Does the serial measurement of known biomarkers help to identify those individuals with cancer who are at the highest risk of thrombosis?

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DECLARATION

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ABSTRACT

Cancer associated thrombosis (CAT) is a serious complication seen in patients with all types of cancer. CAT affects up to 20% of all patients diagnosed with cancer and is associated with poorer outcomes and an increased risk of mortality. Risk assessment models exist which try to predict those cancer individuals who are at the highest risk of thrombosis, though these are not without their limitations.

The aim of this thesis was to investigate novel methods of predicting cancer-associated thrombosis. A variety of approaches were used to address this aim.

First, a retrospective clinical audit was performed on patients (n = 75) with a diagnosis of pancreatic cancer, a cancer with a high prevalence of CAT. The Khorana score, the current standard risk assessment model, was assessed for the prediction of CAT. In this retrospective audit, the Khorana score was not found to predict CAT.

Next, a prospective large-scale meta-analysis of published literature was performed to assess the potential of VEGF as a novel biomarker for CAT. The meta-analysis of eight studies, including 1547 patients with a diagnosis of a variety of primary site cancers, demonstrated that, whilst VEGF was found to be increased in cancer patients at the point of thrombosis, it could not be used for the prediction of CAT.

Finally, a small prospective clinical trial (n = 54) was undertaken to assess the serial measurement at three sampling timepoints (baseline, 1-month and 3-months after the start of chemotherapy) of three biomarkers in participants treated at The

Newcastle Upon Tyne Hospitals NHS Foundation Trust with a diagnosis of cancer of any primary site. This small prospective clinical trial determined that changes in neither D-dimers nor VEGF levels were predictive of CAT. There was however, a statistically significant difference ($p = 0.0310$) was seen between baseline and 1-month in soluble P-selectin levels in those who did and did not experience a thrombotic event.

The findings presented in this thesis demonstrates that adaptation to risk assessment models may be required to improve the prediction of CAT in both pancreatic cancer and local geographical populations. Further work into the predictive capacity of VEGF and serial measurement of soluble P-selectin is required.

Further work is required in larger patient cohorts and in specific cancer types using both VEGF and sP-selectin to elucidate any trends which could be used for the prediction of CAT.

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ADP	Adenosine Diphosphate
ASCO	American Society of Clinical Oncology
ASH	American Society of Hematology
BMI	Body Mass Index
BSH	British Society for Haematology
CAT	Cancer-Associated Thrombosis
CD	Cluster of Differentiation
CP	Cancer Procoagulant
CT	Computerised Tomography
CVA	Cerebrovascular Accident
CVC	Central Venous Catheter
DNA	Deoxyribonucleic Acid
DVT	Deep Vein Thrombosis
ELISA	Enzyme Linked Immunosorbant Assay
ESMO	European Society of Medical Oncology
EPCR	Endothelial Protein C Receptor

ESC	European Society of Cardiology
ET	Essential Thrombocythaemia
FBC	Full Blood Count
FDPs	Fibrin Degradation Products
FGF	Fibroblastic Growth Factor
H3Cit	Citrullinated histone H3
Hb	Haemoglobin
IMWG	International Myeloma Working Group
IQC	Internal Quality Control
ITAC	International Initiative on Thrombosis and Cancer
LMWH	Low Molecular Weight Heparin
MDT	Multi Disciplinary Meeting
MI	Myocardial Infarction
MRI	Magnetic Resonance Imaging
NET	Neutrophil Extracellular Trap
NHS	National Health Service
NICE	National Institute for Health and Care Excellence

NNT	Number Needed to Treat
NOS	Newcastle-Ottawa score
NPV	Negative Predictive Value
NSCLC	Non-Small Cell Lung Cancer
PAI-1	Plasminogen activator inhibitor
PAR	Protease-activated Receptor
PDGF	Platelet Derived Growth Factor
PE	Pulmonary Embolism
PET	Positron Emission Tomography
PF4	Platelet Factor 4
PICC	Peripherally Inserted Central Catheter
Plt	Platelet Count
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PSGL-1	P-selectin glycoprotein ligand-1
PV	Polycythaemia Vera
RAM	Risk Assessment Model

sP-selectin	Soluble P-selectin
SACT	Systemic Anti-Cancer Therapy
tPA	Tissue Plasminogen Activator
TF	Tissue Factor
TFPI	Tissue Factor Pathway Inhibitor
TNF-α	Tumour Necrosis Factor alpha
UKAS	United Kingdom Accreditation Service
VEGF	Vascular Endothelial Growth Factor
VEGF-A	Vascular Endothelial Growth Factor A
VTE	Venous Thromboembolism
vWF	von Willebrand Factor
WBC	White Blood Cell count
WHO	World Health Organisation

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CHAPTER 1. INTRODUCTION

1.1 Cancer associated thrombosis

Cancer is the abnormal, uncontrolled proliferation of mutated cells which causes a tumour. It is one of the most prevalent causes of death in the Western world and can affect any organ in the body. Thrombosis is the formation of a blood clot, which can occur in any blood vessel of the body. When the two events occur together it is known as cancer-associated thrombosis.

The relationship between cancer and thrombosis was first described by Armand Trousseau in 1865, leading to a description of the phenomenon as "Trousseau Syndrome". Although this description is not used in clinical practice, it describes cases where a thrombotic event has occurred in a patient with cancer. The phenomenon is more commonly known as cancer-associated thrombosis, or CAT, and is responsible for up to 9% of deaths in ambulatory cancer patients (Sud and Khorana, 2009).

Cancer-associated thrombosis can either be venous (often resulting in a deep vein thrombosis (DVT) or pulmonary embolism (PE)), or arterial (more commonly resulting in a myocardial infarction (MI, or heart attack), or a cerebrovascular accident (CVA, or stroke) (May and Moll, 2021). Venous events, also known as a venous thromboembolism or VTE, are more common than arterial events in CAT (Falanga and Marchetti, 2023), and some researchers therefore only include VTE events in their

study of cancer-associated thrombosis. For the purposes of this thesis CAT includes venous (including unusual site) and arterial thrombosis.

Occasionally, a seemingly unprovoked thrombotic event can lead to further clinical investigations and result in a diagnosis of cancer. However, the National Institute for Health and Care Excellence (NICE) guidance (NICE, 2023) suggests that extensive cancer screening should not take place after all unprovoked thrombotic events, but rather in individuals where cancer is suspected after a thorough patient history, physical examination and routine blood tests, which is what occurs in the United Kingdom. This is in direct contrast with work by Sørensen et al, 2000 who stated that 'cancer diagnosed at the same time as, or within one year after an episode of venous thromboembolism, is associated with an advanced stage of cancer and a poor prognosis' (Sørensen et al., 2000 pp. 1846), and therefore the author of that paper suggests that extensive cancer screening should occur after a thrombotic event.

Patients diagnosed with CAT have a poor survival rate, with mortality rates significantly different between four cohorts (disease-free – 0.63 per 100 person-years; VTE only – 5.0 per 100 person-years; Cancer (all types) only – 9.2 per 100 person-years, and cancer-related VTE – 45.3 per 100 person-years) studied as part of the Scandinavian Thrombosis and Cancer (STAC) cohort (Crobach et al, 2023). In addition, the 1-year survival for those diagnosed with CAT was 12%, compared to 36% in those with cancer alone (Sørensen et al, 2000). Taken together, these studies illustrate how important it is to be able to predict and prevent a thrombotic episode

in those patients diagnosed with cancer. The prediction of CAT by the serial measurement of biomarkers will be explored in this thesis.

1.2 Incidence

Cancer associated thrombosis (CAT), which in this thesis is where the cancer is from any primary site unless stated otherwise, is said to complicate the clinical journey of 1 in 20 patients with cancer in the United Kingdom (Alikhan et al, 2024).

Whilst the figures above give a good estimation, there have also been several population-based cohort studies who have assessed the risk, and the factors which may predispose a patient with cancer to a higher risk of a thrombotic event than those without cancer.

CAT is associated with a high mortality rate, and poor patient outcomes (Chew et al, 2006). The reasons for this are multi-factorial and include delays to cancer treatment whilst the thrombotic event is treated, with multiple studies confirming that the highest rates of CAT are seen in those with advanced-stage disease, particularly metastatic disease (Blom et al, 2006; Chew et al, 2006; Monreal et al, 2006; Cronin-Fenton et al, 2010; Walker et al, 2013; Mulder et al, 2021; Mahajan et al, 2022).

One of the first such studies, Chew et al, 2006, followed 235,149 patients with cancer of all types in California between 1993 to 1995, with a quoted incidence of VTE in these patients at 1.6% within 2 years. This study overlapped with a Dutch cohort study (Blom et al, 2006), who followed 66,329 patients with cancer of any type in the

Netherlands between 1986 and 2002 and found a cumulative incidence of CAT of 12.3 per 1000 patients (or 1.23%), which roughly agrees with the figures of Chew et al, 2006. Finally, a Danish cohort study (Cronin-Fenton et al, 2010) compared VTE incidence rates between patients with and without cancer of any type between 1997 to 2006, and discovered that the rate in the cancer population was almost double that of the non-cancer population (8.0 per 1000 person- years versus 4.7 per 1000 person-years, 95% confidence intervals 7.6 – 8.5, and 4.3 – 5.1 respectively).

More recent cohort studies have suggested that the incidence of CAT is increasing. Between 1979 and 1999, the VTE rate increased from 1.5 to 3.5% among hospitalized cancer patients, while rates remained stable in patients without cancer (Stein et al, 2006). In more recent years, Mahajan et al (2022) compared the work of Chew et al (2006) and used data from the California Cancer Registry up to 2017. The incidence of CAT, where cancer is of any originating site, in the studied cohort increased from 1.6% in the period 1993 to 1995 up to 6.6% in the period 2014 to 2017, with the 12-month pancreatic cancer exclusively cumulative incidence increasing from 8.92% in the period of 2005 to 2007, to 11.9% in those diagnosed between 2014 to 2017.

These findings were confirmed by Mulder et al (2021), who built on the work of Cronin-Fenton et al (2010), and who followed 499,092 patients with all types of cancer between 1997 to 2017 in Denmark. The 12-month incidence of VTE in these patients was 1% in 1997, but this had increased to 3.4% by 2017 (Mulder et al, 2021).

In addition, Khorana et al (2007a), followed 1,015,598 patients with all types of cancer in the United States of America (USA) between 1995 to 2003. The rate of VTE in this population increased from 3.6% in 1995/6 to 4.6% in 2002/3 (Khorana et al, 2007a).

Walker et al (2013) followed 83,203 patients with cancer (any primary site) in the United Kingdom (UK) between 1997 and 2006. They also observed that rates of CAT were increasing within the UK population. Rates of VTE in the cancer population rose from 10.3 per 1000 person-years in 1997, to 19.0 per 1000 person-years in 2007. There was no such increase in the rates of VTE in the non-cancer population, which acted as a control group (Walker et al, 2013).

Finally, the Vienna CATS Study (Köningsbrügge et al, 2014), a large prospective, observational cohort study which recruited 1544 participants over 10 years (2003 to 2014) and reported a 2-year CAT incidence of 7.4%.

The authors of these cohort studies also theorised why the rate of CAT may appear to be increasing. This includes improved mortality rates, improved cancer-directed therapy (such as immunotherapy, protein kinase inhibitors, hormone therapy, and more intensive chemotherapy regimens), and the more frequent use of high-resolution imaging (and indeed the improvements made in this technology) leading to an increased rate of incidental (asymptomatic) findings of VTE. Indeed, Mulder et al (2021) quotes that the average number of computerised tomography (CT) scans a patient received in the first twelve months after a diagnosis of cancer has increased from 0.17 in 2001, to 1.16 in 2017 (Mulder et al, 2021). Khorana et al (2007a)

summarised this stating that "there is an increased perception in the oncology community that VTE is being diagnosed more frequently, possibly due to an increased awareness and an increased usage of diagnostic procedures" (Khorana et al, 2007a pp.2340).

With increasing incidence of CAT, the impact of CAT on both the patient, and on healthcare service providers is profound. Khorana et al (2008), summarised this into six categories, which are still true to date: Potential for the need of long-term anticoagulation, potential delay in chemotherapy and/or surgery, increased risk of recurrent VTE, increased risk of bleeding whilst on anticoagulant therapy, decrease in the quality of life (QoL) of the individual affected, and an increase in the requirement of healthcare resources. The latter point is of particular importance in a taxpayer-funded healthcare system such as the National Health Service (NHS) in the United Kingdom.

1.3 Risk Factors of CAT

There are multiple risk factors which may increase an individuals' probability of developing CAT. These can be broadly categorised into the following categories; patient-related, cancer-related, and cancer-associated. These are summarised in Table 1.1 and discussed in more detail in the text following.

Patient-related	Cancer-related	Cancer-associated
Age	Timing	Surgery
Sex/ gender	Cancer site	Immobilisation
Ethnicity	Cancer stage and grade	Hospitalisation
Comorbidities	Cancer-causing mutations	Catheters/ indwelling lines
Inherited prothrombotic mutations/ family history of VTE		Chemotherapy
		Hormone therapy
Personal prior history of VTE		Radiotherapy

Table 1.1. **Cancer-associated thrombosis (CAT) risk factors.**

1.3.1 Patient- related risk factors

1.3.1.1 Age

Increasing age is a risk factor for thrombosis. This applies to both the cancer (of any type) and non-cancer population. Individuals over the age of 65 years have a greater likelihood of developing a VTE than those younger than this (Khorana et al, 2006; Khorana et al 2007a). A study in 2013 also found that patients over the age of seventy had a 2-fold increase in the risk of developing a VTE compared to those below 70 years (11% rate versus a 5.6% rate, p = 0.020) (Vergati et al, 2013). The reasons for an increase in thrombosis rate both in the general, and in the cancer population, are multifactorial. Older individuals are more likely to have a chronic low-level inflammatory state which can activate the coagulation system (Silverstein et al, 2007). In addition, immobilisation, dehydration and the increased incidence of comorbid states is more likely in these individuals, which are all independent risk factors for thrombosis (McLendon et al, 2025).

In contrast, whilst individuals over 60 years of age had a higher rate of CAT, a UK population-based cohort study from 1997 to 2006 found that in certain primary site cancer types (pancreas, mesothelioma and lung) the rates of CAT were higher in younger populations (Walker et al, 2013), with no explanation hypothesised.

1.3.1.2 Sex

There is conflicting evidence to suggest that neither males nor females have the highest incidence of CAT. Khorana et al (2007a) suggested that the patients at the highest risk of VTE were females, and that males were at the highest risk of arterial thrombosis. This is in contrast with Mahajan et al (2022) who stated that males were at the highest risk of VTE. However, Chew et al (2006) and Cronin-Fenton (2010) found no differences between the rates of CAT between males and females.

Therefore, the evidence is unclear, with conflicting findings in each paper.

1.3.1.2 Ethnicity

Several population-based cohort studies have suggested that there are differences in the incidence of CAT between ethnicities, the reasons for which are hypothesised, but have not been proven.

Asian-Pacific Islanders have the lowest risk of developing CAT (Chew et al 2006; Khorana et al 2007a; Mahajan et al 2022). The reason hypothesised for this lower rate includes the low prevalence of the inherited thrombophilia mutations such as Factor V Leiden (present in approximately 5% of the European population, and rare in all

other ethnic populations (Arachchillage et al, 2022)) and the Prothrombin G20210A mutation (present in 1-2% of the European population, but very rare or absent in all other ethnicities (Arachchillage et al, 2022)). Further details regarding these mutations can be found further in this thesis chapter (Introduction – Chapter 1).

African Americans are at the highest risk of developing CAT (Chew et al 2006; Khorana et al 2007a; Mahajan et al, 2022). African-Americans with uterine cancer had a 2-fold higher risk of developing VTE (Chew et al, 2006). However, those with lung cancer and non-Hodgkins lymphoma had a lower risk than Caucasians of developing a VTE (Chew et al, 2006).

However, one study (Stein et al, 2006) found no differences in CAT incidence between ethnicities.

1.3.1.4 Comorbidities

The presence of comorbidities also increases the risk of CAT (Khorana et al, 2006; Khorana et al, 2007a; Eichinger et al 2016; Mahajan et al, 2022; Crobach et al 2023).

Comorbidities studied include the presence of an infection, pulmonary disease, hypertension, renal disease, diabetes mellitus, congestive heart failure, hepatic disease, anaemia, obesity, or the use of regular transfusions (Khorana et al, 2007a). In a large California-based population cohort study, Mahajan et al (2022), determined that the incidence of CAT increased with the number of comorbidities present. Where a patient was never admitted to hospital, and the number of comorbidities

could not be determined, the rate was 5.1%, for zero comorbidities it was 6.5%, for 1-2 8.0%, and for 3 or more comorbidities the rate was 8.4% (Mahajan et al, 2022).

Patients with multiple medical comorbidities or other conditions had a 1.5-fold higher rate of VTE compared to those with none (Eichinger et al, 2016).

As a result of this increased risk, the inclusion of specific comorbidities forms part of several risk-assessment models, which aim to predict an individuals' risk of developing CAT. For example, an increased Body Mass Index (BMI), resulting in obesity is one aspect of the Khorana Score (Khorana et al, 2008). Risk assessment models for CAT will be discussed in greater detail later in this Introduction chapter.

1.3.1.5 Inherited prothrombotic mutations, and Family History of VTE

The presence of inherited prothrombotic mutations, for example, Factor V Leiden or the prothrombin G20210A mutation, also confers an increased risk of developing CAT (Pihusch et al, 2002; Blom et al, 2005; Eroglu et al, 2009; Garber et al, 2010; Pabinger et al, 2015; Zöller et al, 2015; Crobach et al, 2023).

The presence of the Factor V Leiden mutation is also a risk factor. 5% of Europeans (or those of European descent) are carriers of the mutation in the heterozygous form. The homozygous form is much rarer (Arachchillage et al, 2022).

The presence of the Factor V Leiden mutation in individuals without cancer, in heterozygous form increases the rate of VTE by 2.5-fold, and in the rare homozygous form by 80-fold (Moore et al, 2010).

Factor V Leiden is due to a point mutation in the Factor V gene, which renders it unable to be cleaved, and therefore inactivated, by activated Protein C (a natural anticoagulant) (Moore et al, 2010). Therefore, patients are an increased risk of developing venous thrombosis. However, not all patients who carry the Factor V Leiden mutation are affected by venous thrombosis, and often other physiological or environmental factors (for example, immobilisation) which increase the probability of venous thrombosis occurring need to be present (Moore et al, 2010).

Pabinger et al (2015), as part of the large Vienna CATS study, determined that the probability of developing VTE in cancer patients was 13% in those with Factor V Leiden, and 5.7% in those without this mutation after 6 months. After 1 year, the risk was 15% for those with Factor V Leiden, and 7.3% without. Thus, corresponding to an approximate 2-fold risk in developing CAT. This echoes work by Blom et al (2005) who determined that carriers (heterozygotes) of the Factor V Leiden mutation who also had cancer had a 12-fold increased risk of developing CAT versus individuals with cancer, but without the Factor V Leiden mutation (Blom et al, 2006). Finally, Eroglu et al (2009) in a small study involving 185 patients found that that 31.7% of those patients with CAT had the Factor V Leiden mutation, whereas only 1.6% of those not affected by CAT did (Eroglu et al, 2009).

The Prothrombin G20210A mutation, leading to increased levels of the coagulation factor prothrombin (Factor II), is present in heterozygous form in approximately 1-2% of Europeans (or those of European descent), and is rare or absent in other ethnic

populations (Arachchillage et al, 2022). The presence of this mutation in the non-cancer population increases the risk of venous thrombosis by 2-3-fold in the heterozygous form. Homozygous are very rare, and therefore data regarding increased relative risk is not available (Moore et al, 2010).

There is less evidence for the role of the prothrombin G20210A mutation in increasing the risk of CAT. Neither Ramacciotti et al (2003) nor Eroglu et al (2009) found an association between the presence of the mutation and the risk of VTE. However, Pihusch et al (2002) did determine that those with this mutation were at an increased risk of CAT.

Finally, a prior family history of VTE is also a risk factor for CAT (Zöller et al, 2015). A family history may indicate the presence of a thrombophilia mutation as discussed above, as well as exposure to the same or similar environmental factors. Zöller et al (2015) in a population-based cohort study in Sweden between 1987 to 2010, found that a family history of VTE was a risk factor for CAT in several cancer types. However, "familial factors are relatively more important in non-cancer than in cancer patients" (Zöller et al, 2015 pp. 573). However, thrombophilia mutations were not tested for in this study.

1.3.1.6 *Prior History of VTE*

A prior history of VTE is also a risk factor the development of CAT. Patients in whom there is a prior history of VTE have a 6-7-fold increased risk of VTE compared to those who have no history (Eichinger et al, 2016). The diagnosis of VTE may also be a

sign of occult cancer, which is yet to be diagnosed. Ovarian and pancreatic cancer had a high incidence of a DVT diagnosis in the year prior to a cancer diagnosis (Blom et al, 2006). The Scandinavian Thrombosis and Cancer Cohort (STAC) study (Crobach et al, 2023) followed patients who had been diagnosed with a VTE up to 1 year prior to their cancer diagnosis and found that 1-year survival rates were lower in those with a previous VTE compared to those who had no previous history (38% versus 47%).

A prior history of VTE is a known risk factor for the development of a further VTE in the non-cancer population (Hansson et al, 2000; Fahrni et al, 2015; Áinle and Kevane, 2020), and therefore it stands to reason that this is the same in the cancer population where a greater number of risk factors are present.

1.3.2 Cancer-related risk factors

There are various elements of the cancer itself, such as site and stage or grade, which may increase an individuals' risk of developing CAT.

1.3.2.1 Timing of cancer diagnosis relative to diagnosis of CAT

Multiple studies have shown that an individual is at the greatest risk of developing CAT in the first 6 months after a cancer diagnosis. For some individuals, the cancer and VTE diagnosis occur at the same time, or within days of each other.

Sørensen et al (2000) compared individuals with cancer of any primary site, but not VTE, and individuals who had cancer and a VTE diagnosed at the same time. The 1-

year survival rate between these two groups was striking, with a 36% survival rate in those with cancer alone, and a 12% survival rate in those with both cancer and VTE diagnosed simultaneously.

The risk of developing CAT is over 8-fold during the first year after cancer diagnosis, 3-fold during the second year, and over 2-fold during subsequent years (Cronin-Fenton et al, 2010). The large observational Vienna CATS Study (Köningsbrügge et al, 2014) also recorded a median time to VTE event of 103.5 days, which confirms that events occur in the first few months after a diagnosis of cancer. The reasons for this are multi-factorial. It is thought that in the first few months after receiving a cancer diagnosis the tumour burden is at its highest and that more interventions are also occurring, for example resection surgery and chemotherapy (Mahajan et al, 2022). The proportion of patients receiving chemotherapy in the first four months following a cancer diagnosis has increased from 17% in 1997 to 33% in 2017 (Mulder et al, 2021), which may also explain the increasing incidence of CAT, as chemotherapy is a strong independent risk factor for CAT, as will be described later in this Introduction chapter.

1.3.2.2 Cancer site

Some primary sites of cancer confer a higher risk of developing CAT than others.

Large population studies have helped to determine the incidence of CAT in various cancer types and have informed multiple risk-assessment models (RAM) to assist in providing a risk score for those at greatest risk of developing CAT.

Cancer sites which have the highest rate of CAT include pancreas, brain, stomach, ovary, bone, bladder, renal and lung (Blom et al 2006; Chew et al 2006; Walker et al, 2013; Eichinger et al, 2016; Mahajan et al 2022). Lower risk malignancies include breast, prostate, testicular, head/neck and melanoma (Blom et al 2006; Chew et al, 2006; Walker et al 2013). Subsequent population studies have confirmed these findings, apart from one study which designated ovarian cancer as a low risk for VTE (Crobach et al, 2023), and with the addition of haematological malignancies, in particular, multiple myeloma (Cronin-Fenton, 2010) as high-risk. The reasons for this are thought to be due to the advent, and increased use, of immunomodulatory drugs, such as thalidomide and lenalidomide, for the treatment of multiple myeloma in these patients (Khorana and Connolly, 2009).

Patients diagnosed with a myeloproliferative disorder are also at an increased risk of developing arterial, as well as venous, thrombotic events (Eichinger et al, 2016).

The highest risk cancer types are all mucin-producing, leading to aberrant expression and altered glycosylation of several mucins. Mucins interact with blood cells via selectins, resulting in the formation of microthrombi, and it is thought that this is which confers the additional risk (Shao et al, 2011; Abdol-Razzak, 2018).

In addition to primary cancer site, histology of the tumour is also thought to play a role. For example, in non-small cell lung cancer (NSCLC), there is an increased risk of CAT in adenocarcinoma versus squamous cell carcinoma (Chew et al, 2006).

Whilst cancer site can determine the probability of developing CAT, it should also be noted that VTE also occurs in those designated as low risk, "highly prevalent cancers with lower rates of VTE can contribute significantly to the overall burden of VTE" (Khorana and Connolly, 2009 pp. 4840).

1.3.2.3 Cancer stage and grade

Both cancer (all sites) stage and tumour grade also play a role in the likelihood for a thrombotic event occurring, with advanced stages, particularly metastatic cancer, have the highest risk of CAT (Mahajan et al, 2022).

In a Californian population study, metastatic disease at the time of diagnosis was found to be the strongest predictor of CAT, with the highest rates recorded in metastatic pancreatic cancer patients (20 per 100 patient-years), and the thromboembolism rate 4 to 13 times higher amongst cases with metastatic disease than cases with localised disease (Chew et al, 2006). Multiple subsequent studies have confirmed that metastatic disease confers a high risk for CAT, with distant metastases said to confer a 1.9-fold increased risk for CAT (Blom et al, 2006). In a Spanish study metastatic disease was independently associated with the occurrence of fatal pulmonary embolism (Monreal et al, 2006).

In addition, the presence of a high-grade tumour can also increase the likelihood of CAT. As part of the Vienna CATS study, tumour grade was also examined. Patients with a high-grade tumour (G3 and G4) had a VTE incidence rate of 8.2%, compared

to low-grade tumours (G1 and G2) with an incidence rate of 4.0% (Ahlbrecht et al, 2012).

The reasons why CAT is more likely to occur in patients with metastatic-stage disease are related to tumour burden, with a greater amount of cancer cells present in the body, which can elicit the processes required for thrombus formation.

1.3.2.4 Cancer-causing mutations

There is evidence that certain oncogenic mutations, present in tumours, can increase the risk of CAT.

Mutations in STK11, KRAS, CTNNB1, KEAP1, CDKN2B and MET all increase the risk of CAT in solid tumours, whereas the presence of a mutation in SETD2 is associated with a lower risk of CAT (Dunbar et al, 2021). Mutations in ALK and ROS1, found in non-small cell lung cancer are also associated with an increased rate of CAT (Zer et al, 2017; Ng et al, 2019).

This indicates that there are many factors at play in the development of CAT, with certain oncogenic mutations, and their relative risk yet to be elucidated.

1.3.3 Cancer-associated risk factors

Risk factors related to all types of cancer, but not due to the cancer itself, can also increase the risk of CAT. These include the treatment of cancer with surgery, chemotherapy, hormone therapy or radiotherapy. In addition, patients with cancer are more likely to be immobilised, hospitalised, have catheters or central lines in situ,

or suffer from dehydration because of the treatments they are receiving. All these factors increase the likelihood of thrombosis and are known risk factors, as outlined below.

1.3.3.1 Surgery

Surgery, and surgical procedures are known risk factors for the development of venous thrombosis. For this reason, all surgical patients treated in the NHS are offered a method for decreasing their risk of this complication. This includes the use of short-term thromboprophylaxis (usually in the form of low molecular weight heparin (Tinzaparin) injections), compression stockings, or the use of an intermittent pneumatic compression device (NICE, 2019)

Surgical procedures in patients with cancer are common, either as part of the diagnostic process (for example taking biopsies), or as part of the treatment, including resection surgery, which may be major surgery. The more major the surgery, the greater the risk of a thrombotic event occurring, with the patients undergoing the longest procedures experiencing a 1.27-fold increased risk of developing a VTE (Kim et al, 2015; Smeets et al, 2023).

This additional risk of thrombosis however is transient, and once the patient is mobile, and able to undertake normal activities the risk is diminished.

1.3.3.2 Immobilisation

Immobilisation, which may be because of surgery as described above, can also increase the risk of CAT (Pottier et al, 2009). Immobilisation may also occur as a side-effect of the cancer treatment, with fatigue, vomiting and gastrointestinal upset being common side-effects (Altun and Sonkaya, 2018; Katta et al, 2023).

Mobility in patients with cancer is clinically assessed by a performance score, where an individual is assessed on their ability to complete everyday tasks such as dressing and washing themselves, preparing meals, distances able to walk etc (NICE, 2007). Details of the World Health Organisation (WHO) performance score are shown in Table 1.2.

Grade	WHO Performance Status
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature e.g. Light housework, office work
2	Ambulatory and capable of all self-care but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of only limited self-care; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry on any self-care: totally confined to bed or chair
5	Dead

Table 1.2. **World Health Organization (WHO) performance status classification (NICE, 2007).**

A poor performance score, or a lack of mobility, increases the chances of VTE by venous stasis, with blood not flowing as normal.

Individuals with a poor performance score are more likely to have an advanced stage disease, and therefore these two risk factors are often linked, therefore risk is difficult to attribute to each individual factor (Khorana and Connolly, 2009).

1.3.3.3 Hospitalisation

Hospitalisation increases the risk of VTE for all patients, with one study reporting a 38-fold increased risk (Jordan Bruno et al, 2022), both in those with a cancer (all types) diagnosis, and those without. All patients should therefore have a VTE risk assessment within 14 hours of admission into a NHS hospital in the UK, as per NICE guidance (NICE, 2019).

Hospitalisation increases the chances of immobilisation as described above, and may have occurred because of surgery, because of the side-effects of the treatment the patient is receiving, or the cancer itself.

Khorana and Connolly (2009) stated that the highest rates of CAT were seen in two cohorts of patients with cancer of any type; hospitalised neutropenic patients (6.4% affected by CAT), and patients admitted to inpatient oncology service (7.8% affected with CAT). This underlines the risk that hospitalisation has on the chances of CAT occurring, and why ambulatory cancer patients have the lowest risk.

1.3.3.4 Presence of central venous catheters (CVC), or indwelling central venous lines

Patients with all types of cancer often have lines inserted, often a peripherally inserted central catheter (PICC) line, for the administration of chemotherapy (Wang et al, 2024).

The presence of a CVC, PICC line or any other indwelling line increases the chance of a VTE by causing damage to the vessel wall, and thus activating both the endothelium and the contact pathway of haemostasis, with the incidence of a symptomatic DVT in these patients ranging from 0.3% to 28% (Khorana and Connolly, 2009), and the incidence of asymptomatic PICC-related thrombosis in patients with cancer ranging from 2 to 66% (Wang et al, 2024). Another study (Decousus et al, 2018) found that 3.8% of the cohort were affected by catheter-associated thrombosis. Factors which may increase the likelihood of a catheter-associated VTE occurring include material used for the catheter, number of attempts it took for insertion, placement of catheter, open-ended catheters, and the substances the catheter transported (with cisplatin and vincristine the highest risk) (Khorana and Connolly, 2009; Wang et al, 2024).

1.3.3.5 Cancer treatments

The treatment a patient receives can also increase the risk of CAT. Surgery as a risk factor is covered elsewhere in this Introduction chapter.

1.3.3.5.1 Chemotherapy

Chemotherapy has been shown to increase the risk of CAT by 2.2-fold (Blom et al, 2006) to 6.5-fold (Khorana et al, 2007a). Some chemotherapy regimens are associated with a higher risk than others, but regardless of type all systemic anti-cancer therapy (SACT) are associated with an increased risk of CAT.

Platinum-containing regimens, particularly cisplatin and carboplatin- based regimens, are associated with an increased risk, with a thrombosis rate of 7.0% seen in patients receiving cisplatin-based chemotherapy in one study (Barni et al, 2011) compared to a rate of 1.1% for those receiving oxaliplatin and 0% for those receiving irinotecan (Barni et al, 2011). Oxaliplatin is also associated with an increased risk, though not as high as cis- or carbo-platin (Starling et al, 2009). Cisplatin-based regimens were also compared to non-cisplatin chemotherapy regimens, with the rates of VTE 1.92% and 0.79% respectively (Seng et al, 2012).

Gemcitabine, a nucleoside metabolic inhibitor, is used to treat many different cancer types including bladder, breast, pancreas, ovary and non-small cell lung, and is also associated with an increased risk of CAT. Gemcitabine is often used together with a platinum-based agent (Ramos et al, 2019). In one study, there was a 15.3% rate of CAT in patients receiving gemcitabine plus cisplatin chemotherapy for urothelial tract cancer (Ramos et al, 2019).

Due to these findings, there have been proposals to add cisplatin, or carboplatin-containing, plus gemcitabine regimens to risk assessment models for the prediction

of CAT. These two agents therefore form part of the PROTECHT score (Verso et al, 2012), which is discussed in more detail within this chapter of the thesis. However, there is conflicting evidence regarding their inclusion into a risk assessment model (RAM), with results from the Vienna CATS Study suggesting that the addition of these agents into a risk assessment model was of limited value and therefore should not be included (Moik et al, 2020).

Other chemotherapy agents which are said to increase the risk of CAT include Vascular Endothelial Growth Factor (VEGF) inhibitors, for example bevacizumab. The use of these agents, which inhibit the formation of blood vessels, and therefore tumour growth, can increase the risk of a myocardial infarction by 3.5-fold, and other arterial thrombotic events by 2-fold (Eichinger et al, 2016). Initially it was thought that these agents only increase the risk of arterial thrombotic events (Scappaticci et al, 2007), though it has since been shown that venous thrombosis events occur as well, with a 11.9% rate of VTE occurring in those on the VEGF inhibitor bevacizumab following a meta-analysis (Nalluri et al, 2008; Khorana and Connolly, 2009).

Immunomodulatory drugs, such as those used in the treatment of multiple myeloma, are one of the main treatment strategies that have been shown to increase the risk of CAT.

These drugs, for example thalidomide or lenalidomide, have high rates of VTE incidence when given with dexamethasone (a corticosteroid) and other various types

of chemotherapy including melphalan, doxorubicin and cyclophosphamide, with rates of 12-34% seen (Palumbo et al, 2008; Khorana and Connolly, 2009).

As a result of this, four different RAMs taking the prescription of immunomodulatory drugs into consideration have been proposed for use exclusively in multiple myeloma patients; International Myeloma Working Group (IMWG) (Palumbo et al, 2007) guidelines, IMPEDE VTE (Sanfilippo et al, 2019), SAVED (Li et al, 2019) and PRISM (Chakraborty et al, 2022). These risk assessment models will be discussed in further detail later in this chapter.

Finally, protein kinase inhibitors (for example, imatinib, erlotinib and cediranib (Bhullar et al, 2018)) and immunotherapy used in lung are also said to risk the risk of CAT (Mulder et al, 2021).

1.3.3.5.2 Hormone therapy

Anti-hormone therapy, such as tamoxifen, used in breast cancer patients, is said to increase the risk of VTE by 1.6-fold (Blom et al, 2006), with a risk ratio quoted at between 2.4 to 7.1 (Kovac et al, 2015). A further study of 16,289 women diagnosed with breast cancer and prescribed tamoxifen in Denmark between 1990 and 2004 concluded that these women were at a higher risk than those not taking tamoxifen. In this study, 1.2% of patients on tamoxifen developed a VTE versus 0.5% in those not on tamoxifen (Hernandez et al, 2009).

Paradoxically, Spyropoulous et al (2020) reported that anti-hormone therapy was associated with an absence of VTE events.

1.3.3.5.3 Radiotherapy

In some studies, the use of radiotherapy was not associated with an increased risk of developing CAT (Khorana and Connolly, 2009). However, there is increasing evidence that patients exposed to radiation do have an increased risk of developing VTE. Cronin-Fenton et al (2010) quote an incidence rate of 10.1% in those receiving radiation only, compared to an incidence rate of 23.1% for chemotherapy only in a large Danish population study. The risk associated with radiotherapy is thought to decrease after 1 year of receiving this treatment (Cronin-Fenton et al, 2010).

1.4 Pathogenesis of cancer

Cancer has been described as a "disease of the genome" and arises from DNA alterations that dysregulate normal gene structure or function, leading to a malignant clone of aberrant cells (Cullen and Breen, 2016). Cancer can originate from any cell type and develop in any organ or tissue in the body often spreading to other sites via the blood or lymphatic system, in a process called metastasis. If left untreated, cancer is ultimately fatal to its host.

Cancer typically occurs due to a gene mutation in a single cell in the body (Phillips et al, 2001). This mutation can be inherited (congenital), or as the result of an environmental agent such as viruses, mutagenic chemical or radiation (Parsa, 2012).

Prolonged and sustained inflammation can also lead to the formation of cancer (Greten and Grivennikov, 2019). Normally there are checks and balances in place to correct any mutations shortly after they occur, and to repair any DNA damage. However, in the case of cancer this does not happen, and the mutant cell can proliferate unchecked and unregulated.

Cancer, or neoplasia (the formation of new tissue), is characterised by the loss of the homeostatic controls which are normally in place to maintain appropriate numbers of cells in normal tissues (Phillips et al, 2001). As these controls are lost, the aberrant cells are free to replicate unchecked, leading to abnormal, continuous growth eventually resulting in the formation of a mass of cells known as a tumour.

1.4.1 Properties of cancer cells

Tumour cells have several characteristics which enable them to become proliferative unchecked, and outside of the control mechanisms normally present.

These include:

- A reduced growth factor dependency as tumour cells can secrete their own growth factor to stimulate their own proliferation.
- An ability to replicate at a higher density than normal cells
- An ability to grow without an attachment to a substratum
- Less adhesive than other cells, which contributes to their invasive and metastatic properties.

- An ability to evade apoptosis (programmed cell death), leading to replicative immortality (Cullen and Breen, 2016).

A summary of some of the properties can be found in Figure 1.1.

1.4.2 Angiogenesis and metastasis

The malignant clone of cells (tumour) can continue replicating and expanding until it reaches approximately 1-2 millimetres in diameter (Folkman 2002; Cullen and Breen, 2016; Jiang et al, 2020). Beyond this point, the tumour requires its own blood supply, and so therefore be able to form new blood vessels in a process known as angiogenesis (Figure 1.1). Tumour cells therefore secrete angiogenic growth factors such as vascular endothelial growth factor A (VEGF-A), fibroblastic growth factors (FGF), and platelet derived growth factor (PDGF) allowing the formation of new blood vessels and sustained tumour growth (Cullen and Breen, 2016; Zuazo-Gaztelu and Casanovas, 2018; Yang et al, 2013).

Eventually, parts of the tumour break off and spread via the lymphatic system or via the blood vessels and form new tumours separate from the original tumour in a distant organ in a process known as metastasis. Common metastatic sites include the liver, lungs and bone (Riihimäki et al, 2018). A diagram summarising these processes can be found in Figure 1.1.

1.4.3 Cancer staging

At diagnosis, a patients' cancer is staged according to how large the tumour is, how aggressive it behaves, if it has spread to the lymph nodes and whether there are any distant metastases. Staging is done by various methods and using various systems, dependent on the cancer site involved, and include histology, and CT (computed tomography), PET (positron emission tomography) or MRI (magnetic resonance imaging) scanning. For most cancer types, staging is from Grade I to IV, IV being the most advanced. Sub-stages, for example a, b and c may also be used e.g. Stage IIIa (NHS, 2022). An alternative system is the TMN system, where T is tumour, N is node, and M is metastasis, with the greater the number on a scale of zero to three, the more advanced a cancer is, or X where it cannot be measured. For example, a TMN stage of T4 NX M1 would indicate a large tumour, which is nearby lymph nodes which cannot be measured, and which has spread to other parts of the body (National Cancer Institute, 2022).

The presence of cancer, originating from any primary site, results in a hypercoagulable state through a variety of mechanisms which will be described herein.

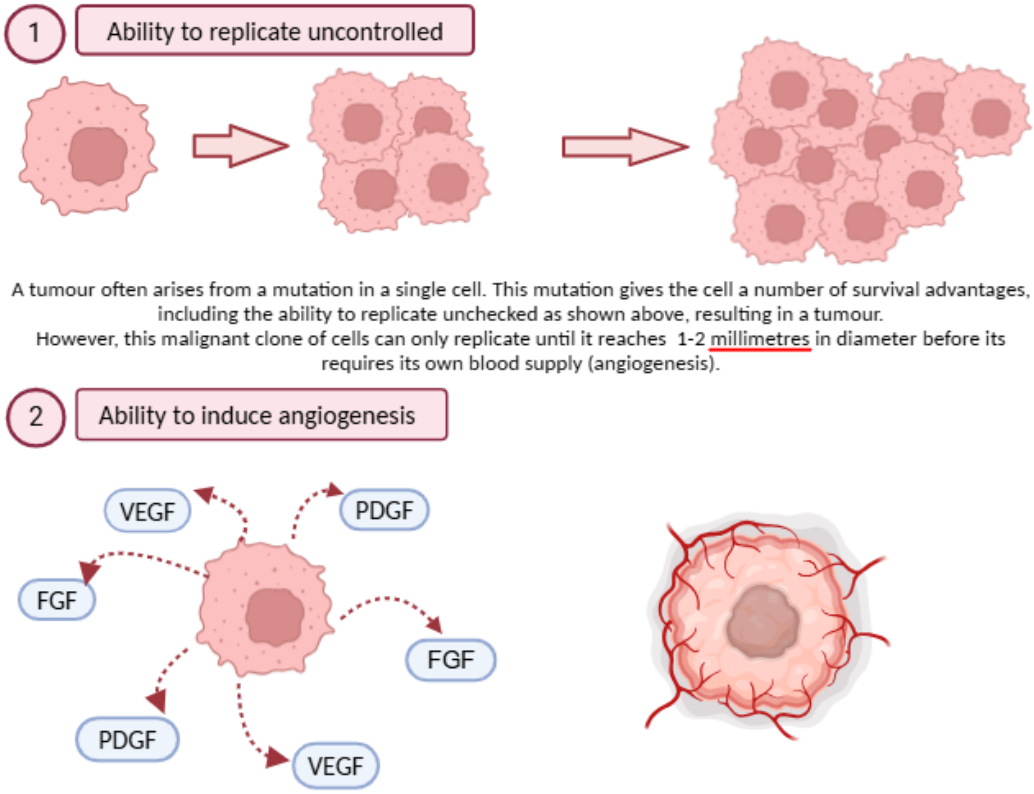


Figure 1.1. **Properties of cancer cells.** Cells carrying cancer mutations have survival advantages allowing them to proliferate unchecked. These include the ability to induce angiogenesis, essential to grow beyond 1-2 mm in diameter. They also can invade other tissues allowing both local, regional and metastasis throughout the body. Created in https://BioRender.com

1.5 Pathogenesis of thrombosis

The process of blood being able to clot is a natural mechanism which occurs in the body in response to an injury or trauma to prevent bleeding.

A thrombosis on the other hand is an unwanted blood clot which can lead to the occlusion of a blood vessel, disruption of blood flow, and in extreme circumstances can lead to a loss or decrease in the function of a limb, organ or other system which it affects. In some cases, this may result in the death of the patient.

In normal health, there are checks and balances in place to ensure that the bleeding can be stopped in the event of a minor injury, and that the blood does not spontaneously clot without this.

Both arteries and veins can be affected by a thrombosis.

1.5.1 Venous thrombosis

Commonly, a thrombosis in the vein presents as either a DVT (deep vein thrombosis) in the limbs, but most frequently in the veins of the lower limb, or as a PE (pulmonary embolism) in the pulmonary circulation of the lungs. 30 to 70% of patients diagnosed with a PE also have an identifiable DVT of the leg (Raskob et al, 2018). Less commonly, a thrombosis can occur in “unusual sites”, for example the splanchnic veins (abdomen, SVT), portal vein (liver, PVT), cerebral sinus vein (brain, CVT) (Tait et al, 2012).

Vein thromboses are thought to predominantly involve the coagulation system, as the thrombi are mainly composed of fibrin and red blood cells, with variable numbers of platelets and leucocytes (Raskob et al, 2018). A VTE can be diagnosed by a variety of tests including an evaluation of the Wells' score (Wells et al, 1995), Doppler ultrasound scan and laboratory measurement of D-dimers, which are present in increased quantities during clot (thrombosis) breakdown. Venous thrombosis is treated with anticoagulants, usually a low molecular weight heparin initially, for example Tinzaparin, followed by a course of oral anticoagulants, for example rivaroxaban or apixaban (NICE, 2023).

1.5.2 Arterial thrombosis

In contrast, a thrombosis in the arteries can result in a cerebrovascular accident (CVA, or stroke), or a myocardial infarction (MI, or heart attack). The thrombosis is predominantly composed of platelets, and a thrombosis occurs when an atherosclerotic plaque ruptures due to the activation of platelets and partially or completely occludes a blood vessel. Treatment of the initial presenting event is the priority, but long term these patients are prescribed anti-platelet medication, such as clopidogrel or aspirin (Buccheri and Angiolillo et al, 2020).

All types of thrombosis can present as a medical emergency and may result in the death of the patient.

1.5.3 Role of platelets in haemostasis and the pathogenesis of thrombosis

The formation of a blood clot is triggered by a variety of mechanisms, and the initial activation of platelets plays an important part in this process, as part of the mechanism known as primary haemostasis. Primary haemostasis is summarised in Figure 1.2.

Platelets are anucleate cells produced by megakaryocytes located in the bone marrow. They are normally around 2μm in diameter, and typically last in the circulation for approximately 7 to 10 days. A normal platelet count in a healthy human is 150 to 450 x 10^9/L (Moore et al, 2010; Hou et al, 2015; Periayah, et al 2017; Scridon, 2022).

Platelets are normally in a quiescent, or resting, state. They naturally marginate towards the periphery of the blood vessels and are in constant communication with the endothelial cells which line the vessel wall. The release of nitric oxide and prostacyclin affects the tone of the vessel and vasodilation which may be required in the event of an injury (Harrison, 2020).

