A STUDY OF CLINICOPATHOLOGICAL CHARACTERISTICS, SYMPTOMS AND PATIENTS EXPERIENCES RELATED TO OUTCOMES IN PEOPLE WITH CANCER AND I-PE

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Abstract

Background: The clinical course of incidental pulmonary embolism in cancer population represents an area of controversy. It presents a growing challenge for clinicians because of a lack of prospective data.

Aim: This research aims to investigate the impact of an incidentally diagnosed pulmonary embolism on cancer population' outcomes and to explore their experience of living with cancer and i-PE. The second aim was to explore the role of the key thrombogenic biomarkers as a predictive biomarker of thrombosis.

Methods: Mixed method research with critical integrative analysis. A systematic literature review and qualitative analysis to examine patients' experience of living with cancer-associated thrombosis. A prospective observational case-controlled cohort study with embedded semi-structured interview study to investigate the quality of life and patients' experience of living with cancer and incidental pulmonary embolism. A retrospective case control-study and scientific analysis of defined biological key factors associated with thrombosis.

Results: The diagnosis of cancer-associated thrombosis including incidental pulmonary embolism negatively affect patients' life, and patients experience this diagnosis in the context of living with cancer. Yet it is a diagnosis that often misattributed, misdiagnosed and associated with lack of information among patients and some of the clinical care professionals. The scientific analysis of the biological biomarkers illustrates the potential role of TF-mRNA as a predictive biomarker for cancer-associated incidental pulmonary embolism and the role of anti-factor ten anticoagulation in reducing the risk of thrombosis.

Conclusion: Awareness of patients and care professionals regarding the high risk of thrombosis among cancer population represent an urgent need. Risk assessment tools to predict patients at increased risk of thrombosis would be of value and help target education and reduce the risk of diagnostic overshadowing**.**

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Table 1 List of abbreviations

ACTS	Anti-Clot Treatment Scale
AKPS	Australian-modified Karnofsky Performance Status
CHH	Castle Hill Hospital
CI	Confidence Interval
CRF	Case Report Form
CRP	C-Reactive Protein
CT	Computerised Tomography
DVT	Deep Vein Thrombosis
ESAS	Edmonton Symptom Assessment Scale
GCP	Good Clinical Practice
HEY	Hull and East Yorkshire
HEYHT	Hull and East Yorkshire Hospitals NHS Trust
HR	Hazard Ratio
HRA	Health Research Authority
HYMS	Hull York Medical School
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
i-PE	Incidental Pulmonary Embolism
LMWH	Low Molecular Weight Heparin
MDCT	Multidetector Computed Tomography
MDT	Multidisciplinary Team
PE	Pulmonary Embolism
PESI	Pulmonary Embolism Severity Index
PI	Principal Investigator
PIS	Patient Information Sheet
PS	Performance Status
QCOH	Queen's Centre for Oncology and Haematology
QoL	Quality of Life
R&D	Research and Development
REC	Research Ethics Committee
SF-12	Short Form-12
SSPE	Sub-Segmental Pulmonary Embolism
VKA	Vitamin K Antagonist
VTE	Venous Thromboembolism

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Dedication

I would like to dedicate this work to my parents, my husband, and my lovely kids.

Author's Declaration

'I confirm that this work is original and that if any passage(s) or diagram(s) have been copied from academic papers, books, the internet or any other sources these are clearly identified by the use of quotation marks and the reference(s) is fully cited. I certify that, other than where indicated, this is my own work and does not breach the regulations of HYMS, the University of Hull or the University of York regarding plagiarism or academic conduct in examinations. I have read the HYMS Code of Practice on Academic Misconduct, and state that this piece of work is my own and does not contain any unacknowledged work from any other sources'. If applicable, the declaration should also include; 'I confirm that any patient information obtained to produce this piece of work has been appropriately anonymised'.

Presentations and publications

Oral presentation

“A systematic review of health-related quality of life of cancer patients who have venous thromboembolism” Hull York Medical School, Postgraduate Research Conference, York 2016

Poster presentations

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“Activation of PAR2 by factor Xa increases the permeability across cultured endothelial cell monolayers” International Society on Thrombosis and Haemostasis, ISTH Congress 2017 Berlin, Germany,

Publications

Cancer patients’ experiences of living with venous thromboembolism: A systematic review and qualitative thematic synthesis. Naima B Benelhaj, Ann Hutchinson, Anthony M Maraveyas, Julie D Seymour, Muhammad Waqas Ilyas and Miriam J Johnson. Palliative Medicine 2018, Vol. 32(5) 1010– 1020.

Alteration in endothelial permeability occurs in response to the activation of PAR2 by factor Xa but not directly by the TF-factor VIIa complex. Naima E. Benelhaj, Anthony Maraveyas, Sophie Featherby, Mary E.W. Collier, Miriam J. Johnson, Camille Ettelaie. Thrombosis Research 2019, Vol. 175, 13–20

Chapter 1 Theses introduction

1.1 Introduction

This thesis presents a preliminary data from a mixed method research undertaken to explore the symptom burden, quality of life, and cancer patients' experience of living with incidental pulmonary embolism treated with self-injection low molecular heparin and the role of thrombogenic proteins (tissue factor and protease activated receptor-2) in the pathophysiology of cancer associated thrombosis as a predictive biomarker.

This chapter will present a road map for the theses. The chapter will start with a summary background of the literature, statement of the problem, the significance of the study, the primary research questions, and hypotheses followed by a summary of the thesis chapters.

1.2 Background

1.2.1 Incidental pulmonary embolism

Various terms have been used to describe incidentally diagnosed PE, such as unexpected, asymptomatic, incidental, silent, and unsuspected. In order to reduce this heterogeneity, a common definition of this condition has been proposed (1). I-PE is defined as pulmonary embolism diagnosed on a CT scan performed for reasons other than a clinical suspicion of PE (2).

Since clinically unsuspected PE does not mean that the patient has no symptoms, the term 'asymptomatic PE' should be avoided (3)(34) I will use 'incidental pulmonary embolism (i-PE) throughout this theses.

Incidental pulmonary embolism (i-PE) in the cancer population, affects nearly 3.1% and it is a growing challenge for clinicians and patients (4). The diagnosis of i-PE in cancer has increased in recent years. This could be explained by the continuous improvement of CT scanners technologies, the use of advanced CT scans (better detection), the practice of whole-body scanning for restaging purpose and/or due to the increased number and lines of anti-cancer treatments. The diagnosis of i-PE in cancer population

complicates management, causing a delay in treatments or surgery for many and is associated with a higher risk of mortality and morbidity (5, 6). It is acknowledged that the diagnosis of cancer and its subsequent treatment can have a profound impact on the equality of patients' life and with some developing post-traumatic distress syndrome (7, 8). Moreover, treatment of i-PE in cancer patients is associated with a high risk of bleeding and inferior VTE recurrence prevention rates (9). Any type of cancer thrombosis [deep vein thrombosis (DVT), pulmonary embolism (PE)] can occur at any stage of the cancer journey, but the risk is higher in the first three months of the diagnosis and in advanced disease. The pathophysiology of thrombosis in cancer (deep vein thrombosis or pulmonary thrombosis) is complex (10). Many risk factors contribute to this increased risk of thrombosis compared to the general population. These factors can be divided into three categories, patient-related, cancer-related and treatment-related factors. Furthermore, many physiological factors can be associated with a higher risk of thrombosis in cancer population including platelets (thrombocytosis), leucocytosis, thrombin, P-selectin, D-dimer, and tissue factor (10-12). The issue of i-PE is discussed in more details in Chapter 2.

1.3 Statement of the problem

The natural history of i-PE in cancer patients has been poorly studied. Given the absence of randomised studies of anticoagulant therapy in cancer patients with incidentally diagnosed venous thrombosis, optimal management remains unknown (13).

The overall prevalence of incidental VTE among cancer population undergoing routine follow up CT scans varies, depending on the study population. A systematic review and meta-analysis of 12 studies report that a weighted mean prevalence of incidental PE of 3.1% (95% CI, 2.2–4.1%)(4). The assumption that these cases are asymptomatic is erroneous. However, patients as well as clinicians, frequently attribute relevant symptomatology to the progression of underlying cancer or the adverse effects of cancer treatments (14, 15). This leads on many occasions to delay in diagnosis and management. Few studies have reported on the correlation between the presence of thrombosis related symptoms and poor outcomes in cancer population with i-PE (14, 16). However, these studies were either retrospective or small in size were the results can't be generalised. The standard of care remains to treat all cancer patients with i-

PE in the same manner as for PE diagnosed in response to presenting symptoms (17). These recommendations for i-PE were extrapolated from studies on symptomatic PE and evidence from retrospective studies. However, the significance of symptoms and clinical outcome of such i-PE in oncology patients represents a big gap in knowledge in the literature. Clinical prognostic scores stratifying patients with i-PE are in widespread use, but they relate predominantly to the symptomatic PE setting (18).

The physiological and psychological effects of cancer diagnosis and its treatments are well recognised. Cancer-related fatigue and loss of physical function have been reported by cancer patients as being a mong the most distressing symptoms (19). Advanced muscle weakness and cachexia, and decreased the independence to perform daily living activities all add to the disease burden for cancer patients (19, 20). The combination of poorer social and psychological well-being have emphasised investigating quality of life (QoL) interventions to help maintain patients' function for as long as possible (21). Determining whether an intervention improved QoL over baseline provides important information to guide clinical decision making (22). However, the effects of the incidentally diagnosed PE, the burden of anticoagulation treatments and the impact on the quality of life of patients that have an i-PE represent a significant knowledge gap in the present literature.

Finally, the incidence of thrombosis among cancer patients varies considerably depending on several factors, the most important being tumour entity and stage. Biomarkers such as circulating D-dimer, P-selectin and tissue factor (TF) have been specially investigated for their capacity of predicting venous thromboembolism (VTE) during the course of cancer (23). Tissue factor has gained significant attention in cancer thrombosis research. Its association with high occurrence of VTE is supported by several studies (24, 25). High level of TF bearing microparticles or (TF-MP) or TF-antigen activity in plasma were associated with higher mortality in some cancer types (24). However, results from studies that investigated circulating TF-bearing MPs as a biomarker for VTE in cancer are inconsistent. The value of TF expression as biomarkers for cancer-associated thrombosis still not fully understood.

Cancer care often concentrates o the state of art of the pharmacological/biomedical treatment and often neglects to address the psychological and social (psychosocial)

problems associated with the cancer diagnosis. This deficiency may compromise the effectiveness of health care and thereby adversely affect the health of cancer patients. Additionally, the literature is lacking prospective studies that investigate these issues in real life, and little is known about the clinical course and impact of i-PE in the cancer population.

1.4 Aims

Given this gap in the literature, the purpose of this theses is two-fold. Firstly, to prospectively capture and describe the clinical outcomes of cancer patients incidentally diagnosed with i-PE and to provide real-life data on the clinical course of such diagnosis. Including gaining an understanding of how patients with cancer experience i-PE in the context of cancer. Secondly, we will explore the potential role of biological biomarkers in this situation, expanding our understanding of the pathophysiology of cancer-associated thrombosis and the role of tissue factor and PAR-2 in cancer thrombosis, and the cancer ability to produce TF could help identify cancer patients who may be at higher risk of developing thrombosis.

1.5 Research questions

The research questions underlying the investigation in this study are as follows:

Q1. What is the experience of people living with cancer-associated thrombosis in regard to their response to the diagnosis, coping with the additional burden and the effects of long-term anticoagulation on their daily life?

Q2. What is the impact of an incidental pulmonary embolism on the clinical outcomes of cancer patients?

Q3. What is the experience of people living with cancer and incidental pulmonary embolism in regard to their response to the diagnosis, coping with the additional burden and the effects of long-term anticoagulation on their daily life?

Q4. Is there a candidate biomarker that can stratify the risk of i-PE and the outcome in patients with cancer?

1.6 Research design

The objectives in relation to these questions were addressed as follows;

First. A mixed-method approach was used;

To gain an understanding of the impact of VTE overall in the context of cancer a systematic literature review and qualitative synthesis were conducted. Then a prospective observational case-control survey study to examine the effects of symptom burden of i-PE on cancer patients' outcome compared to matched controls was planned. Participants in the cohort study were invited to participate in a qualitative semi-structured interview to explore and understand patients' experience of living with cancer and i-PE. The findings of the cohort's quantitative and qualitative data were synthesised following data analysis. A justification of the methodology is will be discussed in detail in chapter 3.

Secondly, the laboratory work includes a retrospective case-control study of cancer tissues to analyse the tissue factor and PAR-2 (mRNA, and proteins) level of patients who developed i-PE compared to matched controls with no thrombosis and an in vitro analysis of the effect of PAR-2 activated by factor X on the permeability of endothelial cell monolayer. The full materials and methods used in this study will be presented in Chapters 7 and 8 respectively.

Finally, the findings of all the studies are brought together in the discussion in Chapter 9.

1.7 Definition of Terms

It is important to define the following terms relating to the topic of this study, which are used throughout this dissertation

1.7.1 Symptom burden

The term symptom burden first reported in 1999 by N.A. Desbiens .et.al in a study of hospitalised patients with terminal illnesses and refers to the relationship between symptom burden and quality of life (26). In the oncology literature, symptom burden has been developed to describe patients' subjective perception of the combined impact of illness and treatment-related symptoms (27). Building on a conceptual analysis of symptom burden, it may be defined as 'the subjective, quantifiable prevalence, frequency and severity of symptoms, placing a physiological burden on

patients and producing multiple negative, physical and emotional patient responses' (28).

1.7.2 Quality of life

QOL is a multidimensional concept that can be difficult to define due to the subjective nature of both positive and negative aspects of life (29). In this thesis, QoL is an overarching concept which refers to the ability to enjoy normal life activity, such as shopping, driving, working and entertaining. Quality-of-life (QoL) Outcomes used for determining the efficacy and impact of cancer care (30).

1.7.3 Performance status

Performance status is an objective measure of how well a person is able to carry on ordinary daily activities, including the ability to care for oneself, engage in physical activity, and whether any assistance or medical care is necessary for the patient while living with cancer. Performance status scales are widely used in assessing the expected outcome of cancer (or prognosis and provides an estimate of what treatments a person may tolerate) (31). The two most commonly used scales are the Karnofsky performance status (KPS) score and the Eastern Cooperative Oncology Group (ECOG) score. The Karnofsky Performance Scale (KPS) has been used as an assessment tool for performance status in oncology since 1948 (32) assesses three dimensions of health status – activity, work and self-care (33). Recently replaced by the Australia-modified version (AKPS) (34), which provide to be much easier and more directive, applicable to both inpatient and community palliative care. The Eastern Cooperative Oncology Group (ECOG) scale (35) is commonly employed in oncology settings as well. The ECOG scale provides a five-point scale in contrast to the 11 points in the Karnofsky scale. It is simpler to use and has a precise message.

1.8 Theses contents

The following is an overview of the organization of this thesis. The following chapters in this dissertation are as follows;

Chapter One presents an outline of the theses in general, including the problem statement, aims, research questions and the research design.

Chapter Two presents a literature review of the problem under investigation. In this chapter, I aimed to present the background and highlight the gap in knowledge. In this chapter, the epidemiology and symptomatology of cancer-associated incidental pulmonary embolism are discussed. The clinical characteristics and current management are covered. Also, the stratification and prognostic tools used in cancer thrombosis are presented. The quality of life and patients experiences of living with cancer are generally discussed. The pathophysiology of cancer-associate thrombosis and thrombogenic biomarkers are covered in this chapter.

Chapter Three, the methodology chapter will cover the study design and justification of the methods used. Because the methods used were different from chapter to another, a brief description of the methods will be presented in this chapter and will be presented in more details in each chapter. The chapter also introduces the research paradigm and the context of the study describes the population and the research sites that were selected for the study. Protection of human subjects and ethical issues are discussed. It also includes various stages of the data collection process and describes the components applied to data analysis as well.

Chapter Four presents a systematic literature review and qualitative synthesis of cancer patients' experience of living with cancer-associated thrombosis both symptomatic and incidental. Because the literature summary in Chapter Two highlighted a major gab in knowledge about this issue in people with i-PE, I thought it was mandatory to try to cover this issue in a systematic manner to avoid any bias in the results reported and to explore relevant work in people with cancer-associated thrombosis who had been diagnosed as a result of symptoms. This systematic literature review has been presented as an abstract in the 15th World Congress of the European Association for Palliative Care (EAPC) 2017 (36) and was published in a peer-reviewed journal as (*N.Benelhaj, at.al. 2018*) (37).

Chapter Five, the methods and preliminary results of a prospective survey study investigating the symptom burden and quality of life of the included participants are presented and findings are discussed.

Chapter Six, the methods and findings of a semi-structured interview exploring patients' experience of living with cancer and i-PE are presented and discussed.

Chapters Seven and Eight present the laboratory work investigating the role of TF and PAR-2 in cancer thrombosis. Chapter seven presents a retrospective case-control study investigating the difference in of TF and PAR-2 (mRNA and protein) levels in tumour tissues in patients with i-PE and matched controls that may be related to cancer thrombosis.

Chapter Eight presents the results of the effects of PAR-2 activated by FXa on the permeability of endothelial cell monolayers and its role in the pathophysiology of thrombosis in cancer. In this chapter, the methods used are presented in details. Also, the analysis and the results are presented. A general discussion of findings is presented. The work in chapter eight has been presented as an abstract in International Society on Thrombosis and Haemostasis XVIII Congress 2017 (38), and was published in a peer-reviewed journal as (*N.Benelhaj, at.al. 2019*) (39).

Chapter Nine discusses the findings of the theses as a whole; the purpose of this chapter is to present a summary of the study, including a brief description of its purpose, a review of the research questions that guided the study, a synopsis of related literature, a description of the methodology, and the findings. The summary is followed by a discussion of the findings, which are presented through a structured review of answers from the research questions as well from the themes that emerged from the mixed-method data analysis. This chapter also includes a discussion of the recommendations for i-PE management in cancer practice and recommendation for further studies. The theoretical contribution of this study to the existing body of literature is also addressed in the implications section of this chapter followed by a brief conclusion of the study.

Appendices include copies of the research instruments such as study protocol, interview guide, informed consent documents, and other documents that were necessary to the development of this study such as ethical approvals.

Chapter 2 Literature review

2.1 Introduction

Venous thromboembolism (VTE) comprising deep vein thrombosis (DVT) and pulmonary embolus (PE) is a common phenomenon worldwide that changes peoples' lives. It affects one in 1,000 patients; 6.5 million people globally each year (40, 41). Approximately 20% of all newly diagnosed cases of VTE are cancer patients (42) and the risk of VTE increases in cancer population compared with the non-cancer population by 6-7 folds (43, 44). Although the risk increases with late-stage and during chemotherapy, over 50% of VTE occurs during the first three months from diagnosis (45)(25) and interferes with cancer management (46). Up to half of the vascular thromboembolic incidents (VTE) in oncology were diagnosed incidentally (2, 15, 47). The discovery of incidental PE (i-PE) on routine restaging scans conducted in cancer patients has increasingly become a concern and a common problem in clinical practice especially with the advent of multidetector row computed tomography (CT) technology (48, 49). i-PE in cancer patients is not a benign condition and as far as the usual endpoint used to assess treatment consequences and efficacy it is associated with high rates of recurrent venous thromboembolic events (50), bleeding and a mortality rate (51) similar to trends seen in cancer patients with symptomatic PE. However, information on the natural history and clinical consequences of i-PE is limited.

2.2 Epidemiology of i-PE

The reported prevalence of incidental VTE among cancer population undergoing routine staging scans varies according to cancer type, stage and site of VTE. In a retrospective study analysed previous scans, i-PE was estimated to represent 40% of diagnosed PE among cancer population and more common in the metastatic group (52). This could be due to the increased frequency of requested scans performed for follow-up and staging in patients with metastatic disease, as well as due to the biology of metastatic cancers compared with localized ones. In a systematic review and meta-analysis of 12 studies including over 10,000 patients, cancer patients had a weighted mean prevalence of i-PE of 3.1% (95% CI, 2.2–4.1%) (4). In another recent systematic review with meta-analysis of fourteen studies, the prevalence was 1.28% (95% CI,1.47-

2.21) (53). G.W Gladish and colleagues (54) reported a prevalence of approximately 4% in the high-risk oncology population (3.8% for out-patients vs 6% for in-patients). These results agreed with previous reports of a higher rate of i-PE in hospitalised patients (55, 56).

In a large retrospective cohort analysis of scans in cancer patients, Douma *et.al* reported a prevalence of an incidental abdominal deep vein thrombosis of 1.1% (95% CI 0.6–2.0), similar to that of a PE or lower extremity DVT (1.3%, 95% CI 0.7–2.3) (57). Singh *et al* in a retrospective analysis of a cohort of 220 consecutive patients with gastrointestinal cancers (higher-risk cancer patients) undergoing active treatment reported that 7.3% of patients undergoing routine staging had unsuspected DVT and visceral venous clots, with i-PE in 2.3% of patients (58). In pancreatic cancer patients, venous thrombosis symptomatic and incidental reported being independently associated with high mortality (59) highlighting the impact of cancer type on the epidemiology and sites of VTE. Generally, the rate of incidental PE in cancer patients has been reported as ranging from 2.6 – 4.4% (54, 60). However, the absolute reported incidence of i-PE is depending on the cancer type and stage and probably represents an underestimation ranges (15). The large disparity found between studies may be explained by several factors; (1) inclusion criteria of ‘‘true’’ asymptomatic patients, (2) cancer type and stage of participants; (3) proportion of inpatients and outpatients; (4) characteristics of CT scanners and imaging protocols (4).

2.3 Clinical characteristics of patients with i-PE

Patients’ demographics and clinical characteristic of cancer patients who developed i-PE were evaluated and compared to matched controls with cancer and symptomatic PE in a few clinical studies aiming to inform patient-centred management. These are shown in Table 2.1. These studies were inconsistent, probably as a result of the sample size or study design used, hence most of them were retrospective studies.

Table 2.1 Patients demographic and clinical characteristic

	history of VTE	recent surgery	recent chemotherapy	tumour type	lower performance status	central venous lines	Hospitalization	metastatic disease	Age/ Gender
Shinagare.et.al.2012 (5)								χ	χ
Exter.et.al.2011(6)				χ					χ
O'Connell CL.2006 (3)	√	√	χ			χ	χ		
DA Thaker. 2017 (52)				√				√	
M.S.D'Izarn. 2012 (61)	√	χ	χ	χ	√	√	χ	√	√
C.O'Connell.20-11 (51)	√	√		χ					
S.Soler. 2012.(62)	√			√					
C.Font.2011 (63)			χ						√
Browne 2010 (64)			√				√	√	

√ =there is a difference / X= there is no difference

2.4 Symptomology

The common perception is that patients with i-PE are asymptomatic, however, a significant proportion of cancer patients with i-PE do have symptoms suggesting pulmonary embolism (PE) (65) However, these can be misattributed to cancer or side effects of cancer treatment, causing a delayed or missed diagnosis (15) and may only be discovered at autopsy (66, 67). It is well acknowledged that the diagnosis of i-PE is difficult as neither the symptoms nor the signs are specific.

This has been previously reported by O'Connell.et.al (51) in analyses of retrospective data from medical charts of cancer patients with i-PE; up to 75% had signs and symptoms possibly related to the presence of PE at the time of the diagnosis. After adjustment for anaemia and lung metastasis, cancer patients with i-PE were significantly more likely than control patients to complain of fatigue (54% v 20%; P=0.0002) and shortness of breath (22% v 8%; P.02). Furthermore, fatigue was more common among cancer patients with i-PE than among the control patients. Fatigue remained a significant predictor of PE even after further adjustment for age and anaemia (P=0.0002) (15, 51).

These findings had been confirmed by Nick van E. 2014 in a retrospective study using data from medical charts of 66 cancer patients with i-PE. This showed that 27 (41%) had one or more symptoms could suggest PE at the time of the scan (61). Therefore, a proportion of cancer patients with incidentally diagnosed PE were symptomatic at the time of diagnosis but the symptoms did not trigger their clinician to order a dedicated CTPA.

Again, the studies are limited by their retrospective nature but there is sufficient evidence of concern to justify the need for prospective robust research to bridge the gap in knowledge regarding this issue.

The outcome of cancer patients with i-PE is appeared to be similar to those who developed asymptomatic (suspected) PE, while their survival appeared to be worse compared with matched controls without PE (4, 9, 51, 63). The relationship between symptoms in cancer patients with i-PE and clinical outcomes is inconsistent (51). In a retrospective study, den Exter et al (9) reported no difference of recurrent pulmonary

emboli (PE), bleeding, or death between cancer patients who diagnosed with i-PE and matching controls with symptomatic PE. However, O'Connell (3) demonstrated that 75% of patients with incidental PE, were symptomatic and in a follow-up study they reported found i-PE adversely affected survival compared to matching controls with no thrombosis. However, the survival rate was shorter among those with symptomatic PE (51). In addition, respiratory symptoms and fatigue have been independently associated with shorter survival in patients with lung cancer (68).

Fatigue and breathlessness are two common symptoms in patients who have advanced cancer (69, 70). Up to 90% of patients undergoing chemotherapy complaining of fatigue, depending on the type of treatment and the type and stage of cancer (71). Furthermore, the aetiology of fatigue or dyspnoea is multifactorial, with many contributing interrelated abnormalities. Recent research reported that patients with cancer who developed incidental pulmonary embolism have high level of fatigue (72). However, no research available explained the increased risk of fatigue among cancer patients who developed incidental pulmonary embolism. Fatigue is a difficult symptom to define, but broadly speaking can be understood as a subjective sensation of weakness, lack of energy or becoming easily tired. and is also a symptom of both physical and mental illnesses (73). Fatigue is sometimes referred to as asthenia, tiredness, lack of energy, weakness, and exhaustion. However, Not all of these terms have the same meaning to all patient populations (74).

Patients with cancer may also define fatigue as tiredness, lack of energy, lack of concentration and motivation, weakness, exhaustion, lethargy, and depression (51).

There is a possibility that some patients may reported breathlessness as fatigue. And since most of the reported studies were retrospective in nature it was unfeasible to go back and clarify if participants meant and perceived breathlessness as fatigue.

Furthermore, even in the study that show that cancer patients with i-PE shown higher rate of fatigue comparing to controls they may have physiological reasons which cannot determine. It is impossible to be able to clarify that because of the retrospective design of the study. Therefore, a prospective study using patients reported outcome measures would be able to determine whether fatigue or breathlessness were meant by patients.

This controversy could be explained that these studies were retrospectively recorded and subjective, thus nonspecific signs and symptoms may be variably documented and interpreted differently depending on the treating physician, are difficult to use as a discriminating or prognostic tool in patients with cancer who are unexpectedly diagnosed with PE.

In summary, PE may be diagnosed incidentally because the signs and symptoms are neither sensitive nor specific, and are misattributed to underlying cancer or the adverse effects of cancer treatments. Most of the data available are a retrospective which contributes to the lack of clarity (3). These findings emphasize the need for prospective robust research to bridge the gap in knowledge about this area.

2.5 Radiology

Two reviews showed that radiologic features of i-PE appear similar to symptomatic PE in the cancer population with some discrepancies between the included studies. Although, one would expect incidental PEs to be more peripheral and sub-segmental compared with symptomatic PEs, this was not the observation reported by the expert reviews. In a narrative review with pooled data of 12 studies (552 patients with i-PE out of 35,990 patients, Donadini, et.al 2014 (53) reported that nearly half of the i-PEs located in central pulmonary arteries and one third involving both lungs: main arteries in 3–31.6 % of cases, lobar in 20–37 % and segmental in 21–60 %. Separate data on the presence of isolated sub-segmental pulmonary embolism were reported in nine studies, ranging from 0 to 34 % of patients (3, 5, 9, 51, 54, 59, 61, 64, 75). Four studies including a control group of patients with SPE found that the embolic burden in i-PE patients is significantly lower than in SPE patients (61, 75, 76), whereas one study reported no significant difference between the two groups (6). In accordance with these results, Nick van Es. 2104, (15) in his review of 11 studies included 609 i-PE patients reported that similar to symptomatic PE, about one-half of i-PE was located in lobar or more central arteries and bilateral lung involvement occurs in 23-46% of the cases. On the other hand, a series of consecutive CT scans in 48 cancer patients with i-PE were reassessed and compared to 113 CTPA scans of consecutive patients (cancer and non-cancer) with symptomatic PE has shown that the median obstruction index, according to the Qanadli scoring system, was significantly higher in patients with symptomatic PE compared to i-PE (30% vs. 18%, p=0.008). However, authors of this

study acknowledged that the embolic burden of i-PE was probably underestimated as none was diagnosed with SSPE which may reflect the challenge of correctly detecting peripheral emboli with CT scans. O'Connell 2011, (14) reported that i-PE identified more proximal than sub-segmental and has a significant negative impact on survival among cancer patients. The hazard ratio (HR) for death among UPE patients was 1.51 (95% CI 1.01–2.27, P = 0.048). However, in a case-control study D'Izaarn (61) reported that patients with i-PE also had fewer proximal clots than patients with symptomatic PE, but with no observed difference s in the rates of recurrent VTE or death were observed between the two groups of patients with PE.

Isolated sub-segmental pulmonary embolism

The clinical relevance of isolated sub-segmental pulmonary embolism is the subject of debate. Increased detection rates of isolated sub-segmental pulmonary embolism have been reported over the last two decades, related to the improvement in the CT scanning techniques (1). However, no concurrent changes in mortality rates are reported raising the question about the significance of SSPE (77). Support for this hypothesis was provided by several retrospective studies that show no recurrent VTE or PE-related deaths during 3 months follow-up among patients with untreated SSPE (78). In contrast, in a combined post hoc analysis of two large prospective cohort studies, Den Exter *et al*, suggested that the prognosis for patients with SSPE may be comparable to patients with more proximally located PE studies (79). Nick van Esa (15) reported no significant difference in the rates of recurrent VTE, bleeding, and mortality between the two groups. The proportion of patients with active malignancy among the 116 patients with SSPE and 632 patients with proximal PE was 18.1% vs. 17.9% respectively.

