE compared to those remaining free of VTE

Aim

To assess whether procoagulants and endothelial adhesion molecules, prior to chemotherapy, are raised in breast cancer patients who subsequently develop VTE, and whether such markers predict for development of VTE

7.3.1 Are procoagulants and endothelial adhesion molecules increased in patients who develop VTE?

Table 30: Procoagulants and endothelial adhesion molecules, prior to chemotherapy, in patients developing VTE compared to those remaining free of VTE

Analysis compares patients with and without VTE at three months following commencement of chemotherapy, and patients with and without VTE at two years following commencement of chemotherapy. Comparison is made using an independent T-test. Geometric mean and 95% confidence interval.

Procoagulant /adhesion molecule	*VTE within 3 months(**n**)*	*No VTE within 3 months (**n**)*	*p*	*VTE within study period (**n**)*	*No VTE in study period (**n**)*	*p*
TF µg/ml, geometric mean (CI)	274 (115-654) *(9)*	107 (86-135) *(122)*	*0.07*	261 (121-561) *(12)*	105 (84-132) *(119)*	*0.03*
CP mU, geometric mean (CI)	30 (27-34) *(9)*	32 (30-34) *(110)*	*1.0*	30 (26-36) *(12)*	32 (30-34) *(107)*	*1.0*

Procoagulant /adhesion molecule	*VTE within 3 months(**n**)*	*No VTE within 3 months (**n**)*	*p*	*VTE within study period (**n**)*	*No VTE in study period (**n**)*	*p*
TSP-1 ng/ml, geometric mean (CI)	4559 (N/A) ***(2)***	*598 (446-802) **(44)***	*0.01*	1093 (124-9655) ***(5)***	614 (454-830) ***(41)***	*0.2*
TNF-α µg/ml, geometric mean (CI)	4.2 (3.3-5.4) ***(5)***	3.2 (2.9-3.6) ***(63)***	*0.2*	4.0 (3.3-4.8) ***(7)***	3.2 (2.9-3.6) ***(61)***	*0.2*
pVEGF µg/ml, geometric mean (CI)	34 (15-79) ***(9)***	16 (14-18) ***(121)***	*0.01*	26 (14-48) ***(13)***	16 (14-18) ***(117)***	*0.05*
sVEGF µg/ml, geometric mean (CI)	357 (158-808) ***(9)***	205 (180-233) ***(122)***	*0.2*	252 (125-509) ***(12)***	209 (184-238) ***(119)***	*0.9*
VCAM-1 ng/ml, geometric mean (CI)	811 (657-1002) ***(9)***	638 (595-684) ***(123)***	*0.2*	723 (588-889) ***(13)***	641 (597-688) ***(119)***	*0.5*
E-sel ng/ml, geometric mean (CI)	31 (17-56) ***(9)***	29 (27-32) ***(123)***	*1.0*	29 (19-43) ***(13)***	30 (27-33) ***(119)***	*0.8*

7.3.2 Do procoagulants and endothelial adhesion molecules, prior to chemotherapy, predict VTE?

Prior to chemotherapy, circulating levels of TF, sVEGF and pVEGF predicted for an increased risk of development of VTE within the first three months following commencement of chemotherapy. A 100µg/ml increase in TF was associated with a 50%

increase risk of VTE (binary logistic regression, p=0.004). A 100μg/ml increase in sVEGF was associated with a 40% increase risk of VTE (p=0.003). A 10μg/ml increase in cVEGF was associated with a 20% increase risk of VTE (p=0.007).

7.4 Response of procoagulants and endothelial adhesion molecules to chemotherapy in patients developing VTE

Table 31: The procoagulant and endothelial response to chemotherapy. Is it altered in patients developing VTE, and can it predict for development of VTE? A summary

Coagulation marker	*Demonstrate a different response to chemotherapy in those developing VTE, compared to VTE-free patients*	*Do early (within 8 days) changes predict for VTE (Binary logistic regression)*
TF	Yes	No
CP	No	No
TSP-1	No	No
TNF-α	No	No
pVEGF	No	Yes
sVEGF	No	No
VCAM-1	No	No
E-selectin	No	No

(Appendix 24 and 25)

The elevated levels of TF that was seen in patients developing VTE, was lost by three months, with a marked decrease in circulating TF levels occurring in patients with VTE, but not in patients without VTE (geometric mean 85.0 and 97.1μg/ml, n=7 and 108, p=0.8). By six months, however, circulating TF levels in patients with VTE had once again increased (geometric mean 345.4 and 115.2, n=6 and 103, p=0.04) (Figure 40 and appendices 24 and 25).

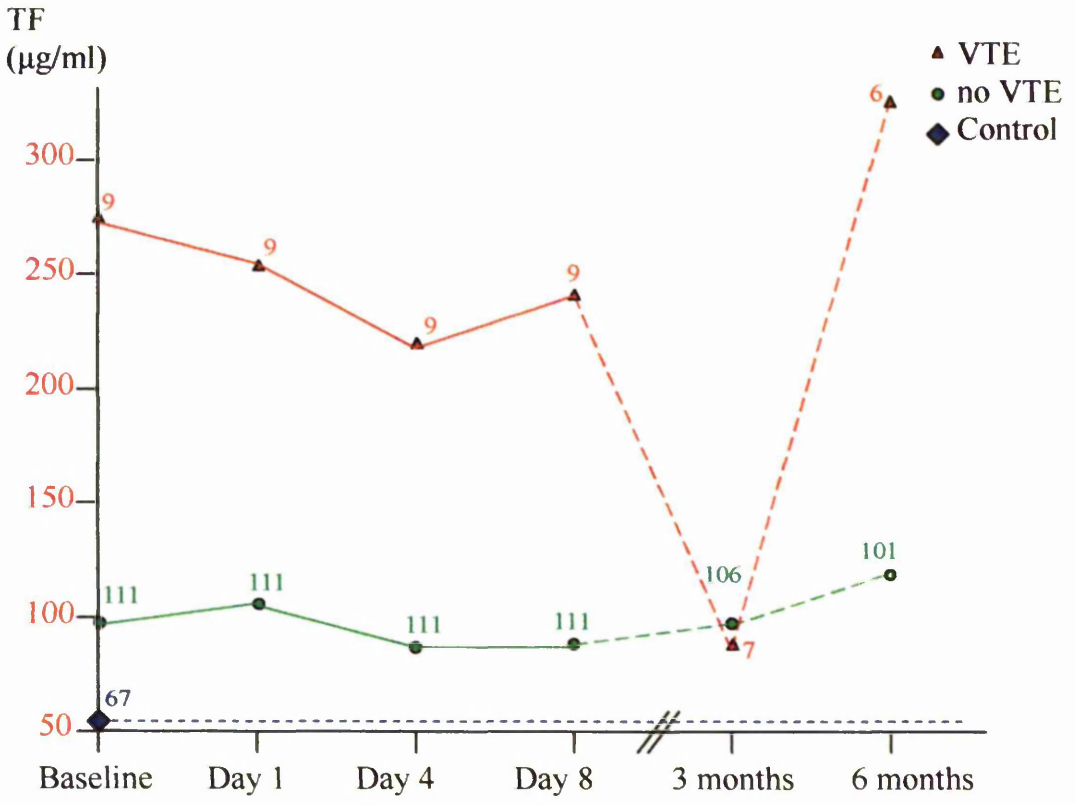

Figure 40: Circulating Tissue Factor in patients developing VTE compared to those remaining free of VTE: Effect of chemotherapy

Geometric mean. Patient numbers given at each timepoint

There was a marked decrease in pVEGF, but not sVEGF, within 24 hours of commencing chemotherapy, in patients developing VTE compared to patients remaining free of VTE (change from pre-chemotherapy levels to 24 hours: -5.0 and -0.4µg/ml, n=9 and 115, p=0.04). (Figure 41 and appendices 22 and 23).

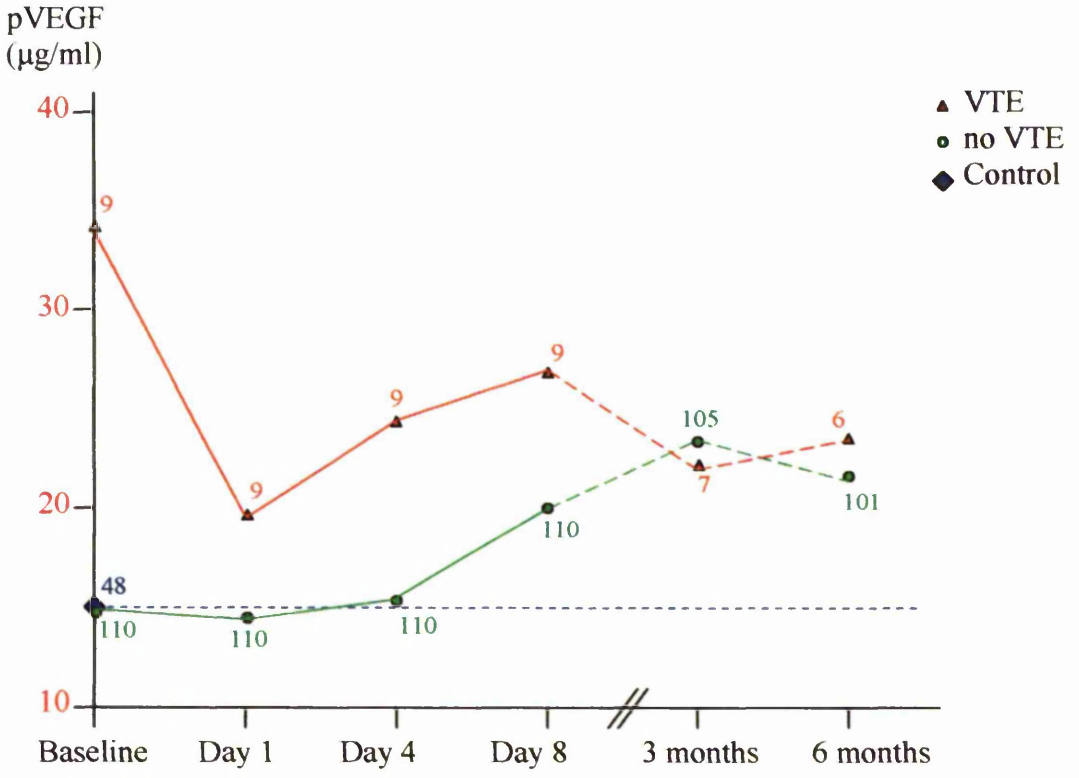

Figure 41: Plasma VEGF in patients developing VTE compared to those remaining free of VTE: Effect of chemotherapy

Geometric mean. Patient numbers given at each timepoint

A decrease in pVEGF within 24 hours was associated with an increased risk of VTE, with a 10µg/ml decrease from baseline, at 24 hours, increasing the risk of VTE by 25% (p=0.05).

7.5 Discussion

7.5.1 Haemostatic markers in patients developing VTE compared to those remaining free of VTE

This study demonstrates a trend towards a shorter APTT in patients that subsequently develop VTE. A predictive value of APTT for VTE is reported. These findings support studies in patients undergoing elective hip surgery (Lowe *et al.* 1999) and general medical inpatients (Reddy, Hall, & MacKintosh 1999). In the former study, pre-operative haemostatic factors were related to the subsequent development of DVT as diagnosed on screening venography in the second post-operative week. Pre-operative elevated PF1+2, TAT, D-dimer and shortened APTT were all associated with DVT (Lowe *et al.* 1999). In the latter study, abnormally fast APTTs were associated with an increased risk of subsequent thrombosis in general medical in-patients (Reddy, Hall, & MacKintosh 1999). The findings in this present study imply that clotting screens may have a role in establishing a high-risk profile for patients undergoing chemotherapy. The power of this study was based on clinical and not molecular endpoints, so such analysis is exploratory in nature.

Other authors have found increased PF1+2 in patients subsequently developing VTE (Corradi *et al.* 1999; Lowe *et al.* 1999). In a similar study to Lowe and co-workers, Corradi found a high correlation between preoperative plasma levels of PF1+2 and postoperative VTE. However in cancer patients undergoing abdominal surgery, both Iversen (Iversen, Okholm, & Thorlacius-Ussing 1996) and Falanga (Falanga *et al.* 1993) failed to find elevated pre-operative PF1+2 in patients developing postoperative VTE compared to those without VTE. The finding, in this present study, of PF1+2 being

predictive for chemotherapy-induced VTE supports the usefulness of this marker in VTE prediction, even in the presence of cancer. In a study of 32 advanced breast cancer patients receiving chemotherapy, randomised to either very low-dose or no warfarin, it is noteworthy that PF1+2 was significantly reduced in the former group (n=16), with a concurrent reduction in VTE rate from 12% to 0% (Falanga *et al.* 1998).

In patients undergoing total hip or knee arthroplasty, Jorgensen, Ginsberg and Cofrancesco independently demonstrate significantly increased levels of pre-operative TAT in those who subsequently develop VTE as compared to those that do not and suggests its use as a possible predictive marker of postoperative VTE (Cofrancesco *et al.* 1997; Jorgensen *et al.* 1990). In contrast to Falanga and colleagues' work, the elevated levels of TAT in patients subsequently developing VTE did not reach significance in this current study (Falanga *et al.* 1993).

We have found, prior to chemotherapy, significantly elevated levels of D-dimer and fibrinogen in patients who subsequently develop VTE. Both markers are predictive for increased risk of VTE. Preoperative plasma levels of soluble fibrin polymers have been found to correlate with development of VTE following elective neurosurgery (Sonaglia *et al.* 1999). Preoperative D-dimer is elevated in patients developing postoperative VTE following major hip surgery (Cofrancesco *et al.* 1997), however its predictive ability for postoperative VTE is limited when surgery is for trauma (Bongard *et al.* 1994; Lowe *et al.* 1999). In critically ill medical and surgical patients, regularly screened for VTE in an ICU department, no predictive hypercoagulable markers, including D-dimer, were found for subsequent development of VTE (Crowther *et al.* 2005). In a large prospective population study, D-dimer was found to be strongly positively related to the occurrence

of future VTE (Cushman *et al.* 2003). In cancer surgery, pre-operative D-dimer prediction of postoperative VTE was not useful in two small studies of gynaecological oncology patients (Olt *et al.* 1990; von Tempelhoff *et al.* 1997). Conversely, total fibrin and fibrinogen degradation products were significantly elevated, preoperatively, in colorectal cancer patients developing postoperative VTE, compared to colorectal cancer patients without postoperative VTE (Okholm *et al.* 1996). In 50 node positive early breast cancer patient receiving adjuvant chemotherapy, von Tempelhoff found elevated pre-chemotherapy D-dimer and fibrinogen in the five patients developing VTE, supporting the findings in the present study (von Tempelhoff *et al.* 1996). In von Templehoff's study, analysis was only performed prior to each chemotherapy cycle (ie three weeks after last chemotherapy), so early haemostatic responses to chemotherapy were not identified. In this study, a possible predictive role for fibrinogen and D-dimer in chemotherapy-induced VTE is established. The sensitivity, specificity, positive and negative predictive values we report for D-dimer and fibrinogen combined (of 67%, 69%, 33% and 31% respectively) are currently less clinically useful than the D-dimer levels reported by Bongard and colleagues for VTE prediction following hip surgery (93%, 23%, 36% and 96% respectively) (Bongard *et al.* 1994). The results of the current study are weakened by combining advanced and early breast cancer patients, as levels of both fibrinogen and D-dimer are elevated in advanced breast cancer. A larger study, allowing separation of these groups, would provide cut-off values for fibrinogen and D-dimer, with greater sensitivity and specificity for predicting VTE.

TPA, uPA but not PAI are increased at baseline in patients who subsequently develop chemotherapy induced VTE. Increased tPA has been shown to correlate with recurrent

VTE, however reduced tPA release in response to veno-occlusion, has also been identified as a risk factor for recurrent VTE (Juhan-Vague *et al.* 1987). This raises the possibility that the upregulation of tPA in these hypercoagulable patients is a response to increased fibrin formation, but may also be balanced by an unmeasured (in this study) tPA inhibitor. Such an inhibitor has previously been reported to be increased in patients up to two months following symptomatic VTE (Wiman *et al.* 1985). Thus it appears there is an enhanced non-specific upregulation of the fibrinolytic system in cancer patients at particular risk of chemotherapy-induced VTE.

There was no difference in pre-chemotherapy platelet count, or platelet release of VEGF (platelet function), in all breast cancer patients with and without subsequent VTE. This supports the findings of Leibovitch, who although reporting reactive thrombocytosis as a common finding following major urological surgery, found no association with thromboembolic complications (Leibovitch *et al.* 1993). Pedersen also found no association between thrombocytosis in primary lung cancer, and development of VTE (Pedersen & Milman 1996). However, thrombocytosis, above the normal range, occurred in the advanced breast cancer patients that subsequently developed VTE. This may allow identification of a high-risk subgroup, through a readily available clinical test. Goodnough, in 159 advanced breast cancer patients, found no incidence of thrombocytosis, prior to chemotherapy, in patients developing VTE compared to patients without VTE (Goodnough *et al.* 1984). However in this analysis, Goodnough included all patients developing VTE, even patients with VTE occurring after completion of treatment. Elevated levels of platelet microparticles (which correlate with platelet activation) *in vivo* have been reported for patients with activated coagulation and

fibrinolysis (Holme *et al.* 1994), however research is minimal on platelet function as a predictor of VTE.

7.5.2 Response of haemostatic markers to chemotherapy in patients developing VTE

It is interesting that PT lengthened following chemotherapy in patients who subsequently developed VTE, but APTT shortened in the same sub-group. PT is a measure of the extrinsic clotting system, and APTT a measure of intrinsic clotting activation. A prolongation of the PT with a normal APTT classically indicates factor VII deficiency, the only factor of the extrinsic system that does not significantly influence the common pathway (measured by PT and APTT) (Colman *et al.* 1994). As such a rapid reduction in the liver-synthesised factor is unlikely, possible alterations in its function may explain this finding, including factor VII activation by thrombin, and interaction with TF. The marked increase in TAT at 24 hours, in the VTE subgroup, may reflect reduced unbound thrombin available to activate factor VII. This early response demonstrates the rapid effect of chemotherapy on the haemostatic system. These haemostatic alterations occurring in high VTE-risk patients may allow a profile of high-risk patients to be developed.

The reduction in D-dimer levels in the VTE group over six months, is likely to simply reflect effective anticoagulation treatment.

Several reports in the literature refer to a fibrinolytic shutdown (due to increased PAI levels), occurring after surgery, which is more pronounced in patients developing VTE.

We do not demonstrate a similar occurrence following chemotherapy (Declerck *et al.* 1994).

7.5.3 Procoagulants and endothelial adhesion molecules in patients developing VTE

Raised levels of TF have been reported in patients with a diagnosis of VTE (Smith *et al.* 1999). The marked increase in pre-chemotherapy levels of TF identifies an at-risk group. In a small study investigating the anti-angiogenic compound SU5415 (an inhibitor of VEGF receptor 1 and 2), pre-treatment TF levels were significantly elevated in the three patients that subsequently developed VTE, compared to the 17 without VTE (Kuenen *et al.* 2002). No previous study has investigated the predictive value of circulating TF for VTE, that we have found in this study.

There has been no published work on CP or TSP-1 levels in VTE. In this current study there was no difference in baseline CP levels in patient with and without VTE, however there was a marked increase in baseline TSP-1 in patients developing VTE within three months. Unfortunately the small number (two) developing VTE in this group limit the value of this result, with further work being required to confirm the finding.

Increased TNF-α levels are associated with raised markers of hypercoagulability in patients with prostate cancer (fibrin degradation products D-dimer and E-fragment), however no studies have investigated circulating levels relating to development of VTE. Recombinant TNF-α is also known to have a procoagulant effect (Bauer *et al.* 1989). In our study, we find no relationship between baseline levels, and development of chemotherapy-induced VTE.

Prior to chemotherapy, plasma VEGF was increased in patients subsequently developing VTE, with a trend for increased sVEGF levels in the VTE patient group. In our study, as with others, sVEGF and pVEGF correlate with the haemostatic markers TAT, fibrinogen and D-dimer (Matsuyama *et al.* 2000). Kim and co-workers report an association between increased sVEGF per platelet and portal vein thrombosis in hepatocellular carcinoma, however in this study the authors do not correct for background (plasma) VEGF levels that may contribute to the reported sVEGF per platelet levels (Kim *et al.* 2004). As previously discussed, sVEGF largely consists of VEGF released from platelets, but pVEGF may also represent a significant tumour contribution. The strong association of pVEGF but not sVEGF with VTE may reflect patients with a greater tumour release of VEGF having an increased risk of VTE.

Neither VCAM-1 or E-selectin (endothelial adhesion molecules) were elevated in patients subsequently developing VTE, although VCAM-1 does demonstrate a trend for increased pre-chemotherapy levels in the VTE sub-group. Elevated levels of VCAM-1 have been reported in patients diagnosed with VTE (Gonzalez-Ordonez *et al.* 2003; Smith *et al.* 1999), however there are no reports in the literature of VCAM-1 predicting for VTE development. E-selectin, as with TF in the study described above, was found to be elevated at baseline in three patients developing VTE following the anti-angiogenic SU5415, compared to the 17 VTE-free patients (Kuenen *et al.* 2002).

7.5.4 Response of procoagulants and endothelial adhesion molecules to chemotherapy in patients developing VTE

In Kuenen's study of SU5415, a marked increase in TF and E-selectin was seen at 14 and 28 days following commencement of treatment in patients developing VTE compared to patients remaining free of VTE. In our study we only demonstrate an alteration in TF levels at three months following commencement of chemotherapy in the VTE group. A marked decline is seen at this point, with levels returning to similar to those of the non-VTE group. This may simply reflect the introduction of successful anticoagulation treatment in those patients who developed VTE. However the marked increase in the TF levels in this hypercoagulable group at six months suggests a possible resistance to treatment.

Of the procoagulants we have studied, only pVEGF demonstrates an early altered response to chemotherapy in patients developing VTE, compared to those without VTE. The marked reduction in pVEGF, but not sVEGF at 24 hours in the VTE group, suggests rapid uptake of background VEGF in the hypercoagulable patients. Whether this uptake is by cancer cells, or perhaps more likely, by altered function of platelets, remains to be established.

There is no altered response of endothelial adhesion molecules in patents with, compared to without, VTE.

7.6 Conclusion

Several haemostatic markers are altered, prior to chemotherapy, in patients that subsequently develop VTE. D-dimer, fibrinogen (and possibly PF1+2, tPA, and uPA), as well as alterations in PT, APTT and TAT as an early response to chemotherapy, may be useful in the development of a biochemical profile to identify patients at increased risk of chemotherapy-induced VTE. Despite almost all of these markers being altered in the presence of cancer, they still have a potential role in the prediction of VTE.

The early alterations in PT, APTT and TAT imply a very rapid haemostatic response, raising the possibility that the maximal at-risk time for the initiation of VTE is within 24 hours of chemotherapy administration.

Raised TF and pVEGF, prior to chemotherapy in patients developing VTE, may point to the procoagulant trigger for cancer-induced VTE. The lack of alteration in TF levels following chemotherapy implies a limited role for TF in chemotherapy-induced VTE. It is possible that chemotherapy-induced alterations in TF are rapid and simply not detected with the timepoints used in this study. The increased baseline pVEGF, but not sVEGF, in patients developing VTE implies tumour VEGF rather than platelet VEGF is the important procoagulant here. However, the rapid reduction in pVEGF following chemotherapy, in the VTE group, suggests an increased VEGF uptake, either by tumour, or perhaps more likely, by platelets.

The absence of changes in levels of circulating endothelial adhesion molecules in response to chemotherapy in patients with and without VTE, implies that chemotherapy induced vascular endothelial cell alteration is not the principle trigger for chemotherapy-induced hypercoagulability.

This study provides evidence that a clinically useful profile could be produced to identify patients at increased risk of VTE. With such a profile, a trial of targeted anticoagulant prophylaxis could be developed. Considering the very rapid response to chemotherapy in PT, APTT and TAT, it may even be possible to provide anticoagulation simply at the high-risk timepoints, for example with administration of each chemotherapy cycle.

As discussed in chapter 6, cancer patients with VTE may still have a promising prognosis. Prevention of VTE in these patients, with a resultant improvement in quality of life, is an important priority.

Chapter 8

Correlation of haemostatic markers, procoagulants and endothelial adhesion molecules with disease progression and survival in breast cancer

Chapter 6 demonstrated

- a hypercoagulable state, and increased circulating procoagulants in advanced breast cancer patients compared to early breast cancer patients and normal controls
- a minimal activation of coagulation, in early breast cancer patients compared to normal controls, supporting the use of this patient group as a "normal control group" receiving chemotherapy
- levels of procoagulants in early breast cancer corresponding to normal controls, supporting the use of this patient group as a "normal control group" receiving chemotherapy
- an altered response of circulating haemostatic markers, procoagulants and endothelial adhesion molecules in advanced breast cancer patients receiving chemotherapy, compared to the early breast cancer "normal control group"

The altered response to chemotherapy, seen in advanced breast cancer patients, may reflect the upregulation of haemostasis, procoagulants and endothelial adhesion molecules that exists prior to chemotherapy. This altered response may also reflect the effect of chemotherapy on cancer cells, triggering cell death, procoagulant release and a

haemostatic response. This molecular response to chemotherapy may thus provide some insight into the effectiveness of chemotherapy.