When a vessel wall is damaged through injury this results in the exposure and expression of several components which lead to the activation of platelets, and ultimately the formation of a blood clot. First, a vessel injury exposes subendothelial collagen. In high-shear conditions, circulating plasma von Willebrand Factor (vWF)

binds to the exposed collagen. As a result of the high-shear stress conditions, the vWF is stretched, exposing binding sites for the GPIb receptor found on platelets (Harrison, 2020). Circulating platelets then bind to vWF via the GPIb receptor (part of the GPIb/ IX/ V complex) in a process known as tethering. In low shear stress conditions, collagen can bind directly to platelets via the GPIb receptor without the requirement for vWF (Dahlbäck, 2005; Moore et al, 2010; Clemetson, 2012).

Von Willebrand Factor can be found circulating in small concentrations, however there are stores in both the Weibel-Palade bodies of the endothelial cells, and in the alpha granules of the platelets. It is this circulating vWF which plays a role in the initiation of the process. Following endothelial damage and platelet activation, other vWF stores help to augment the process (Clemetson, 2012).

However, the interactions via the GPIb receptor between vWF, platelets and collagen are not stable enough to form a clot, and further connections are required. These involve binding directly between the platelet and collagen via the α2β1 and GPVI receptors, both of which are expressed on platelets. The interaction between GPVI and collagen triggers signalling responses which then lead to platelet activation (Moore et al, 2010; Mezouar et al, 2016).

Platelet activation involves several processes, which act in a positive feedback loop, amplified by the release of activators such as ADP (from dense granules), thrombin (produced as part of secondary haemostasis), and thromboxane A2 (produced by the cyclooxygenase pathway) which all serve to recruit further platelets (Dahlbäck, 2005;

Rivera et al, 2009; Harrison, 2020). Processes occurring during platelet activation include; elevation of intra-platelet calcium ions allowing platelet signalling to take place, cytoskeletal rearrangements to facilitate a shape change from discoid to spherical and therefore closer physical interactions with each other and attachment to the vessel wall, the synthesis and release of thromboxane A2 a powerful platelet agonist which recruits further platelets to the site of injury, and the activation of the platelet receptor GPIIbIIIa (αIIbβ3) (Varga-Szabo et al, 2009; Moore et al, 2010; Harrison, 2020). Activation of GPIIbIIIa results in an increased affinity for other ligands including vWF and fibrinogen (Rivera et al, 2009; Clemetson, 2012). In addition, following the translocation of granules to the platelet membrane, a process which is calcium mediated, contents of both the alpha and dense granules can be released (Varga-Szabo et al, 2009). These include additional vWF and GPIIbIIIa, required for further recruitment, binding and aggregation of platelets, but also P-selectin which acts as an adhesive molecule between platelets and white blood cells (leucocytes). Alpha granule secretion also promotes secondary haemostasis by several mechanisms, including the release of microvesicles, or microparticles, which contain platelet membrane and therefore act as additional phospholipid surfaces for coagulation reactions to take place on (Clemetson, 2012), and are said to have a much higher procoagulant activity than activated platelets (Sinauridze et al, 2007). Further alpha granule contents include Factor XIII, fibrinogen, platelet factor 4 (PF4), platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF)

(Moore et al, 2010). Dense granule contents include ADP (adenosine diphosphate), Calcium ions (Ca^{2+}) and serotonin (Moore et al, 2010).

Once platelets have become activated, and the recruitment of further platelets takes place via mechanisms described above, then promotion of platelet aggregation can occur. This is generally via both vWF and fibrinogen via GPIIbIIIa receptors (Moore et al, 2010; Harrison, 2020).

Platelet activation and aggregation are essential for the formation of a blood clot by providing the necessary components for the next stage of haemostasis; a process known as secondary haemostasis or coagulation. Platelets provide the following: a negatively charged phospholipid-rich surface for coagulation reactions to take place on (Clemetson, 2012), an increase in the concentration of calcium ions which are required for the activation of some of the coagulation factors, and a scaffold from which a fibrin mesh, generated in secondary thrombosis, can form.

Primary haemostasis is summarised in Figure 1.2.

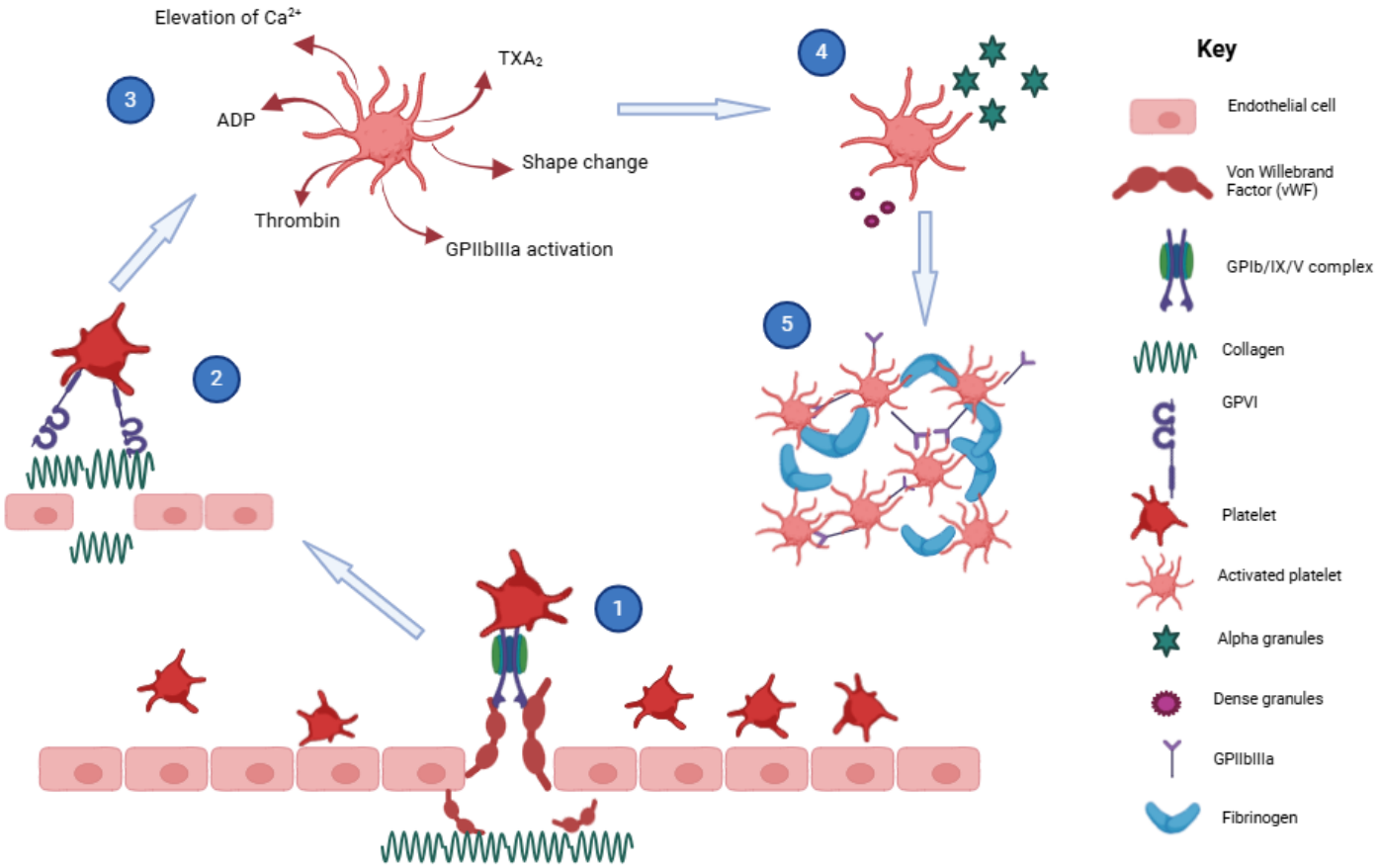


Figure 1.2. **Summary of the role platelets play in haemostasis.** 1. Blood vessel damage exposes subendothelial collagen, which binds to circulating vWF, and to platelets via GPIb (part of the GPIb/ IX/V complex). 2. Direct binding of GPVI to collagen and platelets, which leads to platelet activation. 3. Platelet activation occurs leading to shape change, the release of thromboxane A_2 (TXA_2), Thrombin and ADP, elevation of Ca^{2+} and GPIIbIIIa activation. 4. Platelet activation also leads to the release of both the alpha and dense granules, which contain addition vWF, GPIIbIIIa, PF4, fibrinogen, PDGF, VEGF, ADP Ca^{2+} and serotonin. 5. Platelet aggregation occurs via interactions with both fibrinogen and GPIIbIIIa. Further details are provided in the text. Figure created in https://BioRender.com

1.5.4 Role of coagulation in haemostasis and the pathogenesis of thrombosis

Whilst platelets and primary haemostasis are required to initiate the process of thrombus formation, a clot cannot form without the activation of the coagulation system known as secondary haemostasis. The final product of this process is a fibrin mesh, which traps red blood cells and platelets to form a stable blood clot. This provides a stable structure to the platelet plug formed after aggregation. It also tries to prevent any further blood loss and stays in place until any damage is repaired (Moore et al, 2010).

Therefore, in the formation of a blood clot both primary and secondary haemostasis are required.

Secondary haemostasis in vivo is initiated through the activation of zymogens and a series of enzymatic reactions, which ultimately lead to a 'thrombin burst'. This 'thrombin burst' results in very high plasma levels of thrombin which can then convert fibrinogen to fibrin, which following more reactions will lead to the formation of a fibrin mesh, as previously described.

To achieve thrombin generation, and thus a stable fibrin mesh, a series of reactions must take place, via what is historically known as the extrinsic, intrinsic and common pathways, but now known as the cell-based model. Activation of the extrinsic pathway occurs by the presence of Tissue Factor, which is exposed during vessel

injury, and which originates in the subendothelial matrix. TF has a high affinity for the zymogen Factor VII, which becomes Factor VIIa upon activation. The TF-Factor VIIa complex (also known as the extrinsic tenase complex) then activates Factor X (to become Xa), and Factor IX (to become IXa) (Butenas et al, 2009; Moore et al, 2010).

An alternative route of Factor Xa generation is via the intrinsic pathway. Here, activation is by two pathways; via the TF-FVIIa complex as described above (relatively small amounts of Factor Xa generated this way), or by exposed subendothelial collagen from vessel injury, which is responsible for the majority of Factor Xa generation in this pathway. Factor XII is activated to become Factor XIIa, which then also activates Factor XI to become Factor XIa. Factor XIa, along with the TF-FVIIa complex, plus a cofactor (Factor VIII) all activate Factor IX to IXa. This "intrinsic tenase" complex of Factor VIIIa plus IXa can then also activate Factor X to generate Factor Xa (Dahlbäck, 2005; Moore et al, 2010; Yong and Toh, 2023).

The Factor Xa generated from both the extrinsic and intrinsic pathways then requires phospholipids (provided by the platelet surface), calcium ions (provided by platelet activation) and Factor Va (also provided by platelet granule release) to form the "prothrombinase complex". This complex can convert prothrombin (Factor II) to Thrombin (Factor IIa) (Moore et al, 2010; Yong and Toh, 2023).

Whilst both the extrinsic and intrinsic pathways both play an important role, the extrinsic pathway is said to cause a "thrombin spark", which activates Factors V, VIII and X, and platelets via protease-activated receptors (PARs), and thus provides a

positive feedback loop, and facilitate the intrinsic pathway. However, the extrinsic pathway alone cannot produce fibrin (Yong and Toh, 2023). In contrast, the intrinsic pathway provides a "thrombin burst" ultimately leading to the formation of a fibrin mesh (Yong and Toh, 2023).

Once thrombin is generated, it activates fibrinogen to fibrin. Further reactions involving Factor XIII (released from platelet alpha granules, and activated by thrombin) to form Factor XIIIa, which is required to form stable, cross-linked fibrin which is responsible for the fibrin mesh (Harrison, 2020; Alshehri et al, 2021).

Finally, checks and balances are in place to stop uncontrolled haemostasis and the formation of a massive fibrin mesh leading to venocclusion. These checks include inhibition of the haemostatic pathways via Proteins C and S, and Antithrombin (Dahlbäck, 2005), and fibrinolysis, which is the breakdown of the fibrin clot once healing has occurred.

Fibrinolysis involves the action of the zymogen plasminogen which is converted to plasmin through the action of tissue plasminogen activator (tPA), secreted by endothelial cells. Plasmin then breaks down the stable, cross-linked fibrin in the fibrin mesh into fibrin degradation products (FDPs), which includes D-dimers (Risman et al, 2023). As fibrinolytic activity is always present in plasma, playing a role in the control of the normally occurring low-level activation of coagulation, FDPs, including D-dimers, will be present in low levels in the absence of a thrombotic event. However, raised levels indicate a recent or ongoing thrombotic event (Schutte et al, 2016).

A summary of coagulation can be found in Figure 1.3.

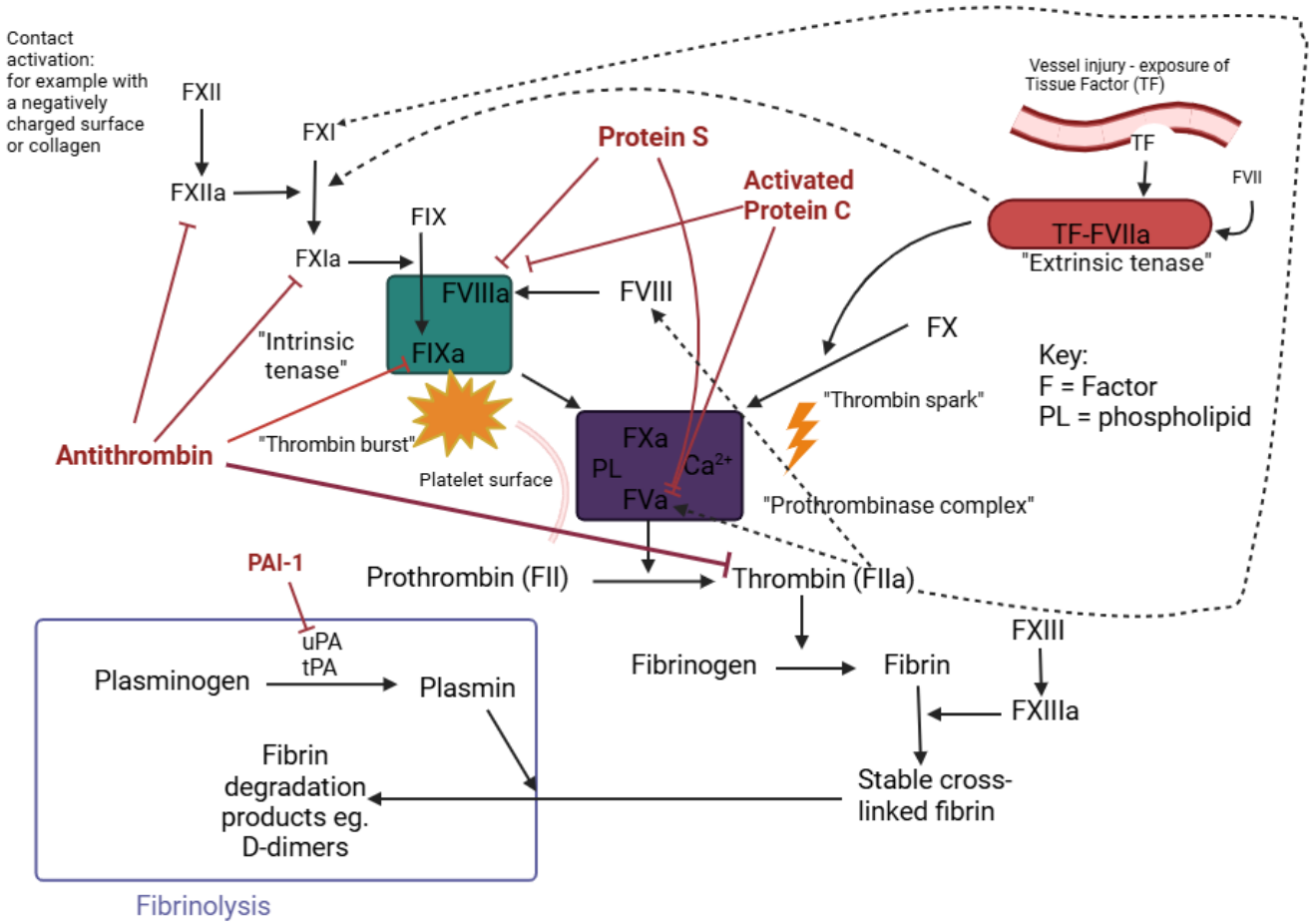


Figure 1.3. **Overview of coagulation.** Vessel injury exposures Tissue Factor which then sets of a cascade of reactions resulting in the generation of a thrombin spark. This thrombin spark ignites the remining pathways resulting in the formation of the intrinsic tenase complex, and the prothrombinase complex which assembles on the surface of an activated platelet, and which results in a thrombin burst, and ultimately the formation of stable, cross-linked fibrin. This is broken down in a series of reaction known as fibrinolysis. Further details are provided in the text. Figure created at https://BioRender.com

1.5.5 Role of the endothelium in haemostasis and the pathogenesis of thrombosis

The endothelium plays a crucial role in maintaining haemostatic balance. It is capable evoking either antithrombotic or prothrombotic agents (Yau et al, 2015).

Healthy, resting endothelial cells secrete both antiplatelet and anticoagulant agents to prevent both platelet activation and aggregation, and the activation of the coagulation system. The endothelium negatively regulates platelets ensuring they do not become activated. It does this by releasing prostacyclin and nitric oxide through elevation of the platelet cyclic nucleotides cAMP and cGMP respectively (Harrison, 2019). In addition, the endothelial expresses various anticoagulants, for example, thrombomodulin, tissue factor pathway inhibitor (TFPI) and EPCR (Endothelial protein C receptor) (Yau et al, 2015), all of which prevent the activation of coagulation in the absence of endothelial injury.

During thrombosis the endothelium also plays an important role. It does this through multiple mechanisms including; the expression of Tissue Factor due to injury or inflammation, which activates the extrinsic pathway of coagulation; and the exposure of collagen which both activates the intrinsic pathway of coagulation and provides a surface to which both vWF and the platelet receptor GPIb can bind, leading to platelet activation. These processes have already been discussed in detail earlier in this Introduction chapter.

1.6 Pathogenesis of cancer-associated thrombosis

Having described the pathogenesis of both cancer and thrombosis, the processes occurring during cancer-associated thrombosis now require exploring.

Patients with cancer of any type are generally in a hypercoagulable or prothrombotic state as described earlier in this thesis. Therefore, as a result, these individuals are more susceptible to a thrombotic event.

Mechanisms of CAT can be either direct or indirect, and they are multifactorial with multiple overlapping pathways (Ay et al, 2017). Inflammation also plays a role in CAT in a process known as immune-thrombosis.

1.6.1 Role of TF, and TF-bearing microparticles

Tissue Factor is constitutively expressed by some tumours (Butenas et al, 2009; Abdol Razzak, 2018), which can lead to the generation of thrombin via the extrinsic coagulation pathway as previously described, Whilst thrombin will convert fibrinogen to fibrin, leading the formation of a fibrin mesh, it can also cleave PAR-1 on the surface of platelets to induce platelet activation and aggregation (Beckendam and Ravid, 2023).

The association between tumour TF expression and the risk of VTE has been observed in both pancreatic and ovarian cancers (Khorana et al, 2007b; Uno et al, 2007).

In addition, microparticles, or microvesicles, can be released from resting malignant cells (Geddings and Mackman, 2013), which are rich in Tissue Factor. Not only do these provide TF to initiate coagulation, but they are also rich is phosphatidylserine, a negatively charged phospholipid, which can be used as a surface for coagulation reactions to take place on. TF-bearing cancer-cell derived microparticles have been shown, when present, to increase the incidence of deep vein thrombosis in mice (Thomas et al, 2015), and the incidence of VTE in humans with either disseminated breast or pancreatic cancer (Tesselaar et al, 2007). Plasma microparticle-associated TF activity increases in CAT (Woei-A Jin et al, 2016). A seminal study by Zwicker et al (2009) showed that the incidence of VTE in patients with TF-bearing microparticles was 34.8%, and those without the incidence was 0% (Zwicker et al, 2009) demonstrating the importance of these particles in the pathogenesis of CAT.

Finally, all tumour cells can secrete both VEGF and FGF (Fibroblast Growth Factor), which can induce TF expression on monocytes and endothelial cells respectively (Abdol Razzak, 2018).

1.6.2 Role of inflammatory cytokines

Inflammatory cytokines, for example Tumour Necrosis Factor - α (TNF-α), Interleukin 6 (IL-6) and Interleukin 1β (IL-1β), can all be secreted by tumour cells (Abdol Razzak, 2018; Greten and Grivennikov, 2019) These cytokines can have several effects, which include the downregulation of thrombomodulin, prostacyclin and nitric oxide (Abdol Razzak, 2018), which are secreted by a resting endothelium to keep platelets and the

coagulation system in a quiescent, or resting state. Therefore, if these elements were not present activation of these systems may occur resulting in the formation of a thrombus. In addition, IL-6 and TNF-α can stimulate the release of both TF and vWF from the endothelium, which can lead to platelet activation (Ay et al, 2017). Finally, the presence of inflammatory cytokines may induce the formation of neutrophil extracellular traps (or NETS)

1.6.3 Role of Neutrophil Extracellular Traps (NETs)

Neutrophil Extracellular Trap (NET) formation is triggered by innate immune receptors including reactive oxygen species (ROS) (Papayannopoulos, 2017) and are released by neutrophils in response to invasion by an infectious agent such as bacteria. They are composed of DNA filaments coated with histones and granule proteins (Thålin et al, 2019). NETs also play a role in coagulation by activating Factor XII, binding vWF, and providing a scaffold for platelet adhesion and fibrin deposition (Mauracher et al, 2018). They can also degrade TFPI, thus allowing haemostasis to commence via the extrinsic pathway (Thålin et al, 2019).

Uncontrolled and excessive NET formation has been thought to contribute to pathological thrombotic disorders (Thålin et al, 2019).

In all types of cancer, excessive NET formation has been observed (Demers et al, 2012) and is thought to contribute to disease pathology by promoting thrombosis, systemic inflammation and relapse of disease (Olsson and Cedervall, 2016). The

release of NETs have also been observed in response to pancreatic cancer derived factors (Abdol Razzak, 2017).

Mauracher et al (2018) as part of the Vienna CATS study observed that citrullinated histone H3 (H3Cit) was raised in patients with cancer-associated VTE. H3Cit is a biomarker for NET formation, suggesting that NETs play a role in CAT. Finally, recently a study by Rosell et al (2023) suggested that the presence of H3Cit could be an independent predictor for the presence of occult cancer within 1 year of a diagnosis of VTE (Rosell et al, 2023).

1.6.4 Role of adhesion molecules

Adhesion molecules, for example P-selectin which is found on the surface of platelets and on endothelial cells, can bind with both leucocytes and tumour cells.

Tumour cells have been found to express specific adhesion molecules which allows for attachment both the vessel wall via endothelial cells, or interaction with platelets and leucocytes via binding with the P-selectin glycoprotein ligand-1 (PSGL-1) (Chen and Geng, 2006). This binding also promotes NETosis, another mechanism of CAT, in mice (Etulain et al, 2015).

These enhanced interactions between tumour cells, endothelial cells, platelets and leucocytes promote the formation of cell aggregates (containing all types of blood cell) leading to a disruption in blood flow, and the promotion of blood clotting (Abdol Razzak, 2018).

Platelet surface P-selectin (CD62P) is elevated in patients diagnosed with the myeloproliferative neoplasms essential thrombocythaemia (ET) and polycythaemia vera (PV), and this correlates with the patients' history of thrombosis (Bekendam and Ravid, 2023).

1.6.5 Inhibition of fibrinolysis

A further mechanism of CAT is the inhibition of fibrinolysis, the process by which a clot breaks down. Plasminogen activator inhibitor (PAI-1) normally inhibits tissue plasminogen activator (tPA) in a controlled fashion, ensuring that clots are broken down in an appropriate way. An excess of PAI-1 leads to a decrease in fibrinolysis meaning that clots are not broken down. This results in an increase in the risk of thrombosis. Raised PAI-1 levels have been found in pancreatic cancer cells (Lupu-Meri, 2012), and in both ovarian cancer and multiple myeloma patients (Ay et al, 2017). Inflammatory cytokines such as TNF-α can also cause an increase in PAI-1 levels (Abdol Razzak, 2018), demonstrating the interplay of multiple different systems in the pathogenesis of CAT.

1.6.6 Role of podoplanin

Podoplanin, a sialomucin-like glycoprotein, is a ligand for the binding of platelet activation receptor C-type lectin receptor type 2 (CLEC-2) (Riedl et al, 2017). The binding of podoplanin to CLEC-2 causes platelet activation and aggregation (Abdol Razzak, 2018) and is expressed by cancer-associated fibroblasts. High podoplanin

expression was found to be associated with an increased risk of VTE in primary brain tumours in a study of 213 patients (Reidl et al, 2017), and tumour-derived microparticles bearing podoplanin have been found in the blood of pancreatic and colorectal cancer patients, suggesting a role in CAT in these patients (Abdol Razzak, 2018).

1.6.7 Role of Cancer Procoagulant (CP)

Cancer procoagulant (CP), a cysteine proteinase produced by malignant tissue, has been found in cancer and can directly induce direct coagulation by directly activating Factor X without the requirement for Factor VII (Abdol Razzak, 2018). In a study of 45 patients diagnosed with a gastrointestinal adenocarcinoma, CP was found to shorten the coagulation time and increase the mean thrombin-antithrombin complex above the normal range, both of which suggest coagulation activation (Kazmierczak et al, 2005). However, CP is thought to play a minor role in the pathogenesis of CAT in this study (Kazmierczak et al, 2005).

1.6.8 Secretion of platelet agonists

Finally, tumour cells themselves can secrete platelet agonists such as ADP (Abdol Razzak, 2018). Pancreatic tumours are also thought to be able to generate thrombin (Haas et al, 2006).

As this section demonstrates there are many different pathways in play with regards to the pathogenesis of CAT, many of which are interlinked. Whilst some directly

affect known platelet or coagulation activation mechanism, for example the expression of TF, others play a more indirect role. The role of inflammatory cytokines, and the formation of NETs, in a process known as NETosis are emerging areas of research.

An overview of the pathogenesis and mechanisms of cancer-associated thrombosis can be found in Figure 1.4.

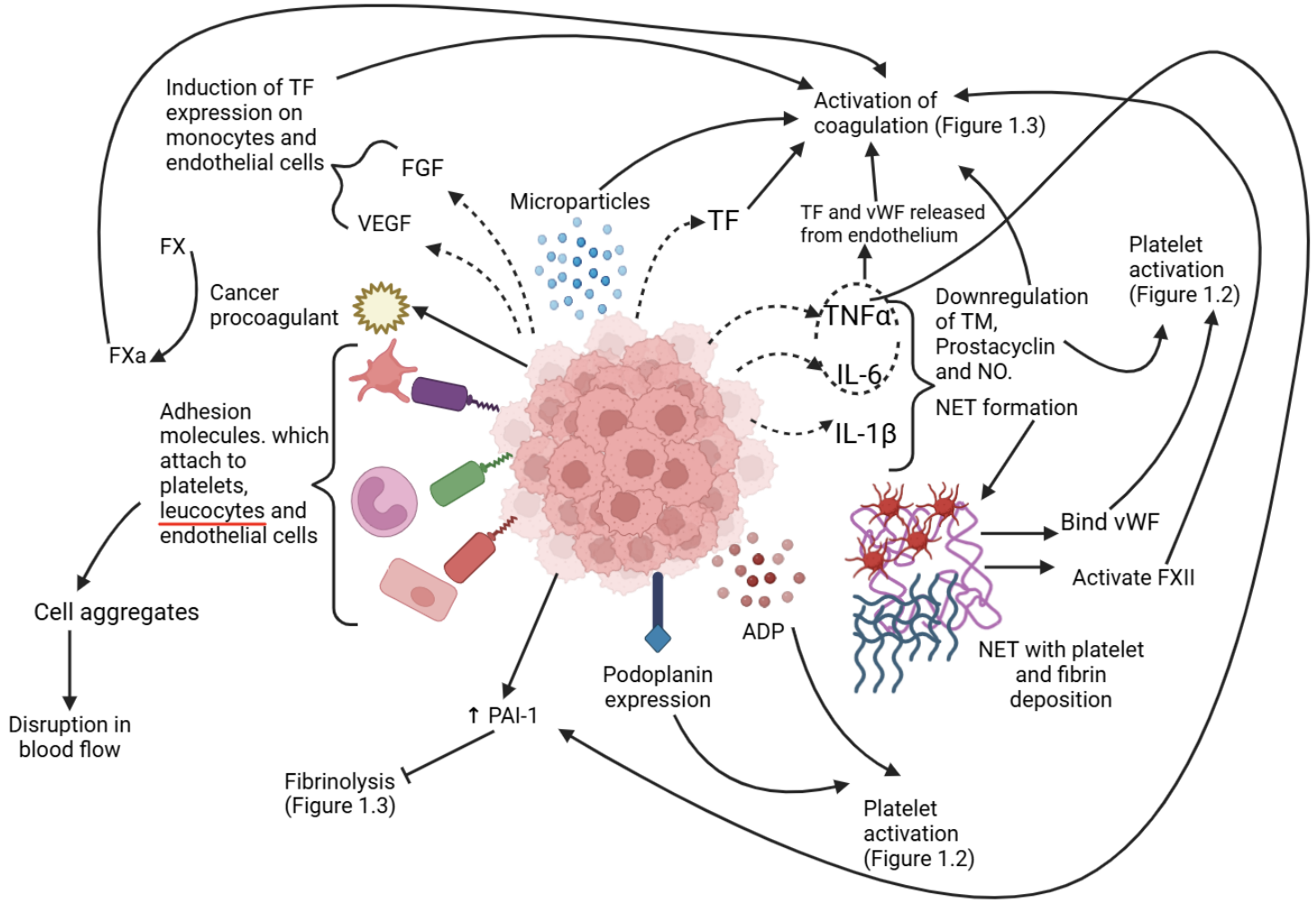


Figure 1.4. **Overview of the processes involved in cancer-associated thrombosis (CAT).** The pathogenesis of CAT is complex with many overlapping and overarching themes ultimately leading to coagulation and/or platelet activation, or the disruption of blood flow. Further details are provided in the text. Figure created in https://BioRender.com

1.7 Current international guidelines for the diagnosis and management of CAT

Cancer-associated thrombosis is a common complication in patients with cancer of any type and is associated with significant morbidity and mortality. Therefore, there are several international guidelines published which aim to provide guidance to clinicians, and other healthcare workers, on how to prevent and treat CAT. The primary focus of all the guidelines is in trying to ascertain which patient groups are at a higher risk of VTE, and to offer these high-risk patients thromboprophylaxis, to prevent a thrombotic episode.

Many of the guidelines suggest using a validated risk assessment model (RAM) to assign a risk profile to a patient, and that all patients should be offered a VTE risk assessment at diagnosis, and before the start of any treatment.

1.7.1 Risk assessment models

There are multiple risk assessment models which can be used to try and predict the risk an individual patient has of developing cancer-associated thrombosis. The main features of each are outlined in Table 1.3. These risk assessment models use various factors to assign risk, and assign individuals to a high-risk, (intermediate-risk), or low-risk category. For many, assignment to a high-risk category should ensure that individual receives thromboprophylaxis

	Khorana (KRS)	Vienna CATS	ONKOTEV	PROTECHT	CONKO	COMPASS-CAT	Tic-ONCO	CATS nomogram	ONCO-THROMB	Thrombo-Nsclc	Ottawa
Site of cancer	Very high risk – gastric, pancreas High risk - lung, lymphoma, gynaecological or genitourinary cancer.	As KRS but addition of brain cancer to very high risk	KRS >2	As KRS	As KRS		High or very high risk (ns) Tumour stage	As KRS	Tumour stage	NSCLC only	Site – lung +1, breast – 1 Tumour stage
Biomarkers	Hb < 100 g/L Platelets ≥ 350 x 109/L WBC >11 x 109/L	As KRS plus D-dimers >1.44 µg/L and sP-selectin > 53.1 ng/mL		As KRS	As KRS	Platelets ≥350 x 109/L		Continuous D-dimer concentrations		Factor VIII > 241 IU/dL sP-selectin > 20.4mADU	
Patient specific factors	BMI > 35 kg/m2		Previous VTE		Performance status >2	Previous VTE Cardiovascular risk factors	Family history of VTE High BMI (ns)		High BMI (ns)		Female sex Previous VTE
Disease-related factors			Metastatic disease Vascular/ lymphatic compression			Metastatic disease Recent hospitalisation					
Treatment			Central venous catheter	Gemcitabine or platinum-based therapy		Antracycline or anti-hormone therapy. Central venous catheter					
Genetics							If high risk SNPs		9 high-risk genetic variants		

Table 1.3. **Overview of the CAT risk assessment models (RAMs) available.** Further details provided in the text. Hb = Haemoglobin, WBC = White Blood cell Count, NSCLC – Non-Small Cell Lung Cancer, ns = not stated/specified.

1.7.1.1 Khorana Score

The most widely recognised, and the most externally validated, risk assessment model (RAM) is the Khorana Score (Khorana et al, 2008). The Khorana Score uses five variables to assign points and then predicts whether an individual is at low (zero points), intermediate (one to two points) or high risk (three or more points) of chemotherapy-associated VTE.

Points are scored based on the following;

- Site of cancer – two points for gastric or pancreatic, one point for lung, lymphoma, gynaecological or genitourinary cancer.
- Platelet count – one point if the platelet count is 350 x 10^9/L or more.
- Haemoglobin (Hb) – one point if the Hb value is less than 100 g/L, or if the patient is on erythropoiesis-stimulating agents (ESAs).
- White Blood cell count (WBC) – one point if the WBC is 11 x 10^9/L or more
- Body Mass Index (BMI) – one point if the BMI is 35 kg/m^2 or more. (Khorana et al, 2008)

The score was developed using patients on chemotherapy, and by using the variables above at the start of chemotherapy to determine a score. The score was derived using a cohort of 2701 patients, and internally validated using 1365 patients, however only a small number of these patients developed a VTE (2.2%) in the observation period (two and a half months).

The score showed good specificity (89.6%), and negative predictive value (NPV) (98.5%), but poor sensitivity (35.7%) and positive predictive value (6.7%). During analysis, neither older age nor the stage of the disease was associated with the development of CAT and were therefore not included. Several cancer types were also not represented in the derivation or validation cohorts including cancers of the brain or haematological cancers.

The major criticisms of the Khorana score are that it only used patients with cancer who were on chemotherapy (Ay et al, 2010), and that the VTE incidence in both the derivation and validation cohorts was low (Cella et al, 2017). Additionally, that the score does not distinguish between those at the highest risk in lung cancer (Mansfield et al, 2016; Kuderer et al, 2018; Spyropoulous et al, 2020; van Es et al, 2020). Finally, most of the VTE events occur outside of the high-risk group, and that if individuals are risk-stratified based on their Khorana score and only those individuals at the highest risk receive thromboprophylaxis, then many individuals would still go on to develop a VTE (Mulder et al, 2019).

One suggestion to improve the sensitivity of the Khorana score is to lower the high-risk category threshold to equal to or greater than two points. A meta-analysis of 34 555 ambulatory cancer patients showed that if this were to occur, then 55.2% of all VTE events would be captured (Mulder et al, 2019).

Despite these limitations, the Khorana score remains one of the most recognised and utilised RAMs, predominantly because of the five easily obtainable variables used to

calculate the score. The Khorana score is specifically recommended for use by the ASCO (American Society of Clinical Oncology) guidelines (Key et al, 2023), ESC (European Society for Cardiology) guidelines (Lyon et al, 2022) and ITAC (International Initiative on Thrombosis and Cancer) guidance (Falanga et al, 2023)

Many of the alternative RAMs are based on the Khorana Score, with additional parameters.

1.7.1.2 Vienna CATS Score

The Vienna CATS (sometimes referred to as CATS) score was developed following the retrospective cohort Vienna CATS Study which followed a total of 819 individuals for two years following their cancer diagnosis (Ay et al, 2010). 7.4% of the cohort developed a VTE, a rate higher than that used for the derivation and validation of the Khorana score (Khorana et al, 2008).

The Vienna CATS score adds two further biomarker variables to the Khorana score:

- D-dimers – one additional point if D-dimer levels are equal to or greater than 1.44 µg/mL (1440 µg/L or ng/mL)
- Soluble P-selectin – one additional point of soluble P-selectin levels are greater than or equal to 53.1 ng/mL (Ay et al, 2010)

In addition, brain tumours were added to the "very high risk" cancer type category and gain an additional two points, and multiple myeloma and renal cancer added to the "high risk" category and gain an additional point (Ay et al, 2010).

Unlike the Khorana score, the cohort of patients used to develop the score included patients recently diagnosed with cancer who may have had or recently started surgery, radiotherapy, chemotherapy or be untreated. In addition, patients were followed for two years (or until their death), which is significantly longer than the Khorana score where individuals were only followed for two and half months.

The Vienna CATS score showed better sensitivity than the Khorana score (96%) and was able to discriminate better between high and low risk patients, but still generally has a poor discriminatory performance (van Es et al, 2017).

One of the major criticisms of the Vienna CATS score is that the measurement of soluble P-selectin is not readily available in many laboratories, requiring analysis by specialist (and potentially research-only) laboratories, and therefore cannot be routinely used.

The Vienna CATS score is mentioned as a potential RAM in the ESMO (European Society of Medical Oncology) guidelines (Falanga et al, 2023).

1.7.1.3 ONKOTEV score

The ONKOTEV score (Cella et al, 2017) adds additional variables to the Khorana score. These include:

- Presence of metastatic disease
- Compression of vascular or lymphatic structures by the tumour
- Personal history of VTE (Cella et al, 2017).

The score used 843 patients, where 73 experienced a VTE, representing a VTE incidence rate of 8.7%.

The ONKOTEV score demonstrated a higher predictive power than the Khorana score with the 12-month VTE incidence in those in the highest risk category 21.7% for the Khorana score, and 33.9% for the ONKOTEV score (Cella et al, 2017).

1.7.1.4 PROTECHT score

The PROTECHT score (Verso et al, 2012) adds the type of chemotherapy to the Khorana score. Platinum-based or gemcitabine-based chemotherapy adds additional points to the score. This score was found to give an improved ability to identify patients at a high risk for VTE in comparison to the Khorana score (Verso et al, 2012). In a large study comparing RAMs, the PROTECHT score was found to discriminate better between those patients who are at low and high risk of developing a VTE, with the 6-month cumulative incidence of those assigned a high-risk score by Khorana 6.5%, compared to 9.6% for the PROTECHT score (van Es et al, 2017).

1.7.1.5 CONKO score

The CONKO score (Pelzer et al, 2013) was validated using 312 patients, all of whom were diagnosed with pancreatic cancer.

The score adds the Karnofsky performance score (also known as the WHO performance score, outlined in Table 1.2) to the Khorana score. The features of performance scores are outlined earlier in this chapter.

1.7.1.6 COMPASS-CAT score

The COMPASS-CAT model (Gerotziafas et al, 2017) was developed using 3814 patients who were diagnosed with breast, ovarian, lung or colorectal cancer. The score uses one element of the Khorana score (platelet count), plus other known risk factors, and consists of the following:

- Cancer related risk factors:
    - Anti-hormonal therapy for women with hormone-receptor positive breast cancer or on anthracycline treatment
    - Time since diagnosis less than six months
    - Presence of a CVC
    - Advanced stage of cancer
- Predisposing risk factors
    - Cardiovascular risk factors (composed by at least two of the following predictors: personal history of peripheral artery disease, ischaemic stroke, coronary artery disease, hypertension, hyperlipidaemia, diabetes, obesity)
    - Recent hospitalisation for acute medical illness
    - Personal history of VTE
- Biomarkers
    - Platelet count greater than 350 x 10^9/L (Gerotziafas et al, 2017).

External validation of this score showed a sensitivity of 95%, specificity of 12%, positive predictive value of 97.73%, and positive predictive value of 6.31% (Spyropoulos et al, 2020).

1.7.1.7 TiC-ONCO score

The TiC-ONCO score (Muñoz-Martin et al, 2018) uses both clinical and genetic risk factors to determine the risk of VTE. 391 ambulatory cancer (colon, pancreas, lung, oesophagus or stomach) patients were used to validate the score. Again, elements of the Khorana score were used, such as a high BMI (although the defined cut-off was determined as >25 kg/m^2, as opposed to 35 kg/m^2), and the site of cancer, and these were built on with known risk factors (family history of VTE). In addition, four genetic risk factors were added which are known to predispose an individual to an increased thrombotic risk, which include Factor V Leiden, Factor XIII variants and a genetic variant encoding Protein Z-dependent protease inhibitor (Muñoz-Martin et al, 2018).

When compared to the Khorana score, the TiC-ONCO score gave better sensitivity (49% versus 22%, and negative (88% versus 82%) and positive predictive values (37% versus 22%) (Muñoz-Martin et al, 2018).

1.7.1.8 CATSCORE nomogram

This nomogram (Pabinger et al, 2018) was developed using the Vienna CATS study cohort and used data from 1423 patients, all of whom were ambulatory. Only two variables are used: Tumour-site risk category (as determined by the Khorana score), and continuous D-dimer concentrations. An advantage of this nomogram is that it is

easy to use, as D-dimer measurement is a routine test and therefore readily available. Neither high-grade gliomas (brain tumour) nor multiple myeloma patients were used to validate the model however.

The score was recently externally validated using 598 patients with the nomogram able to distinguish between low and high-risk groups at study inclusion, and across all three time points (baseline, 3 weeks and 3 months), giving a sensitivity of 76.3% at study inclusion, and 74.4% when all three time points assessed (Englisch et al, 2025).

1.7.1.9 ONCO-THROMB score

The ONCO-THROMB score (Muñoz et al, 2023) uses nine genetic variants known to predispose an individual to an increased prothrombotic risk, plus tumour site, TMN stage and a BMI greater than 25 kg/m^2 to determine the risk of a VTE.

The score showed good specificity and sensitivity when validated, with a NNT (number needed to treat, number of people to be given thromboprophylaxis to prevent one VTE) value of only six patients if all individuals with a high ONCO-THROMB score received thromboprophylaxis.

1.7.1.10 Thrombo-Nsclc risk score

The Thrombo-Nsclc risk score (Castellón-Rubio et al, 2020) was developed for exclusive used in non-small cell lung cancer patients due to the limitations of using the Khorana score in this group of patients. The score adds high levels of Factor VIII (greater than 241% (IU/dL)) and soluble P-selectin (greater than 20.4 mADU) to the

Khorana score. The model shows greater discriminating capacity between low and high-risk patients.

However, the score is only validated in patients diagnosed with non-small cell lung cancer, and sP-selectin measurement was performed using a semi-quantitative method as opposed to a quantitative immunoassay in predominant use elsewhere and utilised in the Vienna CATS study. The two methods are not interchangeable, and one result cannot therefore be converted to another (Castellón-Rubio et al, 2020).

1.7.1.11 Ottawa score

Finally, the Ottawa score (Louzada et al, 2012) can be used to predict the risk of recurrent VTE in patients with cancer. The score uses four independent predictors; sex (with female sex receiving an additional point), primary tumour site (lung cancer receives an additional point, breast cancer loses one point), stage (TMN Stage I loses two points), and previous history of VTE (gains one point).

This score was independently validated and showed good differentiation between low, intermediate and high-risk scores for VTE recurrence, with VTE rates recorded of 2.4%, 8.9% and 15.4% respectively, which is comparable to the original cohort (den Exter et al, 2013).

1.7.1.12 Risk assessment models for use in Multiple Myeloma patients

Multiple myeloma patients are at an increased risk of CAT, predominantly due to the use of immunomodulatory drugs, as outlined earlier in this chapter. In addition, many

of the RAMs outlined above did not use multiple myeloma patients as part of their derivation or validation cohorts. Due to this unmet need, specific risk assessment models have therefore been developed for use in this group of patients.

1.7.1.12.1 International Myeloma Working Group (IMWG) guidelines

The International Myeloma Working Group (IMWG) guidelines (Palumbo et al, 2007) state that individual, myeloma, and therapy-related risk factors should be considered. If two or more of the risk factors are present, then the patient should receive thromboprophylaxis with either LMWH or high-dose warfarin. If less than two risk factors are present, then the patient should still receive aspirin. Specific risk factors include age, obesity, history of VTE, presence of a CVC, comorbidities, recent surgical procedures, presence of inherited thrombophilia mutations, hyper viscosity, receiving high-dose dexamethasone, doxorubicin or multiagent chemotherapy.

1.7.1.12.2 SAVED score

The SAVED RAM (Li et al, 2019) uses five clinical variables to assign risk: **S**urgery within the past 90 days (plus two points), **A**sian race (minus 3 points), **V**TE history (plus three points), Age greater than or equal to 80 years (**E**ighty) (plus one point), and **D**examethasone dose (high dose plus two points, low dose plus one point).

1.7.1.12.3 IMPEDE-VTE score

The IMPEDE-VTE score (Sanfilippo et al, 2019) was derived using a cohort of 4446 patients, and validated using a cohort of 4256 patients. The following are used in the

score; **I**mmunomodulatory agent, Body **M**ass Index > 25 kg/m^2, **P**elvic, hip or femur fracture, **E**rythropoietin stimulating agent, **D**examethasone/ Doxorubicin, Asian **E**thnicity/Race, **V**TE history, **T**unnelled line or CVC and **E**xisting Thromboprophylaxis.

1.7.1.12.4 PRISM score

Finally, the PRISM score (Chakraborty et al, 2022) uses five different variables to determine a risk. These are **P**rior VTE (plus eight points), **R**ace (Black) (plus one point), **I**mmunomodulatory drug (ImiD) use in induction therapy (plus two points), **S**urgery within 90 days (plus five points), and Abnormal **M**etaphase cytogenetics (plus two points).

1.7.1.12.5 Evaluation of myeloma-specific risk assessment models

As with all RAMs, the multiple myeloma-specific RAMs also have their limitations. Amongst all international guidelines, and in practice within the Newcastle upon Tyne Hospitals NHS Foundation Trust, all patients with a multiple myeloma diagnosis therefore receive thromboprophylaxis unless there are contraindications (for example the diagnosis of a bleeding disorder).