Furthermore, only limited data is available on the prognostic relevance of clinically unsuspected SSPE. O'Connell et al et.al.2011, in an analysis median survival rate of 17 cancer patients with unsuspected SSPE of whom 13 were treated with some form of anticoagulation (51) reported significant better survival rate compared to patients with more proximal PE (7 vs. 12 months; HR 1.70; 95% CI 1.06-2.74). However, survival did not differ from the survival of matched control patients without PE, suggesting that unsuspected SSPE in cancer patients is not associated with worse survival.

These data highlight the gap in knowledge in the clinical relevance of isolated sub-segmental PE, and the need to personalise the management of these patients.

2.6 Natural course and outcome of cancer associated i-PE

Patients with cancer and symptomatic VTE have lower survival rated and a higher rate of VTE recurrences or major bleeding events compared to those with cancer but no VTE (80, 81). However, only limited studies available about the natural history of i-PE among patients with cancer. O'Connell et al, reported a high mortality rate among cancer patients with i-PE comparing to matched controls with no VTE (51). While, D'Izarn et al. reported no difference in mortality rate of patients with cancer and i-PE compared to patients with cancer and no VTE, after adjusting of patients' PS and cancer stage (61), While den Exter et al.(2015), reported no significant difference with respect to mean age, gender, cancer type and stage, or additional risk factors for VTE (9). Recent work seems to support the notion that cancer patients with i-PE have similar outcomes to symptomatic (suspected) PE cases, their survival appearing worse compared with matched controls without PE (4, 50, 63, 82, 83).

On the other hand, few studies have compared the clinical course between cancer patients with symptomatic PE and cancer patients with i-PE. A review by Trujillo-Santos reports no significant difference in terms of recurrences, major bleeding or mortality (50). Recently, van der Hulle .et al, (50) in a systematic literature review and a pooled analysis of 926 patients reported that 6-month VTE recurrence risk of 12% (95% CI 4.7–23%) in i-PE cancer patients who were left untreated. In the pre-treatment analysis, the incidence rate of recurrent VTE in patients who did not receive anticoagulant treatment was even 30 % (95% CI 8.2–77). Furthermore, a weighted pooled 6-month mortality of 37% (95% CI 28–47%), mortality varied between cancer type and cancer stage. The weighted pooled 6-month mortality was higher in patients with a centrally located thrombus compared with those with a peripherally located PE. They reported that all-cause mortality at 6 months was significantly higher for patients with a central thrombus (either central or lobar) compared with those with a more peripheral IPE (either segmental or sub-segmental): 42% vs 30% (HR, 1.8). Isolated sub-segmental pulmonary embolism occurred in 7.8% of patients, and central pulmonary embolism was reported in 5.5% of the cohort (HR = 1.1).

Even though the diagnosis of i-PE in cancer population is associated with significant morbidity and lower survival comparable to symptomatic PE has been reported (51, 84, 85) these data may be erroneous due lack of homogeneity in included samples as most of the data available were retrospective. This highlights the importance of the lack of prospective data in the literature regarding the clinical outcome of i-PE in the cancer population.

Although the evidence is largely based on observational, retrospective data, i-PE seems to carry a similarly poor prognosis in terms of recurrent VTE, bleeding and mortality as symptomatic PE (5, 6, 86). Autopsy studies suggest that i-PE represents an unrecognized cause of death in cancer patients (87).

Despite advances in the treatment of cancer-associated thrombosis, many cases of cancer remain complicated and may not benefit from anticoagulation that may add burden to patients' life. Accurate prognostic information can help physicians decide whether to initiate or continue anticoagulation therapy. Patients with advanced cancer and their family members often ask questions related to prognosis, and open, empathic discussions about this topic may improve satisfaction with care.

2.7 Embolic burden

It is reported that incidental pulmonary embolism represents 1–5% of scheduled computed tomography (CT) scans performed in cancer patients for reasons other than pulmonary embolism suspicion (4), representing about half of the pulmonary embolisms currently diagnosed in oncology (5, 6, 16). However, available data about the embolic burden associated with incidental pulmonary embolism in cancer patients are heterogeneous. Data from retrospective and observational studies illustrate that the embolic burden in incidental pulmonary embolism is similar to that in symptomatic pulmonary embolism (15, 16, 76) and that i-PE could have an adverse impact on patient survival [16]. In addition, similar outcomes have been observed in overall mortality, major bleeding and recurrent VTE on comparing patients with UPE to those with symptomatic events [14, 17].

In contrast, Bach et al. reported that the embolic burden of 129 cancer patients with i-PE was significantly lower compared to 111 cancer patients with symptomatic PE (88). Regarding the relevance of embolic burden, den Exter et al. found no association

between the obstruction index in i-PE cancer patients and 6-month survival (76). However, large prospective studies are needed to clarify the prognostic relevance and embolic burden for incidental pulmonary embolism in cancer patients.

2.8 Management of i-PE

Management of i-PE in patients with cancer represents a major therapeutic challenge that further complicated by multiple cancer-related risk factors and comorbidities, which influence the choice of anticoagulation (89-91). In the absence of sufficient evidence to withhold anticoagulant treatment in cancer patients with i-PE, the current international clinical guidelines recommend the same initial and long-term anticoagulation as for comparable patients with symptomatic PE (91-93), but the appropriate treatment and management of i-PE in cancer patients has not been sufficiently investigated.

For the general population, the standard treatment for acute VTE consists of initial therapy with low-molecular-weight heparin (LMWH), followed by longer-term treatment (3 to 6 months) with an oral vitamin K antagonist (VKA) (94). However, due to the substantial and continuing risk of VTE recurrence and haemorrhagic complications in patients with cancer, Evidence-based treatment guidelines recommend low molecular weight heparin (LMWH) monotherapy for cancer-associated venous thromboembolism (CAT) for 3 to 6 months (and possibly indefinitely) (95, 96) demonstrating clinical benefit over VKAs in the secondary prevention of VTE (96, 97).

The 2016 American College of Chest Physicians Guidelines on Antithrombotic for VTE concur with their 2012 recommendation to treat incidental VTE the same as suspected VTE (92, 98). The National Comprehensive Cancer Network also recommends treatment of incidental PE similar to that for symptomatic PE in patients with cancer and recommends against routinely obtaining repeat imaging (98). The American Society of Clinical Oncology (ASCO) recommendations for the management of established VTE in cancer patients (89) can be seen in Table 2.2.

Based on these indications, cancer patients with i-PE would be anticoagulated for at least 6 months or while the disease is active, which in most cases would mean indefinite treatment. Dedicated studies on the treatment of i-PE are lacking, leaving

doubts over the need for such an approach which exposes patients to an increased risk of major bleeding events. However, in cancer patients receiving ongoing cancer therapy, the continued high risk of recurrent VTE may strongly support a decision to initiate anticoagulation. However, in the recent analysis on VTE recurrence from a combined analysis of multiple cohorts of patients with cancer and i-PE, isolated sub-segmental i-PE was not associated with high VTE recurrence risk (50). Given the associated increased risk of bleeding, potential interference with cancer treatments, impact on patient quality of life, and added cost, the decision to initiate anticoagulant therapy is usually made with due consideration of these consequences (86).

Concerns over the need for anticoagulant treatment may especially hold for distal PE since segmental and sub-segmental PE seem to have a more benign course than more proximal embolism. Outpatient care is commonly used but there is little evidence to underpin outpatient approaches, these often being empirical and based on retrospective studies (47). Around half of cancer patients who developed VTE would adhere to the long term of low molecular heparin injection despite strong recommendations from clinical practice guidelines (99) which may be related to the burden of self-injection and medication costs. In recent years, multiple direct oral anticoagulants (DOACs) that target thrombin (dabigatran) or activated factor X (rivaroxaban, apixaban, and edoxaban) have been approved for the management of arterial and venous thrombotic disorders in general population (100). They demonstrated comparable effectiveness and safety over vitamin K antagonists in the non-cancer population (101). However, they are currently not recommended by oncology guidelines for thromboprophylaxis or treatment of cancer-associated thrombosis due to the lack of sufficient clinical data from dedicated trials in patients with cancer (93, 102). Even so, a network meta-analyses based on indirect comparisons also suggest that direct oral anticoagulation may also have similar effectiveness and safety to LMWHs for the management of CAT (103, 104).

The rapid global adaptation of direct oral anticoagulants for management of cancer-associated thrombosis is an emerging treatment trend that needs to be addressed based on the current level of evidence. Direct oral anticoagulants (DOACs), including dabigatran, rivaroxaban, apixaban, and edoxaban, have been approved for the treatment of venous thrombosis in the general population. Current research has

demonstrated comparable effectiveness and safety of direct oral anticoagulants to vitamin K antagonists in the non-selected cancer subpopulation (101). However, many clinical trials tend to exclude cancer patients, or only include high selected cancer patients. Because of that, their results cannot be generalised to the whole cancer population (101, 105). The most appropriate conclusions to draw from this data are that DOACs may have similar efficacy and safety as VKAs in a highly-selected cancer patient population.

Recently, two multicentre, open-label, randomized, controlled trials of direct factor Xa inhibitors (edoxaban and rivaroxaban) and LMWH for the initial therapy of cancer-associated thrombosis were published: The Hokusai VTE Cancer trial (106) and The SELECT-D (107) trial. Both trials included patients with cancer and i-PE. These cancer-specific trials reported that direct oral anticoagulants were more effective than low molecular heparin to prevent recurrent venous thromboembolism albeit associated with higher clinically relevant non-major bleeding, especially gastrointestinal bleeding. However, no survival rate difference was reported suggesting that direct oral anticoagulants are non-inferior to low molecular heparin for the prevention of recurrent venous thromboembolism. Consistent with these results, reviews with meta-analysis also suggest that direct oral anticoagulants may also have similar effectiveness and safety to low molecular heparin for the management of cancer-associated thrombosis (103, 104, 108, 109).

However, clinical guidelines continue to recommend low molecular heparin over direct oral anticoagulants as the preferred initial treatment of cancer-associated thrombosis due to the lack of high-quality data from dedicated trials (91). Despite continued advances in the treatment of cancer-associated thrombosis, many cases of cancer remain complicated and may not benefit from anticoagulation that may add burden to patients' life. Accurate prognostic information can help physicians decide whether to initiate or continue anticoagulation therapy.

Table 2.2 The American Society of Clinical Oncology (ASCO) recommendations for the management of established VTE in cancer patients

a) LMWH is the preferred approach for the initial 5 to 10 days of anticoagulant treatment.
b) LMWH given for at least 6 months is also the preferred approach for long-term anticoagulant therapy. VKAs with a targeted international normalised ratio (INR) of 2 to 3 are acceptable.
c) Indefinite anticoagulant therapy should be considered for selected patients with active cancer, such as those with metastatic disease or those receiving chemotherapy.
d) Vena cava filters can be used in the presence of contraindications for anticoagulation or recurrent VTE while on adequate anticoagulation treatment.
e) Anticoagulation should be avoided in the presence of active intracranial bleeding, recent surgery, pre-existing bleeding diathesis such as thrombocytopenia (platelet count < 50,000/µL) or coagulopathy.
f) For elderly patients, anticoagulation is recommended for established VTE as described for other patients with cancer.

2.9 Stratification and Prognostic assessment; can we predict the patient at risk?

Venous thromboembolism is a significant problem with fatal consequences. Cancer patients who develop venous thromboembolism, especially during chemotherapy are associated with early mortality. Cancer patients with the previous history of venous thromboembolism are more likely to develop recurrent venous thromboembolism; the recurrence rate is 21% in the first year after diagnosis. Furthermore, cancer patients with venous thromboembolism are at an increased risk of bleeding complications. This risk is due, in part, to the anticoagulant therapy necessary to treat VTE. The risk of major bleeding complications is as high as 12% per year following diagnosis and the start of anticoagulation therapy.

The diagnosis of venous thromboembolism impacts chemotherapy delivery, patient quality of life, the cost of hospitalization, and the use of health care resources. Therefore, much emphasis has been placed on clinical factors and predictive biomarkers that identify patients who are at an increased risk for venous thromboembolism (110). The risk of venous thromboembolism in cancer patients needs to be assessed in each patient to determine the clinical and cost-effectiveness of thromboprophylaxis, with the aim of the appropriate use of antithrombotic therapy.

Constantly, clinicians are searching for predictable biomarkers to identify cancer patients who at the higher risk of developing venous thromboembolism. The increased incidence of venous thromboembolism diagnosed in caner population-related in many studies in part, to the use of the newer anticancer treatments that are more thrombogenic, high-resolution imaging studies used to stage cancer patients. The increased risk of venous thromboembolism in the cancer population can be related to many clinical and biological factors, which can be patient-related factors, cancer-related, and treatment-related (25).

Broadly, there are some factors that relate to the patients themselves and also to the type of cancer (older patients, sicker patients, and patients with comorbidities). The most important risk factor is the type of cancer, patients with pancreatic cancer, brain tumours, lung cancer, and lymphoma are much more likely to develop clots than are those with other cancers, such as breast cancer. In addition, treatments such as

chemotherapy, antiangiogenic agents, and surgical procedures as well as hospitalization significantly increase the risk of venous thromboembolism (110). Pulmonary embolism can lead to critical results, and in many cases, this condition often goes undiagnosed in cancer patients despite the presence of symptoms. Prognostication is important for patients, their families, and health care professionals in preparing for the future and in determining eligibility for health care resources. However, clinical prognostication is difficult and fraught with error, often resulting in an overestimation of survival (111).

2.9.1 Risk stratification tools in cancer associated thrombosis

The American Society of Clinical Guidelines recommends that patients with cancer are assessed for venous thrombosis risk at the time of chemotherapy initiation and periodically thereafter (112). Patients' stratification would facilitate patients' education, screening and thromboprophylaxis.

Thromboprophylaxis conceptually appears as a meaningful strategy to reduce the burden of cancer-associated thrombosis, but the identification of patients at high risk remains a clinical issue. For the past decades, many researches have been aimed at finding ways of preventing venous thrombosis events in patients with cancer using anticoagulation. However, anticoagulation in cancer patients associated with increased risk of bleeding compared to non-cancer patients (113).

Studies have identified risk factors for venous thromboembolism in cancer patients. Recently, *Khorana, A. et al* developed a risk score that allows clinicians to predict the risk of venous VTE in cancer patients starting a new chemotherapy regimen (90). Successively, these risk factors are being used in risk assessment models to stratify patients with cancer into low, intermediate and high-risk categories for the occurrence of VTE (90, 114).

Current clinical practice is to use the five readily available clinical and laboratory parameters incorporated in the "Khorana-Score", site of cancer, platelet count, haemoglobin and/or use of erythropoiesis-stimulating agents, leukocyte count, and body mass index (BMI) to predict chemotherapy-associated thrombosis in ambulatory cancer patients. This risk scoring model was independently validated and expanded by Ay et al. by the inclusion of two further biomarkers, sP-selectin and D-dimer(114).

The Khorana score has been designed, based on combinations of the factors mentioned above and others, to assist clinicians with prognostication. However, it tend to be complex, requiring input of laboratory values or calculations that can be time-consuming and impractical for rapid outpatient assessment of prognosis in a cancer clinic (111) this model does not assess patients' performance status, a global assessment of the patient's level of function is not specific for PE. According to the Khorana-Score, patients with low or intermediate risk account for the greatest part of cancer patients and would benefit greatly from a better characterization of their VTE risk and appropriate strategies to prevent VTE (25).

Investigators of CATs have described several biomarkers which were suspected of predicting VTE in cancer patients. Soluble P-selectin (sP-selectin) and D-dimer were both associated with occurrence of VTE in the general population and subsequently were found to be independent risk factors for VTE in cancer patients (115, 116), both parameters were added to an existing risk model for the prediction of VTE in cancer patients by Khorana et al. (90) forming the extended "Vienna CATS Score" (114).

More recently, another modified Khorana risk assessment score (i.e., the Protecht score) (117) was designed by adding platinum- or gemcitabine-based chemotherapy to the five predictive variables for identifying high-risk cancer patients in a post hoc analysis of the Protecht clinical trial. The availability of predictive models can facilitate the management of cancer-associated thrombosis by allowing the identification of patients at the highest thrombotic risk, who have the greatest benefit/ risk ratio from receiving thromboprophylaxis.

Finally, the Ottawa Score has been developed to identify among patients with cancer and thrombosis those at highest risk of recurrent VTE, who may benefit from prolonged anticoagulant treatment (118).

2.9.2 Risk stratification tools in cancer patients with PE

Risk stratification tools exist to predict early post–pulmonary embolism (PE) mortality; however, few were specifically designed for use in patients with cancer. Two prognostic scores (POME-C / RIETE) have been presented in the literature for patients with cancer and acute pulmonary embolism each utilising multiple clinical and laboratory parameters. Both prognostic scores relate predominantly to the

symptomatic PE setting. Pulmonary embolism severity index (PESI) – a tool stratifying risk for all patients with PE (18) has been widely used. Cancer specific POMPE-C score incorporates eight clinical variables; patient weight, respiratory rate, O2 saturation, heart rate, altered mental status, respiratory distress, unilateral limb swelling and "do not resuscitate" status as predictors of 30-day mortality. The POMPE-C score showed better prognostic accuracy than PESI for patients with active cancer (119). The RIETE investigators (99) proposed a score utilising six clinical variables; age > 80 years, heart rate, hypotension, low body weight, recent immobilisation, and metastatic disease, for predicting 30-day mortality. The RIETE investigators also identified two additional laboratory variables namely WBC>11,000/mm3 and creatinine clearance <30ml/min. POMPE-C and RIETE have identified a substantially greater proportion of patients with PE likely to survive to 30 days with comparable sensitivity to the generic tools (120) In risk stratifying PE in patients with active cancer, cancer-specific tools appeared to exhibit better prognostic accuracy than their generic counterparts.

2.9.3 Hull Score

The Hull Score (47)(Table 2.3) was based on a prospective analysis of prognostic factors for early mortality and survival of a 154 cancer patients with i-PE managed uniformly under a standardised diagnostic and management protocol developed and applied in real-life conditions in a single centre. Factors included the presence of new symptoms, PS and the presence of incurable cancer. The most consistent predictors of survival in these analyses were the patient-reported symptomatology (new or worsening) and PS at the time of i-PE, combined with the self-reported presence of new or worsening symptomatology (without further elaboration) was proposed as a tool for risk-stratify cancer patients with an incidental pulmonary embolism. The primary analysis suggests that a simple prognostic score based on the patient-reported symptoms and contemporaneously assessed PS can be used to easily and reliably stratify the mortality outcomes of patients with i-PE and cancer in the clinical setting. The researchers acknowledge the limitations of the current work due to the lack of granularity of patient-reported symptom assessment as well as the lack of follow up of symptomatology which could provide a prognostic assessment of patient-reported outcomes (PRO). This issue will be covered in the survey study Chapter 5.

Table 2.3 Hull Score 1

Variable	Categories	Points
New or worsening symptoms	Yes	1
	No	0
Performance status (ECOG)	0	0
	1 / 2	2
	3 / 4	3

Grouping - Low Risk: 0 / Intermediate Risk: 1–2 / High Risk: 3–4

2.10 Quality of life of cancer patients with i-PE

In health economics, quality of life measures have become the standard means of assessing the results of health care interventions and, more controversially, the means of prioritising funding and many other applications (121). Therefore they can be used to identify patients who need particular care or to screen for psychosocial problems and monitor patients' illness progress, particularly concerning the management of chronic illness; or to determine the type of treatment (121). In clinical trials quality of life assessment, has proved to provide evidence of the effects of interventions.

Patients with cancer experience complex symptoms associated with advancing disease. It is reported that approximately 60–80% of patients will experience pain before death. Furthermore, physical symptoms, as well as psychological symptoms are common issues during the cancer journey. Including; anorexia, nausea, fatigue, dyspnoea, depression or anxiety (122, 123). Patients with advanced cancer experience a range of symptoms that are persistent, discomforting, and intensely limiting, which harms one's well-being with a negative impact on patient's quality of life.

Patients' quality of life is increasingly seen as an important outcome in clinical care (124). Quality of life (QOL) can be defined as "the functional effect of an illness and its consequent therapy upon a patient, as perceived by the patient" (125) furthermore, The World Health Organization (WHO) definition of health is: 'A state of complete physical, mental and social well-being and not merely the absence of disease or infirmity' (125). However, the impact of venous thrombosis on quality of life have studied only by few studies as summarised in a review on the quality of life in patients with the chronic venous disease (126). The review identified a total of 25 papers on the subject of quality of life in patients with chronic venous diseases (126), of which only 4 articles dealt with the assessment of the quality of life in venous thrombosis (127-130). These studies indicate that patients have low perceptions of their general health and high health distress, also found that patients with venous thrombosis report pain and impairment of their physical functioning. Impairment of patients' quality of life appears to be related to symptom severity and the presence of the post-thrombotic syndrome. However, there was no data about cancer patients who developed venous thrombosis.

Instruments used to measure the quality of life can be classified into generic instruments and disease-specific instruments. Generic instruments allow comparison across populations of patients with different diseases, whereas disease-specific instruments are sensitive to key dimensions of quality of life that are impaired by specific diseases. An advantage of disease-specific instruments is that they increase the acceptability of the questionnaire to the patient by including only relevant dimensions. A recommended research approach for assessing the quality of life is the combination of generic and disease-specific instruments to combine the advantages of both methods (131).

In cancer population, performance status is a routine standard measurement of quality of life as it gives a surrogate marker of patient's functional status to gauge if the patient can tolerate potentially toxic therapy (132). Performance status is a score that estimates the patient's ability to perform certain activities of daily living without the help of others, including basic activities such as getting dressed, eating, and bathing, as well as more complex activities such as cleaning the house and working a regular job (132).

Patients' performance status has been utilized in clinical trials as a prognostic tool for survival in many types of cancer. Accordingly, it has been taken into consideration in the planning and evaluation of clinical trials of cancer treatment (31, 132). While patients with poor performance status tend to have more difficulty in tolerating treatment, performance status is an independent predictor of cancer survival. While performance status assessment has traditionally been performed by physicians, this is not necessarily the case in scales measuring the quality of life. Slevin et al.1988 (133) conclude from a questionnaire study that a reliable and consistent method of measuring the quality of life in cancer patients must come from the patients themselves.

Apart from conferring a worse prognosis, the diagnosis of VTE is a physically and emotionally distressing phenomenon that affects patients' experience and quality of life. However, data available on how cancer-associated thrombosis and its treatment affect the cancer patients' experience is scarce compared with that in relation to treatment or prevention. This issue will be covered in more details in chapter Four.

2.11 Thrombogenic biomarkers

The pathogenesis of the prothrombotic state in cancer is complex and multifactorial (134). Recently, it has been established that almost all types of tumour cells can activate the clotting system and cause thrombosis by their ability to produce and release procoagulant substances and inflammatory cytokines, and by direct interaction with host vascular and blood cells through adhesion molecules (135, 136). Many studies have described molecules released by cancer cells that have a direct procoagulant effects, the best characterised were tissue factor (TF) and cancer procoagulant (CP) tumour necrosis factor (TNF), interleukine-1 (IL-1), and vascular endothelial growth factor (VEGF), that act on leukocytes and endothelial cells to further enhance the procoagulant activity (23, 118). In addition, the prothrombotic tendency of cancer patients is enhanced by anticancer therapy, such as surgery, chemotherapy, hormone therapy, radiotherapy and by indwelling central venous catheter (137). There are diverse pathophysiological mechanisms contribute in the hypercoagulable state in the cancer population. (Figure 2.1)

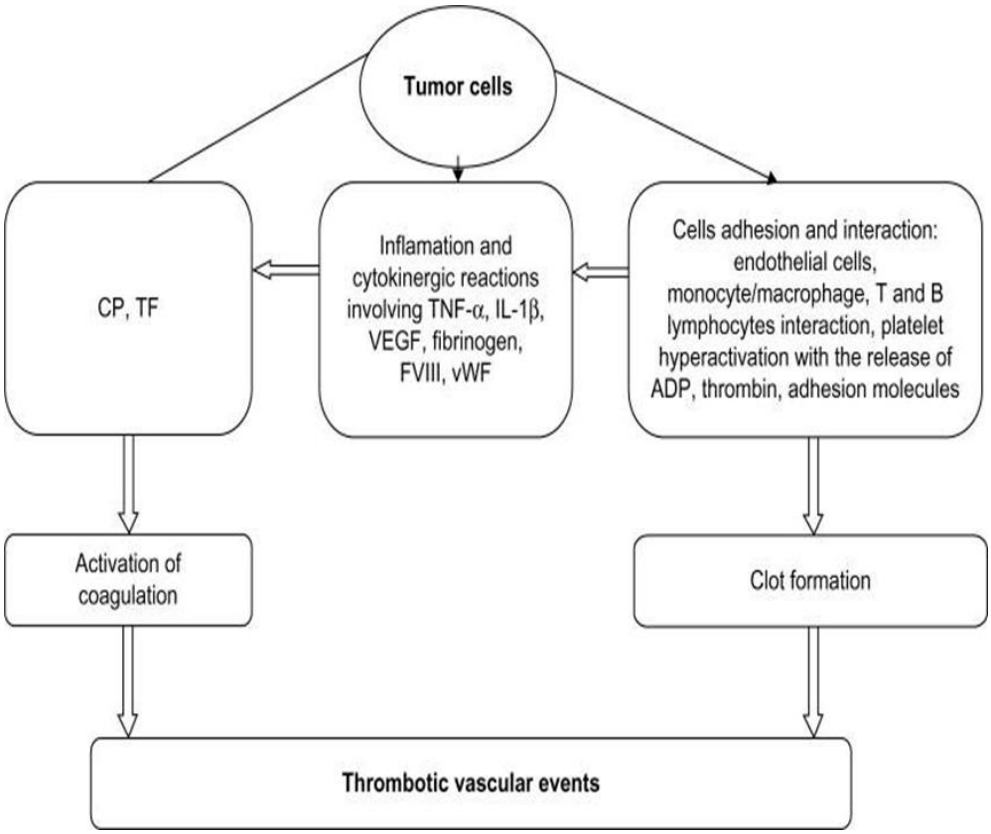


Figure 2.1 Factors involved in CAT.

CAT, cancer associated thrombosis; TF, Tissue factor; CP, Cancer procoagulant; TNF, Tumour necrosis factor; VEGF, Vascular endothelial growth factor; IL-1β, Interleukin-1β; FVIII, Factor VIII; vWF, Von Willebrand factor; ADP, Adenosine diphosphate.
Source: M.Karimi 2010 (138)

2.11.1 Tissue factor and TF positive microvesicles

TF is an integral transmembrane protein expressed by various cells that is essential for the normal haemostasis (139). Under normal physiological circumstances, cells in contact with blood do not express physiologically active tissue factor (140). TF is the physiologic initiator of the extrinsic pathway coagulation (Figure 2.2) (141). In cases of mechanical or chemical damage of the vascular wall occurs, subendothelial tissue factor is expressed/exposed to blood flow and binds plasma factor VIIa, which circulates as an enzyme (142).

The role of TF in haemostasis and coagulation

TF has a principal role in haemostasis as the main initiator of the extrinsic blood coagulation cascade. The coagulation cascade includes two separate pathways; intrinsic and extrinsic pathways which in turn able to activate the common pathway. Activation of the intrinsic pathway by exposure to a negatively charged surface such as collagen leads to factor X activation by FIXa in the presence of FVIIIa. The extrinsic pathway is activated upon disruption of the endothelial barrier which allows the binding of circulating coagulation factor VII (FVII) to the extracellular domain of TF. This results in the activation of FVII to FVIIa. This TF-FVIIa complex is capable of activating both circulating factor IX (FIX) and factor X (FX) (141). Generation of FXa promotes proteolytic conversion of prothrombin to thrombin which digests fibrinogen to fibrin monomers that polymerise to produce the fibrin clot**.** (Figure 2.2)

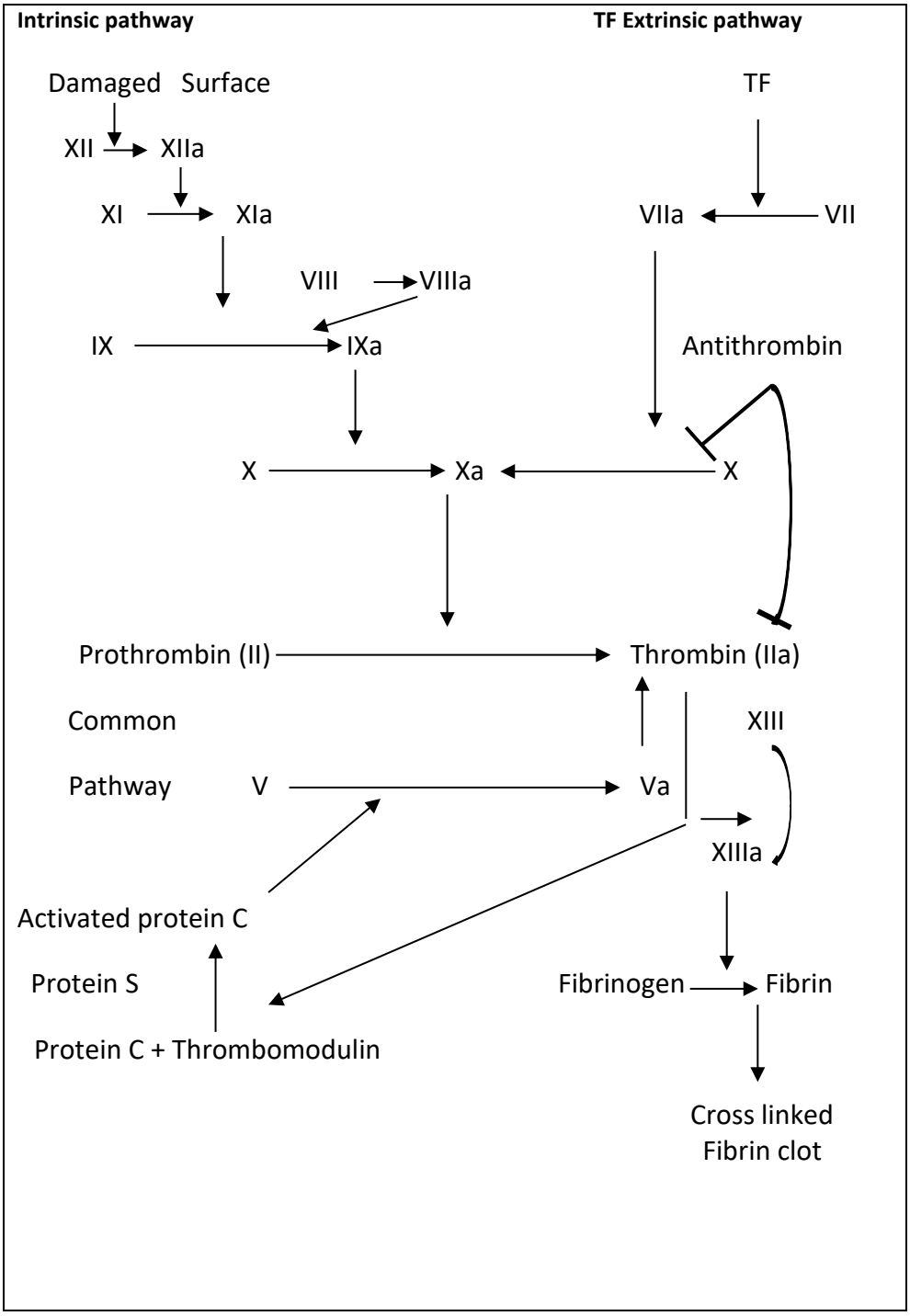


Figure 2.2 Coagulation cascade pathway.