In chapter 8 we aim to establish

- if markers of the haemostatic system, prior to chemotherapy, are increased in patients with poor response to chemotherapy (show early progression)
- if markers of the haemostatic system, prior to chemotherapy can predict for cancer outcome, in both early and advanced breast cancer patients (ie identify more aggressive disease)
- if markers of the haemostatic system demonstrate an altered response to chemotherapy in patients with, compared to without, cancer progression
- if procoagulants and endothelial adhesion molecules, prior to chemotherapy, are increased in patients with poor response to chemotherapy (show early progression)
- if procoagulants and endothelial adhesion molecules, prior to chemotherapy can predict for cancer outcome, in both early and advanced breast cancer patients (ie identify more aggressive disease)
- if procoagulants and endothelial adhesion molecules demonstrate an altered response to chemotherapy in patients with, compared to without, cancer progression

Advanced cancer patients progressing at three and six months will be compared to those remaining stable at three and six months, respectively, following commencement of chemotherapy. Early breast cancer patients progressing at two years will be compared to those remaining stable at two years following commencement of chemotherapy.

8.1 Haemostatic markers in patients with stable compared to progressive breast cancer

Aim

To assess whether haemostatic markers, prior to chemotherapy, are raised in breast cancer patients who subsequently progress, and to establish whether such markers predict for cancer progression or survival

8.1.1 Are haemostatic markers increased in breast cancer patients who subsequently progress?

Table 32: Haemostatic markers in advanced breast cancer: a comparison of patients progressing at three and six months with patients with stable disease

Analysis compares patients with and without cancer progression at three and six months following commencement of chemotherapy. Comparison is made using an independent T-test. Mean and standard deviation, or geometric mean and 95% confidence interval.

Coagulation marker	*Progression within 3 months (n)*	*No progression within 3 months (n)*	*p*	*Progression within 6 months (n)*	*No progression within 6 months (n)*	*p*
PT secs, mean (SD)	12.0 (11.5-12.5) *(10)*	11.6 (11.1-12.1) *(16)*	*0.3*	11.8 (11.5-12.2) *(19)*	11.6 (10.5-12.6 *(7)*	*0.5*
APTT secs, mean (SD)	22.0 (20.1-23.8) *(10)*	23.0 (21.4-24.6) *(16)*	*0.4*	22.7 (21.2-24.2) *(19)*	22.2 (20.2-24.1) *(7)*	*0.7*
PF1+2 nmol/l, geometric mean (CI)	0.92 (0.52-1.61) *(5)*	1.07 (0.86-1.31) *(8)*	*0.4*	0.95 (0.68-1.35) *(7)*	1.08 (0.78-1.51) *(6)*	*0.5*

Coagulation marker	*Progression within 3 months (**n**)*	*No progression within 3 months (**n**)*	*p*	*Progression within 6 months (**n**)*	*No progression within 6 months (**n**)*	*p*
TAT µg/ml, geometric mean (CI)	13.0 (2.3-72.0) *(**6**)*	7.3 (4.0-12.6) *(**8**)*	*0.4*	9.0 (3.8-20.9) *(**11**)*	10.2 (1.0-107.7) *(**3**)*	*0.9*
Fibrinogen g/L, mean (SD)	4.9 (3.0-6.8) *(**7**)*	4.3 (3.3-5.2) *(**14**)*	*0.4*	4.6 (3.5-5.8) *(**15**)*	4.0 (2.8-5.3) *(**6**)*	*0.5*
D-dimer ng/ml, geometric mean (CI)	1718 (1021-2891) *(**14**)*	1128 (738-1725) *(**21**)*	*0.2*	1428(1027-1984) *(**27**)*	1064 (375-3021) *(**8**)*	*0.4*
tPA ng/ml, geometric mean (CI)	13691 (9980-18783) *(**9**)*	8630 (5442-13686) *(**7**)*	*0.06*	10991 (7879-15334)*(**13**)*	12080 (9448-15494) *(**3**)*	*0.8*
uPA ng/ml, geometric mean (CI)	0.89 (0.42-1.89 *(**9**)*	0.32 (0.07-1.34) *(**7**)*	*0.13*	0.58 (0.24-1.43) *(**13**)*	0.50 (0.29-0.86) *(**3**)*	*0.9*
PAI-1 ng/ml, geometric mean (CI)	42.8 (20.5-89.5) *(**5**)*	16.2 (11.9-22.0) *(**8**)*	*0.004*	34.5(19.5-60.9) *(**7**)*	15.0 (9.9-22.8) *(**6**)*	*0.02*
Platelet count $x10^9$/l, mean (CI)	332 (253-411) *(**15**)*	323 (277-368) *(**21**)*	*0.8*	327 (279-376) *(**28**)*	325 (249-401) *(**8**)*	*1.0*
Platelet VEGF / platelet $x10^9$, geo. mean (CI)	1.09 (0.79-1.54 *(**15**)*	0.97 (0.68-1.40) *(**20**)*	*0.7*	1.12 (0.89-1.42) *(**28**)*	0.70 (0.28-1.77) *(**7**)*	*0.11*
CRP mg/l median (range)	17.6 (7.8-226) *(**4**)*	4.5 (1.8-35.7) *(**7**)*	*0.09*	10.0 (4.2-226) *(**6**)*	8.9 (1.8-35.7) *(**5**)*	*0.47*

Table 33: Haemostatic markers in early breast cancer: a comparison of patients progressing at two years with patients with stable disease

Analysis compares patients with and without cancer progression at two years following commencement of chemotherapy. Comparison is made using an independent T-test. Mean and standard deviation, or geometric mean and 95% confidence interval.

Coagulation marker	*Progression within 2 years (n)*	*No progression within 2 years (n)*	*p*
PT secs, mean (SD)	11.9 (11.4-12.4) *(5)*	11.7 (11.5-11.8)*(72)*	*0.4*
APTT secs, mean (SD)	21.9 (18.1-25.7) *(5)*	23.3 (22.7-23.8)*(72)*	*0.2*
PF1+2 nmol/l, geometric mean	*N/A (1)*	*N/A (13)*	*N/A*
TAT μg/ml, geom. mean (CI)	4.0 (2.8-5.8) *(3)*	4.3 (2.4-7.9) *(8)*	*0.9*
Fibrinogen g/L, mean (SD)	3.4 (2.2-4.6) *(5)*	3.3 (3.0-3.5) *(68)*	*0.8*
D-dimer ng/ml, geo. mean (CI)	698 (443-1099) *(5)*	667 (579-768) *(82)*	*0.9*
tPA ng/ml, geometric mean (CI)	5732 (3635-9038) *(2)*	8635 (6972-10695) *(18)*	*0.2*
uPA ng/ml, geom. mean (CI)	0.2 (N/A) *(2)*	0.93 (0.58-1.47) *(17)*	*0.7*
PAI-1 ng/ml, geometric mean	*N/A (1)*	*N/A (13)*	*N/A*
Platelet count $x10^9$/l, mean (SD)	345 (230-460) *(5)*	307 (291-324) *(82)*	*0.3*
Platelet function-VEGF μg/ml per platelet $x10^9$, geometric mean (CI)	0.26 (0.08-0.91) *(5)*	0.55 (0.47-0.65) *(77)*	*0.03*
CRP mg/l geometric mean	*N/A (1)*	*N/A (12)*	*N/A*

8.1.2 Do haemostatic markers, prior to chemotherapy, predict for cancer outcome?

Table 34: Prior to chemotherapy, can haemostatic markers predict for cancer outcome? A summary

Coagulation marker	*Predict for cancer progression*	*Correlate with survival*	*Predict for survival*
PT	No	Yes	No
APTT	No	No	No
PF1+2	No	No	No
TAT	No	No	Yes
Fibrinogen	No	No	No
D-dimer	No	No	No
tPA	No	No	No
uPA	No	No	No
PAI-1	No	Yes	Yes
Platelet count	No	No	No
Platelet function	Yes	No	No
CRP	No	No	Yes

At baseline, prior to chemotherapy, PT had a negative correlation with survival under one year (r^2= -0.53, n=16, p=0.03) (Figure 42).

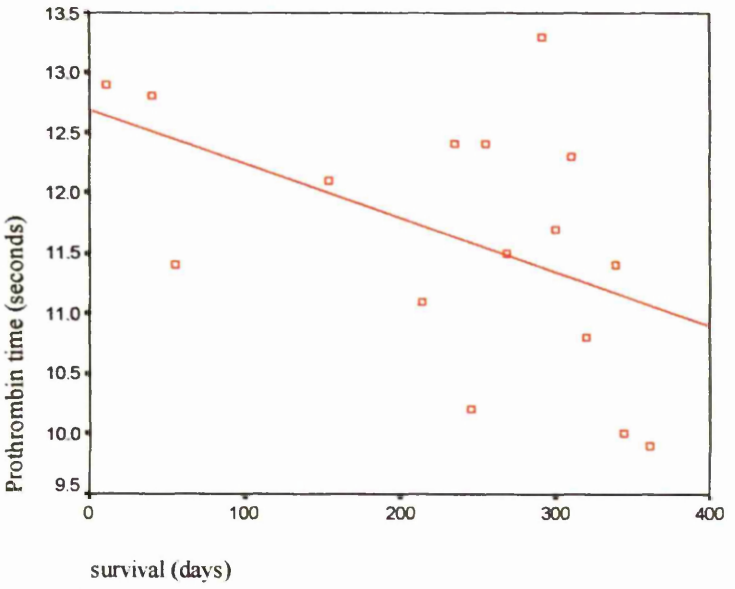

Figure 42: Correlation of prothrombin time, prior to chemotherapy, with survival

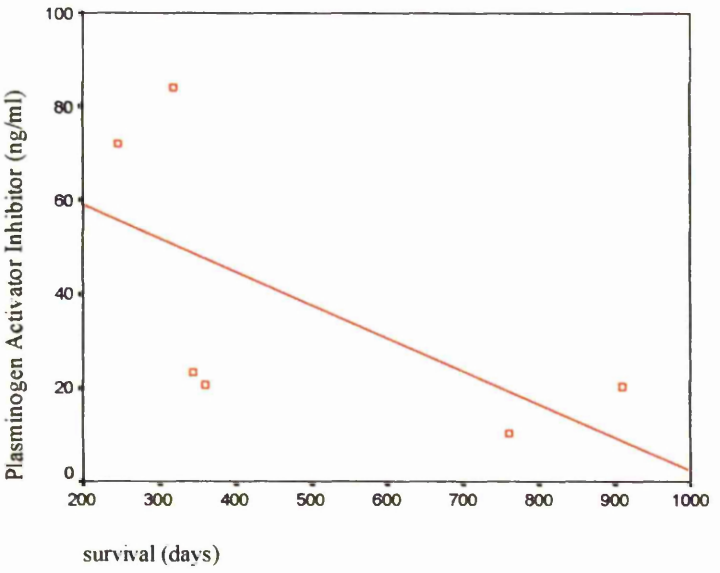

Figure 43: Correlation of plasminogen activator inhibitor-1, prior to chemotherapy, with survival

In advanced breast cancer, PAI-1 also demonstrated a negative correlation with survival (r^2= -0.89, n=6, p=0.02) (Figure 43).

TAT, prior to chemotherapy, was associated with a significant but small increased risk of death. A 10μg/ml increase in TAT was associated with a 1.1 increase in risk of death, independent of age or stage (Cox regression analysis, p=0.05).

Elevated levels of circulating PAI-1 were also associated with an increased risk of death, independent of stage and age. A 10ng/ml increase in baseline PAI-1 was associated with a 2 fold increased risk of death (p=0.01).

There was a trend for reduced survival to be associated with increased baseline CRP, independent of age and stage. A 10mg/ml increase in CRP was associated with a 1.4 increased risk of death (p=0.1).

Platelet release of VEGF demonstrated a possible predictive use for progression in early breast cancer, with, for example, a 2.7 μg/ml (per platelet $x10^9$) decrease in platelet VEGF content at baseline, being associated with a three fold increased risk of progression (binary logistic regression, p=0.05).

8.2 Response of haemostatic markers to chemotherapy in patients with stable compared to progressive breast cancer

8.2.1 Is there an altered haemostatic response to chemotherapy in breast cancer patients that subsequently progress?

Table 35: The haemostatic response to chemotherapy. Is it altered in patients with subsequent cancer progression, and can it predict for progression or survival? A summary

Coagulation marker	*Demonstrate a different response to chemotherapy in those ABC progressing within 6 months, compared to stable disease?*	*Demonstrate a different response to chemotherapy in those EBC progressing within 2 years, compared to stable disease?*	*Does response to chemotherapy predict for cancer progression?*	*Does response to chemotherapy within 3 months correlate with survival?*
PT	No	Yes	No	No
APTT	Yes	No	Yes	No
PF1+2	No	No	No	No
TAT	Yes	No	No	No
Fibrinogen	Yes	No	Yes	No
D-dimer	Yes	No	Yes	No
tPA	Yes	No	No	No
uPA	No	No	No	No
PAI-1	No	No	No	Yes
Platelet count	No	No	No	No
Platelet function	No	No	No	No
CRP	No	No	No	No

(Appendices 26, 27,28,29,30 and 31)

There was a marked prolongation of PT in early breast cancer patients, progressing within two years, by day four following commencement of chemotherapy (p=0.006) (Figure 44 and appendices 30 and 31).

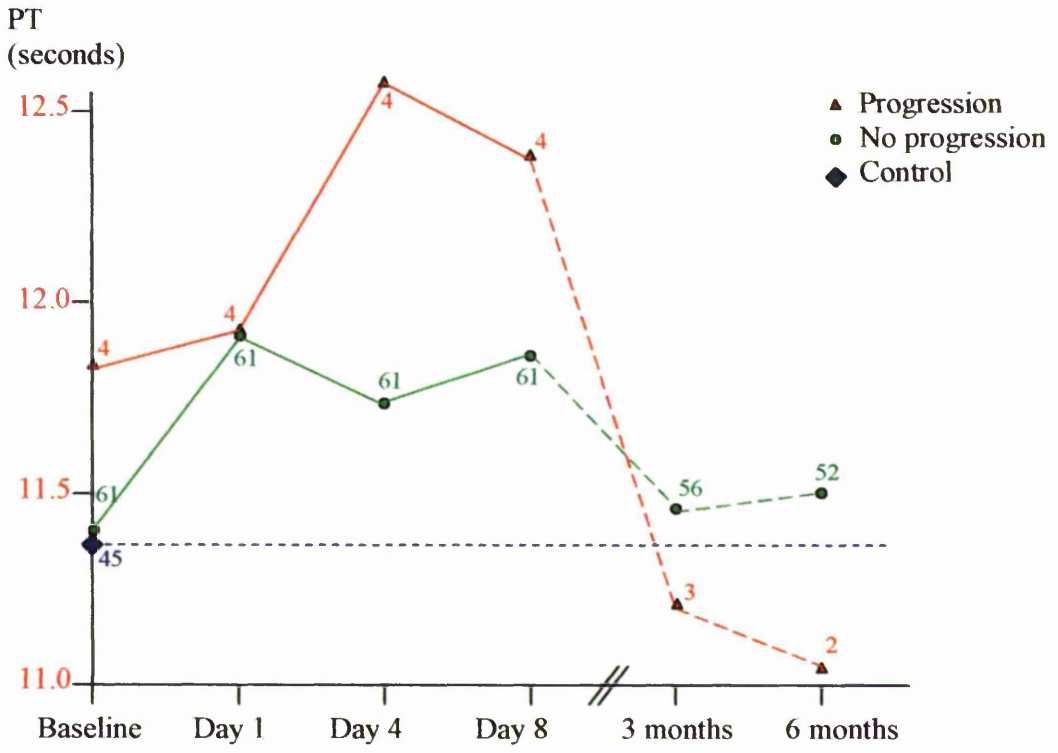

Figure 44: Prothrombin time in early breast cancer patients progressing, compared to not progressing, at two years: Effect of chemotherapy

Mean values. Patient numbers given at each timepoint

In advanced breast cancer, patients that remain stable up to three months following commencement of chemotherapy demonstrated a marked shortening of APTT within four days of starting chemotherapy. This response was not present in patients that subsequently progressed by three months (mean change from pre-chemotherapy to four days following chemotherapy -2.3seconds and 0.5 seconds, n=13 and 9, p=0.05). A

marked prolongation of APTT from baseline to three months was seen in the advanced breast cancer patients that progressed compared to those with stable disease (p=0.04) (Appendices 26 and 27).

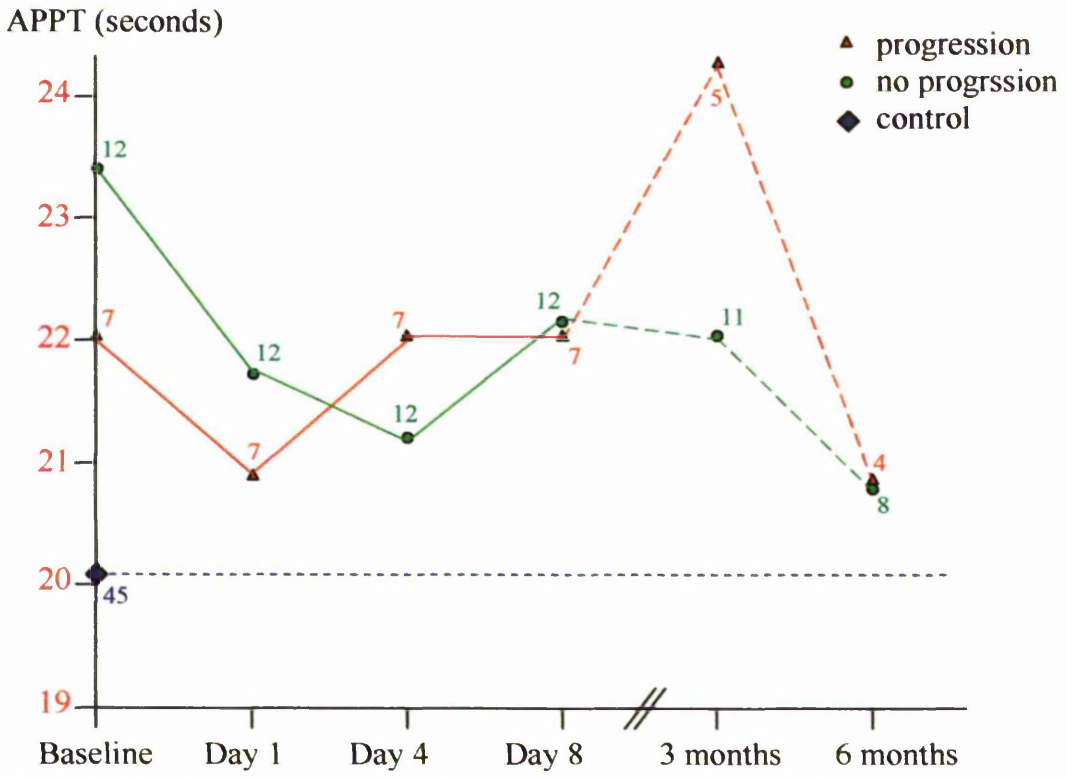

Figure 45: Activated partial thromboplastin time in advanced breast cancer patients progressing, compared to not progressing, at three months: Effect of chemotherapy

The early trend in APTT allowed a prediction of increased risk of progression. A prolongation in APTT of 1 second from pre-chemotherapy to four days following chemotherapy was associated with an increased risk of progression at three months of 60%, compared to patients with no prolongation in APTT (binary logistic regression on change from baseline to day four, p=0.05).

TAT decreased by eight days, from pre-chemotherapy levels, in advanced breast cancer patients progressing within three months. In patients remaining stable at three months, TAT levels increased (change from baseline in patients progressing and not progressing within three months: -2.6 and 5.6μg/ml, n=6 and 7, p=0.05). (Appendices 26 and 27).

Fibrinogen demonstrated a marked decrease, within four days of commencement of chemotherapy, in advanced breast cancer patients progressing at three and six months. This decrease was not seen in patients with stable disease. (Mean change from baseline in patients progressing and not progressing within three months: -0.93 and 0.02g/l, n=6 and 10, p=0.06; mean change from baseline in patients progressing and not progressing within six months: -0.68 and 0.42g/l, n=11 and 5, p=0.03). (Appendices 26, 27, 28 and 29). A decrease in circulating fibrinogen in the four days following chemotherapy was associated with an increased risk of progression. For every 1g/l decrease in fibrinogen from baseline to day four, the risk of progression, at three and six months, increased by 33% and 25% respectively (binary logistic regression on change from baseline to day four, p=0.05 and 0.08 respectively).

A similar decrease in circulating D-dimer was seen within four days, in advanced breast cancer patients progressing within three months (change from baseline in patients progressing and not progressing within three months: -270 and 255ng/ml, n=14 and 19, p=0.02) (Appendices 26 and 27). For every 100ng/ml decrease in D-dimer from baseline to day four, the risk of progression, at three months, increased by 17% (binary logistic regression on change from baseline to day four, p=0.04).

The decrease seen in tPA at eight days following commencement of chemotherapy was more marked in advanced breast cancer patients progressing at six months compared to

advanced breast cancer patients with stable disease (change from baseline in patients progressing and not progressing within six months: -2629 and -2127ng/ml, n=9 and 3, p=0.05) (Appendices 28 and 29).

Only alterations in PAI-1, following chemotherapy, correlated with survival. A decrease in circulating PAI-1 from pre-chemotherapy levels to levels at three months had a negative correlation with survival (r^2=0.69, n=14, p=0.007) (Figure 46).

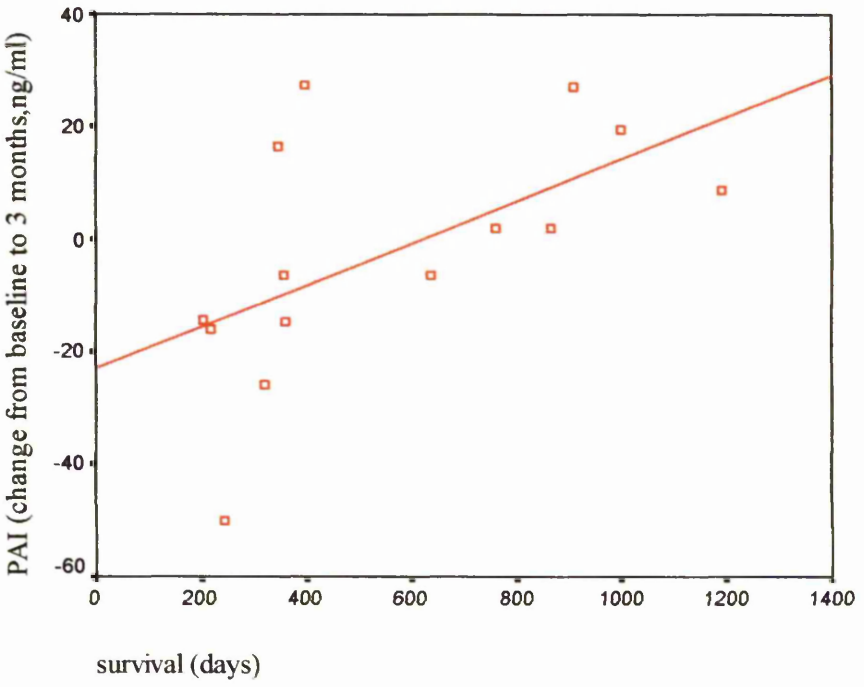

Figure 46: Correlation between change in circulating PAI-1 (from baseline to three months) and survival

8.3 Procoagulants and endothelial adhesion molecules in patients with stable compared to progressive breast cancer

Aim

To establish whether procoagulants and endothelial adhesion molecules are raised in breast cancer patients who subsequently progress, and assess whether such markers can predict for cancer progression or survival

8.3.1 Are procoagulants and endothelial adhesion molecules increased in breast cancer patients who subsequently progress?

Table 36: Procoagulants and endothelial adhesion molecules in advanced breast cancer: a comparison of patients progressing at three and six months with patients with stable disease

Analysis compares patients with and without cancer progression at three and six months following commencement of chemotherapy. Comparison is made using an independent T-test. Geometric mean and 95% confidence interval.

Procoagulant/ adhesion molecule	*Progression within 3 months (n)*	*No progression within 3 months (n)*	*p*	*Progression within 6 months (n)*	*No progression within 6 months (n)*	*p*
TF µg/ml, geometric mean (CI)	195 (125-303) *(15)*	169 (123-232) *(21)*	*0.6*	167 (124-226) *(28)*	228 (146-355) *(8)*	*0.3*
CP mU, geometric mean (CI)	26.2 (20.7-33.1) *(14)*	30.0 (25.4-35.3) *(19)*	*0.3*	27.7 (23.8-32.2) *(27)*	31.0 (21.7-44.4) *(6)*	*0.5*
TSP-1 ng/ml, geometric mean (CI)	939 (414-2129) *(4)*	1195 (863-1655) *(7)*	*0.4*	1150 (777-1702) *(8)*	960 (596-1547) *(3)*	*0.5*

Procoagulant/ adhesion molecule	*Progression within 3 months (**n**)*	*No progression within 3 months (**n**)*	*p*	*Progression within 6 months (**n**)*	*No progression within 6 months (**n**)*	*p*
TNF-α μg/ml, geometric mean (CI)	3.28 (2.52-4.26) ***(8)***	3.58 (2.76-4.65) ***(14)***	*0.6*	3.60 (3.01-4.31) ***(20)***	2.39 (0.02-3.05) ***(2)***	*0.2*
pVEGF μg/ml, geometric mean (CI)	26.0 (16.7-40.6) ***(15)***	20.7 (14.9-28.8) ***(21)***	*0.4*	23.7 (17.4-32.2) ***(28)***	19.8 (11.7-33.6) ***(8)***	*0.6*
sVEGF μg/ml, geometric mean (CI)	359 (239-539) ***(15)***	333 (242-459) ***(20)***	*0.8*	373 (294-474) ***(28)***	248 (103-598) ***(7)***	*0.2*
VCAM-1 ng/ml, geometric mean (CI)	832 (628-1102) ***(15)***	669 (533-841) ***(21)***	*0.2*	778 (651-930) ***(28)***	593 (347-1014) ***(8)***	*0.2*
E-selectin ng/ml, geometric mean (CI)	32.4 (20.6-51.2) ***(14)***	33.4 (25.9-42.9) ***(21)***	*0.9*	31.8 (24.0-42.3) ***(27)***	37.2 (28.0-49.3) ***(8)***	*0.6*

Table 37: Procoagulants and endothelial adhesion molecules in early breast cancer: a comparison of patients progressing at two years with patients with stable disease

Analysis compares patients with and without cancer progression at two years following commencement of chemotherapy. Comparison is made using an independent T-test. Geometric mean and 95% confidence interval.