As shown, despite the many different RAMs for use in all cancer, or specific types, no one RAM can accurately predict the individuals who will develop a VTE. Even if all individuals assigned to a high-risk category received thromboprophylaxis, most venous thrombotic events would still occur (Mulder et al, 2019).

To date, there are no RAMs specifically for the prediction of arterial thrombotic events in patients with cancer, though the COMPASS-CAT score (Gerotziafas et al, 2017) assigns points for known cardiovascular risk factors predisposing to an arterial thrombotic event, and multiple other RAMs assign points for patients with an increased BMI resulting in obesity, another known risk factor for both venous and arterial thrombotic events (Horvei et al, 2016).

In addition, whilst all risk assessment models have been externally validated further validation is required. For example, a RAM may perform better in specific local populations, or with specific cancer types, and this needs to be determined. This is particularly important in a taxpayer-funded healthcare system, such as the NHS, where there are tight financial controls and restrictions. Knowing a RAM performs well in a population or cancer type allows more appropriate management decisions and strategies to be taken.

Further evaluation of the use of risk assessment models for thromboprophylaxis management within The Newcastle upon Tyne Hospitals NHS Foundation Trust in pancreatic cancer will be assessed in more detail in Chapter 4 (Results Chapter 1) of this thesis.

1.7.2 Diagnosis and management

Broadly speaking, the international guidelines are in consensus with each other. The American Society of Hematology (ASH) (Lyman et al, 2021), European Society of

Cardiology (ESC) (Lyon et al, 2022), the International Initiative on Thrombosis and Cancer (ITAC) (Farge et al, 2022), European Society of Medical Oncology (ESMO) (Falanga et al, 2023), the American Society of Clinical Oncology (ASCO) (Key et al, 2023) and the British Society for Haematology (BSH) (Alikhan et al, 2024) guidance all state that ambulatory patients with cancer should not routinely be offered primary thromboprophylaxis.

The reasons for this are multifactorial. Whilst the rate of CAT is high, and appears to be rising as previously discussed, the number-needed-to-treat (NNT) figure is still high. Muñoz-Martin et al (2018) suggests that 12 patients need to receive thromboprophylaxis to prevent one VTE. In addition, patients with cancer, whilst being at a higher risk of a blood clot, are conversely also at an increased risk of bleeding, particularly in gastrointestinal and genitourinary cancers (Angelini et al 2019; Wilks, 2022). A large meta-analysis of 3,283,140 patients with cancer, of whom 435 140 (13.3%) were on anticoagulation also showed that whilst bleeding incidence was higher in cancer patients versus non-cancer patients, there were specific risk factors, which increased the likelihood of bleeding. These included the presence of metastatic disease, primary gastrointestinal cancer, CKD (chronic kidney disease) stage III or greater and a platelet count less than 100 x 10^9/L (Angelini et al, 2019). In addition, one study showed that fatal bleeding occurred in 1.0% of patients with cancer (Monreal et al, 2006). Clearly, if all patients received thromboprophylaxis then this rate would presumably increase, with the Hokusai-Cancer trial showing the risk

of fatal bleeding higher with the treatment with edoxaban (Wilks, 2022). Finally, patients would not want the burden of daily LMWH injections, though the use of oral anticoagulation, particularly the DOACs, is under investigation.

Instead, the international guidelines suggest a more structured and tailored approach to determine those individuals at a greater risk of CAT, and who should therefore receive thromboprophylaxis. The BSH guidelines (Alikhan et al, 2024), for example, state that "the focus should be on trying to identify high-risk oncology patients who would benefit from thromboprophylaxis" (Alikhan et al, 2024 pp.73). The identification of these patients, and the most at-risk groups differs slightly between guidelines.

All guidelines suggest the use of VTE risk assessment scores to aid in identifying these individuals. However, the use of a particular RAM is not suggested by any guideline except the ESC guidelines (Lyon et al, 2022), ITAC guidance (Falanga et al, 2023) and ASCO guidelines (Key et al, 2023), all of which suggest using the Khorana score, but with a modified cut-off of two points or more to guide the use of thromboprophylaxis, which would improve the Khorana score sensitivity as previously discussed, unless that are patient-specific contraindications to the use of thromboprophylaxis (Key et al, 2023). Alternative risk assessment models specifically mentioned in the guidelines include the COMPASS-CAT score (Gerotziafas et al, 2017) (ESC and ESMO guidelines), and the Vienna CATS Score (Ay et al, 2010) (ESMO guidelines).

All other guidelines do not state which risk assessment model to use, and suggest it is up to the treating clinician, dependent upon each individual patients' circumstances.

For lung cancer patients, two of the guidelines significantly differ. ESC guidance (Lyon et al, 2022) state that all individuals diagnosed with metastatic stage lung cancer should be offered thromboprophylaxis. In contrast, ITAC guidance (Farge et al, 2022) states that primary prophylaxis in these patients should not be offered outside of a clinical trial.

All international guidelines agree that all patient's diagnosed with either pancreatic cancer (and receiving systemic anti-cancer therapy (SACT)), or multiple myeloma (and receiving an immunomodulatory drug such as thalidomide or lenalidomide plus steroids) should receive thromboprophylaxis. The reasons why these patients are a higher risk are due to high rates of CAT in both populations.

Finally, all international guidelines agree that if a patient with cancer is hospitalised with any reason, then a VTE risk assessment should take place, and if appropriate thromboprophylaxis should be offered. In the United Kingdom, it is routine practice to perform a VTE risk assessment on all hospitalised patients within 14 hours of admission (NICE, 2019). Patients with cancer would automatically therefore receive thromboprophylaxis unless there were contraindications, for example the patient was bleeding.

1.8 Biomarkers of CAT

There are several biomarkers which appear to be strongly associated with the risk of CAT, and some biomarkers for which further research is required to explore their potential for predicting the risk of CAT. These, and the three biomarkers studied in this thesis, will now be discussed in turn.

1.8.1 Soluble P-Selectin (sP-selectin)

P-selectin (CD62P) is a member of the selectin family of proteins which are all have cell-adhesion properties. The family also includes L-selectin and E-selectin (Chen and Geng, 2006).

P-selectin is stored in both the alpha granules of platelets and the Weibel-Palade bodies of endothelial cells (Chen and Geng, 2006; Ay et al, 2008) and is expressed both on the platelet surface and in soluble form after platelet or endothelial cell activation. Raised sP-selectin levels are therefore indicative of platelet activation (Kannan et al, 2019).

After platelet activation, in which microparticles also expressing P-selectin are also generated, P-selectin interacts with the P-selectin glycoprotein ligand-1 (PSGL-1) found on the surface of white blood cells (leucocytes) (Kannan et al, 2019). Binding of P-selectin to PSGL-1 results in the formation of platelet-leucocyte aggregates, and the recruitment of further leucocytes (Chen and Geng, 2006). In addition, TF expression on monocytes is increased (Chen and Geng, 2006) resulting in the

generation of more thrombin as previously described. Therefore, the binding of P-selectin to PSGL-1 triggers thrombus growth (due to the recruitment of leucocytes) and fibrin formation (due to increased TF expression and therefore leads to a hypercoagulable state (Grilz et al, 2019).

This relationship between P-selectin and thrombosis is demonstrated by P-selectin deficient knockout mice whose bleeding time increased by more than 40%, and who had a two-fold increase in haemorrhage rate (Chen and Geng, 2006). In addition, a mouse model of Haemophilia A showed a correction of haemostasis when there was an over-expression of P-selectin (Hrachovinova et al, 2003). Soluble P-selectin levels were found to be increased in patients with a VTE (Rectenwald et al, 2005; Riva et al, 2018) in non-cancer patients, and are also associated with the risk of arterial thrombosis in non-cancer patients (Grilz et al, 2019).

In patients with all types of cancer, P-selectin has been found to interact with cancer cells, both via activated platelets, and by stimulated endothelial cells. (Chen and Geng, 2006). Cancer cells can enhance the expression of P-selectin on monocytes, macrophages, endothelial cells and platelets (Ay et al, 2008). Neoplastic (cancer) cells can also express CD24, the receptor for P-selectin (Aigner et al, 1997).

Thus, both thrombosis and cancer are associated with elevated levels of P-selectin, in both its soluble and membrane-bound forms.

Ay et al (2008) demonstrated that elevated levels of soluble P-selectin are associated with the development and prediction of CAT, and therefore sP-selectin levels are part of the Vienna CATS score, as previously described. 11.9% of the 687 cancer patients' studied with a sP-selectin level above the 75th percentile developed VTE versus 3.7% in those below the 75th percentile (Ay et al, 2008). As part of the same study group, Grilz et al (2019) also demonstrated that elevated sP-selectin levels were also associated with an increased risk of arterial thrombosis, with a 1.9-fold elevated risk.

1.8.2 D-dimers

D-dimers are formed after the breakdown of a fibrin clot, in a process called fibrinolysis (Moore et al, 2010). The presence of D-dimers is a marker therefore of endogenous fibrinolytic activity (Pulivarthi and Gurrum, 2014), and a global marker of fibrinolysis (Kumar et al, 2020). In this process, the fibrin mesh which contains cross-linked fibrin as well as platelets, red blood cells and white blood cells, is broken down by the enzyme plasmin.

The way in which plasmin digests the cross-linked fibrin is such that the D-dimer (composed of two D fragments of cross-linked fibrin) is the only fibrin degradation product (FDP) which is specific to the presence, or recent breakdown of, cross-linked fibrin and therefore a blood clot (Moore et al, 2010).

D-dimer levels are raised in several clinical scenarios which include thrombosis, inflammation, infection, pregnancy, malignancy, cardiovascular disease, renal disease and liver disease (Pulivarthi and Gurram, 2014).

The measurement of D-dimer levels is used as an initial screening test for the diagnosis or exclusion of VTE, along with the use of the Wells' score (Wells et al, 1995), and a Doppler ultrasound scan, as previously described (NICE, 2023).

The elevation of D-dimer levels has also been shown to be predictive of CAT, with two RAMs using this parameter as a measure of the risk of CAT (Ay et al, 2009; Pabinger et al, 2018). Ay et al (2009) examined the utility of D-dimer concentrations in patients with cancer and showed that levels were significantly higher in those with VTE versus those without. A 2-fold increase in D-dimer levels was associated with a 1.3-fold increase in the hazard ratio for VTE (Ay et al, 2009). Recent work by Verzeroli et al (2024) who studied 189 metastatic breast cancer patients also showed that D-dimer levels above 533 ng/mL were significantly associated with an increased risk of VTE (Verzeroli et al, 2024). Finally, D-dimer levels were used as part of the AVERT trial which aimed to establish if D-dimer levels could be used to target those patients with cancer who would benefit from primary thromboprophylaxis. Patients with a Khorana score greater than or equal to two were randomised to receive Apixaban or a placebo, and risk categories calculated based on the CATS nomogram (Pabinger et al, 2018). If the CATS nomogram was applied, using D-dimer levels, then the number

of patients needed to treat to prevent one VTE significantly dropped (Kumar et al, 2020).

D-dimers are part of both the Vienna CATS score (Ay et al, 2009), and the CATS nomogram (Pabinger et al, 2018).

1.8.3 Vascular Endothelial Growth Factor (VEGF)

Vascular Endothelial Growth Factor (VEGF, or VEGF-A) is a potent angiogenic factor. Angiogenesis, the formation of new blood vessels, is required for the growth and metastases of tumours (Dogan and Demirkazik, 2005). In cancer, the new blood vessels formed as the result of angiogenesis are highly thrombogenic, they have an abnormal structure, and they leak both fibrinogen and plasminogen leading to extracellular fibrin deposition (Dogan and Demirkazik, 2005).

Like P-selectin, VEGF is stored in both the Weibel Palade bodies of endothelial cells, and in the α-granules of platelets (Feroni et al, 2016; Posch et al, 2016), these stores are released upon endothelial or platelet activation respectively. VEGF is 34-50 KDa in weight and is also known as Vascular Permeability Factor. As well as intracellular stores, VEGF is also expressed on a variety of cells including monocytes, lymphocytes, granulocytes and endothelial cells (Posch et al, 2016).

VEGF induces the expression of TF on endothelial cells (Posch et al, 2016) activating the extrinsic pathway of coagulation and promotes the release of vWF from

endothelial cells leading to platelet aggregation and the formation of a blood clot (John et al, 2000).

VEGF plays a role in both cancer and thrombosis and so would therefore be expected to be elevated in both scenarios. Indeed, VEGF has been shown to be overexpressed in a variety of cancer types, including colon, pancreatic, breast and ovarian (Dogan and Demirkazik, 2005), and elevated levels have been linked to decreased survival in bladder cancer patients (John et al, 2020). In cancer, VEGF can be produced by both tumour cells, and by tumour-associated macrophages and neutrophils, which also secrete MMP-9 (matrix metalloproteinase-9) into the tumour microenvironment leading to increased promotion of the liberation of VEGF (John et al, 2020). VEGF inhibitors, for example bevacizumab, are increasingly prescribed for the treatment of cancer, suggesting an important role in the progression of cancer.

With regards to CAT, increased VEGF levels have been demonstrated to be associated with increased incidence of CAT (Posch et al, 2016), with a 2-year cumulative incidence of VTE of 10.2% when VEGF levels were greater than the 75th percentile, compared to a 5.9% incidence in those with lower levels. Feroni et al (2016) also demonstrated that a particular genetic variant of the VEGFA gene (-1154 G/A SNP) is also associated with a higher VTE risk (Feroni et al, 2016).

However, there is also evidence that VEGF is not increased in CAT. Kirwan et al (2008 & 2009) showed that there is no significance difference in VEGF levels between patients who do and do not develop a thrombosis.

Clearly, further research into VEGF is required to assess its utility as both a marker for CAT, and as a biomarker for the prediction of CAT.

Currently, VEGF is not currently a component of any of the CAT risk assessment models, therefore its utility as the potential to be added into one needs to be assessed.

Further analysis of the predictive role of VEGF in cancer-associated thrombosis is examined in depth in Chapter 4 (Results Chapter 2) of this thesis.

1.8.4 Other biomarkers

As well as the three biomarkers outlined in this section, and which are measured as part of this thesis, there are other related biomarkers which are said to predictive of CAT.

1.8.4.1 Tissue Factor (TF) and Microparticles

As previously described, TF is constitutively expressed by some tumours (Butenas et al, 2009; Abdol Razzak, 2018), and this increased expression and a link to the risk of VTE has been observed in both pancreatic and ovarian cancers (Khorana et al, 2007b; Uno et al, 2007).

TF is a 47KDa transmembrane protein, which is expressed by monocytes, macrophages and endothelial cells (Dogan and Demirkazik, 2005). As well as the promotion of thrombosis, a high TF expression has also been associated with increased angiogenesis and advanced disease (Dogan and Demirkazik, 2005).

The presence of TF-bearing microparticles, which indicates platelet activation, have also been studied with regards to their predictive capacity in CAT. Zwicker et al (2009) demonstrated that higher levels of tissue factor-bearing microparticles were present in 34.8% of the study cohort who developed VTE, compared to 0% of those without the microparticles (Zwicker et al, 2009). Further studies have also shown that microparticle levels are higher in patients with cancer who experience a VTE versus those than do not (Yamanaka et al, 2020; Mohamed et al, 2022). This finding was also seen in mice (Thomas et al, 2015).

Despite the evidence, neither Tissue Factor nor the presence of microparticles form part of a risk assessment model for CAT. This is probably due the lack of availability of assays for these components.

1.8.4.2 Markers of NETs and NETosis

As previously described, the formation of neutrophil extracellular traps (NETs), in a process known as NETosis is also associated with CAT.

An increase in citrullinated histone H3 (H3Cit) was observed by Mauracher et al (2018) in patients with cancer who went on to develop a VTE.

However, despite this, this biomarker is not part of any RAM.

This section has outlined many of the biomarkers which show potential to be used as part of a risk assessment model for CAT. There are many biomarkers, for example haemoglobin, platelet count, and white blood cell count which already form part of a

RAM, and these parameters are well established and are routinely performed in most clinical laboratories. However, there are also several emerging biomarkers which could be measured, though these are not routinely performed outside of a research setting.

1.9 Serial measurement of biomarkers in CAT

Most of the work performed with biomarkers to predict cancer-associated thrombosis has concentrated on the measurement of these before the start of chemotherapy, or before the start of other treatments such as radiotherapy, hormone therapy or surgery. Similarly, most RAMs only consider levels of the relevant biomarkers before the administration of chemotherapy, apart from the CATS nomogram, which is not in common use. There are very few studies where repeated, serial or longitudinal measurements of potential biomarkers have been performed after the commencement of cancer treatment to determine whether these change over time and whether these changes are related to the incidence of either venous or arterial thrombosis.

Where studies do exist, they are conflicting, based on data from different patient cohorts and different types of cancer, and investigate a wide range of potential biomarkers making evaluation of the utility of serial measurement of biomarkers difficult. Assessment of the utility of performing serial measurement of CAT biomarkers will be addressed in Chapter 5 in more detail.

A patients' thrombotic risk alters considerably from when they first receive their cancer diagnosis, and one measurement of a biomarker at baseline, before the start of chemotherapy does not accurately reflect this risk. For example, a patients' BMI will alter, as will other variables such as the biomarkers Haemoglobin, platelets and white cell count. Patients with cancer are also likely to undergo minor or major surgical procedures, possibly involving a hospital stay during their treatment.

Therefore, the serial measurement of biomarkers, and other patient variables requires further investigation to assess its utility in the prediction of CAT.

Further analysis of the utility of serial measurements of select biomarkers for the prediction of cancer-associated thrombosis is examined in depth in Chapter 5 (Results Chapter 3) of this thesis.

1.10 Study aims and objectives

This thesis has one main aim:

- To assess current methods of predicting cancer-associated thrombosis to see if any adaptations need to be made within certain populations or localities, and if previous research findings can be replicated within our population.

This will be established by achieving three main objectives:

- To establish if risk assessment models, such as the Khorana score, can be used for the prediction of cancer-associated thrombosis in a select local population, and in a specific high-risk cancer type, pancreatic cancer.
- To assess the utility of VEGF as a biomarker for the assessment and prediction of cancer-associated thrombosis.
- To assess if the serial measurement of certain biomarkers (D-dimers, soluble P-selectin and VEGF) can be used as an additional tool for the prediction of cancer-associated thrombosis in a selected population covering all cancer types.

1.11 Chapter Summary

This Chapter has introduced the topic of cancer-associated thrombosis including its pathogenesis, which is complex and involves many different overlapping pathways and processes. Overall, whilst there are published models to predict CAT occurrence, there is no one universally adopted and widely utilised model. The Vienna CATS model was derived from a large prospective clinical trial, but the availability in routine clinical laboratories of one biomarker, sP-selectin, means it is rarely used. Consequently, CAT continues to be a common complication in patients diagnosed with cancer. If a way of truly predicting CAT could be found it would revolutionise the management and prediction of this condition.

CHAPTER 2. METHODOLOGY

2.1 Ethics statement

Ethical approval for the work outlined in this thesis was sought from a NHS Research Ethics Committee (REC) and granted on the 17th August 2023 (IRAS project ID 301825) (Appendix 1). In addition, the Newcastle upon Tyne Hospitals NHS Foundation Trust conducted a thorough Capacity and Capability review to ensure that this research could take place, and this was granted in April 2024. Finally, ethical approval was sought from Manchester Metropolitan University (EthoS reference 60179) (Appendix 2).

2.1.1 Patient Consent

At recruitment, all participants consented to blood samples being drawn on three separate occasions; before the start of chemotherapy, approximately 1 month afterwards, and 3 months after the start of chemotherapy, when routine blood samples were also being taken for clinical purposes. All participants received a Participant Information Sheet (PIS) (Appendix 4) and were free to withdraw their consent at any time. Signed consent forms are held by the Clinical Trials Team in the Newcastle upon Tyne Hospitals NHS Foundation Trust as per GDPR (General Data Protection Regulation) guidance. A copy of the consent form, and the Participant Information Sheet can be found in Appendix 3 and 4 respectively of this thesis.

2.2 Patients

Participants were recruited at their chemotherapy pre-assessment clinic visit at the Northern Centre for Cancer Care (NCCC) at the Freeman Hospital in Newcastle upon Tyne.

All participants needed to meet the following criteria for inclusion; Over 18 years of age with a current diagnosis of cancer, of any type or stage, and about to commence chemotherapy. Patients with a prior history of cancer were included, as were those who had undergone resection surgery for their current cancer before the start of chemotherapy.

Participants who were prescribed anticoagulant therapy before the commencement of chemotherapy were excluded.

Throughout the study, patients' records were examined for additional information including the diagnosis of a thrombotic event. Hospital electronic records used for this purpose were eRecord (Oracle Health), Chemocare (CIS Oncology), and APEX (Dedalus).

2.3 Blood sampling and collection

Venous blood samples were taken from consented participants by a trained phlebotomist and collected into a BD Vacutainer® containing 3.2% (0.109M) sodium citrate (2.7mL) or silica (5mL).

Samples collected into sodium citrate vacutainers were correctly filled to +/- 10% of the target fill volume, as per the manufacturer's instructions (Becton, Dickinson Limited, Wokingham, United Kingdom) and transported to the Blood Sciences laboratory at the Freeman Hospital, Newcastle upon Tyne Hospitals NHS Foundation Trust shortly afterwards. All samples were hand delivered by Clinical Trial Associates employed by the NHS Trust.

2.4 Sample analysis

2.4.1 D-dimer analysis

Whole blood samples collected into 3.2% (0.109M) sodium citrate were centrifuged for 10 minutes at 2000RCF at 22°c, as per British Society for Haematology (BSH) Guidelines (Baker et al, 2020) for the handling of routine coagulation samples.

Following centrifugation, the sample was analysed on a Werfen TOP 700 analyser (Werfen UK Limited, Warrington, United Kingdom), and D-dimer measurement performed using the HemosIL® D-dimer HS kit (Instrumentation Laboratory, Bedford, MA, USA), which is also used routinely in the Trust, and is described in more detail below.

The HemosIL® D-Dimer HS Kit uses latex agglutination to determine D-dimer levels. It is formed of two reagents – a latex reagent, and a reaction buffer. The latex reagent is reconstituted using 2mL of distilled water and contains a suspension of latex particles which are coated with the $F(ab')_2$ fragment of a mouse monoclonal

antibody (MA-8D3) directed against D-dimers. It also contains bovine serum albumin, buffer, stabilizers and a preservative. The reaction buffer is a phosphate solution which also contains bovine serum albumin, stabilizers and a preservative.

The scientific basis of endpoint determination of this assay is turbidimetric immunoassay. The degree of light absorbance changes as a sample becomes more turbid, or cloudy, which is measured at 671nm. When a plasma containing D-dimer is mixed with the latex reagent and reaction buffer, agglutination with the latex particles occurs, shown in Figure 2.1. The degree of agglutination is directly proportional to the concentration of D-dimer in the sample and is determined by measuring the decrease of the transmitted light (increase in absorbance), measured at 671nm, caused by the aggregates of latex particles formed.

18µl of plasma is added to 150µl of buffer, and 45µl of latex reagent, and incubated at 37ºc for ten seconds, before light absorbance is measured for a further 240 seconds. A graph of light absorbance versus time is plotted with the rate at which the absorbance increases proportional to the amount of D-dimer present. This is shown in Figure 2.2. The rate at which this takes is then converted into a D-dimer concentration as per the calibration curve constructed using the analyser software. D-dimer concentration is recorded as ng/mL. D-dimer is considered elevated when levels are higher than 230 ng/mL.

The analyser is calibrated using five data points with every batch change of reagent, or if the internal quality control shows any obvious trends or bias, by a senior

member of the laboratory team only. Internal quality control (IQC) at two levels is performed every eight hours, or if the reagent is replaced. There are two analysers available, which are rotated into daily use on a weekly basis. Both analysers are set up the same and are serviced by the manufacturer every six months. This test is accredited to UKAS (United Kingdom Accreditation Service) ISO 15189;2012 standards, and the laboratory is inspected to this standard every twelve months.

After analysis the remaining plasma was centrifuged again at 2000RCF for ten minutes to remove any residual platelets, and aliquoted into a labelled microtube for long term storage at -40^{o}c, in case further analysis was required.

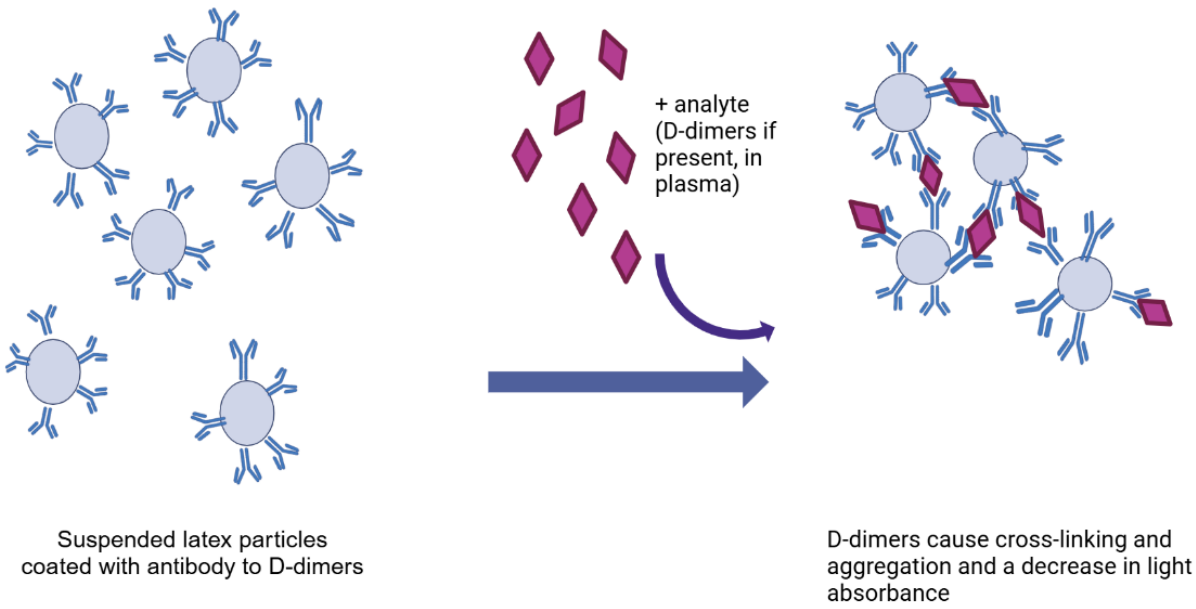


Figure 2.1. **Graphical representation of the principles of latex agglutination used for D-dimer analysis.** Figure created in https://BioRender.com

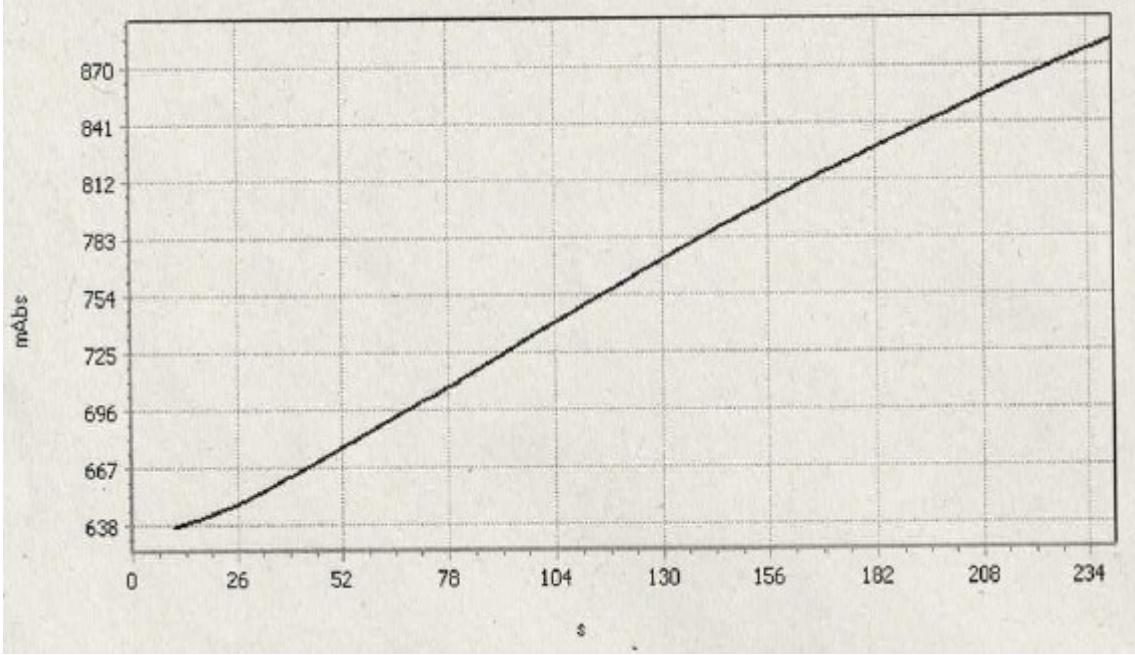


Figure 2.2. **Graphical representation of how the Werfen TOP analysers determine D-dimer concentration in a sample.** The rate at which light absorbance changes is proportional to the amount of D-dimers present in the sample. Image taken from Werfen TOP 700 software.

2.4.2 Plasma and serum VEGF analysis

2.4.2.1 Plasma VEGF sample preparation

Whole blood collected into 3.2% (0.109M) sodium citrate was centrifuged for fifteen minutes at 1000RCF at 25°c.

Following centrifugation, the plasma in the sample was aliquoted into a labelled microtube and stored at -40°c until batch analysis was performed.

2.4.2.2 Serum VEGF sample preparation

Blood samples were collected into a 5.0mL BD Vacutainer® sample tube containing silica, to promote blood clot formation, and an inert serum separation gel which following centrifugation provides a barrier between the serum and the remainder of the blood.

The sample was then centrifuged for fifteen minutes at 1000RCF at 25°c.

Following centrifugation, the serum in the sample was aliquoted into a labelled microtube and stored at -40°c until batch analysis of the samples was performed.

2.4.2.3 VEGF ELISA

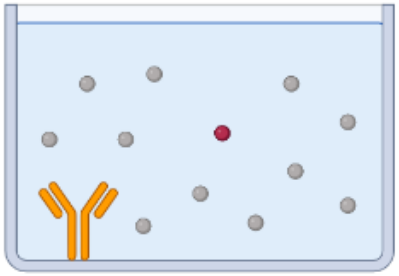
Analysis of VEGF levels was by ELISA (Enzyme Linked Immuno-Sorbant Assay) using the Quantikine® ELISA Human VEGF Immunoassay kit (R&D Systems, Minneapolis, MN, USA).

Sample were analysed using a microplate, which was pre-coated with a monoclonal antibody to VEGF. 100µl of appropriate standards, controls and the participant samples were pipetted into the wells as per the manufacturers instructions and incubated for two hours at room temperature before being washed thoroughly to remove any unbound substances. Next, 200µl of an enzyme-linked polyclonal antibody specific for human VEGF (conjugate) was added followed by a further two hours incubation at room temperature. Finally, following a further washing stage to remove any unbound substances, 200µl of a colour substrate containing tetramethylbenzidine (TMB) was added. The colour developed is proportional to the amount of VEGF in the sample. The reaction was stopped after 25 minutes with the addition of 50µl of 2N sulphuric acid. The plate was then read on the Mulitskan FC microplate reader (Thermo Scientific, Waltham, MA, USA) at 450nm. A standard curve was constructed, and absorbance values of each patient and control derived using the plate reader software. VEGF levels were recorded as pg/mL. The normal reference range for serum VEGF is zero to 770 pg/mL, a normal reference range for plasma VEGF was non-determinable according to the VEGF kit insert (R&D Systems, Minneapolis, MN, USA). A graphical representation of how an ELISA works is shown in Figure 2.3.

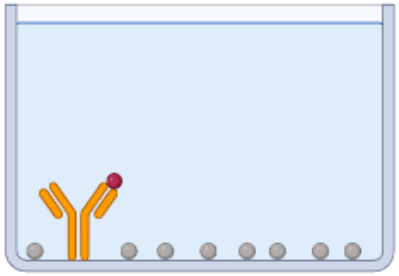
For this thesis, half-plates containing six strips containing a total of 48 wells were used to reduce drift. Four levels of internal quality control were used (high, medium, low, and an in-house made very low (1 in 3 of the Low control)). Controls were

spaced at the start and end of the plate as to assess the amount of drift occurring. All samples, controls and standards were run in duplicate, and the average taken for reporting purposes. If the results differed significantly between replicates, then this sample was repeated.

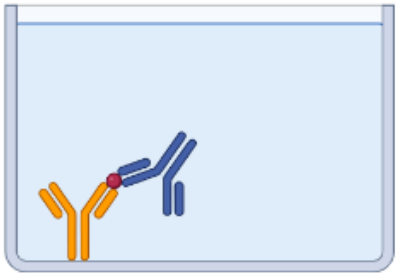
A standard of 2000 pg/mL was provided with each kit. This was serially diluted in calibration diluent. For serum VEGF a standard curve was constructed for each run containing 2000, 1000, 500, 250, 125, 62.5 and 31.25 pg/mL. A blank was also run representing zero pg/mL. As the normal range for plasma VEGF is significantly lower a different set of dilutions were used. The 2000 pg/mL standard was omitted and instead a 15.6 pg/mL standard added.



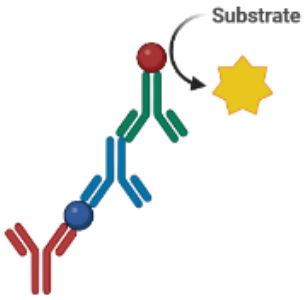
1.Patient sample added to wells on microplate which are coated with antibody to analyte of interest (VEGF or sP-selectin



2. After an incubation period, if present, target analyte will bind to antibody. Washing follow to remove any unbound substances



3. Conjugate added to wells, which will bind to the target analyte. Followed by x3 washes to remove any unbound substances



4. Substrate added (TMB) which induces a colour change. Reaction is stopped with the addition of sulphuric acid after 25 minutes. Colour change is read on a plate reader at 450nm.

Figure 2.3. **Graphical representation of the principles of a sandwich ELISA assay.** Used for both VEGF and sP-Selectin assays. Created in https://BioRender.com

2.4.3 Soluble P-selectin analysis

2.4.3.1 Plasma sample preparation

Whole blood collected into 3.2% (0.109M) sodium citrate was centrifuged for fifteen minutes at 1000RCF at 25^{o}c.

Following centrifugation, the plasma in the sample was aliquoted into a labelled microtube and stored at -40^{o}c until batch analysis of the samples was performed.

2.4.3.2 P-selectin ELISA

Soluble P-selectin plasma levels were measured by an ELISA (R&D Systems, Minneapolis, MN, USA). Samples and controls were diluted 20-fold in P-selectin Sample Diluent (15µl of sample, 285µl of Sample Diluent), and 100µl of this pipetted into the relevant wells of the microplate alongside 100µl of conjugate and incubated at room temperature for 1 hour. 200µl of substrate (tetramethylbenzidine (TMB)) was then added following washing and then the reaction stopped by the addition of 2N sulphuric acid after 15 minutes. The concentration of soluble P-selectin was then determined using the Mulitskan FC microplate reader (Thermo Scientific, Waltham, MA, USA) at 450nm. A standard curve is constructed for each run of samples, with P-selectin concentrations of 50, 25, 12.5, 6.25, 3.13, 1.56 and 0.781 ng/mL, with the P-selectin Sample Diluent acting as a zero standard, and the plasma soluble P-selectin concentration calculated off the standard curve, which constructed by the plate reader software. Soluble P-selectin levels were recorded as ng/mL. This test is not

performed as standard, but reference ranges taken from the kit insert are 0 to 37.9 ng/mL (R&D Systems, Minneapolis, MN, USA).

For this thesis, half-plates containing six strips and 48 wells were used to reduce drift. One level of internal quality control was used, as supplied by the manufacturer, and this was placed at the start and the end of the plate as to assess the amount of drift occurring. All standards, controls and samples were run in duplicate, and the average taken for reporting purposes.

2.5 Clinical Audit

A clinical audit to assess the utility of the Khorana score in predicting a thrombotic event in pancreatic cancer patients was performed as part of this thesis.

2.5.1 Patient Data Collection

Patient electronic health records, described earlier in section 2.2 in this Methods chapter, were retrospectively examined for 75 patients diagnosed with pancreatic cancer. The Khorana score was calculated for each patient using blood results available at the start of the patients' chemotherapy treatment. A patients' Body Mass Index was taken from the electronic health records, and if not available calculated using the following formula: [Weight (in kilograms)/ by height (in metres)^2].

The blood results used as part of the Khorana score include Haemoglobin (Hb), White Blood Cell Count (WBC), and Platelet Count (Plt). The neutrophil count was also recorded. All these parameters form part of the Full Blood Count (FBC).

2.5.2 Full Blood Count analysis

Whole blood was collected into a 4.0mL BD Vacutainer® (Becton, Dickinson Limited, Wokingham, United Kingdom) 7.2 mg K_2EDTA (ethylenediaminetetraacetic acid) tubes. Samples are well mixed and then analysed on a Sysmex XN-10 analyser (Sysmex Corporation, Kobe, Japan). 88µL of whole blood is used to obtain a Full Blood Count result, though the minimum volume required is 1mL due to the dilutional effect EDTA has on results.

Haemoglobin measurement is by spectrophotometry measured at 555nm using a cyanide-free sodium lauryl sulphate (SLS) reagent (Sysmex Sulfolyser) which lyses all cells present. The SLS molecule attaches to the haemoglobin molecules present in the sample, and absorbance is measured at 555nm, with the amount of haemoglobin present proportional to the light transmitted. Haemoglobin levels are recorded as g/L.

The platelet count and White Blood Cell Count (WBC) are performed using impedance technology. Sample are diluted in an isotonic saline solution which allow electrical conductivity, whilst maintaining the characteristics of the cells (size and shape). Cells are then forced through a small aperture to which a current is applied across. Any cells passing through the aperture cause a brief change or “pulse” to the electrical current. The size of the change of the current, or pulse, is proportional to the size of the cell passing through. Thus, platelets, red blood cells and white blood

cells are separated and counted. The normal range for platelet counts are 150-450 x 10^9/L. Platelet counts were recorded as the value x 10^9/L

White blood cells are then further differentiated into their respective types using flow cytometry. The analyser uses two different channels, named WNR and WDF, and different reagents to distinguish between nucleated red blood cells and white blood cells (WNR channel), and then the five types of white cells present in normal individuals (neutrophils, lymphocytes, monocytes, eosinophils and basophils) (WDF channel). First, a reagent is added to perforate the cell membrane (Sysmex Lysercell WNR or WDF) whilst not disrupting its contents. Next a fluorescence dye is added (Fluorocell WNR or WDF) which stains the contents of the cell (both the nucleus and any granules present). Enumeration of cells is then performed using a laser and forward and side scatter to differentiate the cells based on their size, nuclear content, and granular content, with each population of cells displaying different characteristics enabling separation. Normal ranges for white blood cell counts are 4 to 11 x10^9/L. White blood cell counts were recorded as the value x 10^9/L.

Internal quality control (IQC) at three levels is performed once per day, with a precision or drift control run every two hours throughout the day. There are six analysers available, four in the main laboratory, and two at outreach locations within the Northern Centre for Cancer Care department. All analysers are serviced by the manufacturer every six months. This test is accredited to UKAS (United Kingdom

Accreditation Service) ISO 15189;2012 standards, and the laboratory is inspected to this standard every twelve months.

See the Clinical Audit chapter (Chapter 3; Results Chapter 1) of this thesis for further details.

2.6 Meta-analysis

A meta-analysis to examine the potential of VEGF being used as a biomarker for cancer-associated thrombosis was performed as part of this thesis.

2.6.1 Study Design

The meta-analysis conformed to the standard of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). A literature search was performed using specific key words and two different databases (PubMed and OVID).

2.6.2 Risk of Bias, data heterogeneity and statistical analysis

Statistical analysis, including an assessment of heterogeneity using I^2, was performed, and forest plots drawn, using the RevMan software available on the Cochrane website (The Cochrane Collaboration, London, United Kingdom). Risk of bias was assessed using the ROB-ME tool, and quality assessment using the Newcastle-Ottawa Score.

Further details regarding the meta-analysis can be found within Chapter 4 (Results Chapter 2) of this thesis.

2.7 Statistical Analysis

Statistical analysis was performed using GraphPad Prism software version 10.4.1. (GraphPad Software, Boston, MA, USA).

Descriptive statistics including mean, mean, range and 95% confidence limits were determined using this software.

Before any statistical analysis was performed normality testing was undertaken. Again, this was performed using the GraphPad Prism software. Tests for normality included the D'Agostino & Pearson test, Anderson-Darling test, Shapiro-Wilk test and the Kolmogorov-Smirnov test. Where the results of these tests did not agree, the majority was used as the consensus, where there was a tie normality was not assumed, and non-parametric analysis was undertaken. Where statistical analysis was required to compare two populations and where one population passed all normality tests, but the other failed all tests, non-normality was assumed, and non-parametric statistical analysis was performed.

For statistical analysis, Mann-Whitney or Kruskal-Wallis tests were used when the populations failed their normality tests, and an unpaired t-test was used when the populations passed the normality tests. Dunn's post hoc correction was applied when the Kruskal-Wallis performed.

Statistical significance was set at $p = <0.05$.

2.8 Chapter Summary

In this Chapter, I have outlined the methods which will be used for the completion of this thesis. Three biomarkers have been selected for the investigative part of this thesis (Chapter 5) which represent the pathogenesis of CAT, namely platelet activation, endothelial activation, and the activation of the secondary haemostasis pathways. There are, however, many other biomarkers which could have been selected, which may have resulted in novel findings.

CHAPTER 3. RESULTS – CLINICAL AUDIT

Can the Khorana Score predict cancer-associated thrombosis in pancreatic cancer?

A clinical audit of high-risk pancreatic cancer patients at The Newcastle upon Tyne Hospitals NHS Fowundation Trust.

3.1 Introduction

Cancer of the pancreas is the 10th most prevalent cancer in the United Kingdom, accounting for 3% of all cancer types in both the UK and the United States (Turner et al, 2023). Approximately 8800 cases of pancreatic cancer are diagnosed in the United Kingdom each year (Pancreatic Cancer UK). The 10-year survival rate in the UK is less than 5% (Cancer Research UK; Pancreatic Cancer UK), with a median survival of 10 to 12 months with treatment (Wood et al, 2002), but only two to six months if diagnosed at a metastatic stage (Pancreatic Cancer UK). In many cases, symptoms related to the pancreatic cancer do not become apparent until the cancer has disseminated to other organs including the liver, bowel or lungs, with half of patients presenting with metastatic disease, and a further 30 to 35% with locally advanced, unresectable disease (Park et al, 2021; Wood et al, 2022; Turner et al, 2023). Typical symptoms include abdominal pain, jaundice, weight loss, or a new diagnosis of

diabetes (Wood et al, 2022; Turner et al, 2023). Patients with a previous diagnosis of pancreatitis are closely monitored and may present earlier. The most common type of pancreatic cancer is a pancreatic ductal adenocarcinoma, which accounts for more than 90% of all cases (Turner et al, 2023).

Thrombosis occurring in patients with cancer of all types occurs on average in 1 in 20, or 5%, of patients (Alikhan et al, 2024) but this rate is even higher in the pancreatic cancer population (Khorana et al, 2009; Muñoz Martin et al, 2014). A review published in the British Journal of Cancer in 2019 (Campello et al), quoted figures of between 6.9 to 57% for venous thrombosis, and 1.6 to 5.9% for arterial thrombosis. However, despite these striking figures, routine thromboprophylaxis is rarely prescribed in this setting, predominantly due to an inability to differentiate between the highest risk patients within this population (van Es, 2017). Therefore, this audit will seek to address this as The Newcastle upon Tyne Hospitals NHS Foundation Trust is looking to improve its cancer care. This audit set out to establish whether the Khorana score can be used to predict cancer-associated thrombosis in pancreatic cancer patients and review whether thromboprophylaxis would be beneficial.

The Khorana Score is the most widely validated of many published predictive models for cancer-associated thrombosis, however, its clinical use is debatable with several studies demonstrating that it is not effective in all cancer types. The Khorana Score (Khorana et al, 2008) aims to predict those individuals who are at a greater risk of

developing cancer-associated thrombosis and to identify those who should be given thromboprophylaxis in addition to their anti-cancer therapy. The Khorana score is calculated using five main components; site of cancer, prechemotherapy platelet count, haemoglobin level, prechemotherapy leucocyte (white blood cell) count and Body Mass Index (BMI). The Khorana Score is reproduced in full in Table 3.1 and provides an overall score of the risk of thrombosis. A score of zero designates a low risk, one to two points an intermediate risk, and three or more a high risk. The maximum score possible is six.

Pancreatic cancer is designated as a very high-risk cancer by the Khorana Score, receiving two points, and patients are therefore classified in an intermediate category before the addition of any other high- risk factors. There have been several studies that have demonstrated in high-risk cancers, the Khorana score is less effective. A study in 2017 (van Es, 2017) demonstrated that the Khorana score was unable to discriminate between pancreatic cancer patients at intermediate risk and high risk of venous thromboembolism (VTE). Therefore, at The Newcastle upon Tyne Hospitals NHS Foundation Trust, the Khorana score is not currently used as part of pancreatic cancer patients' management. An earlier study, by Pelzer et al (2013), found that the single parameters in the Khorana score can be used independently, and these demonstrated a higher risk towards thrombosis in pancreatic cancer patients, but these associations were not conclusive.

To improve clinical management a system to identify patients at risk of thrombosis is required. For this reason, in addition to the evaluation of the Khorana score, the accuracy of the individual parameters within the model at predicting those that subsequently developed a thrombosis was determined.

At the time of the audit, current practice within The Newcastle upon Tyne Hospitals NHS Foundation Trust is to not prescribe thromboprophylaxis to all pancreatic cancer patients receiving chemotherapy, with prophylaxis only given in cases where other thrombotic risk factors are present, for example smokers, a prior history of a VTE or a family history of VTE.

Prescription of thromboprophylaxis is associated with its risks that can worsen patient outcomes. The most significant of these is the increased risk of bleeding, particularly around the tumour site, which is rich in new, immature blood vessels (angiogenesis) (Johnstone et al, 2018). Therefore, clinicians need to balance the risks, and a predictive tool would be more appropriate by targeting those at the truest highest risk, whilst preventing bleeding episodes in others.