The extrinsic pathway represented by TF and FVII. FXIIa, FXIa, FIXa, and FVIIIa are members of the intrinsic pathway. Both pathways lead to the activation of FXa and the common pathway ultimately results in the formation of a fibrin clot (132)

Tissue Factor Structure:

TF, designated cluster of differentiation (CD) -142; thromboplastin or factor III is a 47 kilo Dalton (KDa) glycoprotein receptor (134). TF is a 263/261 amino acid transmembrane protein containing three domains; [1]. An extracellular domain (residues 1–219) representing the NH2-terminal part of the molecule composed of two fibronectin type III domains. It is involved in complex formation with factor VIIa and increases, in a membrane dependent fashion, the activity of the protease toward its natural substrates factor IX, factor X, and factor VII by several orders of magnitude (143, 144). [2] A transmembrane domain (residues 220–242), which anchors TF to the membrane; [3] A cytoplasmic COOH-terminal domain (residues 243– 263) which is involved in signal transduction. (Figure 2.3)

Bogdanov et al 2003 identified a soluble TF isoform that circulated in the blood. This isoform, derived from alternative splicing of the primary RNA transcript and lacks exon 5 and therefore has no transmembrane or cytoplasmic domain but has a unique C-terminus (145). It has been suggested that this form of TF is procoagulant54 and stimulates clot growth. However, the data about its role as a procoagulant agent is conflicting (145, 146).

Presentation: At physiological condition, remarkably high amount of TF found to be originated from a number of vital organs (140) specifically in the epithelial cells of the lung (bronchial mucosa, alveolar epithelial cells, alveolar macrophages and alveolar septa) (147), in the astrocytes of brain tissue (148), in the cardiomyocytes of the heart, and in the endothelial cells of placenta and blood vessels (vascular smooth muscle cells, adventitial fibroblasts surrounding the blood vessel walls) (149) as well as in the kidney (glomeruli) (140).

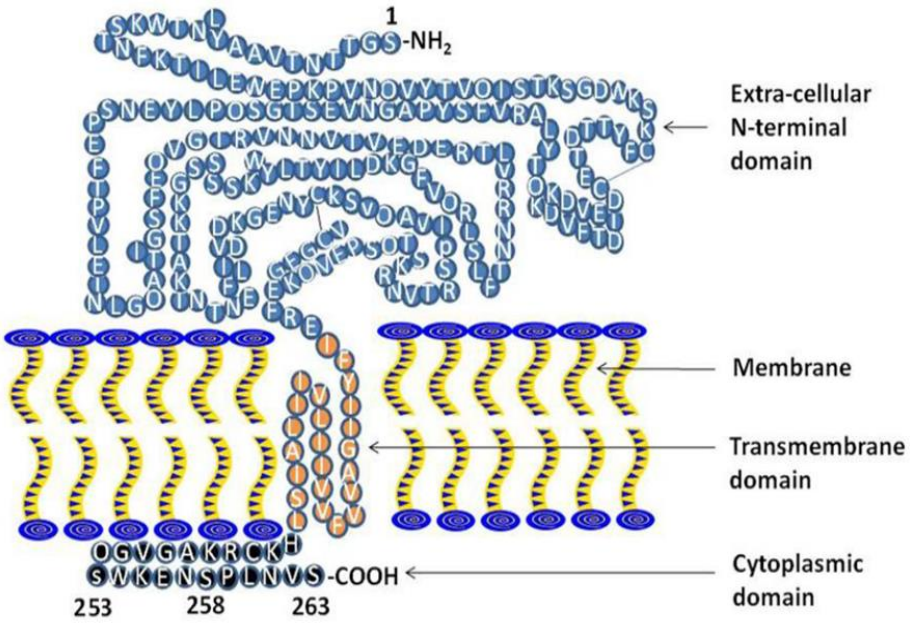


Figure 2.3 Schematic representation of tissue factor adapted from.

The three intracellular serine residues are highlighted.

Source; Nemerson.1998 (150).

TF is also expressed in a number of non-vital organs such as skin (epidermis), gut (mucosa), spleen (trabeculae and capsule) and the peripheral nerve (Schwan cells) (140). Many healthy cells can express a discernible amount of TF under stimulation of various agents, in response to inflammatory stimuli TF has been noted to be expressed normally in host cells such as endothelial cells, macrophage, fibroblasts and monocytes as well as in response to remodelling signals in malignant cases (151).

Concentration in healthy individuals:

In healthy individuals, TF is present in plasma at low levels (152). In a healthy individual, around 100–150 pg/mL of TF circulates within the blood, most of which is thought to be associated with cellular microvesicles (MVs) (153). Aberrant expression of TF contributes to thrombosis associated with various diseases, such as atherosclerosis and cancer (154, 155). In circulation TF reported to be found located on blood cells, platelets and as microvesicles (156, 157). An alternative spliced form of TF has been detected in addition to full-length TF, although its coagulation activity remains undetermined (145). However the concentration of TF in tissues and cells is low, which makes it difficult to detect, quantify and purify enough natural TF for the characterization and use in research and clinical laboratories12/23, that lead to develop a disagreement between studies related to the presence, concentration and the functional activity of TF circulating in blood as a soluble protein and on/in various blood cells and platelets (139).

Presentation of TF in solid tumours:

In patients with cancer, however, there is a wide expression of TF (158). TF is strongly expressed across a wide variety of both solid tumours and hematologic malignancies including; pancreas (159), breast (160), lung (161) and leukaemia (162). Further to its procoagulant activity, it has been reported that tissue factor is an important key factor in angiogenesis and thrombosis in cancer (110).

Studies using immunohistochemistry reported that TF can be expressed in malignant cells as well as in tumour-infiltrating macrophages or endothelial cells (163). Increased expression of TF has been detected in several types of cancers, epithelial ovarian

cancer (EOC) (164), breast cancer (165), glioma (166), gastric cancer, prostate cancer (167), colorectal cancer (CRC) (168), lung cancer (161).

TF Cancer and VTE:

Cancer linked with hypercoagulability and thrombotic risk has long been recognized by Armand Trousseau since (1865) (169). Cancer certainly could be recognized as a prothrombotic risk factor, leading to, venous thromboembolism and its complication of pulmonary embolism and mortality (80). In malignancy, however, high levels of TF expression on the cancer cell surface are believed to contribute to the procoagulant tendencies (170).

Recent studies have concluded that TF cell surface expression was clearly linked to the procoagulant activity (PCA) of tumour cell lines of the pancreas (171), breast, colorectal and head and neck squamous cell carcinoma (HNSCC) (24). TF expression measured by Immunohistochemically staining found to be at the highest level in tumours is known to be associated with a high incidence of venous thromboembolism such as lung, pancreatic cancers, and HNSCC, whereas this level less in breast, renal, and prostate cancers. In addition, cancer patients with VTE had significantly higher levels of tissue factor activity in the blood than cancer patients without VTE. The presence of TF–bearing microvesicles may be predictive of cancer patients developing venous thromboembolism (172, 173), and TF could be as a possible candidate biomarker for venous thromboembolism (174). However, in the meantime there are no current standardized assays available, so the use of tissue factor as a predictive biomarker.

TF is known as the initiator of the extrinsic pathway of coagulation (175), and most tumours carry an increased risk of patients developing venous thromboembolism, which, therefore, may be linked to the TF expression on the tumour itself (176). In addition to their role in fibrin formation, TF/FVIIa complex, as well as the downstream proteases FXa and thrombin, initiate a cellular signal cascade by protease-activated receptors (PARs) (177). PARs are members of a family of seven transmembranes (domain) surface receptors that activate cells via G proteins (178). The family consists of four members, PAR-1 to PAR-4. While thrombin activates PAR-1, -3 and -4, the TF/FVIIa complex and factor Xa activate PAR-2 (179, 180). Activation of PARs

contributes to a variety of biological processes, including inflammation, angiogenesis, metastasis, and cell migration (181).

Microvesicles in cancer

MVs are small membrane vesicles that released from almost all cells upon activation and during apoptosis (182, 183). Microvesicles found to differ in size, number, and antigen composition according to its origin. Microvesicles were discovered in 1969 by Wolf who named it as a “platelet- dust” (184), the reported size of microvesicles is between (0.1–1 μm) (185). Furthermore, the MVs origin can be from different cells including, monocyte (186), platelets (182), and cancer cells which represent the main source of TF activity in cancer (187). The microvesicles are consist of a cytoplasmic component and membrane elements such as PL and cell surface receptors and phospholipid bilayer from the original activated cells (188). Following stimulation, the plasma membrane is rearranged resulting in an outer leaflet rich in phosphatidylserine, essential for TF activity (Figure 2.4). This is followed by the release of MP into the circulation. Microvesicles often possess procoagulant activity which is primarily due to the presence of TF (188).

In cancer, increased level of circulating TF-containing microvesicles has been reported in patients with breast cancer (189). Hrong.G et al 2007 reported a two-fold increase of circulating TF positive microvesicles in patients with advanced colon cancer compared to matched healthy controls (p=0.007) (190). While Zwicker and colleagues found an association between TF positive MVs and cancer-associated thrombosis (191). Another research reported the increased levels of TF positive MVs in patients with cancer and VTE in comparison with cancer patients without VTE, both of which were higher than healthy controls (192). Together, these results indicate that increased levels of MP TF antigen can be predictive of VTE in cancer patients.

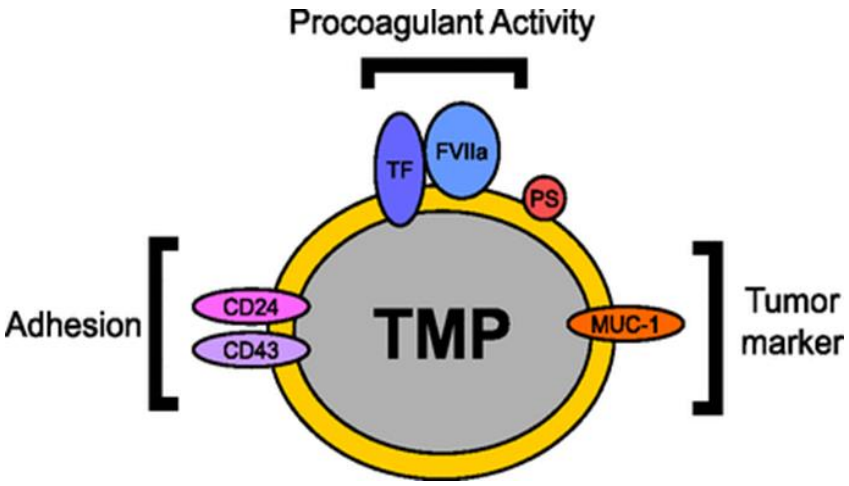


Figure 2.4 TF positive MP (MV).

TMP surface proteins and their functions. TMPs are constitutively released from tumours into the circulation. The procoagulant activity of TMPs is mediated by the expression of TF and the exposure of PS on the MP surface. Adhesion proteins including P-selectin ligand CD24 and E-selectin ligand CD43 have been proposed to be involved in the binding of TMPs to endothelium and thrombosis sites.2,69 Delivery of TMP TF to the site of thrombosis can then initiate thrombosis. This diagram is an example of proteins that can be expressed on the surface of TMPs. Protein expression on the surface of TMPs varies with each tumour. (Source O.Königsbrügge. 2014. (25))

2.11.2 Proteinase-activated receptor 2 (F2RL1) and Thrombosis

Protease-activated receptors (PARs) are a unique class of G protein-coupled receptors that play critical roles in thrombosis, inflammation, and vascular biology (181). These receptors might be activated through proteolytic cleavage by blood coagulation enzymes thus eliciting the production of several pro-humoral factors including cytokines and angiogenic factors.

Each of four PARs—PAR-1, PAR-2, PAR-3, and PAR-4—are encoded by distinct genes. PAR-1, the first receptor to be discovered, was identified in 1991 by two independent laboratories in search of the GPCR that mediated thrombin signalling in human and hamster cells. (193, 194). The presence of the G-protein-coupled thrombin receptor identified at the surface of cancer cells in solid tumours in the late twentieth century (195). In 1994 PAR-2, activated by trypsin, was identified by screening a mouse genomic library for GPCRs with oligos based on conserved transmembrane regions of the bovine substance K receptor (196). Subsequently, PAR-3 and PAR-4 were cloned by mRNA screening of rat platelets and by investigating a human expressed sequence tag database (197) PARs are expressed on nearly all cell types in the blood vessel wall (ECs, fibroblasts, myocytes) and blood (platelets, neutrophils, macrophages, leukemic white cells) with exception of red blood cells (198).

The PARs 1,3, and 4 are activated by thrombin and other proteases at a specific peptide bond to expose a new N-terminus that binds to the body of the receptor in an unusual intramolecular mode (199). PAR-2 is activated by trypsin-like serine proteases or coagulation factors including; trypsin, tryptase factor VIIa, factor Xa, TF-VIIa-Xa ternary complex and TF-VIIa binary complex (200, 201). Activation of PAR-2 by the TF/FVIIa binary complex involves cellular pools of TF with low affinity for FVIIa, whereas high-affinity cell-surface TF mediates coagulation activation and the associated cell signalling of the ternary complex of TF/FVIIa/FXa (194). However, FXa can solely activate PAR-2 (202). (Figure 2.5).

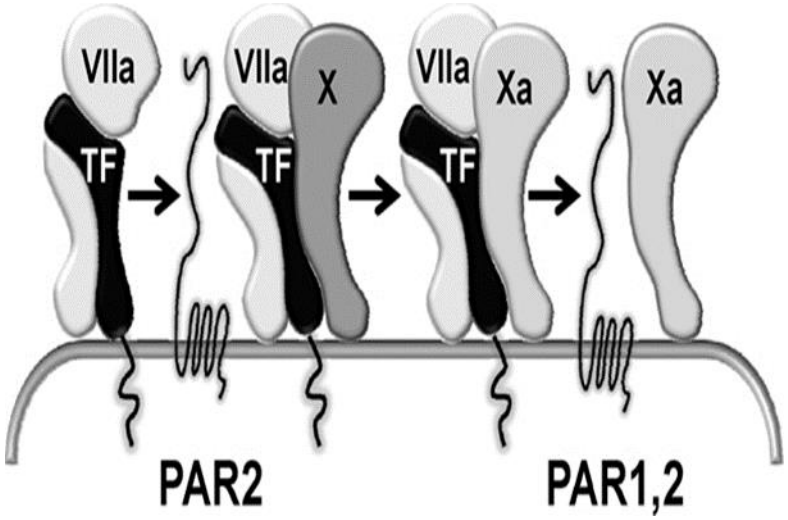


Figure 2.5 Activation of PAR1 and PAR2 by the TF pathway.

In the transient TF-VIIa-Xa complex, Xa is an efficient activator of PAR1 and PAR2. Downstream TF-dependent coagulation occurs after dissociation of Xa from this ternary complex. This essential procoagulant event results in less efficient PAR signalling because either TF-VIIa or free Xa is less capable of PAR activation than the TF-VIIa-Xa complex. (Source, Ruf and Riewald 2003. (203)

FXa can also activate PAR1 and PAR2, although signalling of the ternary TF-FVIIa-FXa complex is at least fivefold more efficient (200). Thus, although TF-FVIIa and FXa can activate PAR2 and PAR1 plus PAR2, respectively, relatively high concentrations are required, which has raised doubts regarding the biologic relevance of signalling by these upstream coagulation proteases. However, FXa within the transient TF-FVIIa-FXa complex is a highly efficient activator of PAR1 and PAR2 at concentrations that are physiologically achievable (203). Notably, because FXa exerts its procoagulant activity only after dissociation from TF-FVIIa, cell signalling by the TF-FVIIa-FXa complex precedes TF-dependent coagulation. As such, cell signalling via PAR1 and PAR2 is directly coupled to the mechanism by which TF initiates coagulation (203).

In cancer cells, increased level of PAR 2 expression compared to normal cells has been reported. In breast tumours, PAR-2 was overexpressed compared with normal breast tissue (181). Experimental animal data reported, PAR-2 induces migration of breast cancer cells in vitro (202, 204), whereas spontaneous breast cancer development and metastasis are reduced in PAR-2-deficient mice (205). While in colon cancer, PAR-2 stimulates both proliferation and migration of colon cancer cells (206, 207) and the proliferation of cervical and pancreatic cancer cells (208, 209). Recently, C.Ettelaie, 2016, (210) in analysis of the potential of cancer cell lines to release tissue factor-containing microvesicles correlation with tissue factor and PAR2 expression reported a strong correlation between TF release and PAR2 mRNA expression, and therefore, quantification of the levels of TF and PAR2 mRNA and possibly PAR2 protein, may prove to be an accurate predictor of risk of thrombosis in vivo.

2.11.3 Factor Xa

Factor X (FX) circulates as a vitamin K–dependent serine protease that is converted to the active form at the point of convergence of the intrinsic and extrinsic coagulation pathways (211). The factor X zymogen serves as a substrate for both the extrinsic (TF/FVIIa) and the intrinsic (FVIIIa/FIXa) tenase enzyme complexes, which generating factor Xa (212). Subsequently, factor Xa interacts with its cofactor, factor Va, phospholipids, and calcium to form a macromolecular prothrombinase complex that converts prothrombin into thrombin (common pathway) (213). Upon vascular injury, FVII forms a complex with its cofactor, the cell surface receptor tissue factor (TF) (TF-VII) complex that converts FX into its active form, FXa (212). Moreover, it is now

evident that FXa utilizes direct effects on a wide variety of cell types by activation of its two main receptors, protease-activated receptor-1 (PAR-1) and PAR-2. Recent data suggest that PAR-2 plays an important role in fibro-proliferative diseases such as fibrosis, and cancer. In cancer, it has been reported FXa as the important mediator coordinating the interface between coagulation and disease progression (214).

2.11.4 C-reactive protein (CRP)

C- reactive protein, a nonspecific marker of inflammation, is induced in tumour tissues by infiltrating lymphocytes and monocytes; it is proposed to be an opsonin and an activator of the complement system (215). Koukourakis.MI, 2009 reported a reduction in CRP after tumour resection which indicates a correlation between tumour size and the level of CRP (216). In a prospective study included lung and gastrointestinal cancer patients with acute venous thrombosis. Kroger. et al, 2006 reported a significantly high level of CRP compared to other factors, (CRP > 5mg/L) was significantly elevated compared to other factors, including; haematocrit (≥0.3675 L/L), lactic acid dehydrogenase (>240 U/L), platelet count (<50 mm–3 or >400 mm3) (217). These preliminary data of some have shown promising results in the utility of CRP as a biomarker for cancer-associated thrombosis. In the same context, univariate analysis of CRP in The Vienna Cancer and Thrombosis Study (CATS) shown that patients with high CRP level (>1.8.mg/dL) had a higher risk of developing VTE during 12 months compared to those with lower CRP level (11.7% vs. 4.9%; p = 0.03) (218). However, in the multivariate analysis incorporating; chemotherapy, radiation, surgery, tumour stage, and sP-selectin levels, the results were insignificant, which means in cancer patients elevated CRP was not independently associated with VTE. Interestingly, sP-selectin remained a significant predictor in this adjusted VTE risk model (hazard ratio [HR] 3.8; 95% confidence interval [CI] 2.0–7.4; p < 0.0001), and CRP and sP-selectin levels did not correlate with each other (218). These data show that whereas CRP may not be an independent predictor of VTE, it may predict decreased survival and is closely related to sP-selectin in malignant thromboembolism (218).

2.11.5 Factor VII

Human factor VII (fVII) is a vitamin K-dependent plasma protein (219). Under physiological conditions, fVII is synthesized by hepatocytes and circulates in the plasma

as an inert zymogen at a molecule of Mr ~50 KDa (219, 220). fVII also circulates in an active enzymatic form (fVIIa) that participates in the extrinsic pathway of coagulation(220, 221). However, it is reported that only ~ 1% of total fVII circulates as an active enzyme (FVIIa), which is insufficient to initiate coagulation under normal physiological conditions (222)

Tissue factor (TF) binds fVIIa with high affinity and potentiates its enzymatic activity (221). The formation of fVIIa/TF complex represents the first step in the extrinsic pathway of blood coagulation (223). The fVIIa/TF complex activates FIX (224) as well as FX (225), which leads to the activation of prothrombin to thrombin (226). On the other hand, coagulation enzymes including FXa, FIXa, and FVIIa can activate FVII (220, 223). TF/fVIIa by proteolytic mechanisms cleaves and activates protease-activated receptor (PAR)-2 (227, 228), and the TF/fVIIa/fXa complex can activating either PAR-2 or PAR-1 (227).

In cancer, recent researches have shown ectopic synthesis of fVII by different cancer cells (229). In ovarian cancer, endogenous synthesis of fVII by ovarian cells have further promoted cell migration and metastasis (229). In addition, TF/fVIIa complex promotes proliferation and migration of hepatoma and colon cancer cells through the activation of protease-activated receptor 2 (PAR2) signalling (230, 231). On the other hand, inhibition of TF-fVIIa complex signalling leads to attenuation of cancer growth in a human breast cancer model.

Increasing evidence suggests that the TF/FVII complex is involved in the pathogenetic mechanisms in cancer, including angiogenesis (232), cell migration and invasion (233), and cell survival (234).

2.11.6 D-dimer

D-dimer is a fibrin degradation product that indicates the activation of haemostasis and fibrinolysis (235). It is plasma levels elevated after clot formation. The measurement of D-dimer is routinely used in conjunction with clinical parameters in the assessment of suspected acute VTE. In cancer patients, several studies reported an association between D-dimer and the risk of VTE (236, 237). Recently CATS study reported that D-dimer was shown to be a significant biomarker for prediction pf VTE in cancer population (209).

2.12 Endothelial cells

The vascular endothelium is strategically located at the interface between tissue and blood. Endothelial cells form a single layer of cells, lining the blood vessels and separating the blood from tissue (238). Endothelial cells functions include nutrient and solute transport across the endothelium; regulate haemostasis, angiogenesis, inflammation and many other processes (239). However, the intimal surface of healthy endothelium is both anticoagulant and antithrombotic: endothelial cells secrete a variety of molecules important for the regulation of blood coagulation and platelet functions. However, vascular damage or exposure to certain cytokines or proinflammatory stimuli shifts the balance towards a procoagulant/prothrombotic phenotype of the endothelial cells (240). Furthermore, endothelial cells contribute to the regulation of blood pressure and blood flow by releasing vasodilators such as nitric oxide and prostacyclin, as well as vasoconstrictors, including endothelin and platelet-activating factor (241). Specific functions and adaptations of endothelial cells aim at maintaining blood fluidity and preventing thrombus formation. The endothelium inhibits coagulation by synthesising and displaying anti-thrombogenic substances including TFPI, heparan sulphate proteoglycans and nitric oxide. These, in turn, prevent platelet adhesion and clot formation (239). This is only possible because the endothelium also actively controls the extravasation of fluid, solutes, hormones, and macromolecules (181), as well as that of platelets and blood cells. This guarantees the availability of appropriate amounts of clotting factors and platelets. Another important feature of endothelial cells is that they can become activated by inflammatory cytokines, and by other stresses such as hypoxia and metabolic stress that trigger the innate and acquired immune response (242, 243). In response to these activations, endothelial cells offer a new repertoire of activities and receptors by up-regulating the expression of various pro-inflammatory and pro-coagulant receptors and molecules (244). However, activation of endothelial cells in pathological conditions, lead to express a potent pro-coagulant molecule tissue factor (TF) on the cell surface or in a form of microvesicles (180). TF-bearing microvesicles are capable of activating the coagulation mechanism, resulting in the precipitation of a hypercoagulable state and an increased tendency to thrombosis (245). The blood coagulation categorized into three phases: initiation, amplification, and propagation. Initiation occurs upon vascular injury with resultant activation of the endothelium. Activated endothelial cells express

tissue factor (TF), which binds with circulating coagulation factor VII (FVII) to become active FVII (FVIIa), wherein TF also binds with FVIIa to form the TF/FVIIa complex. The TF/FVIIa complex then goes on to proteolytically cleave FIX and FX into FIXa and FXa, respectively. FIXa serves to generate more FXa and FXa serves to generate thrombin (211). Endothelial cells express several types of integral membrane protein receptors, including a family of G-protein, coupled receptors, termed protease-activated receptors (PAR1–4), which activation by their coagulation proteases, initiate and modulate a diverse array of cellular activities under various pathophysiological conditions (246). Thrombin is able to activate PAR-1, -3 and -4, FVIIa activates PAR-2 only and FXa mediates intracellular signalling via activation of PAR-1 and/or PAR-2 (143, 214).

2.13 Summary

This chapter presents a summary of the available literature data regarding i-PE in cancer patients. I have included; definition of i-PE, the epidemiology of i-PE in oncology, clinical course and outcomes, quality of life, and a summary of related thrombogenic biomarkers.

This review of the literature illustrates the gap in knowledge regarding clinical outcomes among cancer population diagnosed with i-P, and need for this type of research to understand the diversity of this subject. Chapter Three will describe and provide the rationale for the methodology used to address the research questions of this study.

Chapter 3 Methodology

3.1 Introduction

This chapter details and justifies the choice of the methodology used to address the research questions of this theses. An overview of the study design will be presented. A description of the methods used to collect the data will be presented in more detail in corresponding chapters.

Research questions:

- Q1. What is the experience of people living with cancer associated thrombosis in regard to their response to the diagnosis, coping with the additional burden and the effects of long-term anticoagulation on their daily life?

- Q2. What is the impact of incidental pulmonary embolism (i-PE) on the clinical outcomes of cancer patients?

- Q3. What is the experience of people living with cancer and i-PE in regard to their response to the diagnosis, coping with the additional burden and the effects of long-term anticoagulation on their daily life?

- Q4. What is the relationship between thrombogenic proteins (TF, PAR2) level in cancer tissues and the risk of thrombosis?

3.2 Methodological approach for the theses

This thesis explores a diverse set of research questions, and therefore the methods I will need to use to approach these questions and answer these questions are also diverse. I used a systematic review of the literature to address question one and an observational prospective cohort study (quantitative approach) to address question two. To address question three, I used a semi-structured in-depth interview study (qualitative approach). To address question four, I used quantitative analysis of laboratory assays.

Combining such a wide range of methodological approaches is unusual with no agreed standard method of data synthesis. I have therefore adapted an approach, Critical

Interpretive Synthesis, developed to synthesise findings from quantitative and qualitative studies in systematic literature reviews to synthesise the quantitative and qualitative results (247).

3.2.1 Experience of people living with cancer associated thrombosis

In order to answer the research question one; what is the experience of people living with cancer-associated thrombosis in regard to their response to the diagnosis, coping with the additional burden and the effects of long term anticoagulation on their daily life? I used a systematic literature review approach.

Scoping of the literature showed that systematic reviews and meta-analyses about cancer-associated thrombosis appeared to be limited to studies of biomolecular markers associated with cancer-associated thrombosis (136, 248), risk assessment of cancer-associated thrombosis (249, 250), or both (251), clinical outcomes, thromboprophylaxis (252) and risk stratification (253). Therefore, the aim of this review was to find out and understand what is known in the literature about the experience of patients with cancer of living with venous thrombosis in order to identify gaps in knowledge.

Why a systematic review?

It is crucial to avoid risk of bias when performing a literature review that may arise from many reasons (e.g. selection bias, publication bias, outcome bias, etc.) (254), thus systematic reviews apply scientific strategies in ways that limit bias and use critical appraisal, and synthesis of relevant studies that address a specific clinical question (254). Therefore a systematic search was performed as recommended by the Centre for Reviews and Dissemination (255) (details given in Chapter Four).

Traditionally, to guide clinical decision-making, the narrative review has been used as a means of providing a summary of the available evidence. However, narrative reviews are subjective and therefore prone to bias and error, that would risk losing the opportunity to provide a synthesis which goes beyond the individual findings of the papers and enable a more generalisable and powerful conclusion to be drawn (256). Systematic literature reviews are more focused and they may be qualitative (which may include qualitative synthesis), or quantitative (which may include meta-analysis)

(257). The systematic review provides a summary of medical reports on a specific clinical question, by using defined methods to search, critically appraise, and searching the available literature systematically (258). It is useful in bringing together a number of separately conducted studies, sometimes with conflicting findings, and synthesising their results (259).

As with any scientific research, systematic literature reviews are prone to the risk of bias at different levels, recognising these risks and working to eliminate or reduce them is a crucial step. Risks of bias may present at the selection step, and by developing a well-defined research strategy (inclusion and exclusion criteria), that used by more than one researcher would reduce this type of risk. Furthermore, searching the grey literature, as well as contacting authors about unpublished data reduces the risk of publication and outcome bias. Assessing included studies against appropriate quality appraisal tools works to reduce, or at least understand, the risk of bias at the study level. Furthermore, it is known that when conducting a systematic literature review the included articles that may contain insufficient primary data that would affect the final analysis and implications. In this case, contacting the authors would reduce the risk of bias of including insufficient data. Lastly, is the risk of language bias, language restrictions may represent a source of bias, which can be eliminated by avoiding language limits by searching non-English literature, including and translating articles in other languages (254). However, in this systematic review, the only publications in English were included due to limited access to translation.