Procoagulant/adhesion molecule	*Progression within 2 years (**n**)*	*No progression within 2 years (**n**)*	*p*
TF μg/ml, geometric mean (CI)	166 (30.1-912) *(**5**)*	89 (65-122) *(**80**)*	*0.3*
CP mU, geometric mean (CI)	29.5 (19.4-45.0) *(**4**)*	33.3 (30.8-36.0) *(**74**)*	*0.5*
TSP-1 ng/ml, geometric mean (CI)	272 *(**2**)*	643 (427-970) *(**30**)*	*0.3*
TNF-α μg/ml, geometric mean (CI)	*N/A (**1**)*	*N/A (**41**)*	*N/A*
pVEGF μg/ml, geometric mean (CI)	13.8 (6.4-29.7) *(**5**)*	14.2 (12.1-16.8) *(**79**)*	*0.9*
sVEGF μg/ml, geometric mean (CI)	111 (38-326) *(**5**)*	187 (160-217) *(**81**)*	*0.1*
VCAM-1 ng/ml, geometric mean (CI)	766 (518-1131) *(**5**)*	621 (578-667) *(**81**)*	*0.2*
E-selectin ng/ml, geometric mean (CI)	25.4 (14.6-44.4) *(**5**)*	28.2 (25.4-31.2) *(**82**)*	*0.6*

8.3.2 Do procoagulants and endothelial adhesion molecules, prior to chemotherapy, predict for cancer outcome?

Table 38: Prior to chemotherapy, can procoagulants and endothelial adhesion molecules predict for cancer outcome? A summary

Procoagulants/ endothelial adhesion molecules	*Predict for cancer progression*	*Correlate with survival*	*Predict for survival*
TF	No	No	No
CP	No	No	Yes
TSP-1	No	No	No
TNF-α	No	No	No
pVEGF	No	No	No
sVEGF	No	No	No
VCAM-1	No	No	Yes
E-selectin	No	No	No

CP had borderline significance for prediction of survival, with lower levels being associated with reduced survival (Cox regression, p=0.06). For example, pre-chemotherapy CP levels of 20mU had a three fold increased risk of death compared to 55mU.

At all timepoints except baseline, sVEGF was significantly decreased in early breast cancer patients that progressed within two years (binary logistic regression, p=0.02-0.05, Appendix 36 and 37), however reduced sVEGF did not predict for cancer progression in early breast cancer.

Increased baseline levels of VCAM-1 were associated with a reduced survival (Cox regression, p=0.006). A 200ng/ml increase in VCAM-1 was associated with a 40% increased risk of death.

8.4 Response of procoagulants and endothelial adhesion molecules to chemotherapy in patients with stable compared to progressive breast cancer

Table 39: Procoagulant and endothelial response to chemotherapy. Is it altered in patients who progress, and can it predict for cancer outcome? A summary

Procoagulants/ endothelial adhesion molecules	*Demonstrate a different response to chemotherapy in ABC progressing by 6 months, compared to stable disease?*	*Demonstrate a different response to chemotherapy in EBC progressing by 2 years, compared to stable disease?*	*Does response to chemotherapy predict for cancer progression?*	*Does response to chemotherapy correlate with or predict for survival?*
TF	No	No	No	No
CP	No	No	No	No
TSP-1	No	No	No	No
TNF-α	No	No	No	Yes
pVEGF	No	No	No	No
sVEGF	Yes	No	Yes	No
VCAM-1	No	Yes	Yes	No
E-selectin	No	No	No	No

(Appendices 32-37)

The decrease in sVEGF within eight days of commencing chemotherapy, previously reported in advanced but not early breast cancer patients (section 3, chapter 6), was more marked in advanced breast cancer patients progressing at six months compared to advanced breast cancer patients with stable disease (change from baseline to four days in patients progressing and not progressing within six months: -0.35 and -0.19μg/ml, n=26 and 7, p=0.04; change from baseline to eight days in patients progressing and not progressing within six months: -0.31 and 0.27μg/ml, n=26 and 7, p=0.03) (Appendices 34 and 35). Reduced sVEGF following chemotherapy only showed a trend to predict for increased risk of progression (binary logistic regression, p=0.09).

Early breast cancer patient that progressed within two years had a greater reduction in circulating VCAM-1 levels at three months, compared to patients with stable disease (change from baseline to three months in early breast cancer patients progressing and not progressing within two years: -104 and 26ng/ml, n=4 and 78, p=0.02) (Appendices 36 and 37). A decrease in VCAM-1 at three months was associated with an increased risk of progression (binary logistic regression, p=0.05).

The change in TNF-α from pre-chemotherapy levels to levels at both four days and six months had an inverse correlation with survival. Thus, patients with increasing TNF-α had a shortened survival (r^2=-0.53 and -0.86, n=19 and 13, p=0.02 and <0.001 respectively) (Figure 47).

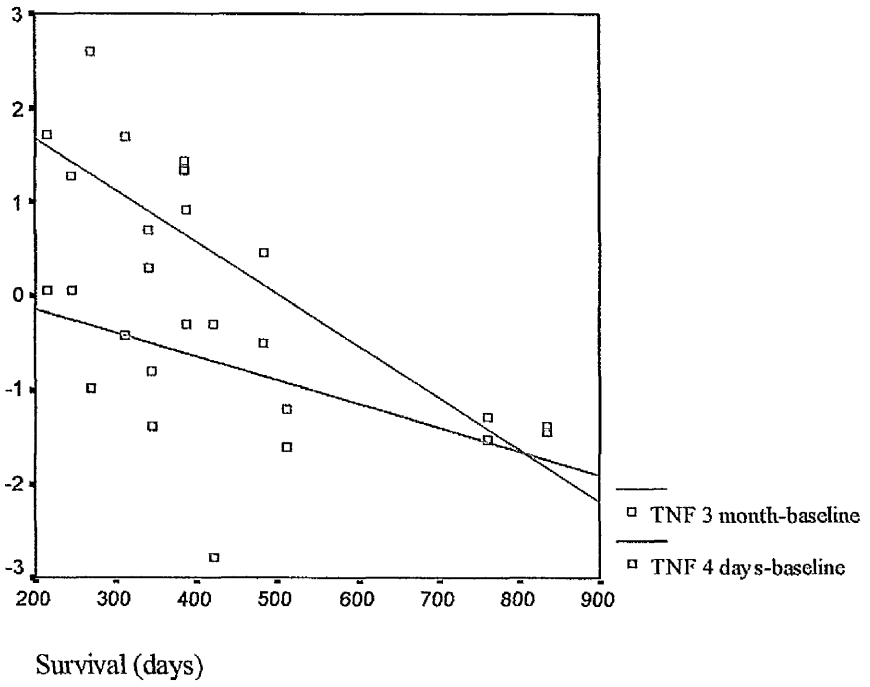

Figure 47: Correlation of change in TNF-α levels from baseline to four days, and from baseline to three months, with survival

8.5 Discussion

8.5.1 Haemostatic markers in patients with stable compared to progressive breast cancer

Prolonged APTT is an independent predictor of reduced survival in both disseminated small cell and non-small cell lung cancer, but not colon cancer (Wojtukiewicz *et al.* 1992). Prolonged PT is also associated with a poor prognosis in lung cancer (Buccheri *et al.* 1997). This current study, although not powered for such sub-group analysis, identified an association between prolonged PT and reduced survival, supporting the previous findings in lung cancer.

Low levels of circulating TAT are associated with an increased chance of remission in 99 newly-diagnosed lung cancer patients prior to treatment (Seitz *et al.* 1997). In this present study, the association of increased baseline TAT with shortened survival, provides further evidence for TAT as a predictive marker of cancer outcome.

Increased fibrinogen and D-dimer have also been associated with reduced survival in early (Buccheri *et al.* 1997) and advanced lung cancer (Wojtukiewicz *et al.* 1992), advanced colorectal (Wojtukiewicz *et al.* 1992) and ovarian (Koh *et al.* 2001; von Tempelhoff *et al.* 1997) cancer. The latter two studies are weakened by the inclusion of early as well as advanced ovarian cancer patients. In the present study a significant increase of baseline levels of intravascular fibrin formation, in advanced breast cancer with early progression, compared to advanced breast cancer patients with stable disease is not detected. Numbers in this sub-group are limited, however a possible trend for increased levels is apparent.

High *tissue* levels of tPA have been reported as an independent predictor of disease-free and overall survival in breast cancer patients (Duffy *et al.* 1988). Conversely, high levels of tissue uPA is established as a poor prognostic indicator in primary breast cancer (Duffy 2002). High tissue concentrations of PAI-1, an endogenous inhibitor of uPA, also correlate with poor prognosis in patients with breast cancer (Duffy 2002). Increased *circulating* uPA receptor has been found to correlate with reduced survival in colorectal cancer (Stephens *et al.* 1999), and is an independent predictor of reduced survival in rectal cancer (Fernebro *et al.* 2001). There is currently no published data on the prognostic significance of circulating tPA, uPA or PAI-1. In this study a trend for increased levels of uPA, and particularly tPA, in advanced breast cancer patients progressing within three months is identified. As patient numbers are limited, no predictive value for progression is established.

A significant increase in PAI-1 in advanced cancer patients progressing within three and six months, compared to those with stable disease is identified. Increased levels of PAI-1 correlated with reduced survival. An upregulation of the fibrinolytic system in advanced cancer patients with a poor prognosis is apparent from these results.

Thrombocytosis has previously been reported to be associated with poor survival in cancer. Pedersen and co-workers, in a large cohort of 1115 consecutive primary lung cancer patients, found thrombocytosis to be an independent prognostic factor for survival (Pedersen & Milman 1996). Cox found a raised preoperative platelet count to be associated with poor prognosis in non-small cell lung cancer, however in Cox's study no correction was made for cancer stage (Cox *et al.* 2000). In the current study no alteration in platelet count or function (as expressed by VEGF release per platelet) in advanced

breast cancer patients progressing within six months, compared to advanced breast cancer patients with stable disease, was detected. There was a significant reduction in platelet release of VEGF in early breast cancer patients progressing within two years compared to those in remission. This finding is based on five of 77 early breast cancer patients progressing. With these small numbers, definitive conclusions should be made with caution.

CRP approached significance for increased levels in those advanced cancer patients progressing within three months. Elevated CRP is reported to have poor prognostic significance heralding the recurrence of cancer (Nowacki, Janik, & Nowacki 1996). Raised CRP is a predictor of reduced survival in ovarian and colorectal cancer (Kodama *et al.* 1999; Nozoe *et al.* 1998) and an independent predictor of poor survival in both pancreatic and renal cell carcinoma (Barber *et al.* 1999; Hoffmann *et al.* 1999). Albuquerque and colleagues found serum CRP to be predictive of survival in 85 consecutive newly diagnosed metastatic breast cancer patients, however there was no correlation between CRP levels and response to therapy (Albuquerque *et al.* 1995).

8.5.2 Response of haemostatic markers to chemotherapy in patients with stable compared to progressive breast cancer

In advanced cancer patients with more aggressive disease, there is an absence of an early reduction in APTT in response to chemotherapy (this early reduction in APTT occuring in both advanced breast cancer patients with stable disease, and early breast cancer patients). The subsequent further prolongation by three months, in advanced cancer patients with more aggressive disease, is in keeping with Wojtukiewicz' previous

findings in lung cancer patients (Wojtukiewicz *et al.* 1992). It is interesting, however, that in patients with responsive or stable disease, the prolonged baseline APTT is rapidly shortened by treatment. This shortening of APTT appears to predict for tumour response to treatment. The trend in APTT in response to treatment in advanced breast cancer patients with stable disease is similar to that of early breast cancer patients (ie cancer-free controls), implying the early decrease in APTT is induced by chemotherapy, and the prolongation of APTT at three months, in patients with progression, appears to be a cancer induced effect.

The marked decrease in TAT, fibrinogen and D-dimer (and to a lesser extent, tPA) in patient progressing within six months indicates a significantly different haemostatic reaction in advanced breast cancer patients with stable/responsive disease compared to those with aggressive disease, resistant to treatment. This response occurs within four days of treatment and may represent consumption of haemostatic factors, in keeping with the ongoing, relatively prolonged APTT. However, decreases in TAT, fibrinogen and D-dimer are seen as a response to chemotherapy in early breast cancer patients. It is the early *relative* increase in TAT, fibrinogen and D-dimer, occurring in advanced breast cancer patients responding to chemotherapy, that is unusual. These results suggest that the *chemotherapy* induced haemostatic changes may be masked in the presence of advanced breast cancer patients responding to treatment, and there may be a relative upregulation of haemostatic markers in response to chemotherapy- induced cancer cell death.

Although pre-chemotherapy levels of PAI-1 are raised in patients who subsequently progress, it is surprising that a decrease in these levels, at three months, is associated with reduced survival. This finding warrants further clarification.

8.5.3 Procoagulants and endothelial adhesion molecules in patients with stable compared to progressive breast cancer

There was no significant difference in TF and TSP-1 levels in either advanced or early breast cancer patients with progressive compared to stable disease.

CP activity in leukaemic cells has previously been reported to parallel the course of disease, with an increase in activity prior to disease relapse (Donati *et al.* 1990). In the current study groups, reduced CP levels were found in patients who subsequently progressed, however this finding was not statistically significant. Nevertheless, the finding is consistent with reduced CP correlating with reduced survival, and implies that CP activity decreases with more aggressive or end-stage disease.

Normal levels of TNF-α have been reported as a good prognostic indicator in previously untreated renal cell carcinoma patients (Dosquet *et al.* 1997). In the present study no prognostic value for TNF-α prior to chemotherapy in either early or advanced breast cancer patients was identified.

In this study, although both pVEGF and sVEGF are increased in advanced breast cancer patients with progressive disease, compared to stable disease, the increased levels are not significant, and do not predict for progression or survival. Dirix has previously demonstrated increased levels of sVEGF in metastatic cancer patients showing

subsequent rapid compared to slow progression, however his study group of 132 patients contained colorectal, breast, ovarian and renal carcinomas (Dirix *et al.* 1997).

Increased VCAM-1 has been correlated with poor survival in metastatic malignant melanoma (Franzke *et al.* 1998), gastric (Velikova *et al.* 1997) and colorectal cancer (Alexiou *et al.* 2001), however in the latter two studies analysis was not performed independently of tumour stage. Similar results have been found with E-selectin. Elevated serum levels are associated with poor prognosis in gastric (Benekli *et al.* 1998) and non-small cell lung cancer (Tsumatori *et al.* 1999), however only the latter study confirms this finding independently of cancer stage. In 456 node-negative breast cancer patients, raised (pre-surgery) circulating E-selectin was found to be a strong prognostic factor for reduced survival on multivariate analysis (Hebbar *et al.* 1999). In a study performed by the same group, E-selectin did not predict for reduced survival in 113 metastatic breast cancer patients (Hebbar & Peyrat 2000). In the present study VCAM-1, but not E-selectin, demonstrated a trend for increased levels in advanced breast cancer patients progressing within six months, and early breast cancer patients progressing within two years. VCAM-1 also demonstrated potential as a marker for reduced survival.

8.5.4 Response of procoagulants and endothelial adhesion molecules to chemotherapy in patients with stable compared to progressive breast cancer

The correlation of reduced survival with increasing TNF-α from baseline to six months is in keeping with progressive disease, as end-stage cancer with the onset of cachexia, is associated with increased TNF-α (Knapp *et al.* 1991). The correlation of reduced survival with increased TNF-α from baseline to four days implies release of TNF-α following

chemotherapy is associated with a poor outcome, or conversely, uptake of TNF-α is associated with an improved outcome. A possible early response of TNF-α may provide predictive information on response to chemotherapy and cancer outcome.

The more marked decrease in sVEGF in patients with progressive disease, in response to chemotherapy, implies a reduction in platelet content of VEGF (the major source of sVEGF). Background pVEGF does not appear to have a different response to chemotherapy in patients responding or resistant to treatment. The reduction in platelet content, therefore, is not secondary to release of VEGF from platelets into the plasma. However, it is possible a difference is not detected in pVEGF, as levels are much lower and thus greater patient numbers may be required to reach significance. Plasma VEGF increases in the eight days following chemotherapy, irrespective of cancer stage, possibly as a result of chemotherapy-induced increased platelet release of VEGF. The decrease in sVEGF in the progressive cancers may reflect a greater release of VEGF from platelets (not detected as an increase in pVEGF in this study, possibly due to small numbers), and hence a more labile platelet response to chemotherapy in such patients. The lack of alteration in pVEGF may be a true finding, in which case the decrease in sVEGF is not simply due to platelet release into plasma, but may also reflect increased tumour cell uptake of this released VEGF by the more aggressive cancers in response to chemotherapy.

The significant decrease in sVEGF in patients with progressive disease predicts for cancer progression and thus may have a role as a surrogate marker of the response or resistance of cancer cells to treatment.

A hypothesis of this study was that the altered response to chemotherapy seen in advanced breast cancer patients would be greatest in patients with increased cell death (ie stable disease/response to treatment), with subsequent tumour-procoagulant release into the circulation. Here we provide evidence that the greatest alteration in procoagulants is seen with sVEGF and TNF-α only. This occurs in patients with progressive disease, and so does not support the hypothesis.

The decrease in VCAM-1 between baseline and three months in early breast cancer patients that progress by two years warrants further investigation as a potential marker of high risk patients. This study was not powered to identify such changes, and with our limited numbers of early breast cancer patients progressing, these results have to be viewed as exploratory in nature and requiring further confirmation.

8.6 Conclusion

Prolonged PT, increased TAT, uPA, tPA and PAI-1, and a trend for increased fibrinogen and D-dimer were all associated with progression or reduced survival in advanced breast cancer. This demonstrates a more marked upregulation of the haemostatic and especially fibrinolytic, systems in patients with more aggressive and poor prognostic breast cancer. However, in this study there is no identified increased rate of VTE in patients with more aggressive cancers. A biochemical profile to identify patients with a poorer prognosis may allow targeting of specific treatment.

The marked decrease in markers of coagulation in advanced breast cancer with progressive disease supports the hypothesis that in the presence of effective

chemotherapy (ie response to treatment/stable disease) there is an altered haemostatsic response compared to patients in whom chemotherapy is ineffective. The marked alterations in the haemostatic system occurring in advanced breast cancer with progressive disease are similar to chemotherapy-induced changes in the early breast cancer control group. The patients with advanced breast cancer responsive to chemotherapy (despite less marked decreases from baseline) have a different haemostatic response to chemotherapy than the early breast cancer control group. However this is not reflected in an increased rate of VTE in our study. The altered haemostatic response in advanced breast cancer patients responding to treatment supports the hypothesis that chemotherapy-induced cancer-cell death stimulates an upregulated haemostatic response, possibly through tumour procoagulant release.

Only VEGF (plasma and serum) and VCAM-1 demonstrated trends for increased levels at baseline in advanced breast cancer patients who subsequently progress. Both VEGF and VCAM-1 may have a role as predictive markers for cancer outcome.

Unlike the haemostatic markers, none of the procoagulants or endothelial adhesion molecules in this study demonstrated a relatively upregulated response to chemotherapy in advanced breast cancer patients responding to chemotherapy. A tumour procoagulant released from cancer cells as a response to chemotherapy-induced cell death is not identified in this study.

There is an altered response of sVEGF (possibly due to alteration in platelet function or tumour uptake of VEGF) in advanced breast cancer patients not responding to chemotherapy.

SECTION 4

CONCLUSION

In this thesis, national and regional surveys were performed to establish current thromboprophylaxis practice in breast surgery and in oncology respectively. A general understanding of the relative risks for the development of VTE following breast surgery exists amongst surgeons, with most employing risk-stratification. However a lack of consensus exists in prescribing policy for VTE prophylaxis amongst surgeons operating on women with breast disease within the UK, with up to 6% not using prophylaxis even on high-risk cases. Only 1% of respondents referred to a hospital policy. Respondents believed a high percentage of their patients received prophylaxis, however this is dependent on effective prescription of medical prophylaxis by junior doctors, or mechanical prophylaxis by nurses, theatre staff and compliant patients.

Over one quarter of oncologists surveyed do not recognise the significant thrombogenic effects of cancer treatments. Thromboprophylaxis is rarely used in cancer patients undergoing treatment.

In both surveys, risk of VTE was perceived to be low. Overt VTE in breast surgery or oncology is rare. VTE is often clinically silent or has a delayed presentation to other physicians. A reduction in rate of VTE would be difficult to appreciate in an individual clinician's practice however a bleeding complication is less likely to occur unnoticed.

VTE as a complication of breast cancer surgery or chemotherapy is still poorly documented, with no formal method of reporting. A national database for recording confirmed VTE would allow identification of risk factors and thus stratification of at risk patients into a specifically designed prophylaxis protocol.

The prospective study of chemotherapy in advanced and early breast cancer patient receiving chemotherapy identified a VTE rate of 17% and 8% respectively, with VTE-

related mortality of 8% in advanced breast cancer patients. Two thirds of VTEs occurred within three months of commencing chemotherapy. This study confirms previously published data despite the lower than expected recruitment rate. Chemotherapy is therefore a significant risk factor for VTE, but this risk is substantially elevated in the presence of cancer.

Retrospective evidence supports a worse outcome when VTE and cancer coexist as compared to either diagnosis alone (Morgan *et al.* 2002; Seward *et al.* 1999; Sorensen *et al.* 2000). This current study provides some evidence to support this. VTE within three months following cancer chemotherapy suggests a poorer outcome ($p=0.07$). However chemotherapy-induced VTE also occurs in early breast cancer patients with a good prognosis. VTE has substantial associated morbidity and mortality. In the presence of an otherwise good prognostic cancer, thromboprophylactic measures may prevent this serious complication.

An upregulation of the haemostatic system (markers of both coagulation and fibrinolysis) is demonstrated in advanced breast cancer, in keeping with the increased VTE rate in these patients. The procoagulants TSP-1, TF and VEGF are increased in advanced breast cancer, with the latter two correlating with markers of haemostasis. The endothelial adhesion molecule VCAM-1 is also increased in advanced breast cancer.

The minimal upregulation of markers of haemostasis, as well as procoagulants and endothelial adhesion molecules, in the early breast cancer group supports early breast cancer patients as a suitable "cancer-free group" receiving chemotherapy. Despite this minimal upregulation of haemostasis, the prolonged correlation of D-dimer with tumour

stage, and days since surgery in early breast cancer demonstrates that recent surgery may be an added VTE risk-factor for up to six weeks following surgery.

Chemotherapy stimulates a rapid alteration in markers of haemostasis, occurring within 24 hours, in both early and advanced breast cancer. This indicates the almost instantaneous hypercoagulable effect of chemotherapy, irrespective of the presence of cancer, or chemotherapy-induced cancer cell lysis. Several markers of haemostasis (D-dimer, fibrinogen, PF1+2, tPA and uPA) are elevated prior to chemotherapy, in patients developing VTE. Other haemostatic markers (PT, APTT and TAT) demonstrate an, almost immediate, altered response to chemotherapy in patients developing VTE. This indicates that a biochemical profile could be developed to identify patients at high risk of VTE, allowing targeted prophylaxis. It also demonstrates that the hypercoagulable effect occurs very rapidly. This raises the possibility that negating this hypercoagulable reaction at chemotherapy induction, with a single administration of anticoagulant, may be sufficient to inhibit the coagulation response. Plasma VEGF may provide insight into the trigger for chemotherapy-induced hypercoagulability. Levels are increased prior to chemotherapy, and rapidly decrease following chemotherapy, in patients developing VTE. This may reflect tumour or platelet uptake of VEGF, and hence a prothrombotic alteration in cellular (tumour or platelet) function.

Chemotherapy stimulates a marked reduction in circulating endothelial adhesion molecules, possibly due to increased uptake by vascular endothelial cells. The decrease in both VCAM-1 and E-selectin, is not significantly different in patients developing VTE compared to those remaining free of VTE. This provides evidence that chemotherapy-

induced vascular endothelial cell activation is not responsible for chemotherapy-induced hypercoagulability.

The upregulation of the haemostatic system is most marked in advanced breast cancer patients with rapidly progressive disease. Markers of coagulation (TAT, fibrinogen and D-dimer) show an overall decrease in the four to eight days following chemotherapy in the early breast cancer (control) group, however this decrease is absent in advanced breast cancer patients with disease responsive to chemotherapy. The relative upregulation of haemostatic markers in patients with chemotherapy-induced cell death provides evidence that apoptotic or lysed cells may initiate chemotherapy induced VTE in the presence of cancer.