Validation of the Khorana Score, or identification of one of its components that can predict thrombosis in pancreatic cancer patients, would be useful in this setting to determine which patients should be prescribed prophylaxis. If a parameter, or parameters, could be identified then this could allow more individualised and appropriate patient care and treatment with regards to thrombosis. It could also

potentially save financial resources by reducing the number of prescriptions and possibly duration of a hospital stay.

Patient characteristic	*Risk score*
Site of cancer:	
Very high risk (stomach, pancreas)	2
High risk (lung, lymphoma, gynaecological, bladder, testicular)	1
Prechemotherapy platelet count 350 x 10^9/L or more	1
Haemoglobin level less than 100 g/L or use of red cell growth factors	1
Prechemotherapy leucocyte count more than 11 x 10^9/L	1
BMI 35 kg/m^2 or more	1

Table 3.1. **Khorana score** (Khorana et al, 2008)

3.2 Aims

The first aim of the clinical audit was to establish whether, if the Khorana Score had been applied to patients with pancreatic cancer, it could have been used to predict those individuals who were later diagnosed with a thrombosis, with the intention of driving changes in clinical management of these patients.

The second aim of this clinical audit was to determine whether thromboprophylaxis reduces thrombosis in pancreatic cancer patients, with the intention of driving changes in clinical management of pancreatic cancer patients at The Newcastle upon Tyne Hospitals NHS Foundation Trust.

3.3 Approach

A clinical audit was conducted using seventy-five consecutive patients diagnosed with pancreatic cancer at The Newcastle upon Tyne Hospitals NHS Foundation Trust between July 2020 and July 2023. All patients were discussed at a Multi Disciplinary Meeting (MDT), and a formal diagnosis and staging made including recommendations regarding treatment.

This retrospective audit examines the clinical history of each of the 75 patients up to May 2024, including any incidences of a proven thrombosis (either arterial or venous). Using data collected prior to administration of chemotherapy, the Khorana Score was calculated for each of the 75 patients.

3.4 Methods

National Health Service (NHS) numbers and patient demographics (forename and surname) were obtained for seventy-five patients who received a diagnosis of pancreatic cancer between July 2020 and July 2023. Of the seventy-five, eleven patients were already on prescribed thromboprophylaxis and were therefore excluded from the initial study and investigation of the Khorana score, leaving 64 patients. The eleven patients on thromboprophylaxis were subsequently used as comparators to the remaining 64 patients for rates of thrombosis. The data for the audit was gathered in May 2024 therefore each patient was followed up for a minimum of ten months, or until their death, after their diagnosis.

From this information, the Trust electronic medical records systems (eRecord (Oracle Health), Chemocare (CIS Oncology), and APEX (Dedalus)) were interrogated to collect the following information.

- Age
- Date of MDT and stage of pancreatic cancer at diagnosis (if known)
- Chemotherapy regimens prescribed and their start date
- Patient Body Mass Index (BMI) at start of chemotherapy
- White blood cell count (WBC), Platelet count, Haemoglobin (Hb) and Neutrophil count at the start of chemotherapy (as established during pre-assessment chemotherapy clinic)
- Details of any proven, medically confirmed thrombosis, and
- Details of any thrombophylactic regimes, including whether this was prescribed before or after the diagnosis of a thrombotic event.

Where the patients' BMI was unavailable in the records, it was calculated using the following formula; [Weight (in kilograms)/ by height (in metres)^2]. In each case, the height and weight were available in the health records.

Inclusion Criteria; all patients receiving chemotherapy for pancreatic cancer at the Northern Centre for Cancer Care (NCCC) at the Freeman Hospital were included, some (n = 18) patients had recently received resection surgery following their diagnosis.

Exclusion criteria; patients less than 18 years of age and patients prescribed thromboprophylaxis for other medical reasons, a previous VTE, or because they were deemed at a higher risk of thrombosis.

Patients were split into two groups for analysis: those with thrombosis within 10 months of diagnosis (or before their death if less than 10 months) and those without thrombosis (within 10 months of diagnosis).

3.5 Results

3.5.1 Can the Khorana score be used to predict thrombosis in pancreatic cancer patients?

The main patient characteristics of the 64 patients included in this analysis are summarised in Table 3.2.

Of the 64 patients examined as part of this audit, 31.3% (20/64), developed a thrombosis within ten months of diagnosis. This is higher than the figures reported for general cancer-associated thrombosis rates (Alikhan et al, 2024) but agrees with observational data and statistics previously published for pancreatic cancer which are higher (Muñoz Martin et al, 2014). All thrombosis documented was venous and included deep vein thrombosis (DVT), pulmonary embolism (PE), portal vein thrombosis and a brachial thrombosis.

All 20 patients within the audit who developed a thrombosis were subsequently prescribed thromboprophylaxis (Low Molecular Weight Heparin (LMWH) either Tinzaparin or Enoxaparin) followed by a Direct Oral Anticoagulant (Apixaban or Rivaroxaban) as per NICE (National Institute of Health and Care Excellence) guidance (NICE, 2020).

With regards to the types of treatment prescribed, of the 64 patients included in this audit, seven different chemotherapy treatment regimens were documented, though some patients received more than one type. One patient did not receive any chemotherapy, passing away before any could be administered. However, they had had pre-assessment chemotherapy bloods done and so are therefore included in the statistics.

Number of patients	All	Without a thrombosis	With a thrombosis
	N = 64	N = 44 (68.7%)	N = 20 (31.3%)
Sex	M = 34 (53%) F = 30 (47%)	N = 21 N = 23	N = 13 N = 7
Ethnicity *			
White British	N= 56 (87.5%)	N = 36	N = 19
White Other	N = 2 (3%)	N = 2	N = 0
Not stated	N = 5 (8%)	N = 5	N = 1
Not known	N = 1 (1.5%)	N = 1	N = 0
Mean age (range):	65.9 years (46 to 83)	66.2 years (48 to 83)	65.2 years (46 to 77)
Male	65.3 years (46 to 78)	66.7 years (51 to 78)	63.1 years (46 to 74)
Female	66.6 years (48 to 83)	65.8 years (48 to 83)	69.1 years (63 to 77)
BMI (range)	25.78 (18 to 36.6)	25.77 (18 to 35.5)	25.80 (18.5 to 36.6)
Mean Khorana Score (range)	2.48 (2 to 5)	2.47 (2 to 4)	2.50 (2 to 5)
Low risk (0 points)	N = 0 (0%)	N = 0 (0%)	N = 0 (0%)
Intermediate risk (1-2 points)	N = 37 (57.8%)	N = 24 (54.5%)	N = 13 (65%)
High risk (3 or more points)	N = 27 (42.2%)	N = 20 (45.5%)	N = 7 (35%)

Chemotherapy regimes [$]			
FOLFIRINOX	N = 33		
FOLFOX	N = 8	N = 21 (63.6%)	N = 12 (36.4%)
FOLFIRI	N = 2	N = 5 (62.5%)	N = 3 (37.5%)
Gemcitabine, Abraxane	N = 20	N = 1 (50%)	N = 1 (50%)
Gemcitabine, capecitabine		N = 12 (60%)	N = 8 (40%)
Gemcitabine only	N = 8		
Radiotherapy plus capecitabine	N = 24	N = 7 (87.5%)	N = 1 (12.5%)
	N = 11	N = 15 (62.5%)	N = (37.5%)
		N = 5 (45.5%)	N = 6 (54.5%)

Table 3.2. **Summary of clinical audit patient characteristics** * = as recorded in eRecord. [$] = many patients received more than one line of treatment.

3.5.1.1 The Khorana Score is not predictive of thrombosis in pancreatic cancer patients

Whilst a useful tool in other cancer types, it has been suggested that the Khorana score is not as effective at identifying those patients with pancreatic cancer, or other high-risk cancers such as lung cancer, that are at risk of thrombosis (Muñoz Martin et al, 2014) (van Es et al, 2017) (van Es et al, 2020). To investigate whether this is the case in this cohort of pancreatic cancer patients at The Newcastle upon Tyne Hospitals NHS Foundation Trust, Khorana scores were calculated for all 75 patients and compared between those patients that had thrombosis and those that did not.

A diagnosis of pancreatic cancer equates to a score of two, and so therefore all patients within this audit were assigned to an intermediate category or above (2+). Patients were also split by risk category – 57.8% (37/64) were assigned into the intermediate category, and 42.2% (27/64) into the high-risk category. Of those who developed a thrombosis, 65% (13/20) were in an intermediate category, and the

remaining 35% (7/20) in the highest risk category. Of those assigned the high-risk category only 25.9% (7/27) developed a thrombosis, compared to 35.1% (13/37) in the intermediate category. This suggests that the Khorana score is not predictive of thrombosis, with more individuals in the intermediate category, compared to the high-risk category, developing a thrombosis.

In addition, the population represents an abnormal distribution, and as a result non-parametric statistical analysis (the Mann-Whitney test) was also used to determine if there was a statistical difference between the Khorana scores of those patients who subsequently developed a thrombosis, and those who did not.

In support of observations made in other studies, as shown in Figure 3.1, comparison of Khorana scores between those patients who did not experience a thrombosis versus those who did, demonstrated no statistical difference between the two populations ($p = 0.6353$).

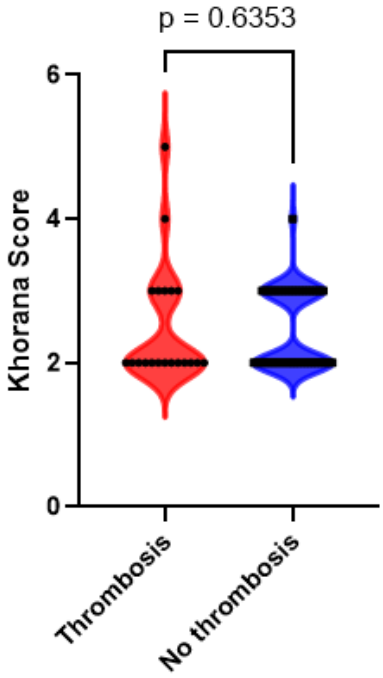


Figure 3.1. **No difference in the Khorana score in pancreatic cancer patients who develop a thrombosis versus those who do not.** Khorana scores were calculated for 64 patients with pancreatic cancer and compared between groups of patients with (n = 20) and without (n =44) thrombosis. Data presented as a Violin plot showing the thrombosis and no thrombosis populations within the audited group. Statistical analysis performed using Mann Whitney tests. p=0.6353.

Further analysis was performed separating the males and female patients, to investigate whether the Khorana score may be more effective when stratified by sex. Similar to that observed with the whole population, no statistical difference in those who developed a thrombosis versus those who did not was observed (males p = 0.9753, females p = 0.6746).

Taken together, these findings further support previous observations in other studies and demonstrates that the Khorana score, is unable to predict those pancreatic

cancer patients who will develop cancer-associated thrombosis versus those who will not.

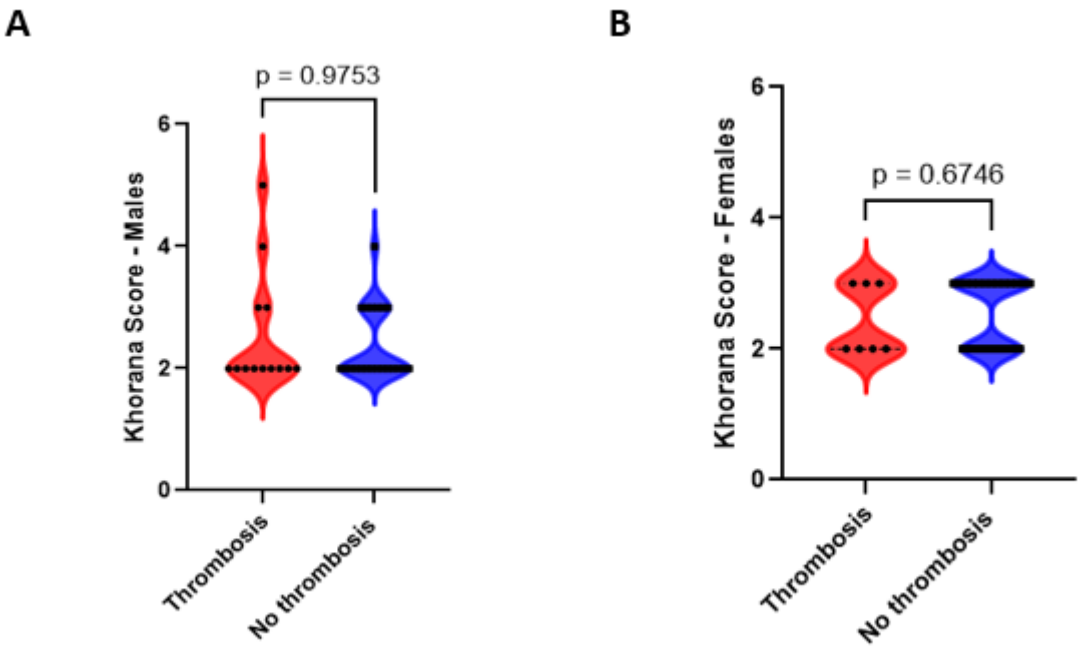


Figure 3.2. **No difference in the Khorana score in pancreatic cancer patients who develop a thrombosis versus those who do not when data analysed by sex.** Khorana score were calculated for males (n = 34) (Panel A) and females (n = 30) (Panel B) with and without a thrombosis. Data presented as a violin plot showing the thrombosis and no thrombosis populations within the audited groups. Statistical analysis performed using Mann Whitney tests. p = 0.9753 for males, and p = 0.6746 for females.

As a result of these findings, the individual components of the Khorana score were further examined to establish if any parameters, when applied individually, could predict thrombosis in this group of patients.

3.5.1.2 The Body Mass Index (BMI) is not predictive of thrombosis in pancreatic cancer patients

Obesity, resulting in a high BMI, is associated with an increased risk for VTE (Eichinger et al, 2008) (Hotoleanu 2020). The reasons for this are predominantly due to inactivity, with immobilisation being a thrombotic risk factor. In addition, obesity is also associated with raised intra-abdominal pressure, a chronic low-grade inflammatory state (potentially leading to immune-thrombosis), elevated levels of fibrinogen, von Willebrand factor and Factor VIII all of which lead to a prothrombotic state and increased risk of thrombosis (Eichinger et al, 2008) (Hotoleanu 2020). The additional risk factor of a cancer diagnosis further increases this risk.

Body Mass Index is one of the component parameters of the Khorana Score. However, to count towards the final score, a patient must have a BMI of 35 kg/m^2 or more (Khorana et al, 2008). In our audited population, the mean BMI was 25.78 kg/m^2, with a range of 18.0 to 36.6. Only two individuals in our audit had a BMI of 35 kg/m^2 or more; one of these experienced a thrombosis, the other did not.

When separated out between those with or without a thrombosis diagnosis after the commencement of chemotherapy, the mean BMI is very similar – 25.80 kg/m^2 versus 25.77 kg/m^2.

Analysis was performed to determine to see if there were any statistical differences between the two groups. Data was found to be normally distributed. As shown in

Figure 3.3, with a p-value of 0.9840 no statistically significant difference was found in BMI between the group with thrombosis versus without.

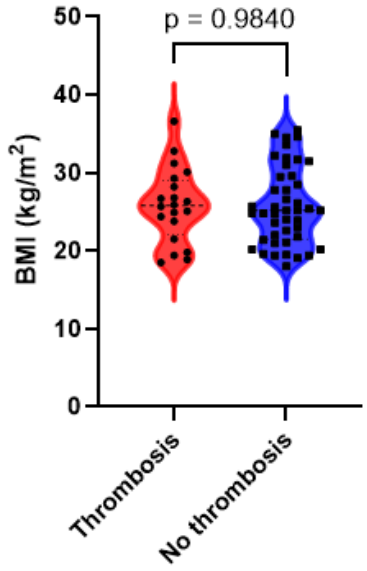


Figure 3.3. **No difference in Body Mass Index (BMI) in pancreatic cancer patients who develop a thrombosis versus those who do not.** BMI was calculated for 64 patients with pancreatic cancer using [weight (kg)/ height (m)^2] and compared between groups of patients with (n=20) and without (n = 44) thrombosis. Data presented as a Violin plot showing the thrombosis and no thrombosis populations within the audited group. Statistical analysis performed using an unpaired t-test. p = 0.9840.

Therefore, these findings indicate BMI cannot predict cancer-associated thrombosis, either alone or as part of the Khorana Score.

3.5.1.3 Platelet Count is not predictive of thrombosis in pancreatic cancer patients

Platelets are key contributors to thrombosis pathology. A pre-chemotherapy platelet count above 350 x10^9/L is predictive of cancer-associated thrombosis as indicated by the Khorana Score (Khorana et al, 2008). Whether platelet counts could be predictive of thrombosis in pancreatic cancer patients was therefore investigated. Within the

audited population, the average (mean) platelet count was 309.9 x10^9/L, with a range of 91 to 1137 x 10^9/L.

A mean platelet count of 325.5 x 10^9/L was observed for those patients with thrombosis, and a mean count of 302.9 x 10^9/L within the non-thrombosis group (Figure 3.4). A Mann-Whitney test, gave a p value of 0.7438, suggesting that there are no differences in platelet count between the two and that the platelet count cannot be used to predict thrombotic events. 25% (5/20) of patients in the thrombosis group had counts over 350 x10^9/L and 31.8% (14/44) of patients without thrombosis did. The individual with the highest platelet count of 1137 x 10^9/L did experience a thrombosis. Further retrospective audits or studies, with a larger auditable population are required to establish if an extremely high platelet count could be predictive of a thrombosis in pancreatic cancer patients.

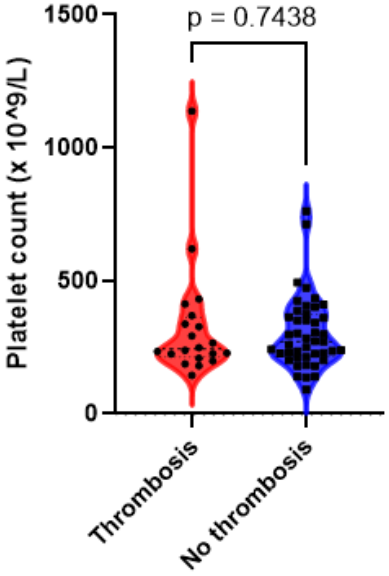


Figure 3.4. **No difference in platelet count in pancreatic cancer patients who develop a thrombosis versus those who do not.** Platelet count gathered for 64 patients with pancreatic cancer and compared between groups of patients with (n=20) and without (n = 44) thrombosis. Data presented as a Violin plot showing the thrombosis and no thrombosis populations within the audited group. Statistical analysis performed using Mann-Whitney tests. p = 0.7438.

3.5.1.4 Haemoglobin concentration is not predictive of thrombosis in pancreatic cancer patients

Haemoglobin values less than 100 g/L, or the use of red cell growth factors (such as erythropoietin), also contribute to a high Khorana score, and thus a higher risk of cancer-associated thrombosis. Whether haemoglobin levels could be predictive of thrombosis in pancreatic cancer patients was therefore investigated. Within our population, the average (mean) haemoglobin concentration was 129.5 g/L, with a range of 99 to 176 g/L. Only one patient within our audit hit the haemoglobin threshold for an additional point on the Khorana Score, but this patient did subsequently develop a thrombosis (although they also gained points for their WBC

and Platelet counts too, resulting in a Khorana score of 5). Within the thrombosis and non-thrombosis populations, the mean haemoglobin was 132.6 g/L for thrombosis, and 128.1 g/L for non-thrombosis. Statistical significance was determined by an unpaired t-test and no statistical differences between the two groups observed (p = 0.2653) (Figure 3.5). Therefore, in this cohort, haemoglobin concentration alone cannot be used to predict thrombosis in pancreatic cancer patients.

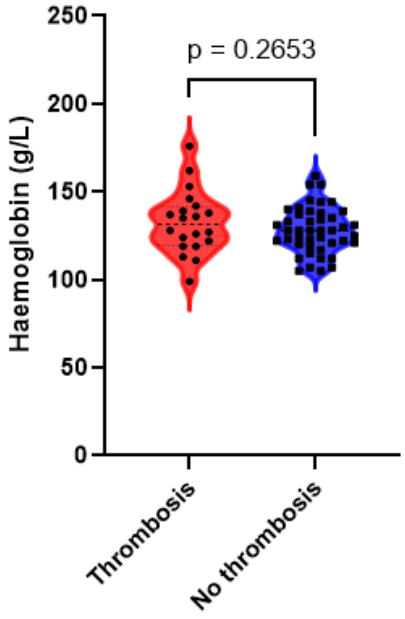


Figure 3.5. **No difference in haemoglobin concentration in pancreatic cancer patients who develop a thrombosis versus those who do not.** Haemoglobin concentration gathered for 64 patients with pancreatic cancer and compared between groups of patients with (n=20) and without (n = 44) thrombosis. Data presented as a Violin plot showing the thrombosis and no thrombosis populations within the audited group. Statistical analysis performed using an unpaired t-test. p = 0.2653.

3.5.1.5 White Blood Cell Count (WBC) is not predictive of thrombosis in pancreatic cancer patients

The final parameter which forms part of the Khorana Score is the leucocyte, or white blood cell, count. Whether white blood cell counts could be predictive of thrombosis in pancreatic cancer patients was therefore investigated. Within the audited population, the average (mean) WBC was 8.29 x 10^9/L, with a range of 4.08 to 21.85 10^9/L. Again, between the thrombosis and non-thrombosis populations there was little difference, with a mean of 8.88 x 10^9/L and 8.02 x 10^9/L respectively. Further statistical analysis by the Mann-Whitney test confirms no difference (p = 0.1668) in WBC between patients with thrombosis versus without. Therefore, WBC cannot be used to predict thrombosis in pancreatic cancer patients.

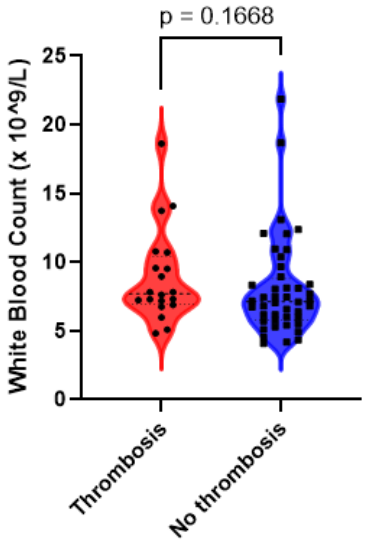


Figure 3.6. **No difference in white blood cell count (WBC) (Leucocyte) in pancreatic cancer patients who develop a thrombosis versus those who do not.** WBC gathered for 64 patients with pancreatic cancer and compared between groups of patients with (n=20) and without (n = 44) thrombosis. Data presented as a Violin plot showing the thrombosis and no thrombosis populations within the audited group. Statistical analysis performed using Mann-Whitney tests. p = 0.1668.

Overall analysis of the components of Khorana score shows that collectively, or individually, none are predictive of cancer-associated thrombosis within our audited population.

As a result, additional parameters and information gathered as part of the audit were examined in addition to see if any statistically significant parameters could be found.

3.5.1.6 Additional parameters

3.5.1.6.1 Neutrophil count is not predictive of thrombosis in pancreatic cancer patients

Neutrophils play an important part in the pathogenesis of thrombosis mainly through the generation and subsequent release of neutrophil extracellular traps, or NETs (Xu et al, 2022). The main role of NETs is to work to prevent widespread infection, by both trapping pathogens and undergoing programmed cell death, or apoptosis. In an inflammatory setting however, such as cancer and other inflammatory conditions, neutrophil activation occurs resulting in the formation of NETs outside of an infection.

Therefore, an increased neutrophil count, and the activation of neutrophils through an inflammatory process leading to the formation of NETs, could increase the likelihood of a thrombosis occurring.

The neutrophil count is not part of the Khorana Score, however with regards to a patient receiving chemotherapy they are closely monitored, to prevent neutropenic

sepsis, a potentially fatal complication if a patient is immunosuppressed because of chemotherapy administration.

Therefore, neutrophil counts were compared between the patients with thrombosis versus those without. The average (mean) neutrophil within the audited population was 5.42 x 10^9/L, with a range of between 1.26 and 17.20 x 10^9/L. A neutrophil count was unavailable for one patient, and so 63 sets of data were examined. Within the thrombosis population, the mean was 5.35 x 10^9/L, and in the no thrombosis population it was 5.49 x 10^9/L. Statistical analysis using the Mann-Whitney test revealed that there was no statistically significant difference between the two populations ($p = 0.7077$). This data is represented in Figure 3.7 below.

Therefore, with respect to neutrophil counts, there is no association between the neutrophil count and the risk of thrombosis, and the neutrophil count cannot be used to predict who will develop a thrombosis and who will not.

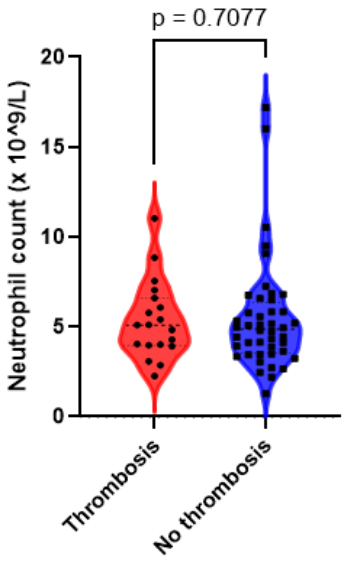


Figure 3.7. **No difference in neutrophil count in pancreatic cancer patients who develop a thrombosis versus those who do not.** Neutrophil count gathered for 63 patients with pancreatic cancer and compared between groups of patients with (n=19) and without (n = 44) thrombosis. Data presented as a Violin plot showing the thrombosis and no thrombosis populations within the audited group. Statistical analysis performed using Mann-Whitney tests. p = 0.7077.

3.5.2 Chemotherapy regimens cannot be used to predict thrombosis in pancreatic cancer patients

Some types of chemotherapy are known to increase an individual's thrombotic risk. These include gemcitabine (Verso et al, 2012), cisplatin or carboplatin-based drugs (Verso et al, 2012) and immunomodulatory drugs such as thalidomide and lenalidomide (De Stefano et al, 2022).

Therefore, chemotherapy regimens were also examined within the audited population to see if there were any associations with the type of chemotherapy (or

radiotherapy) received and the development of thrombosis in pancreatic cancer patients.

The most widely used regime was FOLFIRINOX (33 patients), which consists of folinic acid (leucovorin), fluorouracil, irinotecan and oxaliplatin. Oxaliplatin is in the same family of drugs as cisplatin or carboplatin drugs, and so is known to increase an individual's thrombotic risk (Verso et al, 2012). Derivatives from this regime include FOLFOX (no irinotecan) (8 patients), and FOLFIRI (no oxaliplatin) (2 patients). As shown in Table 3.2, the incidence of thrombosis for patients receiving these therapies was between 36.4-50% which compared to 31.3% of the total population.

Gemcitabine was also widely used (47 patients), which is an antimetabolite drug. This was used either alone (24 patients) or in combination with capecitabine (8 patients) or Abraxane (20 patients). As shown in Table 3.2, the incidence of thrombosis for patients receiving gemcitabine alone was 37.5%, in combination with capecitabine was 12.5%, and in combination with Abraxane was 40%, which compares to 31.3% of the total population.

The final treatment used in these patients was radiotherapy plus capecitabine (12 patients). As shown in Table 3.2, the incidence of thrombosis for patients receiving radiotherapy in combination with capecitabine was 54.5%.

One patient did not receive any chemotherapy, unfortunately they passed away before any chemotherapy administration. They did not develop a documented thrombosis.

The overall rate of thrombosis in the study was 31.3%. Several of the chemotherapy regimens have a higher thrombosis rate than the average – FOLFIRINOX, FOLFOX, FOLFIRI, gemcitabine only, gemcitabine plus Abraxane, and radiotherapy plus capecitabine. Only gemcitabine plus capecitabine (12.5%) gave a low thrombosis rate than the overall mean incidence for the study.

However, it is difficult to draw any conclusions from the data presented, as many of the audited population received more than one type of chemotherapy treatment, and therefore those patients who developed a thrombosis, but who received more than one line of treatment, are therefore counted more than once. In addition, all patients who received chemotherapy received drugs which are associated with a high risk of thrombosis (either gemcitabine and/or FOLFIRINOX or FOLFOX) (Verso et al, 2012).

Therefore, it is difficult to establish with respect to the date of the diagnosis of a thrombosis, how each chemotherapy may or may not have contributed to the thrombus formation, and the relative risk of each type of chemotherapy regime.

3.5.3 Should pancreatic cancer patients be prescribed thromboprophylaxis?

Within The Newcastle upon Tyne Hospitals Foundation NHS Trust, the current policy is to not routinely prescribe thromboprophylaxis to pancreatic cancer patients. However, eleven patients originally identified within the audit were receiving thromboprophylaxis at diagnosis, or shortly afterwards, prior to the start of systemic chemotherapy administration. The main patient characteristics of the 11 patients taking thromboprophylaxis are summarised in Table 3.3.

Patient characteristics are similar between the audited population, and those excluded due to thromboprophylaxis. However, as there are only eleven patients in the thromboprophylaxis group, not all chemotherapy regimens are represented, and it is therefore difficult to draw any conclusions from this. One patient in this group did not receive any chemotherapy, passing away before any was administered, but they attended pre-chemotherapy assessment and bloods were taken, so they are included in the table. The mean BMI in this population is interestingly lower than that of the audited population (22.90 versus 25.78) the reasons for this unknown, though the small population size is thought to be the main factor. However, the mean Khorana score is similar (2.36 versus 2.48). Three patients had a high-risk Khorana score (1 patient with a score of 4, and two with a score of 3), all other patients had an intermediate risk score of 2 points.

Of the 11 patients receiving thromboprophylaxis, 9.1% (1/11), developed a thrombosis within ten months of diagnosis. This is lower than the incidence of thrombosis observed in the 64 patients not taking thromboprophylaxis of 31.3% (20/64). In support of our earlier findings, the one patient that developed a thrombosis whilst on thromboprophylaxis had a Khorana Score of 3 which whilst considered high risk, is not dissimilar to the mean Khorana score of the other 10 patients that did not experience a thrombosis (mean Khorana score = 2.30), further demonstrating the Khorana score is not effective at predicting thrombotic risk in pancreatic cancer patients.

Number of patients	All	Without a thrombosis	With a thrombosis
	N = 11 (100%)	N = 10 (90.9%)	N = 1 (9.1%)
Sex	M = 8 (72.7%)	N = 7	N = 1
	F = 3 (27.3%)	N = 3	N = 0
Ethnicity *			
White British	N = 9 (81.8%)	N = 8	N = 1
Black British or African	N = 1 (9.1%)	N = 0	N = 0
Asian or Asian British Pakistani	N = 1 (9.1%)	N = 0	N = 0
Mean age (range):	67.3 years (30 to 84)	67.7 years (30 to 84)	63 years (N/A)
Male	66.9 years (30 to 84)	67.4 years (30 to 84)	63 years (N/A)
Female	68.3 years (62 to 76)	68.3 years (62 to 76)	N/A
BMI (range)	22.90 (19.2 to 26.7)	22.54 (19.2 to 26.7)	26.50 (N/A)
Mean Khorana Score (range)	2.36 (2 to 4)	2.30 (2 to 4)	3.00 (N/A)
Low risk (0 points)	N = 0 (0%)	N = 0 (0%)	N = 0 (0%)
Intermediate risk (1-2 points)	N = 8 (72.7%)	N = 8 (80%)	N = 0 (0%)
High risk (3 or more points)	N = 3 (27.3%)	N = 2 (20%)	N = 1 (100%)
Chemotherapy regimes $			
FOLFIRINOX	N = 5	N = 4 (80%)	N = 1 (20%)
FOLFOX	N = 0	N/A	N/A
FOLFIRI	N = 0	N/A	N/A
Gemcitabine, Abraxane	N = 3	N = 3 (100%)	N = 0 (0%)
Gemcitabine, capecitabine	N = 1	N = 1 (100%)	N = 0 (0%)
Gemcitabine only	N = 2	N = 2 (100%)	N = 0 (0%)
Radiotherapy plus capecitabine	N = 3	N = 2 (66.7%)	N = 1 (33.3%)

Table 3.3. **Summary of the clinical audit patient characteristics - those prescribed thromboprophylaxis only** * = as recorded in eRecord. $ = many patients received more than one line of treatment. N/A = Not Applicable.

3.6 Discussion

This clinical audit set out to evaluate whether the Khorana score could be used to predict thrombosis in patients with pancreatic cancer undergoing treatment at The Newcastle upon Tyne Hospitals NHS Foundation Trust.

3.6.1 Methodology

Seventy-five consecutive patients who were diagnosed with pancreatic cancer between July 2020 and July 2023 and treated at The Newcastle upon Tyne Hospitals NHS Foundation Trust were retrospectively audited to determine the rates of thrombosis. Eleven patients were excluded from the analysis due to receiving thromboprophylaxis at diagnosis.

For the remaining 64 patients, the Khorana score was calculated and each individual parameter of the Khorana score, as well as additional parameters, including the neutrophil count and the type of chemotherapy received, were examined to see if any could be used to predict cancer-associated thrombosis in this cohort of pancreatic cancer patients.

3.6.2 Use of the Khorana Score in pancreatic cancer patients

This retrospective audit has indicated that the Khorana score cannot be used in this population to predict cancer-associated thrombosis. There was no statistically significant difference between the Khorana scores between those who developed a thrombosis and those who did not in this audit of pancreatic cancer patients (Figure

3.1). Patients were also split by risk category, with more patients assigned an intermediate risk developing a thrombosis (35.1%) than those assigned into the higher risk category (25.9%).

This confirms findings from previous studies (Ruch et al 2012; Muñoz-Martin et al 2014; van Es et al, 2017; Kruger et al 2017) who also concluded that the Khorana score cannot predict the individuals who will develop a thrombosis in high-risk cancers, including pancreatic cancer.

Ruch et al (2012) audited 89 patients diagnosed with pancreatic cancer and followed these up for a median of 268 days (range 18 to 2433 days). The utility of both the Khorana score (Khorana et al, 2008) and the Vienna CATS score (Ay et al, 2010) were assessed to predict VTE in pancreatic cancer. A total of 22% of the patients studied developed a VTE in the study period, which is less than the figures seen in this audit. Within the Khorana score VTE risk categories, 20.8% of intermediate risk (scoring 1 to 2 points) patients developed a VTE, whilst 24.3% of high risk (scoring 3 or more points) patients developed a VTE. This is the opposite to this audit where higher rates of thrombosis were seen in the intermediate risk category. For the Vienna CATS score, whilst the patients were separated between the different risk categories, there was no difference in VTE incidence between the groups. Univariate analysis demonstrated that whilst neither the Khorana score nor the Vienna CATS score could accurately predict VTE in this population, a raised BMI and a low platelet count could.

This is in direct contrast with the Khorana and Vienna CATS scores where a high platelet count receives extra points in the prediction of VTE in cancer patients.

Muñoz-Martin et al (2014) audited 84 patients, all of whom were diagnosed with a pancreatic adenocarcinoma. 35.7% (30 patients) of the audited population developed a VTE, 66% of these were within the first 6 months after diagnosis. 33.3% of patients in the intermediate Khorana score (2 points) developed a VTE, whilst 37.5% in a high-risk category (3 or more points) did. Therefore, the study concluded that the Khorana score is not useful to discriminate between high and intermediate risk populations. This audit supports the findings from Muñoz-Martin et al in that the rate of thrombosis was similar, however only patients diagnosed with pancreatic adenocarcinoma were examined, though the cohort used was larger. Rates of thrombosis at 6 months were recorded, whilst this audit retrospectively examined up to a maximum of 10 months post-diagnosis. In addition, higher rates of thrombosis were seen in the intermediate risk category compared to the high-risk category in our audit.

Van Es et al (2017) followed 178 patients over a 2-year period. All were diagnosed with pancreatic cancer, and 12.4% (22 patients) developed either a DVT or PE. This work had a much lower rate of thrombosis in comparison to both this audit and that of the Muñoz-Martin study. The reasons for this are unclear but could be explained by limiting the types of thrombosis to only a DVT or PE. Again, Khorana scores were applied which showed that after a 2-year follow up period the incidence of a DVT or

PE was higher in the intermediate risk category (15.3%) (1-2 points) than the high-risk category (10.1%) (3 or more points), which agrees with our audit. However, the opposite is true after a follow up period of only 6 months. Therefore, the Khorana score was unable to discriminate between pancreatic cancer patients at intermediate and high risk of VTE. Similar to this audit, the authors suggest that a possible explanation for the poor performance of the Khorana score is the absence of the predictive value of severe obesity (manifesting in a high BMI), or the use of erythropoiesis- stimulating agents. In this audit, the same theory and suggestion can be applied as no patients received erythropoiesis-stimulating agents, and only two patients reached the threshold for an additional point for BMI in the Khorana score and therefore these parameters were rarely applied and therefore had a poor predictive value.

Kruger et al (2017) followed 172 patients, and retrospectively calculated the Khorana score, CONKO score (Pelzer et al, 2013) and the APTT (Activated Partial Thromboplastin Time) ratio for each patient who had received a pancreatic cancer diagnosis. Patients receiving thromboprophylaxis were excluded from the study. 41.3% (n = 71) of patients in the study developed a VTE, which is higher than the figure reported in this audit. The study found that each of the predictive scores (Khorana and CONKO) could not be used alone to predict VTE in pancreatic cancer, but if used alongside the APTT ratio it could be. An APTT ratio below the median was

an independent predictor for the future development of VTE. APTT ratios are not available for this audit and have therefore not been calculated.

In conclusion, the findings from this audit and previous studies suggest that the Khorana score cannot be used in very high-risk cancer patients such as those diagnosed with pancreatic cancer. The Khorana score is also unable to discriminate between those at an intermediate or higher risk of VTE.

These findings are likely to reflect that in the initial studies that were involved in the development and validation of the Khorana score very few patients (less than 2%; 19 patients) had been diagnosed with pancreatic cancer, with these patients underrepresented (Khorana et al, 2008; Ay et al, 2010; Munoz-Martin et al, 2014). It has been demonstrated that the score may be more clinically useful in other, lower-risk cancer types (Overvad et al, 2022).

3.6.3 The use of the individual component parts of the Khorana score to predict thrombosis

As the overall Khorana score cannot be used to predict a thrombosis in pancreatic cancer patients, as demonstrated both in our audit, and in previous studies, the utility of each individual component was investigated.

In this audit, none of the individual components were predictive of thrombosis.

A high BMI was examined individually, but not found to be predictive of thrombosis. A high BMI may result in conditions within the body which are favourable to the

formation of a thrombosis. This includes a chronic low-grade inflammatory state, immobilisation, and increased levels of fibrinogen, vWF and Factor VIII (Eichinger et al, 2008; Hotoleanu 2020). However, whilst this was not found to be predictive within this audit, only one patient reached the threshold for an extra point to be assigned by the Khorana score, therefore the true predictive value of this parameter was not tested in this audit.

Haemoglobin values, platelet counts, WBC count and neutrophil counts were also examined individually for their predictive value in this audit. None of these parameters were found to be predictive.

Platelets play a pivotal role in the formation of a thrombus, by playing a vital part in primary haemostasis and are the major component of a fibrin plug (Simanek et al, 2010; Warny et al, 2019). They also contribute by providing a negatively charged phospholipid surface on which coagulation reactions can take place (Moore et al, 2010). There is a known association between the risk of thrombosis and the myeloproliferative neoplasm Essential Thrombocythaemia (ET) where high platelet counts are found (Vannucchi et al, 2007; Mancuso et al, 2020). Therefore, a high platelet count combined with an inflammatory background would suggest it would be more likely that a thrombosis could be formed. However, this audit has shown that the platelet count cannot be used to predict thrombosis in pancreatic cancer patients.

White blood cells, and more specifically neutrophils, contribute to thrombus formation primarily through the action of NETs. Neutrophil extracellular traps (or NETs), and their component parts, can directly activate the contact (extrinsic) coagulation system, and activate platelets (Kapoor et al, 2018; Zhou et al, 2022). NETs promote the formation of a thrombus by several mechanisms. Intact NETs are not thought to directly activate coagulation, but their components do (Noubouossie et al, 2019). For example, free neutrophil DNA directly activates coagulation through the contact pathway, and free histones induce a procoagulant phenotype on blood and endothelial cells (Noubouossie et al, 2019). Intact NETs provide a scaffold for the deposition of platelets, erythrocytes, fibrinogen and von Willebrand Factor (vWF) (Zhou et al, 2022), leading to the close interaction of these components and the activation of coagulation pathways. In addition, NETs interact with fibronectin and vWF to promote platelet adhesion and activation (Zhou et al, 2022), the first stages of primary haemostasis. Kushnir et al (2016) demonstrated that a persistently high neutrophil count has been shown to be a marker for an increased risk of venous thrombosis, and so for this reason the parameter was chosen to be studied for this audit. Peng et al (2023) also reported an association between the risk of pulmonary embolism (PE) and the neutrophil count of patients upon admission to hospital. It is also readily available and forms part of the Full Blood Count, whose parameters also include Haemoglobin, Platelets and White Blood Count, all part of the Khorana Score.

However, again this audit has shown that neither the white blood count nor the neutrophil count can be used to predict thrombosis in pancreatic cancer patients.

These findings are both in agreement and at odds with those from the work of Pelzer et al (2013), who found that single parameters in the Khorana score can be used independently, and these demonstrated a higher risk towards thrombosis in pancreatic cancer patients, but these associations were not statistically significant. In this study no trends (significant or otherwise) were observed demonstrating higher risk. Pelzer et al (2013) suggested that the BMI could be replaced with a performance score measure, such as the Eastern Cooperative Oncology Group (ECOG) score, or the WHO performance status score (Table 1.2) in a cancer-associated thrombosis predictive model, specifically for pancreatic cancer. This is known as the CONKO score.

3.6.4 Chemotherapy regimens as predictive of cancer-associated thrombosis

Various chemotherapy regimens were used within the audited population, and most of the audited patients received more than one line of treatment. The intent of chemotherapy is to slow or stop the growth of the cancerous cells or tumour. Most chemotherapy agents exert this effect by interfering or disrupting the DNA in the nucleus of the tumour cells.

FOLFIRINOX consists of folinic acid (leucovorin), fluorouracil, irinotecan and oxaliplatin. FOLFIRINOX is licensed by NICE as a first-line treatment for unresectable metastatic pancreatic cancer (NICE, 2018). Fluorouracil is an antimetabolite, which disrupts the ability of the cancer cells to make DNA and proteins (National Cancer Institute, 2024). Folinic acid is added to enhance its effect. Irinotecan is a topoisomerase I inhibitor (National Cancer Institute, 2024) which stops the growth of the cancer cells, and oxaliplatin is a platinum-based drug, which causes DNA damage. Derivatives of this regime include FOLFOX (no irinotecan) and FOLFIRI (no oxaliplatin).

Gemcitabine is also an antimetabolite and interferes with DNA synthesis (National Cancer Institute, 2024). Within the audited population it was either used alone, in combination with capecitabine (which is converted to fluorouracil in the circulation), or with Abraxane (blocks the action of microtubules, thus preventing the formation of new cells).

Finally, radiotherapy works by damaging DNA either by killing or slowing the growth of cancer cells.

Within the types of chemotherapy used, both gemcitabine and platinum-based drugs such as oxaliplatin are considered high-risk with regards to thrombosis incidence, and form part of the PROTECHT score (Verso et al, 2012). The PROTECHT score is an alternative cancer-associated thrombosis predictive model, where the patients' chemotherapy regime is included. An additional point is given to both

gemcitabine and cisplatin or carboplatin-based drugs such as oxaliplatin. All the 64 patients in this audit received either or both drugs, as they are the standard of care in pancreatic cancer treatment (NICE, 2018). It is therefore unlikely that the type of chemotherapy is confounding our observations, however the treatments which patients receive may also help to explain why such high rates of thrombosis are seen in pancreatic cancer, as compared to other cancer types.

Within this audit it is difficult to establish what role the chemotherapy regimens played in the development of thrombosis, if any. All the audited population received at least one of the two designated (by the PROTECHT score) high-risk drugs. Only one patient received no chemotherapy, passing away before any was administered. It is difficult to come to any conclusions with regards to the timings of the administration of the chemotherapy and the thrombosis diagnosis.

Guman et al (2021) compared the Khorana, PROTECHT and 5-SNP scores for the prediction of thrombosis in all cancer types. The 5-SNP score is not discussed further within this audit, but it involves assigning additional points for patients with specific genetic polymorphisms, such as Factor V Leiden, which are associated with an increased risk of thrombosis. This retrospective audit looked at 2729 patients, of whom 60 (or 2.2%) were diagnosed with pancreatic cancer. 160 (5.9%) of the total numbers of patients developed a VTE. However, only 63.2% of the total audited patients had all three predictive scores calculated due to a lack of data. The authors concluded however that the PROTECHT and 5-SNP scores were not superior to the

Khorana score for the prediction of cancer-associated thrombosis. They did however, state that some of the components, namely; WBC greater than 11 x 10^9/L, Haemoglobin < 100 g/L and the use of cisplatin or carboplatin-based drugs were significantly associated with the 6-month risk of VTE. In addition, they also stated that the overall performance of the clinical risk scores decreases over time, which is explained by the dynamic nature of laboratory variables, a patients' BMI throughout treatment, and the type of chemotherapy they are receiving. These findings indicate that the PROTECHT score (and Khorana score) are changing as lines of treatment are added, laboratory parameters change, and a patients' BMI alters and are therefore not static and as a result the risk of thrombosis is also constantly changing.

In conclusion, further research is needed in this area to further investigate the utility of alternative biomarkers and predictive scores exclusively in pancreatic cancer patients.

3.6.5 The use of thromboprophylaxis in pancreatic cancer patients

Historically, there has been a reluctance to prescribe thromboprophylaxis to every pancreatic cancer patient due to the perceived high risk of bleeding associated with these patients. Within the Newcastle upon Tyne Hospitals NHS Trust this is also the case, with a reluctance to change procedure. The aim of this audit is to alter this perception.