Health care professionals are increasingly required to base their practice on the best available evidence. It is therefore important that health care decisions are not based solely on one or two studies without account being taken of the whole range of research information available on that topic (255). Evidence-based medicine (EBM) "represents an integration of clinical expertise, patient's values and best available evidence in the process of decision making related to patients health care" (260, 261). The revised and improved definition of evidence-based medicine is a systematic approach to clinical problem solving which allows the integration of the best available research evidence with clinical expertise and patient values (261). Because of this, systematic reviews are being increasingly valued and utilized in the medical profession and physicians can integrate research findings into practice in a timely way (262).

Therefore, clinical or health policy decisions are facilitated by reviewing the available evidence, understanding reasons why some studies differ in their results (heterogeneity among the primary studies), coming up with an assessment of the expected effect of an intervention or exposure (for questions of therapy or aetiology), and then integrating the new information with other relevant treatment, patient, and health care system factors.

Justification for the study designs included

As the aim of the review was to identify and synthesise studies that explore patients' experience of living with cancer associated thrombosis and address the gap in knowledge, only qualitative studies were included.

Approach to data synthesis in the systematic review

Data synthesis is an important tool for the evidence-informed policy, that seeks to inform the practice and decision-making bodies, where decisions cannot be made based on an individual research study. Research synthesis is a general term used to describe the 'bringing together' of a body of research on a particular topic. The aim is usually to describe, analyse and draw conclusions on the research evidence and is often used to make decisions about the effectiveness of healthcare interventions (263). A systematic review summarises the results of available from healthcare studies and provides a high level of evidence on the effectiveness of healthcare procedures (264). Quantitative data synthesis methods are well known generating meta-synthesis that combine and summarize the results of several primary studies (265). However, methods for qualitative data synthesis are still growing and proved to be more complex regardless of being more recognised method in the health sciences (266, 267).

Recently there is a growing recognition of the significance of synthesising qualitative research in the evidence base in order to inform health-related policy and practice (268). The Cochrane Library now includes qualitative syntheses (269). This systematic review will include qualitative papers and therefore a qualitative synthesis will be applied.

Various methods can be used to conduct qualitative syntheses, including narrative description, meta-ethnography, critical interpretive synthesis, thematic synthesis, realist review and meta-aggregation (270). For this systematic review, I could have used a narrative description analysis, but this way of synthesis is very limited and does not generate any new themes or thoughts so, therefore, I chose a deductive philosophy analysis synthesised using qualitative thematic synthesis as described by Thomas and Harden within the theoretical framework of uncertainty in order to generate new knowledge and to increase the generalisability of findings (271). Thematic synthesis is recommended for use as a realist method of synthesis which can develop a final product capable of informing health policy and practice (270). (Clarke, V. and Braun, V. 2006) (272)argued that thematic synthesis offers an accessible and theoretically-flexible approach to analysing qualitative data. It is suited to a wide range of research interests and theoretical perspectives, and is useful as a 'basic' method because: a) it works with a wide range of research questions, from those about people's experiences or understandings to those about the representation and construction of particular phenomena in particular contexts; b) it can be used to analyse different types of data, from secondary sources such as media to transcripts of focus groups or interviews; c) it works with large or small data-sets; and d) it can be applied to produce data-driven or theory-driven analyses (273).

3.2.2 The impact of i-PE on patients' clinical outcome and experience

In order to address the research question two and three, I used a mixed-methods approach. First, I used a quantitative research approach, a prospective observational survey study, to answer research question two and a qualitative approach, in-depth semi-structured interviews, was used in the same cohort to answer the research question three. Then I synthesised the findings of both methods to address the variety of research data formulated from the preliminary data generated from each.

First, I will describe the methodology used in the cohort study to gain the quantitative data with regard to the impact of i-PE on patients' clinical outcome. Then I will describe the methodological approach to gain the qualitative data with regard the patients' experience of living with cancer and i-PE, then I will describe the mixed methods approach to synthesise the findings with regard the impact of i-PE on patients' clinical outcome and experiences.

3.2.2.1 The methodological approach to the quantitative data collection

I describe here the methodological approach to the quantitative data collection with regard to the impact of i-PE on clinical outcome. I did this by using an observational prospective cohort study. The description and justification of using this approach are presented.

3.2.2.1.1 Description and justification of observational studies

Observational studies often referred to as cohort, cross-sectional, and control studies, are becoming increasingly important and considered a practical method of studying different problems where a randomised controlled trial could be considered unethical, difficult to conduct or where preliminary data are needed in order to inform future study designs for specific research questions (274-276). Simply observed, no interventions are carried out by the investigator and usual care is unaffected (277). The objective of most observational studies is to address; prevalence, incidence, cause, or prognosis (277). In case of little evidence available about an issue, observational studies are cost-effective ways of producing and investigating hypotheses before larger and more expensive study designs are embarked upon.

Cohort studies

Cohort studies (prospective, retrospective and sometimes two cohorts are compared) are effective when the objective is determining the natural history and incidence of a condition (277). In a prospective single cohort study, a sample of the same patients is studied and followed over time and observed for developing the outcome of interest. While in two cohort studies, two cohorts are used, where one group has been exposed to or treated with the agent of interest and the other has not, thereby acting as an external control aiming to eliminate confounding and gain efficiency. This type of study enables the investigator to measure a number of variables that might be relevant to the condition under investigation. In the retrospective cohort studies, usually using data which have been already collected, often for other purposes, uses the same methodology but retrospectively. Observational studies may be relatively straightforward (especially retrospective studies or those with only short-term follow up) but do not permit distinction between cause and effect, although longitudinal follow up may allow causal hypotheses to be made (278).

Table 3.1 Advantages and disadvantages of cohort studies

Advantages	Disadvantages
Used when RCT is not applicable.	Risk of missing data in retrospective cohort studies. Risk of recall bias.
Prospective cohorts can measure potential causes before the outcome occurred.	Risk of loss follow up of participants in prospective cohort study.
Retrospective studies are much cheaper as the data have already been collected.	Prospective studies could be expensive and time consuming.
Can examine various outcome variables using a single study and determine the relative risk of each variable.	Inability to control the confounding factors where two groups are used.

Cross-sectional studies

Cross-sectional studies are generally used to detect prevalence or to infer causation (277). The advantage of these studies is they are relatively cost-effective and quick where only one group is used. Data are collected only at a one-time point, nevertheless, different outcomes can be studied. Furthermore, because no intervention used, there are hardly any ethical difficulties. The disadvantage of this type of study is the inability to differentiate cause and effect from the simple association, therefore cannot provide an explanation for the findings (275).

Table 3.2 Advantages and disadvantages of cross-sectional studies

Advantages	Disadvantages
Less expensive and quick	Weaker evidence of causality than cohort studies
Ability to determine the prevalence of outcome and odds ratio	Low accuracy when studying rare conditions.

Case-control studies

Case-control studies are usually used to investigate a particular outcome where people with the outcome of interest are matched with a control group who do not (277). Researchers can then look at factors in each group to identify any risk factors that are associated with developing or not developing the outcome and help the estimation of odds ratios (275). Case-control studies are valuable in investigating rare or chronic illness which may result from long-term exposure to specific risk factors, and when long term follow up is needed (275). However, because case-control studies are usually retrospective, this exposes it to recall bias and it may be associated with sampling bias.

Table 3.3 Advantages and disadvantages of case-controlled studies

Advantages	Disadvantages
Ability to generate information from small sample size	Risk of sampling bias (patients or controls)
Useful for generating hypotheses that can then be tested using other types of studies	Risk of recall bias in retrospective design.
Relatively cheap	Only one outcome is studied.

Justification of the quantitative approach

In this thesis for the quantitative part I used a prospective case-control cohort study to address the research question; what is the impact of incidental pulmonary embolism (i-PE) on the clinical outcomes of cancer patients?

The aims of the study are to compare performance status (PS), symptoms, quality of life (QoL), and other key clinical outcomes such as (VTE o(re)currence, haemorrhage, days in hospital, i-PE treatment-related complications) between people with cancer and i-PE to people with cancer and no thrombosis, to identify clinical and demographic predictors of symptom burden, VTE o(re)currence, haemorrhage, QoL and PS. To achieve that I used a prospective survey study using validated generic and disease-specific questionnaires. I chose a prospective approach because the data needed are not routinely collected in the department. A cohort of patients with cancer and i-PE were studied and followed over certain time points. A cohort of matched control patients was identified and followed for the same time points aiming to reduce confounding and gain efficiency (increase the study's efficiency by forcing the case and control samples to have similar distributions across confounding variables) (279).

It is acknowledged that observational studies are not experimental, and it is hard to control all confounding factors, therefore, *Bradford Hill criteria* (280), have provided a background framework when assessing the causal nature of an observed association in epidemiological studies. These are criteria to be used in decision making, not a list on which every box has to be ticked. We may not have enough evidence to deal with all the points, or some of them may not be applicable. Not all the answers may even point in the same direction, then we will have to decide how much importance to give it. Bradford Hill criteria include (1) Strength of the association (2) Consistency (3) Specificity (4) Temporality (5) Dose-response (6) Plausibility (7) Coherence (8) Experiment (9) Analogy. (Table 3.4)

Table 3.4 Bradford Hill criteria of causality

Bradford Criteria	
1. Strength of the association.	Bradford Hill suggests that a strong association supports causality.
2. Consistency.	Bradford Hill suggests that causation is more likely if the results from various research studies are consistent.
3. Temporality or study design suitability.	Bradford Hill describes that there must be a temporal relation between the exposure and outcome.
4. Biological gradient.	As described by Bradford Hill, a biological gradient between an exposure and the magnitude of an effect increases the confidence in causality.
5. Specificity.	According to Bradford Hill, causation is more likely if there is a specific outcome related to a specific exposure in that altering the cause alters the disease outcome.
6. Biological plausibility.	Whether the association is plausible or not influences causality in the Bradford Hill approach.
7. Coherence.	According to Bradford Hill, causation is more likely if what is observed is supported by and in agreement with the natural history of the disease.
8. Experimental evidence.	Experimental evidence enhances the probability of causation. GRADE places emphasis on rigorous experimental designs and this criterion is directly considered.
9. Reasoning by analogy.	Bradford Hill suggests that existing similar associations would support causation.

3.2.2.2 Data collection instruments used in the survey study

Data collection instruments refer to devices used to collect data such as questionnaires, tests, structured interview schedules and checklists.

Patient-reported outcome (PRO) instruments are rapidly becoming the primary or secondary outcome measures of preference in pivotal clinical practice and trials research, which means that PRO data now have a key role in patient care and policy-making(281). PROs are assessed using PRO instruments that may be generic or condition-specific measures.

Generic scales assess constructs that are common to a wide range of individuals, such as the commonly used medical outcomes study short-form (SF36) health survey12 (282) and Short Form 12 Questionnaire (SF12) (283). Generic instruments can assess a range of outcomes that apply across multiple diseases, treatments/health care interventions, and facilitate comparisons with populations across disease states and intervention. On the other hand, condition-specific scales are intended to assess outcomes that directly relate to a particular condition (284). However, the challenges for clinicians and researchers are choosing the appropriate instrument applicable to suit the research questions context, and constraints (285).

It is well acknowledged that both approaches have their strengths and weakness, generic scales can provide information that is broad although not necessarily detailed where condition-specific scales can provide information detailed but not necessarily broad

In this study, I used two generic questionnaires namely the generic SF-12 and the Edmonton Symptom Assessment System (ESAS), and condition-specific questionnaires, the Anti-Clot Treatment Score (ACTS). A summary of the questionnaires used and justification is presented here.

SF-12 questionnaire

It is a short-form multidimensional generic tool designed to provide a measure of a patient's health-related quality of life (286). The 12-item Short-Form (SF-12) questionnaire is a shortened version of the SF-36 multi-dimensional generic measure

of health-related quality of life which make up the MCS (Mental Component Summary) and PCS (Physical Component Summary) scales (287). It is widely used in clinical trials and outcome assessments due to its brevity and alleged psychometric comparability with the longer SF-36. Two summary measures, MCS and PCS, derived from the SF-12, were used to analyse this survey which is able to satisfactorily gauge the general health of the population. The two scores range between 0 and 100, with increasing values equating to better health.

Edmonton Symptom Assessment System (ESAS)

The Edmonton Symptom Assessment System (ESAS) is a valid and reliable assessment tool commonly used for advanced cancer and palliative care patients (288). This one-page screening tool has been acknowledged for its ease of use that can be used to provide a clinical profile of the severity of nine commonly experienced symptoms over time including; pain, tiredness, nausea, depression, anxiety, drowsiness, appetite, wellbeing, and shortness of breath (288-290). Patients' own assessments of their psychological and physical distress considered as a gold standard in palliative care. The ESAS was designed so that the patient, or his/her family caregiver, could self-administer the tool. The ESAS provides a clinical profile of symptom severity over time. It provides a context within which symptoms can be understood. However, it is not a complete assessment in itself. The original tool was developed by the Regional Palliative Care Program, Capital Health in Edmonton (288). The assumption of scores for all symptoms is defined as the symptom distress score. ESAE considered being time-saving, not expensive, and easy to complete by patients themselves or with help from a carer (290).

Anti-Clot Treatment Scale

The Anti-Clot Treatment Scale ACTS measures (ACTS Burdens and ACTS Benefits) were confirmed to meet widely accepted reliability and validity criteria(291), in line with the US Food and Drug Administration (FDA) recommendations for evaluation of patient-reported outcome instruments (292). As treatment satisfaction should be measured using instruments that have been both specifically designed for its purpose and crossly validated (293). Both the ACTS Burdens and ACTS Benefits scales satisfied traditional reliability and validity criteria across multiple language datasets, advocate it as a

clinically useful patient-reported instrument of satisfaction with anticoagulant treatment in clinical trials (291).

The Anti-Clot Treatment Scale (ACTS) is a 15-item patient-reported instrument of satisfaction with anticoagulant treatment representing negative and positive aspects of anticoagulation treatment: ACTS Burdens (12 items) and ACTS Benefits (3 items) (291). The ACTS is a modification of the Duke Anticoagulation Satisfaction Scale (DASS) (294, 295). Patient experience with anticoagulation treatment was rated on a five-point scale from 'Not at all' to 'Extremely', with higher scores indicating greater satisfaction with treatment. The 12 items of ACTS Burdens were reverse coded (scored 5 to 1), whereas the three items of ACTS Benefits were coded normally (scored 1 to 5) so that higher scores indicate greater patient satisfaction. Item scores are summed across domains to give an ACTS Burdens score ranging from 12 to 60 and an ACTS Benefits score ranging from 3 to 15.

Justification:

The use of two measures offers advantages – generic measures of quality of life (SF-12) and the generic measure of symptoms (ESAS) allow comparisons across studies, thus enhancing the generalisability of findings, while specific measures provide better content validity, by measuring the frequency and severity of specific symptoms of a specific (296). For ACTS scale regardless of the model of care, there are a number of characteristics of anticoagulation that can potentially induce dissatisfaction and reduce the quality of life. Among these characteristics are the need for regular blood testing and other contacts with the medical system, lifestyle limitations (e.g., restrictions on diet and activities), and possible worry about bleeding and/or bruising.

Anticoagulation might also have a number of positive effects; for example, the reassurance provided by effective treatment and contact with support providers. There are relatively few extant condition-specific scales that measure the quality of life and satisfaction with anticoagulation, and to our knowledge, none of these scales can be generalized across models of medical care. For research purposes, having such a scale would be particularly important in support of studies designed to determine which approach to anticoagulation management is superior. In clinical practice, being able to measure the quality of life and satisfaction with anticoagulation management

could help support interventions that increase time in therapeutic range and reduce adverse thromboembolic or bleeding events (293). *Brink and Wood* (297) state that the following aspects characterise a questionnaire

Table 3.5 characteristic of a questionnaire

- Each participant enters his/her responses on the questionnaire, saving the researcher's time, compared to the time required to conduct personal interviews.
- It is less expensive than conducting personal interviews.
- Respondents feel that they remain anonymous and can express themselves in their own words without fear of identification.
- Data on a broad range of topics may be collected within a limited period.
- The format is standard for all subjects and is independent of the interviewer's mood.

3.2.3 Qualitative methodology used in this thesis

3.2.3.1 Rational of qualitative research

A qualitative method was used to support the quantitative data and to explore the patients' experience of living with i-PE that cannot be captured using quantitative research. The qualitative approach aimed to provide an in-depth understanding of the context in which the i-PE and antithrombotic treatments were experienced by patients with cancer. One of the goals that many forms of qualitative research share is understanding of the data, and understanding the context from which the data are derived.

3.2.3.2 Semi-structured interviews methodology

To answer the research question three and gain more understanding of patients' perception and experience of the diagnosis of i-PE in the context of the cancer journey, a qualitative approach was chosen. A semi-structured interview with patients and their carers (if available) was chosen for methodological and practical reasons. The advantage of the semi-structured interview relies on the ability to cover many relevant areas under research. As Rubin & Rubin argue "it allows depth to be achieved by providing the opportunity on the part of the interviewer to probe and expand the interviewee's responses" (298). Although other qualitative approaches could be used. For example, an ethnographic approach could have been used, but given this fairly narrow topic area which focused on patient experience rather than looking at more complex relationships such as the patient consultation, I felt an interview would identify relevant findings, and be less burdensome for participants. In addition, an ethnographic approach would have been difficult to do as I would have been restricted in the ability to be present during clinical encounters due to laboratory commitments in particular. Focus group discussions could have been used, but I thought it was not feasible to invite all participants to the same time and date if I was to minimise patient participation burden which was a significant ethical consideration in this study. Most interviews took place during a participant's routine visit to the clinic rather than expecting the participant to make an additional journey.

3.2.3.3 Philosophical approach

Choosing an appropriate research philosophy is an important part of the research methodology. In fact as *Guba & Lincoln,1982* (299) proposed, "a research philosophy is a belief about the way in which data about a phenomenon should be gathered, analysed and used". Two major research philosophies have been identified in the western tradition of science, namely positivist (sometimes called scientific) and interpretivism (also known as anti-positivist) (300).

Positivists believe that reality is stable and can be observed and described from an objective viewpoint, i.e. without interfering with the phenomena being studied (301). Predictions can be made on the basis of the previously observed and explained realities and their inter-relationships. Interpretivists argue that only through the subjective interpretation of an intervention in reality can that reality be fully understood. The study of phenomena in their natural environment is key to the interpretivist philosophy, together with the acknowledgement that scientists cannot avoid affecting those phenomena they study.

Qualitative research is a diverse field situated within a series of debates with quantification; e.g. natural vs. artificial settings, induction vs. deduction (302). However, a dominant theme in qualitative research is the understanding of linguistic meaning within textual material.

The theoretical approach of a modified Grounded Theory approach was chosen as the most appropriate for the study as it is well documented that used when little is known about a phenomenon (303) and as the data were approached with a set of specific research questions so the aim was to develop categories and themes to highlight the data collected, generating new insight into the participant's experience of living with the condition under investigation and finally, qualitative methods are particularly useful when describing a phenomenon from the participant's point of view (304, 305).

Interviewing methodology gains its value in its flexibility that enables interviewees to "talk in their own voice and express their own thoughts and feelings" (306). The review of patients' experience of cancer-associated thrombosis in Chapter 3 has shown that there was a significant burden physically and psychologically and information needs, that shaped patients' response and coping to cancer-associated thrombosis. With this

in mind, the nature of the research presented in this chapter is exploratory, building on existing knowledge and theories, but also being receptive to any new or as yet unthought of relationships or phenomena. It seeks to explore possible relationships between the patients' experience of i-PE and a range of internal and external forces noted in the literature as presented in chapter 2 and 3.

3.2.3.4 Topic guide

The semi-structured interviews followed a topic guide that developed from the literature and with help of academic supervisor to ensure the structure and content of the interviews are aligned with the research questions, as well as guiding me as a researcher. (Appendix G. Topic guide). As Berg, 2007 (306) considers, topic guide "allows for in-depth probing while permitting the interviewer to keep the interview within the parameters traced out by the aim of the study"

The topic guide questions were about how the participant's life before the diagnosis of i-PE was and how they perceive the new diagnosis in the context of their cancer journey as well as their experience and coping with the new incidental diagnosis. Although the topic guide was informed by the research questions, the questions were open-ended and flexible to allow the opening of another avenue of questioning if an unexpected answer were given.

3.2.3.5 Analysis approach

The method of thematic analysis was chosen as the method of choice to analyse the data related to the research questions. As first described by *Boyatzis* (307) and lately extended *by Braun and Clarke* (272). The advantages of thematic analysis rely on its flexibility that can be used with the size of the sample chosen to give a maximum variation of views from included participants, whereas other methods of analysis would not be sufficient to do so.

3.2.3.6 Limitations of qualitative design and methods

While a structured interview has a rigorous set of questions which does not allow one to divert, a semi-structured interview is flexible, allowing new ideas to be brought up during the interview as a result of what the interviewee says (308). *Jennifer Mason* (309) argues that the variations in style and tradition, they have certain core features

in common, ie the interactional exchange of dialogue, topic-centred approach where the researcher has topics, themes or issues they wish to cover, but with a fluid and flexible structure, a perspective regarding knowledge as situated and contextual.

Cohen et al, 2007 (310) add that interviewing is "a valuable method for exploring the construction and negotiation of meanings in a natural setting". The benefit of interviewing relies on its flexibility that enables interviewees to "speak in their own voice and express their own thoughts and feelings" (306).

However, any research method can be criticized from different perspectives. The common features of criticism rely upon its anecdotal, illustrative, lacks rigour, descriptive, is unsystematic, biased, not replicable and not generalizable (308). interviews have been criticised by *Robson* (311) as being time-consuming with regard to both data collection and analysis. It requires arrangements to visit, scheduling appointments. Interviews need to be transcribed, coded and analysed which takes time. Semi-structured interviews are known as a subjective method that is open to bias from both the researcher and the participant.

In this study, the interview data represent the participant's experience of the diagnosis of i-PE in the context of cancer. The interviews took place after around four weeks from the diagnosis of i-PE which did not provide the opportunity to observe the participant's response immediately. Therefore, the findings were based on the participant's memory and their ability to accurately reconstruct events which may not fully reflect the "true" patient's experience with the diagnosis and anticoagulation treatments representing recall bias. It is also acknowledged that using a pre-set of questions may influence the participant's answers that they may give only what they are prepared to reveal about their perceptions of events and opinions (312).

However, although the subjective component of patients' experience of the diagnosis of i-PE in context of cancer may change and influence the patient's ability to cope or manage everyday activity, the qualitative data gained in this research was then integrated with the quantitative results from the survey study. Therefore, by synthesising these data sets together may provide valuable results that would inform future research.

3.2.4 Mixed methods research

The mixed-methods approach chosen to address the different research questions in this thesis explored the impact of the diagnosis of i-PE on cancer patients. In health research combining qualitative and quantitative methods has gained a lot of interest and accepted into "mainstream" medical research due to the complexity of many different factors that influence health (313). *Creswell et al.* put it as, "A mixed methods study involves the collection or analysis of both quantitative and/or qualitative data in a single study in which the data are collected concurrently or sequentially, are given a priority and involve the integration of the data at one or more stages in the process" (314). Mixed methods research can address some research questions that tend to be broad and complex more comprehensively than by using either quantitative or qualitative methods alone (315, 316).

3.2.4.1 Justification of using a mixed method research

Traditionally, research was often dichotomized as quantitative or qualitative according to the purposes and methods used to generate data. The quantitative research methodology is based on positivism and is associated with the observation of a single external reality, such as clinical trials or observational studies, focused on research goals that typically (deductive, objective and general) that generates numerical data. Quantitative research usually associated with a positivist stance and a belief that reality can be measured and observed objectively (317). Generally, it can test a priori hypothesis and is therefore conventionally described as "deductive" and produce generalizable finding when it is based on large and representative samples. However, this approach is less suited to generating hypotheses about how or why things are happening or explaining complex social or cultural phenomena. In health services, researchers use quantitative methodologies to address research questions about causality, generalizability, or magnitude of effects (317).

On the other hand, qualitative approaches tend to generate non-numerical data, using methods such as semi-structured interviews, focus group discussions and participant observation, focused on research goals that typically (inductive, subjective, and contextual). Qualitative research is concerned more about personal perspectives and based on the constructionist epistemological paradigm. In health research qualitative

methodologies are applied to research questions to explore why or how a phenomenon occurs, to develop a theory, or to describe the nature of an individual's experience (318). In this thesis a mixed-methods approach used to answer the variety of research questions.

These two methodological paradigms can be viewed as mutually exclusive and incompatible if the nature of the difference between them is seen from an epistemological perspective, in which the nature of knowledge and how it can be generated is of paramount concern. However, if the nature of the difference between qualitative and quantitative research methodologies is seen from a technical perspective, in which the emphasis is on the relative strengths of the research methods generated by each paradigm, then the two paradigms can be seen as mutually supportive and thus a mixed methods approach can be justified as it results in a greater understanding of the subject under study that would be gained with either method alone (319).

In this thesis, the research question two was asked in order to understand the impact of the diagnosis of i-PE on cancer patients' QoL and their clinical outcomes in comparison to matched controls with no VTE. This research question can be best answered by using quantitative methods and for this reason, a prospective case-control survey study was performed. However, quantitative results may provide statistical analysis although it does not answer the third research question of this theses regarding patients' experiences of living with cancer and diagnosed with i-PE. Therefore, qualitative semi-structured interviews for the same participants were conducted to enable an understanding of the ways in which these patients experience and cope with the new diagnosis.

Thus, the theses research questions can be approached using quantitative and qualitative methods and then synthesised to obtain a clearer picture of the situation. Therefore, a mixed methods approach was adapted, and the findings were integrated to obtain a more complete understanding of the area researched.

3.2.4.2 Methods of integration

The integration of quantitative and qualitative data can enhance the value of mixed methods research (320). Qualitative data can be used to assess the validity of

quantitative findings. Quantitative data can also be used to help generate the qualitative sample or explain findings from the qualitative data. Qualitative inquiry can inform development or refinement of quantitative instruments or interventions, or generate hypotheses in the qualitative component for testing in the quantitative component (321). Although there are many potential gains from data integration, the extent to which mixed methods studies implement integration remains limited (321). Nevertheless, there are specific approaches that can be used to integrate qualitative and quantitative research procedures and data. These approaches can be implemented at the design, methods, interpretation and reporting levels of research Table 3.6).

Table 3.6 Levels of integration in mixed method research

Levels of Integration in Mixed Methods Research		
Integration Level	Approaches	
Design	Basic designs	Sequential exploratory/explanatory Convergent
	Advanced frameworks	Multistage Intervention Case study Participatory—Community-based Participatory research, and transformative
Methods	Connecting Building Merging Embedding	
Interpretation and Reporting	Narrative—Weaving, contiguous and staged Data transformation Joint display	

Adapted from, Achieving Integration in Mixed Methods Designs—Principles and Practices (317).

3.2.4.2.1 Integration at the Study Design Level

Basic designs include sequential exploratory or explanatory where the intention is to have one phase of the mixed methods study build on the other. In exploratory sequential design, the qualitative data collected and analysed first, and the finding informs the quantitative data collection (322). Whereas in sequential explanatory design the quantitative data collected and analysed, then the findings inform qualitative data collection and analysis (323).

In the convergent designs the intent is to merge the phases in order that the quantitative and qualitative results can be compared (318), the quantitative and qualitative data are collected in parallel and analysis for integration occurs after completion of data collection. The purpose of this design is to better understand or develop a more complete understanding of the research problem by obtaining different but complementary data.

3.2.4.2.2 Integration at the method level

Creswell et al. 2011 (324), conceptualize integration to occur through linking the methods of data collection and analysis. Including (1) connecting; (2) building; (3) merging; and (4) embedding.

Integration through connecting occurs when one type of data links with the other through the sampling frame, whereas, integration through building occurs when results from one data collection procedure informs the data collection approach of the other procedure, and integration through merging of data occurs when researchers bring the two databases together for analysis and for comparison. Integration through embedding occurs when data collection and analysis are being linked at multiple points and is especially important in interventional advanced designs, but it can also occur in other designs. Embedding may involve any combination of connecting, building, or merging, but the hallmark is recurrently linking qualitative data collection to quantitative data collection at multiple points.

3.2.4.2.3 Integration at the Interpretation and Reporting Level

Integration occurs at the interpretation and reporting level, through; narrative, or data transformation, and joint displays (325).

Integrating through narrative includes describing the qualitative and quantitative findings in a single or series of reports. Different approaches used to integration through narrative include; the weaving approach that involves presenting both data qualitative and quantitative findings together on a theme-by-theme or concept-by-concept basis. The contiguous approach involves a presentation of quantitative and qualitative findings within a single report but in different sections. The staged approach often occurs in multistage mixed methods studies when the results of each step are reported in stages as the data are analyzed and published separately. Integrating through joint displays by bringing the data together through a visual means (figure, table, matrix, or graph) to draw out new insights beyond the information gained from the separate quantitative and qualitative results.

In this thesis, the data are synthesised at the interpretation level, once the data collection is complete.

3.2.5 Thesis synthesis

The research questions posed in this thesis cannot be answered by a single research paradigm. Some require a quantitative health services research approach, others a qualitative approach and still others, a laboratory bench science approach. However, all approaches shed light on the central issue of i-PE in people with cancer.

To synthesise the whole theses, I adapted the approach of Critical Interpretative Synthesis developed by Dixon-Woods (2). This was originally developed to address the issue of combining diverse forms of evidence retrieved through systematic reviewing, but the approach is relevant to this thesis where methods of data collection vary widely.

3.2.5.1 Critical Interpretative Synthesis

In health care research the complexity surrounds clinical decision making requires synthesis of different forms of evidence (262, 268). Synthesizing qualitative and quantitative research produces findings relevant to clinical practice (263). Therefore, different methods for undertaking the synthesis of diverse research methods have been developed (326), one of which is Critical Interpretive Synthesis (CIS) (263). Critical interpretive synthesis (CIS) was developed in the context of a systematic review that

incorporates qualitative synthesis that enables the generation of theory with strong explanatory power (247). CIS allows the integration of qualitative and quantitative evidence through an interpretive process (327).