There is an altered response of sVEGF, and therefore possibly platelet function or tumour uptake of VEGF, in patients with more aggressive cancer. However, in this study procoagulants or endothelial adhesion molecules do not demonstrate a greater release or uptake in advanced breast cancer patients responding to chemotherapy, compared to those resistant to chemotherapy. We do not identify a procoagulant molecule, released into the circulation and initiating chemotherapy-induced hypercoagulability as a result of chemotherapy-induced cell death.

VTE is a common and potentially preventable consequence of breast cancer chemotherapy, especially in advanced disease. A biochemical profile based on haemostatic markers such as D-dimer, fibrinogen and early alterations in PT and APTT may allow identification of high-risk patients to target prophylaxis. The rapid initiation of chemotherapy-induced hypercoagulability may facilitate the use of very short-term or

single dose thromboprophylaxis with chemotherapy administration. The hypercoagulability of cancer appears closely related to more aggressive disease. Chemotherapy-induced hypercoagulability is not caused by endothelial cell activation, but may be enhanced by chemotherapy-induced cell death.

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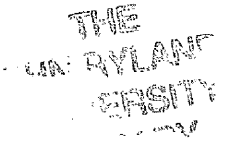

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APPENDIX 1

Venous Thromboembolism (VTE) Prophylaxis in Breast Cancer Surgery

A Questionnaire

Consultants name (optional).....

Type of Hospital DGH ☐ Teaching ☐

Years in post..... **Speciality** Breast ☐ Mixed ☐

Number of breast patients operated on per year (estimate) .....

1. In the following three clinical scenarios please select the VTE prophylactic protocol used for your patients (please tick).

i) A 35 year old lady, previously fit and well, undergoing excision of a breast lump that is a fibroadenoma on clinical examination.

No prophylaxis ☐ Subcutaneous Heparin ☐ TED stockings ☐

Aspirin ☐ Pneumatic compression stockings in theatre ☐

Other (please specify) .....

ii) A 55 year old lady, previously fit and well, undergoing wide local excision of a breast lump that is a TII, NI carcinoma on clinical examination.

No prophylaxis ☐ Subcutaneous Heparin ☐ TED stockings ☐

Aspirin ☐ Pneumatic compression stockings in theatre ☐

Other (please specify) .....

iii) A 70 year old lady, with known ischaemic heart disease, undergoing mastectomy and axillary node clearance following a partial response to neoadjuvant chemotherapy for an inflammatory carcinoma.

No prophylaxis ☐ Subcutaneous Heparin ☐ TED stockings ☐

Aspirin ☐ Pneumatic compression stockings in theatre ☐

Other (please specify) .....

2. Does risk of haematoma influence your choice of VTE prophylaxis?

Yes - I do not use prophylaxis ☐

Yes - I use non-anticoagulant prophylaxis (eg stockings) ☐

No ☐

3. Do you use pre-operative hormone therapy (prior to definitive ER status)?

Yes ☐ No ☐

4. Do you stop hormone therapy preoperatively if patients require further surgery?

Yes ☐ No ☐

5. Which of the following VTE risk factors would make you alter your prophylaxis practise? (Please grade as 0=not influence practice, 1=minor influence, 2=intermediate, 3=major influence)

Never use VTE prophylaxis ☐ Previous VTE ☐ Thrombophilia ☐

Family history of VTE ☐ Obesity ☐ Hormone therapy ☐

Immobility ☐ Age ☐ Other (Please specify).....

What is your estimate of the number of postoperative VTE events in your breast patients in one year.....

Please estimate the percentage of your breast patients receiving VTE prophylaxis for surgery.....

Thank you for your time.

APPENDIX 2

Venous Thromboembolism (VTE) Prophylaxis in Cancer Treatment

A Questionnaire

Please tick the relevant box

Consultant's name (optional).....

Years in post..... Speciality/Subspeciality.....

Type of Hospital DGH ☐ Teaching ☐

What are the main tumours that you treat?
Haematological ☐ Specify.....
Solid ☐ Specify.....

What are the main cancer treatments you use (1=most frequent, 3= least frequent)?
Chemotherapy ☐ Hormone ☐ Radiotherapy ☐

Do you consider VTE to be a significant risk in your patients during treatment?
Yes ☐ No ☐

Do you think the following treatments increase the risk of VTE?
1. Chemotherapy:
Not at all ☐ a little ☐ substantially ☐

2. Hormone treatment:
Not at all ☐ a little ☐ substantially ☐

3. Radiotherapy:
Not at all ☐ a little ☐ substantially ☐

Do you routinely prescribe VTE prophylaxis for patients undergoing treatment?
1. Chemotherapy: Yes ☐ No ☐
2. Hormone treatment: Yes ☐ No ☐
3. Radiotherapy: Yes ☐ No ☐

If yes, what do you use (may mark more than one)
Aspirin ☐ Stockings ☐ S/c heparin ☐ Warfarin ☐
Other ☐
Specify.....

If no, what indications would you routinely use for VTE prophylaxis? (may tick more than one)
Never use VTE prophylaxis ☐ Previous VTE ☐
Family history of VTE ☐ Thrombophilia ☐
Obesity ☐ Hormone therapy ☐ Immobility ☐
Age ☐ Other ☐
Specify.....

Please estimate the percentage of your patients on VTE prophylaxis .....

Thank you for your time.

Appendix 3: WHO Performance Status in cancer patients (World Health Organisation (WHO) 1979)

Status	*Description*
0	Asymptomatic, fully active and able to carry out all pre-disease performance without restrictions
1	Symptomatic, fully ambulatory but restricted in physically strenuous activity, able to carry out performance activity of a light or sedentary nature e.g. light housework, office work
2	Symptomatic, ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours: in bed less than 50% of the day
3	Symptomatic, capable of only limited self-care, confined to bed or chair more than 50% of the waking hours, but not bedridden
4	Completely disabled. Cannot carry out any self-care. Totally bedridden

Appendix 4: Assessment of response to chemotherapy

Complete response	No clinically detectable tumour
Partial response	Reduction by at least 50% of a measurable lesion
Stabilisation of disease	Decrease of less than 50% or an increase of less than 25% over the original tumour measurements
Subjective response	Patient felt better and significant pain relief was obtained
Progression of disease	Increase by 25% or greater of the original measurements or significant deterioration of symptoms and performance status

Appendix 5: Definition of Nottingham Prognostic Index (Miller *et al.* 1994)

NPI = (0.2 x size, cm) + lymph node stage + grade

NPI 1=<3.4 (good prognosis)

NPI 2=3.5-5.3 (moderate prognosis)

NPI 3=>5.4 (poor prognosis)

Lymph node stage:

1=none

2=low axillary

3=apical / internal mammary

Grade:

1=well differentiated

2=intermediate differentiation

3=poorly differentiated

Appendix 6: Chemotherapies used in breast cancer treatment, divided into class

Alkylating	*Cytotoxic antibiotic*	*Antimetabolite*	*Vinca alkaloid*	*Taxane*
Cyclophoshamide	Epirubicin	Methotrexate	Vinorelbine	Paclitaxel
	Doxorubicin	Fluorouracil		Docetaxel
	Mitomycin			

Appendix 7: Association between age, menopausal status and markers of the haemostatic system, prior to chemotherapy

Pre and post menopausal levels compared using an independent T-test

Coagulation marker	*Pre menopause* ***(n)***	*Post menopause* ***(n)***	*p*	*Correlation with age* ***(n)*** *(Spearman coefficient)*
PT secs, mean (SD)	11.6 (11.4-11.8) ***(76)***	11.6 (11.4-11.7) ***(81)***	*0.8*	0.3 ***(157)***
APTT secs, mean (SD)	22.1 (21.6-22.6) ***(76)***	22.6 (21.9-23.2) ***(81)***	*0.2*	0.5 ***(157)***
PF1+2 nmol/l, geometric mean (CI)	0.89 (0.53-1.52) ***(8)***	1.04 (0.82-1.32) ***(13)***	*0.5*	0.3 ***(21)***
TAT µg/ml, median (range)	3.0 (2.0-25.0) ***(10)***	4.7 (2.4-28.1) ***(31)***	*0.05*	0.15 ***(41)***
Fibrinogen g/L, mean (SD)	3.14 (2.92-3.37) ***(74)***	3.47 (3.16-3.77) ***(74)***	*0.09*	0.08 ***(148)***
D-dimer ng/ml, geometric mean (CI)	439 (372-519) ***(98)***	691 (582-819) ***(102)***	*<0.001*	<0.001 ***(200)*** (0.27)
tPA ng/ml, geometric mean (CI)	6694 (5505-8138) ***(28)***	8760 (7677-9995) ***(39)***	*0.2*	0.06 ***(67)***
uPA ng/ml, geometric mean (CI)	0.40 (0.21-0.76) ***(24)***	0.46 (0.27-0.77) ***(35)***	*0.7*	0.5 ***(59)***
PAI-1 ng/ml, geometric mean (CI)	24.0 (10.4-55.5) ***(8)***	16.1 (12.9-20.0) ***(13)***	*0.2*	0.4 ***(21)***
Platelet count $x10^9$/l, mean (SD)	297 (280-314) ***(92)***	314 (296-332) ***(95)***	*0.2*	0.2 ***(187)***

Coagulation marker	*Pre menopause* ***(n)***	*Post menopause* ***(n)***	*p*	*Correlation with age* ***(n)*** *(Spearman coefficient)*
Platelet function-VEGF μg/ml per platelet $x10^9$, geometric mean (CI)	0.53 (0.43-0.64) ***(84)***	0.66 (0.55-0.79) ***(91)***	*0.09*	0.3 ***(175)***
CRP mg/l, median (range)	3.1 (0.8-16.0) *(7)*	6.7 (0.5-226) ***(10)***	*0.8*	0.06 *(17)*

Appendix 8: Association between oestrogen receptor status and markers of the haemostatic system

Analysis compares markers of haemostasis, prior to chemotherapy, in patients with oestrogen receptor positive and oestrogen receptor negative primary tumours. Comparison is made using a Mann-Whitney or independent T-test. Mean and geometric mean and 95% confidence interval, or median and range.

Coagulation marker	*Oestrogen receptor positive (n)*	*Oestrogen receptor negative (n)*	*p*
PT secs, mean (CI)	11.8 (11.6-12.0) *(72)*	11.5 (11.3-11.7) *(40)*	*0.06*
APTT secs, mean (CI)	22.8 (22.2-23.4) *(72)*	23.2 (22.4-24.1) *(40)*	*0.4*
PF1+2 nmol/l, geometric mean (CI)	0.98 (0.65-1.48) *(9)*	0.68 (0.49-0.95) *(5)*	*0.2*
TAT µg/ml, median (range)	4.3 (2.2-280.6) *(17)*	4.9 (2.5-25.0) *(11)*	*0.7*
Fibrinogen g/L, mean (CI)	3.5 (3.2-3.8) *(68)*	3.7 (3.2-4.1) *(35)*	*0.4*
D-dimer ng/ml, geometric mean (CI)	756 (629-908) *(84)*	792 (632-992) *(48)*	*0.8*
tPA ng/ml, geometric mean (CI)	9896 (8165-11994) *(24)*	8932 (6634-11325) *(16)*	*0.4*
uPA ng/ml, geometric mean (CI)	0.74 (0.43-1.29) *(24)*	0.60 (0.29-1.22) *(15)*	*0.6*
PAI-1 ng/ml, geometric mean (CI)	19.2 (9.9-37.1) *(9)*	19.4 (6.5-68.1) *(5)*	*1.0*
Platelet count $x10^9$/l, mean (CI)	317 (296-338) *(85)*	315 (293-337) *(49)*	*0.9*
Platelet function-VEGF µg/ml per platelet $x10^9$, geometric mean (CI)	0.60 (0.50-0.72) *(82)*	0.67 (0.52-0.85) *(45)*	*0.5*
CRP mg/l, median (range)	2.1 (0.5-35.7) *(8)*	5.6 (0.8-10.5) *(2)*	*0.7*

Appendix 9: Association between progesterone receptor status and markers of the haemostatic system

Analysis compares markers of haemostasis, prior to chemotherapy, in patients with progesterone receptor positive and progesterone receptor negative primary tumours. Comparison is made using a Mann-Whitney or independent T-test. Mean and geometric mean and 95% confidence interval, or median and range.

Coagulation marker	*Progesterone receptor positive (n)*	*Progesterone receptor negative (n)*	*p*
PT secs, mean (CI)	11.7 (11.5-11.9) ***(55)***	11.6 (11.4-11.8) ***(56)***	*0.3*
APTT secs, mean (CI)	22.9 (22.3-23.6) ***(55)***	23.0 (22.2-23.7) ***(56)***	*0.9*
PF1+2 nmol/l, geometric mean (CI)	1.05 (0.62-1.80) *(7)*	0.70 (0.56-0.89) *(7)*	*0.1*
TAT μg/ml, median (range)	4.1 (2.2-280.6) ***(14)***	4.7 (2.5-24.9) ***(13)***	*0.8*
Fibrinogen g/L, mean (CI)	3.5 (3.2-3.8) ***(53)***	3.6 (3.2-4.0) ***(50)***	*0.8*
D-dimer ng/ml, geometric mean (CI)	720 (581-894) ***(66)***	800 (667-960) ***(65)***	*0.5*
tPA ng/ml, geometric mean (CI)	9561 (7625-20568) ***(20)***	9213 (7381-11500) ***(20)***	*0.8*
uPA ng/ml, geometric mean (CI)	0.64 (0.36-1.13) ***(20)***	0.73 (0.37-1.43) ***(19)***	*0.8*
PAI-1 ng/ml, geometric mean (CI)	17.2 (7.2-41.2) *(7)*	21.6 (10.7-43.7) *(7)*	*0.6*
Platelet count $x10^9$/l, mean (CI)	314 (290-339) ***(67)***	316 (297-336) ***(66)***	*0.9*
Platelet function-VEGF μg/ml per platelet $x10^9$, geometric mean (CI)	0.61 (0.50-0.74) ***(64)***	0.64 (0.52-0.79) ***(62)***	*0.7*
CRP mg/l, median (range)	1.1 (0.5-35.7) *(7)*	3.1 (0.8-10.5) *(3)*	*0.8*

Appendix 10: Association between Her 2 neu receptor status and markers of the haemostatic system

Analysis compares markers of haemostasis, prior to chemotherapy, in patients with Her 2 neu receptor positive and Her 2 neu receptor negative primary tumours. Comparison is made using a Mann-Whitney or independent T-test. Mean and geometric mean and 95% confidence interval, or median and range.

Coagulation marker	*Her 2 neu receptor positive (n)*	*Her 2 neu receptor negative (n)*	*p*
PT secs, mean (CI)	11.7 (11.5-12.0) ***(26)***	11.6 (11.4-11.8) ***(45)***	*0.5*
APTT secs, mean (CI)	23.2 (22.2-24.1) ***(26)***	22.2 (21.4-23.0) ***(45)***	*0.1*
PF1+2 nmol/l, geometric mean (CI)	0.74 (0.43-0.77) ***(3)***	0.76 (0.43-1.33) ***(5)***	*1.0*
TAT µg/ml, median (range)	4.6 (2.5-24.9) ***(8)***	7.0 (3.5-23.4) ***(9)***	*0.2*
Fibrinogen g/L, mean (CI)	3.2 (2.8-3.6) ***(22)***	3.9 (3.5-4.4) ***(43)***	*0.03*
D-dimer ng/ml, geometric mean (CI)	768 (573-1028) ***(30)***	865 (676-1106) ***(55)***	*0.5*
tPA ng/ml, geometric mean (CI)	8127 (6292-10495) ***(12)***	10528 (7937-13963) ***(16)***	*0.2*
uPA ng/ml, geometric mean (CI)	0.56 (0.22-1.45) ***(12)***	0.73 (0.32-1.67) ***(16)***	*0.7*
PAI-1 ng/ml, geometric mean (CI)	20.9 (4.0-108.7) ***(3)***	28.0 (8.5-92.7) ***(5)***	*0.7*
Platelet count $x10^9$/l, mean (CI)	312 (284-341) ***(30)***	322 (296-348) ***(55)***	*0.6*
Platelet function-VEGF µg/ml per platelet $x10^9$, geometric mean (CI)	0.67 (0.47-0.95) ***(27)***	0.69 (0.56-0.86) ***(54)***	*0.9*
CRP mg/l, median (range)	*N/A(1)*	*N/A(4)*	*N/A*

Appendix 11: Association between Nottingham Prognostic Index, tumour size and markers of the haemostatic system (early breast cancer patients only)

Grades compared using an independent T-test

Coagulation marker	*NPI Grade 1 and 2 (**n**)*	*NPI Grade 3 (**n**)*	*p*	*Correlation with tumour size-p (**n**)(Spearman coefficient)*
PT secs, mean (CI)	11.7 (11.4-11.9) *(**44**)*	11.7 (11.4-12.0) *(**33**)*	*0.8*	0.5 *(**77**)*
APTT secs, mean (CI)	23.5 (22.7-24.3) *(**44**)*	22.7 (22.0-23.5) *(**33**)*	*0.2*	0.7 *(**77**)*
PF1+2 nmol/l, geometric mean (CI)	1.05 (0.47-2.36) *(**5**)*	0.84 *(**2**)*	*0.7*	0.6 *(**7**)*
TAT µg/ml, median (range)	3.6 (2.5-22.2) *(**6**)*	4.1 (2.5-5.6) *(**5**)*	*0.8*	0.2 *(**11**)*
Fibrinogen g/L, mean (CI)	3.5 (3.1-3.8) *(**41**)*	3.0 (2.8-3.3) *(**32**)*	*0.04*	0.2 *(**73**)*
D-dimer ng/ml, geometric mean (CI)	585 (492-695) *(**51**)*	809 (659-993) *(**36**)*	*0.02*	0.01 *(**85**)* (0.27)
tPA ng/ml, geometric mean (CI)	9240 (6656-12827) *(**11**)*	7258 (5712-9222) *(**9**)*	*0.2*	0.6 *(**20**)*
uPA ng/ml, geometric mean (CI)	1.07 (0.47-2.44) *(**10**)*	0.57 (0.17-1.95) *(**9**)*	*0.3*	0.5 *(**19**)*

Coagulation marker	*NPI Grade 1 and 2 (n)*	*NPI Grade 3 (n)*	*p*	*Correlation with tumour size-p (n)(Spearman coefficient)*
PAI-1 ng/ml, geometric mean (CI)	12.5 (4.5-34.9) *(5)*	15.8 *(2)*	*0.7*	0.4 *(7)*
Platelet count $x10^9$/l, mean (CI)	303 (283-323) *(51)*	319 (290-348) *(36)*	*0.4*	0.3 *(87)*
Platelet function-VEGF µg/ml per platelet $x10^9$, geometric mean (CI)	0.52 (0.42-0.64) *(50)*	0.55 (0.42-0.73) *(32)*	*0.7*	0.8 *(83)*
CRP mg/l, median (range)	*N/A(5)*	*N/A(1)*		0.2 *(6)*

Appendix 12: Alterations in haemostatic markers induced by chemotherapy in breast cancer patients

Coagulation marker	*Pre-chemotherapy (n)*	*Day 1 (post chemotherapy) (n)*	*Day 4 (post chemotherapy) (n)*	*Day 8 (post chemotherapy) (n)*	*3 months (post chemotherapy) (n)*	*6 months (post chemotherapy) (n)*	*Significant trend over time? p(repeated measures),(n) i) pre-chemo to day 8 ii) pre-chemo to 3 months iii) pre-chemo to 6 months*
PT secs, mean (CI)	11.7 (11.5-11.8) *(112)*	11.9 (11.7-12.0) *(116)*	11.7 (11.6-11.9) *(113)*	11.8 (11.7-12.0) *(116)*	11.5 (11.3-11.6) *(106)*	11.6 (11.4-11.8) *(99)*	i) 0.002 (88) ii) 0.01 (79) iii) 0.01 (70)
APTT secs, mean (CI)	22.9 (22.5-23.4) *(112)*	21.6 (21.1-22.0) *(116)*	21.4 (21.0-21.8) *(113)*	21.8 (21.3-22.2) *(116)*	21.1 (20.6-21.6) *(108)*	21.4 (20.9-21.9) *(100)*	i) <0.001(88) ii) <0.001(80) iii) <0.001(71)
PF1+2 nmol/l, geometric mean (CI)	1.08 (0.90-1.30) *(30)*	1.14 (0.97-1.35) *(30)*	1.02 (0.86-1.22) *(28)*	1.16 (0.94-1.43) *(29)*	1.09 (0.90-1.31) *(29)*	1.24 (0.94-1.63) *(19)*	i) 0.4 (27) ii) 0.6 (26) iii) 0.3 (15)
TAT µg/ml, geometric mean (CI)	6.5 (4.3-9.6) *(28)*	10.5 (5.8-18.9) *(27)*	6.5 (4.6-9.1) *(26)*	5.5 (4.0-7.4) *(26)*	5.9 (3.6-9.8) *(23)*	5.4 (3.3-9.0) *(21)*	i) 0.1 (26) ii) 0.1 (22) iii) 0.2 (21)
Fibrinogen g/L mean (CI)	3.5 (3.3-3.8) *(103)*	3.3 (3.0-3.5) *(106)*	3.2 (2.9-3.4) *(105)*	3.5 (3.2-3.7) *(112)*	4.1 (3.8-4.4) *(99)*	3.4 (3.2-3.7) *(96)*	i) <0.001(74) ii) <0.001(62) iii) <0.001(53)
D-dimer ng/ml, geometric mean (CI)	567 (659-880) *(127)*	788 (679-916) *(119)*	735 (633-863) *(118)*	750 (645-863) *(119)*	765 (659-889) *(113)*	518 (446-608) *(106)*	i) 0.3 (115) ii) 0.3 (109) iii) <0.001 (103)

tPA ng/ml, geometric mean (CI)	9414 (8103-10938) *(40)*	10405 (8691-12457) *(38)*	8111 (3793-9680) *(36)*	9160 (7739-10829) *(37)*	8946 (7281-11004) *(33)*	9750 (7864-12125) *(21)*	i) <0.001(36) ii) 0.002(31) iii) 0.03(21)
uPA ng/ml, geometric mean (CI)	0.68 (0.45-1.04) *(39)*	0.47 (0.29-0.79) *(37)*	0.46 (0.29-0.73) *(35)*	0.53 (0.36-0.78) *(36)*	0.82 (0.62-1.08) *(33)*	1.00 (0.67-1.48) *(23)*	i) 0.2 (35) ii) 0.01 (31) iii) 0.01 (22)
PAI-1 ng/ml, geometric mean (CI)	18.7 (14.8-23.6) *(30)*	17.5 (13.8-22.3 *(29)*	14.7 (11.4-18.9) *(24)*	16.5 (12.3-22.2) *(29)*	17.6 (13.4-23.1) *(29)*	11.2 (8.2-15.5) *(20)*	i) 0.3 (23) ii) 0.4 (22) iii) 0.06 (13)
Platelet count x10^9/l mean (CI)	316 (301-332) *(134)*	308 (290-327) *(106)*	(265 (252-279) *(114)*	244 (230-258) *(126)*	320 (296-345) *(122)*	260 (245-275) *(118)*	i) <0.001(97) ii) <0.001(89) iii) <0.001(87)
Platelet function geometric mean (CI)	0.62 (0.54-0.72) *(127)*	0.61 (0.52-0.71) *(102)*	0.51 (0.43-0.61) *(109)*	0.53 (0.45-0.64) *(117)*	0.72 (0.62-0.83) *(111)*	0.59 (0.50-0.62) *(107)*	i) <0.001(91) ii) <0.001(84) iii) <0.001(80)
CRP mg/l, geometric mean (CI)	5.0 (2.7-9.3) *(26)*	5.0 (2.8-8.8) *(26)*	3.8 (2.0-7.2) *(24)*	3.4 (1.8-6.5) *(25)*	2.5 (1.3-4.6) *(25)*	2.9 (1.0-7.8) *(15)*	i) 0.1 (23) ii) 0.05 (22) iii) 0.2 (14)

Appendix 13: Alterations in haemostatic markers induced by chemotherapy in advanced breast cancer patients

Coagulation marker	*Pre-chemo (n)*	*Day 1 (post chemo) (n)*	*Day 4 (post chemo) (n)*	*Day 8 (post chemo) (n)*	*3 months (post chemo) (n)*	*6 months (post chemo) (n)*	*Significant difference in trend compared to early breast cancer patients? p, (n-advanced, early)* *i) pre-chemo to day 8* *ii) pre-chemo to 3 months* *iii) pre-chemo to 6 months*
PT secs, mean (CI)	11.7 (11.4-12.1) *(26)*	12.1 (11.8-12.4) *(28)*	11.9 (11.5-12.3) *(31)*	11.9 (11.5-12.3) *(31)*	11.9 (11.5-12.3) *(22)*	11.9 (11.5-12.2) *(17)*	i) 0.1 (19,65) ii) 0.004 (16,59) iii) 0.03 (12,54)
APTT secs, mean (CI)	22.6 (21.4-23.7) *(26)*	21.5 (20.7-22.3) *(28)*	21.9 (20.8-21.7) *(29)*	22.5 (21.5-23.5) *(31)*	22.3 (21.0-23.7) *(23)*	21.7 (20.1-23.3) *(18)*	i) 0.2 (19,65) ii) 0.002 (16,60) iii) 0.001 (12,55)
PF1+2 nmol/l, geometric mean (CI)	0.93 (0.77-1.13) *(16)*	1.03 (0.87-1.23) *(16)*	1.09 (0.90-1.31) *(15)*	1.24 (0.89-1.73) *(15)*	1.15 (0.86-1.52) *(15)*	1.50 (0.83-2.73) *(8)*	i) 0.02 (14,13) ii) 0.1 (13,13) iii) 0.1 (5,10)
TAT µg/ml, geometric mean (CI)	9.2 (4.7-18.2) *(14)*	12.4 (6.1-25.5) *(13)*	8.0 (4.8-13.3) *(13)*	8.0 (4.7-13.6) *(13)*	4.9 (3.5-6.8) *(9)*	8.8 (2.3-5.5) *(8)*	i) 0.6 (13,11) ii) 0.6 (9,11) iii) 0.7 (8,11)
Fibrinogen g/L mean (CI)	4.5 (3.7-5.3) *(21)*	4.5 (3.9-5.1) *(25)*	4.6 (4.0-5.1) *(27)*	4.4 (3.9-5.0) *(29)*	4.9 (4.0-5.8) *(18)*	4.8 (4.0-5.7) *(17)*	i) 0.04 (13,57) ii) 0.01(9,49) iii) 0.6 (6,43)