Eleven patients were identified during this audit that were receiving thromboprophylaxis at diagnosis, or shortly afterwards, before the start of systemic chemotherapy administration. The reasons for this are if the patient has a pre-existing medical condition, for example atrial fibrillation, or they were considered at a higher risk of developing a thrombosis due to lifestyle factors, a prior history of VTE, or family history of VTE.

Of the eleven patients on prescribed thromboprophylaxis, the incidence of thrombosis was 9.1% (1/11) with only one patient developing a thrombosis despite the thromboprophylaxis. This is lower that the incidence of thrombosis observed in the cohort of 64 patients not on thromboprophylaxis (31.3% 20/64). This patient had a Khorana score of 3 and was therefore designated at a high risk of thrombosis. A further patient developed minor bleeding, and was removed from the thromboprophylaxis, but then subsequently developed a SMV (superior mesenteric vein) thrombosis. Demonstrating that thromboprophylaxis can reduce thrombosis but needs to be carefully managed.

Two randomised control trials (RCTs) studied the effect of routine thromboprophylaxis (high dose LMWH) on the thrombosis rate in the pancreatic cancer population.

The CONKO 004 trial (Reiss et al, 2010) examined administering enoxaparin (another LMWH) at a high dose for 3 months, followed by a lower dose for the following three months. Lower rates of VTE were seen at both 3 months (10.6% in the control arm vs

1.3% in the enoxaparin arm) and at 12 months (15% vs 5%). In both arms there were incidences of severe bleeding suggesting that this would have taken place regardless of the thromboprophylaxis.

The FRAGEM trial (Maravegas et al, 2012) studied advanced pancreatic cancer patients receiving gemcitabine alone versus with a full dose of dalteparin (a Low Molecular Weight Heparin (LMWH)). Those receiving dalteparin had an 85% risk reduction of VTE compared to those who did not in the 12-week treatment period. There was also a 58% risk reduction in the incidence of VTE during the follow up period of 1 year.

The AVERT and CASSINI clinical trials further examined the use of thromboprophylaxis in ambulatory cancer patients looking specifically at apixaban (AVERT) and rivaroxaban (CASSINI) and helped to inform guidelines (Maravegas 2020). Although pancreatic cancer patients were not specifically examined these clinical trials helped to shape practice, particularly with regards to high-risk cancers and the use of an oral anticoagulant which requires no laboratory monitoring.

A Cochrane review of the many clinical trials, initially published in 2012, but most recently updated in 2020 (Rutjes et al, 2020) has also helped to inform practice and influence the contents of several international guidelines, which all suggest that routine thromboprophylaxis should be considered in the pancreatic cancer population.

Taken together, these studies and the findings of this audit, highlight the importance of thromboprophylaxis in this population of patients.

The American Society for Haematology (ASH) (Lyman et al, 2021) does not specifically state any guidance regarding pancreatic cancer patients. However, patients at an intermediate risk are suggested to not receive thromboprophylaxis, whereas those at a high risk it is suggested should receive thromboprophylaxis. Risk should be assigned using a validated risk assessment tool such as the Khorana score. This aligns with advice from The American Society for Clinical Oncology (ASCO) (Key et al, 2023) suggests that any high-risk patient (defined as any patient with a Khorana score greater than 2 and therefore all pancreatic cancer patients) should be offered thromboprophylaxis before starting systemic chemotherapy with apixaban, rivaroxaban or LMWH providing there are no significant risk factors for bleeding or drug interactions.

Similar to the US, The European Society of Cardiology (ESC) (Lyon et al, 2022) recommend that 'ambulatory patients with cancer at high risk of thrombosis receiving systemic therapy primary thromboprophylaxis with a NOAC (apixaban or rivaroxaban) or LMWH may be considered, provided there are no significant contraindications' and The European Society for Medical Oncology (ESMO) (Falanga et al, 2023) state that validated risk-assessment models, such as the Khorana score, Vienna CATS score, or COMPASS-CAT score, should be used to assign a risk to

patients. Ambulatory pancreatic cancer patients on first-line systemic anti-cancer treatment LMWH at a higher dose for a maximum of 3 months should be considered.

These international guidelines are also mirrored in UK guidelines, published by NICE (National Institute for Health and Care Excellence) in 2019, which states that in pancreatic cancer patients receiving chemotherapy, the use of pharmacological VTE prophylaxis with LMWH (Low Molecular Weight Heparin) should be considered. The British Society for Haematology (BSH) recently updated its guidance on cancer-associated venous thrombosis (Alikhan et al, 2024) and states that "Pancreatic cancer patients, receiving SACT, should be offered pharmacological thromboprophylaxis with an anticoagulant in line with the dosage used in clinical trials".

Taken together, this clinical audit and previous studies support current national and international guidelines suggesting that thromboprophylaxis should be prescribed to all pancreatic cancer patients at diagnosis and before the start of systemic chemotherapy. It is hoped that as a results of this audit and review of current guidelines that practice within The Newcastle upon Tyne Hospitals NHS Foundation Trust may be altered to reflect these guidelines and to decrease the risk of VTE in this high-risk population.

3.6.6 Questions which remain to be answered, and the potential use of alternative biomarkers or risk assessment scores to predict cancer-associated thrombosis in pancreatic cancer

There is still a need for more effective biomarkers or risk assessment scores to predict cancer-associated thrombosis in pancreatic cancer.

In this study use of both the PROTECHT and Khorana scores have been evaluated as approaches to predict the risk of thrombosis in pancreatic cancer patients. In agreement with other previous studies, neither are effective at predicting those individuals at high risk of thrombotic events. Alternative risk assessment scores have been developed. These include the CONKO score (Pelzer at al, 2013) which uses the performance score (a measure of how active a patient is and their ability to care for themselves) of a patient as opposed to the BMI, and the Vienna CATS score (Ay et al, 2010), which adds two biomarkers (D-dimer and soluble P-selectin) to the Khorana score. However, as with the Khorana score only a small number of pancreatic cancer patients (47 out of 819, or 5.7%) were used to validate the Vienna CATS score. There have been no further independent studies to validate the Vienna CATS score in pancreatic cancer alone. D-dimer, whilst a routine test within The Newcastle upon Tyne Hospitals NHS Foundation Trust, was not routinely performed pre-chemotherapy in the audited population and therefore results are not available for analysis in this audit. Soluble P-selectin is not performed within the Trust and was therefore not available for additional analysis.

In this study, the individual components of the Khorana score were not found to be predictive of thrombosis in pancreatic cancer, however, perhaps more mechanistically relevant or more specific markers could be? For example, instead of BMI, another marker or obesity and poor dietary health could be used, for example, cholesterol. Even though neither a platelet count nor a high neutrophil/ white blood cell count was predictive, we know that once activated these cells contribute the thrombosis pathology. Therefore, could markers of both platelet and neutrophil activation be used as an alternative? Examples of platelet activation markers include P-selectin, platelet microparticles and CD40L (Kannan et al, 2019). Soluble P-selectin has been demonstrated to be linked to the development and prediction of cancer-associated thrombosis and forms part of the Vienna CATS score (Ay et al, 2008; Ay et al, 2010), an alternative predictive model. Neutrophil activation markers include the presence of cell-free DNA (cfDNA), neutrophil elastase and citrullinated Histone H3 (H3cit) (Boettcher et al, 2020). Mauracher et al (2018) showed that citrullinated histone H3 can predict venous thromboembolism in cancer patients, though it does not form part of any predictive scores. Markers of both platelet and neutrophil activation are not routinely measured in UK laboratories and so would therefore be difficult to assay routinely in pancreatic (or other types) cancer patients.

There are also no markers of endothelial activation, for example vascular endothelial growth factor (VEGF), a marker of endothelial activation is not included within any predictive model but may be mechanistically relevant.

The potential utility of other biomarkers, including both serum and plasma VEGF will be addressed in the following chapters of this thesis.

3.6.7 Limitations of this audit

As with any audit, there were limitations associated with this investigation. The Newcastle upon Tyne Hospitals NHS Foundation Trust acts as the tertiary centre for the North East and North Cumbria region for oncology. Therefore, many of the audited population were not local to Newcastle upon Tyne. The electronic health records system only records encounters with the patient occurring within The Newcastle upon Tyne Hospitals NHS Foundation Trust, and not those occurring in others. Encounters occurring in neighbouring hospitals are occasionally documented during a clinic visit, but not on every occasion. Therefore, as a result, it is possible that some thrombosis diagnoses may have been missed, and the incidence of thrombotic events in this patient cohort may have been higher.

By the same token, the blood results closest in time to the start of chemotherapy were recorded. It is possible that a neighbouring NHS Trust may have blood results closer to the start of chemotherapy date. Results after the commencement of chemotherapy should not be used as chemotherapy induces changes in the blood and thus would potentially affect the Khorana scores calculated.

Follow up periods also varied. Every patient was monitored for a minimum of ten months, or until their death, whichever was soonest. However, patients can develop a thrombosis several years after the start of their chemotherapy.

Only 75 patients were examined, eleven of which were excluded due to the use of thromboprophylaxis, and in one NHS Trust in England. The catchment area of this area is one of the most deprived areas in England with one of the country's weakest and most deprived regional economies (Bennet Institute for Public Policy, 2019; Office for National Statistics, 2021). With regards to ethnicity not all the local population were represented. According to the 2021 National Census, 91.7% of the population are white, in this audit 90.5% were white. However, the remainder of the audit population were recorded as "Not stated" or "Not known". There were no individuals from an Asian or Black background within this audit, though there were one of each in the excluded thromboprophylaxis population. Gender was represented equally, but there was a skew towards an older population, however this reflects the age at which individuals are diagnosed with cancer. Therefore, the results from this audit may not be reflected elsewhere however they provide a good snapshot to see how practice may be changed throughout the United Kingdom.

3.7 Conclusions

This audit has demonstrated that the Khorana score and its individual components in isolation cannot be used to predict thrombosis in pancreatic cancer patients. This

supports findings from previous studies and suggests that an alternative predictive score or biomarker is required for pancreatic cancer patients, and likely other high-risk patients. This will be explored in the following chapters.

Pancreatic cancer is designated a very high-risk cancer with regards to the incidence of thrombosis, and this was confirmed in this audit with 31.3% of the patients not taking thromboprophylaxis experiencing a thrombosis within ten months of diagnosis. In comparison the incidence of thrombosis was reduced to 9.1% in a small subset of patients receiving thromboprophylaxis. Whilst national and international guidelines suggest that routine thromboprophylaxis should be considered for all pancreatic cancer patients at diagnosis and before the start of systemic chemotherapy. The findings of this audit indicate that The Newcastle upon Tyne Hospitals NHS Foundation Trust should consider changing its current practice to prescribe routine thromboprophylaxis.

An alternative predictive score for pancreatic cancer patients could include additional biomarkers, and this is something which will be explored in this thesis.

3.8 Chapter Summary

Overall, this retrospective audit has demonstrated that one predictive model, the Khorana score, does not predict CAT in a group of patients with a pancreatic cancer diagnosis. Published literature suggests that alternative risk assessment models may also not improve the predictive ability, however these were not examined in this

audit which would have been of interest. The results of this small audit suggest that alternative risk assessment models should be sought which will improve the prediction of CAT, this may include the addition of alternative biomarkers, or the derivation of risk assessment models which are dependent on primary cancer type. There is evidence that specific cancer-type risk assessment models may behave better in specific cancer-type populations, and it is the authors' opinion that these specific models should also be explored for pancreatic cancer.

CHAPTER 4. – RESULTS – META-ANALYSIS

Vascular Endothelial Growth Factor (VEGF) as a biomarker for Cancer Associated Thrombosis: A Meta-analysis.

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Please see Appendix 6 for a screenshot of the PDF of the published article.

4.1 Introduction

Cancer-associated thrombosis (CAT) affects up to 20% of patients with cancer and is associated with a poorer prognosis (Dogan and Demirkazik, 2005; Chew et al, 2006; Van Es et al, 2017). The use of thromboprophylaxis has been shown to reduce the risk of thrombosis but also increases the risk of bleeding (Lyman et al, 2021), which complicates the clinical picture and does not allow routine thromboprophylaxis to be given to all ambulatory patients with cancer (Watson et al, 2015).

Clinicians need to target the use of thromboprophylaxis and offer it to those at highest risk of thrombosis. A way of predicting those who are a higher risk of developing a thrombosis, has been a long sought-after clinical decision-making tool.

To address this, numerous risk assessment scores (RAMs) have been proposed, some of which use circulating levels of biomarkers at the time of diagnosis of the cancer, before the start of any chemotherapeutic regimes. The most validated is the Khorana score (Khorana et al, 2008) which uses the major parameters of a full blood count - haemoglobin, white cell count and platelets, along with patient factors such as cancer site and Body Mass Index (BMI), to determine the likelihood of a thrombosis occurring. The Vienna CATS score (Ay et al, 2010) goes further and has added two additional biomarkers – soluble P-selectin (sP-selectin) and D-dimers, to predict those individuals at a greater risk of thrombosis.

However, whilst these prediction scores demonstrate a strong association with VTE, in that those assigned to a high-risk category are more likely to develop a thrombosis, these scores can identify only a proportion of all individuals who will develop a thrombosis (Van Es et al, 2017), and the majority of individuals who do develop a thrombosis are outside of this high-risk category. Published RAMs also have limited discriminatory power (Moik et al, 2020). 90% of patients who are in either the intermediate or high-risk categories based on the Khorana score do not develop a thrombosis after 6 months (Moik et al, 2020). Therefore, these risk assessment scores need to be improved to truly distinguish the patients who are a

higher risk of developing a thrombosis, and who would benefit from receiving thromboprophylaxis.

Vascular Endothelial Growth Factor (VEGF or VEGF-A) is a potent angiogenic factor (Li et al, 2004) that is also thought to promote thrombosis. Angiogenesis, the formation of new blood vessels, is essential for the growth, invasion, progression, and metastasis of tumour tissue (Dogan et al, 2006). As a result, VEGF has been shown to be overexpressed in breast, colorectal, lung, pancreatic, ovarian, and cervical cancers (Dogan and Demirkazik, 2005; Dogan et al, 2006).

In health and disease, VEGF is expressed on the surface of many different cell types, including monocytes, endothelial cells, lymphocytes, and granulocytes (Dogan and Demirkazik, 2005; Posch et al, 2016), but it is thought that VEGF levels on these cells are higher in cancer than in healthy individuals (Salven et al, 1999). VEGF is stored in platelets, within their alpha granules, and within the Weibel-Palade bodies of endothelial cells (Dogan and Demirkazik, 2005). In cancer, both radiotherapy and chemotherapy have been shown to increase VEGF within tumours (Wang et al, 2020).

Despite its association with both cancer and thrombosis, the predictive value of VEGF, in cancer-associated thrombosis events, is less well defined.

Therefore, we sought to investigate the predictive potential of VEGF in cancer-associated thrombosis by conducting a meta-analysis of the previously published data.

4.2 Methods

4.2.1 Search strategy and eligibility criteria

This meta-analysis complies with the standard of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Page et al, 2021).

A literature search was performed using two databases: PubMed and OVID until 9th July 2023. To represent recent research, only papers published after the year 2000 were included. One paper (Musolino et al, 2002) was found by examining the references of another paper. Using the same format, two authors (for publication) refined the literature search and evaluated the quality of the studies independently.

Keywords included: “cancer”, “thrombosis” and “VEGF”. The following search terms were also used: (“cancer” OR “neoplasms”) AND (“VEGF” OR “vascular endothelial growth factor” OR “vascular endothelial growth factors” [Mesh Major Topic] OR “vascular permeability factor” OR “biomarkers/ analysis” [Mesh] OR “biomarkers/ blood” [Mesh]) AND “thrombosis” OR “vte” OR "Thrombosis/blood"[Mesh] OR "Thrombosis/complications"[Mesh] OR "Thrombosis/diagnosis"[Mesh] OR "Thrombosis/epidemiology"[Mesh] OR "Thrombosis/etiology"[Mesh] OR "Thrombosis/immunology"[Mesh] OR "Thrombosis/pathology"[Mesh]).

Inclusion criteria: 1) patients with cancer studied, 2) studies reported either plasma or serum VEGF levels in patients with cancer in both those with a thrombosis and those without quantitatively, 3) VEGF measured before or during the thrombotic event, 4)

adults over the age of 18 studied, 5) full text available, and 6) studies written in English.

Exclusion criteria: 1) Paediatric population studied, 2) review article, case report or conference abstract, 3) Cell lines and not patients studied, 4) full text not available, 5) not written in English, 6) study did not have figures for thrombosis and no thrombosis and 7) subjects studied were not humans.

Both venous and arterial thrombosis were included as were unusual site thrombosis including portal vein thrombosis. The references of relevant studies and review articles were also studied and checked for relevance to identify additional studies. The searches were validated for publication by two additional authors.

4.2.2 Data extraction and quality assessment

Following the inclusion and exclusion criteria above, and data selection, studies were further examined for suitability. All VEGF values were converted to pg/mL irrespective of the values used originally in the study to allow an easier comparison between them. Two studies (Kirwan et al, 2008; Kirwan et al, 2009) quoted VEGF values as µg/mL, representing a 10^6 difference between these results, and other comparable studies. Attempts were made to verify these values. As the values given were comparable to those which were pg/mL and based on the sensitivity and range of the ELISA assay used (9 pg/mL), these values were subsequently assumed to be pg/mL and are represented as such.

Studies where thrombosis had already occurred at the sampling point were also included. All studies measured VEGF by an ELISA (Enzyme Linked Immunosorbant Assay) method. Further details of the studies included within this meta-analysis and their design are shown in Table 4.1.

In instances where research papers contained qualitative findings and no comparable quantitative data, the studies were included in a qualitative manner.

Quality assessment of the included studies was performed using the Newcastle-Ottawa score (NOS) (Wells et al, 2021). The Agency for Healthcare Research and Quality (AHRQ)'s 11-item criteria were used to evaluate each of the studies. A score of 6 or more was considered to indicate good quality.

4.2.3 Statistical analysis

The association of VEGF with cancer associated thrombosis was evaluated by calculating the mean and SD values for plasma and serum VEGF levels for each study. Therefore, in this meta-analysis, studies looking at plasma and serum levels of VEGF have been separated into different forest plots to allow easier comparisons to be drawn. Currently, there is no consensus on which is the better VEGF parameter to measure.

Meta analysis of the mean difference for random effects was performed using Rev Man software. Random effects as opposed to fixed effects was used due to high heterogeneity between included studies. Heterogeneity between the included

studies was tested using the Rev Man software and I^2 values. We chose to set statistical significance at $p = <0.05$.

The risk of bias for this meta-analysis was assessed using the ROB-ME tool (Risk Of Bias due to Missing Evidence in a meta-analysis) (Page et al, 2023). This tool identified that there was a low risk of bias with this meta-analysis.

Study (year published)	Geographical location of study	Study design	Total number of participants	Age in years (range) (Mean or Median)	Sex	Cancer type (s) and stage	Type of thrombosis	Control group?	Newcastle-Ottawa Quality Assessment Score	VEGF biomarker measured
Cacciola et al (2002)	Italy	Retrospective case-control	19	63.11 +/- 15.69 (mean)	M = 13 F = 6	Polycythaemia vera, all stages	All	10 healthy controls	8	Serum VEGF
Dogan et al (2006)	Turkey	Prospective cohort	31	56.74 +/- 16.06 (mean)	M = 13 F = 18	All types and stages	Venous	51 matched pairs (all had cancer)	7	Serum VEGF
Kim et al (2004)	Korea	Prospective cohort	52	57 (35-80) (median)	M = 39 F = 13	Hepatocellular carcinoma (HCC), all stages	Portal vein	30 healthy, 26 liver cirrhosis	9	Serum VEGF, and serum VEGF per platelet count
Kirwan et al (2008)	United Kingdom	Prospective cohort	123	52 (31-78) (median)	M = 0 F = 123	Breast, early and advanced stages	Venous	68 healthy controls	9	Plasma VEGF
Kirwan et al (2) (2009)	United Kingdom	Prospective cohort	123	52 (31-78) (median)	M = 0 F = 123	Breast, early and advanced stages	Venous	68 healthy controls	9	Plasma VEGF, serum VEGF and platelet release of VEGF
Li at al (2004)*	China	Prospective cohort	45	50 (29-77) (mean)	M = 37 F = 8	Hepatocellular carcinoma (HCC), all stages	Portal vein	17 healthy, 20 benign liver lesions	9	Plasma VEGF
Malaponte et al (2015)	Italy	Retrospective case-control	385	62 +/- 9 (mean) no DVT. 64 +/- 10 (mean) with DVT	M = 185 F = 200	All types and stages	DVT only	100 healthy controls	7	Plasma VEGF
Musolino et al (2002)*	Italy	Retrospective cohort	55	60 (median)	M = 17 F = 38	Myeloproliferative neoplasms	All	20 healthy	4	Plasma VEGF
Nazari et al (2019)*	Austria	Prospective cohort	76	54 (46-67) (median)	M = 41 F = 35	Glioma	Venous	No	7	Unclear if plasma or serum VEGF
Posch et al (2016)	Austria	Prospective cohort	804	63.1 (54.2 – 69.2) (median)	M = 371 F = 433	All types and stages	Venous	No	7	Plasma VEGF
Ramadan et al (2021)	Egypt	Prospective cohort	87			Hepatocellular carcinoma (HCC), all stages	Portal vein	No	7	Serum VEGF

Table 4.1. **Summary of the study designs included in meta-analysis** (*denotes not included in forest plots due to lack of availability of data)

4.3 Results

4.3.1 PRISMA protocol

801 records were identified through screening of two databases; PubMed and OVID. After duplicates were removed, 556 papers remained. Review of the paper title and abstract, reduced the number of papers to 33. For these remaining papers the full text was accessed and assessed for eligibility. Once the inclusion and exclusion criteria were applied, 11 records remained. Of the remaining papers, only eight of these could be included in the meta-analysis due to the lack of data (Figure 4.1). The remaining three are still included in the meta-analysis but qualitatively rather than quantitatively. This is due to the raw data either not being available, (Nazari et al (2019)), presented in a different format which did not allow inclusion in the forest plots (a median value only was provided by Li et al (2004), and Musolino et al (2002) did not present the figures for thrombosis and no thrombosis as two separate populations. Attempts were made to contact the authors where data was missing, though in two cases the paper was published 20 and 22 years ago respectively.

The main characteristics of the eight papers used for the meta-analysis, plus the three used qualitatively are summarised in Table 4.1.

4.3.2 Patient characteristics

The overall population included in the meta-analysis consisted of 1547 participants, 221 of which were patients with cancer who were affected by thrombosis. The

remaining 1326 were patients with cancer who were not affected by thrombosis, representing a 14% rate of cancer-associated thrombosis in the study population. This figure agrees with the widely reported rates of cancer-associated thrombosis (Dogan and Demirkazik, 2005; Chew et al, 2006; Van Es et al 2014). In some cases, the nature of the thrombosis was recorded, but in others it was not.

All types of cancer, and all stages of the disease were represented in the data studied. Of the eight papers with quantitative data, three examined thrombosis in all patients, regardless of primary cancer site, whereas the work of Kirwan et al (2008, 2009) studied exclusively breast cancer. Ramadan et al (2021) and Kim et al (2004) studied hepatocellular carcinoma (HCC) patients, and Cacciola et al (2002) studied patients diagnosed with polycythaemia vera (PV, a haematological malignancy). The eight studies represent a wide geographical area (Table 4.1) and the median age of participants across the eight studies was 62 years.

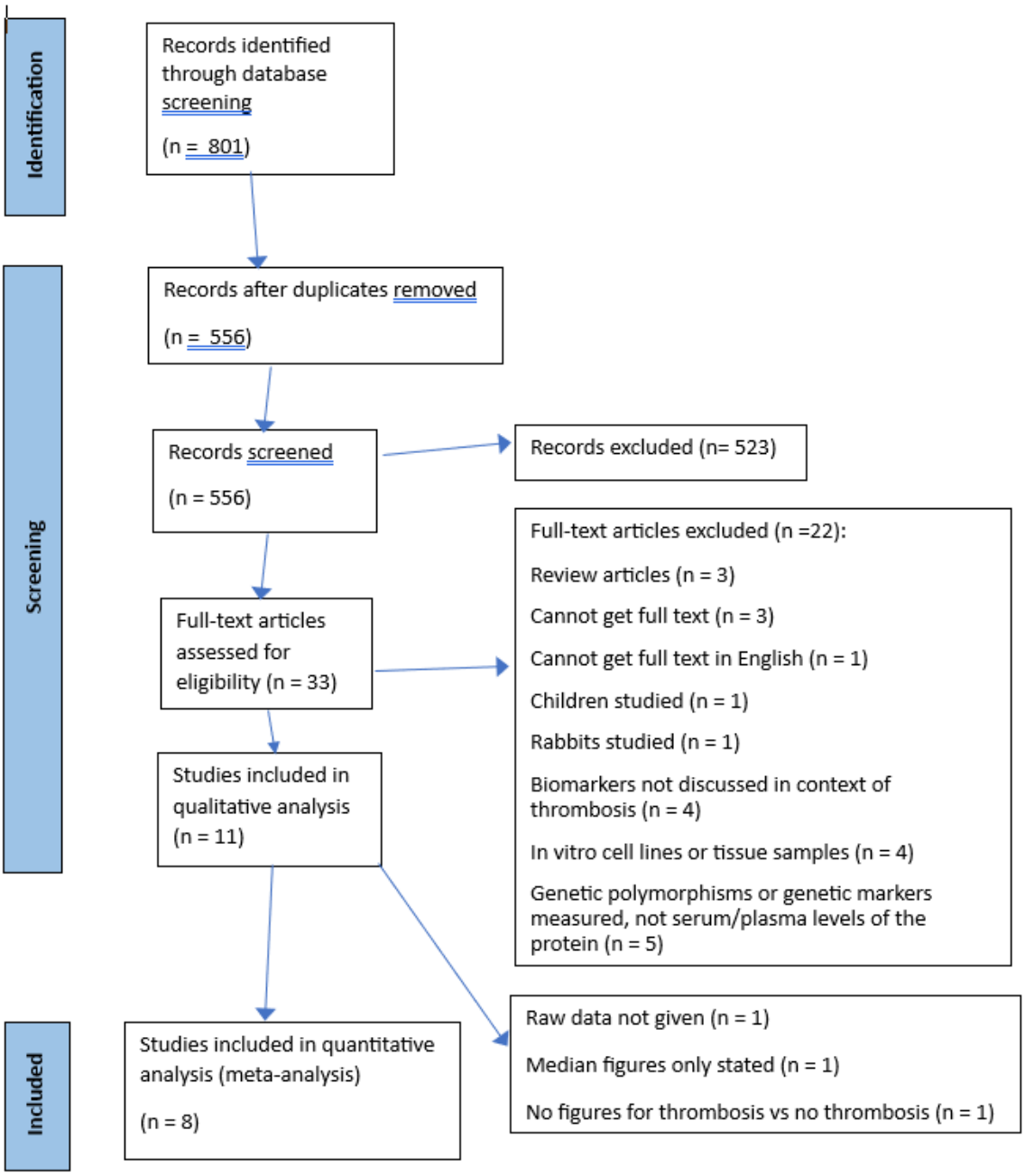

Figure 4.1. **Flow diagram of the inclusion and exclusion procedures.** PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses

4.3.3 Quality assessment and risk of bias

Quality assessment of the eleven included studies was performed using the NOS scale (Wells et al, 2021). Ten of the eleven studies were assessed to have scores greater than 6 and therefore of good quality, with the remaining study (Musolino et al, 2002) considered to be of moderate quality (score of 4).

4.3.4 Meta analysis of VEGF levels on thrombotic events in cancer

VEGF levels at the time of thrombosis are increased in patients with cancer

Five studies, with 625 patients (154 with thrombosis), assessed VEGF levels at the time of the thrombotic event, four analysing serum VEGF levels (Cacciola (2002), Dogan et al (2006), Kim et al (2004), Ramadan et al (2021)), and one study analysed plasma VEGF levels (Malaponte et al (2015)). Analysis of the five studies, identified significantly higher levels of VEGF in patients with thrombosis versus those patients without (mean difference 184.01 pg/mL, 95% CI; 85.45-282.58, p = 0.0003) (Figure 4.2). Heterogeneity was assessed with a I^2 value of 91%. All five papers demonstrated that VEGF significantly rises at the time of a thrombotic event. Taken together this indicates a positive association of VEGF levels with thrombosis in cancer patients and identifies VEGF as a marker of cancer-associated thrombosis, where a thrombosis has already occurred.

These findings are further supported by the work of Musolino et al (2002) who showed that increased plasma VEGF levels were seen in patients with

myeloproliferative neoplasms who had had a thrombotic event within the preceding month, and by the work of Li et al (2004) who also showed that the presence of portal vein thrombosis in patients with hepatocellular carcinoma was associated with a higher plasma VEGF level.

Taken together these findings indicate a positive association of VEGF levels with thrombosis in cancer patients and identifies increased VEGF as a marker of cancer-associated thrombosis at the time of thrombosis.

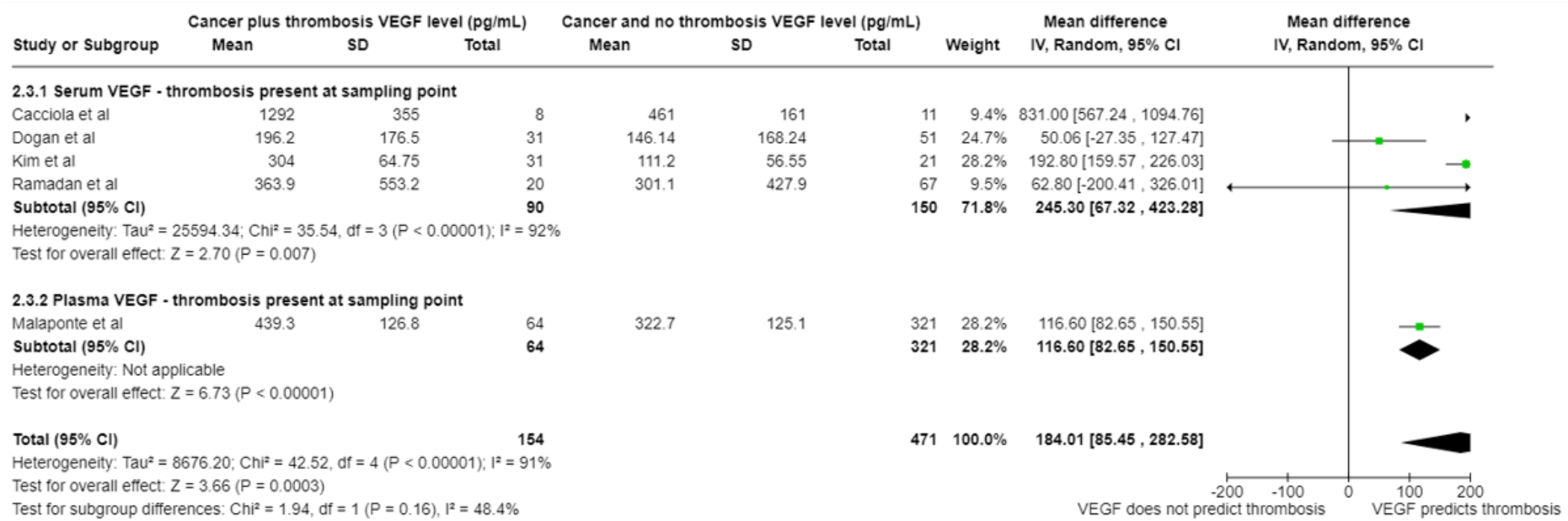

Figure 4.2. **Forest plot for VEGF levels among cancer-associated thrombosis and patients with cancer and no thrombosis.** Figure generated using RevMan software (https://revman.cochrane.org). p = 0.0003 overall, suggesting that there is a statistically significant difference between those who did and did not experience a thrombosis. VEGF levels analysed at the point of thrombosis. Statistical significance set at p = <0.05.

VEGF levels prior to a thrombotic event are not associated with cancer-induced thrombosis

Having identified an association of VEGF levels with thrombosis post thrombotic event, we next analysed the three remaining studies, where VEGF was measured prior to a thrombotic event occurring, to determine whether VEGF could be used as a predictive biomarker of thrombosis. Three studies including 922 participants examined the role of VEGF as a predictor of thrombosis (serum VEGF; (Kirwan et al (2009), plasma VEGF; (Kirwan et al (2008) and (2009) – data only included once and Posch et al (2016)). The 3-month cumulative incidence of VTE in the Kirwan et al studies population was 9.8%, whilst the 6-month cumulative incidence in the Posch et al study population was 5.0%. Analysis of data from these studies show that whilst pre-event plasma VEGF or serum VEGF levels are higher in patients that go on to experience CAT there is no significant difference in VEGF levels between patients who develop thrombosis versus those who do not (mean difference 11.68 pg/mL, 95% CI; -2.39 – 25.73, p=0.10 (Figure 4.3). Heterogeneity was assessed, giving an I^2 value of 0%, this is possibly due to the papers included.

These findings are further supported by the work of Nazari et al (2019), which also showed no association of serum VEGF levels and the prediction of VTE in patients with glioma (brain cancer) (Hazard ratio per double increase: 0.995, 95% CI 0.640 – 1.548, p = 0.983).

Taken together these observations indicate that whilst VEGF levels are increased in cancer patients at the time of thrombosis (Figure 4.2) VEGF levels in cancer patients are not predictive of thrombosis.

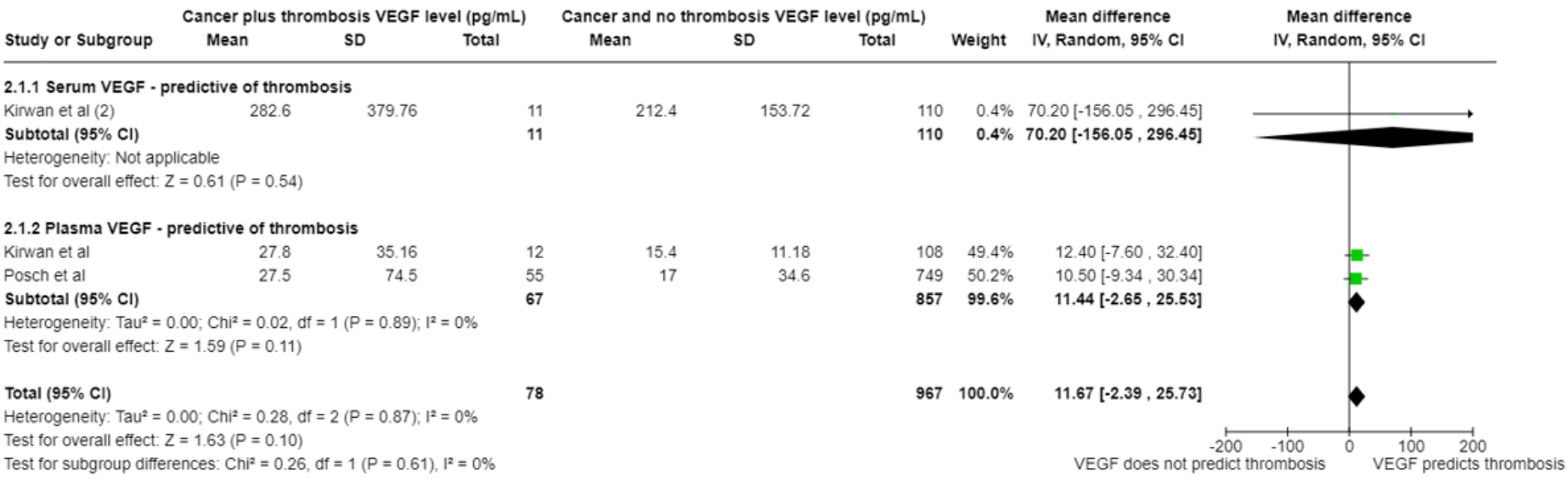

Figure 4.3. **Forest plot for VEGF levels, collected prior to thrombosis among cancer-associated thrombosis and patients with cancer and no thrombosis.** Figure generated using RevMan software (https://revman.cochrane.org). p = 0.10 overall, suggesting that there is not a statistically significant difference between those who did and did not experience a thrombosis. VEGF levels analysed before the incidence of thrombosis. Statistical significance set at p = <0.05.

4.4 Discussion

Cancer is the uncontrolled proliferation of genetically aberrant cells, which is a leading cause of death throughout the world. It can occur in any tissue of the body, including the blood. For the cancer tumour to grow and proliferate, certain conditions need to be in place, one of which is the ability for angiogenesis to occur, which is the formation of new blood vessels (Dogan and Demirkazik, 2005). VEGF is a potent angiogenesis stimulator, and so therefore we would expect VEGF to be raised in patients with cancer (Dogan and Demirkazik, 2005).

Thrombosis is the presence of a blood clot, which can occur in either the veins or the arteries.

Compared to the general population, patients with cancer are at an increased risk of developing a thrombosis, between 1 and 20% of patients develop this complication, which is associated with a higher mortality rate (Dogan and Demirkazik, 2005; Chew et al, 2006; Van Es et al, 2017).

VEGF is raised in patients with cancer (Dogan and Demirkazik, 2005; Dogan et al, 2006; Posch et al, 2016) and is thought to play a role in thrombosis (Dogan and Demirkazik, 2005) by promoting both the release of tissue factor, and platelet activation and adhesion (Posch et al, 2016)

Tissue Factor, released from endothelial cells, is one of the main initiators of coagulation (Dogan and Demirkazik, 2005; Posch et al, 2016). VEGF is also stored

within endothelial cells in the Weibel-Palade bodies (Feroni et al, 2016). Tissue Factor may also play a role in angiogenesis, by upregulating VEGF, and downregulating the angiogenesis inhibitor thrombospondin (Khorana et al, 2007b; Zhang et al, 1994), a mechanism which is independent of coagulation activation (Khorana et al, 2007b; Echrish et al, 2014).

For thrombosis to take place platelets must be both activated and adhere to each other. Activated platelets release further VEGF from their alpha granules (Posch et al, 2016) into the circulation enhancing thrombosis via these mechanisms. Platelets can also act as a transporter of tumour-originated VEGF (Verheul et al, 1997), further contributing to tumour angiogenesis and progression, as well as the risk of thrombosis.

Therefore, we hypothesized that VEGF shows excellent theoretical potential to be used as a biomarker for cancer-associated thrombosis. In this meta-analysis we investigated whether plasma or serum VEGF levels are associated with thrombotic events in cancer patients, pre and post thrombosis.

Eight papers (seven patient cohorts) were included in this meta-analysis. The findings shown within this chapter indicate that VEGF levels are increased at the time of a thrombotic event, indicating VEGF may play a role during a thrombotic event and in addition to its role in the pathogenesis of a malignancy but does not appear to be predictive of thrombosis.

This meta-analysis included five studies where the thrombosis was present at the blood sampling point, to determine whether VEGF was associated with thrombus formation. All the studies included showed increased mean differences between patient groups who had a thrombosis versus those who had not ($p = 0.0003$). Most stark was the data collected by Cacciola et al (2002), which showed a mean serum VEGF level of 1292 pg/mL in those with a thrombosis, against a mean serum VEGF level of 461 pg/mL in those who did not have a thrombosis. However, a limitation of this data is that the VEGF levels measured by this study appear to be significantly different to others. The reasons for this are unknown. These findings were confirmed by the work of Musolino et al (2002) and Li et al (2004), whose data was not included in the forest plots for reasons stated earlier in this chapter.

Activated platelets release VEGF (Posch et al, 2016), and therefore it is not unexpected that VEGF levels were observed to be increased at the time of a thrombosis. Platelet activation is an essential part of primary haemostasis, which is required in the formation of a thrombus. VEGF is also found in higher levels in patients with cancer compared to healthy controls (Dogan and Demirkazik, 2005), due to ongoing angiogenesis required for tumour growth and survival (Dogan and Demirkazik, 2005). Interestingly Musolino et al (2002) showed that in patients with myeloproliferative neoplasms increased plasma VEGF levels were seen up to one month post thrombotic event, possibly indicating a state of platelet hyper-activation and/or indicating a more global contribution of VEGF to thrombosis.

Having identified an association of VEGF with CAT at the time or post thrombosis, this meta-analysis set out to investigate whether VEGF can be used as a biomarker to predict cancer-associated thrombosis. Three studies identified by our search strategy, collected blood samples for VEGF level measurement from cancer patients before a thrombosis had occurred. The incidence of CAT in these was 9.8% (3-month cumulative; Kirwan studies (2008 and 2009) and 5.0% (6-month cumulative; Posch et al (2016). This reflects typical CAT incidence (Dogan and Demirkazik, 2005; Chew et al, 2006; Van Es et al, 2017), and the two study populations characteristics, as the Kirwan et al studies include exclusively breast cancer patients, associated with a higher risk of CAT, whereas Posch et al studies a variety of cancer types, with various differing risk profiles. Whilst all three studies showed a trend towards higher levels of VEGF in those patients who subsequently developed a thrombosis versus those who did not, this difference was not statistically significant (P-value of 0.10). Reasons hypothesised for this include not knowing how long prior to the thrombotic event the samples were taken for example, which we suggest may impact the study's conclusions. Posch et al 2015 [11], for example, followed patients for thrombotic events for two years following initial sampling as part of the large Vienna CATS Study, so it not inconceivable that VEGF would not be raised up to two years before a thrombotic event occurred. The work of Nazari et al (2019) was also part of the same study cohort (though only patients with glioma were examined) and so the same conclusions can be drawn. In contrast, the two remaining studies, Kirwan et al (2008

and 2009), which used plasma and serum samples collected from the same cohort of 123 patients (120 for plasma, and 121 for serum) only followed patients for three months after blood sampling, these differences in follow up time may be confounding the results. In addition, different cancer types were studied, at different stages, which may also be impacting the findings. It is also difficult to compare studies however, as plasma (Kirwan et al, 2008) and serum (Kirwan et al, 2009) VEGF levels were included from two publications that include the same patient population, which inevitably leads to bias. Overall, the lack of independent studies will have had an impact on the results obtained and highlights that further work in this area is required.

As part of this meta-analysis, we included studies measuring VEGF from both serum and plasma. This has consequences for our interpretation as serum and plasma VEGF have very different normal reference ranges. However, Malaponte et al (2015) appears to be an outlier with the measurement plasma VEGF, recording VEGF levels much higher than the other groups also measuring these biomarkers, and more like levels expected for serum VEGF measurement, even in those individuals with no thrombosis. The reasons for this are unclear, with the original paper being checked on multiple occasions for any transcription errors. However, the percentage difference in mean plasma VEGF values between individuals with and without a VTE was 26.5% in this study, which is comparable to that of other studies in the same category (25.5% in Dogan et al (2006), 17.3% in Ramadan et al (2021), with Kim et al

(2004) being an outlier with a 63.4% difference). Therefore, all studies show that VEGF levels are higher in those with a thrombosis compared to those without.

Normal plasma and serum VEGF reference ranges differ significantly, with the serum level being 10 to 15 times higher than that of the plasma level (D'Souza et al (2011)). This is because the platelets will have become activated during centrifugation in the serum sample, but they remain intact in plasma samples due to the presence of anticoagulant in the sample tube. Serum VEGF analysis therefore gives a measure of how much VEGF there is in platelets, whereas plasma VEGF analysis does not, and instead represents VEGF released from platelets which is indicative of platelet activation.

By examining the forest plots we can see that the measurement of serum VEGF is much more variable than that of plasma, and this is possibly affecting the significance of our findings. The difference in the values could also explain why serum VEGF was found to be associated with occurring thrombosis but not found to be predictive of thrombosis. Activated platelets secrete VEGF, indicating that they are prothrombotic, and therefore a thrombosis may occur. However, by analysing a serum sample, where these 'naturally- activated' platelets are present, plus those platelets 'artificially-activated' by centrifugation, it is unlikely that we are truly representing the predictive value of VEGF measurement in serum samples. Plasma samples may therefore give a more accurate representation of the predictive value of

VEGF in thrombosis in patients with cancer, and further studies are therefore needed to investigate this.

VEGF is a potent angiogenic factor that has been shown to be overexpressed in breast, colorectal, lung, pancreatic, ovarian and cervical cancers (Dogan and Demirkazik, 2005; Dogan et al, 2006), where it promotes the formation of new blood vessels (angiogenesis), and is essential for the growth, invasion, progression, and metastasis of tumour tissue (Dogan et al, 2006). Several of the studies included in this analysis demonstrated increased VEGF levels in cancer patients versus healthy controls (Li et al, 2004; Musolino et al, 2002; Kim et al, 2004; Kirwan et al, 2018; Kirwan et al, 2019).

VEGF levels also increase as a cancer develops. Patients with more advanced stages of cancer therefore can have higher levels of VEGF (Kraft et al, 1999). In the studies examined this was acknowledged by all, but not considered with regards to the VEGF level and reported thrombosis rates. However, Dogan et al (2006) matched controls according to cancer stage, which showed that those who experienced a thrombotic event still had higher VEGF levels than the matched controls, suggesting that the thrombotic process was an additional factor for an increase in VEGF levels. Posch et al (2015) also addressed this, using multivariable analysis to adjust for tumour stage in their analysis and showed that the association between VEGF and risk of VTE prevailed after adjustment.

The role of VEGF in initiating thrombus formation is also not well established. There is little to no evidence to suggest that VEGF alone can trigger thrombotic events, which may explain why our analysis found it not to be predictive of thrombosis. It is possible, however, that VEGF plays a role along with other prothrombotic factors to initiate thrombus formation (Khorana et al, 2008).

Given the association of increased VEGF levels at the time of, or after, the thrombotic event, some consideration should be made as to whether adding VEGF as a biomarker to an existing risk-assessment model (RAM), could be useful. Other biomarkers such as D-dimer levels are already part of the Vienna CATS score (Ay et al, 2010) with strong evidence available demonstrating increased D-dimer levels associated with both current and future thrombotic events (Cohen et al, 2014; Tan et al, 2017; Linkens et al, 2017; Hansen et al, 2021). Interestingly, the Kirwan studies (2008), show significantly higher D-dimer levels in patients who subsequently went on to experience a thrombotic event versus those who did not (1655 (834-3273) ng ml-1 vs 727 (631-836) ng ml-1, p = 0.003), in the same cohort, VEGF tended to be higher, but this difference was not statistically significant.