CIS includes a two-stage process, the assembly of 'synthetic constructs' which result from the transformation of the underlying evidence into a new conceptual form, and the creation of an "argument' (263). *Dixon and colleagues* (326) argue that interpretive syntheses can be carried out on all types of evidence, both qualitative and quantitative and the choice of the form of synthesis is likely to be crucially related to the form and nature of the research question being asked (326). In this thesis, CIS was used and the findings presented in the discussion chapter.

3.3 Summary of the methodology chapter

This chapter illustrated and justified the mixed method approach chosen to answer the research questions arising from the literature review. The research methods used in this thesis were diverse and unique for every research question. The details of the methods are presented in corresponding chapters.

In the following chapter, the methods used to gather the research data for the systematic literature review and the qualitative synthesis of the data will be presented in detail.

Chapter 4 Systematic literature review and qualitative analysis of cancer patients' experience of living with cancer associated thrombosis

4.1 Introduction

Findings from the literature review presented in chapter 2 have highlighted a significant gap in knowledge about the patient's experience of living with cancer and thrombosis. Most of the research about the effects of thrombosis on patients' life did not include cancer patients, especially with advanced stages. While in cancer research, systematic reviews and meta-analyses available were limited to biomolecular markers associated with cancer thrombosis (136, 328), risk assessment of VTE in cancer patients (249, 250) or both (251). Others focused on clinical outcomes in terms of survival and disease progression, and the importance of thromboprophylaxis (252), or management of cancer-associated thrombosis (329-331) and some focused on risk stratification (253).

Apart from conferring a worse prognosis, the diagnosis of venous thromboembolism is a physically and emotionally distressing phenomenon that affects patients' experience and quality of life (332, 333). However, data available on how cancer-associated thrombosis and its treatment affect the cancer patients' experience is scarce compared with that in relation to treatment or prevention.

At the time of writing of this review, no systematic reviews or meta-analyses were found that comprehensively examined cancer patients' experience of living with cancer and venous thromboembolism.

This review aimed to bridge the gap in the literature and raise awareness of cancer-associated thrombosis and to stimulate improvements in the supportive care of cancer patients by systematically reviewing and analysing all available qualitative studies on cancer patient's experience of living with venous thromboembolism.

Chapter Three illustrated the methodology chosen to answer the research questions in this thesis. This chapter presents the methods used, the findings and a discussion of the findings from the systematic review and qualitative synthesis to answer the research question:

What are the experiences of people living with cancer and thrombosis?

4.2 Aim of Systematic review:

The aim of this systematic review is to summarise the data available about the experience of cancer patients who have cancer-associated thrombosis including those treated with anticoagulation, in an effort to improve our understanding and raise awareness of cancer-associated thrombosis and to stimulate improvements in the supportive care of cancer patients

4.3 Review objective

The objective of this systematic review is to systematically identify, appraise and synthesise the studies that investigate the effect of venous thromboembolism on the experience of cancer patients.

4.4 Systematic literature review methods

4.4.1 Search strategy

The two independent researchers conduct the search, were Naima Benelhaj (NB) and Illyas Waqas (IW), (a registrar in medical oncology at Castle Hill Hospital) according to a pre-constructed protocol as illustrated by Cochrane Handbook for Systematic Reviews of Interventions(334). The protocol was developed with the supervision of Prof. Miriam Johnson (MJ). Combined Mesh terms and text words for cancer, VTE, QoL as presented in Appendix A. The search was performed in electronic databases: EMBASE, MEDLINE, CINAHL, and PsychINFO, from start to October 2016 and limited to English language. In addition, an internet-based search was performed of the following journals: Journal of thrombosis haemostasis (Isth/JTH), thrombosis research and Haematologica journal. Bibliographies of relevant articles were then used to obtain further related studies.

4.4.2 Inclusion criteria

Population and exposure; Studies of adult cancer patients with VTE, with or without treatment for the VTE were included. The review included qualitative studies or mixed methods that assessed the experience of this group of patients. Inclusion criteria are presented in Appendix B.

4.4.3 Study selection

Two reviewers (NB, IW) independently screened the titles and abstracts retrieved from the search against inclusion and exclusion criteria. The disagreement was resolved by discussion with access to a third opinion (MJ). The abstract and studies were screened then those did not fit the selection criteria has been excluded from the review. Full articles that matched the inclusion criteria were assessed independently by the two researchers (NB/ IL) then the final decision was made in agreement with (MJ). Studies that match the selection criteria were retrieved and their full-text version analysed. Only studies published in the English language were included.

4.5 Data extraction

Data extraction is done by (NB) manually. Contextual data were extracted from all primary studies, including patients' quotes and research interpretation of the results and discussion parts.

4.6 Quality Appraisal

Hill and Spittlehouse defined the quality appraisal as "the process of systematically examining research evidence to assess its validity, results and relevance before using it to inform a decision"(335).

Quality of the included studies was assessed using the Critical Appraisal Skills Programme (CASP) tool for appraising qualitative research (336) (Appendix C. CASP). A widely used tool that has been employed in previous syntheses of qualitative studies to inform decisions about the exclusion of poor-quality papers (337) it is typical of many checklist-style approaches, consisting of a series of 10 questions.

4.7 Analysis

The review sought to locate and analyse any qualitative data that reflected the cancer patients' experience who were diagnosed with VTE. Data from the qualitative studies found were synthesised to identify common themes using the approach used by (J. Thomas. 2008)(271). In mixed-methods studies, the qualitative data were extracted and included in the synthesis.

The primary quotation data were synthesised by NB using thematic synthesis (271) and the principles of thematic analysis to explore the understanding of long term effects of VTE on cancer patient's life quality(338). This allows the context of each study to be taken into account whilst aiming to produce a generalizable synthesis(266). Direct quotes from patients and the researcher comments under the headings "results, findings, or discussion" from each study were extracted for coding.

Thematic synthesis involved: line by line coding of the findings of primary articles after reading and rereading of the papers to get familiarised with the data included, then the codes were discussed with MJ, and a coding framework formed which was used to code all papers followed by the development of descriptive and analytical themes from the codes, in discussion with MJ and JS (339). Both inductive (allowing themes to arise from the specific observations) and deductive (working within existing knowledge about the effect of VTE on people without cancer, looking specifically within our data for similarities and differences) processes were involved.

4.8 Results

4.8.1 Overview of articles

The search identified a total 13197 articles, Embase (11632); MEDLINE (1272); CINAHL (254); and PsycINFO (38) articles. One additional article was identified through searches of relevant bibliographies. Titles and abstracts were independently reviewed by two researchers (NB and IW) against the inclusion and exclusion criteria. Disagreements resolved in discussion with the supervisor (MJ). Full-text articles of all included studies were accessed. The reference lists of all included studies were checked for further studies and any relevant titles were screened against the inclusion and exclusion criteria.

Eleven full articles were retrieved and assessed for eligibility; six articles were excluded following review. This is summarised in the PRISMA flow chart (Appendix D).

Five qualitative studies published between 2005 and 2015, met the inclusion criteria. Four of them were conducted in the UK. The key characteristics of the included studies are summarised in (Appendix E).

4.8.2 Study populations

A total of 92 cancer patients with VTE were included in these studies. All were adult patients of mixed gender with a mean age of 58 years (range 32-84). Participants represent a wide variety of cancer types and stage. The most cancers were: breast, colorectal, ovary, lung, prostate, pancreas, and renal.

4.9 Findings synthesis

Four major themes were identified as being central to the experience of cancer patients with cancer associated thrombosis: Knowledge deficit (among cancer patients, among care providers), effects of cancer associated thrombosis (diagnosis process, response to the diagnosis, physical effects, psychological effects), effects of cancer associated thrombosis treatments (patients' perception toward anticoagulation, patients' perception toward self-injected LMWH, patients' experience with warfarin, Patients' perception toward DOACs), and finally employment of coping mechanism (moving on, employment of personalised routines).

4.9.1 Knowledge deficit

The theme Knowledge deficit described the shortness of knowledge among cancer patients and some of the care providers regarding the association between cancer and thrombosis. Participants reported some knowledge about the causes and the seriousness of thrombosis, but they were not aware of its association with cancer.

Two studies investigated the patients' knowledge about cancer-associated thrombosis in the context of cancer journey (332, 340).

Although some participants reported a prior knowledge about the VTE as a complication of long flights and about the dangerous effect it may have of peoples' life, non-recognise the association between cancer and thrombosis.

Despite the high rate of VTE in cancer patients receiving chemotherapy, patients expressed disappointment that they have not received any education about the symptoms or signs of VTE to look for. Therefore, when patients experienced symptoms signifying DVT or PE, several attributed them to the side effects of chemotherapy and did not acknowledge thrombosis as a cause which in some cases led to delay presentation and consequently delaying in receiving management.

In addition, There was also evidence of limited awareness about venous thromboembolism and cancer amongst health care professionals (332). This is consistent with patient reports of delayed diagnosis of the venous thromboembolism; on many occasions, alternative causes were considered first.

Moreover, patients on chemotherapy usually experienced different side effects, when they develop venous thromboembolism they associate it with chemotherapy and do not recognise that their symptoms are symptoms of venous thromboembolism.

"[…] it's only when you start reading up about it, you sort of realize just how serious erm, you know, sort of blood clots are [...] I was very lucky that you know, it was a fatal, you know attack, so er which is a little bit erm, scary". [VCC05](332)

"One patient presenting with unilateral leg swelling was treated for several months with escalating doses of diuretics even when this did not lead to improvement"(332)

"I went to the doctor and she listened and whatever and said it was probably pleurisy". [VCC12]"(332)

"It [patient's leg] just got bigger and bigger and bigger, over months really […] then they doubled them [diuretics], and then they trebled them". [RG05](332)

"During my cancer treatments. I was never told that there was a risk of getting a blood clot. I didn't know about it….I was pretty shaken up"(340)

"[…] but um this time again first set of chemo, she felt terrible and the thing is, when we

went back to hospital really desperate, the only problem we thought was that it was the chemotherapy that was causing It". [RG02]"(332)

"[…] but I didn't realize that that was what causing it like, obviously a clot like you know, I didn't have any pain or anything, I just thought I

was getting short of breath anyway like, do you know what I mean, because of the chemo and everything". [VCC04](332)

"[…] but they don't tell you you're gonna get clots after chemo, that's the one thing they haven't, they never said but we, we just put it down to, it's just my breathing […] just that one item of information that we weren't aware of. [VCC07]"(332)

"[…] that it could happen yeah, cos you're half expecting it then, in a way, but er, and I have learnt that more people die from clots in the lungs and everything else after cancer than they do from cancer, and you're never told that, so I thought well it is a major point, you know these clots". [VCC07](332)

"[…] cos I do long-haul flights […] I do all the things, the long socks, I walk up and down the plane, you know so I'm aware of the seriousness of a blood clot. [VCC02]"(332)

"[…] they're in the news quite a bit with people dropping dead when getting off an aeroplane and things like that. [VCC12](332)

Nobody really explained, […] 'coz they need the bed, you know. So you don't feel as though erm, you know, I think if it was a little bit more relaxed er, they probably would've got somebody you know, from a department to come and explain it more. [VCC05](332)

On the other hand, participants with prior knowledge about venous thromboembolism respond in calm and seek medical help immediately.

"I was out of breath and I said to my partner, 'I think we are going to hospital' without panic because I knew that it was something that could be rectified effectively)(340)

"Knowing that it is a PE reassures you a little. Nevertheless, I knew I need to go to other hospital as fast as possible"(340)

4.9.2 Effects of cancer associate thrombosis

The effects of cancer-associated thrombosis theme include three subthemes (responses to venous thromboembolism diagnosis, psychological and physical effects).

4.9.2.1 Effects of the diagnosis process

Patients' perspective on cancer-associated thrombosis diagnosis varied. Some participants reacted to the diagnosis of cancer-associated thrombosis as an entity distinct to cancer, while others considered cancer-associated thrombosis as a complication of their cancer. However, in both cases the diagnosis of cancer-associated thrombosis had a negative impact; it led to delays in cancer treatment and added more burden to their health.

"Having the cancer and then the thrombosis on top of it, not knowing how bad it was"(332)
"The fact that there were clots meant we couldn't operate on my leg. Not being able to operate my leg pushed back my radiation and chemotherapy. So everything was shifted in time"(340)
"*If I had just a thrombosis, it would not have been complicated, it was a cherry on sundae"(340)*
"It was something on top of all problems I was having and it wasn't necessary"(340)
"I've had more trouble with the chest part of me than I have with the bottom part of me (cholorectal cancer)"(341)
"During chemotherapy I didn't have any great nausea and brachytherapy went well too, so I told myself well I'm going to overcome the cancer but then I started to go down again"(340)
"I didn't really on upswing, I had just finished the chemotherapy and I was saying "yes, it's gone. But why aren't I feeling like I should?"(340)

4.9.2.2 Response to cancer associated thrombosis diagnosis

The experience of a VTE, and particularly a PE, associated with a state of shock and trauma. Participants report a feeling of threat and dissociation at the time of the VTE.

"….I was pretty shaken up"(340)

"I have never heard of VTE, so that's why I was so shocked"(340)

"[…] like everything else, it's a shock at first". [RG05](332)

"It frightened the life out of me; I was more scared of that than the cancer. You know blood clots can kill you like that (clicks fingers), cancer you've got a little bit of chance, you know". [PT13](341)

"it was frightening to be honest". [VCC01](332)

"the cancer to a point they can treat, hold it back – blood clots they go so quick and that frightened me, it was the only time I broke down". [PT13](341)

"I was like, Oh, something more to deal with"(340)

"[…] and you think ah crumbs, what's next, you know, what's going to happen next?" [RG06](332)

"I think we both thought oh God, what else? […] what else is going to happen?" [VCC11](332)

"I was having a pain in my leg and so I went to my doctor and I said, "Doc, look, they're going to cure me of cancer but I'm going to die of a clot." [PT2](341)

"What he said to me, "It's the clot on your lung that's going to kill you, not the cancer the way it's going" – that's the way he put it to me like". [PT8](341)

"I almost died because I did not know I had PE. SO thank God the medical team found it"(340)

4.9.2.3 Physical effects

The acute and chronic symptoms of cancer-associated thrombosis were profound and negatively affected patients' lives. *Mockler 2012* and *Seaman 2014* described the negative impact of symptoms that interfered with patients' daily living (340, 341).

In particular participants with PE described that being short of breath prevented them from completing even small tasks at home. Symptoms from cancer-associated thrombosis prevented them from returning to normal life and activities; unable to do daily activities around the house or to mobilize unaided.

> *"I couldn't breathe; I literally couldn't breathe and couldn't talk". [PT6](341)*
> *"I had a terrible pain in my chest which I thought was indigestion […] and I'd started coughing up a little bit of blood". [PT13](341)*
> *"I couldn't breathe; I literally couldn't breathe and couldn't talk". [PT6](341)*
> *"All of the sudden it was like something hit me right there (makes a fist and hits centre of chest over sternum). I just went back and I'm like this (breathes heavily as if gasping for breath) breathing in through the nose out through the mouth I was doing. Anyway, it passed off but it scared me I can tell you, it was like something I haven't had, so the following day I had trouble with … I was out of breath and the rest of it". [PT5](341)*
> *"You get up to go to the toilet and you're all huffing and puffing when you get back". [PT11](341)*
> *"I was getting awful shortage of breath. I think the clot moved from the leg to my lung. I was having terrible shortage, I couldn't walk, and I was going to hospital in a wheelchair – that's how bad it was, I never done that in my life". [PT8](341)*
> *"My leg had started aching and it was swelling up and I thought 'this isn't right' […] It was burning and when you touched it was sort of sinking into it, you know, leaving finger marks". [PT3](341)*

"My leg had started aching and it was swelling up and I thought 'this isn't right' […] It was burning and when you touched it was sort of sinking into it, you know, leaving finger marks". [PT3](341)
"The lake of energy and being out of breath…it's just so frustrating. Frustration of not being able to be where O should be. In my mind, you know?"(340)
"I cannot do anything…will I always continue heading in this regression"(340)
"Oh, I was very breathless. Oh my goodness, even bending down to the washing machine to put a wash in I was gasping for air". [PT11](341)
"I couldn't do anything, I couldn't talk on the telephone or anything – it was that bad". [PT12](341)

4.9.2.4 Psychological effect

Four studies reported that the diagnosis of cancer-associated thrombosis was distressing, especially in those without prior knowledge of the symptoms, had a major impact on patients' lives and was perceived as life-threatening (322,340, 341, 343)

Moreover, the distressing symptoms of VTE with the knowledge that VTE is potentially fatal increased the stress level among patients. The emotions of shock and fright were more prominent in patients who have no previous experience or knowledge regarding VTE. This anxiety was heightened when they found out themselves that VTE was potentially fatal.

"*Knowing that it is a PE reassures you a little. Nevertheless…I know I need to go to the hospital as fast as possible*"(340)

"[…] having the cancer and then the thrombosis on top of it, erm, not knowing how bad it was when I went in, I know I was in terrific pain with my chest and that erm, it was frightening to be honest". [VCC01](332)

Anyway, it passed off but it scared me I can tell you[P15](341)

"Knowing that it is a PE reassures you a little. Nevertheless…I know I need to go to the hospital as fast as possible. (340)

"*I was out of breath and I said to my partner, I think we are going to the hospital without panic because I knew that it was something that could be rectified effectively"*(340)

"I have never heard of VTE, so that's why I was so shocked"(340)

"[…] it's only when you start reading up about it, you sort of realize just how serious erm, you know, sort of blood clots are [...] I was very lucky that you know, it was a fatal, you know attack, so er which is a little bit erm, scary". [VCC05](332)

"It frightened the life out of me; I was more scared of that than the cancer. You know blood clots can kill you like that (clicks fingers), cancer you've got a little bit of chance, you know". [PT13](341)

"All of the sudden it was like something hit me right there (makes a fist and hits centre of chest over sternum). I just went back and I'm like this (breathes heavily as if gasping for breath) breathing in through the nose out through the mouth I was doing.[PT5]"(341)

"The breathlessness was very striking. I asked myself whether I was going to die"(340)

"What he said to me, "It's the clot on your lung that's going to kill you, not the cancer the way it's going" – that's the way he put it to me like". [PT8](341)

"All of sudden I couldn't breathe… I didn't know that was happening. They wrote in the chart that it was a possible heart attack"(340)

"I felt I was having a heart attack… the stress made(the symptoms) worse"(340)

4.9.3 Effects of cancer associated thrombosis treatments

This theme captured patients' perspective toward anticoagulation treatment (self-injected precalculated LMWH and toward warfarin) and their anticipation.

4.9.3.1 Patients' perspective toward self-injected precalculated LMWH

Participants found LMWH to be an acceptable intervention for the treatment of CAT despite reporting a variety of symptoms associated with injecting (341). Patients understood why they were on LMWH and considered it an acceptable treatment. None of the participants reported distress or anxiety related to the injections (342). This includes necessity, tolerability, (freedom and control) and downsides of LMWH.

Many participants considered anticoagulant injection as an obligatory process, however unpleasant in treating a potentially life-threatening condition. Moreover, many reported the downside of a daily injection to be acceptable, the necessary trade-off to keep them free of thrombotic recurrence. This is likely to reflect their knowledge of the potentially fatal complications of VTE, coupled with the experience of distressing symptoms at presentation. In addition, it suggests that some patients will comply with indefinite anticoagulation therapy if required (341).

"I really don't feel like to pricking myself, but if that or dying well I'd rather pick myself" (340)
"I don't like to pick myself, but I do it and the needles it's painful but you have to do it" (340)
"It's not a problem to inject myself, I'll do that for as long as I have to.[PT9](22)
"I've got used to it like we all do everything in life, but it's … whilst it's only a small injection, it's quite painful". [PT10] (341)
"I'll stick the needles in until doomsday – it doesn't make any odds to me as long as they're keeping me going". [PT2] (341)

"Well I've got to, haven't I? It's either that or I peg out, like. So you see that it's doing you good. I've got to do it, simple as that, no argument". [PT8] (341)

"Well, if it keeps me alive it's as simple as that. I'd take poison to stay alive. It's not nice, you're tired every morning, pants down and injection but there you go – that's life, isn't it, and as I say, it's keeping me alive, so that's the important bit". [PT9] (341)

Additionally, the self-injection of precalculated weight adjusted of LMWH means no need for frequent blood tests, which gave patients more freedom, thus gaining more control of their lives.

"The heparin is more than acceptable.... so much of my treatment has been sitting back and having things done to you ... I prefer this ... I feel that I have got control back in my life." (1 ICS) (342)

"[…] they give me the option of doing 'em myself, that's when I decided to do 'em myself. And now I do 'em myself, I do 'em, you know, it's to suit me. So I do 'em in the morning. And the day's my own then like, do you know what I mean like?" [VCC05] (332)

"I used to spend my life travelling to hospital for a warfarin check ... sat in the car ... sat in the waiting room ... not much of a life really" (19CS) (342)

"The use of LMWH did not come without price many patients reported pain associated with the injection, and bruising is common. Plus, Long-term use is associated with the development of subcutaneous lumps and further bruising, making it harder to find suitable places for injection" (341).

"Sometimes when the needle goes in you don't even feel the injection; other times the needle, it's as if the needle's blunt and it won't go through the skin … you know, you do it and then you bleed. Other than that there's no discomfort at all". [PT9]. (341)

"I always bruise on my belly – I did when I was in hospital – doesn't worry me though" (8CS) (342)
"I don't worry whether it is at the right level in my blood ... I know its getting into my body" (342)
"I do 'em, you know, it's to suit me. So I do 'em in the morning. And the day's my own then like, do you know what I mean like?" [VCC05] (332)
"Because I do it as soon as I get up and then if I've got to go anywhere it's all done. Done and dusted". [VCC02] (332)
"You try to spend your what's left of your life as actively as possible .... You can't do that if you have to wait at home for the district nurse to come round and check your blood each day. . then you have to wait for the result to see what dose of warfarin you need to be on" (332)

Patients' perspective toward anticoagulation (necessary). Many participants illustrate a state of necessity to take anticoagulation to prevent VTE reoccurrence (340).

However, some participants want to discontinue the anticoagulation treatment and expressed the wish to stop the treatment as soon as possible aiming to return to normal life and in some cases because of its side effects.

"But then they found out there was a everything was alright and there was no clots so I thought I'd see how it went without it. If I'm not getting any symptoms or anything there's no point in having treatment is there? Just have regular check-ups".(343)
"I just wanted to see if I how I went without it, you know, because it if I can cope with without all, you know, all that every day I can get on with my life then, cos I couldn't imagine taking dozens of syringes on holidays, you know, I don't mind half a dozen . . . . . until I stopped the injections I didn't know whether they were doing me any more good or whether there was, you know, once they said the clots were gone, but there was a risk of them coming back, well I they don't

know and I didn't know wh whether until I tried for myself, you know" (343)

"And I think that's part of having had so many things pumped into you I wanted, you know, I suppose I just wanted to know how my body was really because you it it's not you anymore you know .And so I was very keen I have to say, I was predisposed I don't want any further injections once the treatments finished I just want to try to get back to as much normality as I can" (343)

"I was just happy to get off of it to be honest with you, um it was more or less the same time every night, um and the pain as I said eh to me was terrible, horrific and a lot of bruising and things" (343)

4.9.3.2 Patients' perspective toward warfarin

Disruptive, Participants reported that frequent alteration of warfarin doses was inconvenient and disruptive (342). The inconvenience of frequent blood tests was one of the reasons given for their negative attitude toward warfarin. Time-consuming and distressing, when warfarin therapy is complicated, patients report experiencing distress, discomfort and feeling inhibited in day-to-day activities (342).

"Every day my blood was checked and every day the dose of warfarin was changed ... Eventually they started me on heparin. I wish they'd done it sooner ... a quick injection and then you're done ... no blood tests no hassle" (SCA) (342)

*"I used to dread the blood tests ... I ran out of veins. The heparin is so much simpler than all the ****ing about with warfarin" (22CS) (342)*

"With the warfarin what was kind of crappy was that I had to do blood tests every two weeks. But with LMWH no need for draws."(340)

"With warfarin you have to avoid eating all kinds of cancer-fighting vegetables. Given that I have cancer and I can't eat like I should… you Know? With the LMWH it's a bit more logical" (340)

"I used to spend my life travelling to hospital for a warfarin check ... sat in the car ... sat in the waiting room ... not much of a life really" (19CS) (342)

"You try to spend what's left of your life as actively as possible .... You can't do that if you have to wait at home for the district nurse to come round and check your blood each day…. then you have to wait for the result to see what dose of warfarin you need to be on" (342)

"It was never far from your mind. What's the INR going to be today? What dose of warfarin will I need? Do I have enough 1 mg tablets?" (23CS) (342)

4.9.3.3 Patients' perception toward DOAC

Although most patients would prefer the oral route to the subcutaneous route, this would only be if there were equal efficacy and safety. Because none of the DOACs have demonstrated non-inferiority against LMWH, it would seem counterintuitive to recommend DOACs routinely as first-line treatment of CAT (341). Participants favoured efficacy over convenience, expressing a preference for injections over a theoretical trade-off of reduced efficacy with oral medication. The distressing symptoms associated with VTE are likely to have influenced their satisfaction with LMWHs (341).

"It would have to be the tablet. Most definitely". [PT10] (341)

"I don't mind either, I suppose. I'm not fussed on tablets, I take so many a day". [PT4] (341)

"Well I don't know. It would be probably … well, I can't say ... I've got used to injecting, but who's to say?" [PT5] (341)

"Having been through what I know now, I suppose if I was asked that question at the start, "Look, tablet form but it's not clinically trialled or whatever, we haven't got any data on it or an injection which we believe at this moment in time is more effective," I probably would have gone for the injection". [PT10 (341)]

"I'd opt for the injection … as I said, I don't mind trying new things, but with reservations. I've gone through this now for nearly 11, so I

think I know a little bit more than somebody who's just had it and I would have to be reassured". [PT13](341)

4.9.3.4 Anticipation (optimism)

The feeling that something active was being done and the optimism that accompanied it was a recurrent theme Many participants had experienced attitudes of futility in the later stages of their illness and an overall feeling of being given up on (342). Participants who experienced problems with control of the INR viewed the LMWH as a proactive therapy that improved their outlook on the future and their view of those looking after them (342).

Underlying this duty was the belief that the anticoagulation is working and would resolve the lingering symptoms (341).

"I know I'm going to die. I know that the doctors don't have any more chemo to give ... you don't like feeling that you've been put on the scrap heap ... the injection isn't stopping the cancer but it is stopping the blood clots' (14CS) (342)

"It is important to know that people are still doing something".(342)

"The doctors were having problems with my warfarin... but they didn't give up on me". (21CA) (342)

"I can't beat the tumour but I can fight its effects ... the heparin helps me face the future. I choose to inject myself because I feel I can face the future on my own terms ... the DVT was terrible, I couldn't face the day". (17CS) (342)

"The blood thinner is working" (341)

"I have to trust that the dedication is working even though I have been on it for two months and it doesn't seem to be" (341)

4.9.4 Employment of coping mechanisms

Moving on (Needs to recover), the treatment of VTE brings with it symptomatic relief, reassuring patients that their condition is improving. This reduces distress and allows patients, over time, to get back to 'some sort of normality'(332)

> *"You want to try to recover and get back to some sort of um, you know, some sort of normality". [RG06](332)*
> *"Because I do it as soon as I get up and then if I've got to go anywhere it's all done. Done and dusted" [VCC02](332)*
> *"I mean I just treat it as one illness to be honest [...] because you know, you've got it at the same time and the one was caused by the other". [RG03] (1)*

Participants described the development of strict routines and rituals to ensure LMWH was administered on time and without fail.(341) Participants developed systems to continue injecting in a way that would allow bruised areas to recover. They reflected confidence to be pragmatic in finding ways to continue with their injections (341). Many participants described the development of systems and rituals around their daily LMWH injection (332). They described the development of specific personalized routines, which they would strictly adhere to (332).

> *"I usually take them between 8 and half past 8. And then I know it's done, and I don't forget for the day, then, because someone I was talking to, he was saying "You don't do it in the night, do you?" and I said, "No, I get up, have my cup of tea then 8, half past 8 do it." [PT13](341)*
> *"This side is the odd dates and this side is the even dates. So the even date had its share today, other than that you're … well, not everyone's like me but I'd be, "Now what date was it yesterday when I done it?" And it's a routine now, you see. It's better. You know. I keep between 8 and quarter past, so I have my ablution, then I give my injection, then I get dressed". [PT5](341)*

"I'm using the tops of my legs now so it isn't as painful. I was using my stomach but after a while your stomach gets really hard and then you've got to really force them in". [PT8](341)

"[…] is a ritual now". [VCC10](332)

"Right we sit here and I say I've got to have me jab. I go in the bedroom […] shut the door, 'coz I got to pull this up, pull that down. It's only a little thing like that". [VCC01](332)

"My husband's got his phone on alarm and when the alarm goes off he gives me the injection". [PT4](341)

"I've got my routine. I'm usually in bed by 10 o'clock. So, I've got them all upstairs, so I take my tablet, get my injection, do it, put it in the thing, then my husband fetches them down and puts them in the burn bin". [PT2](341)

4.10 Discussion

This systematic review and qualitative synthesis of the literature relating to the experience of cancer patients living with VTE found four distinct but related major themes. The four themes from this synthesis of primary qualitative studies (knowledge deficit, effects of cancer-associated thrombosis, effects of anticoagulation and employment of coping mechanisms) illustrate the ways in which cancer- associated thrombosis affects participants' quality of life.

Thrombosis with its complex presentation, diagnosis and treatment was seen by many patients as a significant additional, frightening and unexpected burden affecting cancer treatment and which impose psychosocial and functional limitations.

4.10.1 Lack of information

The theme "lack of information" appeared to be the Key feature of the review findings. It's relation to the miss-interpretation of the symptoms and signs, delay in diagnosis, patients' response to the VTE, and coping with the effects of the VTE, its treatment and their cancer.

Despite being at high risk for thrombosis, participants frequently reported being neither aware of the increased risk of DVT/PE, nor about the symptoms and signs to look for or prophylaxis measures.

It is very common for patients with cancer to experience complications during their cancer journey, due to either disease progression or treatment's side effects. Signs and symptoms are usually a typical and patients may attribute them to cancer progression and do not seek medical advice. Not seeking medical help and delayed diagnosis may cost patients their life or long life complications, namely pulmonary hypertension or post-thrombotic syndrome. It is widely recognized that cancer journey is very difficult and patients continue to experience a burdensome time with the cancer diagnosis, starting cancer treatments, and living low quality of life, so having this life-threatening diagnosis would add more constraints to their life.