D-dimer ng/ml, geometric mean (CI)	1339 (973-1845) *(35)*	1510 (1097-2059) *(33)*	1556 (1153-2101) *(33)*	1353 (982-1863) *(33)*	1394 (992-1939) *(26)*	1176 (773-1772) *(22)*	i) 0.05 (31,76) ii) 0.4 (25,76) iii) 0.2 (21,75)
tPA ng/ml, geometric mean (CI)	11188 (8591-14574) *(16)*	11499 (8425-15709) *(15)*	8835 (6555-11908) *(15)*	9349 (6905-12645) *(15)*	10625 (7122-15851) *(11)*	12577 (4770-33153) *(5)*	i) 0.08 (15,18) ii) 0.3 (11,17) iii) 0.7 (5,15)
uPA ng/ml, geometric mean (CI)	0.57 (0.28-1.15 *(16)*	0.59 (0.28-1.25) *(15)*	0.50 (0.22-1.15) *(14)*	0.48 (0.23-1.0) *(15)*	1.00 (0.57-1.75) *(11)*	0.98 (0.58-1.65) *(7)*	i) 0.2 (14,18) ii) 0.2 (10,18) iii) 0.2 (6,15)
PAI-1 ng/ml, geometric mean (CI)	22.2 (15.6-31.5) *(16)*	11.8 (12.8-27.7 *(15)*	17.8 (12.1-26.4) *(14)*	21.3 (13.2-34.4) *(15)*	21.1 (14.2-31.3) *(15)*	13.2 (7.2-24.3) *(8)*	i) 0.8 (13,10) ii) 0.9 (12,10) iii) 0.05 (5,8)
Platelet count x10^9/l mean (CI)	327 (287-366) *(36)*	336 (284-387) *(28)*	(300 (281-319) *(31)*	250 (220-280) *(33)*	302 (195-410) *(26)*	242 (226-257) *(24)*	i) 0.01 (25,67) ii) 0.06 (17,67) iii) 0.007 (15,67)
Platelet function geometric mean (CI)	1.02 (0.80-1.30) *(35)*	0.92 (0.68-1.23) *(28)*	0.85 (0.61-1.17) *(30)*	0.92 (0.72-1.19) *(31)*	1.03 (0.77-1.39) *(25)*	1.01 (0.73-1.40) *(22)*	i) 0.007 (23,63) ii) 0.03 (16,62) iii) 0.05 (14,62)
CRP mg/l, geometric mean (CI)	9.7 (4.6-20.6) *(13)*	8.8 (4.2-18.3) *(13)*	6.8 (2.5-18.4) *(12)*	7.7 (3.1-19.3) *(12)*	3.4 (1.4-8.2) *(12)*	11.3 (2.8-46.5) *(7)*	i) 0.3 (11,12) ii) 0.4 (10,12) iii) 0.2 (6,8)

Appendix 14: Alterations in haemostatic markers induced by chemotherapy in early breast cancer patients

Coagulation marker	*Pre-chemo (n)*	*Day 1 (post chemo) (n)*	*Day 4 (post chemo) (n)*	*Day 8 (post chemo) (n)*	*3 months (post chemo) (n)*	*6 months (post chemo) (n)*	*Significant difference in trend compared to advanced breast cancer patients? p (repeated measures, (n-advanced, early)* *i) pre-chemo to day 8* *ii) pre-chemo to 3 months* *iii) pre-chemo to 6 months*
PT secs, mean (CI)	11.7 (11.5-11.8) *(77)*	11.8 (11.7-11.9) *(80)*	11.8 (11.6-12.0) *(78)*	11.8 (11.7-12.0) *(77)*	11.4 (11.2-11.6) *(76)*	11.6 (11.4-11.7) *(75)*	i) 0.1 (19,65) ii) 0.004 (16,59) iii) 0.03 (12,54)
APTT secs, mean (CI)	23.2 (22.6-23.7) *(77)*	21.6 (21.0-22.1) *(80)*	21.3 (20.8-21.7) *(78)*	21.5 (21.0-22.0) *(77)*	20.6 (20.1-21.1) *(77)*	21.4 (20.9-21.9) *(75)*	i) 0.2 (19,65) ii) 0.002 (16,60) iii) 0.001 (12,55)
PF1+2 nmol/l, geometric mean (CI)	1.28 (0.91-1.81) *(14)*	1.29 (0.95-1.75) *(14)*	0.95 (0.67-1.35) *(13)*	1.07 (0.80-1.44) *(14)*	1.02 (0.78-1.35) *(14)*	1.08 (0.82-1.42) *(11)*	i) 0.02 (14,13) ii) 0.1 (13,13) iii) 0.1 (5,10)
TAT µg/ml, geometric mean (CI)	4.2 (2.8-6.4) *(11)*	10.0 (2.8-35.5) *(11)*	5.3 (2.8-9.8) *(11)*	3.6 (2.7-4.8) *(11)*	4.9 (2.1-11.7) *(11)*	3.9 (2.8-34.8) *(11)*	i) 0.6 (13,11) ii) 0.6 (9,11) iii) 0.7 (8,11)
Fibrinogen g/L mean (CI)	3.3 (3.1-3.5) *(73)*	2.8 (2.7-3.0) *(73)*	2.7 (2.5-2.8) *(72)*	3.6 (2.7-4.8) *(75)*	3.9 (3.6-4.1) *(73)*	3.1 (2.8-3.3) *(73)*	i) 0.04 (13,57) ii) 0.01(9,49) iii) 0.6 (6,43)

D-dimer ng/ml, geometric mean (CI)	652 (572-750) *(82)*	652 (567-750) *(77)*	590 (508-685) *(77)*	633 (550-728) *(78)*	645 (545-765) *(78)*	407 (268-464) *(77)*	i) 0.05 (31,76) ii) 0.4 (25,76) iii) 0.2 (21,75)
tPA ng/ml, geometric mean (CI)	8288 (6790-10117) *(20)*	10270 (8029-13148) *(19)*	7303 (5590-9643) *(18)*	8928 (6956-11458) *(19)*	8193 (6021-11148) *(18)*	9015 (7428-10938) *(15)*	i) 0.08 (15,18) ii) 0.3 (11,17) iii) 0.7 (5,15)
uPA ng/ml, geometric mean (CI)	0.79 (0.41-1.54 *(19)*	0.38 (0.16-0.92) *(18)*	0.44 (0.22-0.90) *(18)*	0.60 (0.35-1.03) *(18)*	0.81 (0.54-1.22) *(18)*	1.02 (0.56-1.86) *(15)*	i) 0.2 (14,18) ii) 0.2 (10,18) iii) 0.2 (6,15)
PAI-1 ng/ml, geometric mean (CI)	15.4 (11.3-21.0) *(14)*	16.3 (11.8-22.6 *(14)*	11.2 (8.9-14.2) *(10)*	12.6 (9.1-17.6) *(14)*	14.6 (9.8-21.8) *(14)*	10.2 (6.7-15.5) *(12)*	i) 0.8 (13,10) ii) 0.9 (12,10) iii) 0.05 (5,8)
Platelet count $x10^9$/l mean (CI)	310 (293-326) *(87)*	321 (273-370) *(71)*	(339 (234-444) *(77)*	269 (254-284) *(84)*	244 (207-281) *(86)*	268 (205-331) *(85)*	i) 0.01 (25,67) ii) 0.06 (17,67) iii) 0.007 (15,67)
Platelet function geometric mean (CI)	0.53 (0.45-0.62) *(82)*	0.51 (0.42-0.63) *(67)*	0.43 (0.35-0.52) *(73)*	0.43 (0.34-0.53) *(78)*	0.65 (0.54-0.77) *(78)*	0.52 (0.43-0.63) *(78)*	i) 0.007 (23,63) ii) 0.03 (16,62) iii) 0.05 (14,62)
CRP mg/l, geometric mean (CI)	2.6 (1.0-6.6) *(13)*	2.8 (1.2-6.6) *(13)*	2.1 (0.9-4.9) *(12)*	1.6 (0.7-3.5) *(13)*	1.8 (0.7-4.7) *(13)*	0.9 (0.4-2.0) *(8)*	i) 0.3 (11,12) ii) 0.4 (10,12) iii) 0.2 (6,8)

Appendix 15: Association between oestrogen receptor status and procoagulants and endothelial adhesion molecules

Analysis compares circulating procoagulants and endothelial adhesion molecules, prior to chemotherapy, in patients with oestrogen receptor positive and oestrogen receptor negative primary tumours. Comparison is made using an independent T-test. Geometric mean and 95% confidence interval.

Procoagulant/ adhesion molecule	*Oestrogen receptor positive (**n**)*	*Oestrogen receptor negative (**n**)*	*p*
TF μg/ml, geometric mean (CI)	115.4 (88.1-151.2) *(84)*	112.8 (76.5-166.2) *(47)*	*0.9*
CP mU, geometric mean (CI)	32.3 (29.7-35.1) *(76)*	31.1 (28.0-34.6) *(43)*	*0.6*
TSP-1 ng/ml, geometric mean (CI)	497 (334-739) *(32)*	1220 (856-1739) *(14)*	*0.007*
TNF-α μg/ml, geometric mean (CI)	3.14 (2.77-3.57) *(45)*	3.64 (3.13-4.25) *(23)*	*0.2*
pVEGF μg/ml, geometric mean (CI)	17.4 (14.6-20.7) *(83)*	14.9 (12.3-18.2) *(47)*	*0.3*
sVEGF μg/ml, geometric mean (CI)	204 (174-240) *(85)*	230 (182-289) *(46)*	*0.4*
VCAM-1 ng/ml, geometric mean (CI)	629 (576-688) *(84)*	684 (618-756) *(48)*	*0.2*
E-selectin ng/ml, geometric mean (CI)	29.9 (26.7-33.5) *(85)*	28.7 (24.4-33.7) *(47)*	*0.7*

Appendix 16: Association between progesterone receptor status and procoagulants and endothelial adhesion molecules

Analysis compares circulating procoagulants and endothelial adhesion molecules, prior to chemotherapy, in patients with progesterone receptor positive and progesterone receptor negative primary tumours. Comparison is made using an independent T-test. Geometric mean and 95% confidence interval.

Procoagulant/ adhesion molecule	*Progesterone receptor positive (**n**)*	*Progesterone receptor negative (**n**)*	*p*
TF μg/ml, geometric mean (CI)	119.7 (90.3-158.8) ***(66)***	107.9 (76.2-152.7) ***(64)***	*0.6*
CP mU, geometric mean (CI)	32.0 (29.1-35.1) ***(58)***	32.0 (29.1-35.0) ***(60)***	*1.0*
TSP-1 ng/ml, geometric mean (CI)	546 (335-891) ***(24)***	785 (512-1204) ***(21)***	*0.3*
TNF-α μg/ml, geometric mean (CI)	3.19 (2.78-3.65) ***(36)***	3.44 (2.96-3.99) ***(32)***	*0.4*
pVEGF μg/ml, geometric mean (CI)	18.7 (15.2-23.0) ***(65)***	14.3 (12.2-16.9) ***(64)***	*0.05*
sVEGF μg/ml, geometric mean (CI)	206 (171-249) ***(67)***	220 (182-266) ***(63)***	*0.6*
VCAM-1 ng/ml, geometric mean (CI)	617 (559-681) ***(66)***	678 (619-742) ***(65)***	*0.2*
E-selectin ng/ml, geometric mean (CI)	30.3 (27.0-34.0) ***(67)***	28.4 (24.5-32.9) ***(64)***	*0.5*

Appendix 17: Association between Her 2 neu receptor status and procoagulants and endothelial adhesion molecules

Analysis compares circulating procoagulants and endothelial adhesion molecules, prior to chemotherapy, in patients with Her 2 neu receptor positive and Her 2 neu receptor negative primary tumours. Comparison is made using an independent T-test. Geometric mean and 95% confidence interval.

Procoagulant/ adhesion molecule	*Her 2 neu receptor positive (**n**)*	*Her 2 neu receptor negative (**n**)*	*p*
TF μg/ml, geometric mean (CI)	141.4 (105.2-190.0) ***(29)***	106.5 (72.9-155.7) ***(55)***	*0.3*
CP mU, geometric mean (CI)	33.7 (28.6-39.7) ***(25)***	30.2 (27.8-32.9) ***(51)***	*0.2*
TSP-1 ng/ml, geometric mean (CI)	927 (438-1962) ***(9)***	756 (380-1501) ***(16)***	*0.7*
TNF-α μg/ml, geometric mean (CI)	3.27 (2.63-4.06) ***(17)***	3.36 (2.89-3.91) ***(29)***	*0.8*
pVEGF μg/ml, geometric mean (CI)	15.3 (12.5-18.9) ***(28)***	15.9 (12.9-19.7) ***(54)***	*0.8*
sVEGF μg/ml, geometric mean (CI)	231 (172-312) ***(29)***	238 (194-291) ***(55)***	*0.9*
VCAM-1 ng/ml, geometric mean (CI)	741 (636-863) ***(30)***	617 (556-684) ***(55)***	*0.04*
E-selectin ng/ml, geometric mean (CI)	28.1 (22.8-34.6) ***(30)***	30.3 (26.0-35.3) ***(54)***	*0.6*

Appendix 18: Association between Nottingham Prognostic Index, tumour size and procoagulants and endothelial adhesion molecules (early breast cancer only)

Grade compared using an independent T-test

Procoagulant/ adhesion molecule	*NPI Grade 1 and 2 (**n**)*	*NPI Grade 3 (**n**)*	*p*	*Correlation with tumour size-p (**n**)(Spearman coefficient)*
TF μg/ml, geometric mean (CI)	96.7 (61.5-152.0) ***(50)***	85.8 (57.8-127.6) ***(35)***	*0.7*	0.9 ***(85)***
CP mU, geometric mean (CI)	31.4 (28.4-34.7) ***(46)***	35.7 (31.8-40.0) ***(32)***	*0.09*	0.6 ***(78)***
TSP-1 ng/ml, geometric mean (CI)	651 (368-1153) ***(19)***	553 (295-1036) ***(13)***	*0.7*	0.5 ***(32)***
TNF-α μg/ml, geometric mean (CI)	3.30 (2.90-3.76) ***(25)***	3.04 (2.29-4.04) ***(17)***	*0.5*	0.5 ***(42)***
pVEGF μg/ml, geometric mean (CI)	14.0 (11.3-17.3) ***(50)***	14.5 (11.3-18.7) ***(34)***	*0.8*	0.6 ***(86)***
sVEGF μg/ml, geometric mean (CI)	172 (141-211) ***(51)***	194 (153-246) ***(35)***	*0.4*	1.0 ***(87)***
VCAM-1 ng/ml, geometric mean (CI)	616 (560-678) ***(50)***	645 (581-718) ***(36)***	*0.5*	0.4 ***(86)***
E-selectin ng/ml, geometric mean (CI)	30.0 (26.7-33.9) ***(51)***	25.3 (21.2-30.1) ***(36)***	*0.08*	0.7 ***(87)***

Appendix 19: Alterations in procoagulants and endothelial adhesion molecules induced by chemotherapy in breast cancer patients

Procoagulant/ adhesion molecule	*Pre-chemotherapy (n)*	*Day 1 (post chemotherapy) (n)*	*Day 4 (post chemotherapy) (n)*	*Day 8 (post chemotherapy) (n)*	*3 months (post chemotherapy) (n)*	*6 months (post chemotherapy) (n)*	*Significant trend over time? p (repeated measures,(n)* *i) pre-chemo to day 8* *ii) pre-chemo to 3 months* *iii) pre-chemo to 6 months*
TF µg/ml, geometric mean (CI)	114.4 (92-142) *(131)*	115 (91-145) *(125)*	97 (77-123) *(121)*	98 (79-123) *(124)*	96 (76-122) *(115)*	122 (96-156) *(109)*	i) 0.3 (120) ii) 0.5 (113) iii) 0.06 (107)
CP mU, geometric mean (CI)	31.8 (29.9-34.0) *(119)*	36.9 (34.7-39.3) *(117)*	34.7 (32.7-37.0) *(111)*	32.3 (30.2-34.4) *(115)*	31.3 (29.3-33.4) *(108)*	33.8 (31.4-36.3) *(101)*	i) 0.001 (110) ii) <0.001 (103) iii) 0.002 (97)
TSP-1 ng/ml, geometric mean (CI)	652 (478-898) *(46)*	652 (455-944) *(49)*	602 (441-821) *(47)*	443 (321-608) *(44)*	796 (578-1097) *(50)*	679 (469-992) *(39)*	i) 0.3 (38) ii) 0.5 (34) iii) 0.8 (25)
TNF-α µg/ml, geometric mean (CI)	3.30 (3.00-3.64) *(28)*	3.16 (2.83-3.53) *(27)*	2.97 (2.67-3.30 *(26)*	2.90 (2.62-3.22) *(26)*	3.47 (3.09-3.90) *(23)*	3.40 (3.03-3.80) *(21)*	i) 0.002 (63) ii) <0.001 (60) iii) 0.001 (54)

Procoagulant/ adhesion molecule	*Pre-chemotherapy (n)*	*Day 1 (post chemotherapy) (n)*	*Day 4 (post chemotherapy) (n)*	*Day 8 (post chemotherapy) (n)*	*3 months (post chemotherapy) (n)*	*6 months (post chemotherapy) (n)*	*Significant trend over time? p (repeated measures),(n) i) pre-chemo to day 8 ii) pre-chemo to 3 months iii) pre-chemo to 6 months*
pVEGF µg/ml, geometric mean (CI)	16.4 (14.4-18.8) *(130)*	14.6 (12.9-16.5) *(124)*	15.9 (13.7-18.4) *(121)*	20.8 (18.6-23.3) *(122)*	22.1 (19.1-25.5) *(114)*	20.9 (18.3-24.0) *(109)*	i) <0.001(119) ii) <0.001(112) iii) <0.001(107)
sVEGF µg/ml, geometric mean (CI)	213 (187-243) *(131)*	203 (177-232) *(123)*	158 (137-183) *(122)*	155 (134-180) *(124)*	245 (214-281) *(118)*	179 (156-204) *(111)*	i) <0.001(118) ii) <0.001(111) iii) <0.001(105)
VCAM-1 ng/ml, geometric mean (CI)	649 (607-693) *(132)*	589 (548-633) *(124)*	603 (563-645) *(121)*	581 (545-620) *(124)*	651 (599-708) *(114)*	655 (602-713) *(110)*	i) <0.001(118) ii) <0.001(110) iii) <0.001(105)
E-selectin ng/ml, geometric mean (CI)	29.5 (26.9-32.3) *(132)*	28.3 (25.7-31.1) *(126)*	25.1 (22.9-27.6) *(122)*	22.3 (20.3-24.5) *(124)*	26.6 (24.2-29.2) *(117)*	30.4 (27.3-33.9) *(110)*	i) <0.001(121) ii) <0.001(114) iii) <0.001(108)

Appendix 20: Alterations in procoagulants and endothelial adhesion molecules induced by chemotherapy in advanced breast cancer patients

Procoagulant/ adhesion molecule	*Pre-chemotherapy (n)*	*Day 1 (post chemotherapy) (n)*	*Day 4 (post chemotherapy) (n)*	*Day 8 (post chemotherapy) (n)*	*3 months (post chemotherapy) (n)*	*6 months (post chemotherapy) (n)*	*Significant difference in trend compared to early breast cancer patients? p,(repeated measures) (n-advanced, early)* *i) pre-chemo to day 8* *ii) pre-chemo to 3 months* *iii) pre-chemo to 6 months*
TF μg/ml, geometric mean (CI)	179 (140-230) *(36)*	173 (132-228) *(35)*	150 (117-193) *(34)*	166 (131-210) *(34)*	133 (86-205) *(26)*	213 (158-287) *(21)*	i) 0.7 (33,79) ii) 0.8 (26,79) iii) 0.8 (21,78)
CP mU, geometric mean (CI)	28.3 (24.8-32.3) *(33)*	33.2 (28.9-38.2) *(32)*	28.4 (24.6-32.7) *(30)*	27.4 (23.3-32.2) *(31)*	27.0 (22.1-33.1) *(24)*	24.4 (20.7-28.8) *(19)*	i) 0.3 (29,74) ii) 0.4 (23,73) iii) 0.02 (18,73)
TSP-1 ng/ml, geometric mean (CI)	1094 (829-1437) *(11)*	852 (459-1572) *(11)*	756 (324-1755) *(11)*	659 (279-1556) *(9)*	1826 (770-4359) *(8)*	821 (578-1176) *(5)*	i) 0.6 (7,28) ii) 0.6 (4,27) iii) 0.8 (2,21)
TNF-α μg/ml, geometric mean (CI)	3.46 (2.90-4.14) *(22)*	3.83 (3.20-4.59) *(21)*	3.47 (2.95-4.09) *(19)*	3.13 (2.58-3.82) *(21)*	3.95 (3.08-5.05) *(16)*	4.21 (3.29-5.41) *(13)*	i) 0.06 (19,41) ii) 0.2 (16,41) iii) 0.6 (13,39)

Procoagulant/ adhesion molecule	*Pre-chemotherapy (n)*	*Day 1 (post chemotherapy) (n)*	*Day 4 (post chemotherapy) (n)*	*Day 8 (post chemotherapy) (n)*	*3 months (post chemotherapy) (n)*	*6 months (post chemotherapy) (n)*	*Significant difference in trend compared to early breast cancer patients? p, (n-advanced, early) i) pre-chemo to day 8 ii) pre-chemo to 3 months iii) pre-chemo to 6 months*
pVEGF µg/ml, geometric mean (CI)	22.8 (17.6-29.4) *(36)*	19.7 (15.6-25.2) *(35)*	22.0 (15.8-30.6) *(34)*	27.5 (23.1-32.7) *(34)*	24.0 (18.5-31.1) *(26)*	31.5 (23.1-42.8) *(22)*	i) 0.8 (33,78) ii) 0.1 (26,78) iii) 0.3 (22,77)
sVEGF µg/ml, geometric mean (CI)	344 (271-437) *(35)*	287 (219-376) *(33)*	240 (182-317) *(33)*	253 (196-327) *(33)*	344 (248-477) *(26)*	283 (203-394) *(22)*	i) 0.01 (31,79) ii) 0.2 (24,79) iii) 0.2 (20,78)
VCAM-1 ng/ml, geometric mean (CI)	733 (617-870) *(36)*	688 (572-827) *(34)*	694 (589-819) *(33)*	619 (531-722) *(33)*	642 (499-826) *(25)*	767 (583-1010) *(21)*	i) 0.2 (31,79) ii) 0.07 (24,79) iii) 0.01 (20,78
E-sel ng/ml, geometric mean (CI)	33.0 (26.4-41.2) *(35)*	33.3 (26.5-41.8) *(34)*	32.2 (26.3-39.4) *(33)*	24.0 (19.0-30.3) *(33)*	30.1 (23.2-38.9) *(25)*	34.5 (24.8-47.8) *(21)*	i) <0.001 (32,81) ii) 0.09 (25,81) iii) 0.2 (21,80)

Appendix 21: Alterations in procoagulants and endothelial adhesion molecules induced by chemotherapy in early breast cancer patients

Procoagulant/ adhesion molecule	*Pre-chemotherapy (n)*	*Day 1 (post chemotherapy) (n)*	*Day 4 (post chemotherapy) (n)*	*Day 8 (post chemotherapy) (n)*	*3 months (post chemotherapy) (n)*	*6 months (post chemotherapy) (n)*	*Significant difference in trend compared to advanced breast cancer patients? p,(repeated measures) (n- advanced, early) i) pre-chemo to day 8 ii) pre-chemo to 3 months iii) pre-chemo to 6 months*
TF µg/ml, geometric mean (CI)	92 (68-125) *(85)*	98 (71-135) *(81)*	81 (59-112) *(79)*	78 (58-105) *(81)*	84 (63-112) *(81)*	103 (76-138) *(80)*	i) 0.7 (33,79) ii) 0.8 (26,79) iii) 0.8 (21,78)
CP mU, geometric mean (CI)	33.1 (30.7-35.7) *(78)*	37.8 (35.2-40.6) *(77)*	37.2 (34.9-39.7) *(74)*	34.0 (31.7-36.5) *(77)*	32.2 (30.1-34.4) *(76)*	36.3 (33.6-39.1) *(76)*	i) 0.3 (29,74) ii) 0.4 (23,73) iii) 0.02 (18,73)
TSP-1 ng/ml, geometric mean (CI)	610 (407-911) *(32)*	706 (450-1108) *(35)*	567 (392-812) *(33)*	402 (279-584) *(32)*	713 (503-1012) *(39)*	659 (416-1033) *(32)*	i) 0.6 (7,28) ii) 0.6 (4,27) iii) 0.8 (2,21)
TNF-α µg/ml, geometric mean (CI)	3.19 (2.80-3.64) *(42)*	2.87 (2.50-3.30) *(41)*	2.83 (2.49-3.23) *(41)*	2.85 (2.52-3.22) *(41)*	3.41 (2.97-3.92) *(41)*	3.24 (2.87-3.66) *(39)*	i) 0.06 (19,41) ii) 0.2 (16,41) iii) 0.6 (13,39)