At this time, the analysis of predictive studies demonstrates that there is not sufficient evidence that VEGF can be used to predict cancer-associated thrombosis independently. However, it is possible that VEGF levels may increase predictive capacity in combination with other established markers and risk scores, such as cancer type (Khorana et al, 2008; Ay et al, 2020; Verso et al, 2012), BMI (Khorana et al,

2008; Ay et al, 2020; Verso et al, 2012) and D-dimers (Ay et al, 2010), or alongside other novel biomarkers such as soluble P-selectin (Ay et al, 2010; Swamy et al, 2023). The study by Posch et al (2016), demonstrated a positive interaction between soluble VEGF levels and D-dimer indicating that the predictive potential of VEGF might be enhanced in combination with D-dimer, particularly in individuals with high levels of both biomarkers. Further investigation and studies are required.

4.5 Conclusions

In this Chapter a meta-analysis approach has been used to investigate whether VEGF has the potential to be used as biomarker for cancer associated thrombosis. This has identified that high plasma and serum VEGF levels are associated with current thrombosis in samples taken at the time of or post thrombotic event, however, plasma and serum VEGF levels were not found to be associated with or predictive of thrombosis when collected prior to thrombotic events in cancer patients. In the future, more prospective cohort studies in specific cancer types and stages are needed to ascertain whether VEGF could be used as a predictive biomarker of cancer associated thrombosis.

4.6 Chapter Summary

Overall, further work is required in this area to establish if VEGF can be used to predict cancer-associated thrombosis. Whilst eight studies were examined during this meta-analysis all were very different from each other with regards to study design

and patients selected. Suggested further work to be performed in this area could include other specific cancer types to see if the predictive capacity of VEGF is improved in a particular cancer type or types.

CHAPTER 5. RESULTS – INVESTIGATION

Can the serial measurement of biomarkers be used to predict cancer-associated thrombosis?

5.1 Introduction

Cancer-associated thrombosis contributes to the morbidity and mortality of patients diagnosed with cancer. Many risk assessment models have been proposed which aim to stratify those individuals at the highest risk of CAT. However, they are poorly predictive with many thrombotic events occurring outside of the high-risk categories and are therefore poorly utilised in clinical practice.

The majority of historical research in this area has concentrated on the measurement of biomarkers at baseline, before the commencement of chemotherapy, or before the start of other treatments for cancer including surgery, radiotherapy and hormone therapy. The only risk-assessment model which considers a change in biomarker level is the CATS nomogram (Pabinger et al, 2018), which is rarely used in clinical practice.

The thrombotic risk in a patient with cancer changes throughout their journey. The treatment of cancer, including chemotherapy, can induce cytopenia's, and side-effects of treatment including vomiting, diarrhoea and a loss of appetite can lead to dehydration and weight loss. Tumour burden, and therefore the potential of cancer to cause a thrombotic event may also change. However, none of these alterations are

considered within the current risk assessment models (RAMs). This could be addressed with the serial measurement of biomarkers in these patients.

The aim of this chapter was to assess whether serial measurement of biomarkers associated with cancer and/or thrombosis can be useful for the prediction of CAT. This chapter focusses on the measurement of four biomarkers (D-dimers, soluble P-selectin, serum VEGF and plasma VEGF). Studies of serial biomarker measurements to date are conflicting with very little consensus. These biomarkers were chosen to reflect to the haemostatic processes occurring during thrombosis, as outlined in the Introduction chapter (Chapter 1) of this thesis. In addition, whilst both D-dimers and sP-selectin are established biomarkers within CAT risk assessment models, VEGF is not and reflects new research. Both serum and plasma VEGF were measured due to the findings outlined in the meta-analysis covered in Chapter 4, Results Chapter 2, where there was no consensus on whether plasma or serum VEGF should be measured. Serum VEGF will capture VEGF present after both platelet and endothelial activation, whereas plasma measurement will only capture that present after endothelial activation (Hagn et al, 2024).

First though we need to consider the research which has already been performed on the serial measurement of biomarkers.

Reitter et al (2016) measured both D-dimers and soluble P-selectin (sP-selectin) in all cancer types. Increasing D-dimers and sP-selectin were seen in the last blood

sampling point before the diagnosis of a VTE, 93% of patients had a D-dimer level above the median, with 50% above the 75th percentile (Reitter et al, 2016).

Posch et al (2020) measured D-dimers monthly in all cancer types and showed that D-dimer levels increased by an average of 34% in those who developed a VTE, versus an average increase of 2.6% in those who did not (Posch et al, 2020). Furthermore, a doubling of D-dimer levels was associated with a 2.8-fold increase in the risk of VTE (Posch et al, 2020).

Van Es (2018) measured D-dimer and sP-selectin levels in all cancer types at baseline, 1 week, 4-, 5-, 12- and 24-weeks post treatment initiation and could not find any association between the changes in biomarkers levels in this period and an association with recurrent VTE. However, all patients had a current VTE at enrolment and were currently being treated with LMWH (van Es et al, 2018).

Kirwan et al (2008 & 2009) studied biomarker levels in 123 breast cancer patients, and measured levels at baseline, 24 hours, 4 days, 8 days and 3 months after the commencement of chemotherapy. Baseline levels of both D-dimers and VEGF were increased in those who subsequently developed a VTE.

Finally, Von Tempelhoff et al (1998) studied 47 patients diagnosed with ovarian cancer but found that no parameter was significantly different in those who developed a DVT versus those who did not. Parameters were measured before the

start of chemotherapy, but after surgery, and in a 2 month follow up appointment following the completion of chemotherapy.

Therefore, this study has sought to address some of the gaps in the relevant research, and to establish if serial measurement of D-dimers, sP-selectin, and VEGF can be used for the prediction of CAT in this study population.

5.2 Study dates and details

This was an observational cohort study of individuals who were starting a new course of chemotherapy following a diagnosis of cancer. Some participants had had resection surgery following their cancer diagnosis and before enrolment (n = 10, 18.5%), and four participants (9.3%) had been diagnosed with cancer in the past, but a different primary site than this diagnosis. All cancer types were included, and all cancer stages.

With regards to personal or family history of thrombosis, three participants (5.6%) had a personal history of thrombosis, and five participants had a family history (9.3%). One of these five participants had a family history of CAT, their father had a pulmonary embolism during chemotherapy for a cancer diagnosis.

Recruitment for the study commenced on the 20th May 2024 at the Northern Centre for Cancer Care, which is housed at the Freeman Hospital, part of The Newcastle upon Tyne Hospitals NHS Foundation Trust. Each participant had baseline bloods before the commencement of chemotherapy, bloods after one month of

chemotherapy, and three months. Each participant was monitored for a minimum of three months after the start of their chemotherapy.

Ethical approval granted stated that participants would only have bloods taken whilst attending hospital for care purposes. Participants would not attend solely for the purposes of the study, but bloods could be taken even if no other bloods were required for care purposes providing the participant consented.

As a result, the three sampling time points differed from participant to participant, with the average (mean) number of days from baseline to one month being 29.5 days, with a range of 14 to 58 days. The mean number of days from baseline to three months was 100 days, with a range of 48 to 148 days. For the purposes of this study, data was only included where the 1-month sampling point was 1 month +/- 10 days (20 to 40 days after recruitment), and for the 3-month sampling point +/- 2 weeks (76 to 104 days after recruitment). Whilst this reduced variability in sampling time points and strengthened the study design, it did reduce the number of full serial measurement data sets collected.

Fifty-four eligible participants were recruited and consented to the study. As described in Chapter 2 – Methods Section 2.1.1, once consented and recruited, bloods were hand delivered to the laboratory by a Clinical Trial Associate and analysed. At recruitment a medical information sheet was completed by a Clinical Trial Associate, employed by The Newcastle upon Tyne Hospitals NHS Foundation Trust, following a clinical interview. a copy of which can be found in Appendix 5.

In addition, not all participants had three sets of bloods taken. Some were missing, and other had to be excluded due to the time constraints as discussed previously. Thirteen (n = 13) participants had all three sets, eleven (n =11) had baseline only, twenty-four (n=24) had baseline and 1 month, and six (n = 6) had baseline and 3-month bloods taken. Ten sets of 1-month bloods were excluded due to being taken outside of the 20-to-40-day range, and seventeen sets of 3-month bloods excluded due to being outside of the 76-to-104-day range.

Two participants died during the study period. One of these had baseline only bloods, did not experience a thrombotic event, and died from progression of cancer. The other had baseline and 1-month bloods experienced a thrombotic event and passed away a few days later.

Sample analysis was completed on the 18th January 2025.

5.3 Demographics of participants

Fifty-four participants were recruited to the study. Of these, six participants (6/54) (11.1%) developed a thrombosis in the study period. This figure is higher than that reported by many other studies (Chew et al, 2006; Stein et al, 2006; Khorana et al, 2007a; Cronin-Fenton et al, 2010; Walker et al, 2013; Köningsbrügge et al, 2014; Mahajan et al, 2022), and which are outlined in the Introduction chapter of this thesis. The reasons for this are unclear, but the size of the cohort will have influenced this.

The main patient characteristics of the 54 participants are summarised in Table 5.1.

Patients were split into two groups for analysis: those with thrombosis within the stated study period of 3 months plus 2 weeks, and a further 30 days of observation (up to 134 days after recruitment), and those without thrombosis.

All six participants who developed a thrombosis in the study period were female and were on average older than the total study population (65.83 years versus 60.34 years) but this was not statistically significant ($p = 0.2484$). All documented thromboses were venous and included deep vein thrombosis (DVT), pulmonary embolism (PE), brachial thrombosis (in the arm), catheter-associated thrombosis, superficial thrombophlebitis and splenic vein thrombosis. A thrombotic event was diagnosed an average of 68.6 days (range 12 to 124 days) after baseline bloods were taken. Of those who developed a thrombosis, two participants had a personal history of thrombosis (33%) (10 years and 28 years prior to enrolment), and one participant (17%) had a family history of thrombosis.

All thrombotic events occurred in female participants, with none recorded in male participants.

Sites of cancer were grouped together anatomically (K. Musgrave, personal communication). Thus, 11 groups and types of primary cancer sites are represented, as shown in Table 5.1.

Within those participants who developed a thrombosis, there were five different primary cancer sites (cervix, lung, colon, pancreas and breast). Both high and low risk cancers for CAT, as according to the Khorana score, were represented (Khorana et al, 2008).

The Trust electronic medical records were interrogated to find additional information where required or if this was not provided on the Medical Information Sheet. This included a calculation of the participants' Body Mass Index by the formula: [Weight (in kilograms)/ by height (in metres)^2] which was found to be not statistically significant ($p = 0.4392$) between the thrombosis and no thrombosis populations. The stage of cancer at diagnosis was also determined by these records. To avoid ambiguity with regards to the various staging systems used for different cancer types, the distinction between metastatic and non-metastatic was used. Metastatic cancer is defined as where the cancer has already spread to organs distant from the primary tumour, for example the lungs, liver or brain (Welch and Hurst, 2019).

Number of patients	All	Without a thrombosis	With a thrombosis
	N = 54	N = 48	N = 6
Sex	F = 37 (68.52%) M = 17 (31.48%)	F = 31 (64.58%) M = 17 (35.42%)	F = 6 (100%) M = 0 (0%)
Mean age (range)*	60.34 years (36 to 84) F = 60.95 years (36 to 84) M = 58.8 (38 to 78)	59.69 years (36 to 84) F = 60.00 years (36 to 84) M = 59.12 years (38 to 78)	65.83 years (39 to 78) F = 65.83 years (39 to 78) M = N/A
Ethnicity $ **White British** **White Irish** **Other-Not stated** **Other-Not known**	 N = 51 (94.40%) N = 1 (1.85%) N = 1 (1.85%) N = 1 (1.85%)	 N = 46 (95.8%) N = 1 (2.08%) N = 1 (2.08%) N = 0 (0%)	 N = 5 (83.33%) N = 0 (0%) N = 0 (0%) N = 1 (16.67%)
BMI (kg/m^2) (range) *	28.5 (14.9 to 42.1) F = 28.7 (14.9 to 42.1) M = 28.1 (22.8 to 33.3)	28.30 (14.9 to 42.1) F = 28.40 (14.9 to 42.1) M = 27.90 (22.8 to 33.3)	30.23 (20.1 to 38.9) F = 30.23 (20.1 to 38.9) M = N/A
Site of cancer **Breast** **Head and neck (Head and neck, throat, tonsil, larynx, tongue)** **Lower GI (Colorectal, rectal, bowel)** **Pancreas** **Upper GI (Gastric, oesophagus, cholangiocarcinoma)** **Gynaecological (cervix, ovarian)** **Renal (kidney, bladder)** **Lung** **Sarcoma** **Brain (brain, glioma)** **Lymphoma**	 N = 10 (18.52%) N = 8 (14.81%) N = 8 (14.81%) N = 6 (11.11%) N = 4 (7.40%) N = 4 (7.40%) N = 4 (7.40%) N = 4 (7.40%) N = 3 (5.55%) N = 2 (3.70%) N = 1 (1.11%)	 N = 9 (18.75%) N = 8 (16.67%) N = 6 (12.50%) N = 5 (10.42%) N = 4 (8.33%) N = 3 (6.25%) N = 4 (8.33%) N = 3 (6.25%) N = 3 (6.25%) N = 2 (4.17%) N = 1 (2.08%)	 N = 1 (16.67%) N = 2 (33.33%) N = 1 (16.67%) N = 1 (16.67%)
Stage of cancer **Non-metastatic** **Metastatic**	 N = 37 (68.52%) N = 17 (31.48%)	 N = 35 (72.92%) N = 13 (27.08%)	 N = 2 (33.33%) N = 4 (66.67%)

Table 5.1. **Demographics of the study population** $=as recorded in eRecord. N/A – not applicable. Note numbers may not equal 100% due to rounding. *Statistical analysis performed using an unpaired t-test (p = 0.2484 for age, and p = 0.4392 for BMI) suggesting that both are not statistically significantly different between thrombosis and non-thrombosis populations (statistical significance limit set at P = <0.05).

5.4 Can the biomarker levels at baseline predict who will develop a thrombosis, and who will not?

Four biomarkers were measured for this study at three sampling points: baseline, 1-month and 3-months.

D-dimers were measured by latex immunoassay on the Werfen TOP 700 analysers. Both soluble P-selectin and VEGF (both serum and plasma) were measured by ELISA.

Further details can be found within Chapter 2 Methods section 2.4 of this thesis.

First, we sought to investigate if the baseline biomarker levels only could have predicted who would have developed a thrombosis, and who would not in our study population, and whether there was any statistically significant difference between the two groups. Current risk assessment models (RAMs) which include biomarker levels to assess the risk of a patient developing CAT use biomarker levels at baseline only, with one exception (CATS nomogram). This includes the Khorana score (Khorana et al, 2008) which uses haemoglobin, white blood cell counts and platelet count, and the Vienna CATS score (Ay et al, 2010), which uses D-dimers and soluble P-selectin in addition to the biomarker parameters of the Khorana score.

A clinical audit assessing the utility of the Khorana score in a population of pancreatic cancer patients was undertaken as part of this thesis and is presented in Chapter 3 (Results Chapter 1).

The average (mean) value of the four biomarkers at baseline (n = 54) is shown on Table 5.2.

The population for all four biomarkers represents an abnormal distribution, and as a result non-parametric statistical analysis (the Mann-Whitney U test) was used to determine if there was a statistically significant difference between the baseline levels of D-dimer, soluble P-selectin, serum VEGF or plasma VEGF in those who did and did not develop a thrombosis. Additional scatter plots showing these results are shown in Figure 5.1. Statistical significance was set at p = < 0.05.

	All (n = 54)	**Without a thrombosis (n = 48)**	**With a thrombosis (n = 6)**	**p-value**
D-dimers (ng/mL)	334.7 (202.4 – 466.9)	339.9 (192.5 – 487.2)	293.1 (21.4 – 564.8)	0.7988
Soluble P-selectin (ng/mL)	78.4 (63.6 – 93.2)	81.4 (65.8 – 97.1)	54.3 (-4.3 – 112.9)	0.1081
Serum VEGF (pg/mL)	416.6 (303.6 – 529.7)	412.4 (287.5 – 537.3)	450.5 (157.9 – 743.0)	0.3237
Plasma VEGF (pg/mL)	51.4 (38.6 – 64.3)	50.4 (36.8 – 64.0)	60.1 (5.5 – 114.6)	0.6363

Table 5.2. **Mean values of D-dimers, soluble P-selectin, and serum and plasma VEGF at baseline.** 95% confidence intervals of the mean are shown in brackets afterwards. Statistical analysis by Mann-Whitney tests with statistical significance set at p=<0.05.

As demonstrated in Table 5.2 and Figure 5.1 no significant differences in baseline values were observed between those who subsequently developed a thrombosis and

those who did not. This analysis shows that there is no statistically significant difference between baseline levels of either D-dimers ($p = 0.7988$), soluble P-selectin ($p = 0.1081$), serum VEGF ($p = 0.3237$) or plasma VEGF ($p = 0.6363$) in those who did or did not develop a thrombosis in the study period, and for this study population.

These findings demonstrate that the measurement of these biomarkers at baseline only, and for each individual biomarker in isolation, cannot be used to predict CAT. These observations are similar to those made with full blood count data in pancreatic cancer patients (Chapter 3) and observations with VEGF (Chapter 4). This is further supported by the findings of many other studies (van Es et al, 2017; Guman et al, 2021; Mosaad et al, 2021) which have demonstrated the limited predictive power and potential of many of the published risk assessment models, where predictive power is only established at baseline, before any chemotherapy is administered.

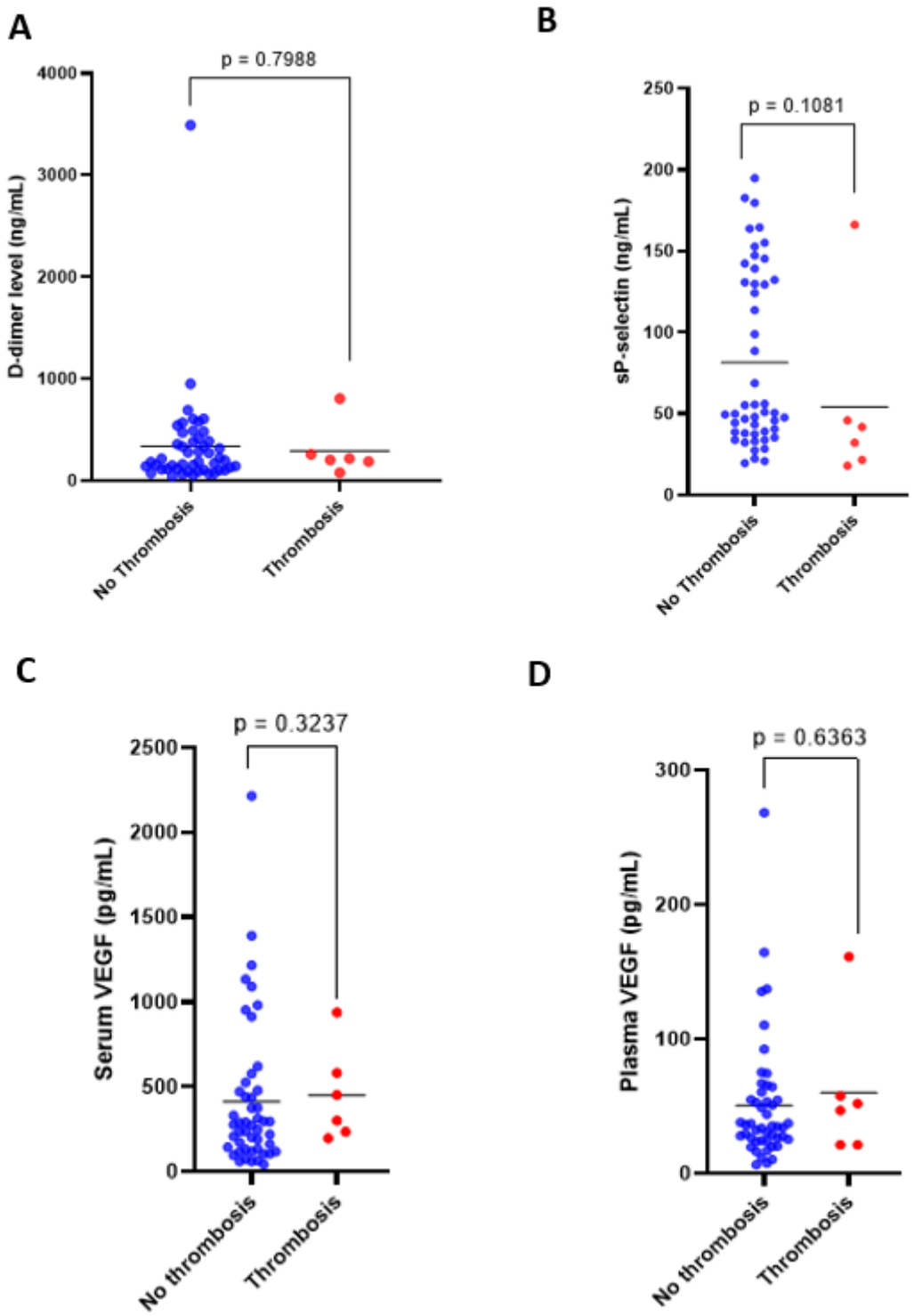

Figure 5.1. No difference in baseline levels of D-dimers, soluble P-selectin, serum VEGF or plasma VEGF in those who develop a thrombosis versus those who do not. Baseline levels for 54 participants plotted for those with (n = 6) and without (n = 48) thrombosis. Data represented as a scatter plot. Statistical analysis using Mann-Whitney tests. P = 0.7988 (D-dimers) (Panel A), p = 0.1081 (P-selectin) (Panel B), p = 0.3237 (serum VEGF) (Panel C), and p = 0.6363 (plasma VEGF) (Panel D).

Having established that in this study population baseline biomarker levels in isolation cannot be used to predict those who will develop a thrombosis versus those who will not, we next sought to investigate if 1-month or 3-month biomarker levels could be used in isolation to predict cancer-associated thrombosis.

5.5 Can biomarker levels at the 1-month or 3-months sampling point predict who will have a thrombosis and who will not?

Next, we sought to establish if the sampling points at 1-month or 3-months could be used in the prediction of CAT.

The numbers of samples at these sampling points, however, are different to that of the overall study population. This is because participants have either not attended, or the sampling point fell outside the remit of 20 to 40 days for 1-month, and/or 76 to 104 days for the 3-months sampling point after baseline.

In addition, those participants who have already experienced a thrombosis prior to the time point were excluded from analysis. For those diagnosed with a thrombosis on the same day as the sampling point, these points were also excluded. Table 5.3 outlines the mean values for all four biomarkers at the 1-month sampling point, whilst Table 5.4 does the same for the 3-months sampling point.

Note that numbers are very low, particularly in the thrombosis population (n = 2 for 1-month, and n = 1 for 3 months) and therefore whilst p-values could be obtained for most, they could not for the 3-month sampling point.

Table 5.3 shows that there is a statistically significant difference, where values are higher, between the D-dimer values at 1-month between those that subsequently developed a thrombosis and those that did not. However, numbers are very small and so this should be interpreted with caution.

	All (n = 36)	**Without a thrombosis (n = 34)**	**With a thrombosis (n = 2)**	**p-value**
D-dimers (ng/mL)	370.1 (202.8 – 537.4)	282.4 (206.4 – 358.4)	1861 (-11720 - 15443)	0.0127
Soluble P-selectin (ng/mL)	78.87 (59.66 – 98.08)	73.91 (54.95 – 92.86)	163.2 (-14.69 – 341.1)	0.0635
Serum VEGF (pg/mL)	437.3 (291.6 – 583.0)	433.0 (281.9 – 584.0)	510.9 (-4400 – 5422)	0.7143
Plasma VEGF (pg/mL)	56.02 (32.69 – 79.34)	56.67 (31.94 – 81.39)	44.95 (-127.2 – 217.1)	0.7651

Table 5.3. **Mean values of D-dimers, soluble P-selectin, and serum and plasma VEGF at the 1-month sampling point.** 95% confidence intervals of the mean are shown in brackets afterwards. Statistical analysis by Mann-Whitney tests with statistical significance set at p=<0.05.

	All (n =17)	Without a thrombosis (n = 16)	With a thrombosis (n = 1)	p-value
D-dimers (ng/mL)	353.7 (94.98 – 612.5)	360.9 (84.44 – 637.4)	238.6 (N/A)	Unable to perform
Soluble P-selectin (ng/mL)	55.3 (29.65 – 80.96)	56.54 (29.22 – 83.85)	35.6 (N/A)	Unable to perform
Serum VEGF (pg/mL)	264.8 (150.6 – 379.1)	252.2 (133.3 – 371.2)	466.2 (N/A)	Unable to perform
Plasma VEGF (pg/mL)	38.59 (26.53 – 50.65)	37.64 (24.91 – 50.36)	53.8 (N/A)	Unable to perform

Table 5.5. **Mean values of D-dimers, soluble P-selectin, and serum and plasma VEGF at the 3-months sampling point.** 95% confidence intervals of the mean are shown in brackets afterwards. Statistical analysis by Mann-Whitney tests with statistical significance set at p=<0.05.

5.6 Can the serial measurement of biomarkers predict those who will develop a thrombosis, and those who will not?

The previous sections have established that a single sample taken before the start of chemotherapy (baseline) does not predict those who will experience a thrombotic event in this study population. The 1-month and 3-months sampling points were also examined in isolation, and whilst D-dimer levels at the 1-month point were statistically significantly different, numbers are too small to form any firm conclusions.

Next, we examined the serial measurement of these biomarkers, by comparing levels at each sampling point, and analysing any trend or patterns sought to establish if

there are any patterns or trends in each of the biomarkers in turn could be found in those who develop a thrombosis versus those who did not.

Unlike previous analysis, all data points were included where applicable, unless they fell outside the sampling point windows, as previously described. This was to ensure that all data could be captured, and trends could be assessed. For example, one participant developed a thrombosis four days prior to the 1-month sampling point. If we had excluded this 1-month data point, we would not have been able to assess the trend in biomarkers up to the point of the thrombotic event.

5.6.1 D-dimer serial measurement

First, the serial measurement of D-dimer was examined to determine whether changes in D-dimer levels were associated with thrombosis (Table 5.6, Figure 5.2).

	Baseline (n = 54)	**1 month (n = 37)**	**3 months (n = 18)**	**P-value**
All participants (n = 54)	334.7 (202.4 – 466.9)	378.8 (215.3 – 542.4)	341.9 (97.86 – 586.0)	0.4022
No thrombosis (n = 48)	339.9 (192.5 – 487.2)	282.4 (206.4 – 358.4)	360.9 (84.44 – 637.4)	
Thrombosis (n = 6)	293.1 (21.4 – 564.8)	1472 (-1668 – 4612)	190.0 (-428.2 – 808.1)	

Table 5.6. **Average (mean) D-dimer level (ng/mL) at each of the three sampling points in those who did and did not experience a thrombosis.** 95% confidence levels are shown in brackets. P-value significance set at p = <0.05. Kruskal-Wallis tests with Dunn's correction used to determine statistical significance.

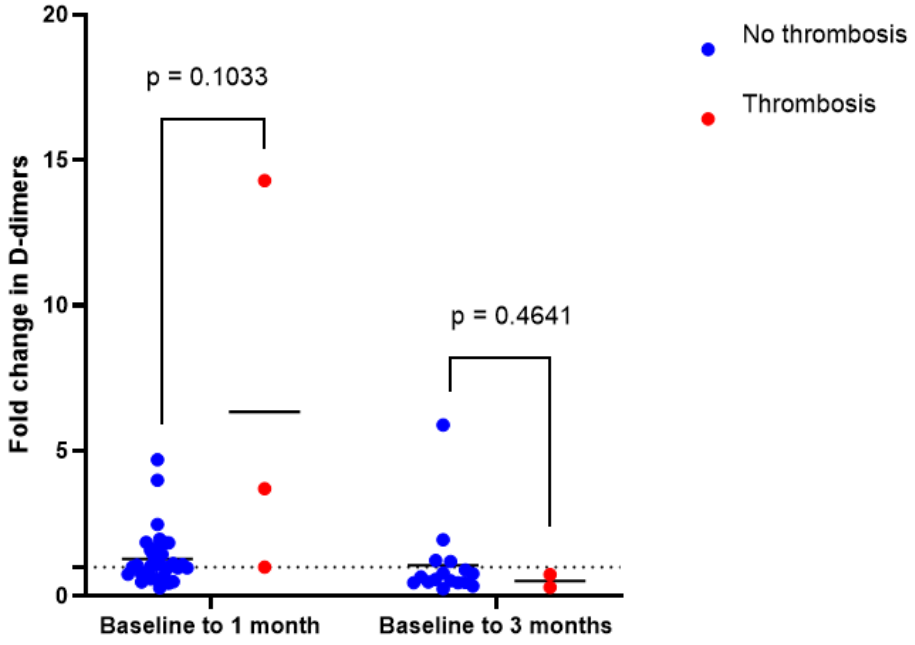

Figure 5.2. **Scatter graph showing fold change in D-dimers between the sampling points.** Mann-Whitney statistical analysis performed with statistical significance set at p = <0.05.

Statistical analysis was performed to see if there was any statistically difference between the results at the three sampling points in the non-thrombosis, and thrombosis populations, which gave a p-value of 0.4022 (Table 5.5), demonstrating no statistically significant difference between the populations at all three timepoints. To investigate whether individual changes in D-Dimer levels were associated with thrombosis, fold change was calculated to examine the difference in results from baseline to 1 month, and from baseline to 3 months in turn (Figure 5.2). No significant differences in fold change of D-Dimer levels between baseline and 1 month (p = 0.1033) and baseline and 3 month (p = 0.4641) was observed between the thrombosis and non-thrombosis groups. Taken together these observations

indicate that D-dimer levels and changes in D-dimer levels collected at 1 month and 3 months are not predictive of cancer associated thrombosis.

5.6.2 Soluble P-selectin serial measurement

Next, sP-selectin serial measurement was examined to see if there were any differences between the thrombosis and non-thrombosis populations (Table 5.6, Figure 5.3).

	Baseline (n = 54)	**1 month (n = 37)**	**3 months (n = 18)**	**p-value**
All participants (n = 54)	78.42 (63.63 – 93.20)	83.02 (62.54 – 103.5)	54.19 (30.01 – 78.38)	0.0787
No thrombosis (n = 48)	81.43 (65.81 – 97.06)	73.91 (54.95 – 92.86)	56.54 (29.22 – 83.85)	
Thrombosis (n = 6)	54.28 (55.86 – 22.80)	186.3 (81.00 – 291.6)	35.45 (33.54 – 37.36)	

Table 5.6. **Average (mean) sP-selectin level (ng/mL) at each of the three sampling points in those who did and did not experience a thrombosis.** 95% confidence levels are shown in brackets. P-value significance set at p = <0.05. Kruskal-Wallis test with Dunn's post hoc correction used to determine statistical significance.

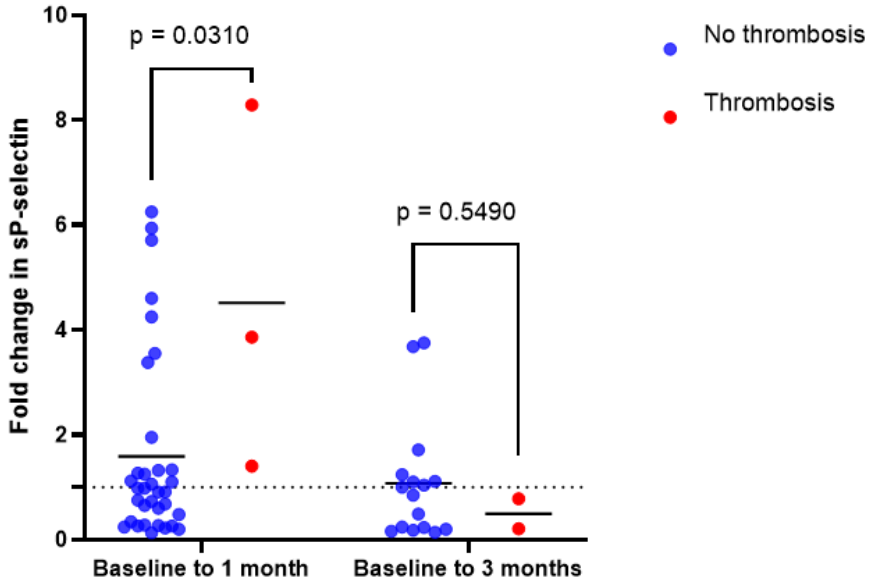

Figure 5.3. **Scatter graph showing fold change in sP-selectin between the sampling points.** Mann-Whitney statistical analysis performed with statistical significance set at p = <0.05.

Statistical analysis was performed to see if there was any statistically difference between the results at the three sampling points in the non-thrombosis, and thrombosis populations, which gave a p-value of 0.0787 (Table 5.6), demonstrating no statistically significant difference between the populations at all three timepoints. To investigate whether individual changes in sP-selectin levels were associated with thrombosis, fold change was calculated to examine the difference in results from baseline to 1 month, and from baseline to 3 months in turn (Figure 5.3). Mann Whitney tests showed that there is a statistically significant difference between the fold change from baseline to 1 month between the thrombosis and non-thrombosis populations (p = 0.0310), but not between baseline and 3 months (p = 0.5490).

Taken together, these observations suggest that sP-selectin levels and changes in sP-selectin levels collected at 1 month and 3 months are not predictive of cancer associated thrombosis.

5.6.3 Serum VEGF serial measurement

During the meta-analysis described in Chapter 4 (Results Chapter 2), VEGF levels were found to be higher in individuals experiencing a thrombosis at the time of thrombosis but were not predictive of thrombosis at baseline. To determine whether the serial measurement of VEGF levels over 3 months could predict CAT we examined the serial measurement of serum VEGF, to see if there were any differences between the thrombosis and non-thrombosis populations (Table 5.7, Figure 5.4).

	Baseline (n = 54)	**1 month (n = 37)**	**3 months (n = 18)**	**p-value**
All participants (n = 54)	416.6 (303.6 – 529.7)	467.5 (313.2 – 621.8)	281.3 (168.6 – 394.0)	0.4156
No thrombosis (n = 48)	412.4 (287.5 – 537.3)	433.0 (281.9 – 584.0)	252.2 (133.3 – 371.2)	
Thrombosis (n = 6)	450.5 (157.9 – 743.0)	859.1 (-920.3 – 2639)	513.6 (-88.67 – 1116)	

Table 5.7. **Average (mean) serum VEGF level (pg/mL) at each of the three sampling points in those who did and did not experience a thrombosis.** 95% confidence levels are shown in brackets. P-value significance set at p = <0.05. Statistical analysis by Kruskal-Wallis test with post hoc Dunn's correction.

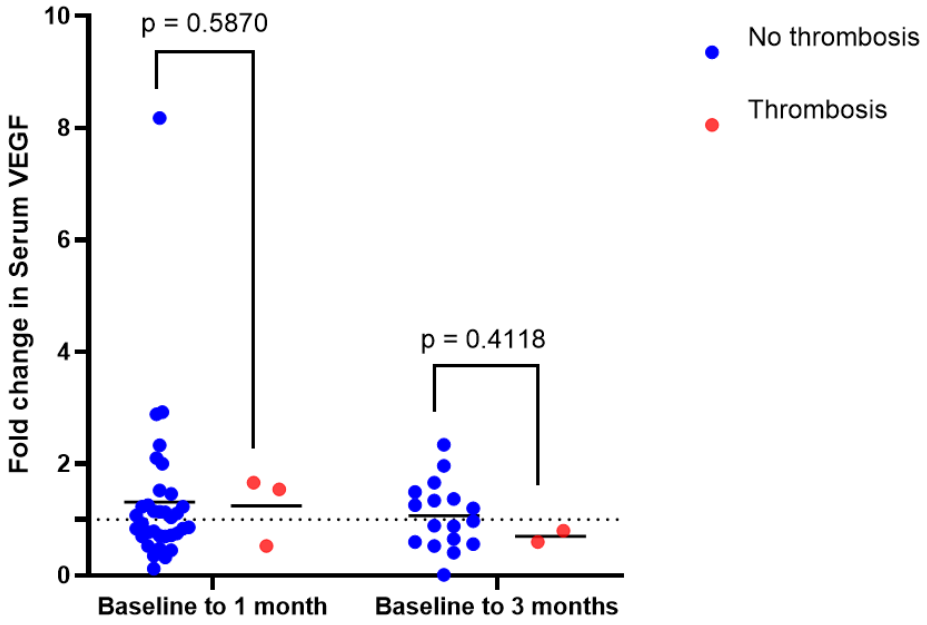

Figure 5.4. **Scatter graph showing fold change in serum VEGF between the sampling points.** Mann-Whitney statistical analysis performed for baseline to 1 month, and an unpaired t-test for baseline to 3 months with statistical significance set at p = <0.05.

Statistical analysis was performed to see if there was any statistically difference between the results at the three sampling points in the non-thrombosis, and thrombosis populations, which gave a p-value of 0.4156 (Table 5.7), demonstrating no statistically significant difference between the populations at all three timepoints. Next, we calculated fold change between baseline and 1 month, and baseline and 3 months (Figure 5.4) which showed no statistically significant difference between either baseline to 1 month (p = 0.5870) nor baseline to 3 months (p = 0.4118) between the two populations. Taken together these observations indicate that serum VEGF levels and changes in serum VEGF levels collected at 1 month and 3 months are not predictive of cancer associated thrombosis.

5.6.4 Plasma VEGF serial measurement

Finally, we analysed the serial measurement of plasma VEGF to see if there were any differences between the thrombosis and non-thrombosis populations (Table 5.8, Figure 5.5).

Again, during the meta-analysis described in Chapter 4 (Results Chapter 2) VEGF levels were found to be higher in individuals experiencing a thrombosis at the time of thrombosis but were not predictive of thrombosis at baseline.

	Baseline (n = 54)	**1 month (n = 37)**	**3 months (n = 18)**	**p-value**
All participants (n = 54)	51.44 (38.62 – 64.26)	56.31 (33.64 – 78.98)	40.81 (28.56 – 53.06)	0.8381
No thrombosis (n = 48)	50.36 (36.78 – 63.95)	56.67 (31.94 – 81.39)	37.64 (24.91 – 50.36)	
Thrombosis (n = 6)	60.05 (5.518 – 114.6)	52.20 (6.308 – 98.09)	66.20 (-91.36 – 223.8)	

Table 5.8. **Average (mean) plasma VEGF level (pg/mL) at each of the three sampling points in those who did and did not experience a thrombosis.** 95% confidence levels are shown in brackets. P-value significance set at p = <0.05. Kruskal-Wallis test with post hoc Dunn's correction used to determine statistical significance.

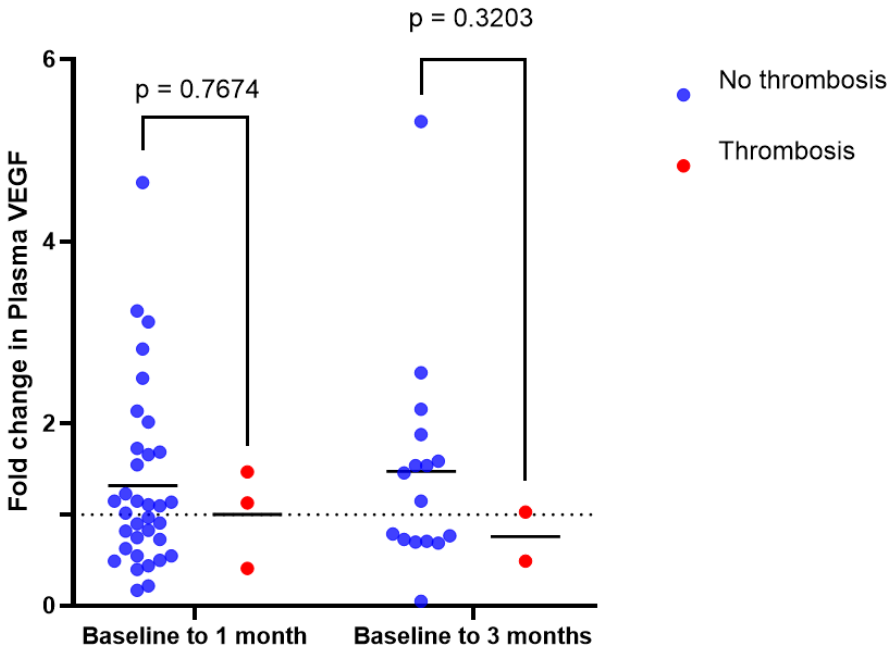

Figure 5.5. **Scatter graph showing fold change in plasma VEGF between the sampling points.** Mann-Whitney statistical analysis performed for baseline to 1 month, and an unpaired t-test for baseline to 3 months with statistical significance set at p = <0.05.

Statistical analysis was performed to see if there was any statistically significant difference between the results at the three sampling points in the non-thrombosis, and thrombosis populations, which gave a p-value of 0.8381 (Table 5.8), demonstrating no statistically significant difference between the populations at all three timepoints.

Further investigation was conducted to see if there were changes in plasma VEGF levels. Fold change was calculated to examine the difference in results from baseline to 1 month, and from baseline to 3 months in turn (Figure 5.5). No significant differences in fold change of plasma VEGF levels between baseline and 1 month (p = 0.7674) and baseline and 3 month (p = 0.3203) was observed between the

thrombosis and non-thrombosis groups. Taken together these observations indicate that plasma VEGF levels and changes in plasma VEGF levels collected at 1 month and 3 months are not predictive of cancer associated thrombosis.

5.7 Discussion

The aim of this chapter was to establish if any patterns or trends could be found in four biomarker levels; D-dimers, sP-selectin, serum VEGF and plasma VEGF in those who experienced a thrombosis, and those who did not.

Fifty-four participants were recruited to the study, six of whom experienced a thrombotic event during the study period. However, some data had to be excluded due to falling outside of the defined time limits for each sampling point (20 to 40 days after baseline for 1-month, and 76 to 104 days after baseline for 3-months).

The mean level of D-dimers, sP-selectin, serum VEGF and plasma VEGF was calculated for at each sampling point in both the thrombosis and non-thrombosis populations. Following this, we conducted statistical analysis to determine if there were any statistically significant differences between each of the sampling points, or any patterns or trends could be determined.

5.7.1 D-dimers for the prediction of CAT

D-dimers are formed as the result of the degradation of a fibrin clot (Moore et al, 2010), and their presence in high numbers outside of the reference range is therefore indicative of recent and/or ongoing clot breakdown. D-dimers have been extensively

studied regarding thrombosis, and cancer-associated thrombosis. D-dimer measurement is featured in two CAT risk assessment models: the Vienna CATS score (Ay et al, 2010), and the CATS nomogram (Pabinger et al, 2018).

For D-dimers we found no statistically significant difference, with D-dimer levels fluctuating in both populations with no consistent patterns or trends seen in this study cohort. For 33% (2/6) of those who experienced a thrombotic event D-dimer levels were rising at thrombosis diagnosis, whereas for one participant (1/6) (17%) D-dimer levels were decreasing. For three participants (50%) patterns could not be determined due to a lack of data. For participants who did not experience a thrombotic event, there were similar patterns of fluctuating D-dimer levels. There was however, a statistically significant difference ($p = 0.0127$) between levels at 1-month between the thrombosis and no thrombosis populations, though caution should be exercised as there were only two data points.

This was somewhat surprising, as D-dimer levels are known to increase in the presence of a thrombosis (Wells et al, 1995; Moore et al, 2010). However, how far the sampling point is away from the diagnosis of a thrombosis is a determinant. Statistical analysis of fold change from baseline to 1 or 3 months also revealed no difference between the thrombosis and non-thrombosis populations.

In previous research done on the serial measurement of D-dimers, Reitter et al (2016) in a cohort of 112 patients with either brain, lung, colon or pancreatic cancer, observed that in the last blood sampling point before the diagnosis of a VTE, 93% of

patients had a D-dimer level above the median, with 50% above the 75th percentile (Reitter et al, 2016). D-dimer levels also decreased in those patients who finished the study in complete remission, an observation reflective of the biology of cancer and suggesting that the hypercoagulability state observed in many patients with cancer has diminished in line with tumour burden.

This confirms the work of Posch et al (2020) who measured D-dimers on a monthly basis in a cohort of 167 patients with various cancer types, and determined that D-dimer levels rose an average of 34% a month in those who subsequently developed a VTE, whereas more modest monthly increases of 2.6% were seen in those who did not develop a VTE (Posch et al, 2020).

This study confirms the findings of several other studies however, who did not detect any trends or patterns in D-dimer levels in patients with cancer prior to the diagnosis of a thrombotic event. These include the work of von Tempelhoff et al (1998) who studied 47 patients diagnosed with ovarian cancer, two papers by Kirwan et al (2008 and 2009), who studied 123 patients diagnosed with breast cancer, and the work of van Es et al (2018) (117 patients, all cancer types), and finally the ROADMAP-CAT study (Syrigos et al (2018) (150 patients, lung cancer).

In this study, D-dimers could not be used for the prediction of CAT.

5.7.2 sP-selectin for the prediction of CAT

Soluble P-selectin (sP-selectin) is a cell adhesion molecule which is found in soluble form after platelet and endothelial cell activation. It is therefore thought that levels increase at the time of a thrombosis. sP-selectin features on two risk assessment models for CAT; the Vienna CATS score (Ay et al, 2010) and the Thrombo-Nsclc score (Castellón-Rubio et al, 2020).

For sP-selectin we found no statistical significance in either baseline, 1 month or 3 months levels. However, the fold change between baseline and 1 month was found to be statistically significant ($p = 0.0310$) with all results increasing, whereas the fold change from baseline to 3 months was not, despite all results decreasing from baseline. Further analysis showed that at the point of thrombosis sP-selectin was rising for 2 participants (2/6) (33%) and decreasing for one participant (1/6) (17%). For three participants (3/6) (50%) the trend could not be established. Two of these three participants had 1-month and 3-month sampling bloods taken but figures were excluded due to falling outside of the sampling point parameters. In the non-thrombosis population, there is a similar number of fluctuations in sP-selectin levels, with high 95% confidence limits demonstrating a high variation in data (Table 5.6).