The presented data showed that knowledge and awareness of patients regarding cancer associated thrombosis were highly influencing patients' responses and their ability to deal with this new turn in their cancer journey.

Unawareness about the high risk of thrombosis has led to a significant state of shock, fear, and the sense of life threating, combined with the disappointment of being not prepared for this and not educated about preventable measures in advance. On the other hand, the effects of being acknowledged about the issue were very clear in a response of subgroup of patients who have a previous history of VTE, they were less distressed and knew the symptoms to watch for, consequently sought medical help more quickly (340). This affirms the importance of improving cancer patients' awareness and education about VTE.

Research has indicated that the majority of cancer patients prefer to be informed about their illness, although, it is varied in how much information they need and that may change over the course of their illness (344). Consequently, in the absence of that from their caregivers, patients tend to seek information from other sources (internet, charities brochures, etc.) accessing information without enough support from their carers, which may add to their distress and anxiety. Moreover, the combination information deficiency and poor communication are likely to aggravate patients' concerns of feeling alone and abandoned (345).

Even though the cancer-associated thrombosis is not a new phenomenon, a lack of clinician awareness of cancer associated thrombosis appears to compound the lack of patient knowledge (346). The practice of venous thromboembolism management varies worldwide, a survey study reported that over 25% of British oncologists in northern England were unaware of the associated risks of venous thromboembolism with cancer (347). Another study of 12 patients who had venous thromboembolism reported being felt let down by services, they were angry and lost trust in doctors when they repeatedly misdiagnosed (345).

On a global level, it has been reported that public awareness regarding thrombosis overall, and VTE in particular is low (348). In recent years, the issue of lack of information and awareness about the high risk of VTE among cancer population has been raised by many organisations. (*Sousou.T 2010)* in a survey included 190 ambulatory cancer patients with VTE found a better level of knowledge of cancer-associated thrombosis risk than those in this review, but still nearly half of the participants (53%) were unaware of the increased risk of VTE with cancer, although

they were aware that it is a preventable disease (349). While a survey released by *Aggarwal. A.et al 2015* included 500 cancer patients (inpatients and outpatients) found a difference in term awareness between patients, they were more aware of the term blood clot that the term of PE or DVT, with 19% and 17% of them could name signs and symptoms of DVT and PE respectively. However, regarding risk factors, awareness only 4% reported chemotherapy, radiotherapy and other cancer treatments(350). Moreover, it reported that inpatients were more aware of the risk, the treatments, and the prophylaxis measures of VTE than ambulatory patients(350).

In oncology sitting, it is essential that patients are aware of the potential risk of VTE and the symptoms and signs in the context of cancer so they can seek medical help earlier. Organisations such as NICE, CQUIN and the All-Party Parliamentary Thrombosis Group have been pivotal in raising national awareness about VTE. The National Institute for Health and Care Excellence (NICE) in 2010 published guidelines recommended that VTE risk assessment be undertaken at admission (and repeated after 24 h) and appropriate prophylaxis be provided where indicated (351). Also, recommend that "Patients/carers are offered verbal and written information on VTE prevention as part of the admission and discharge process".

In this context, (CQUIN/2010) Commissioning for Quality and Innovation agreements was introduced. Required that at least 90% of patients who attend acute trusts in England to be VTE risk assessed to avoid financial penalties (352).

Alongside these initiatives, an All Party Parliamentary Thrombosis Group (APPTG), in their 2016 report, has also highlighted that potentially avoidable hospital deaths due to VTE in England and Wales could be prevented by increased clinical awareness and by providing patients with basic information. "*At least two-thirds of cases of hospital-associated thrombosis are preventable through VTE risk assessment and the administration of appropriate thromboprophylaxis"(353).*

Furthermore, the European Commission has taken a major step to reduce cancer-associated thrombosis related death. By developing an action plan aiming to raise the awareness level of health care professionals, policy-makers, health authorities, patients and patient associations about the impact of VTE on cancer patients'

morbidity and mortality, emphasise the need for early diagnosis and treatment and the means of preventing this complication (354, 355).

The ASCO guidelines for treatment and prophylaxis of cancer-associated thrombosis and based on consensus, the Panel recommends that patients with cancer be assessed for VTE risk at the time of chemotherapy initiation and periodically thereafter. For the outpatient setting, the risk assessment can be conducted based on a validated risk assessment tool. The Panel recommends that oncologists educate patients regarding VTE, particularly in settings that increase risk such as major surgery, hospitalization, and while receiving systemic antineoplastic therapy (356).

Patients' lack of information either because these recommendations been overlooked or not implemented. The busy environment in the NHS, where there is not enough time to explain to patients or provide enough information at diagnosis time. And low level of awareness among some of the caregiver professionals of VTE and lack of clinicians' knowledge about management of this complex issue, particularly in advanced cancer, which in many cases professionals may find clinical-decision making difficult (343). As a result lack of clinician awareness of VTE compounded the lack of patient knowledge (343) (357).

Moreover, in palliative sitting, defining what is appropriate and what is not seems to be intrinsically related to the individual viewpoints of different doctors, in absence of immediate benefit for DVT treatment comparing to other medication. In addition to the absence of clear guild to facilitate decision especially for patients at their end of life. These difficulties have sometimes influenced the dissension of some clinician to not to treat and considering a PE is a good way to go (358).

Given the prevalence of cancer-associated thrombosis, it is surprising that there is no routine education about VTE for in and outpatients with cancer. This lack of routine information-giving is in contrast to other cancer-related complications such as malignant spinal cord compression and post-chemotherapy neutropenia, where there are clear guidelines, systematically applied for treatment with education of cancer patients, their family, and carers about symptoms and signs to look for, when to seek medical help, and who to contact (359, 360).

One of the common complications of cancer is malignant spinal cord compression where we found a well-developed guidelines management, compared to CAT. The guideline included patient's education and advice where to seek help, whom to contact in case experiencing any symptom or signs and every trust has MSCC coordinator, this was not implemented by many clinicians even in the presence of recommendations from NICE, ITCA, and ASCO (356).

These findings reinforce the importance of increased patients' education and awareness as well as health care professionals. Increased awareness of the risks of thrombosis among cancer patients (Inpatients and ambulatory) how to recognize them, and targeted support during the diagnostic process would be a priority for improving the management of this common oncological complication.

Overall, many studies suggest that cancer patients continue to experience high levels of unmet needs across a range of domains including health system and information (361-363), psychosocial (364), physical and daily living(365-367).

4.10.2 Effects of cancer associated thrombosis

The review shows that the scale of VTE burdensome is very significant (physically and psychologically), from the onset of the symptoms through the diagnosis process to the treatments and coping.

Response; The patients' initial responses on many occasions were overwhelming, it was a mix of shock, frightening, and uncertainty The response could be partially explained by unawareness of patients about the symptoms and signs of thrombosis and to the nature of the sudden onset of the symptoms where described in many cases as a life-threatening condition. Participants were able to give a vivid description of their symptoms experienced in reference to the scale of the effect of VTE. These findings were accordant with a qualitative study described a "loss of self" owing to the physical restriction of people admitted to hospital with a PE due to a variety of causes (368)

Physically, it was clear that participants were having difficulties dealing with the physical burden of their VET symptoms. Being too ill to carry out simple daily living requirements, participants who were previously independent became unable to carry

on simple activities without help. Pains, suffering from even simple movements were apparent across the review findings. Physical pain has typically experienced from the beginning of the symptoms with PE and DVT that continue in many cases even after treatments. These findings were in accordance to a previous study reported that patients with DVT tend to have lower physical health status than adults norm after 1 and 4 months, that is lower or similar to patients with chronic conditions such as arthritis, chronic lung disease, or angina(369).

One of the common features that concerned participants in this review was breathlessness among patients with PE that associated with a significant negative impact on patients' life quality.

It is well known that breathlessness is a frightening, restricting symptom, that reduces the quality of life (370) limits activities of daily life,(371) and is strongly associated with increased health care utilization (372), higher rates of depression, anxiety as well as shorter survival.(371, 373, 374).

It has been reported that persistent dyspnoea among patients with PE comparing to controls represents an independent predictor of reduced QoL, and in many occasion, patients suffer from a reduced functional capacity that persists for many years after the event (375). These findings were supported by findings of other studies (Noble.2014) (368)and (Punekar 2016) (23) they described the negative impact of symptomatic PE has on patients' life including modification of physical activity and exertion.

Building upon evidence and NICE guidance, breathlessness services have been successfully developed, and evidence suggests that such services are successful in the palliation of this disabling symptom (376-380)

Patients with cancer frequently experienced breathlessness as the disease progress, and the absence of single effective palliative treatment, complex interventions may need (381). One of the known programs is Breathlessness Intervention Service (BIS) that proved to be effective in reducing distress and cost among patients with advanced cancer (381). Breathlessness Intervention Service (BIS) is a multi-disciplinary complex intervention incorporating pharmacological and non-pharmacological interventions

that support patients with breathlessness, theoretically underpinned by a palliative care approach (381, 382).

To provide good quality health care, recognising these symptoms are important for their broad effects on patient's life emphasizing the requirements for supportive treatments aiming to improve these complications. In this context, the review findings may support the hypothesis that cancer patients with PE and persistent dyspnoea may benefit from cardiopulmonary rehabilitation programmes.

It is well documented that in cancer patients with VTE, symptoms can take weeks to improve despite receiving optimal anticoagulation. Moreover, patients with VTE are at high risk of developing post-thrombotic syndrome, suffering long-lasting morbidity and increased risk of recurrent VTE (383, 384)

Post-thrombotic syndrome (PTS) is a chronic condition that may occur in patients subsequent to the development of deep venous thrombosis (DVT). Characterised by; pain, cramps, heaviness, pruritus, and paraesthesia, oedema, skin induration, hyperpigmentation, venous ectasia, redness, and pain during calf compression and presence of a venous ulcer(385, 386), and associated with lower QoL as has been reported by (van Korlaar.2004) (387)

These findings indicate that the life quality of cancer patients with VTE impaired in all domains. They report physical dysfunction because of pain, they have low perceptions of their general health and experience a high level of distress struggling to cope with lingering symptoms and with a high prevalence of trauma been reported as well (345, 368).

Research has consistently shown that cancer patients have low or reduced levels of QoL from initial diagnosis often for several years post-treatment, in addition to significant psychological distress commonly manifested as depression and/or anxiety (388, 389). Among those patients, the diagnosis of thrombosis added more burden to their life.

While the physical and medical consequences of VTE have been widely researched, the psychological and emotional impact has not. This is despite clinician and patient

reports in hospital and by leading VTE support charity, (Thrombosis UK) indicating the potential for significant psychosocial difficulties following VTE.

Psychologically; beyond the physical restriction, VTE has profound psychological effects. The long-term outcomes following acute VTE continue beyond the physical burden. The response to VTE diagnosis was individual among cancer patients. In the context of cancer, for some patients, the diagnosis with VTE came as a greater shock associated with intense distress. In the absence of previous knowledge many cancer patients concern about cancer progression or recurrence and were not prepared for such complication

Cancer itself considered to be a chronic illness and for many patients in this review the experience of the sudden onset of VTE (DVT or PE), coupled with the knowledge of the seriousness of the diagnosis reinforce the distress level. Consequently, it is also likely to cause high levels of fear and shock and considered to be a life-changing event.

These finding corresponded to studies explored psychosocial impact of VTE in non-cancer patients They reported elevated levels of anxiety and depression among VTE patients, with impaired quality of life one month after diagnosis, the effect that continue after two years post-VTE (368, 390, 391).

These finding highlighted the need for social psychology interference from the beginning to provide support and aid.

The experience of VTE, and in particular a PE, presents a high number of pre-traumatic risk factors, in that it is a sudden, life-threatening and acute onset illness over which the patient has no control. Therefore, it is also likely to cause high levels of fear and often dissociation at the time of the event.

Since VTE is a potentially life-threatening condition that often occurs in previously well patients, coupled with the uncertainty of recurrence it is possible that such patients are at increased risk of emotional distress, especially those who have experienced major PE (368) .

In the non-cancer population, several studies had investigated the long term psychological impact of VTE reported a high level of anxiety and depression, with impaired quality of life (390, 391). Findings that supported by a qualitative study by

Noble et al. (2014) (368) in a small number of VTE patients who reported the experience of symptomatic PE to be a life-changing distressing event leading to changes in behaviour.

Life-threatening and traumatic medical events are those most likely to cause psychological distress and behavioural changes associated with symptoms of PTDS (392). In patients with cancer, the psychological impact of a cancer diagnosis can take many forms such as; depression, anxiety, fear, and cognitive defects (393). Effectively addressing the psychosocial impacts of cancer has the opportunity for patients to lead a better quality of life (394).

PTSD is a psychiatric disorder that can develop after experiencing a traumatic event, involving physical harm or the threat of physical harm. Symptoms of PTSD grouped into four broad categories, including heightened arousal, intrusive thoughts (re-experiencing the traumatic event), avoidance, and negative changes in cognition and mood (395). These symptoms cause clinically significant distress and impairment in important domains of function (395).

The experience of any health-related crisis is likely to result in some form of emotional distress presenting in a variety of forms including worry, anxiety, intrusive ideation and dysphoric mood, among other negative emotional reactions (371) . Manifests as anxiety, anger, depression and even symptoms of post-traumatic stress disorder (PTSD). The diagnosis of cancer recognised as an event capable of eliciting PTSD by the American Psychiatric Association (396). However, cancer-associated thrombosis may have greater potential to produce PTSD compared to other complications given that the disease is not only life-threatening but also associated with a high recurrence rate despite being on anticoagulation.

In addition, patients expected to return to normal after their cancer treatment, interrupting with VTE considered as a setback on the road to cancer recovery. Given this information, possessing knowledge about VTE might mitigate a decline in well-being for patients with cancer. This further represents another argument in favour of patient education on VTE in cancer.

4.10.3 Effects of cancer-associated thrombosis Treatments

It was clear that anticoagulation treatment of VTE associated with symptomatic relief, which would improve patients' life quality. However, it is well documented that the management of VTE in cancer sitting is very challenging, especially in advanced cases (397). Needs long term anticoagulation that associated with a high risk of bleeding and reoccurrence. Furthermore, the choice of anticoagulation in cancer population has usually influenced by many factors, including the type of cancer, stage, presence of metastasis, type of anticancer and doctors attitude (398).

The population in this review were mixed with many of them were in palliative care. Generally, there was clear evidence of the acceptability of anticoagulation as a treatment of thrombosis among this population. This acceptability could be explained by the experience of the life-threatening symptoms of venous thromboembolism. It is worth noting that, patients in this review consider themselves as cancer patients than venous thromboembolism patients. They were more concern about their cancer journey and consider the diagnosis of venous thromboembolism as a complication of cancer more than a unique form.

The finding regarding patients' attitude about anticoagulation reported that they would prefer treatments that; do not interfere with or delay their cancer treatment; that it reduces venous thromboembolism recurrence and associated with a low major bleeding rate regardless route of administration.

Even though, the low molecular heparin injections were acceptable, simple, and gave them more freedom and control on their life. Many reported accepting the daily injection of a pre-calculated dose of low molecular heparin injections to the frequent blood test and dose alteration of warfarin. This finding in contrast to articles reported that daily injection of low molecular heparin would increase patients' burden in palliative sitting(399). Important point state here that many of those in palliative care see their treatment of VTE means that their doctors did not give up on them, influencing their optimism and expectation about their doctors.

As with any other treatment, patients on anticoagulation experienced many side effects, with low molecular heparin; haematoma, fibrosis at the site of injection, and bruising were common. While with warfarin, frequent hospital visits for blood tests,

dose alteration, diet restriction, and interfering with some other medication. Patients in this review show an ability to develop ways that enabled them to continue their medication, especially with low molecular heparin. Including changing the site of injections, sitting the alarm to remind them and family help. The findings of patients' attitude about anticoagulation were clearly in favourable of LMWH.

Evidence of improved clinical outcomes for cancer patients receiving low molecular heparin versus warfarin has been well documented (400). However, many healthcare providers still questioning the tolerability of daily injection in patients with cancer (401). A study of registry data from the United Kingdom reported that despite the evidence to support the use of long-term low molecular heparin for secondary prevention of venous thromboembolism in cancer patients, yet patients still receive suboptimal doses with only 9% received low molecular heparin for longer than 30 days and only 5% for longer than 90 days(402) (398).

Furthermore, the preference of tablet over injection has been reported. In accordance with that, when patients perceptions toward direct oral anticoagulation assessed it revealed that preference of tablets with the condition that it provide the same efficacy (403).

The necessity of anticoagulation to keep them clot free was clear, that may power patients' acceptance to anticoagulation, and develop their specific ways to adapt and ritualize. Even though, many clinicians, especially in palliative sitting, argue that although LMWH is good for managing physical symptoms of PE it also provided daily reminders, triggering negative thoughts among patients regarding their illness and future (345).

4.10.4 Employment of coping mechanisms

The diagnosis of VTE is life-changing illness, moreover, living with the physical symptoms of cancer-associated thrombosis and maintaining the treatment regimen is usually stressful, demanding one to cope with these progressing situations.

The lack of educational interventions for participants with cancer-associated thrombosis is surprising, considering the life-changing nature of the diagnosis and its treatment. Patients experience a variety of physical and psychological symptoms,

including post-traumatic syndrome, fatigue, anxiety, and depression plus treatment side effects all of these associated with increased stress and changes in a person's lifestyle.

However, even in the absence of clear guidelines about education and support for cancer patients with cancer-associated thrombosis, participants in this review have shown the ability to develop different ways of coping strategies to adapt the new changes in their life allowed them to maintain some sort of normal life activities and continue on their treatments.

Coping has been defined as "constantly changing cognitive and behavioural efforts to manage specific external and/or internal demands that are appraised as taxing or exceeding the resources of the person" (404). Coping strategies have been categorized in different ways; although, the meaning is similar(405)

Active coping (adaptive, favourable, problem-focused) refers to a situation where individual accept and actively attempt to deal with any new situation. Strategies of active coping (or engagement-focused strategies) include; active problem solving, seeking emotional support, and planning. The other category is avoidant and maladaptive coping. These refer to strategies where individuals try to avoid dealing with problems by cognitively and physically distancing themselves from the situation. Avoidant coping strategies characterized by escape from the stressor including denial, behavioural disengagement, and alcohol/drug use (406).

Another coping strategy that related to poor psychological adjustment is self-blame. Behavioural self-blame can occur when individuals perceive that they could have done something differently to prevent or change their situation(407).
Generally, active coping is viewed as positive and avoidant coping is viewed as negative in these conceptualizations. However, adapting one strategy is not necessarily good or bad, rather it depends on the situation and outcome that follows its particular use.

A systematic review about the coping style associated with heart failure outcome reported that adopting a specific style or other of coping is more influenced by many factors that related to concepts contribute to the physical and psychological quality of

life. For example, knowledge of one's illness, patients' perception of chronic heart failure (408) and individual personality (409). Moreover, an individual's quality of life may actually influence the style of coping utilized as well (410).
Available research suggests that engagement-focused coping strategies are related to positive outcomes, including benefit finding (411, 412) and lower distress (413). Conversely, the use of disengagement/ avoidant coping strategies have been related to a low quality of life, anxiety, and depression (414, 415)

In this review, we did not aim to locate any studies that examined the coping strategies in patients with cancer-associated thrombosis. Even though, coping quotations in this review signal the use of active coping yet emphasise the need for more support and information to minimize the consequences of side effects of venous thromboembolism, and of anticoagulation treatments.

Participants in the included articles were able to develop confidence in their ability to care for their own management needs and sometimes with help from a family member. Self-care practices take time to learn (410), and participants in these articles were able to care for their own management needs and sometimes with help from a family member.

These findings were in parallel with the findings of (Pedersen.B 2003) (416) investigating cancer patients' approaches to minimize the impact of the side effects of cancer treatments to maintain a sense of control in their new life situation like engaging in many simple activities (cooking, walking a dog) that kept them busy and helped them controlling of treatments' side effects.

Given the increasing numbers of individuals having to learn how to live with cancer-associated thrombosis, educational programs tailored to cancer patients with thrombosis definitely needed. Information on a cancer-related thrombosis topic including understanding anticoagulation treatments, managing the side effects of illness and treatment, communication, resources and support, as well as strategies for self-care would be very helpful for patients.

One of the earliest reported educational group programmes for people with cancer was the 'I Can Cope' (ICC) programme, which consists of six 90-minute structured

educational sessions conducted over 6 weeks (417). Topics include: learning about the disease; coping with daily health problems; communicating with others; liking oneself; living with limits; and identifying helpful resources. The results of evaluation studies (418) showed that the programme had a significant impact on participants' level of anxiety, disease-related knowledge and sense of meaning in their lives, with participants reporting high satisfaction with the structure and content of the programme. Another programme was developed to help cancer patients cope with the illness was the LWCEP (Live with Cancer Education Program) including core features of information provision, support and facilitation of coping, ongoing monitoring and evaluation of treatment provided.

Adapting similar programs would benefit cancer patients to recognise the risk of thrombosis, and how to live with it.

Hunter 2016, in a study about the post-thrombotic panic syndrome, reported that life after VTE marked a new chapter of the participant's life. Defined by efforts to cope with the physical impact, health anxieties, which integrate their experience along with new restrictions, fears, and uncertainties (419). For many, this led to high levels of stress in this early phase and affected their lives significantly. Participants respond in two different ways, some engaged in avoidance behaviours, withdrawing and isolating, where others found it hard to reflect on how they were coping, reflecting different coping styles.

Such experiences require further exploration and understanding if we are to promote coping and positive adjustment following traumatic-onset conditions such as venous thromboembolism.

The data reported in this study (Hunter,2016) also clearly suggest that people who have experienced a venous thromboembolism would benefit from some form of psychosocial intervention, perhaps utilizing a triage approach ranging from psycho-education to post-traumatic counselling, identifying and supporting individuals at risk of post-traumatic stress or significant psychological distress could enable early intervention. The developments of targeted interventions to enhance psychological well-being, promote adjustment and growth and reduce distress. Providing such

intervention to cancer patients could, in turn, improve patient's wider emotional and physical health outcomes (345).

4.10.5 Uncertainty and information

Uncertainty has been recognised as an integral element of health and illness (420-422). As Babrow (420) (423) demonstrate that uncertainty occurs when situations are shaped with ambiguity, complexity, unpredictable, or probabilistic; and more when information is not available or inconsistent; and when people feel insecure in their own state of knowledge or the general state of knowledge.

The varying responses to the threat of cancer-associated thrombosis and its treatment seem to be related to uncertainty as to whether cancer-associated thrombosis will recur, whether it will resolve, whether the treatment will be effective and/or harmful.

Uncertainty management theory (UMT) (422, 424) is one theoretical framework to help the understanding of how patients encounter, appraise levels of danger, seek information, respond to and cope with health-related threats. Uncertainty management theory has been established on the hypothesis that individuals for its meaning appraise uncertainty, such appraisals may lead to information seeking efforts aimed at change or maintain one's uncertainty (425).

One strategy of uncertainty management theory is information seeking that been frequently documented in previous researches as an important component of coping with illness and illness-related uncertainty (425, 426).

Given the prevalence and importance of information seeking in Uncertainty management theory, it is essential to consider how individuals search for information to manage uncertainty (427). The differing needs for information, ways of seeking it and success in receiving it was seen within these data presented. Likewise, some patients appraised cancer-associated thrombosis as very dangerous, whereas others (often those with previous experience and better information) were able to appraise it as less dangerous because they knew what to look for and how to act.

Although, the relationship between uncertainty and danger appraisal is complex, tailored and accessible information seems to play a key part in reducing anxiety even if absolute reassurances cannot be given(427, 428). As *Brashers* states, uncertainty

occurs when “information is unavailable or inconsistent, and when people feel insecure in their own state of knowledge or the state of knowledge in general” (422). Recognizing the kinds of information and information-seeking behaviour can offer a more nuanced comprehension of uncertainty management processes (425).

For health care practitioners, such experience could be important in creating systems for helping patients to deal with their health-related uncertainty more viable. A better understanding of the patients’ experience and the impact of cancer-associated thrombosis and its therapy on the patients’ experience may enhance supportive care for cancer patients and influence the decisions about the effectiveness of the joint patient-clinician decision making about anticoagulation.

4.11 Strengths and limitations

As with any systematic review, it is possible to miss relevant studies. The included studies were qualitative research which is designed to give insights from the patients involved rather than to be generalizable. However, through synthesis more generalizable findings can be derived(271). Only one included study came from outside the UK, however, they were from different centres, but still indicated similar concerns.

Only limited papers were found, illustrating that this area has been under-researched. The serious concerns highlighted by this review show that further work is needed.

4.12 Implications for clinical practice and policy makers

Raised clinical awareness and the provision of basic information for patients about the risk of cancer-associated thrombosis is a policy priority in the UK (353). Information about cancer-associated thrombosis, both written and verbal, should be provided routinely for patients at diagnosis.

Cancer-associated thrombosis should be part of standard training and education for all clinicians caring for people with cancer, including those in primary and palliative care. Recent initiatives such as the International Initiative on Thrombosis and Cancer (429) should help raise awareness and help with high-quality training. Streamlined clinical services for diagnosis and treatment of cancer-associated thrombosis aiming to minimise the time in hospital awaiting tests, especially for those with advanced disease, should improve clinical decision making.(358, 398, 430)

4.13 Conclusion

The cancer journey is difficult enough. However, setbacks from complications of cancer or its treatments make a day-to-day living even harder. Thrombosis in cancer, one such complication with multiple causes affects up to 20% of patients during their cancer journey and is one of the common causes of cancer death. Associated with significant impact on patient symptoms, may delay or prevent the delivery of cancer treatments and decrease quality of life and span.

Incorporating patients' experience into standard clinical practise holds great promise for improving communication with health care providers, with a resultant improvement in patient outcomes.

This systematic review highlights the impact of cancer-associated thrombosis on the lives of cancer patients and calls for education for patients and clinicians to be part of routine care, and further work to address this patient priority equal to that of other cancer complications such as spinal cord compression or neutropenic sepsis. Finally, educational initiatives to increase awareness among patients and health providers would be of great value. Data suggests a need to implement patient and clinician education programs on cancer-associated thrombosis in an effort to explain risk factors and to teach venous thromboembolism management and coping strategies to cancer patients and their carers. Raising the emphasis of cancer-associated thrombosis to equal that of other cancer complications such as spinal cord compression or neutropenic sepsis in needed.

Chapter 5 Symptom burden and quality of life of cancer patients diagnosed with incidental pulmonary embolism treated with low molecular weight heparin: findings from a prospective case-control cohort study

5.1 Introduction

Since March 2010, in the oncology department at the [Queen's Centre for Oncology and Haematology, Castle Hill Hospital, Cottingham, UK, Hull and East Yorkshire Hospitals NHS Trust] all cancer patients diagnosed with i-PE are managed under a specifically developed nurse-led service (431). The pathway includes; 1) diagnosis of i-PE at the radiography department, 2) referral to the nurse specialist team, 3) clinical assessment for bleeding risk by nurse (or doctor if needed) using Pulmonary Embolism Severity Index (BESI) score and Performance status (PS), and order the blood tests needed, 4) anticoagulation started as an outpatients for those at lower risk of bleeding, or during admission for those at higher risk, 5) follow up investigations at one week to monitor for anticoagulation side effects including heparin-induced thrombocytopenia (HIT) syndrome (heparin-induced thrombocytopenia), 6) phone-call follow up at one and three months. This i-PE pathway used in Hull and East Yorkshire Hospitals NHS Trust (HEYHT) has been implemented since 2010. The clinic receives around 50-80 i-PE cancer patients per year with around 80% of them treated as outpatients.

The first 234 patients managed under this service between March 2010 and December 2014 were assessed for symptoms. Most were found to have pulmonary related symptoms, such as dyspnoea, and fatigue (47). The presence of new or worsening of symptoms was found to be an independent risk factor for mortality and the Hull score was developed (new/worsening symptoms, performance status, presence of metastatic cancer) (47). In March 2013, the Hull score was introduced to the clinic to inform the patient's management decisions. However, the patient-reported symptomatology at baseline was recorded through a simple dichotomous questionnaire (14, 432). The incorporated risk-assessment algorithm, which guides

hospital admission decisions, is described in a previous publication (431) and was based on a modified PESI score (433, 434).

To capture and analyse the type of symptoms (at baseline only), the patient-reported outcome questionnaires were added to the clinic assessment from December 2016, including SF-12 and ESAS in addition to Hull Score at the same time.

This chapter presents data of preliminary results of a prospective case-control cohort study investigating the impact of i-PE on the clinical outcomes of cancer population compared to matched controls with no thrombosis.

5.2 Hypothesis and aims

Patients with cancer diagnosed with i-PE do have PE related symptoms. Symptoms associated with the diagnosis of i-PE negatively affect cancer patients' quality of life and their clinical outcomes comparing to those who have PE.

Aim

- To compare and evaluate symptom burden and quality of life between cancer patients with i-PE and matched controls with no thrombosis over time
- To determine the survival rate compared to controls and its relation to symptom burden
- To determine the secondary endpoints (clinically significant events), such as the re(o)currence of PE/VTE; cancer progression or recurrence; the number of days in hospital; haemorrhage; and anticoagulant-related QoL; of the i-PE cancer patients compared to cancer patients without VTE. And investigate the related demographic factors

5.3 Methods

5.3.1 Survey study summary design

This is a prospective case-controlled cohort study. The methodological approach is described in more detail in the methodology Chapter Three. The study protocol is presented in Appendix G.