Procoagulant/ adhesion molecule	Pre-chemotherapy (n)	Day 1 (post chemotherapy) (n)	Day 4 (post chemotherapy) (n)	Day 8 (post chemotherapy) (n)	3 months (post chemotherapy) (n)	6 months (post chemotherapy) (n)	Significant difference in trend compared to advanced breast cancer patients? p, (n-advanced, early) i) pre-chemo to day 8 ii) pre-chemo to 3 months iii) pre-chemo to 6 months
pVEGF µg/ml, geometric mean (CI)	14.2 (12.1-16.6) *(84)*	13.1 (11.5-15.1) *(80)*	14.3 (12.1-16.8) *(78)*	18.1 (15.7-20.9) *(80)*	20.9 (17.6-24.8) *(80)*	19.4 (16.6-22.6) *(79)*	i) 0.8 (33,78) ii) 0.1 (26,78) iii) 0.3 (22,77)
sVEGF µg/ml, geometric mean (CI)	181 (156-210) *(86)*	177 (151-207) *(81)*	134 (114-157) *(81)*	127 (107-151) *(83)*	211 (181-246) *(83)*	158 (137-183) *(82)*	i) 0.01 (31,79) ii) 0.2 (24,79) iii) 0.2 (20,78)
VCAM-1 ng/ml, geometric mean (CI)	628 (586-675) *(86)*	572 (524-608) *(81)*	583 (541-627) *(80)*	572 (533-618) *(82)*	663 (607-724) *(82)*	640 (584-700) *(81)*	i) 0.2 (31,79) ii) 0.07 (24,79) iii) 0.01 (20,78
E-sel ng/ml, geometric mean (CI)	28.0 (25.3-30.9) *(87)*	26.1 (23.5-29.0) *(83)*	22.9 (20.6-25.4) *(81)*	21.2 (19.1-23.6) *(83)*	25.3 (22.8-28.2) *(83)*	29.0 (25.8-32.8) *(82)*	i) <0.001 (32,81) ii) 0.09 (25,81) iii) 0.2 (21,80)

Appendix 22: Alterations in haemostatic markers induced by chemotherapy in breast cancer patients developing VTE within three months of commencing chemotherapy

Coagulation marker	*Pre-chemo (n)*	*Day 1 (post chemo) (n)*	*Day 4 (post chemo) (n)*	*Day 8 (post chemo) (n)*	*3 months (post chemo) (n)*	*6 months (post chemo) (n)*	*Significant difference in trend compared to breast cancer patients remaining free of VTE? p, (n- VTE, no VTE) i) pre-chemo to day 8 ii) pre-chemo to 3 months iii) pre-chemo to 6 months*
PT secs, mean (CI)	11.6 (11.0-12.3) *(7)*	11.9 (11.4-12.4) *(9)*	11.9 (11.3-12.6) *(9)*	12.2 (11.3-13.1) *(8)*	12.1 (11.0-13.1) *(5)*	11.9 (10.7-13.2) *(5)*	i) 0.005 (6,82) ii) 0.03 (4,75) iii) 0.07 (4,66)
APTT secs, mean (CI)	21.5 (17.2-25.8) *(7)*	19.6 (17.7-21.6) *(9)*	20.7 (18.7-22.6) *(9)*	21.5 (19.0-24.0) *(8)*	21.4 (18.4-24.4) *(7)*	21.5 (18.3-24.7) *(6)*	i) 0.2 (6,82) ii) 0.2 (5,75) iii) 0.1 (5,66)
PF1+2 nmol/l, geometric mean (CI)	1.92 (0.91-4.07) *(5)*	1.89 (0.831-4.26) *(5)*	1.79 (0.19-16.6) *(3)*	1.81 (0.48-6.89) *(5)*	0.75 (0.25-2.18) *(5)*	1.05 (0.54-2.04) *(5)*	i) 0.6 (3,24) ii) 0.001 (3,23) iii) 0.001 (3,12)
TAT µg/ml, geometric mean (CI)	12.9 (2.3-72.2) *(6)*	22.9 (3.9-135.6) *(6)*	5.2 (2.1-12.9) *(6)*	5.9 (2.0-17.1) *(6)*	3.7 (1.9-7.4) *(5)*	4.8 (1.3-17.5) *(4)*	i) 0.1 (6,20) ii) 0.1 (5,17) iii) 0.1 (4,17)
Fibrinogen g/L mean(CI)	5.1 (2.8-7.5) *(6)*	4.7 (3.2-6.2) *(7)*	4.4 (3.0-5.8) *(9)*	4.4 (3.0-5.7) *(7)*	6.0 (4.5-7.5) *(6)*	4.5 (2.0-7.0) *(5)*	i) 0.9 (4,70) ii) 0.7(3,59) iii) 0.2 (2,51)
D-dimer ng/ml, geometric mean (CI)	1652 (837-3262) *(9)*	1808 (963-3361) *(9)*	1588 (626-4024) *(9)*	1588 (699-3605) *(9)*	1097 (464-2592) *(7)*	441 (284-692) *(6)*	i) 0.9 (9,109) ii) 0.4 (7,105) iii) 0.02 (6,100)

tPA ng/ml, geometric mean (CI)	14412 (8982-23126) *(4)*	15678 (8708-28396) *(4)*	13812 (6227-30638) *(4)*	15057 (7911-28653) *(4)*	16269 (1056-250697) *(3)*	11294 (1686-75358) *(3)*	i) 0.7 (4,32) ii) 0.7 (3,28) iii) 0.08 (3,18)
uPA ng/ml, geometric mean (CI)	2.13 (0.70-6.49 *(4)*	1.58 (0.19-13.1) *(4)*	1.41 (0.17-11.4) *(4)*	1.43 (0.34-6.06) *(4)*	1.01 (0.56-1.81) *(3)*	1.73 (0.25-12.0) *(3)*	i) 0.9 (4,31) ii) 0.6 (3,28) iii) 0.7 (3,19)
PAI-1 ng/ml, geometric mean (CI)	16.3 (8.9-29.7) *(5)*	21.6 (13.4-35.0 *(5)*	11.7 (2.6-53.0) *(2)*	21.9 (12.6-38.1) *(5)*	16.9 (8.4-34.1) *(5)*	17.3 (7.1-41.7) *(5)*	i) 0.6 (2,21) ii) 0.7 (2,20) iii) 0.5 (2,11)
Platelet count $x10^9$/l mean (CI)	348 (251-446) *(9)*	353 (254-452) *(9)*	278 (230-326) *(9)*	292 (221-362) *(8)*	402 (173-630) *(7)*	319 (157-481) *(6)*	i) 0.4 (8,89) ii) 0.5 (6,83) iii) 0.2 (5,82)
Platelet function geometric mean (CI)	0.89 (0.42-1.87) *(9)*	0.89 (0.47-1.68) *(9)*	0.86 (0.39-1.86) *(9)*	0.83 (0.39-1.75) *(8)*	1.02 (0.53-1.97) *(7)*	0.66 (0.36-1.24) *(6)*	i) 0.4 (8,84) ii) 0.3 (6,80) iii) 0.3 (5,77)
CRP mg/l, geometric mean (CI)	5.9 (1.7-20.7) *(5)*	5.3 (1.7-16.9) *(5)*	3.0 (0.2-38.1) *(3)*	5.8 (1.4-23.8) *(5)*	3.9 (0.3-55.9) *(5)*	2.1 *(2)*	i) 0.9 (3,20) ii) 1.0 (3,19) iii) 1.0 (2,12)

Appendix 23: Alterations in haemostatic markers induced by chemotherapy in breast cancer patients remaining free of VTE within the first three months of chemotherapy

Coagulation marker	*Pre-chemo (n)*	*Day 1 (post chemo) (n)*	*Day 4 (post chemo) (n)*	*Day 8 (post chemo) (n)*	*3 months (post chemo) (n)*	*6 months (post chemo) (n)*	*Significant difference in trend compared to breast cancer patients developing VTE?* *p, (n- VTE, no VTE)* *i) pre-chemo to day 8* *ii) pre-chemo to 3 months* *iii) pre-chemo to 6 months*
PT secs, mean (CI)	11.7 (11.5-11.8) *(105)*	11.9 (11.7-12.0) *(107)*	11.7 (11.5-11.8) *(104)*	11.8 (11.6-11.9) *(108)*	11.5 (11.3-11.6) *(101)*	11.6 (11.4-11.7) *(94)*	i) 0.005 (6,82) ii) 0.03 (4,75) iii) 0.07 (4,66)
APTT secs, mean (CI)	23.0 (22.6-23.5) *(105)*	21.7 (21.3-22.1) *(107)*	21.5 (21.1-21.9) *(104)*	21.8 (21.3-22.3) *(108)*	21.1 (20.6-21.5) *(101)*	21.4 (20.9-21.9) *(94)*	i) 0.2 (6,82) ii) 0.2 (5,75) iii) 0.1 (5,66)
PF1+2 nmol/l, geometric mean (CI)	0.96 (0.82-1.14) *(25)*	1.04 (0.91-1.18) *(25)*	0.96 (0.83-1.11) *(25)*	1.05 (0.92-1.22) *(24)*	1.17 (1.01-1.36) *(24)*	1.32 (0.94-1.85) *(14)*	i) 0.6 (3,24) ii) 0.001 (3,23) iii) 0.001 (3,12)
TAT µg/ml, geometric mean (CI)	5.4 (3.9-7.4) *(22)*	8.3 (4.4-15.8) *(21)*	6.9 (4.6-10.4) *(20)*	5.3 (3.8-7.4) *(20)*	6.8 (3.7-12.6) *(18)*	5.6 (3.0-10.4) *(17)*	i) 0.1 (6,20) ii) 0.1 (5,17) iii) 0.1 (4,17)
Fibrinogen g/L mean(CI)	3.4 (3.2-3.7) *(97)*	3.2 (3.0-3.4) *(99)*	3.0 (2.8-3.3) *(96)*	3.4 (3.2-3.6) *(105)*	4.0 (3.7-4.2) *(93)*	3.4 (3.1-3.6) *(91)*	i) 0.9 (4,70) ii) 0.7(3,59) iii) 0.2 (2,51)

D-dimer ng/ml, geometric mean (CI)	721 (626-829) *(118)*	735 (633-854) *(110)*	692 (596-804) *(109)*	699 (608-812) *(110)*	750 (639-871) *(106)*	523 (450-614) *(100)*	i) 0.9 (9,109) ii) 0.4 (7,105) iii) 0.02 (6,100)
tPA ng/ml, geometric mean (CI)	8948 (7640-10478) *(36)*	9897 (8185-11980) *(34)*	7589 (6352-9065) *(32)*	8624 (7258-10250) *(33)*	8425 (6995-10158) *(30)*	9519 (7631-11849) *(18)*	i) 0.7 (4,32) ii) 0.7 (3,28) iii) 0.08 (3,18)
uPA ng/ml, geometric mean (CI)	0.60 (0.38-0.93 *(35)*	0.41 (0.24-0.70) *(33)*	0.40 (0.25-0.64) *(31)*	0.47 (0.31-0.70) *(32)*	0.80 (0.59-1.09) *(30)*	0.92 (0.59-1.42) *(20)*	i) 0.9 (4,31) ii) 0.6 (3,28) iii) 0.7 (3,19)
PAI-1 ng/ml, geometric mean (CI)	19.2 (14.7-25.2) *(25)*	16.8 (12.7-22.3 *(24)*	15.0 (11.4-19.8) *(22)*	15.6 (11.0-22.0) *(24)*	17.8 (12.9-24.5) *(24)*	9.8 (6.9-14.0) *(15)*	i) 0.6 (2,21) ii) 0.7 (2,20) iii) 0.5 (2,11)
Platelet count $x10^9$/l mean (CI)	314 (299-329) *(125)*	304 (286-323) *(97)*	(264 (250-278) *(105)*	241 (226-256) *(118)*	315 (292-339) *(115)*	257 (243-272) *(112)*	i) 0.4 (8,89) ii) 0.5 (6,83) iii) 0.2 (5,82)
Platelet function geometric mean (CI)	0.61 (0.52-0.70) *(118)*	0.58 (0.49-0.69) *(93)*	0.49 (0.41-0.58) *(100)*	0.52 (0.43-0.62) *(109)*	0.70 (0.61-0.81) *(104)*	0.59 (0.49-0.70) *(101)*	i) 0.4 (8,84) ii) 0.3 (6,80) iii) 0.3 (5,77)
CRP mg/l, geometric mean (CI)	4.8 (2.3-10.3) *(21)*	4.9 (2.4-9.8) *(21)*	3.9 (1.9-8.2) *(21)*	3.0 (1.4-6.5) *(20)*	2.2 (1.2-4.1) *(20)*	3.0 (0.9-9.5) *(13)*	i) 0.9 (3,20) ii) 1.0 (3,19) iii) 1.0 (2,12)

Appendix 24: Alterations in procoagulants and endothelial adhesion molecules induced by chemotherapy in breast cancer patients developing VTE within the first three months of chemotherapy

Procoagulant/ adhesion molecule	*Pre-chemotherapy (n)*	*Day 1 (post chemotherapy) (n)*	*Day 4 (post chemotherapy) (n)*	*Day 8 (post chemotherapy) (n)*	*3 months (post chemotherapy) (n)*	*6 months (post chemotherapy) (n)*	*Significant difference in trend compared to breast cancer patients remaining VTE-free? p, (n-VTE, no VTE) i) pre-chemo to day 8 ii) pre-chemo to 3 months iii) pre-chemo to 6 months*
TF µg/ml, geometric mean (CI)	273 (114-652) *(9)*	261 (118-578) *(9)*	219 (111-437) *(9)*	247 (118-518) *(9)*	85 (19-384) *(7)*	345 (85-1408) *(6)*	i) 1.0 (9,111) ii) 0.02 (7,106) iii) 0.03 (6,101)
CP mU, geometric mean (CI)	29.8 (26.6-33.7) *(9)*	36.9 (28.7-47.3 *(9)*	31.8 (24.3-41.6) *(9)*	27.9 (21.5-36.3) *(9)*	26.3 (18.4-37.7) *(7)*	27.0 (18.8-38.9) *(6)*	i) 0.7 (9,101) ii) 0.8 (7,96) iii) 0.8 (6,91)
TSP-1 ng/ml, geometric mean (CI)	4537 *(2)*	699 (72-6701) *(4)*	821 (503-1339) *(4)*	626 (7-4902) *(3)*	1249 *(2)*	*(1)*	i) 0.1 (2,36) ii) 0.2 (2,32) iii) (1,24)
TNF-α µg/ml, geometric mean (CI)	4.20 (3.25-5.42) *(5)*	4.47 (2.23-8.94) *(5)*	4.02 (3.24-5.00) *(5)*	3.60 (2.48-5.23) *(5)*	5.00 (3.78-6.61) *(5)*	3.82 (3.20-4.56) *(4)*	i) 0.6 (5,58) ii) 0.7 (5,55) iii) 0.8 (4,50)

Procoagulant/ adhesion molecule	*Pre-chemotherapy (n)*	*Day 1 (post chemotherapy) (n)*	*Day 4 (post chemotherapy) (n)*	*Day 8 (post chemotherapy) (n)*	*3 months (post chemotherapy) (n)*	*6 months (post chemotherapy) (n)*	*Significant difference in trend compared to breast cancer patients remaining VTE-free? p, (n-VTE, no VTE) i) pre-chemo to day 8 ii) pre-chemo to 3 months iii) pre-chemo to 6 months*
pVEGF µg/ml, geometric mean (CI)	34.0 (14.6-79.2) *(9)*	19.6 (9.02-42.7) *(9)*	24.4 (12.4-48.1) *(9)*	27.2 (14.0-29.6) *(9)*	19.9 (13.4-29.6) *(7)*	22.0 (10.9-44.4) *(6)*	i) 0.1 (9,110) ii) 0.1 (7,105) iii) 0.3 (6,101)
sVEGF µg/ml, geometric mean (CI)	357 (158-808) *(9)*	335 (159-708) *(9)*	270 (123-590) *(9)*	276 (130-588) *(9)*	396 (139-1128) *(7)*	221 (99-492) *(6)*	i) 0.8 (9,109) ii) 0.6 (7,104) iii) 0.5 (6,99)
VCAM-1 ng/ml, geometric mean (CI)	812 (657-1002) *(9)*	748 (600-934) *(9)*	740 (608-901) *(9)*	683 (546-854) *(9)*	718 (325-1583) *(7)*	830 (463-1489) *(6)*	i) 0.5 (9,109) ii) 0.6 (7,103) iii) 0.5 (6,99)
E-selectin ng/ml, geometric mean (CI)	31.0 (17.3-55.8) *(9)*	29.0 (15.7-53.5) *(9)*	27.1 (16.2-45.5) *(9)*	20.3 (11.9-34.7) *(9)*	22.1 (14.2-34.4) *(7)*	33.9 (12.4-92.2) *(6)*	i) 0.08 (9,112) ii) 0.06 (7,107) iii) 0.1 (6,102)

Appendix 25: Alterations in procoagulants and endothelial adhesion molecules induced by chemotherapy in breast cancer patients remaining free of VTE within the first three months of chemotherapy

Procoagulant/ adhesion molecule	*Pre-chemotherapy (n)*	*Day 1 (post chemotherapy) (n)*	*Day 4 (post chemotherapy) (n)*	*Day 8 (post chemotherapy) (n)*	*3 months (post chemotherapy) (n)*	*6 months (post chemotherapy) (n)*	*Significant difference in trend compared to breast cancer patients developing VTE? p, (n- VTE, no VTE) i) pre-chemo to day 8 ii) pre-chemo to 3 months iii) pre-chemo to 6 months*
TF µg/ml, geometric mean (CI)	108 (86-134) *(122)*	108 (85-137) *(116)*	91 (71-116) *(112)*	92 (73-116) *(115)*	98 (76-124) *(108)*	116 (91-147) *(103)*	i) 1.0 (9,111) ii) 0.02 (7,106) iii) 0.03 (6,101)
CP mU, geometric mean (CI)	32.0 (29.9-34.3) *(110)*	36.9 (34.6-39.4) *(108)*	35.0 (32.9-37.4) *(102)*	32.7 (30.5-35.0) *(106)*	31.6 (29.6-33.9) *(101)*	34.2 (31.8-36.9) *(95)*	i) 0.7 (9,101) ii) 0.8 (7,96) iii) 0.8 (6,91)
TSP-1 ng/ml, geometric mean (CI)	596 (446-804) *(44)*	652 (441-953) *(45)*	590 (420-821) *(43)*	433 (314-596) *(41)*	781 (561-1086) *(48)*	713 (498-1033) *(38)*	i) 0.1 (2,36) ii) 0.2 (2,32) iii) (1,24)
TNF-α µg/ml, geometric mean (CI)	3.24 (2.92-3.60) *(63)*	3.07 (2.76-3.42) *(61)*	2.89 (2.59-3.23) *(58)*	2.85 (2.56-3.18) *(61)*	3.36 (2.97-3.80) *(55)*	3.36 (2.98-3.80) *(50)*	i) 0.6 (5,58) ii) 0.7 (5,55) iii) 0.8 (4,50)

Procoagulant/ adhesion molecule	*Pre-chemotherapy (n)*	*Day 1 (post chemotherapy) (n)*	*Day 4 (post chemotherapy) (n)*	*Day 8 (post chemotherapy) (n)*	*3 months (post chemotherapy) (n)*	*6 months (post chemotherapy) (n)*	*Significant difference in trend compared to breast cancer patients developing VTE? p, (n- VTE, no VTE) i) pre-chemo to day 8 ii) pre-chemo to 3 months iii) pre-chemo to 6 months*
pVEGF µg/ml, geometric mean (CI)	15.6 (13.7-17.7) *(121)*	14.2 (12.6-16.1) *(115)*	15.3 (13.2-17.9) *(112)*	20.3 (18.2-22.9) *(113)*	22.3 (19.1-25.9) *(107)*	20.9 (18.1-24.1) *(103)*	i) 0.1 (9,110) ii) 0.1 (7,105) iii) 0.3 (6,101)
sVEGF µg/ml, geometric mean (CI)	205 (180-233) *(122)*	195 (171-223) *(114)*	152 (132-175) *(113)*	149 (128-172) *(115)*	238 (208-272) *(111)*	177 (154-203) *(105)*	i) 0.8 (9,109) ii) 0.6 (7,104) iii) 0.5 (6,99)
VCAM-1 ng/ml, geometric mean (CI)	638 (595-684) *(123)*	578 (536-623) *(115)*	593 (552-637) *(112)*	574 (536-614) *(115)*	647 (598-701) *(107)*	646 (593-704) *(104)*	i) 0.5 (9,109) ii) 0.6 (7,103) iii) 0.5 (6,99)
E-selectin ng/ml, geometric mean (CI)	29.4 (26.8-32.2) *(123)*	28.2 (25.7-31.0) *(117)*	25.0 (22.7-27.5) *(113)*	22.4 (20.4-24.7) *(115)*	26.9 (24.4-29.7) *(110)*	30.2 (27.1-33.6) *(104)*	i) 0.08 (9,112) ii) 0.06 (7,107) iii) 0.1 (6,102)

Appendix 26: Alterations in haemostatic markers induced by chemotherapy in advanced breast cancer patients remaining stable or responding to chemotherapy within the first three months of commencement of treatment

Coagulation marker	*Pre-chemo (n)*	*Day 1 (post chemo) (n)*	*Day 4 (post chemo) (n)*	*Day 8 (post chemo) (n)*	*3 months (post chemo) (n)*	*6 months (post chemo) (n)*	*Significant difference in trend compared to advanced breast cancer patients progressing within 3 months? p, (n- progress, stable)* *i) pre-chemo to day 8* *ii) pre-chemo to 3 months* *iii) pre-chemo to 6 months*
PT secs, mean (CI)	11.6 (11.1-12.1) *(16)*	11.9 (11.4-12.3) *(16)*	11.5 (11.0-12.1) *(16)*	11.9 (11.3-12.5) *(19)*	11.8 (11.4-12.3) *(15)*	11.9 (11.4-12.4) *(12)*	i) 0.6 (7,12) ii) 0.9 (5,11) iii) 0.8 (4,8)
APTT secs, mean (CI)	23.0 (21.4-24.6) *(16)*	21.5 (20.7-22.3) *(16)*	21.3 (20.2-22.3) *(16)*	22.5 (21.1-23.9) *(19)*	21.3 (20.1-22.5) *(15)*	21.8 (19.5-24.1) *(12)*	i) 0.2 (7,12) ii) 0.04 (5,11) iii) 0.09 (4,8)
PF1+2 nmol/l, geometric mean (CI)	1.07 (0.86-1.34) *(8)*	1.13 (0.93-1.37) *(8)*	1.25 (0.92-1.70) *(7)*	1.60 (0.85-3.00) *(7)*	1.14 (0.66-1.95) *(6)*	*(5)*	i) 0.6 (5,9) ii) 0.7 (5,8) iii) 0.8 (1,4)
TAT µg/ml, geometric mean (CI)	7.1 (4.1-12.6) *(8)*	14.2 (5.6-35.9) *(7)*	12.6 (5.6-28.2) *(7)*	12.9 (6.0-27.9) *(7)*	5.9 (3.9-8.8) *(6)*	15.6 (1.6-156.0) *(5)*	i) 0.2 (6,7) ii) 0.5 (3,6) iii) 0.6 (3,5)
Fibrinogen g/L mean(CI)	4.3 (3.3-5.2) *(14)*	4.6 (3.7-5.5) *(14)*	4.8 (4.1-5.5) *(15)*	4.6 (3.9-5.3) *(18)*	4.8 (3.9-5.8) *(12)*	4.5 (3.4-5.5) *(11)*	i) 0.4 (5,8) ii) 0.5 (3,6) iii) 0.3 (2,4)