Our findings agree with those seen in previous studies, with no correlation in sP-selectin levels between those with or without thrombosis seen by either Reitter et al (2016), van Es (2018) or Syrigos et al (2018). In both the work of Reitter et al (2016) and Syrigos et al (2018) sP-selectin values decreased over the course of the study,

which was also seen in this study. This could represent a decrease in the hypercoagulability state of these patients and likely indicates that their treatment regime is working, and that the cancer burden is diminishing and reflects what is known about cancer biology.

In this study, sP-selectin levels showed a statistically significant increase from baseline to 1 month in thrombosis patients. However, the sample number of patients experiencing a thrombosis was small (n = 3) (5.5% of total study population), limiting the power that these findings have, and therefore further work is required. Over the length of the study however, sP-selectin levels decreased as has been observed in other studies.

5.7.3 Serum and plasma VEGF for the prediction of CAT

Vascular Endothelial Growth Factor (VEGF) is a potent growth factor, which is released from its stores by both endothelial and platelet activation. Its measurement does not feature in any CAT risk assessment models. In this study, VEGF was measured in both serum and plasma as previous work did not indicate that one parameter was superior to another for the prediction of CAT. See Chapter 4 (Results Chapter 2) for a meta-analysis examining this.

We observed no statistically significant differences between either serum or plasma VEGF across each of the sampling points, nor the fold changes between each

sampling point between the two groups. No changes in VEGF levels were observed in either the thrombosis population, or the non-thrombosis population.

At the point of thrombosis, for one participant (1/6) (17%) both serum and plasma VEGF levels were increasing, and for two participants (2/6) (33%) both serum and plasma VEGF levels were decreasing. For three participants (3/6) (50%) patterns could not be determined for either serum or plasma VEGF due to a lack of data.

Only one previous study has examined the serial measurement of VEGF for the prediction of CAT (Kirwan et al, 2008 & 2009) and found that increased levels at baseline were predictive of CAT and that an increase from baseline levels was associated with an increased risk of VTE. However, this association was not found in this study. In addition, an increase from baseline levels was associated with an increased risk of VTE. Again, in this study that association was not found.

In this study, neither serum nor plasma VEGF could be used for the prediction of CAT.

5.7.4 Limitations

There are several limitations to our study which require comment. The study population is small, with only fifty-four participants and encompasses a range of cancer types and stages. Whilst the total number of samples collected was greater, several sets had to be excluded from analysis due falling outside the defined sample-collection windows. Whilst this strengthened our study design by decreasing the

amount of variability in the data, it also decreased the amount of data which was available for analysis. In total, once exclusion had taken place, this left a total of only thirteen sets of complete data, where the participant had baseline, 1-month and 3-months bloods analysed.

In addition, the gender balance was not equal, with more female participants (37/54) (68.52% of the total), and all of those who experienced a thrombotic event were female. The removal of males from the total study population was therefore considered to ensure that no skewing of data or bias would occur but ultimately it was decided to include them. The main reasoning for this was a lack of sex-specific reference ranges for the parameters examined, and therefore no evidence that the biomarker levels would be different between sexes, and therefore potentially skew any findings.

In addition, the ethnicity make-up of the study population does not reflect the local population. 94.4% (51/54) of the study population was White British, with the remainder of the study population made up of White Irish, and Other – Not stated, or Not known. According to the 2021 National Census, 91.7% of the population of Newcastle upon Tyne is White, whereas in this study 96.3% (52/54) are. There is no representation from either the Black, Asian or Mixed communities which make up 1.2%, 4.4% and 1.4% of the local population respectively. Therefore, whilst this study has examined a local population, the cohort is not accurately representative of this.

The small numbers of samples involved meant that whilst statistical analysis could be performed in many cases, and statistically significant results could be found, further work involving a greater number of participants will be required to confirm or repute these findings.

5.7.5 Conclusions

The aim of this study was to determine if the serial measurement of four biomarkers, D-dimers, sP-selectin, serum VEGF and plasma VEGF could be used to predict cancer-associated thrombosis.

Firstly, each of the three sampling points was examined in turn to establish if any one in isolation could predict CAT. We found that there was a statistically significant difference between D-dimer results in the thrombosis and non-thrombosis population at the 1-month sampling point ($p = 0.0127$). All other biomarkers showed no statistically significant differences.

Next, we used fold change to standardise any changes seen in the four biomarkers between each of the sampling points. Only sP-selectin between baseline and 1-month sampling points showed a statistically significant difference ($p = 0.0310$), with results increasing from baseline to 1-month in those who experienced a thrombosis. All other biomarkers, and fold changes between sampling points were not statistically significant.

Whilst we found these statistically significant findings however our study design has limitations and suggests that further work is required.

To improve and confirm the findings in this study, a larger study population is required. Once we had excluded several data points due to previously outlined flaws, our six participants had only a few data points available for statistical analysis. 50% (3/6) of the thrombosis population had only baseline bloods eligible for analysis and therefore fold change could not be performed. This meant that any findings require confirming with further studies.

In addition, our study population was not wholly representative of the local population, with a gender imbalance and ethnic minorities not represented. This will have created biases in our findings.

Therefore, further work is required to assess whether the serial measurement of biomarkers is useful in predicting cancer-associated thrombosis.

5.8 Chapter Summary

Overall, this small prospective clinical trial has identified one statistically significant observation, and larger studies are required to determine the significance of this finding. Some primary cancer sites were not represented, and numbers of participants were small. Suggestions for further work could be to see if the findings presented here could be replicated in a larger group of patients diagnosed with cancer, and to see if there are any observations made with specific cancer types.

CHAPTER 6. DISCUSSION

6.1 Overall summary of findings

This thesis had an overall aim of evaluating current methods of predicting cancer-associated thrombosis and to assess whether these were effective for patients.

Throughout this thesis we have seen that there are many different proposed methods of predicting CAT, but that these do not appear to be able to predict all cases.

Cancer-associated thrombosis is sadly an increasingly common complication in patients who are diagnosed with cancer. Rates are thought to be increasing (Mahajan et al, 2022; Mulder et al 2021), and whilst research has allowed us to determine those at the highest risk, unfortunately many cases still occur in those outside of this group (Mulder et al, 2019). When CAT occurs, it is associated with poor survival (Sørensen et al, 2000; Crobach et al, 2023). Therefore, if we could improve prediction of CAT, and prescription of thromboprophylaxis to those at the highest risk, then this could reduce mortality, and the demand on healthcare resources. This is of particular importance within a taxpayer-funded healthcare system such as the NHS.

6.1.1 Evaluation of current risk assessment models

In Chapter 3 of this thesis, we sought to evaluate one widely used risk-assessment model, the Khorana score (Khorana et al, 2008), in a group of pancreatic cancer

patients classified by the Khorana score as at a high risk of CAT. This retrospective clinical audit examined the medical records of 75 patients who received treatment for pancreatic cancer within The Newcastle upon Tyne Hospitals NHS Foundation Trust between July 2020 and July 2023. This particular risk assessment model was chosen for several reasons; the ease of calculations of the score, the small number of parameters required, routine clinical measurement of the required parameters and the availability of the scoring parameters for retrospective analysis, and its inclusion in several international guidelines - the European Society of Cardiology (ESC) (Lyon et al, 2022), European Society for Medical Oncology (ESMO) (Falanga et al, 2023) and American Society of Clinical Oncology (ASCO) (Key et al, 2023) for the prediction of CAT. In addition, whilst multiple international guidelines (American Society of Haematology (ASH) (Lyman et al, 2021), ESC (Lyon et al, 2022), ESMO (Falanga et al, 2023), ASCO (Key et al, 2023) and the British Society for Haematology (BSH) (Alikhan et al, 2024)) recommend the routine prescribing of thromboprophylaxis in this high-risk group of patients, it is not yet standard practice within The Newcastle upon Tyne Hospitals NHS Foundation Trust. The aim of the audit was to inform practice within The Newcastle upon Tyne Hospitals NHS Foundation Trust.

Overall, the rates of CAT were very high in this small audit of 75 pancreatic cancer patients (31.3%) supporting evidence that these patients are at a very high risk of CAT. This figure is higher than those reported overall in many cancer types within the published literature (Chew et al, 2006; Blom et al, 2006; Stein et al, 2006; Cronin-

Fenton et al, 2010; Walker et al, 2013; Köningsbrügge et al, 2014; Mulder et al, 2021; Mahajan et al, 2022). Several factors could be at play regarding this including the socioeconomic status of the local region, with the North East of England being one of the most deprived regions in England (Benet Institute for Public Policy, 2019; Office for National Statistics, 2021). Despite the high incidence of CAT in the population none of the parameters that contribute to the Khorana score nor the full score, were observed to be different between those who developed a thrombosis and those that did not. Therefore, analysis of the audit data demonstrated that the Khorana score could not be used to predict CAT in this high-risk audit population, and that thromboprophylaxis should be considered for all patients with a pancreatic cancer diagnosis, as per published guidelines.

Unlike the Khorana score, other RAMs typically require non-standard clinical and laboratory data. Due to a lack of recorded clinical and laboratory data for, for example, D-dimers and soluble P-selectin (sP-selectin), we could not evaluate any of the other published risk assessment models and compare them to the Khorana score in our patient population. However, the overall population of patients diagnosed with cancer used to derive the Khorana Score was small and only included a handful of patients who had a pancreatic cancer diagnosis (<2% of the total cohort; 19 patients) (Khorana et al, 2008) and therefore a criticism of it is that is not representative of all patients with cancer, and therefore might not be that effective in specific cancer populations.

Alternative risk assessment scores exist and include other, alternative biomarkers. These include the Vienna CATS score (Ay et al, 2010) which utilises the measurement of D-dimers and sP-selectin at baseline. The Vienna CATS score appears to be able to discriminate between high and low risk populations better than the Khorana score (van Es et al, 2017b; Mosaad et al, 2021) with the 6-month VTE risk in high-risk patients 9.1% with the Vienna CATS score versus 6.5% with the Khorana score (van Es et al, 2017b). However, the incidence of VTE was greater in the low-risk population than it was in the high-risk population for both the Khorana score and the Vienna CATS score (van Es et al, 2017b; Mulder et al, 2019) suggesting that the additional biomarkers make little difference with regards to predictive power, and that the current risk assessment models are not very good for use in high-risk populations.

These alternative risk assessment models have also been assessed in a variety of other cancer types. The Khorana score was derived using a cohort of just 2701 patients with a variety of cancer types, breast cancer being the most numerous (34.6% of the total cohort; 936 patients) (Khorana et al, 2008), and as a result has been criticised for not been specific enough and not considering the varying biology's of different cancer types (Mulder et al, 2019). Therefore, RAMs for specific cancer types have been proposed, the most numerous of which include lung cancer (Gerotziafas et al, 2018; Syrigos et al, 2018) and multiple myeloma (Palumbo et al, 2007; Li et al, 2019; Sanfilippo et al, 2019; Chakraborty et al, 2022). This suggests the

need for cancer-type specific RAMs and may explain why the Khorana score performed so poorly in the audit population examined in this thesis.

Taken together, the data presented has indicated that alternative novel biomarkers or measures of biomarker levels need to be sought which may have more predictive potential than those examined in the high-risk audit population.

6.1.2 Novel biomarkers associated with CAT

Having established that the Khorana score could not predict CAT in our audit population, assessment of novel biomarkers with potential use in the prediction of CAT was conducted. Vascular Endothelial Growth Factor (VEGF) is a marker of both endothelial and platelet activation, similar to the currently used soluble P-selectin (sP-selectin), a biomarker which appears in both the Vienna CATS score (Ay et al, 2010) and the Thrombo-Nsclc score (Castellón-Rubio, 2020).

VEGF is a potent angiogenic factor, which is required for the growth and metastases of tumours (Dogan and Demirkazik, 2005) and therefore plays an important role in the pathogenesis of cancer.

VEGF also plays a role in haemostasis, both within endothelial cells, by inducing the release of Tissue Factor and von Willebrand Factor (vWF), but also in platelets, being released from the alpha granules upon platelet activation.

Its dual role in both cancer and thrombosis therefore make VEGF an excellent candidate as a biomarker for CAT. However, despite this VEGF does not currently used in any risk assessment model for CAT.

VEGF as a biomarker of CAT was therefore investigated. A systematic review and meta-analysis of published studies and data investigating the potential for VEGF to be used as a biomarker for the prediction of CAT is presented in Chapter 4.

Data from eight studies was synthesised to examine if serum or plasma VEGF showed any statistically significant differences in levels between those who experienced a thrombosis, and those who did not.

This meta-analysis established that whilst VEGF was found to be increased at the time of active thrombosis, there was not enough evidence to suggest that it could be used to predict a thrombotic event. However, as outlined within the meta-analysis (Chapter 4) there is very little published research which examines the potential for VEGF to be used as biomarker to predict thrombosis (Kirwan et al, 2008 and 2009; Posch et al, 2016).

As previously discussed, there is evidence to suggest that specific, tailored risk assessment models may be derived for a specific population to improve its predictive power, for example, a specific cancer type. Within the meta-analysis the data used came from a variety of cancer types, though some, rarer cancer types will not have

been represented. Therefore, it is plausible that VEGF may have more predictive potential when used for a specific cancer type.

Therefore, further studies with a variety of cancer types are required to see if this biomarker could be used in the prediction of CAT.

6.1.3 Serial Measurement of Biomarkers for CAT prediction

Despite D-dimers, sP-selectin and VEGF all having been shown to be associated with thrombosis, individually they do not appear to be predictive of CAT or add additional benefit to current published RAMs. Therefore, we next sought to establish if the serial measurement of four biomarkers, including both serum and plasma VEGF, but also D-dimers and sP-selectin could help to predict those individuals at the highest risk of CAT. Following our meta-analysis, previous research indicated that there is no consensus with regards to sample type for VEGF analysis, and therefore both were analysed in a cohort of 54 cancer patients at The Newcastle upon Tyne Hospitals NHS Foundation Trust.

Neither levels nor fold change of D-dimer, sP-selectin and VEGF from baseline to 1-month and 3-months from diagnosis could predict who would develop a thrombosis.

Our evaluation of the serial measurement of biomarkers has shown that, in our study population, serial measurement of D-dimers, sP-selectin and serum and plasma VEGF cannot be used to predict CAT.

6.1.4 Conclusions of work performed for thesis

Taken together, the results obtained in this thesis have shown that the Khorana Score RAM and individual measurement of VEGF, D-dimer and soluble P-selectin biomarkers are not effective at predicting CAT in the different populations studied. Whilst some studies have concentrated on one cancer type, and have seen improvements in the prediction of CAT, they are still not without their limitations, and further studies and evaluations are required to improve their predictive power.

The work presented in this thesis has established that the prediction of CAT is not without difficulties. The predictive model used to predict CAT in a high-risk population showed limited predictive power, and both established and novel biomarkers, both in isolation and serially, could not predict CAT in a small cohort. Whilst some risk assessment models have been established for only a specific cancer type (for example, ONCO-THROMB for non-small cell lung cancer (Muñoz et al, 2023) and the Myeloma-specific RAMs – IWMG (Palumbo et al, 2017), SAVED (Li et al, 2019), IMPEDE-VTE (Sanfilippo et al, 2019) and PRISM (Chakraborty et al, 2022)) and appear to work better in that individual scenario, these still cannot predict all cases of CAT.

Guidelines recommend targeted thromboprophylaxis for those at the highest risk. However, this is difficult to implement, as, as demonstrated by the tools used in this thesis, there does not appear to be a universal method for establishing risk. This thesis has demonstrated that one risk assessment model, the Khorana score, could

not predict CAT in a high-risk population, nor the use of a novel biomarker, nor the serial measurement of known and novel biomarkers could predict those who would develop a thrombosis. However, the addition of VEGF, with a suggested cut-off of results falling outside of the reference range (greater than 770 pg/mL) to a risk assessment model may enhance prediction.

6.2 Role of thromboprophylaxis

Having established that the Khorana score in a high-risk population, the use of a novel biomarker (VEGF), and the serial measurement of VEGF, D-dimers and sP-selectin cannot be used for the prediction of CAT in this study population, a challenge remains for the clinical management of thrombotic risk in patients.

Thromboprophylaxis, the administration of anticoagulants to prevent a thrombotic event, is well established in several other disease groups. These include patients with atrial fibrillation (NICE, 2021), congenital heart disease (Paiva et al, 2025) those with antiphospholipid syndrome (NICE, 2023), and in other high-risk groups such as those who have recently undergone surgery (NICE, 2019). Its use within patients with cancer however is poorly established and is largely driven by international guidelines which suggests its use in high-risk patients, as established by risk assessment scores. However, within this thesis we have seen that these do not accurately predict those who will experience a thrombotic event in all populations.

Both the AVERT (Carrier et al, 2019) and CASSINI (Khorana et al, 2019) clinical trials examined the role of the Khorana score to assess risk, and the use of thromboprophylaxis (Apixaban and Rivaroxaban respectively) in a variety of cancer types.

The AVERT trial (Carrier et al, 2019) prescribed apixaban to 288 ambulatory patients with cancer of all types who had a Khorana score greater than or equal to two. 275 patients with the same criteria received a placebo. 4.2% (12/288) of patients receiving apixaban experienced a VTE, whereas 10.2% (28/275) of patients in the placebo group did. Major bleeding rates were higher in the apixaban group than the placebo group (3.5% versus 1.8%). The number of individuals needed to treat (NNT) to prevent one VTE was 17 (Carrier et al, 2019).

In the CASSINI trial (Khorana et al, 2019), 841 ambulatory patients with high-risk cancers (32.6% pancreatic cancer, 54.5% metastatic disease) and a Khorana score equal to or greater than 2 were assigned to receive rivaroxaban (420 patients) or a placebo (421 patients). 6.0% (25/420) of those in the rivaroxaban group experienced a VTE, whereas 8.8% (37/421) of those in the placebo group did (hazard ratio, 0.66; 95% confidence interval 0.40 to 1.99; p = 0.10). Major bleeding occurred in 2.0% of the rivaroxaban group and in 1.0% of the placebo group. Therefore, the authors concluded that treatment with rivaroxaban did not result in a significant lowering of the VTE incidence in a high-risk population. In addition, the number of individuals needed to treat (NNT) to prevent one VTE episode was 36 (Khorana et al, 2019).

Taken together, the results of these two clinical trials suggest that whilst the use of thromboprophylaxis does reduce the rate of VTE as compared to a placebo, it does not prevent all episodes, and the rates of bleeding are higher in the thromboprophylaxis groups. The NNT figures are also high for both trials, suggesting that the Khorana score may not be able to accurately identify individuals who are at the highest risk, which is supported by the findings described in Chapter 3. Rates of VTE for patients designated as low risk by the Khorana score were not recorded.

Alternative approaches to determine the efficacy of thromboprophylaxis in patients with cancer in large clinical trials have included blanket use to all participants regardless of perceived risk, and the use of alternative risk assessment models derived by the authors.

Two large studies, the PROTECHT (Agnelli et al, 2009) and SAVE-ONCO (Agnelli et al, 2012) trials compared low molecular weight heparin (LMWH) prophylaxis to placebo.

In a randomised, placebo-controlled, double-blind study, the PROTECHT trial (Agnelli et al, 2009), 769 patients with cancer of various types (lung, gastrointestinal, pancreatic, breast, ovarian, hand and neck) received nadroparin (a LWMH derivative), and 381 patients with the same characteristics received a placebo. 2.0% (15/769) of patients receiving nadroparin experienced a thrombotic event, whilst 3.9% (15/381) of patients receiving the placebo did. Incidences of minor bleeding and serious adverse events were similar in the two groups (7.4% and 15.7% versus 7.9% and 17.6%).

In the SAVE-ONCO trial (Agnelli et al, 2012) 1608 patients with cancer of various types (lung, pancreas, stomach, colon, rectum, bladder or ovary) and stages receiving chemotherapy received semuloparin (a LMWH derivative), and 1604 patients with the same characteristics a placebo. 1.2% (20/1608) patients on semuloparin developed a VTE, compared to 3.4% (55/1604) receiving a placebo (hazard ratio 1.40; 95% CI 0.21 to 0.60; p = <0.001). Bleeding rates were 2.8% and 2.0% respectively.

Taken together, these two studies suggest that thromboprophylaxis as prescribed widely can reduce the incidence of thrombotic events whilst the rate of bleeding is not significantly increased. Risk assessment models were not used in either clinical trial.

The TARGET-TP clinical trial (Alexander et al, 2023) studied 328 patients in Australia with a diagnosis of either lung or colorectal cancer. Patients were stratified into two groups based on their fibrinogen and D-dimer levels, taken at baseline, and after one cycle of chemotherapy. High-risk patients were defined as those with; a baseline fibrinogen greater than 4 g/L plus D-dimer levels greater than 500 ng/mL OR baseline D-dimer levels greater than 1500 ng/mL OR post 1 cycle D-dimers greater than 1500 ng/mL. High-risk patients were then assigned into one of two groups; to receive enoxaparin (a LMWH) (100 patients) or to receive a placebo (100 patients). Patients who did not meet the above criteria (128 patients) were observed only. Between the three groups, rates of VTE varied; 8% in the low-risk observation cohort, 8% in those received enoxaparin and 23% in the placebo group. Bleeding occurred in

12% (40/328) of all participants, with major bleeding in 3 of the low-risk observational group, 1 in the enoxaparin group, and 2 in the placebo group (Alexander et al, 2023). In this cohort, the use of targeted thromboprophylaxis based on biomarkers (D-dimers and fibrinogen) was found to reduce the rate of VTE to similar levels of those considered low risk, though rates were still high. Again, this demonstrates, that in this study, the use of a tailored risk assessment model for a specific cancer type in a local population may be of use.

Current opinion is to establish the group of patients who are at the greatest risk of CAT, and to prescribe thromboprophylaxis to these high-risk patients. Most international guidelines recommend routine thromboprophylaxis for select groups of individuals thought to be at the highest risk. For example, the British Society for Haematology guidelines (Alikhan et al, 2024) recommends thromboprophylaxis be offered to all pancreatic cancer patients, and intermediate to high-risk multiple myeloma patients following a risk assessment but does not recommend a specific risk assessment tool to use. Others however specifically suggest using the Khorana score to assign a risk including the European Society of Cardiology (ESC) (Lyon et al, 2022), International Initiative on Thrombosis and Cancer (ITAC) guidance (Falanga et al, 2023) and American Society of Clinical Oncology (ASCO) (Key et al, 2023) However, there is a reluctance to adhere to this for several reasons, which include a perceived higher risk of bleeding in these patients (despite the evidence presented here), and the knowledge that most thrombotic events occur outside of this high-risk

category. This was seen in practice in the clinical audit (Chapter 3), with a reluctance to alter practice for these high-risk individuals. In addition, despite risk assessment models being available, most thrombotic events in cancer continue to occur outside of these highest-risk populations. When comparing four RAMs (Khorana, Vienna CATS, PROTECHT and CONKO), van Es et al (2017b) noted that the cumulative incidence of CAT was highest in those assigned into a low-risk category. Further evidence is provided by a systematic review of the Khorana score, where Mulder et al (2019) found that only 23.4% of all thrombotic events occurring in patients with cancer were in those who had been classified as high-risk by the Khorana score (Mulder et al, 2019).

Therefore, further work is required to prevent CAT, as offering standard thromboprophylaxis to all ambulatory patients with cancer is not without its risks, nor is cost effective.

6.3 The use of biomarkers to predict thrombotic events in a non-cancer setting

Outside of the setting of cancer, there have been many clinical trials and studies which have examined the use of biomarkers, particularly D-dimers, to predict a thrombotic episode.

The use of D-dimers has been studied extensively with regards to the prediction of recurrent VTE outside of the setting of cancer, with one predictive model (the DASH

score) using D-dimer measurement to assign risk. This score contains four elements; **D**-dimers (abnormal levels after stopping anticoagulation), **A**ge (greater than 50 years), **S**ex (male) and **H**ormone therapy (was this associated with the VTE), with a higher score associated with a higher risk of recurrence (Tostetto et al, 2012). This score is used clinically to guide anticoagulation duration.

The PROLONG study (Legnani et al, 2008) measured D-dimers in 321 patients twenty to forty days after stopping warfarin following a VTE and found that D-dimer levels at this point could predict VTE recurrence.

Briz et al (2008) studied the use of the serial measurement of D-dimer levels to predict VTE recurrence in a non-cancer setting of 216 patients after stopping anticoagulation. D-dimer levels were measured at the point of stopping anticoagulation, four weeks later, and on each subsequent clinic visit. For those who had a recurrence of their VTE (10.7%, 23/216), 8 patients had repeatedly high D-dimer levels after stopping anticoagulation, 6 had normal D-dimer levels, then high at the last sampling point before recurrence, and three patients had D-dimer levels which were consistently within the normal range.

The DULCIS study (Palareti et al, 2014) used D-dimer levels to guide the duration of thromboprophylaxis in patients after a VTE. If D-dimers levels were persistently negative after stopping 'standard therapy' the risk of recurrence was low. The authors hypothesised that serial D-dimer measurement may be useful in clinical practice for

the identification of patients with VTE in whom anticoagulation can be safely discontinued (Palareti et al, 2014).

Outside of this setting, biomarkers have been studied to develop predictive models for the occurrence of VTE. Wu et al (2025) used the Caprini score plus molecular markers in 342 patients in China to predict DVT in patients with bone fractures. The Caprini score (Wilson et al, 2022) uses known risk factors for VTE to assign a risk for patients following surgery. Components include; BMI greater than 40 kg/m^2, current or history of malignancy, smoking, insulin-dependent diabetes, recent history of blood transfusion, history of miscarriages, family history of thrombosis, leg oedema, presence of a central venous catheter and recent stroke of myocardial infarction (Wilson et al, 2022). The efficacy of known thrombotic biomarkers was assessed, with D-dimer levels shown to be statistically significant (Wu et al, 2025).

D-dimers have also been shown to be associated with thrombotic risk in COVID-19 (COronaVirus Disease of 2019) patients. Woller et al (2022) produced a systematic review of biomarkers and their use in predicting VTE in COVID-19 patients and found that D-dimer levels were associated with VTE during hospitalisation (Woller et al, 2022). Five studies analysed in this paper using both univariate and multivariate analysis, and in both European and USA settings, found that the occurrence of VTE was associated with high D-dimer levels at admission.

Despite the observations made in this thesis in regards to CAT, previous work has shown that D-dimers show excellent predictive potential for thrombotic events.

Whilst these studies have not guided the use of thromboprophylaxis in these settings, in certain settings, such as in COVID-19 patients, thromboprophylaxis is utilised despite biomarker levels.

The role of the remaining two biomarkers studied in this thesis for predicting thrombotic events in the absence of cancer setting is less well studied.

Rectenwald et al (2004) studied sP-selectin, microparticles and D-dimers for the prediction of DVT. The single variable most predictive for thrombosis was sP-selectin, and with a threshold level of 0.68 ng/mL of total protein showed a sensitivity of 68%, specificity of 81% and accuracy of 74% (Rectenwald et al, 2004).

Swamy et al (2023) observed that in individuals following an DVT high sP-selectin levels in women were associated with DVT recurrence. However, the opposite was observed in men, with higher sP-selectin levels, associated with a lower chance of recurrence. This indicates potential sex differences, which should be considered for future investigation of CAT.

There is very little work performed on the use of VEGF as a predictive biomarker for thrombosis. Guerra-López et al (2022) examined the role of VEGF in predicting thrombosis in COVID-19 patients. 139 patients diagnosed with COVID-19 were comparted to 38 healthy controls. Similar to that observed in CAT as described in Chapter 4, patients with COVID-19 were found to have elevated VEGF levels compared to controls. 20% of the study population experienced a thrombotic event,

and although VEGF levels were correlated with inflammatory markers such as C-reactive protein (CRP), fibrinogen and ferritin they were not with the occurrence of a thrombotic event and were unable to predict these (Guerra-López et al (2022).

These studies indicate that further research is required to determine whether sP-selectin and VEGF have potential to be used for the prediction of thrombotic events outside of the setting of cancer.

In conclusion, whilst D-dimers have been shown to be useful in the prediction of thrombotic events in other settings, sP-selectin and VEGF have not. Therefore, findings from non-cancer settings are unlikely to be able to be transferred to a cancer setting.

6.4 Future developments for the prediction of CAT

Throughout this thesis we have established that the current risk assessment models show poor predictive power and have identified that whilst some new biomarkers show potential, insufficient evidence exists to demonstrate their utility in current RAMs and predictive models. Further work is therefore needed.

Exciting novel areas of research into methods for predicting CAT, will be reviewed herein.

6.4.1 Alternative risk assessment models

We have established within this thesis that published risk assessment models have limited predictive power and that most thrombotic events occur outside of the high-risk categories. Current studies are taking place to improve current risk assessment models.

Li et al (2023) used retrospective data from 9769 patients diagnosed with cancer in the USA to derive a modified Khorana score. The original Khorana score consists of five variables; cancer type, Body Mass Index (BMI), Haemoglobin, White Blood cell count and platelet count. In this modified score, the "high-risk" category was adapted, gaining two points instead of the current one point, and cancer types were modified. This included the inclusion of ovarian and uterine cancer (instead of the broader gynaecological term), multiple myeloma, aggressive non-Hodgkins lymphoma and soft tissue sarcoma. Cholangiocarcinoma and cancer of the gallbladder were added to the "very high-risk" category along with stomach and pancreas and gained 3 points. In addition, colorectal cancer was added as a specific cancer type, gaining one point. Finally, cancer stage, targeted or endocrine monotherapy, a personal history of VTE, history of paralysis or immobility in the past 12 months, recent hospitalisation, and Asian or Pacific Islander ethnicity were all added (Li et al, 2023). 79,517 patients in the USA were used to validate this score with the c statistic outperforming the Khorana score in both the derivation (9.769 patients) and validation cohorts (0.71 and 0.68 for the new RAM versus 0.65 and 0.60

for the Khorana score (Li et al, 2023). However, this new RAM is but it is yet to be externally validated.

Gomez-Rosas et al (2023) also proposed a modified RAM, known as the lung-HYPERCAN VTE risk score, for use in ambulatory lung cancer patients. This score uses D-dimer levels plus Eastern Cooperative Oncology Group (ECOG) performance score status to assign a risk (Gomez-Rosas et al, 2023). It has been externally validated and compared to the Khorana, CATS nomogram, PROTECHT and CONKO score, outperforming both the Khorana, PROTECHT and CONKO scores with regards to sensitivity (63% versus 21%, 59% and 26% respectively) and both the CATS nomogram and PROTECHT score with regards to specificity (74% versus 43% and 42% respectively) in 568 patients in Italy with non-small cell lung cancer (Gomez-Rosas et al, 2023).

These two new proposed risk assessment models suggest that adaptations to risk assessment models may be required for specific cancer types and populations to improve predictive power.

6.4.2 MicroRNAs

MicroRNAS (miRNA) are involved in a range of biological processes including haemostasis and are commonly dysregulated in disease states (Anjis et al, 2023). They are small, non-coding RNAs which regulate gene function and expression by binding to mRNAs (Anjis et al, 2023). miRNAs are found in the blood and easily

accessible in a blood sample, and other body fluids such as urine and saliva making it easy to gather samples for analysis. There have been several studies (Starikova et al, 2015; Wang et al, 2019; Thibord et al, 2020; Rodriguez-Ruis et al, 2020) which have looked at the potential use of miRNAs as predictive biomarkers for VTE in the absence of cancer and identified several circulating miRNAs which appear to be associated with VTE. However, only two of the miRNAs (has-miR-27b-3p and hsa-miR-222-3p) which are said to associated with VTE appear to overlap between two of the four studies (Wang et al, 2019 and Thirbord et al, 2020), suggesting that further work is required. Despite this, Rodriguez-Ruis et al (2020) developed a risk model based on four miRNAs (has-miR-885-5p, has-miR-194-5p, has-miR-126-3p and has-miR-192-5p), age and sex to determine an individual's risk of VTE (Rodriguez-Rius et al, 2020), and which when validated in 935 Spanish patients produced a sensitivity of 85.7%, and a specificity of 41.1% (Rodriguez-Ruis et al, 2020). Building on these findings, studies investigating miRNAs for the prediction of CAT have been published and which have identified multiple different miRNAs which are downregulated. Whilst Starikova et al (2017) and Oto et al (2021) studied patients with various types of cancer, Oto et al (2020a) measured miRNAs in both pancreatic cancer and cholangiocarcinoma patients, Oto et al (2020b) studied only patients with intracranial tumours, whereas Kim et al (2019) and Anjis et al (2022) studied colorectal cancer patients, which may explain the variety of findings published. All the miRNAs associated with CAT are involved in either the pathogenesis of cancer, or

complement or haemostatic pathways, which is understandable. Again, however, there are very few overlapping findings between studies, suggesting that further research in this area is required.

This area looks to be promising, with several miRNAs found to be downregulated in CAT, although further work in a variety of cancers is required before predictive models can be established.

6.4.3 "Liquid biopsies"

The role of "liquid biopsies", testing for the presence of circulating tumour DNA (ctDNA) is also promising for cancer diagnosis. The presence of ctDNA also is associated with more advanced stages of disease, and these biopsies have been widely implemented for cancer diagnosis and detection (Connal et al, 2023; Ma et al, 2024), but their use for VTE prediction is unknown (Jee et al, 2024).

Cell free DNA (cfDNA) is associated with the formation of neutrophil extracellular traps (NETs) which are also associated with CAT as described in Chapter 1 (Introduction) of this thesis. Thus, the presence of cfDNA (which could originate either from the tumour (ctDNA) or be wild-type (original non-cancer DNA) could indicate the likelihood, or prediction, of CAT.

Jee et al (2024) analysed three cohorts of patients with a view to establishing if the presence of ctDNA could be used in the setting of CAT. They found that ctDNA (tumour DNA) detection was associated with VTE independent of clinical or

radiographic features (Hazard ratio (HR) = 2.49, 95% confidence interval (CI): 1.99-3.11, P = <0.001 (Jee et al, 2024)). To go further, a machine learning model trained on liquid biopsy data and this was compared to the Khorana score in all three cohorts, with the liquid biopsy model outperforming the Khorana score in all three cohorts (discovery, validation and generalisability), with the c-indices for the three cohorts 0.74, 0.73 and 0.67 for the machine-learning model versus 0.57, 0.61 and 0.54 for the Khorana score (Jee et al, 2024).

In further evidence, Ma et al (2025) also found in a cohort of 1038 patients with all cancer types that the presence of ctDNA predicts CAT and was independently associated with VTE even after adjusting for clinical variables (Ma et al, 2025 (abstract only)).

The potential of using ctDNA as a means of predicting CAT is exciting, though research is still ongoing.

6.4.4 Role of machine learning and artificial intelligence

Finally, the role of machine learning and artificial intelligence is also worth exploring. Machine learning "uses computer algorithms to recognise patterns in data and make predictions without preprogrammed instructions" (Chen et al, 2025 pp. 610). Therefore, it appears to be well-suited to be able to identify patterns and risk factors that will support the prediction of CAT.

As previously discussed throughout this thesis, there are multiple different factors at play in being able to predict cancer-associated thrombosis. These include cancer-related factors such as type and stage, cancer-associated factors such as treatments including chemotherapy, radiotherapy, hormone therapy and surgery, and patient-related factors including age, sex, BMI, and personal or family history of VTE. Some of these factors are constantly changing, and where a biomarker may be at one level before the start of chemotherapy it is likely to have changed throughout the course of treatment. This is summarised by the following statement "CAT risk is dynamic and continuous risk assessment would be beneficial to account for variations in risk with time" (Patell et al, 2024 pp.3). Currently used risk assessment models do not take changing parameters into account. However, the recently proposed CATS nomogram RAM does. The CATS nomogram (Pabinger et al, 2018) uses tumour-site risk category and continuous D-dimer concentration to constantly predict the risk of CAT. The RAM was constructed using machine learning, demonstrating its potential.

A machine learning model could be capable of accessing and constantly updating information regarding a patients' risk, and therefore, once trained, could provide a real-time risk for individuals', allowing thromboprophylaxis to be administered where required. It is envisaged that machine learning could be applied to the field of CAT in three different ways; through the identification of thrombotic complications by nature language processing, using computer vision to classify radiology images, and using predictive machine learning models to predict CAT (Patell et al, 2024).

Jin et al (2022) compared five machine-learning algorithms (linear discriminant analysis, logistic regression, classification tree, random forest and support vector machine and compared these to the Khorana score, using a random selection of 2100 patients in China. They found that eleven variables or predictors could be used to develop a training set for the model; length of stay in hospital, Charlson Comorbidity Index (which predicts 10-tear survival in patients with multiple comorbidities), type of chemotherapy, presence of a Port-Cath, administration of NSAIDs (Non-Steroidal Anti Inflammatory Drugs), VTE history, length of time on bedrest, if the patient was in plaster, and two biomarkers – D-dimers and WBC. Thus, this machine-learning model used many elements of previously published risk assessment models, but in a state which was constantly evolving, and considering a changing thrombotic risk. Two out of the five machine learning models (linear discriminant analysis and logistic regression, AUC = 0.773 and 0.772 respectively) outperformed the Khorana score (AUC = 0.642) (Jin et al, 2022).

He et al (2024) used a longitudinal machine learning system to predict the risk of CAT throughout cancer treatment. The cohort studied was thoracic and gastrointestinal cancers only, and the system was trained to examine CT scans and Doppler scans for the presence of CAT using open-source large language models (He et al, 2014). Following this, longitudinal machine learning systems were trained to predict CAT within 90 days of each cancer treatment, and achieved a, AUROC of 0.651 (95% CI 0.620 – 0.678) (He et al, 2014).

Mantha et al (2024) used dynamic modelling with deep learning to construct a neural network model to predict CAT. Predictors included age, sex, cancer type, time since cancer diagnosis, follow-up time, chemotherapy received, presence of metastatic disease, WBC, haemoglobin, platelet count, mean cell volume and all components of the complete metabolic profile (Mantha et al, 2024). Using two cohorts, one for derivation and the other for validation, Harrel's c-index was 0.75 and 0.73 for the two cohorts after 28 days of observation, suggesting that the model showed good predictive ability, although further research is required and alongside more detailed comparison to other risk assessment models.

Therefore, the role of machine learning and artificial intelligence may be an option in the future when predicting the risk of CAT.

Whilst these methods show potential and promise however, they are also expensive, with estimated costs from $40,000 USD to $100,000 USD for a modest machine learning model (Alkhaldi, 2024). It remains to be seen, when further research is performed and completed, if a health service, such as the National Health Service in the United Kingdom, would be able to afford this. Therefore, other options may have to be relied upon.

6.5 Conclusions

This thesis has examined the role of current and novel approaches for predicting cancer-associated thrombosis and has established that the Khorana score, serum and plasma VEGF and serial measurement of D-dimers, sP-selectin and VEGF levels have

limited to no predictive potential in the populations studied. The widely used Khorana score was not found to be sensitive enough especially in high-risk patients and likely misses many cases of CAT. New or novel biomarkers, such as VEGF, and repeat sampling strategies, have predictive potential, but further investigations, particularly in larger scale single cancer populations are required to provide confirmative evidence of their utility.

6.6 Chapter Summary

This Chapter has examined possible future directions which could be taken in the prediction of cancer-associated thrombosis. Whilst some statistically significant observations were made during this thesis, further work in required in a larger group of patients diagnosed with cancer. Further work is also suggested in specific cancer types, to see if any novel observations can be seen.

It is the author's opinion that the most exciting area of potential future development in the prediction of cancer-associated thrombosis lies with machine learning. The mechanisms surrounding cancer-associated thrombosis are very varied, and risks change over time in a patients' cancer journey. Machine learning would take this into account and provide an up-to-date risk profile for a patient, thus allowing the administration of thromboprophylaxis, and thus potentially saving lives. However, the technology comes at a cost, and it would therefore be difficult to pursue in a taxpayer-funded system such as the NHS.

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APPENDIX 1. HEALTH RESEARCH AUTHORITY

(HRA) APPROVAL LETTER

Ymchwil Iechyd a Gofal Cymru
Health and Care Research Wales

NHS
Health Research Authority

Dr Kathryn M Musgrave
Consultant Haematologist & Honorary Clinical Senior Lecturer
The Newcastle upon Tyne Hospital NHS Foundation Trust
Department of Haematology
Royal Victoria Infirmary
Queen Victoria Road, Newcastle upon Tyne
NE4 1LP

Email: approvals@hra.nhs.uk
HCRW.approvals@wales.nhs.uk

17 August 2023

Dear Dr Musgrave

HRA and Health and Care Research Wales (HCRW) Approval Letter

Study title:	**Identifying Thrombotic Markers of Cancer-Associated Thrombosis (TM-CAT)**
IRAS project ID:	**301825**
REC reference:	**23/PR/0729**
Sponsor	**The Newcastle upon Tyne Hospitals NHS Foundation Trust**

I am pleased to confirm that **HRA and Health and Care Research Wales (HCRW) Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

Please now work with participating NHS organisations to confirm capacity and capability, in line with the instructions provided in the "Information to support study set up" section towards the end of this letter.

How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?
HRA and HCRW Approval does not apply to NHS/HSC organisations within Northern Ireland and Scotland.

If you indicated in your IRAS form that you do have participating organisations in either of these devolved administrations, the final document set and the study wide governance report

(including this letter) have been sent to the coordinating centre of each participating nation. The relevant national coordinating function/s will contact you as appropriate.

Please see IRAS Help for information on working with NHS/HSC organisations in Northern Ireland and Scotland.

How should I work with participating non-NHS organisations?
HRA and HCRW Approval does not apply to non-NHS organisations. You should work with your non-NHS organisations to obtain local agreement in accordance with their procedures.

What are my notification responsibilities during the study?

The standard conditions document "*After Ethical Review – guidance for sponsors and investigators*", issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

Who should I contact for further information?
Please do not hesitate to contact me for assistance with this application. My contact details are below.

Your IRAS project ID is **301825**. Please quote this on all correspondence.
Yours sincerely,

Harriet Wood
Approvals Specialist
Email: approvals@hra.nhs.uk

Copy to: *Elaine Chapman, Newcastle upon Tyne Hospitals (NuTH) NHS Foundation Trust*

APPENDIX 2. ETHOS APPROVAL LETTER

11/02/2025

Project Title: Identifying Thrombotic Markers of Cancer-Associated Thrombosis (TM-CAT)

EthOS Reference Number: 60179

Certification

Dear Brown,

The above application was reviewed by the Research Ethics and Governance Team and on the 11/02/2025, was certified. The certification is in place until 01.05.2025 and is based on the IRAS documentation submitted with your application.

Application Documents

Document Type	File Name	Date	Version
External Approval Supporting Information	23PR0729 301825 Favourable_opinion_on_further_information 17 August 2023	17/08/2023	1
External Approval Supporting Information	HEE Letter re Training Allowance for the Higher Specialist Scientist Training Programme	22/12/2024	1
External Approval Supporting Information	A Unsworth CV 2024	01/01/2024	1
External Approval Supporting Information	Kate Musgrave CV research 2024 10 11	11/10/2024	1
External Approval Supporting Information	22-23 TWIMC Combined Letter - University of Newcastle - NHE-08CA03-0013	06/01/2025	1
External Approval Supporting Information	TM-CAT Consent 1.0 2023-08-02	02/08/2023	1.0
External Approval Supporting Information	TM-CAT Consent 1.0 2023-08-02 TC	02/08/2023	1.0
External Approval Supporting Information	TM-CaT Protocol 1.0 2023-08-02 no TC	02/08/2023	1.0
External Approval Supporting Information	TM-CaT Protocol 1.0 2023-08-02	02/08/2023	1.0
External Approval Supporting Information	TM-CaT PIS 1.0 2023-08-02	02/08/2023	1.0
External Approval Supporting Information	TM-CaT PIS 1.0 2023-08-02 TC	02/08/2023	1.0
External Approval Letter	301825 Letter_of_HRA_Approval 17 August 2023	17/08/2023	1
External Approval Application Form	IRASForm (2)	22/06/2023	1.0

Conditions of certification

The Research Ethics and Governance Team would like to highlight the following conditions

Adherence to Manchester Metropolitan University's Policies and procedures

This certification is conditional on adherence to Manchester Metropolitan University's Policies, Procedures, guidance and Standard Operating procedures. These can be found on the Manchester Metropolitan University Research Ethics and Governance webpages.

Amendments

If you wish to make a change to this approved application, you will be required to submit an amendment in accordance with **HRA and REC** guidelines. Please contact the Research Ethics and Governance team for advice around how to do this.

We wish you every success with your project.

Research Ethics and Governance Team

APPENDIX 3. PARTICIPANT CONSENT FORM

CONSENT FORM: Thrombotic Markers of Cancer-associated Thrombosis

Participant Number: _____

Researchers: Dr Kathryn Musgrave and Prof John Simpson

	Please initial in the box
1. I confirm that I have read and understood the information sheet dated 19/01/2024 (version 1.1) for the above study. I have had the opportunity to consider the information, ask questions, and have had these answered satisfactorily.	☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected.	☐
3. I understand that relevant sections of any of my medical notes and data collected during the study may be looked at by responsible individuals from regulatory authorities or from the NHS Trust. I give permission for these individuals to have access to my records for up to 2 years and understand that all information will be treated as confidential and in a way compliant with the Data Protection Act.	☐
4. I agree to a 19 mL blood sample being taken on three occasions.	☐
5. I agree to storage, in an anonymised fashion, of my blood samples for 5 years	☐
6. I agree to storage of information about my medical condition for 5 years	☐
7. I agree that my samples may be used in future studies, on condition that I understand the nature of any further research and the types of tests that will be done, that I cannot be identified from my samples, and that new ethical approval is granted for those studies.	☐
8. If I become more unwell and I am no longer able to understand that the study is continuing, I agree that all the information and samples already collected can still be used.	☐

Name of Patient	Signature	Date
Name of Person taking consent	Signature	Date

Filing instructions: 1-partcipant copy; 1-site file; 1-medical records

APPENDIX 4. PARTICIPANT INFORMATION SHEET

Identifying thrombotic markers of cancer-associated thrombosis

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Ask us if there is anything that is not clear or if you would like more information.
Thank you for reading.

PART ONE

Researchers: Dr Kathryn Musgrave and Prof John Simpson

Study Sponsor:

This study is sponsored by the

Newcastle Joint Research Office (JRO)

1st Floor Regent Point
Regent Farm Road
Gosforth
Newcastle upon Tyne
NE3 3HD

The sponsor holds an adequate insurance policy for this study.