5.3.2 Ethical approvals

The study was conducted in accordance with the International Conference for Harmonisation of Good Clinical Practice (ICH GCP) (435) guidelines. The protocol, procedures, patient information sheets, consent forms and questionnaires were approved by the East Midlands - Nottingham Research Ethics Committee HRA (REC ref: 16/EM/0474) (Appendix F)Appendix F CLOTS-QoL study ethical approval document. (IRAS project ID: 216188) approved 21/12/2016, including for the method of consent and level of participant burden. Patients' data were stored and handled in accordance with data protection law to preserve anonymity and confidentiality. The researcher (NB) had an honorary contract with the HEYHT prior to the start of the study allowing the researcher to contact the participants and invite them to participate in the study. Ethical approval is presented in Appendix F. Institutional permission were granted by the R&D Department, Hull and East Yorkshire Hospitals NHS Trust (R&D)

5.3.3 Sample size calculation

The calculation of the sample size required was overseen by a statistician (VA) at HYMS. For the SF-12, to detect a minimal clinically important difference (MCID) of 5 between the i-PE group and the control group at 90 days, for PCS (Physical Component Summary) we require 55 i-PE patients and 110 matched controls (in total 165 participants) and for MCS (Mental Component Summary) we require 42 i-PE patient and84 matched controls (in total 126 participants) using scores from data in an Australian study

Sample calculation is based on the survival difference seen in a small retrospective study by Dentali and colleagues (436); based on a log-rank test with a 5% significance level, this will have 80% power to detect the difference between an expected survival rate of 45% in the i-PE group and one of 30% in the control group at 6 months when the sample sizes are 77 and 154, respectively (a total sample size of 231) (calculated using State/SE 14.0). Hence to maximise the power the total sample size will be 231.

5.3.4 Participants:

5.3.4.1 i-PE patients

All participants were recruited from a single regional oncology tertiary centre. The aim was to recruit a maximum of 77 patients with incidentally diagnosed PE (Study Arm) according to inclusion criteria.

5.3.4.1.1 i-PE patients inclusion criteria

1) Adults ≥ 18 years

2) Active cancer

3) Have i-PE

4) Able to provide written informed consent

5) Able to complete study assessments

5.3.4.1.2 Exclusion criteria

1) No current cancer

2) Known other current thrombosis (either receiving treatment or within the last 12 months)

3) Inability to provide informed consent or complete study assessments

5.3.4.2 Participants: control patients

Two matched controls identified through the HEY cancer multidisciplinary team (MDT) database for each cancer patient with i-PE recruited. Participants were matched according to their: age (± 5 years), gender, performance status, and cancer type/stage.

5.3.4.3 Inclusion criteria for the control group

1) Adults ≥ 18 years

2) Active cancer

3) Able to provide written informed consent

4) Able to complete study assessments

5.3.4.4 Exclusion criteria for the control group

1) No current cancer

2) Known other current thrombosis (either receiving treatment or within the last 12 months)

3) Inability to provide informed consent or complete study assessments.

5.3.5 Methods of recruitment

5.3.5.1 Recruitment method for i-PE participants

A patient information sheet (PIS) (Appendix I) was given to all potential participants when they attended their first clinic appointment by a member of the research team. Interested participants were contacted by the researcher (NB) to offer the opportunity to discuss the study and answer any questions. The researcher (NB) had a GCP training and an honorary contract with the HEYHT in place prior to the start of recruitment. Those willing to participate were consented by NB in accordance with GCP guidance. As the SF-12 health survey and ESAS questionnaire are given to patients as part of routine clinical i-PE first clinic attendance, this group signed a consent to provide data during follow up, and for use of the data already documented in the clinical record (baseline measures).

5.3.5.2 Recruitment method for controls

For every patient with i-PE two matched control were identified through the multidisciplinary team (MDT) database. Identified patients were invited to participate at their next regular oncology outpatient's appointment by a member of their clinical team. A patient information sheet was offered (Appendix J), followed by a similar recruitment method to the i-PE group thereafter to those expressing interest. In this group, participants provided both baseline and follow up data following consent.

5.3.5.3 Method of follow up

For i-PE patients, the first clinical assessment data was used as the baseline data. Follow up was conducted at one week, and at one and three months. At each visit, participants were asked to complete the symptoms and quality of life questionnaires; the SF-12 (Appendix K), ESAS (Appendix L) and Anti-Clot Treatment Scale (ACTS)

(Appendix M). Control participants were asked to complete the SF-12 and ESAS questionnaires only. All participants were offered the option of completing the questionnaires in the clinic with the researcher during their regular visits or at home and returned by post using a free pre-stamped envelope.

5.3.5.4 Presenting the questionnaires

Patients were offered help with filling in the questionnaires, and a choice of completing the questionnaires at the clinic or taking them home and posting the completed questionnaires back to NB in a pre-stamped envelope.

5.3.5.5 Statistical analysis plan for the full study

According to the study protocol, data would be analysed by the study or control group at baseline, even if control patients subsequently developed i-PE and then attended the i-PE clinic. In addition to descriptive data presentation of participant characteristics the following analyses are planned:

1) ANCOVA will be used to compare the two groups with regard to symptom burden and QoL at +90 days, adjusted for baseline QoL. A further generalised linear model allows adjustment for confounding variables, including baseline QoL, age, gender, disease stage, comorbidities and PS.

2) Log-rank test will be used to compare overall survival between the cancer patients with i-PE and cancer patients without i-PE. Survival at 6 months will also be reported as a surrogate VTE-related outcome. And to use Cox regression to measure the effects of other variables (confounders). And to draw the Kaplan-Meier curve.

3) Log-rank tests, chi-square tests and t-tests will be used as appropriate to compare the secondary outcomes (clinically significant events), such as the recurrence of PE/VTE; cancer progression or recurrence; the number of days in hospital; haemorrhage; and anticoagulant-related QoL between the cancer patients with i-PE and cancer patients without thrombosis.

4) Multilevel models to investigate the demographic factors that can be used to predict QoL, symptoms, hospitalization, and survival. Summary statistics are presented as mean (standard deviation, SD) or n (%).

5.3.6 Data analysis used in this exploratory data presentation

Due to the difficulties in recruiting enough patients and time constraints to generate enough data from this prospective study, a descriptive data analysis only applied. Descriptive statistics are presented as mean (standard deviation (SD)) and median (Inter-quartile range (IQR)) or n (%) for all sociodemographic, clinical and symptom data. The ACTS questionnaire was scored in accordance with the developers' guidelines (291). The 12 items of ACTS Burdens were reverse coded (scored 5 to 1), whereas the three items of ACTS Benefits were coded normally (scored 1 to 5) so that higher scores indicate greater patient satisfaction. Item scores are summed across domains to give an ACTS Burdens score ranging from 12 to 60 and an ACTS Benefits score ranging from 3 to 15.

5.4 Results

5.4.1 i-PE patients descriptions and demographic characteristics

During the study period from Feb 2017 to Oct 2017, twenty-two eligible patients were contacted and invited to participate in the study. Thirteen patients agreed to participate (59%) of whom four (30%) were women. Mean age was 57 y, range 32 to 82 years. One patient died during the first week of the study, but otherwise, 12 completed follow up, data presented in Figure 5.1. Participants were, 7 gastrointestinal (53.8 %), 3 lung and mesothelioma (23.07 %), 3 genitourinary (23.07 %), twelve were advanced stage and one participant with stage 2B. Performance stat was; PS 0 for six participants, PS 1 for three participants, and two participants with PS 2. Median Hull score was 2.

5.4.2 Matched control patients' descriptions and demographic characteristics

Database of 1677 oncology patients was screened for potential matched controls. Screening process presented in Figure 5.1 It was obvious that due to the nature of illness under research most of the eligible patients were deceased at the time of recruitments. Performance status was another barrier for matching. Six participants met the matching criteria for three urology i-PE patients were invited to participate in the study by their key worker. However, only two patients returned the QoL

questionnaires. Those patients were between 35- 45 years old, males with stage IV testicular cancer.

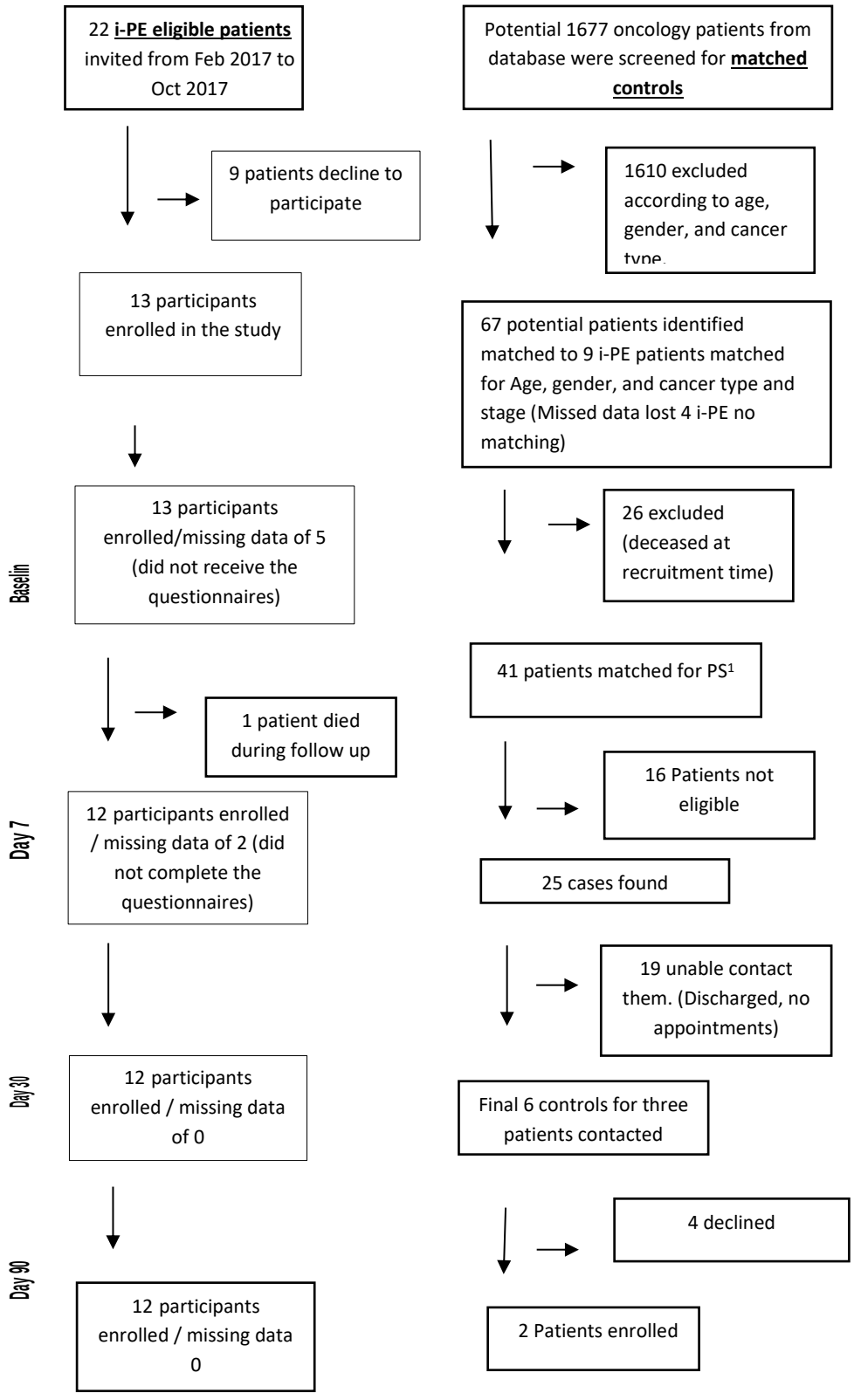


Figure 5.1 i-PE patients and matched control recruitment flow diagram

[1] Performance Status

5.4.3 iPE- Participants' reported outcome measures

Table 5.1 provides summary measures at baseline and follow up for i-PE participants.

SF-12 Questionnaire:

The baseline mean of the physical component score of (SF-12) was (43.6 ± 11), which decreased by day 7 and 30 to (35.4 ± 10 / 35.7 ± 9.3) respectively, then slightly improved by day 90 to reach (37.6 ± 9.2).

The baseline mean of the mental component score of (SF-12) was (55.3 ± 6), that declined throughout the follow-up period at (50.8 ± 9, 48.9 ± 9, and 47.4 ± 9.8) at day 7, 30 and 90 respectively. As shown in figure 5.2.

ESAS Questionnaire:

The ESAS symptom total score varied during the study period. The mean score at baseline was (24 ± 16.2) that declined by day 7 to reach (19.8 ± 13.7), then improve by day 30 the declined again by day 90 (25.4 ± 17.3, 19.2 ± 13.6) respectively. The mean score of symptoms recorded during the follow up are presented in table 5.2.

ESAS symptom score measures varied all symptoms improved over the first 7 days but although tiredness (baseline at 5.57 ± 3.1then dropped to 4.4 ± 3.2) and drowsiness (baseline at 4.7 ± 3.4 that decreased to 3.25 ± 3.2) continued to improve till day 30, pain and breathlessness started to increase again. By day 90, tiredness, drowsiness and breathlessness were increasing (5 ± 3.1, 3.5 ± 3.3, 4 ± 3.5 respectively) although had not reached baseline levels. Pain, on the other hand, increased steadily from day 7 exceeding baseline levels by day 30 and continued to rise (1.14 ± 1.9 – 3.6 ± 3.1) (Figure 5.3).

ACTS Questionnaire:

Patients with i-PE received Low molecular weight heparin reported a better satisfaction at day 7 and continued to day 90 as recorded by ACTS questionnaire. The mean ACTS burden/ ACTS Benefit at day 7 was (43.91 ± 9.45/ 9.5 ± 5.5) respectively, which improved by day 9 to reach (47.63 ± 11.43/ 12 ± 2.1) table 5.2 and figure 5.4.

5.4.4 Control patients reported outcome measures summary:

Tow matched controls, stage VI testicular cancer patients matched to one i-PE patient completed the QoL questionnaires.

The SF -12 scores for both participants were above the average for both physical and mental score at baseline and continued during the follow-up period. Baseline score for PC and MC score for the Participants 1 PC were (56.57/ 55.25) and for participant 2 (56.77/ 55.84). At day 90 the scores were (54.83/ 59.77 for participant 1 and 55.85/ 55.85 for participant 2).

The ESAS questionnaire for the control patients hardly reported any symptoms apart from patient 1 who reported pain during the follow-up period at score 1 of 10.

Table 5.1 i-PE Patients' baseline and follow up reported outcome measures

	Baseline (i-PE=13)	Day 7 (i-PE=12)	Day 30 (i-PE= 12)	Day 90 (i-PE=12)
(PCS[2]-12) mean ± SD/ range/ median/ IQR	43.6 ± 11/ (24.8-56.5)/ 45.58/26.99	35.4 ±10/ (21.1-53.1)/ 34.7/ 20.0	35.7 ± 9.3/ (24.1-55.5)/ 33.49/ 16.58	37.6 ± 9.2 (23.4-47.2)/ 37.37/ 7.37
(MCS[3]-12) mean ± SD/ range/ median/ IQR	55.3 ± 6 (39.6-61.5)/ 57.58/7.79	50.8± 9 (37.1-63.6)/ 49.7/20.6	48.9 ± 9.7 (34.5-64.3)/ 44.96/18.85	47.4 ± 9.8 (36.1-59.7)/ 46.7/ 20.7
ESAS[4]	24 ± 16.2 (0-43)	19.8 ± 13.7 (0-44)	25.4 ±17.3 (0-50)	19.2 ± 13.6 (3-51)
Median PS[5]	1			
ACTS[6] Burden scale	NA[7]	43.91 ± 9.45 (30-55)	43.67± 9.36 (30-55)	47.63 ± 11.43 (30-60)
ACTS Benefit scale	NA[7]	9.5 ± 5.5 (3-15)	12.7 ± 2.6 (6-15)	12 ± 2.1 (9-15)
Missing data	5	3	0	0

[2] Physical Component Score.
[3] Mental Component Score.
[4] Edmonton Symptom Assessment Scale.
[5] Performance Status
[6] Anti- Clot treatment Scale.
[7] Not Applicable

Table 5.2.ESAS symptom scores (Means ± SD)

Symptom	Baseline	Day 7	Day 30	Day 90
Pain score (mean ± SD)	1.1 ± 2.1/ 0/ 1	0.9 ± 1.7/ 0 / 1	1.7 ± 2.9/ 0.5 / 2	3.2 ± 1.9/ 0 / 3
Tiredness score (mean ± SD)	5.5 ± 3.1/ 7/ 5	4.9 ± 3.3/ 5.5 / 8	4.4 ± 3.3/ 4.5 / 8	5 ± 3 /5.5/ 5
Drowsiness score (mean ± SD)	4.7 ± 3.4 5.5 / 7	4.2 ± 3.3/ 4/ 6	3.3 ± 3.3/ 3.5 / 7	3.5 ± 2.9/ 3.5 / 6
Nausea score (mean ± SD)	0.71 ±1.25/ 0 / 3	1.1 ± 1.5/ 0 / 2	0.9 ± 1.6 / 0 / 3	0.9 ± 1.2 / 0 / 3
Lack of appetite score (mean ± SD)	1.57 ± 2.29 / 0 / 5	2.1 ± 2.3/ 0/ 2	1.1 ± 1.6 / 0/ 2	0.58 ± 0.6 / 0 / 1
Depression score (mean)	1.28 ± 1.7/ 0 / 3	0.9 ± 1.5 / 0 / 2	2 ± 2.7 0.5 / 5	1.3 ±1.3/ 0.5 / 3
Anxiety score (mean ± SD)	2 ± 2 / 3 / 3	2.2 ± 2 / 2 / 4	2.4 ± 2.4 2.5 / 5	1.6 ± 1.3 / 1 / 3
Breathlessness score (mean ± SD)	4.7 ±3.7 / 5 / 7	4 ± 3.1 / 3 / 6	4.8 ± 3.7 / 5.5 / 8	4 ± 3.1 / 4.5 / 8
Wellbeing score (mean ± SD)	4 ± 2.4 / 5/ 4	2.3 ± 2.4	3.4 ± 2.6	2.8 ± 2.2
Other problems	0	0	0	0

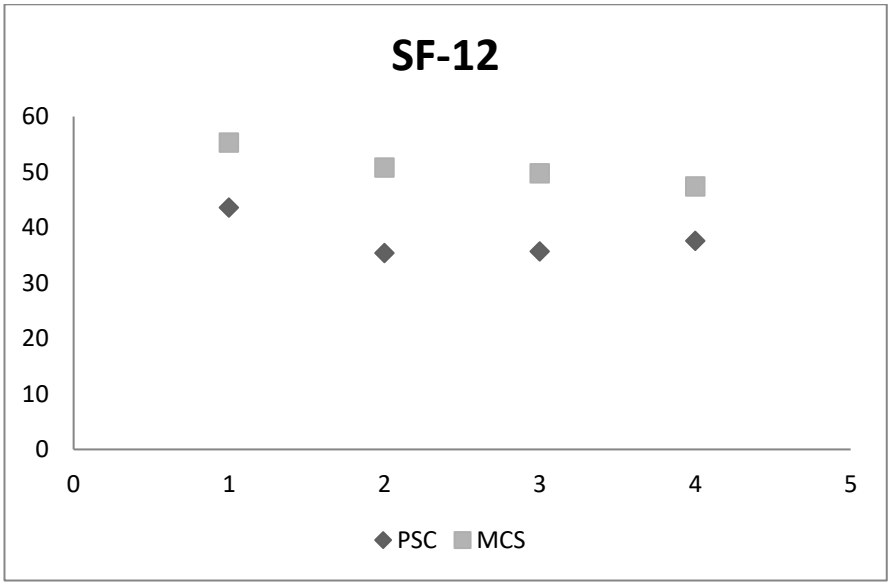


Figure 5.2 i-PE patients Physical and mental scale summary of SF-12, baseline, day 7, 30, and 90.

The data show steady decrease in the MCS measures over the study time, while the PSC was decreased during the first week then gradually improved over the study time.

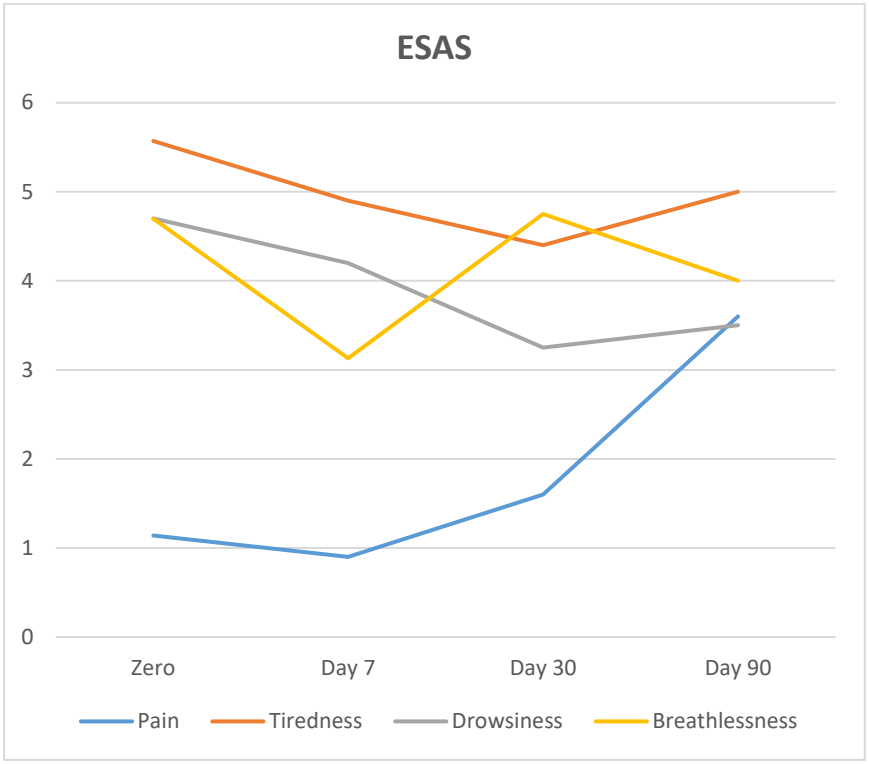


Figure 5.3 Common symptoms reported by i-PE patients over 90 days.

Pain was slightly decreased during the first week then continue to increase throughout the study time, breathlessness improved during the first week after treatment then increased during the first month then slightly improved at day 90. Tiredness and drowsiness data show steady improvement during the first month flowed by gradually increase at day 90.

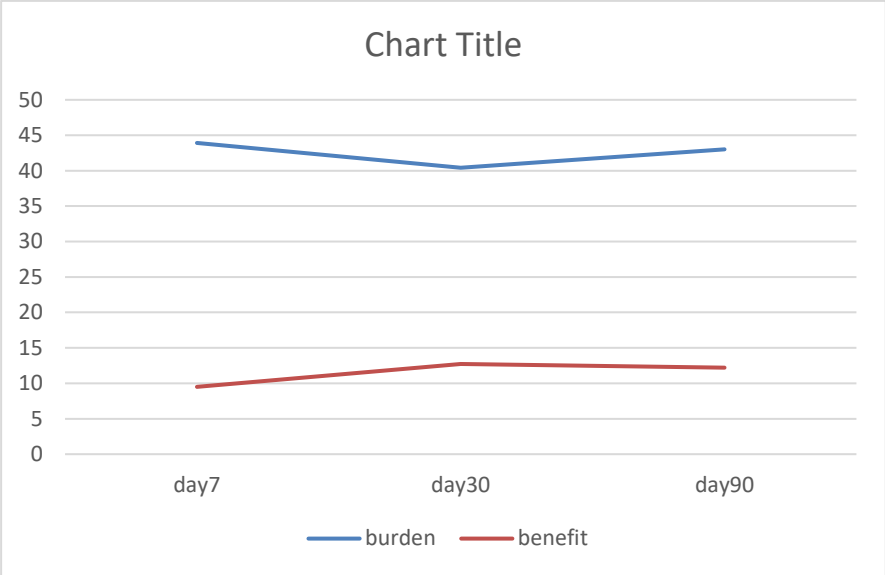


Figure 5.4 Summary of the ACTS Burden/ Benefit mean scale of i-PE patients at day 7, 30, and 90.

5.5 Discussion

This chapter presents preliminary data from a small prospective case-control survey study that examined symptom burden and health-related quality of life in cancer patients diagnosed with i-PE compared to matched controls with no thrombosis. Although the sample size is small and only one i-PE participant had matched controls, these initial data indicates lower quality of life and symptom burden among people with cancer and i-PE.

Patients with cancer and i-PE treated with low molecular weight heparin injections reported a lower quality of life compared to the general population. The physical summary component of PCSF-12 was lower than the norm at baseline at 43.6 and progressively decreased over the study period of three months to 37.6 at day 90. The mental component summary started at a higher score than the norm at 55.3 but the score declined over the time of study period to 47.3. These findings, however, were in contrast to a recent study the QUAVITEC study included symptomatic VTE (24.7%), reported an improvement in health-related QoL over 6 months among cancer patients treated with LMWH for VTE (437). These discrepancies may indicate that the presence of pulmonary embolism is responsible in some way for the lower QoL in these patients as indicated by the results of ESAS total score.

The ESAS total score data of this study shows that cancer patients who incidentally diagnosed with i-PE actually had a PE related symptom at baseline. Symptoms including shortness of breath, tiredness and drowsiness (means of 4.7, 4.7 and 5.5 respectively) reported being higher than others. These results are in consistent with the study by Abdel-Razeq 2011(84), who reported patients with cancer and i-PE did have symptoms or signs that could have been attributed to PE. Symptoms including respiratory symptoms in the form of shortness of breath, chest pain, and haemoptysis were identified in ≥ 53% of the patients. Respiratory symptoms and fatigue have been independently associated with shorter survival in patients with cancer (68), this confirms the notion that incidental pulmonary embolism does not mean asymptomatic.

Throughout the study, participants reported better ACTS treatment satisfaction scores across visits these results may reflect patients' adaptation to and acceptance of anticoagulation therapy.

It is well known that cancer-associated thrombosis is more common with advanced cancer and it is associated with poor prognosis. Therefore, appropriate management of cancer-associated thrombosis in patients with cancer is essential to improved outcomes, including both morbidity and mortality, and could have a significant impact on health care resources. In this study, all patients managed uniformly under the care of i-PE clinic. All patients started on low molecular weight heparin and continued for the rest of the study time except one who transferred to DOACs due to drug interaction. LMWH is recommended than warfarin in many studies given the fact that it is easy to administer and does not need frequent investigation which give patients more freedom and control over their life. Although, the study population was too small ACTS benefit show steady improving over study time that may refer to patients' satisfaction.

Furthermore, although the number of control patients enrolled was too small but on a closer look at the questionnaires, they reported a higher level of QoL even than the norm in both the physical and mental components. They were less symptomatic than i-PE, this may highlight the effect of PE on patients reported symptoms and their QoL in general.

This could indicate that the presence of pulmonary clot and associated symptoms may be responsible in some way for the lower QoL in these patients which may present valuable data for identifying risk factors and predicting long term clinical outcome in this group of patients.

Research has consistently shown that cancer patients frequently reported physiological, psychological or social problems including; pain, tiredness, stress, anxiety, depression, social isolation, role and function loss; and, eventually, a worsened quality of life (388). All that makes the differential diagnosis of these symptoms difficult. However, the included control patients were less symptomatic than i-PE participants, which may indicate the relation between the presence of pulmonary embolism and worsening symptoms as previously stated (3, 61, 86). It is not

uncommon that clinicians and patients themselves frequently attribute relevant symptoms to the progression of underlying cancer or the adverse effect of cancer treatments, and often is unclear indeed whether these symptoms might be attributable to the PE, especially cancer patients (37).

Recent work seems to support the notion that cancer patients with i-PE have similar outcomes to symptomatic (acute) PE cases, their survival appearing worse compared with matched controls without PE (4, 9, 82). Moreover, the presence of symptoms in cancer patients with i-PE has been correlated with poorer outcome (14). However. In this preliminary data, one patient died during 30 days follow up representing 7.7% of the study population.

Although QoL is recognized as an important indicator of the course of a disease, the available data evaluating the outcomes of patients with cancer and i-PE are scares and usually from retrospective designs. This highlighting a shortage in research and a gap in knowledge about QoL in this population. I acknowledge that the findings represent a preliminary data of an ongoing study, however, the findings of this study could not be compared to the same study type and it is not generalizable in the meantime.

It is worth mentioning that similar to what has been reported on symptomatic VTE in cancer patients, most of the i-PE patients were diagnosed during receiving active cancer treatment (chemotherapy or radiotherapy and during their initial staging workup or to evaluate response to active treatment. Additionally, given the relatively small number of patients recruited, it would be difficult in the meantime to state that incidental PE is more common in certain gender or tumour types or to suggest or recommend routine evaluation or even closer follow-up.

Finally, Cancer and its treatment have a substantial impact on mental and social health and in conclusion, on quality of life of patients, and the development of cancer-associated thrombosis has been reported to be associated with more physiological and psychological. The clinical spectrum of PE ranges from potentially fatal events to incidental findings on computed tomography (CT) scans. Pulmonary embolism entails specific issues in patients with cancer, such as a greater risk of recurrent thrombosis or major bleeding, and therefore calls for a specific classification. Prognostic multivariate models have been created, such as the RIETE registry scale and POMPE-C score, that predict 30-day mortality probability following PE, although, at best, they are marginally

superior to other classifications developed for PE in the general population (e.g., PESI or sPESI) (438) and their suitability for incidental PE has yet to be proven to assist in decision-making (119, 439). Furthermore, they are not sensitive to competitive risks, such as increased bleeding, responsible for some 10% of early mortality, or cancer progression, which accounts for 50% of 30-day mortality after a pulmonary embolism event (439).

Within this context, and in the absence of adequate prognostic stratification method for i- PE a clinical prognostic scores Hull Score utilising PS, the presence of new or worsening symptoms and the presence of metastatic incurable disease has been introduced for assisting clinical decision-making at the i-PE clinic at the (Queen's Centre for Oncology and Haematology, Castle Hill Hospital, Cottingham, UK, HEY Hospitals NHS Trust) (47). However, lack of follow-up of symptomatology which could provide a prognostic assessment of patient-reported outcomes (PRO) represented one of the limitations that this study aimed to overcome.

5.6 Strengths and Limitations

As with any observational prospective cohort study this study has strengths and limitations. The main strength of this study is the prospective approach used to collect the data. This approach enabled me to collect data that are not routinely recorded which would minimise the recall bias. Using self-administered PRO measures means that I as a researcher had no influence on the recorded data. All patients were recruited from one centre and were uniformly managed under a nurse-led service.