D-dimer ng/ml, geometric mean (CI)	1130 (735-1720) *(21)*	1353 (871-2122) *(19)*	1556 (1012-2392) *(19)*	1300 (829-2039) *(20)*	1480 (1022-2165) *(18)*	982 (590-1636) *(15)*	i) 0.1 (13,18) ii) 0.2 (8,17) iii) 0.5 (7,14)
tPA ng/ml, geometric mean (CI)	8630 (5432-13684) *(7)*	8848 (4163-18788) *(6)*	7044 (3771-13161) *(6)*	7165 (3887-13194) *(6)*	10037 (5943-16933) *(5)*	14073 *(2)*	i) 0.9 (9,6) ii) 0.4 (6,5) iii) 0.2 (3,2)
uPA ng/ml, geometric mean (CI)	0.32 (0.07-1.34 *(7)*	0.30 (0.05-1.83) *(6)*	0.23 (0.02-2.20) *(5)*	0.27 (0.04-1.65) *(6)*	1.13 (0.30-4.26) *(5)*	0.75 (0.28-2.00) *(3)*	i) 0.8 (9,5) ii) 0.3 (6,4) iii) 0.3 (4,2)
PAI-1 ng/ml, geometric mean (CI)	16.4 (12.2-22.1) *(11)*	15.4 (9.8-24.4 *(10)*	13.5 (8.9-20.5) *(9)*	16.0 (9.0-28.7) *(10)*	20.0 (12.1-33.1) *(11)*	12.2 (7.4-20.4) *(6)*	i) 0.7 (5,8) ii) 0.4 (4,8) iii) 0.3 (3,2)
Platelet count $x10^9$/l mean (CI)	323 (277-368) *(21)*	322 (270-374) *(16)*	(258 (220-296) *(16)*	240 (188-292) *(19)*	327 (242-412) *(18)*	262 (219-304) *(17)*	i) 0.9 (11,14) ii) 0.3 (6,11) iii) 0.5 (6,9)
Platelet function geometric mean (CI)	0.97 (0.68-1.40) *(20)*	0.73 (0.47-1.12) *(16)*	0.80 (0.49-1.32) *(15)*	0.98 (0.59-1.43) *(18)*	1.06 (0.70-1.60) *(17)*	0.99 (0.68-1.45) *(15)*	i) 0.2 (10,13) ii) 0.7 (6,11) iii) 0.8 (6,8)
CRP mg/l, geometric mean (CI)	6.2 (3.2-12.1) *(9)*	5.3 (3.1-9.2) *(9)*	4.1 (1.1-15.6) *(8)*	5.9 (1.5-24.3) *(8)*	2.4 (0.9-6.8) *(9)*	11.4 (2.0-66.0) *(6)*	i) 0.5 (4,7) ii) 0.7 (3,7) iii) 0.9 (1,5)

Appendix 27: Alterations in haemostatic markers induced by chemotherapy in advanced breast cancer patients progressing within three months of commencement of treatment

Coagulation marker	*Pre-chemo (n)*	*Day 1 (post chemo) (n)*	*Day 4 (post chemo) (n)*	*Day 8 (post chemo) (n)*	*3 months (post chemo) (n)*	*6 months (post chemo) (n)*	*Significant difference in trend compared to advanced breast cancer patients remaining stable within 3 months? p, (n-progress, stable)* *i) pre-chemo to day 8* *ii) pre-chemo to 3 months* *iii) pre-chemo to 6 months*
PT secs, mean (CI)	12.0 (11.5-12.5) *(10)*	12.4 (11.9-12.9) *(12)*	11.7 (11.3-12.2) *(13)*	12.0 (11.4-12.5) *(12)*	12.1 (11.3-12.9) *(7)*	11.8 (11.1-12.6) *(5)*	i) 0.6 (7,12) ii) 0.9 (5,11) iii) 0.8 (4,8)
APTT secs, mean (CI)	22.0 (20.1-23.8) *(10)*	21.5 (19.6-23.1) *(12)*	22.6 (21.1-24.2) *(13)*	22.5 (21.0-24.1) *(12)*	24.3 (21.0-27.6) *(8)*	21.6 (19.0-24.1) *(6)*	i) 0.2 (7,12) ii) 0.04 (5,11) iii) 0.09 (4,8)
PF1+2 nmol/l, geometric mean (CI)	0.92 (0.52-1.61) *(5)*	1.07 (0.63-1.80) *(5)*	0.99 (0.61-1.60) *(5)*	1.03 (0.53-2.04) *(5)*	1.24 (0.80-1.94) *(5)*	*(1)*	i) 0.6 (5,9) ii) 0.7 (5,8) iii) 0.8 (1,4)
TAT µg/ml, geometric mean (CI)	12.9 (2.3-72.2) *(6)*	10.8 (2.3-49.9) *(6)*	4.7 (3.1-7.1) *(6)*	4.6 (2.6-8.1) *(6)*	3.4 (2.1-5.5) *(3)*	3.4 (0.9-13.5) *(3)*	i) 0.2 (6,7) ii) 0.5 (3,6) iii) 0.6 (3,5)
Fibrinogen g/L mean(CI)	4.9 (3.0-6.8) *(7)*	4.4 (3.3-5.4) *(11)*	4.2 (3.4-5.1) *(12)*	4.2 (3.4-5.0) *(11)*	5.1 (2.7-7.4) *(6)*	5.5 (3.8-7.3) *(6)*	i) 0.4 (5,8) ii) 0.5 (3,6) iii) 0.3 (2,4)

D-dimer ng/ml, geometric mean (CI)	1720 (1022-2893) *(14)*	1720 (1064-2807) *(14)*	1541 (963-2465) *(14)*	1451 (863-2441) *(13)*	1188 (523-2724) *(8)*	1703 (735-3944) *(7)*	i) 0.1 (13,18) ii) 0.2 (8,17) iii) 0.5 (7,14)
tPA ng/ml, geometric mean (CI)	13698 (9997-18788) *(9)*	13705 (10231-18398) *(9)*	10277 (7151-14765) *(9)*	11159 (7767-16030) *(9)*	11137 (5080-24441) *(6)*	11673 (1339-100710) *(3)*	i) 0.9 (9,6) ii) 0.4 (6,5) iii) 0.2 (3,2)
uPA ng/ml, geometric mean (CI)	0.89 (0.42-1.89 *(9)*	0.92 (0.46-1.85) *(9)*	0.78 (0.36-1.73) *(9)*	0.70 (0.36-1.39) *(9)*	1.90 (0.44-1.84) *(6)*	1.20 (0.43-3.31) *(4)*	i) 0.8 (9,5) ii) 0.3 (6,4) iii) 0.3 (4,2)
PAI-1 ng/ml, geometric mean (CI)	42.8 (20.5-89.5) *(5)*	27.9 (11.8-66.7 *(5)*	29.4 (13.1-65.8) *(5)*	37.7 (15.4-92.2) *(5)*	24.2 (7.9-73.7) *(4)*	16.4 *(2)*	i) 0.7 (5,8) ii) 0.4 (4,8) iii) 0.3 (3,2)
Platelet count $x10^9$/l mean (CI)	332 (253-411) *(15)*	321 (219-422) *(12)*	(241 (189-293) *(15)*	250 (190-310) *(14)*	397 (170-624) *(8)*	312 (149-475) *(7)*	i) 0.9 (11,14) ii) 0.3 (6,11) iii) 0.5 (6,9)
Platelet function geometric mean (CI)	1.09 (0.77-1.54) *(15)*	1.24 (0.84-1.83) *(12)*	0.90 (0.55-1.45) *(15)*	0.85 (0.59-1.23) *(13)*	0.98 (0.63-1.54) *(8)*	1.04 (0.45-2.41) *(7)*	i) 0.2 (10,13) ii) 0.7 (6,11) iii) 0.8 (6,8)
CRP mg/l, geometric mean (CI)	26.6 (2.4-287) *(4)*	26.6 (2.4-296) *(4)*	18.5 (3.4-102) *(4)*	12.9 (4.4-38.1) *(4)*	9.5 (0.7-122) *(3)*	*(1)*	i) 0.5 (4,7) ii) 0.7 (3,7) iii) 0.9 (1,5)

Appendix 28: Alterations in haemostatic markers induced by chemotherapy in advanced breast cancer patients remaining stable or responding to chemotherapy within the first six months of commencement of treatment

Coagulation marker	*Pre-chemo (n)*	*Day 1 (post chemo) (n)*	*Day 4 (post chemo) (n)*	*Day 8 (post chemo) (n)*	*3 months (post chemo) (n)*	*6 months (post chemo) (n)*	*Significant difference in trend compared to advanced breast cancer patients progressing within 6 months? p, (n- progress, stable)* *i) pre-chemo to day 8* *ii) pre-chemo to 3 months* *iii) pre-chemo to 6 months*
PT secs, mean (CI)	11.6 (10.5-12.6) *(7)*	12.3 (11.6-13.0) *(5)*	11.5 (10.1-12.9) *(6)*	11.7 (10.7-12.7) *(7)*	11.7 (10.9-12.5) *(7)*	11.9 (11.4-12.4) *(6)*	i) 0.6 (5,14) ii) 0.7 (11,5) iii) 0.7 (8,4)
APTT secs, mean (CI)	22.2 (20.2-24.1) *(7)*	21.2 (19.8-22.6) *(5)*	20.9 (18.1-23.6) *(6)*	21.7 (18.7-24.6) *(7)*	21.2 (19.9-22.5) *(7)*	20.9 (18.2-23.6) *(6)*	i) 0.9 (14,5) ii) 0.8 (11,5) iii) 0.6 (8,4)
PF1+2 nmol/l, geometric mean (CI)	1.08 (0.78-1.51) *(6)*	1.18 (0.91-1.54) *(6)*	1.28 (0.87-1.86) *(6)*	1.18 (0.87-1.60) *(5)*	1.12 (0.52-2.44) *(6)*	1.88 (0.69-5.1) *(4)*	i) 0.6 (6,8) ii) 0.6 (6,7) iii) 0.9 (2,3)
TAT µg/ml, geometric mean (CI)	10.3 (1.0-108) *(3)*	23.8 (0.7-812) *(3)*	12.8 (4.4-37.0) *(3)*	10.8 (1.9-62.2) *(3)*	6.6 (2.8-15.6) *(3)*	7.5 (2.1-27.4) *(3)*	i) 0.8 (10,3) ii) 0.9 (6,3) iii) 0.7 (5,3)
Fibrinogen g/L mean(CI)	4.1 (2.8-5.3) *(6)*	4.1 (1.1-7.0) *(4)*	4.7 (3.2-6.2) *(6)*	4.9 (3.4-6.3) *(7)*	4.5 (2.7-6.2) *(6)*	4.4 (3.0-5.7) *(5)*	i) 0.2 (10,3) ii) 0.6 (7,2) iii) 0.3 (5,1)

D-dimer ng/ml, geometric mean (CI)	1064 (376-3011) *(8)*	1339 (420-4316) *(7)*	1394 (602-3229) *(8)*	1261 (513-3072) *(8)*	1556 (773-3165) *(8)*	1064 (488-2298) *(8)*	i) 0.6 (24,7) ii) 0.5 (18,7) iii) 0.6 (14,7)
tPA ng/ml, geometric mean (CI)	12080 (9452-15444) *(3)*	13161 (4546-38063) *(3)*	8103 (4024-16482) *(3)*	7187 (5340-9838) *(3)*	10711 (3940-29115) *(3)*	*(0)*	i) 0.07 (12,3) ii) 0.4 (8,3) iii) (5,0)
uPA ng/ml, geometric mean (CI)	0.50 (0.29-0.86 *(3)*	0.50 (0.14-1.75) *(3)*	0.49 *(2)*	0.52 (0.10-2.88) *(3)*	0.79 (0.07-8.45) *(3)*	*(1)*	i) 0.8 (12,2) ii) 0.7 (8,2) iii) (6,0)
PAI-1 ng/ml, geometric mean (CI)	15.7 (10.9-22.6) *(9)*	15.2 (8.3-27.9 *(8)*	13.7 (8.5-22.2) *(8)*	16.1 (7.5-34.5) *(8)*	19.7 (10.9-35.3) *(9)*	12.2 (5.0-30.0) *(4)*	i) 0.7 (6,7) ii) 0.7 (5,7) iii) 0.4 (3,2)
Platelet count $x10^9$/l mean (CI)	325 (249-401) *(8)*	319 (206-432) *(5)*	(260 (180-339) *(6)*	251 (129-373) *(8)*	311 (226-396) *(7)*	252 (184-320) *(8)*	i) 0.9 (11,14) ii) 0.3 (6,11) iii) 0.5 (6,9)
Platelet function geometric mean (CI)	0.70 (0.28-1.77) *(7)*	0.43 (0.11-1.65) *(5)*	0.60 (0.17-2.10) *(6)*	0.83 (0.30-2.27) *(7)*	0.92 (0.31-2.73) *(7)*	0.77 (0.39-1.52) *(8)*	i) 0.2 (18,5) ii) 0.9 (13,4) iii) 0.8 (10,4)
CRP mg/l, geometric mean (CI)	6.8 (2.8-16.9) *(7)*	4.8 (2.3-9.8) *(7)*	4.1 (0.8-20.1) *(7)*	5.7 (0.8-42.1) *(6)*	2.8 (0.7-11.2) *(7)*	14.6 (1.7-128) *(5)*	i) 0.5 (5,6) ii) 0.5 (4,6) iii) 0.5 (2,4)

Appendix 29: Alterations in haemostatic markers induced by chemotherapy in advanced breast cancer patients progressing within six months of commencement of treatment

Coagulation marker	*Pre-chemo (n)*	*Day 1 (post chemo) (n)*	*Day 4 (post chemo) (n)*	*Day 8 (post chemo) (n)*	*3 months (post chemo) (n)*	*6 months (post chemo) (n)*	*Significant difference in trend compared to advanced breast cancer patients remaining stable within 6 months? p, (n-progress, stable)* *i) pre-chemo to day 8* *ii) pre-chemo to 3 months* *iii) pre-chemo to 6 months*
PT secs, mean (CI)	11.8 (11.5-12.2) *(19)*	12.0 (11.6-12.4) *(23)*	11.6 (11.3-12.0) *(23)*	12.0 (11.5-12.5) *(24)*	12.0 (11.6-12.4) *(15)*	11.8 (11.3-12.4) *(11)*	i) 0.6 (5,14) ii) 0.7 (11,5) iii) 0.7 (8,4)
APTT secs, mean (CI)	22.7 (21.2-24.2) *(19)*	21.5 (20.5-22.5) *(23)*	22.1 (21.2-23.1) *(23)*	22.8 (21.7-23.8) *(24)*	22.9 (20.9-24.8) *(16)*	22.1 (18.2-23.6) *(12)*	i) 0.9 (14,5) ii) 0.8 (11,5) iii) 0.6 (8,4)
PF1+2 nmol/l, geometric mean (CI)	0.95 (0.68-1.35) *(7)*	1.04 (0.75-1.43) *(7)*	1.00 (0.69-1.44) *(6)*	1.46 (0.68-3.16) *(7)*	1.23 (0.89-1.69) *(7)*	1.45 (0.16-13.1) *(3)*	i) 0.6 (6,8) ii) 0.6 (6,7) iii) 0.9 (2,3)
TAT µg/ml, geometric mean (CI)	8.9 (3.8-20.9) *(11)*	10.3 (4.6-22.9) *(10)*	7.0 (3.7-13.2) *(10)*	7.3 (3.7-14.3) *(10)*	4.2 (2.8-6.4) *(6)*	10.0 (0.7-134) *(5)*	i) 0.8 (10,3) ii) 0.9 (6,3) iii) 0.7 (5,3)
Fibrinogen g/L mean(CI)	4.6 (3.5-5.8) *(15)*	4.6 (3.9-5.2) *(21)*	4.5 (3.9-5.1) *(21)*	4.3 (3.8-4.9) *(22)*	5.1 (4.0-6.3) *(12)*	5.0 (3.9-6.2) *(12)*	i) 0.2 (10,3) ii) 0.6 (7,2) iii) 0.3 (5,1)

D-dimer ng/ml, geometric mean (CI)	1422 (1022-1978) *(27)*	1556 (1130-2122) *(26)*	1604 (1141-2231) *(25)*	1394 (973-1998) *(25)*	1313 (871-1978) *(18)*	1236 (713-2165) *(14)*	i) 0.6 (24,7) ii) 0.5 (18,7) iii) 0.6 (14,7)
tPA ng/ml, geometric mean (CI)	10993 (7879-15367) *(13)*	11123 (7586-16301) *(12)*	9018 (6186-13148) *(12)*	9997 (6816-14545) *(12)*	10594 (6003-18676) *(8)*	*(5)*	i) 0.07 (12,3) ii) 0.4 (8,3) iii) (5,0)
uPA ng/ml, geometric mean (CI)	0.58 (0.24-1.43 *(13)*	0.61 (0.23-1.61) *(12)*	0.51 (0.19-1.35) *(12)*	0.47 (0.19-1.19) *(12)*	1.09 (0.53-2.21) *(8)*	*1.07 (0.59-1.94) (6)*	i) 0.8 (12,2) ii) 0.7 (8,2) iii) (6,0)
PAI-1 ng/ml, geometric mean (CI)	345 (19.5-60.9) *(7)*	24.0 (13.4-42.9 *(7)*	25.3 (12.3-52.0) *(6)*	29.4 (14.9-57.9) *(7)*	23.4 (11.3-48.3) *(6)*	14.3 (3.1-65.3) *(4)*	i) 0.7 (6,7) ii) 0.7 (5,7) iii) 0.4 (3,2)
Platelet count $x10^9$/l mean (CI)	327 (279-376) *(28)*	322 (264-379) *(23)*	(247 (212-282) *(25)*	242 (204-280) *(25)*	362 (250-474) *(19)*	289 (219-358) *(16)*	i) 0.9 (11,14) ii) 0.3 (6,11) iii) 0.5 (6,9)
Platelet function geometric mean (CI)	1.12 (0.89-1.42) *(28)*	1.08 (0.83-1.39) *(23)*	0.93 (0.67-1.29) *(24)*	0.95 (0.75-1.21) *(24)*	1.08 (0.86-1.37) *(18)*	1.17 (0.79-1.73) *(14)*	i) 0.2 (18,5) ii) 0.9 (13,4) iii) 0.8 (10,4)
CRP mg/l, geometric mean (CI)	14.5 (3.0-69.4) *(6)*	17.6 (4.3-71.5) *(6)*	13.7 (3.3-56.5) *(5)*	10.5 (4.3-25.3) *(6)*	4.5 (1.0-21.5) *(5)*	5.9 *(2)*	i) 0.5 (5,6) ii) 0.5 (4,6) iii) 0.5 (2,4)

Appendix 30: Alterations in haemostatic markers induced by chemotherapy in early breast cancer patients remaining stable within the first two years of commencement of chemotherapy

Coagulation marker	*Pre-chemo (n)*	*Day 1 (post chemo) (n)*	*Day 4 (post chemo) (n)*	*Day 8 (post chemo) (n)*	*3 months (post chemo) (n)*	*6 months (post chemo) (n)*	*Significant difference in trend compared to early breast cancer patients progressing within 2 years? p, (n- progress, stable) i) pre-chemo to day 8 ii) pre-chemo to 3 months iii) pre-chemo to 6 months*
PT secs, mean (CI)	11.7 (11.5-11.8) *(72)*	11.8 (11.6-11.9) *(76)*	11.8 (11.6-11.9) *(74)*	11.8 (11.6-12.0) *(73)*	11.4 (11.2-11.6) *(73)*	11.6 (11.4-11.8) *(72)*	i) 0.01 (4,61) ii) 0.09 (3,56) iii) 0.1 (2,52)
APTT secs, mean (CI)	23.2 (22.7-23.8) *(72)*	21.7 (21.2-22.2) *(76)*	21.3 (20.9-21.8) *(74)*	21.6 (21.0-22.1) *(73)*	20.7 (20.2-21.1) *(74)*	21.5 (21.0-22.0) *(72)*	i) 0.7 (4,61) ii) 0.5 (3,56) iii) 0.3 (2,53)
PF1+2 nmol/l, geometric mean (CI)	*(13)*	*(13)*	*(12)*	*(13)*	*(13)*	*(10)*	i) (1,12) ii) (1,12) iii) (1,9)
TAT µg/ml, geometric mean (CI)	4.3 (2.4-7.8) *(8)*	8.1 (1.6-40.4) *(8)*	4.9 (2.1-11.5) *(8)*	3.6 (2.8-4.6) *(8)*	3.8 (2.7-5.2) *(8)*	3.6 (2.7-4.7) *(8)*	i) 0.7 (3,8) ii) 0.7 (3,8) iii) 0.8 (3,8)
Fibrinogen g/L mean(CI)	3.3 (3.0-3.5) *(68)*	2.8 (2.7-3.0) *(70)*	2.7 (2.5-2.9) *(68)*	3.0 (2.8-3.3) *(71)*	3.9 (3.6-4.1) *(70)*	3.0 (2.8-3.3) *(70)*	i) 0.9 (3,54) ii) 0.8 (2,47) iii) 0.3 (1,42)

D-dimer ng/ml, geometric mean (CI)	652 (567-750) *(77)*	652 (561-750) *(73)*	590 (503-685) *(73)*	626 (545-728) *(74)*	645 (545-765) *(74)*	412 (361-469) *(73)*	i) 0.9 (5,74) ii) 1.0 (5,74) iii) 1.0 (5,73)
tPA ng/ml, geometric mean (CI)	8639 (6974-10689) *(18)*	*(18)*	*(17)*	*(18)*	*(17)*	*(14)*	i) (1,17) ii) (1,16) iii) (1,14)
uPA ng/ml, geometric mean (CI)	0.93 (0.58-1.47 *(17)*	*(17)*	*(17)*	*(17)*	*(17)*	*(14)*	i) (1,17) ii) (1,17) iii) (1,14)
PAI-1 ng/ml, geometric mean (CI)	*(13)*	*(13)*	*(10)*	*(13)*	*(13)*	*(11)*	i) (0,10) ii) (0,10) iii) (0,8)
Platelet count $x10^9$/l mean (CI)	307 (291-324) *(82)*	299 (279-319) *(67)*	268 (253-284) *(73)*	242 (226-259) *(80)*	299 (278-320) *(81)*	247 (232-262) *(80)*	i) 0.8 (4,60) ii) 0.8 (4,60) iii) 0.4 (4,63)
Platelet function geometric mean (CI)	0.55 (0.45-0.65) *(77)*	0.54 (0.45-0.66) *(63)*	0.45 (0.37-0.55) *(69)*	0.45 (0.36-0.56) *(74)*	0.68 (0.57-0.80) *(74)*	0.55 (0.46-0.66) *(74)*	i) 0.007 (23,63) ii) 0.03 (16,62) iii) 0.9 (4,60)
CRP mg/l, geometric mean (CI)	*(12)*	*(12)*	*(11)*	*(12)*	*(12)*	*(7)*	i) (1,11) ii) (1,11) iii) (1,7)

Appendix 31: Alterations in haemostatic markers induced by chemotherapy in early breast cancer patients progressing within the first two years of commencement of chemotherapy

Coagulation marker	*Pre-chemo (n)*	*Day 1 (post chemo) (n)*	*Day 4 (post chemo) (n)*	*Day 8 (post chemo) (n)*	*3 months (post chemo) (n)*	*6 months (post chemo) (n)*	*Significant difference in trend compared to early breast cancer patients remaining stable within 2 years? p, (n- progress, stable)* *i) pre-chemo to day 8* *ii) pre-chemo to 3 months* *iii) pre-chemo to 6 months*
PT secs, mean (CI)	11.9 (11.4-12.4) *(5)*	11.9 (11.1-12.6) *(4)*	12.6 (10.9-14.3) *(4)*	12.3 (11.2-13.4) *(4)*	11.2 (10.5-12.0) *(3)*	11.2 (9.9-12.4) *(3)*	i) 0.01 (4,61) ii) 0.09 (3,56) iii) 0.1 (2,52)
APTT secs, mean (CI)	21.9 (18.1-25.7) *(5)*	19.8 (13.9-25.7) *(4)*	20.0 (16.8-23.3) *(4)*	20.5 (18.0-23.1) *(4)*	20.0 (11.1-28.9) *(3)*	19.3 (13.4-25.2) *(3)*	i) 0.7 (4,61) ii) 0.5 (3,56) iii) 0.3 (2,53)
PF1+2 nmol/l, geometric mean (CI)	*(1)*	*(1)*	*(1)*	*(1)*	*(1)*	*(1)*	i) (1,12) ii) (1,12) iii) (1,9)
TAT µg/ml, geometric mean (CI)	4.1 (2.8-5.8) *(3)*	17.5 (0.1-29.8) *(3)*	6.3 (1.0-41.3) *(3)*	3.6 (0.6-22.4) *(3)*	9.9 (0.0-600) *(3)*	5.0 (0.6-43.8) *(3)*	i) 0.7 (3,8) ii) 0.7 (3,8) iii) 0.8 (3,8)
Fibrinogen g/L mean(CI)	3.4 (2.2-4.6) *(5)*	2.8 (1.9-3.7) *(3)*	2.2 (1.1-3.4) *(4)*	2.8 (2.2-3.3) *(4)*	3.7 (2.8-4.5) *(3)*	4.1 (2.7-5.4) *(3)*	i) 0.9 (3,54) ii) 0.8 (2,47) iii) 0.3 (1,42)

D-dimer ng/ml, geometric mean (CI)	699 (441-1097) *(5)*	721 (347-1495) *(4)*	614 (198-1882) *(4)*	679 (183-2540) *(4)*	706 (164-3041) *(4)*	351 (181-679) *(4)*	i) 0.9 (5,74) ii) 1.0 (5,74) iii) 1.0 (5,73)
tPA ng/ml, geometric mean (CI)	5710 (3634-9036) *(2)*	*(1)*	*(1)*	*(1)*	*(1)*	*(1)*	i) (1,17) ii) (1,16) iii) (1,14)
uPA ng/ml, geometric mean (CI)	*(2)*	*(1)*	*(1)*	*(1)*	*(1)*	*(1)*	i) (1,17) ii) (1,17) iii) (1,14)
PAI-1 ng/ml, geometric mean (CI)	*(1)*	*(1)*	*(0)*	*(1)*	*(1)*	*(1)*	i) (0,10) ii) (0,10) iii) (0,8)
Platelet count $x10^9$/l mean (CI)	345 (230-460) *(5)*	321 (247-394) *(4)*	279 (192-367) *(4)*	224 (177-271) *(4)*	293 (203-382) *(5)*	282 (198-366) *(5)*	i) 0.8 (4,60) ii) 0.8 (4,60) iii) 0.4 (4,63)
Platelet function geometric mean (CI)	0.26 (0.08-0.91) *(5)*	0.22 (0.04-1.20) *(4)*	0.16 (0.05-0.59) *(4)*	0.17 (0.02-1.17) *(4)*	0.26 (0.05-1.29) *(4)*	0.17 (0.02-1.79) *(4)*	i) 0.007 (23,63) ii) 0.03 (16,62) iii) 0.9 (4,60)
CRP mg/l, geometric mean (CI)	*(1)*	*(1)*	*(1)*	*(1)*	*(1)*	*(1)*	i) (1,11) ii) (1,11) iii) (1,7)