Ethical Review

This study has been reviewed by the London - Hampstead Research Ethics Committee

What is the background and purpose of the study?
People with cancer are more likely to develop blood clots, particularly in the blood vessels in their legs and lungs. Currently, there are some preventative treatments available to prevent blood clots, but these are not without risk.

We need to be able to identify which patients would benefit most. This study will try to identify markers in the blood that could help us discover which patients are at the highest risk of blood clots and should therefore be given the preventative treatment.
The markers which we are testing look at various aspects which contribute to how a blood clot forms, for example the blood proteins which help form a clot (D-dimers), your platelets (soluble P-selectin), and the cells lining your blood vessels (VEGF).

This research forms part of an educational doctorate project.

Why have you been invited to take part?
You have been asked to take part because you are currently a patient at the Northern Centre for Cancer Care and are about to start chemotherapy treatment.

Do you have to take part?
It is up to you to decide whether to take part. If you do, you will be given this information sheet to keep and be asked to sign a consent form. You are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care received by you now or at any stage in the future.

How long can you take to decide?

Please let your clinical team know if you would like to participate within 7 days of receiving this information.

What will happen to you if you take part?
If you decide to take part in the study, you will be asked to provide written consent.

What do I have to do?
The study will involve three visits from a researcher. The first visit will take about 30 minutes and will involve a discussion about your medical history and a blood sample will be taken. The later visits will take only a few minutes, they will involve the taking of an extra blood sample when you are already having a blood test performed as part of your usual clinical care.

What are the possible disadvantages and risks of taking part?
The taking of blood samples and may cause mild discomfort and bruising but wherever possible a tube already present in a blood vessel will be used. We will try very hard to make sure that the blood sample will be taken at the same time as the blood tests being taken for your usual clinical care to avoid the need for extra blood tests.

What are the possible benefits of taking part?
There will be no direct benefits to you.

Is there any reimbursement for taking part?
No.

What will happen when the study ends?
You will continue with your usual care at the hospital.

What if there is a problem?
Any complaint about the way you have been dealt with during the study or any possible harm you might suffer will be addressed.

How will we use your personal data?
In this research study we will use information from you and your medical records. We will only use information that we need for the research study. We will let very few people know your name or contact details, and only if they really need it for this study.

Everyone involved in this study will keep your data safe and secure. We will also follow all privacy rules.

At the end of the study we will save some of the data in case we need to check it and for future research. We will make sure no-one can work out who you are from the reports we write.

The information pack tells you more about this.

This completes Part 1 of the Information Sheet.

If the information in Part 1 has interested you and you are considering participation, please continue to read the additional information in Part 2 before making your decision.

Thank you.

PART TWO

What will happen if I wish to take part in the study?
This study involves a total of five visits.

First visit
This occurs once you have given consent to join the study. This first visit will take approximately 30 minutes. This first visit includes two parts.

Part 1. This a discussion with a member of the research team where details are collected relating to your age, medication you are taking and other health problems.

Part 2. A blood sample of 19 mL (about a tablespoon) will be taken from either your left or right arm. This is similar to the amount of blood taken during a routine blood test. We will try and take this sample when you already have a blood test planned as part of your clinical care. If you have an indwelling line we will wherever possible use this to take the sample. The sample will be taken but a doctor or nurse who are qualified to take blood samples and use lines.

Later visits
These visits will include only the taking of an extra blood sample.

As before a 19 mL blood sample will be taken. We will try and take these samples at the same times as other bloods test you will need for your clinical care. If you have an indwelling line, we will use this to take the sample whenever possible.

These visits will take place at approximately 1 and 3 months following the first visit.

Access to medical records
Doctors and nurses who are part of the study will have access to your medical records for up to 2 years following the study. This is so we can identify people who develop blood clots after joining the study. It is also useful to record other information such as medication you may start or other blood tests your clinical team may perform during this time.

Blood samples
Blood samples will be taken to the university laboratory and labelled with a unique anonymous identifier. The sample will be processed to isolate white blood cells from other parts of the bloods called plasma and serum. Some of the sample will be frozen to be used for later experiments. Sample will be frozen and stored for a maximum of 5 years.

The markers we are measuring are not anticipated to affect your clinical care. In the unlikely event that the researchers come across any abnormal, incidental findings as part of their work, you and your clinical team will be informed.

What will happen if I don't want to carry on with the study?
Participation in this study is completely voluntary and you can decide to withdraw from the study at any time. Withdrawing from the study will not affect the care that you get from the NHS at any stage in the future.

What if there is a problem?
If you have a concern about your treatment by members of staff during the study, you should ask to speak with the researchers who will do their best to answer your concerns (a contact number is at the end of Part 1 of the Information Sheet). If you remain unhappy and wish to

complain formally, you can do this through the NHS Complaints Procedure. Details can be obtained from your hospital.

In the unlikely event that something goes wrong and you are harmed during the study there are no special compensation arrangements. If you are harmed and this is due to someone's negligence then you may have grounds for a legal action for compensation against the NHS/Newcastle University but you may have to pay for your legal costs. The normal NHS complaints mechanisms will still be available to you.

How will we use information about you?
We will need to use information from you and from your medical records for this research project.

This information will include your:
- name
- date of birth
- hospital identifier

People will use this information to do the research or to check your records to make sure that the research is being done properly. People who do not need to know who you are will not be able to see your name or contact details. Your data will have a code number instead. We will keep all information about you safe and secure.

Once we have finished the study, we will keep some of the data so we can check the results. We will write our reports in a way that no-one can work out that you took part in the study.

What are your choices about how your information is used?
You can stop being part of the study at any time, without giving a reason, but we will keep information about you that we already have.

We need to manage your records in specific ways for the research to be reliable. This means that we won't be able to let you see or change the data we hold about you.

Where can you find out more about how your information is used?
You can find out more about how we use your information
- at www.hra.nhs.uk/information-about-patients/
- our leaflet available from www.hra.nhs.uk/patientdataandresearch
- by asking one of the research team
- by sending an email to the JRO data protection officer Julia Scott julia.scott12@nhs.net, or
- by ringing us on 0191 282 4743

NOTE: At least one of these sources must be able to point people directly to the sponsor's Data Protection Officer.

Will your GP be informed that you are taking part in the study?
No.

Will any genetic tests be done?
Yes. The genetic tests will help us understand how blood cells respond to illness. We shall not, at any point, be testing for genes associated with specific medical conditions.

Will any information and material be stored?
Yes, but only with your permission. Information about you will be collected and entered onto a secure database. Access to this database will be password protected and only available to

your doctors and the research staff. All data stored on computers will not use your name – you will be given a unique study number under which all data and test results will be entered.

Any blood that we obtain as part of the research will be processed in Professor Simpson's research laboratory at Newcastle University. We shall store the liquid component of blood (called serum or plasma) in freezers. The samples will only be labelled with your unique study number (i.e. your name will not appear).

It is possible that in the future new tests will become available that will help to predict the development of blood clots. Should this situation arise we may use your samples again, but this would be on condition that you agree to this, that you could not be identified from the sample except by our research team, and that we obtain fresh and separate permission from a Research Ethics Committee. The Ethics Committee is completely independent from this study.

We may share samples with other investigators or commercial organisations in the UK or internationally, to help further understanding of severe infection. If this is happens, the samples shared would be anonymous and external investigators or organisations would not be able to identify you. The anonymised data collected as part of the study may also be used to understand the sample analyses.

This study is being overseen by Newcastle upon Tyne Hospitals NHS Foundation Trust. Authorised persons from the Trust or from other legally authorised regulatory bodies may look at some parts of your medical records and the data collected for the study. This is to ensure the quality of the work being carried out. All will have a duty of confidentiality to you as a research participant and nothing that could reveal your identity will be disclosed outside the research site.

What will happen to the results of the research study?
We intend for the results of this study to be published in medical/scientific journals and presented at medical/scientific meetings. All information in the public domain will be anonymous and it will not be possible to identify you from these publications/presentations.

Who is organising and funding the research?
This study has been funded by Professor Simpson's research group through an educational grant and will be overseen by the Newcastle Upon Tyne Hospitals NHS Foundation Trust and Newcastle University.

Who has reviewed the study?
This study has been reviewed by the Newcastle and North Tyneside Research Ethics Committee.

What if something goes wrong?
If you have any concerns about any aspect of this study, you should contact the Chief Investigator (Dr Kate Musgrave, telephone number 0191 282 4743), who will do their best to answer your questions. If you remain unhappy and wish to complain formally, you can do this through the normal NHS Complaints Procedure.

If something does go wrong and you are harmed due to someone's negligence, then they you may have grounds for a legal action against their NHS Trust, but you may have to pay legal costs.

Is there an independent doctor you can approach for further information?

If you would like to discuss any aspect of this research with an experienced researcher who is not linked in any way to this study, please feel free to contact Dr Wendy Funston (telephone 0191 28 24634).

Alternatively, you may prefer to raise your concerns through the Patient Advise and Liaison Service (PALS). This service is confidential and can be contacted on Freephone: 0800 032 0202

Alternatively, if you wish to make a formal complaint you can contact the Patient Relations Department through any of the details below:

Telephone: 0191 223 1382 or 0191 223 1454
Email: patient.relations@nuth.nhs.uk
Address: Patient Relations Department
The Newcastle upon Tyne Hospitals NHS Foundation Trust
The Freeman Hospital
Newcastle upon Tyne
NE7 7DN

Thank you for taking the time to read this information.

If you agree to take part in the study you will be given a copy of this information to keep, along with a copy of your signed consent form.

APPENDIX 5. MEDICAL INFORMATION SHEET

The Newcastle upon Tyne Hospitals
NHS Foundation Trust

Medical questionnaire for TM-CAT study

Please complete questionnaire as fully as possible

PARTICIPANT DETAILS

Participant number_____

Gender (delete as appropriate) Male/Female

Height_____

Weight_____

INFORMATION ABOUT CANCER

Cancer type_____

Cancer stage_____

Cancer treatment in the previous 6 months (delete as appropriate):

Radiotherapy Yes/No (Add date if applicable_____)

Surgery Yes/No (Add date if applicable_____)

Previous chemotherapy immunotherapy or targeted therapy Yes/No

(Add date if applicable _____)

(type of previous chemotherapy if applicable_____)

Chemotherapy course that is about the begin:

Type of chemotherapy_____

Number of courses planned_____

Current oncologist_____

PAST MEDICAL HISTORY

Previous personal history of blood clots (VTE: DVT, PE, STP, venous clot in unusual site). Please record date of blood clot, site, anticoagulation given, and cause of clot if known

Previous personal history of arterial clots (MI, Stroke, TIA). Please record date of event, site, anti-platelet or anticoagulation given

Family history of VTE (delete as applicable) Yes/No

Family member _____

Age they had the blood clot _____

Cause of blood clot if known_____

Other past medical history

Current medication

BLOOD TESTS PRIOR TO CHEMOTHERAPY

Hb	Sodium	Bilirubin
WBC	Potassium	ALP
Neut	Urea	ALT
PLT	Creatinine	Albumin

END OF QUESTIONS

APPENDIX 6. PUBLISHED META-ANALYSIS

THIEME OPEN ACCESS

Vascular Endothelial Growth Factor (VEGF) as a Biomarker for Cancer-Associated Venous Thrombosis: A Meta-analysis

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TH Open 2025;9:a25134381.

Abstract

Cancer-associated thrombosis affects between 1 and 20% of all patients diagnosed with cancer and is associated with significant morbidity and a poorer prognosis. Risk assessment scores exist which include the measurement of biomarkers, and which aim to identify patients at a higher risk of developing thrombotic events, but these are poor predictors and rarely used in routine clinical practice.

VEGF is a potent angiogenic factor, produced by tumour cells, and released by platelets and is essential for tumour growth and progression. It also plays a role in the promotion of thrombosis through platelet activation and adhesion, and by inducing the expression of tissue factor. Therefore, the potential of VEGF to be used as a biomarker to predict cancer-associated thrombosis requires further investigation.

This study reviewed the published literature to determine whether circulating VEGF levels are associated with increased risk of venous thromboembolism in patients with cancer.

PubMed and OVID databases were systematically searched according to PRISMA guidelines for relevant papers using the keywords "cancer" AND "thrombosis" AND "VEGF" up to July 2023. Inclusion and exclusion criteria were applied.

Seven papers (1,528 participants) were identified and included in the meta-analysis, three of which (922 participants) measured VEGF before a thrombotic event, and the remaining four (606 participants) measured VEGF at the time of the thrombosis. Our results showed that although plasma and serum VEGF tended to be higher in those who subsequently developed thrombosis than those who did not (mean difference 70.2 pg/mL for serum, and 11.44 pg/mL for plasma VEGF, 95% CI −2.39–25.73, $p = 0.10$), this was not found to be statistically significant. However, analysis of VEGF following blood sampling at the time of thrombosis showed a stronger statistically significant association between increased VEGF levels and presence of thrombosis (mean

Keywords
- VEGF
- cancer
- thrombosis
- biomarker

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difference 117.02 pg/mL for serum, and 116.6 pg/mL for plasma VEGF, 95% CI 55.42–190.82, $p = 0.0004$).
Based on current studies, whilst it is increased at the time of thrombosis, VEGF is not effective as a predictive biomarker of CAT.

Introduction

Cancer-associated thrombosis (CAT) affects up to 20% of patients with cancer and is associated with a poorer prognosis.[1–3] The use of low-dose anticoagulation (thromboprophylaxis) has been shown to not only reduce the risk of venous thrombosis but also increases the risk of bleeding,[4] which complicates the clinical picture and does not allow routine thromboprophylaxis to be given to all people with cancer in the outpatient setting.[5]

Clinicians need to target the use of thromboprophylaxis and offer it to those at highest risk of thrombosis. A way of predicting those who are a higher risk of developing a venous thromboembolism (VTE) has been a long sought-after clinical decision-making tool.

To address this, numerous risk assessment scores have been proposed, some of which use circulating levels of biomarkers at the time of diagnosis of the cancer. The most validated is the Khorana score[6] which uses the major parameters of a full blood count—haemoglobin, white cell count and platelets, along with patient factors such as cancer site and body mass index (BMI)—to determine the likelihood of a thrombosis. The Vienna CATS score[7] goes further and has added two additional biomarkers, namely, soluble P-selectin and D-dimers, to predict those individuals at a greater risk of thrombosis.

However, whilst these prediction scores demonstrate a strong association with VTE, in that those assigned to a high-risk category are more likely to develop a thrombosis, these scores can identify only a proportion of all individuals who will develop a thrombosis[3] and have limited discriminatory power.[8] About 90% of patients who are in either the intermediate- or high-risk categories based on the Khorana score do not develop a thrombosis after 6 months.[8] Therefore, these risk assessment scores need to be improved to truly distinguish the patients who are at a higher risk of developing a thrombosis, and who would benefit from receiving thromboprophylaxis.

Vascular endothelial growth factor (VEGF or VEGF-A) is a potent angiogenic factor[9] that is also thought to promote thrombosis. Angiogenesis, the formation of new blood vessels, is essential for the growth, invasion, progression, and metastasis of tumour tissue.[10] As a result, VEGF has been shown to be overexpressed in breast, colorectal, lung, pancreatic, ovarian, and cervical cancers.[1,10]

In health and disease, VEGF is expressed on the surface of many different cell types, including monocytes, endothelial cells, lymphocytes, and granulocytes,[1,11] but it is thought that VEGF levels in these cells are higher in cancer than in healthy individuals.[12] Platelets, cells that are essential for thrombosis, are also rich in VEGF, which is stored within their alpha granules.[1] In cancer, both radiotherapy and chemotherapy have been shown to increase VEGF within tumours.[13]

Despite its association with both cancer and thrombosis, the predictive value of VEGF, in CAT events, is less well defined.

Herein we present a meta-analysis of previously published data to assess the predictive potential of VEGF in CAT.

Methods

Search Strategy and Eligibility Criteria

This meta-analysis complies with the standard of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).[14]

A literature search was performed using two databases, PubMed and OVID, until 9 July 2023. Papers were included only if published after the year 2000. This time frame was chosen to represent recent research. One paper (Musolino et al, 2002[21]) was found by examining the references of another paper.

Keywords included: 'cancer', 'thrombosis', and 'VEGF'. The following search terms were also used: ('cancer' OR 'neoplasms') AND ('VEGF' OR 'vascular endothelial growth factor' OR 'vascular endothelial growth factors' [Mesh Major Topic] OR 'vascular permeability factor' OR 'biomarkers/analysis' [Mesh] OR 'biomarkers/blood' [Mesh]) AND 'thrombosis' OR 'vte' OR 'Thrombosis/blood' [Mesh] OR 'Thrombosis/complications' [Mesh] OR 'Thrombosis/diagnosis' [Mesh] OR 'Thrombosis/epidemiology' [Mesh] OR 'Thrombosis/etiology' [Mesh] OR 'Thrombosis/immunology' [Mesh] OR 'Thrombosis/pathology' [Mesh]).

Inclusion criteria: (1) patients with cancer being studied, (2) studies reporting either plasma or serum VEGF levels in patients with cancer, in both those with a thrombosis and those without, quantitatively, (3) VEGF measured before or during the thrombotic event, (4) adults over the age of 18 studied, (5) full text available, and (6) studies written in English.

Exclusion criteria: (1) Paediatric population being studied, (2) review article, case report, or conference abstract, (3) cell lines and not patients studied, (4) full text not available, (5) not written in English, (6) study did not have figures for thrombosis and no thrombosis, and (7) subjects studied were not humans.

This study focussed on venous thrombosis, including unusual site thrombosis such as portal vein thrombosis. The references of relevant studies and review articles were also studied and checked for relevance to identify additional studies. Two additional authors (SN and AU) validated the search and assessed the articles and abstracts.

Data Extraction and Quality Assessment

Following the inclusion and exclusion criteria above, and data selection, studies were further examined for suitability. Data extraction was performed by AB. All VEGF values were converted to pg/mL irrespective of the values used originally in the study to allow an easier comparison between them. Two studies (Kirwan et al, 2008[15]; Kirwan et al, 2009[16]) quoted VEGF values as µg/mL, representing a 10^6 difference between these results, and other comparable studies. Attempts were made to verify these values. As the values given were comparable to those which were given in pg/mL, and based on the sensitivity and range of the enzyme-linked immunosorbent assay (ELISA) used (9 pg/mL), these values were subsequently assumed to be pg/mL and are represented as such.

Studies where thrombosis had already occurred at the sampling point were also included. All studies measured VEGF by an ELISA method. Further details of the studies were included, and their design are shown in ►**Table 1**.

Patient characteristics from the included studies are shown in ►**Table 2**.

In instances where research papers contained qualitative findings and no comparable quantitative data, the studies were included in a qualitative manner.

Two authors (AB and SN) evaluated the quality of the studies independently. If a disagreement occurred, a third investigator made the final decision. Quality assessment of the included studies was performed using the Newcastle-Ottawa score (NOS).[17] The Agency for Healthcare Research and Quality's (AHRQ) 11-item criteria were used to evaluate each of the studies. A score of 6 or more was considered to indicate good quality.

Statistical Analysis

The association of VEGF with CAT was evaluated by calculating the mean and SD values for plasma and serum VEGF levels for each study. Therefore, in this meta-analysis, studies looking at plasma and serum levels of VEGF have been separated into different forest plots to allow easier comparisons to be drawn. Currently, there is no consensus on which is the better VEGF parameter to measure.

Meta analysis of the mean difference for random effects was performed using Rev Man software. Random effects as opposed to fixed effects were used due to high heterogeneity between included studies. Heterogeneity between the included studies was tested using the Rev Man software and I^2 values. The chosen statistical significance threshold was set at $p < 0.05$.

The risk of bias for this meta-analysis was assessed using the ROB-ME tool (Risk Of Bias due to Missing Evidence in a meta-analysis).[18] This tool identified that there was a low risk of bias with this meta-analysis.

Results

PRISMA Protocol

A total of 801 records were identified through screening of two databases: PubMed and OVID. After duplicates were removed, 556 papers remained. Review of the paper title and abstract reduced the number of papers to 33. For these remaining papers the full text was accessed and assessed for eligibility. Once the inclusion and exclusion criteria were applied, 11 records remained. A further study was excluded as it mainly described arterial thrombotic events (Cacciola et al, 2002[19]). Of the remaining, only seven of those could be included in the meta-analysis due to the lack of data (►**Fig. 1**). The remaining three are still included in the meta-analysis but qualitatively rather than quantitatively. This is due to the raw data either not being available (Nazari et al, 2019[20]) or presented in a different format which did not allow inclusion in the forest plots (only a median value was provided by Li et al, 2004,[9] and Musolino et al, 2002[21] did not present the figures for thrombosis and no thrombosis as two separate populations). Attempts were made to contact the authors where data were missing, though in two cases the papers were published 20 and 22 years ago.

The main characteristics of the seven papers used for the meta-analysis, plus the three used qualitatively, are summarized in ►**Table 1**.

Patient Characteristics

The overall population included in the meta-analysis consisted of 1,528 participants, 213 of which were patients with cancer who were affected by thrombosis. The remaining 1,315 were patients with cancer who were not affected by thrombosis, representing a 14% rate of CAT in the study population. This figure agrees with the widely reported rates of CAT.[1–3] In some cases, the nature of the thrombosis was recorded, but in others it was not.

All types of cancer and all stages of the disease were represented in the data studied. The seven studies represent a wide geographical area (►**Table 1**) and the median age of participants across the seven studies was 57.82 years. Individual studies' participant characteristics are shown in ►**Table 2**.

Quality Assessment and Risk of Bias

Quality assessment of the 11 included studies was performed using the NOS scale.[17] Of the 11 studies 10 were assessed to have scores greater 6 and therefore of good quality, with the remaining study (Musolino et al, 2002[21]) considered to be of moderate quality (score of 4).

Meta-analysis of VEGF Levels on Thrombotic Events in Cancer

VEGF Levels at the Time of Thrombosis are Increased in Cancer Patients

Four studies, with 606 patients (146 with thrombosis), assessed VEGF levels at the time of the thrombotic event, three analyzed serum VEGF levels (Dogan et al, 2006,[10] Kim et al, 2004,[22] Ramadan et al, 2021[23]), and one study analyzed plasma VEGF levels (Malaponte et al, 2015[24]). Our analysis of the four studies identified significantly higher levels of VEGF in patients with thrombosis versus those patients without (mean difference 123.12 pg/mL, 95% CI 55.42–190.82, $p = 0.0004$) (►**Fig. 2**). Heterogeneity was assessed with a I^2 value of 82%. All four papers demonstrated that VEGF

Table 1 Summary of the study designs included in meta-analysis (* denotes not included in forest plots due to lack of availability of data)

Study (year published)	Geographical location of study	Study design	Total number of participants	Cancer type(s) and stage	Type of thrombosis	Control group?	Newcastle-Ottawa Quality Assessment Score	VEGF biomarker measured
Dogan et al (2006)[10]	Turkey	Prospective cohort	31	All types and stages	Venous	51 matched pairs (all had cancer)	7	Serum VEGF
Kim et al (2004)[22]	Korea	Prospective cohort	52	Hepatocellular carcinoma (HCC), all stages	Portal vein	30 healthy, 26 liver cirrhosis	9	Serum VEGF, and serum VEGF per platelet count
Kirwan et al (2008)[15]	United Kingdom	Prospective cohort	123	Breast, early and advanced stages	Venous	68 healthy controls	9	Plasma VEGF
Kirwan et al (2009)[16]	United Kingdom	Prospective cohort	123	Breast, early and advanced stages	Venous	68 healthy controls	9	Plasma VEGF, serum VEGF and platelet release of VEGF
Li et al (2004)*[9]	China	Prospective cohort	45	Hepatocellular carcinoma (HCC), all stages	Portal vein	17 healthy, 20 benign liver lesions	9	Plasma VEGF
Malaponte et al (2015)[24]	Italy	Retrospective case-control	385	All types and stages	DVT only	100 healthy controls	7	Plasma VEGF
Musolino et al (2002)*[21]	Italy	Retrospective cohort	55	Myeloproliferative neoplasms	All	20 healthy	4	Plasma VEGF
Nazari et al (2019)*[20]	Austria	Prospective cohort	76	Glioma	Venous	No	7	Unclear if plasma or serum VEGF
Posch et al (2016)[11]	Austria	Prospective cohort	804	All types and stages	Venous	No	7	Plasma VEGF
Ramadan et al (2021)[23]	Egypt	Prospective cohort	87	Hepatocellular carcinoma (HCC), all stages	Portal vein	No	7	Serum VEGF

Abbreviations: DVT, deep vein thrombosis; VEGF, vascular endothelial growth factor.

Table 2 Summary of the patient characteristics used in meta-analysis where available

Study (year published)	Total number of participants	Age of participants in years (range) (Mean or median)	Sex	Body mass index (BMI) (range)	Blood cell count: Platelets ($\times 10^9$/L) Haemoglobin (g/L) White blood cell count ($\times 10^9$/L)	D-dimer levels (ng/mL) (range)	Fibrinogen level (g/L)	Cancer type(s) and stage
Dogan et al (2006)[10]	31	56.74 +/− 16.06 (mean)	Male = 13, Female = 18	Not stated	Not stated	960.71 +/− 1,066.85	Not stated	All types and stages
Kim et al (2004)[22]	52	57 (35–80) (median)	Male = 39, Female = 13	Not stated	Platelet count: 130 (76.4–217.3)	Not stated	Not stated	Hepatocellular carcinoma (HCC), all stages
Kirwan et al (2008)[15]	123	52 (31–78) (median)	Female = 123	Not stated	Platelet count: 314.3 (287.2–325)	1,618.6 (979–2,676.1) with thrombosis 815.3 (707.8–989.3) without thrombosis	3.6 (3.3–3.8) with thrombosis 4.9 (3.0–6.9) without thrombosis	Breast, early and advanced stages
Kirwan et al (2009)[16]	123	52 (31–78) (median)	Female = 123	Not stated	Platelet count: 314.3 (287.2–325)	1,618.6 (979–2,676.1) with thrombosis 815.3 (707.8–989.3) without thrombosis	3.6 (3.3–3.8) with thrombosis 4.9 (3.0–6.9) without thrombosis	Breast, early and advanced stages
Li et al (2004)*[9]	45	50 (29–77) (mean)	Male = 37, Female = 8	Not stated	Not stated	Not stated	Not stated	Hepatocellular carcinoma (HCC), all stages
Malaponte et al (2015)[24]	385	62 +/− 9 (mean) no DVT 64 +/− 10 (mean) with DVT	Male = 185, Female = 200	25.85 +/− 8.3	Not stated	Not stated	413.7 +/− 87.7 with thrombosis 404.2 +/− 71.1 without thrombosis (Units not stated)	All types and stages
Musolino et al (2002)*[21]	55	60 (median)	Male = 17, Female = 38	Not stated	Not stated	Not stated	Not stated	Myeloproliferative neoplasms
Nazari et al (2019)*[20]	76	54 (46–67) (median)	Male = 41, Female = 35	Not stated	Not stated	Not stated	Not stated	Glioma
Posch et al (2016)[11]	804	63.1 (54.2–69.2) (median)	Male = 371, Female = 433	25.0 (22.3–28.1)	Platelet count 245 (199–302) Haemoglobin 131 (120–141) White blood cell count 7.2 (5.7–9.4)	710 (360–1,320)	3.94 (3.25–4.83)	All types and stages
Ramadan et al (2021)[23]	87	61.93 +/− 6.99 (mean) Group 1, 64.42 +/− 8.87 Group 2	Male = 68, Female = 19		Platelet count 141.7 +/− 80.2 Haemoglobin 112.1 +/− 24.9 White blood cell count 6.90 +/− 3.77	Not stated	Not stated	Hepatocellular carcinoma (HCC), all stages

Abbreviation: DVT, deep vein thrombosis.
Note: Chemotherapy regimens and antithrombotic treatments not included due to a lack of information.

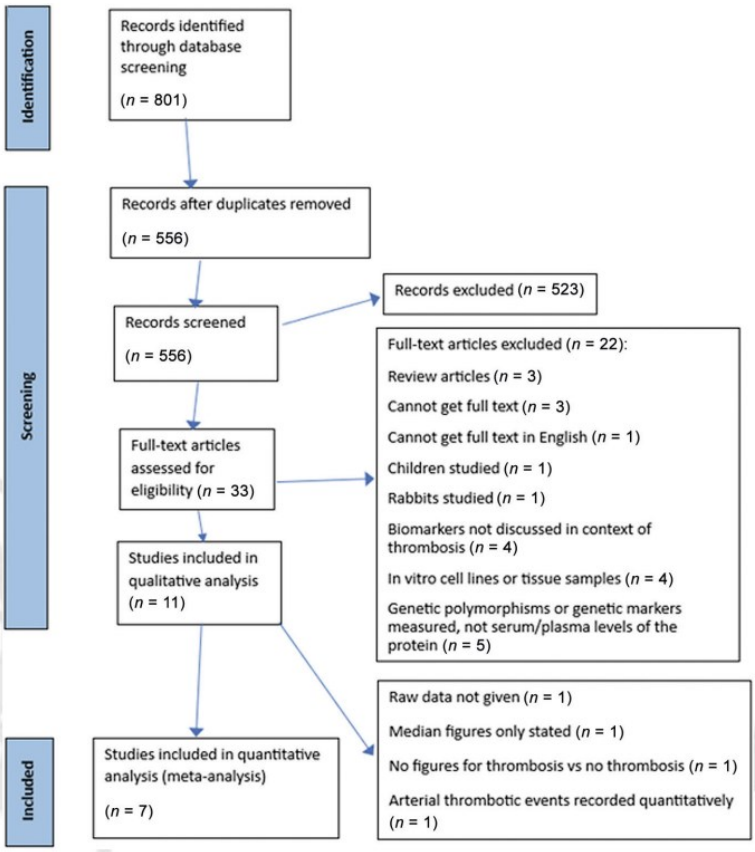

Fig. 1 Flow diagram of the inclusion and exclusion procedures. PRISMA, Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

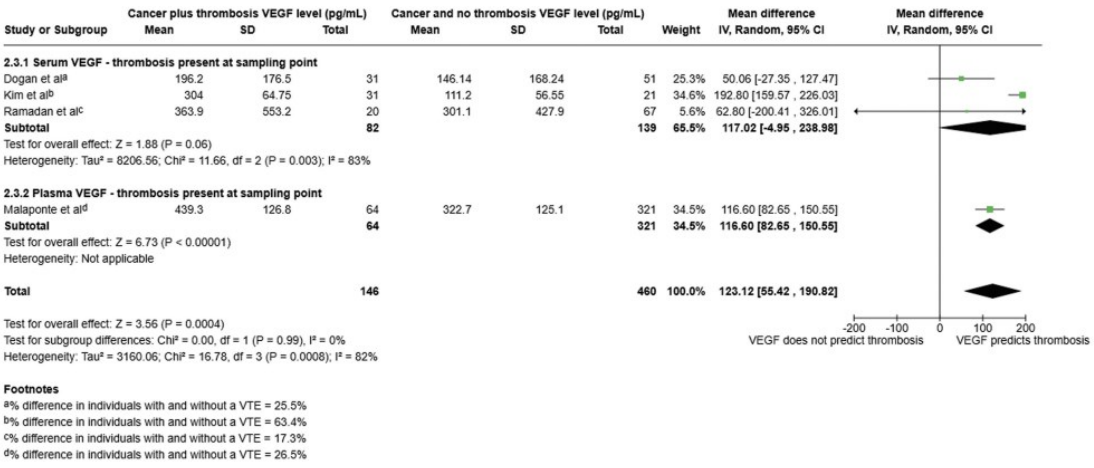

Fig. 2 Forest plot for vascular endothelial growth factor (VEGF) levels among cancer-associated thrombosis and patients with cancer and no thrombosis.

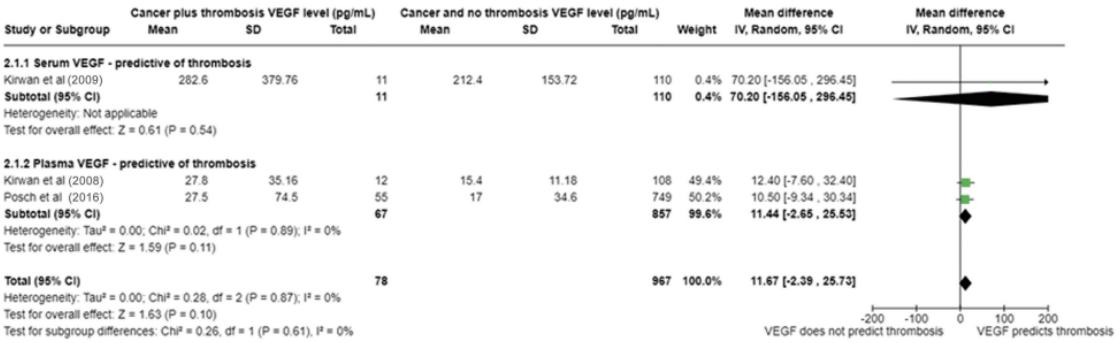

Fig. 3 Forest plot for vascular endothelial growth factor (VEGF) levels, collected prior to thrombosis among cancer-associated thrombosis and patients with cancer and no thrombosis.

significantly rises at the time of a thrombotic event, with the percentage difference in VEGF levels between those with and without thrombosis of 17.3 and 63.4% across the four studies.

These findings are further supported by the work of Musolino et al[21] who showed that increased plasma VEGF levels were seen in patients with myeloproliferative neoplasms who had had a thrombotic event within the preceding month, and by the work of Li et al[9] who also showed that the presence of portal vein thrombosis in patients with hepatocellular carcinoma was associated with a higher plasma VEGF level.

Taken together these findings indicate a positive association of VEGF levels with thrombosis in cancer patients and identifies increased VEGF as a marker of CAT at the time of thrombosis.

VEGF Levels Prior to a Thrombotic Event are not Associated with Cancer-Induced Thrombosis

Having identified an association of VEGF levels with thrombosis post thrombotic event, we analyzed the three remaining studies, which measured VEGF levels prior to thrombotic event occurring, to determine whether VEGF could be used as a predictive biomarker of thrombosis. Three studies involving 922 participants examined the role of VEGF as a predictor of thrombosis (serum VEGF, Kirwan et al, 2009[16] plasma VEGF, Kirwan et al, 2008[15] and 2009[16]—data only included once—and Posch et al, 2016[11]). The 3-month cumulative incidence of VTE in the Kirwan et al studies' population was 9.8%, whilst the 6-month cumulative incidence in the Posch et al study's population was 5.0%. Analysis of data from these studies show that whilst pre-event plasma VEGF or serum VEGF levels are higher in patients who go on to experience CAT there is no significant difference in VEGF levels between patients who develop thrombosis versus those who do not (mean difference 11.68 pg/mL, 95% CI −2.39–25.73, $p = 0.10$; ►**Fig. 3**). Heterogeneity was assessed, giving an I^2 value of 0%; this is possibly due to the papers included.

These findings are further supported by the work of Nazari et al,[20] which also showed no association of serum VEGF levels and the prediction of VTE in patients with glioma (hazard ratio per double increase: 0.995, 95% CI 0.640–1.548, $p = 0.983$).

Taken together these observations indicate that whilst VEGF levels are increased in cancer patients at the time of thrombosis (►**Fig. 2**) VEGF levels in cancer patients are not predictive of thrombosis.

Discussion

Cancer is the uncontrolled proliferation of genetically aberrant cells, which is a leading cause of death throughout the world. It can occur in any tissue of the body, including the blood. For proliferation of the cancer cells to take place, certain conditions need to be in place, one of which is the ability for angiogenesis to occur, which is the formation of new blood vessels.[1] VEGF is a potent angiogenesis stimulator, and therefore we would expect VEGF to be raised in patients with cancer.[1]

Compared to the general population, patients with cancer are at an increased risk of developing a thrombosis; between 1 and 20% of patients develop this complication, which is associated with a higher mortality rate.[1–3] Whilst both venous and arterial thrombotic events can occur in CAT, the incidence of VTE is widely considered to be equivalent to the incidence of CAT in patients diagnosed with cancer.

VEGF is raised in patients with cancer[1,10,12] and is thought to play a role in thrombosis[1] by promoting both the release of tissue factor, and platelet activation and adhesion.[11]

Tissue factor, released from endothelial cells, is one of the main initiators of coagulation.[1,11] It may also play a role in angiogenesis, by upregulating VEGF, and downregulating the angiogenesis inhibitor thrombospondin,[25,26] a mechanism which is independent of coagulation activation.[25,27]

Platelet adhesion and activation are involved in the thrombotic process. Activated platelets release further VEGF from their alpha granules[11] into the circulation enhancing thrombosis via these mechanisms. Platelets can also act as a transporter of tumour-originated VEGF,[28] further contributing to tumour angiogenesis and progression, as well as the risk of thrombosis.

Therefore, we hypothesized that VEGF shows excellent theoretical potential to be used as a biomarker for CAT. In this

analysis we investigated whether plasma or serum VEGF levels are associated with thrombotic events in cancer patients, pre and post thrombosis.

Seven papers (six patient cohorts) were included in this meta-analysis. The findings presented here indicates that VEGF levels are increased at the time of a thrombotic event, indicating VEGF may play a role during a thrombotic event, in addition to its role in the pathogenesis of a malignancy, but it does not appear to be predictive of CAT/thrombosis.

Our meta-analysis included four studies where the thrombosis was present at the blood sampling point, to determine whether VEGF was associated with thrombus formation. All of these studies showed increased mean differences between patient groups who had a thrombosis versus those who did not ($p = 0.0004$). These findings were further supported by the work of Musolino et al[21] and Li et al,[9] which demonstrated increased plasma VEGF in patients with thrombosis versus those with no thrombosis, but whose data were not compatible to be included in our forest plots analysis. Taken together these findings demonstrate that VEGF levels are significantly increased and associated with the presence of thrombosis in patients with cancer.

Activated platelets release VEGF,[11] and therefore it is not unexpected that VEGF levels were observed to be increased at the time of a thrombosis. Platelet activation is an essential part of primary haemostasis, which is required in the formation of a thrombus. VEGF is also found in higher levels in patients with cancer compared to healthy controls,[1] due to ongoing angiogenesis required for tumour growth and survival.[1] Interestingly Musolino et al[21] showed that in patients with myeloproliferative neoplasms increased plasma VEGF levels were seen up to 1 month post thrombotic event, possibly indicating a state of platelet hyperactivation and/or indicating a more global contribution of VEGF to thrombosis.

Having identified an association of VEGF with CAT at the time or post thrombosis, this meta-analysis set out to investigate whether VEGF can be used as a biomarker to predict thrombosis. Three studies identified by our search strategy collected blood samples for VEGF level measurement from cancer patients before thrombosis had occurred. The 3-month cumulative incidence of VTE was 9.8% for the Kirwan studies,[15,16] and the 6-month cumulative incidence in the Posch et al study population was 5.0%. This reflects typical CAT incidence,[1–3] and the two study populations' characteristics, as the Kirwan et al's studies include exclusively breast cancer patients associated with a higher risk of VTE, whereas Posch et al studied a variety of cancer types, with various differing risk profiles. Although all three studies showed a trend towards higher levels of VEGF in those patients who subsequently developed a thrombosis versus those who did not, this difference was not statistically significant (*P*-value of 0.10). There are many reasons for this, including not knowing how long prior to the thrombotic event the samples were taken for example, which we hypothesize may impact the study's conclusions. Posch et al,[11] for example, followed patients for thrombotic events for 2 years following initial sampling as part of the large Vienna CATS Study, so it not inconceivable that VEGF would not be raised up to 2 years before a thrombotic event occurred. The work of Nazari et al[20] was also part of the same study and so the same conclusions can be drawn. In contrast, the two remaining studies, Kirwan et al, 2008 and 2009,[15,16] which used plasma and serum samples collected from the same cohort of 123 patients (120 for plasma, and 121 for serum), only followed patients for 3 months after blood sampling. These differences in follow-up time may be confounding the results. In addition, different cancer types were studied, at different stages, which may also be impacting the findings. It is also difficult to compare studies as plasma[15] and serum[16] VEGF levels were included from two publications that include the same patient population, which inevitably leads to bias. Overall, the lack of independent studies will have had an impact on the results obtained and highlights that further work in this area is required.

As part of this meta-analysis, we included studies measuring VEGF from both serum and plasma. This has consequences for our interpretation as serum and plasma VEGF have very different normal reference ranges. In this respect study by Malaponte et al[24] appears to be an outlier with the measurement of plasma VEGF recording VEGF levels much higher than the other groups also measuring these biomarkers, even in those individuals with no thrombosis. The reasons for this are unclear. However, the percentage difference in mean plasma VEGF values between individuals with and without a VTE was 26.5% in this study, which is comparable to that of other studies in the same category (25.5% in Dogan et al,[10] 17.3% in Ramadan et al,[23] with Kim et al[22] being an outlier with a 63.4% difference). Therefore, all studies show that VEGF levels are higher in those with a thrombosis compared to those without.

Normal plasma and serum VEGF reference ranges differ significantly, with the serum level being 10 to 15 times higher than that of the plasma level (D'Souza et al[29]). This is because the platelets will have become activated during centrifugation in the serum sample, but they remain intact in plasma samples due to the presence of anticoagulant in the sample tube. Serum VEGF analysis therefore gives a measure of how much VEGF there is in platelets, whereas plasma VEGF analysis does not, and instead represents VEGF released from platelets which is indicative of platelet activation.

By examining the forest plots, we can see that the measurement of serum VEGF is much more variable than that of plasma, and this is possibly affecting the significance of our findings. The difference in the values could also explain why serum VEGF was found to be associated with occurrence of thrombosis but not found to be predictive of thrombosis. Activated platelets secrete VEGF, indicating that they are prothrombotic, and therefore a thrombosis may occur. However, by analyzing a serum sample, where these 'naturally-activated' platelets are present, plus those platelets 'artificially-activated' by centrifugation, it is unlikely that we are truly representing the predictive value of VEGF measurement in serum samples. Plasma samples may therefore give a

more accurate representation of the predictive value of VEGF in thrombosis in patients with cancer, and further studies are therefore needed to investigate this.

VEGF is a potent angiogenic factor that has been shown to be overexpressed in breast, colorectal, lung, pancreatic, ovarian, and cervical cancers,[1,10] where it promotes the formation of new blood vessels, and is essential for the growth, invasion, progression, and metastasis of tumour tissue.[10] Several of the studies included in this analysis demonstrated increased VEGF levels in cancer patients versus healthy controls.[9,15,16,21,22]

VEGF levels also increase as a cancer develops. Patients with more advanced stages of cancer therefore can have higher levels of VEGF.[30] In the studies examined, this was acknowledged by all, but not considered with regards to the VEGF level and reported thrombosis rates. However, Dogan et al[10] matched controls according to cancer stage, which showed that those who experienced VTE still had higher VEGF levels than the matched controls, suggesting that the thrombotic process was an additional factor for an increase in VEGF levels. Posch et al[11] also addressed this, using multivariable analysis to adjust for tumour stage in their analysis, and showed that the association between VEGF and risk of VTE prevailed after adjustment.

The role of VEGF in initiating thrombus formation is also not well established. There is little to no evidence to suggest that VEGF alone can trigger thrombotic events, which may explain why our analysis found it not to be predictive of thrombosis. It is possible, however, that VEGF plays a role along with other prothrombotic factors to initiate thrombus formation.[6]

Given the association of increased VEGF levels at the time of, or after, the thrombotic event, some consideration should be made as to whether adding VEGF as a biomarker to an existing risk-assessment model (RAM) could be useful. Other biomarkers such as D-dimer levels are already part of the Vienna CATS score,[7] with strong evidence available demonstrating increased D-dimer levels associated with both current and future thrombotic events.[31–34] Interestingly, the Kirwan studies (2008) show significantly higher D-dimer levels in patients who subsequently went on to experience a VTE versus those who did not (1,655 (834–3,273) ng ml^{-1} vs. 727 (631–836) ng ml^{-1}, $P = 0.003$); in the same cohort, VEGF tended to be higher, but this difference was not statistically significant.

At this time, our analysis of predictive studies demonstrates that there is not sufficient evidence that VEGF can be used to predict CAT independently. However, it is possible that VEGF levels may increase predictive capacity in combination with other established markers and risk scores, such as cancer type,[6,7,35] BMI,[6,7,35] and D-dimers,[7] or alongside other novel biomarkers such as soluble P-selectin.[7,36] The study by Posch et al[11] demonstrated a positive interaction between soluble VEGF levels and D-dimer, indicating that the predictive potential of VEGF might be enhanced in combination with D-dimer, particularly in individuals with high levels of both biomarkers. Further investigation and studies are required.

Conclusion

We present here a meta-analysis approach to investigate whether VEGF has the potential to be used as biomarker for CAT. We identify that high plasma and serum VEGF levels are associated with current thrombosis in samples taken at the time of or post thrombotic event; however, plasma and serum VEGF levels were not found to be associated with or predictive of thrombosis when collected prior to thrombotic events in cancer patients. In the future, more prospective cohort studies in specific cancer types and stages are needed to ascertain whether VEGF could be used as a predictive biomarker of CAT.

What is Known on This Topic?

- Patients with cancer are at an increased risk of developing a thrombosis, which are associated with a poorer prognosis.
- Prediction scores for cancer-associated thrombosis exist but although they have a strong association with VTE in cancer they typically have moderate to poor discrimination.

What Does This Paper Add?

- Vascular endothelial growth factor (VEGF) is increased at the time of thrombosis.
- Although increased at the time of thrombosis, further work is required to determine if a rise in VEGF levels could predict a thrombotic episode.

Authors' Contributions

A.M.B., K.M., and A.J.U. were responsible for the conceptualization of the study, while resources were provided by A.M.B. The original draft was prepared by A.M.B. and A.J.U., with review and editing contributions from A.M.B., S.N., K.M., and A.J.U. Visualization was carried out by A.M.B., and the project was supervised by K.M. and A.J.U. Project administration and funding acquisition were managed by A.M.B. All authors have reviewed, read, and approved the final version of the manuscript. Furthermore, none of the authors have any competing interests to declare.

Conflict of Interest

None declared.

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This review was not registered, and nor was a protocol prepared or amended. No aspects are publicly available.

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