Appendix 32: Alterations in procoagulants and endothelial adhesion molecules induced by chemotherapy in advanced breast cancer patients remaining stable or responding to chemotherapy within the first three months of commencement of treatment

Procoagulant/ adhesion molecule	*Pre-chemotherapy (n)*	*Day 1 (post chemotherapy) (n)*	*Day 4 (post chemotherapy) (n)*	*Day 8 (post chemotherapy) (n)*	*3 months (post chemotherapy) (n)*	*6 months (post chemotherapy) (n)*	*Significant difference in trend compared to advanced breast cancer patients progressing within 3 months? p, (n-progress, stable) i) pre-chemo to day 8 ii) pre-chemo to 3 months iii) pre-chemo to 6 months*
TF µg/ml, geometric mean (CI)	169 (123-233) *(21)*	179 (137-233) *(20)*	147 (106-204) *(19)*	167 (129-219) *(20)*	161 (111-233) *(18)*	206 (138-305) *(14)*	i) 0.5 (14,19) ii) 0.4 (8,18) iii) 0.5 (7,14)
CP mU, geometric mean (CI)	30.0 (25.4-35.3) *(19)*	35.9 (28.8-44.7) *(18)*	25.9 (21.6-31.2) *(17)*	28.0 (22.3-35.2) *(18)*	26.5 (19.9-35.3) *(17)*	24.3 (19.5-30.1) *(14)*	i) 0.3 (12,17) ii) 0.6 (7,16) iii) 0.5 (5,13)
TSP-1 ng/ml, geometric mean (CI)	1195 (863-1652) *(7)*	706 (204-2441) *(6)*	590 (224-1541) *(6)*	846 (334-2122) *(7)*	1737 (626-4817) *(7)*	821 (578-1176) *(5)*	i) 0.4 (2,5) ii) (1,3) iii) (0,2)
TNF-α µg/ml, geometric mean (CI)	3.58 (2.76-4.65) *(14)*	4.18 (3.28-5.32) *(13)*	3.82 (3.12-4.68) *(12)*	3.37 (2.52-4.50) *(13)*	4.05 (2.88-5.70) *(11)*	4.29 (3.22-5.73) *(9)*	i) 0.9(7,12) ii) 0.6 (5,11) iii) 0.4 (4,9)

Procoagulant/ adhesion molecule	*Pre-chemotherapy (n)*	*Day 1 (post chemotherapy) (n)*	*Day 4 (post chemotherapy) (n)*	*Day 8 (post chemotherapy) (n)*	*3 months (post chemo) (n)*	*6 months (post chemo) (n)*	*Significant difference in trend compared to advanced breast cancer patients progressing within 3 months? p, (n- progress, stable)* *i) pre-chemo to day 8* *ii) pre-chemo to 3 months* *iii) pre-chemo to 6 months*
pVEGF µg/ml, geometric mean (CI)	20.7 (14.9-28.8) *(21)*	19.9 (13.4-29.5) *(20)*	19.8 (11.3-35.0) *(19)*	27.6 (21.5-35.4) *(20)*	24.2 (17.4-33.8) *(18)*	35.2 (23.8-52.0) *(15)*	i) 0.5 (14,19) ii) 0.6 (8,18) iii) 0.5 (7,15)
sVEGF µg/ml, geometric mean (CI)	333 (242-459) *(20)*	263 (179-389) *(18)*	243 (165-359) *(18)*	260 (184-368) *(19)*	344 (238-496) *(18)*	281 (187-423) *(15)*	i) 0.4 (14,17) ii) 0.5 (8,16) iii) 0.8 (7,13)
VCAM-1 ng/ml, geometric mean (CI)	669 (533-841) *(21)*	608 (484-764) *(19)*	640 (514-797) *(18)*	546 (442-675) *(19)*	650 (484-873) *(17)*	698 (486-1002) *(14)*	i) 0.2 (14,17) ii) 0.09 (8,16) iii) 0.3 (7,13)
E-selectin ng/ml, geometric mean (CI)	33.3 (25.9-42.9) *(21)*	34.5 (25.8-46.1) *(20)*	33.7 (26.1-43.5) *(19)*	25.0 (18.8-33.2) *(20)*	33.2 (24.4-45.2) *(18)*	36.9 (24.8-54.9) *(15)*	i) 0.6 (13,19) ii) 0.4 (7,18) iii) 0.6 (6,15)

Appendix 33: Alterations in procoagulants and endothelial adhesion molecules induced by chemotherapy in advanced breast cancer patients progressing within three months of commencement of treatment

Procoagulant/ adhesion molecule	*Pre-chemotherapy (n)*	*Day 1 (post chemotherapy) (n)*	*Day 4 (post chemotherapy) (n)*	*Day 8 (post chemotherapy) (n)*	*3 months (post chemotherapy) (n)*	*6 months (post chemotherapy) (n)*	*Significant difference in trend compared to advanced breast cancer patients remaining stable within 3 months? p, (n-progress, stable) i) pre-chemo to day 8 ii) pre-chemo to 3 months iii) pre-chemo to 6 months*
TF µg/ml, geometric mean (CI)	194 (125-302) *(15)*	166 (93-296) *(15)*	154 (98-240) *(15)*	162 (101-262) *(14)*	86 (23-317) *(8)*	228 (125-416) *(7)*	i) 0.5 (14,19) ii) 0.4 (8,18) iii) 0.5 (7,14)
CP mU, geometric mean (CI)	26.2 (20.7-33.1) *(14)*	30.1 (25.5-35.6) *(14)*	31.9 (25.2-40.4) *(13)*	26.7 (20.6-34.5) *(13)*	28.4 (24.0-33.6) *(7)*	25.0 (17.8-35.0) *(5)*	i) 0.3 (12,17) ii) 0.6 (7,16) iii) 0.5 (5,13)
TSP-1 ng/ml, geometric mean (CI)	944 (416-2122) *(4)*	1064 (589-1920) *(5)*	1022 (133-7864) *(5)*	276 *(2)*	*(1)*	*(0)*	i) 0.4 (2,5) ii) (1,3) iii) (0,2)
TNF-α µg/ml, geometric mean (CI)	3.28 (2.52-4.26) *(8)*	3.33 (2.45-4.54) *(8)*	2.94 (2.18-3.99) *(7)*	2.80 (2.13-3.68) *(8)*	3.72 (2.31-6.02) *(5)*	4.03 (1.66-9.80) *(4)*	i) 0.9(7,12) ii) 0.6 (5,11) iii) 0.4 (4,9)

Procoagulant/ adhesion molecule	*Pre-chemotherapy (n)*	*Day 1 (post chemotherapy) (n)*	*Day 4 (post chemotherapy) (n)*	*Day 8 (post chemotherapy) (n)*	*3 months (post chemo) (n)*	*6 months (post chemo) (n)*	*Significant difference in trend compared to advanced breast cancer patients remaining stable within 3 months? p, (n- progress, stable)* *i) pre-chemo to day 8* *ii) pre-chemo to 3 months* *iii) pre-chemo to 6 months*
pVEGF µg/ml, geometric mean (CI)	26.0 (16.7-40.6) *(15)*	19.8 (15.4-25.5) *(15)*	25.1 (18.7-33.7) *(15)*	27.4 (21.0-35.6) *(14)*	23.5 (14.1-39.3) *(8)*	24.7 (13.5-45.4) *(7)*	i) 0.5 (14,19) ii) 0.6 (8,18) iii) 0.5 (7,15)
sVEGF µg/ml, geometric mean (CI)	359 (239-539) *(15)*	317 (208-484) *(15)*	236 (151-371) *(15)*	244 (159-375) *(14)*	345 (148-804) *(8)*	286 (132-619) *(7)*	i) 0.4 (14,17) ii) 0.5 (8,16) iii) 0.8 (7,13)
VCAM-1 ng/ml, geometric mean (CI)	832 (628-1102) *(15)*	805 (588-1101) *(15)*	766 (583-1004) *(15)*	733 (588-914) *(14)*	624 (341-1141) *(8)*	928 (558-1541) *(7)*	i) 0.2 (14,17) ii) 0.09 (8,16) iii) 0.3 (7,13)
E-selectin ng/ml, geometric mean (CI)	32.5 (20.6-51.2) *(14)*	31.7 (20.8-48.2) *(14)*	30.3 (20.9-43.9) *(14)*	22.5 (14.4-35.3) *(13)*	23.2 (13.3-40.7) *(7)*	29.1 (13.0-65.1) *(6)*	i) 0.6 (13,19) ii) 0.4 (7,18) iii) 0.6 (6,15)

Appendix 34: Alterations in procoagulants and endothelial adhesion molecules induced by chemotherapy in advanced breast cancer patients remaining stable or responding to chemotherapy within the first six months of commencement of treatment

Procoagulant/ adhesion molecule	*Pre-chemotherapy (n)*	*Day 1 (post chemotherapy) (n)*	*Day 4 (post chemotherapy) (n)*	*Day 8 (post chemotherapy) (n)*	*3 months (post chemotherapy) (n)*	*6 months (post chemotherapy) (n)*	*Significant difference in trend compared to advanced breast cancer patients progressing within 6 months? p, (n-progress, stable) i) pre-chemo to day 8 ii) pre-chemo to 3 months iii) pre-chemo to 6 months*
TF µg/ml, geometric mean (CI)	228 (147-354) *(8)*	204 (129-327) *(8)*	187 (106-327) *(8)*	194 (117-322) *(8)*	185 (120-284) *(8)*	196 (128-302) *(7)*	i) 1.0 (25,8) ii) 0.9 (18, 8) iii) 0.5 (14,7)
CP mU, geometric mean (CI)	31.0 (21.7-44.4) *(6)*	26.5 (15.0-47.0) *(6)*	25.9 (17.3-38.9) *(6)*	23.7 (12.9-43.5) *(6)*	29.1 (19.5-43.4) *(7)*	25.2 (19.8-32.1) *(7)*	i) 0.4 (23,6) ii) 0.5 (17,6) iii) 0.6 (12,6)
TSP-1 ng/ml, geometric mean (CI)	963 (596-1541) *(3)*	171 *(2)*	679 (392-1188) *(3)*	399 (33-4915) *(3)*	750 (459-1224) *(3)*	742 (645-854) *(3)*	i) 0.1 (5,2) ii) (3,1) iii) (1,1)
TNF-α µg/ml, geometric mean (CI)	2.39 *(2)*	3.77 *(2)*	3.74 *(2)*	2.35 *(2)*	2.71 *(2)*	3.52 *(2)*	i) 0.04 (17,2) ii) 0.04 (14,2) iii) 0.06 (11,2)

Procoagulant/ adhesion molecule	*Pre-chemotherapy (n)*	*Day 1 (post chemotherapy) (n)*	*Day 4 (post chemotherapy) (n)*	*Day 8 (post chemotherapy) (n)*	*3 months (post chemo) (n)*	*6 months (post chemo) (n)*	*Significant difference in trend compared to advanced breast cancer patients progressing within 6 months? p, (n- progress, stable)* *i) pre-chemo to day 8* *ii) pre-chemo to 3 months* *iii) pre-chemo to 6 months*
pVEGF µg/ml, geometric mean (CI)	19.8 (11.7-33.6) *(8)*	17.2 (10.1-29.1) *(8)*	13.7 (5.3-35.1) *(8)*	26.4 (19.0-36.7) *(8)*	21.3 (11.3-40.2) *(8)*	34.0 (19.1-60.5) *(8)*	i) 0.3 (25,8) ii) 0.4 (18,8) iii) 0.4 (14,8)
sVEGF µg/ml, geometric mean (CI)	248 (103-598) *(7)*	188 (54-660) *(6)*	203 (73-562) *(7)*	229 (89-590) *(7)*	308 (140-678) *(8)*	224 (109-458) *(8)*	i) 0.4 (25,6) ii) 0.7 (18,6) iii) 0.4 (14,6)
VCAM-1 ng/ml, geometric mean (CI)	593 (347-1013) *(8)*	519 (296-909) *(7)*	559 (361-865) *(7)*	468 (290-755) *(7)*	583 (315-1080) *(7)*	604 (349-1045) *(7)*	i) 0.8 (25,6) ii) 0.4 (18,6) iii) 0.4 (14,6)
E-selectin ng/ml, geometric mean (CI)	37.2 (28.0-43.4) *(8)*	39.2 (26.1-58.9) *(8)*	35.7 (24.8-51.3) *(8)*	26.8 (17.4-41.3) *(8)*	37.2 (23.0-60.0) *(8)*	33.3 (19.0-58.2) *(8)*	i) 0.7 (24,8) ii) 0.7 (17,8) iii) 0.3 (13,8)

Appendix 35: Alterations in procoagulants and endothelial adhesion molecules induced by chemotherapy in advanced breast cancer patients progressing within six months of commencement of treatment

Procoagulant/ adhesion molecule	*Pre-chemotherapy (n)*	*Day 1 (post chemotherapy) (n)*	*Day 4 (post chemotherapy) (n)*	*Day 8 (post chemotherapy) (n)*	*3 months (post chemotherapy) (n)*	*6 months (post chemotherapy) (n)*	*Significant difference in trend compared to advanced breast cancer patients remaining stable within 6 months? p, (n-progress, stable) i) pre-chemo to day 8 ii) pre-chemo to 3 months iii) pre-chemo to 6 months*
TF µg/ml, geometric mean (CI)	167 (124-226) *(28)*	165 (117-230) *(27)*	140 (104-189) *(26)*	158 (119-209) *(26)*	114 (62-211) *(18)*	221 (144-344) *(14)*	i) 1.0 (25,8) ii) 0.9 (18, 8) iii) 0.5 (14,7)
CP mU, geometric mean (CI)	27.7 (23.8-32.2) *(27)*	35.0 (30.7-40.0) *(26)*	29.0 (24.7-34.1) *(24)*	28.4 (24.0-33.6) *(25)*	26.3 (20.2-34.0) *(17)*	24.0 (18.7-30.8) *(12)*	i) 0.4 (23,6) ii) 0.5 (17,6) iii) 0.6 (12,6)
TSP-1 ng/ml, geometric mean (CI)	1153 (781-1703) *(8)*	1212 (871-1686) *(9)*	788 (226-2752) *(8)*	846 (247-2893) *(6)*	3134 (963-10199) *(5)*	953 *(2)*	i) 0.1 (5,2) ii) (3,1) iii) (1,1)
TNF-α µg/ml, geometric mean (CI)	3.60 (3.01-4.31) *(20)*	3.84 (3.14-4.70) *(19)*	3.44 (2.87-4.12) *(17)*	3.24 (2.64-3.97(*(19)*	4.16 (3.21-5.40) *(14)*	4.35 (3.23-5.86) *(11)*	i) 0.04 (17,2) ii) 0.04 (14,2) iii) 0.06 (11,2)

Procoagulant/ adhesion molecule	*Pre-chemotherapy (n)*	*Day 1 (post chemotherapy) (n)*	*Day 4 (post chemotherapy) (n)*	*Day 8 (post chemotherapy) (n)*	*3 months (post chemo) (n)*	*6 months (post chemo) (n)*	*Significant difference in trend compared to advanced breast cancer patients remaining stable within 6 months? p, (n- progress, stable)* *i) pre-chemo to day 8* *ii) pre-chemo to 3 months* *iii) pre-chemo to 6 months*
pVEGF µg/ml, geometric mean (CI)	23.7 (17.4-32.2) *(28)*	20.7 (15.6-27.5) *(27)*	25.5 (18.1-35.9) *(26)*	27.9 (22.5-34.5) *(26)*	25.3 (18.8-34.1) *(18)*	30.1 (19.8-45.6) *(14)*	i) 0.3 (25,8) ii) 0.4 (18,8) iii) 0.4 (14,8)
sVEGF µg/ml, geometric mean (CI)	373 (294-473) *(28)*	315 (245-403) *(27)*	251 (189-332) *(26)*	260 (201-337) *(26)*	361 (247-530) *(18)*	323 (219-478) *(14)*	i) 0.4 (25,6) ii) 0.7 (18,6) iii) 0.4 (14,6)
VCAM-1 ng/ml, geometric mean (CI)	778 (651-931) *(18)*	740 (610-900) *(27)*	736 (612-884) *(26)*	667 (570-781) *(26)*	666 (494-898) *(18)*	865 (614-1219) *(14)*	i) 0.8 (25,6) ii) 0.4 (18,6) iii) 0.4 (14,6)
E-selectin ng/ml, geometric mean (CI)	31.8 (24.0-42.3) *(27)*	31.7 (23.9-42.0) *(26)*	31.2 (24.2-40.2) *(25)*	23.2 (17.4-30.9) *(25)*	27.2 (19.6-37.9) *(17)*	35.2 (22.0-56.3) *(13)*	i) 0.7 (24,8) ii) 0.7 (17,8) iii) 0.3 (13,8)

Appendix 36: Alterations in procoagulants and endothelial adhesion molecules induced by chemotherapy in advanced breast cancer patients remaining stable within the first two years following commencement of chemotherapy

Procoagulant/ adhesion molecule	*Pre-chemotherapy (n)*	*Day 1 (post chemotherapy) (n)*	*Day 4 (post chemotherapy) (n)*	*Day 8 (post chemotherapy) (n)*	*3 months (post chemotherapy) (n)*	*6 months (post chemotherapy) (n)*	*Significant difference in trend compared to early breast cancer patients progressing within 2 years? p, (n- progress, stable) i) pre-chemo to day 8 ii) pre-chemo to 3 months iii) pre-chemo to 6 months*
TF µg/ml, geometric mean (CI)	89 (65-122) *(80)*	96 (68-133) *(77)*	79 (57-110) *(75)*	75 (55-103) *(77)*	82 (61-110) *(77)*	103 (75-140) *(76)*	i) 1.0 (4,75) ii) 1.0 (4,75) iii) 0.9 (4,74)
CP mU, geometric mean (CI)	33.3 (30.8-36.0) *(74)*	37.9 (35.3-40.8) *(73)*	37.3 (34.9-40.0) *(70)*	34.2 (31.9-36.8) *(73)*	32.0 (29.9-34.3) *(72)*	36.0 (33.3-38.9) *(72)*	i) 0.9 (4,70) ii) 0.6 (4,69) iii) 0.5 (4,69)
TSP-1 ng/ml, geometric mean (CI)	645 (428-973) *(30)*	721 (446-1176) *(31)*	557 (372-837) *(30)*	369 (242-561) *(28)*	713 (498-1033) *(37)*	633 (392-1022) *(30)*	i) (1,27) ii) (1,26) iii) (1,20)
TNF-α µg/ml, geometric mean (CI)	*(41)*	*(40)*	*(40)*	(*(40)*	*(40)*	*(38)*	i) (1,40) ii) (1,40) iii) (1,38)

Procoagulant/ adhesion molecule	*Pre-chemotherapy (n)*	*Day 1 (post chemotherapy) (n)*	*Day 4 (post chemotherapy) (n)*	*Day 8 (post chemotherapy) (n)*	*3 months (post chemo) (n)*	*6 months (post chemo) (n)*	*Significant difference in trend compared to early breast cancer patients progressing within 2 years? p, (n- progress, stable) i) pre-chemo to day 8 ii) pre-chemo to 3 months iii) pre-chemo to 6 months*
pVEGF µg/ml, geometric mean (CI)	14.2 (12.0-16.8) *(79)*	13.5 (11.8-15.5) *(76)*	14.5 (12.2-17.2) *(74)*	18.7 (16.2-21.6) *(76)*	21.1 (17.7-25.2) *(76)*	19.8 (16.8-23.2) *(75)*	i) 0.5 (4,74) ii) 0.6 (4,74) iii) 0.8 (4,73)
sVEGF µg/ml, geometric mean (CI)	187 (160-217) *(81)*	183 (156-215) *(77)*	139 (118-164) *(77)*	132 (111-157) *(79)*	219 (188-255) *(79)*	164 (142-189) *(78)*	i) 0.5 (4,75) ii) 0.8 (4,75) iii) 0.8 (4,74)
VCAM-1 ng/ml, geometric mean (CI)	621 (578-667) *(81)*	559 (518-604) *(77)*	580 (538-625) *(76)*	572 (530-616) *(78)*	662 (604-726) *(78)*	639 (582-701) *(77)*	i) 0.1 (4,75) ii) 0.2 (4,75) iii) 0.3 (4,74)
E-selectin ng/ml, geometric mean (CI)	28.2 (25.4-31.2) *(82)*	25.9 (23.3-28.8) *(79)*	22.8 (20.5-25.3) *(77)*	21.1 (19.0-23.5) *(79)*	25.3 (22.7-28.3) *(79)*	28.9 (25.5-32.8) *(78)*	i) 0.5 (4,77) ii) 0.6 (4,77) iii) 0.8 (4,76)

Appendix 37: Alterations in procoagulants and endothelial adhesion molecules induced by chemotherapy in early breast cancer patients progressing within two years of commencement of chemotherapy

Procoagulant/ adhesion molecule	*Pre-chemotherapy (n)*	*Day 1 (post chemotherapy) (n)*	*Day 4 (post chemotherapy) (n)*	*Day 8 (post chemotherapy) (n)*	*3 months (post chemotherapy) (n)*	*6 months (post chemotherapy) (n)*	*Significant difference in trend compared to early breast cancer patients remaining stable within 2 years? p, (n- progress, stable) i) pre-chemo to day 8 ii) pre-chemo to 3 months iii) pre-chemo to 6 months*
TF µg/ml, geometric mean (CI)	166 (30-916 *(5)*	167 (23-1236) *(4)*	145 (15-1422) *(4)*	150 (19-1176) *(4)*	136 (17-1075) *(4)*	106 (15-728) *(4)*	i) 1.0 (4,75) ii) 1.0 (4,75) iii) 0.9 (4,74)
CP mU, geometric mean (CI)	29.5 (19.4-45.0) *(4)*	35.6 (18.8-67.4) *(4)*	34.7 (26.6-45.2) *(4)*	29.8 (17.8-50.1) *(4)*	35.6 (18.1-70.1) *(4)*	41.4 (25.9-66.2) *(4)*	i) 0.9 (4,70) ii) 0.6 (4,69) iii) 0.5 (4,69)
TSP-1 ng/ml, geometric mean (CI)	273 *(2)*	602 (72-5014) *(4)*	639 (420-982) *(3)*	742 (672-812) *(4)*	679 (469-992) *(2)*	1153 *(2)*	i) (1,27) ii) (1,26) iii) (1,20)
TNF-α µg/ml, geometric mean (CI)	*(1)*	*(1)*	*(1)*	*(1)*	*(1)*	*(1)*	i) (1,40) ii) (1,40) iii) (1,38)

Procoagulant/ adhesion molecule	*Pre-chemotherapy (n)*	*Day 1 (post chemotherapy) (n)*	*Day 4 (post chemotherapy) (n)*	*Day 8 (post chemotherapy) (n)*	*3 months (post chemo) (n)*	*6 months (post chemo) (n)*	*Significant difference in trend compared to early breast cancer patients remaining stable within 2 years? p, (n- progress, stable) i) pre-chemo to day 8 ii) pre-chemo to 3 months iii) pre-chemo to 6 months*
pVEGF µg/ml, geometric mean (CI)	13.8 (6.4-29.8) *(5)*	7.8 (2.0-30.3) *(4)*	10.9 (6.0-15.4) *(4)*	10.0 (6.6-15.4) *(4)*	16.8 (7.9-35.5) *(4)*	13.1 (4.5-38.3) *(4)*	i) 0.5 (4,74) ii) 0.6 (4,74) iii) 0.8 (4,73)
sVEGF µg/ml, geometric mean (CI)	111 (38-326) *(5)*	91 (30-277) *(4)*	60 (27-134) *(4)*	54 (14-213) *(4)*	94 (24-359) *(4)*	82 (22-303) *(4)*	i) 0.5 (4,75) ii) 0.8 (4,75) iii) 0.8 (4,74)
VCAM-1 ng/ml, geometric mean (CI)	766 (519-1131) *(5)*	668 (359-1244) *(4)*	642 (362-1136) *(4)*	616 (320-1188) *(4)*	675 (392-1160) *(4)*	647 (339-1236) *(4)*	i) 0.1 (4,75) ii) 0.2 (4,75) iii) 0.3 (4,74)
E-selectin ng/ml, geometric mean (CI)	25.4 (14.6-44.4) *(5)*	31.1 (14.2-68.0) *(4)*	25.1 (10.7-59.1) *(4)*	23.4 (12.7-43.1) *(4)*	25.4 (14.2-45.4) *(4)*	31.9 (19.7-51.8) *(4)*	i) 0.5 (4,77) ii) 0.6 (4,77) iii) 0.8 (4,76)