This study has some limitations that must be considered when interpreting the results. As in most cohort studies, a large number of participants were needed to recruit and follow for a substantial time, which was not feasible for me due to time constraints, therefore the data are presented as preliminary results. Recruiting control patients represents another limitation. It has proved to be extremely difficult. Due to the nature of the illness most of the patients who fulfilled the inclusion criteria were either deceased or difficult to reach. Whilst pertinent risk factors would have been recorded, I cannot confirm that all confounding factors have been eliminated.

It is well known that recruiting the target number of research participants in clinical trials is difficult (440). Many research projects fail to recruit a sufficient number of

participants (441). These challenges could be summarised in six categories, including trial design, obtaining consent, approach to participants, financial incentives, training for recruiters and trial coordination (442).

In this study, the patients with i-PE were prospectively recruited from the Hull i-PE database, and when the study was designed it was expected that the matching control patients would be found from the contemporaneous or historical group of patients that come through the Trust and are collected for management purposes on the relevant MDT database. However, trying to match patients for performance status at diagnosis of I-PE within a case-mix that included very many patients with advanced malignancies (e.g liver, pancreas lung cancer etc.) to achieve the sample size for the study was not feasible. These 'contemporaneous' patients with the relevant characteristics had either become unwell or had a substantial change of the performance status or had died from when the MDT entry was made.

A feasibility study would have allowed the demonstration of a feasible endpoint and a stronger rationale for moving forward with a larger trial. It became apparent that the only patients we could recruit would-be patients with prostate cancer or breast cancer but the number of these patients in the data-base were very low pointing to the futility of this approach and leading to early termination.

5.7 Recommendation

Since the methods and design used in this study were relevant, a future multi-site prospective study would be able to recruit the sample required (patients and control) to present enough data. Future work would also recommend recruiting controls in a prospective manner to ensure that patients are available and not deceased.

5.8 Conclusion

These results represent primarily data investigating the symptom burden and the effects of i-PE on the cancer-patients outcome using PRO measures. The results show that patients with cancer who incidentally diagnosed with PE reported lower QoL, had PE related symptoms and the benefits of anticoagulation (in this study is self-injection LMWH) outweigh its burden.

The next chapter investigates the cancer patients' experience of living with i-PE treated with LMWH a using semi-structured interview with the same i-PE patients included in this chapter.

Chapter 6 Cancer patients' experiences of the diagnosis and treatments of i-PE interview study

6.1 Introduction

In Chapter Four, the systematic review demonstrated how the diagnosis of cancer-associated thrombosis often caused distress and alarm for patients, especially for those who did not know about the risk, or the signs and symptoms to look out for. The educational needs for those patients as well as their clinicians were highlighted, and we called for further work to address this issue with a priority equal to that of other cancer complications such as spinal cord compression or neutropenic sepsis. However, only a few patients diagnosed with i-PE in the review were included, and thus a greater insight about how the incidental nature of the diagnosis experienced was not possible.

Therefore, I conducted a qualitative study using semi-structured interviews to explore cancer patients' experience of the diagnosis of, and life with, i-PE.

This chapter presents the methods used, the findings and the discussion of the findings of semi-structured interviews of patients with cancer diagnosed with i-PE.

6.2 Research Question

What is the experience of people living with cancer and incidental pulmonary embolism in regard to their response to the diagnosis, coping with the additional burden and the effects of long-term anticoagulation on their daily life?

The overall aim of this study is to understand the patient's views and experience of the diagnosis of i-PE in the context of cancer.

The objectives are to gain knowledge of, and to understand, the impact of i-PE on the lives of people with cancer.

6.3 Ethical approval

Ethical approval was granted by the East Midlands - Nottingham Research Ethics Committee and HRA (REC ref: 16/EM/0474) (IRAS project ID: 216188) approved 21/12/2016, including for the method of consent and level of participant burden. Patients' data were stored and handled in accordance with data protection law to preserve anonymity and confidentiality. The researcher (NB) had an honorary contract

with the HEYHT prior to the start of the study allowing the researcher to contact the participants and invite them to participate in the study.

6.4 Study methods

Summary design

This is a qualitative study using semi-structured interviews, nested as part of an observational questionnaire study (See Chapter five). The methodological approach is described in details in the methodology Chapter Three.

6.5 Setting, recruitment, eligibility, and consent

Eligible interview participants were those who had participated in the observational questionnaire study. The details of setting, recruitment, eligibility and consent for the observational study are given in Chapter five.

6.5.1 Sampling approach for the interviews

This was a convenience sample of all patient participants completing the observational questionnaire study; all gave consent to interview as part of the consent process for the questionnaire study were interviewed.

6.5.2 Consent

The consent was obtained alongside the consent for the questionnaire study (Chapter five). Written consent included permission to audio- record the interview and to use anonymised quotes in presentation of findings including publication. On the day of the interview the researcher (NB) again explained the study process to the participants and informed them about their choice to withdraw from participation in the study at any time without reprisal to confirm consent prior to data collection.

6.5.3 Data collection

I interviewed consented participants within one month of the survey study's baseline. Participants were offered the choice of the interview site either at their home or at the Queen's Centre to coincide with their next clinic visit. A topic guide (Appendix N) was developed from the literature and expertise of the research team to ensure that the same issues were explored with all participants whilst allowing individuals to raise unanticipated issues. The guided questions were about their experience of i) life

before the diagnosis of i-PE, ii) being diagnosed with i-PE, iii) anticoagulation in the context of incidental diagnosis, iv) the effect of the i-PE on their life and their cancer journey, v) everyday life living with cancer and i-PE. Interviews lasted for 15-30 minutes, were audio-recorded and verbatim transcribed. The interviews were deliberately focussed and short to minimise the patients' burden. However, those who wished to talk for longer were able to and breaks were offered as required.

Data management

To ensure confidentiality, participants' details were coded and stored in a master file accessed only by the researcher (NB), the anonymised recordings were stored on an encrypted hard drive at the Hull University and a password protected computer. Only authorised researcher (NB) could access identifiable data. Once the anonymised transcription had been completed the audio recorders were deleted from the recorder.

6.6 Analysis plan

Thematic analysis as described by Braun and Clarke (253) was chosen to analyse the study results (for justification of this approach see (Chapter Three). The analysis involved five phases as shown in Table **6.1**. First, I familiarised myself with the data by listening to the recordings and read and re-read all the interview transcripts. Then NB and MJ independently conducted a line by line coding of two transcripts. Following a discussion between NB and MJ, a coding framework was formed and used by NB to code all transcriptions, being alert to new codes. Codes were then compared and with a discussion between NB and MJ generated an initial thematic map. Final defined themes were then formed to "tell the story" of the participants' experience.

Table 6.1 Phases of thematic analysis

Phase	Description of the process
1. Familiarising yourself with your data	Transcribing data (if necessary), reading and re-reading the data, noting down initial ideas
2. Generating initial codes	Coding interesting features of the data in a systematic fashion across the entire data set, collating data relevant to each code
3. Searching for themes	Collating codes into potential themes, gathering all data relevant to each potential theme
4. Reviewing themes	Checking if the themes work in relation to the coded extracts (Phase 1) and the entire data set (Phase 2), generating a thematic map of the analysis
5. Defining and naming themes	Ongoing analysis to refine the specifics of each theme and the overall story the analysis tells, generating clear definitions and names for each theme

6.7 Results

6.7.1 Participants

The flow chart for the observational study is shown in Chapter Five. Thirteen survey participants were invited to participate in the interview study, but only eleven patients (36.4% women, 63.6% men) agreed to take part in the interview study at the initial consenting process of the observational study. Patients involved in the interview study had mean age of 68.3y, age range (38-82). The most common primary tumour site was gastrointestinal cancer (54.5%), 27.3% had lung cancer and 27.3 had genitourinary cancer. Ten patients had advanced stage and one participant had stage 2B. All participants were white British. Interviews took place between (February and September 2017).

6.7.2 Themes

Three major themes with one overarching theme were generated from the data and a summary of illustrative quotes sample are presented in (Table 6.2). Codes and themes are presented in **(Appendix O).**

Table 6.2 Summary of themes, subthemes and related quotes from the interview study

Themes	Subthemes	Quotes
Theme 1. i-PE in the context of living with a disease	Life with cancer	*"Cancer was doing fine until the blood clot came a long". (P01)* *"Well it was upsetting to find out it was growing again" (P02)*
	cancer associated thrombosis in context of cancer	*"If I get a pain I immediately related to cancer, if I am a bit fluctuant or wind in my stomach I put it down to cancer" (P01)* *"I have heard of it, but I did not know it is associated with cancer treatment". (P02)*
	i-PE in context of comorbidity	*"I am asthmatic (but under control) I have felt short of breath like going upstairs" (P02)*
Theme 2. Diagnosis of i-PE	Delayed diagnosis as a consequence of theme 1	*""After the first session of chemotherapy, I got really bad chest pain, I was referred to the cardiology department"(P05)*
	Incidental does not mean asymptomatic	*""the only symptom I had a cold a heavy cough, and I was rather short of breath, and I was wheezing, which I have never wheezed in my life" (P01)*
	Response to i-PE diagnosis	*""It was a big shock. Well, I think I was such a chock I did not really take things in"(P02)*
	Need for information	*"So I did what you shouldn't do and as soon as I get home I went on Google and I found out all about the symptoms and what it could be" (P07)*
Theme 3. Living with i-PE treatments	Impact of injection	*"Because no pleasure in sticking needle in myself"(P01)* *"I think the breathlessness has improved since I've been on fragmin."(P10)*

	Coping, more broadly with i-PE and treatment	*"The first couple were a bit a bit hmm (Laughing) nervous, but now it is alright"(P11)*
Overarching theme. Lack of knowledge and uncertainty	*"not blaming the staff, I think I've should have more information, but it is not there if you know what I mean, it is not" (P01)* *"I mean, it is going to be a long while if the blood clot will ever disappear, which is worry for life really" (P01)* *"Each time you think Why me? Is it something I Have done wrong, or is it my life style"(P02)*	

6.7.3 Theme 1. i-PE in the context of living with cancer

This theme describes patients' perception of the diagnosis of i-PE in the context of living with cancer and other comorbidities; how they understand the issues in the shadow of current serious illness.

6.7.3.1 Life with cancer

Participants described living with cancer as a journey of ups and downs, hopes and disappointments.

> *"I thought cancer was getting better, till I was told that cancer was growing again. It [tumour marker] was quite high, quite aggressive and it came down by hormone treatments, and then it grew again, so it got to a level where I had to have radiotherapy, and that is seems knocked it right back. Well it was upsetting to find out it was growing again before Christmas. "(P02)*

Despite this, participants tried to live as normally as they could with cancer. Many described ways to overcome the side effects of chemotherapy, and other restrictions where possible and even when these were extensive.

> *"I am very fortunate, although I've got a cancer for a 78 year old I am doing as much as I think I would do if I was with no cancer. You know I am just carrying on a normal life. (P01)*

> *"Well it was strange really because I managed to cope well with it a part from the sickness, and now I got that sorted, so I am feeling a lot better." (P08)*

> *"I went to the first four [chemotherapy treatments] with very little of after effect, perhaps on day 5 or 6 and I get over that and I was OK, after I had treatment number(5 I started to have trouble with my left shoulder, now my legs been a bit weak for some time and you are dependent on your arms to pull yourself out of a chair, … I had to get my friend to come and look after me for two weeks, and she arranged for me to get a walking frame, and a high seat for the toilet." (P06)*

> Some showed great fortitude in the face of bad news.

"I saw the surgeon and I saw Prof X and the both told me there was no cure it is because how it is spread from liver, pancreas, and into my lung. You know, I have to be strong both for my husband and for myself, you know it is not easy -- - it is not when we were first told, we have tears, and hugged and kissed and all thought… but, we have to get on with it." (P04)

"… You just accepted you've got the cancer. "(P11)

6.7.3.2 I-PE in context of cancer

Living with cancer formed the context for patients' response and their interpretation of new symptoms. Symptoms would be related to either cancer or to the side-effects of the treatment.

"I woke up in the morning with the top of my calf red, a bit sore and feeling hot. I do cough but I associate that more to the cancer than the blood clot." (P06)

For some, even though the diagnosis of PE was an incidental finding on CT scans ordered for other reasons, participants reported new or worsening of old symptoms.

"Really breathless. She goes to the toilet and the shower with help and she is always gasping when she comes back out."(P11)

For others, they reported no new/worsening symptoms and thus they had no warning of any new problem. Misattribution of symptoms was also true for some health care professionals, even when some were following established DVT protocols. One patient subsequently found to have an i-PE was brought back for a 7-day repeat ultrasound to exclude DVT only to have the symptoms misattributed and mistreated to cellulitis and the scan cancelled:

"I woke up in the morning with the top of my calf, red a bit sore and feeling hot. I saw a doctor and he said you've better go and have an ultra-scan to see if there is any sign of a clot in your leg. So I did that they did not find anything, he said the symptoms are there but we can't find anything, so come back in 7 days and we will have another look. I went back 7 days later "and saw a different medical person and she said oh no I don't think it is that, she said because my

legs in a bit of a state and she said it more likely cellulitis, so put me on a course of antibiotics 2 or 3 weeks." (P 06)

Another patient was referred to cardiology and reviewed subsequently at a number of chemotherapy treatments before they were diagnosed with i-PE:

"*After the first session of chemotherapy, I got really bad chest pain, so I was referred to the cardiology department to check on my heart. So the chemotherapy continued and every session get progressively worse." (P 05)*

Eventually, the patient went to the GP for help for the persisting symptoms, who arranged a CT scan for him:

"So when my last session of my chemotherapy was finished, the GP had arranged for me, because I've been to see him telling him that I got this persistent cough, he arranged for me to have a scan. (P05)"

Some had a stable condition and were pleased that they were doing well with their cancer. For these patients, it was disappointing to find out that an otherwise unsuspected complication had developed, but others took it in their stride, pleased that the cancer was responding to treatment:

"I was also over of the moon because he told me that my cancer has shrunk by 50% so I wasn't bothered by the clot, I was so happy that the cancer has shrunk." (P10)

For most participants the diagnosis of i-PE was at the same time of the diagnosis of cancer or just at the start of chemotherapy. Although this was not perceived as a setback just when life was becoming better for them, it was a significant additional burden on them at an already difficult time which sometimes contributed to not seeking help.

"After I had the first session of the chemotherapy, I got the severe chest pain, I didn't do anything about it for a week, I wasn't, I should have done probably." (P05)

6.7.3.3 I-PE in context of comorbidity

The participants in this study not only had cancer, some of them were old and had other chronic conditions to contend with. The presence of other illnesses provided more conditions to attribute symptoms of cancer associated thrombosis and made distinguishing the cause of symptoms very difficult.

> *"…and I do occasionally feel out of breath and I have used my (inhaler) I am asthmatic (but under control) I have felt short of breath like going upstairs, …even now I feel little out of breath, but is that the clots." (P02)*

> *"I have a lot of problems before I had a stroke, and a heart attack. I have had some chest pains, but this is the effect of stroke, and I do get such pins and needles things in my arm and sometimes I get pains in this side of my chest" (P02)*

> *"But also she got Lupus as well, before, and your breathing could be bad because of lupus, in fact it is better after the operation."(P08)*

Even intercurrent simple conditions made identifying symptoms of cancer associated thrombosis as something to be concerned about difficult:

> *"… the only symptom I had a cold a heavy cough, and I was rather short of breath, and I was wheezing, which I have never wheezed in my life, but I put it down to the cold. So one presumes it could be happened with cold, I've no idea" (P01)*

6.7.4 Theme 2. Diagnosis of i-PE

This theme describing participants' response to the diagnosis, diagnosis process, and delayed diagnosis.

6.7.4.1 Response to i-PE diagnosis

Patients stated that receiving the diagnosis of PE was shocking and distressing situation, especially in the absence of prior information identifying it as life threatening condition.

"So I was still waiting to find out results of the cancer search, and then to find out I got this It was in a big shock. It came out of blue. Is was quite a shock. It was a big shock". (P02)

"I said Oh what else can you see, can't do much can you?" (P09)

Some patients, were concerned and distressed by the unexpected finding of PE and worried about the consequences of having a blood clot.

"Well, the only thing worried me was the chance of having a stroke". (P06)

Others, could not describe their response, as they felt uncertain in the absence of enough information, and reported demanding for more and immediate information and support.

" *Ahh, (he sigh) I wasn't, I didn't know how sever having a blood clot, I mean no one said, they did not say oh a blood clot in your lung or in the bottom of both lungs. May be that would worried me more if they said that, that is why they don't". (P05)*

"Upset, I wanted to speak to a consultant in here, ideally you want to speak to Dr XX , to get some advice". (P07)

"…So you start sort of you want to rehear really, but you absorbed it and get on really. Get on with treatment, which what I have done".(P03)

6.7.4.2 Process of diagnosis

This subtheme illustrates the process of the diagnosis and the communication between patients and health care providers. The diagnosis process happened in two ways, some had their diagnosis on the same day while they were waiting for the results of their scan, while others received their diagnosis over the phone after they went home.

Those who had their diagnosis on the same day expressed appreciation for the service, as they were able to talk to a member of staff who explained things to them and referred them to the i-PE clinic.

"Yes, he was very good, he was very good as I said the radiographer was waiting in the room and he explained everything for me. And the following day, the following call I received it all went as he said would". (P04)

"Once I came in and I have my blood taken and once I have been reassured about there been no chance of having a stroke then I wasn't bothered at all". (P06)

Those who received their diagnosis over the phone after they went home, described it as an inappropriate way of breaking bad news. They felt rushed with instruction to come the next day to start treatment with not enough information over the phone which made them more worried. That led to increased levels of uncertainty regarding the course of the i-PE, and increasing stress with no sources for trusted information.

"I went for CT scan, and they found it then. They did not tell me then, then they rang me up then I had to come here (Castle Hill) and they put me on Fragmin". (P08)

"Which a bit worrying because, being rushed when you got cancer you would say Oh what else?". (P01)

"They called me for a CT scan, and then, I get contacted to say you got these blood clots in the lungs. But I did not know the size of them and the extent of them". (P03)

"I am not depressed about having the clot I was only worried in case the clot moved as I do not understand these things in case the clot moved to my brain and have a stroke". (P06)

One patient reported looking for information about PE in the Macmillan leaflets about his cancer but found none. In response to inadequate information, patients turned to the internet or to relatives who have medical background.

"I looked at Macmillan leaflets in the hospital, did not find anything, so I did what you shouldn't do and as soon as I get home I went on Google and I found out all about the symptoms and what it could be" (P07)

"As I said I went on the internet to look up pulmonary embolism to find out about it, and my sister who is now lived in Suffolk for many years, her daughter is pharmacy assistant in hospital in Great Yarmouth, and she said Ah! it is quite common, and usually 6 months injection treatment with Fragmin often clears up, but nobody else". (P02)

6.7.4.3 Delayed diagnosis

Although the diagnosis of pulmonary embolism was incidental finding discovered on a CT scan ordered for cancer staging or follow up, patients reported having thrombosis (PE/DVT) related symptoms. These new or worsening symptoms were largely ignored by patients due to unfamiliarity of symptoms or signs, relating them to side effects of cancer or its treatment and did not seek any medical help.

"After I had the first session of the chemotherapy, I got the severe chest pain, I didn't do anything about it for a week". (P05)

"• I woke up on morning and my right calf was really hurting, I assumed that I just the way I slept on the sofa uncomfortably" (P07)

Not only patients misattributed their symptoms. Even health care professionals did. That not only delayed diagnosis of PE but put patients at risk of having other medication for incorrect diagnosis.

"I got really bad chest pain, so I was referred to the cardiology department to check on my heart. I was put on various medications, for my pain in my chest which I am still taking". (P05)

"I woke up in the morning with top of my calf red, a bit sore and feeling hot… she [doctor] said oh no I don't think it is that [cancer associated thrombosis], she said because my legs in a bit of a state and she said it more likely cellulitis, so put me on a course of antibiotics but then of course 2 or 3 weeks. (P06)

6.7.5 Theme 3. Living with i-PE treatments

This theme describes participants' response to the anticoagulation treatments and its effects on their life. All patients started on low molecular heparin and continued for six months. One patient transferred to DOACs due to a drug interaction. There was

general agreement by patients that low molecular weight heparin was an acceptable treatment, however unpleasant.

6.7.5.1 Impact of anticoagulation injections

The patients understood why they were on LMWH and considered it acceptable. However, there were comments about the long duration of anticoagulation treatments needed for treating the i-PE. Many expressed their dislike of injection and a discomfort at the point of injection, although this was short-lived. None of the patients reported distress or anxiety related to the injections.

> *"You know six months, 18 days, 186 times I've got to have this, and again you will adapt to these things".(P10)*

> *"The lady doctor diagnosed me said Oh you might have to inject for life, which… was a big shock for me". (P02)*

> *"I mean I do not like needles, I've just been having a blood test and I just look away". (P04)*

One patient highlighted the difficulties of doing the injection by himself due to concomitant comorbidity.

> "*Because no pleasure in sticking needle in myself, but if my hands won't shake I would have no worries what so ever". (P01)*

Adhering to the treatment represent a challenge to some patients, remembering it, taking treatments with them when traveling and even finding a place to do the injection if they were out and about. All these had add more burden to the patients' lives.

> *"Well, I hate having to do it (the injection) I found myself messing round with things.* It *is just unpleasant, and I feel I got in the bathroom and close the door because I feel a bit fearful of it like I am injecting Heroin or anything like that. My stomach is blue and bruised". (P02)*

"Just remember to do it some days, and some days else you say Oh well… that is the only thing. And I suppose if I go away or anywhere I have to take a supply with me". (P05)

Patients reported notable improvement in their symptoms after receiving anticoagulation that positively affects their life.

"Now it has had a massive effect on my shortness of breath. Now in the last three or four days is got remarkably better , so climbing the stairs now and doing few activities in the garden actually cut the grass and I am not being out of breath".(P05)

"I think Fragmin injection is working so I am not coughing at all actually at the moment I've got no cough, my life doesn't hurt I have got no tightness no shortness of breath, so I currently assumed it is working". (P07)

The common side effects of the injection that almost all patients described was bruising. For most of them this was acceptable. Even for the two patients who reported major bruising they seemed tp accept this as necessary and were not particularly distressed by it. One patient was on hormonal injections and the other had a colostomy bag.

"*I am all bruised all around here (abdomen), it is black and blue, the whole of my stomach, but this side effects do not affect me". (P05)*

"Oh my abdomen is blue and bruised all over. Doesn't hurt only when it goes in because it stings, but for just 5 seconds that is all. I got a lot of bruise, so but you can't see them can you". (P09)

"She got a colostomy bag, and she got bruises, few ones here, and we did one in her leg". (P08)

"I am on hormone Zoladex that is why I am trying to leave a space on my stomach" (P02)

Patients were aware of the risk of bleeding while on anticoagulation but expressed no distress about that, rather than of needing to be more careful.

"Just need to be careful, if I am going anywhere, and wrap cloves if I work in the garden. It is not worrying me too much, you know I cut myself a couple of times and it did not bleed excessively. It is not like an awful or something. I am aware but is not worrying me it is not going to stopping me doing anything". (P03)

6.7.5.2 Coping with i-PE and treatment

This theme describes ways participants used to cope with cancer associated i-PE and its treatments. The need to stay positive when dealing with these complication (PE) especially that there was nothing to do about it. Patients stated that once they get over the shock they were able to find ways to cope.

"But you have to put trust in the doctors here and say; yes well I've got that so let's get on with that and what can we do about it and get on with it". (P03)

"You know, I have to be strong both for my husband and for myself". (P04)

"…being negative, because does not do any good, just making you feel worse, just got to be positive all the time, I was fine by it, I was fine". (P08)

Although, some participants expressed their dislike of the injections but they were willing to take the treatment for as long as it needed. They were able to find ways to take their injections and make sure that they did not miss a dose.

"It has to be done hasn't it, when you have to deal with this you have to deal with this don't you. What's the alternative, say no? Just you get on and do it. So it is not a hassle, just get on with it". (P03)

"I do it by myself. It's silly as it sound. I forget which side I've done, I done it on my mobile L or R, just as a reminder. but it has just become a norm". (P07)

"District nurse came and do one side one day and then the other side the other day". (10)

6.7.6 Overarching theme. Uncertainty and lack of information

This theme was common to all the other themes, namely lack of information and uncertainty. The overwhelming majority of participants reported lack of knowledge

about the association between cancer, cancer treatment and the risk of thrombosis. One of the participants had a medical back ground but nevertheless was unprepared for such complication. Participants were well aware and supported about the side effects of chemotherapy and knew the measures to take to reduce the effects, but, despite the study setting being hospital with a special interest in cancer associated thrombosis, none remembered being informed about the risk of cancer associated thrombosis, or the symptoms and signs to watch for.

> *"No. It would be nice if I've been told I mean I know there are the normal side effects of having chemotherapy, you know tiredness, whatever of loss of appetite, and bad mouth and that sort of things, experience all of those, loss of taste, finding difficult to eat, mouth very sore, hair loss, and all those, but nothing about the blood clot. "(P05)*

> *"No, I was not told that. After the scan, I was told that often when you have chemotherapy blood clots can appear." (P04)*

> *"No point during my chemotherapy has anybody ever mentioned the possibility that it could cause a blood clot". (P05)*

Patients often reported that when information was provided it was unclear and not enough. This made them seek more information from other sources, such as the internet. This resulted in some participants receiving alarming information in an unsupported way and in sometimes conflicting with their health professional opinion.

> *"And I did something that never normally do I did look at the internet." (P01)*

> *"So I did what you shouldn't do and as soon as I get home I went on Google and I found out all about the symptoms and what it could be." (P07)*

Others were left feeling it was their fault for not being more proactive in seeking information in clinic:

> "*II think, I think some of it is my fault. I've should asked, if I asked it would be there,…I'm not palming the staff, but think I've should have more information, but it is not there if you know what I mean." (P01)*

When the diagnosis of i-PE was associated with low levels of information, this increased the level of uncertainty. Participants were uncertain about the causes of i-PE, were not sure why they (in particular) had developed this complication, what was the likely clinical course of i-PE, what if they developed further blood clot and how would they know if it did, and would the anticoagulation treat the i-PE successfully.

> "*..and each time you think Why me?". (P02)*
>
> *"When you think emboli, what if it then moves, my mind was not happy". (P03)*
>
> *"Well, the only thing worried me was the chance of having a stroke". (P06)*

6.8 Discussion

Further to the results of the systematic literature review in Chapter Four, the aim of this study was to explore the cancer patients' experience of the diagnosis of pulmonary embolism in the context of incidental diagnosis. Describing the illness-story from a patient perspective could increase understanding of living with illness for health professionals and others, facilitate decision-making about treatment and enhance information about the outcome from a patient perspective.

The qualitative thematic analysis generated four major themes which illustrated patients' experience of living with cancer and i-PE: i) i-PE in the context of living with cancer, ii) diagnosis of i-PE and iii) living with i-PE treatments, with iv) an overarching theme of lack of information and uncertainty.

The findings of these interviews are similar to those from the systematic literature review, and previous research. However, it provides some unique features as well.

Misattribution, misdiagnosis

It is well recognised that the diagnosis of cancer-associated thrombosis adds more burden on patients' lives physically and psychologically. We reported previously in the systematic review that although cancer patients are at high risk of developing venous thrombosis, they do not routinely receive enough, or any, education about the warning signs and symptoms related to venous thromboembolism which led in many

cases to misattribution of these symptoms by patients and clinician to cancer or its treatment. The presence of other comorbidities aggravated the difficulties in identifying these symptoms as due to cancer-associated thrombosis.

This interview study highlights these issues and adds that even patients were completely unaware of the possibility of incidentally diagnosed with venous thromboembolism when attending for their routine cancer care scans. Patients being investigated for possible thromboembolism where this was suspected by the clinician at least had some warning that this was a possibility. Although patients in this study often reported symptoms consistent with cancer-associated thrombosis, they were not being investigated for thrombosis as a possible explanation for their symptoms. Therefore, the diagnosis came out of the blue adding to the distress already recognised with finding out about cancer-associated thrombosis.

Lack of information among the interviewees shaped their response, and in some cases led to delayed diagnosis. Uncertainty and lack of information theme from the interview study mirror the theme uncertainty and information from the systematic literature review analysis. This theme considerably overlapped with all the other themes. It is well documented that patients living with cancer experience an uncertain situation and confusion that might trigger mixed feelings, including anxiety, fear, hope and despair (443, 444).

These findings were consistent with the qualitative synthesis of the systematic literature review. In this study, although the diagnosis of pulmonary embolism was incidental, patients had often experienced venous thrombosis related symptoms. In this study, patients presented with chest pain, persistent cough, wheezing chest, and some of them with a painful swollen leg, which should have prompted further evaluation for the possibility of pulmonary embolism or deep vein thrombosis. However, having advanced cancer and being on chemotherapy overshadowed the possible diagnosis of pulmonary embolism /deep vein thrombosis.

Diagnosis overshadowing, first described in 1982 (445), diagnostic overshadowing is related to clinicians' tendency to misattribute symptoms of patients with mental health to their cognitive deficits, leading to under-diagnosis of other comorbid illness. Diagnostic overshadowing also manifests as the process by which patients receive

inadequate or delayed care because the treating provider inaccurately attributes physical symptoms to mental illness. These diagnostic errors contribute to the increased mortality among patients with mental health (446). In general, medical errors and adverse events are of concern and contributes to serious effects (447, 448). In acute care Canadian hospitals, errors or adverse events were estimated to occur in 7.5% of patient admissions each year (449), and between 44 000 and 98 000 deaths yearly occurred in US hospitals because of errors or adverse events (450). Researchers reported that diagnostic error may involve missed diagnosis, a wrong diagnosis may be provided, or delayed diagnosis, all of which can lead to harm from delayed or inappropriate treatments and tests (451).

Other factors contributing to misdiagnosis could be related to clinicians ignoring patients' knowledge, or to difficulties in communication or challenging behaviour by some patients or related to the complexity of illness (452). This misattribution can prevent or delay providers from making accurate or complete diagnoses, even if such conclusions would seem obvious in patients without behavioural health conditions.

Miscommunication and information giving

The way patients were told about their i-PE affected their levels of distress. Patients who were given the diagnosis of i-PE and treatment *in person* were more relaxed and assured, but those who received the diagnosis by telephone had a much poorer experience. The lack of information in the context of an urgent request for clinic attendance for treatment left patients anxious and “in limbo”, driving them to inappropriate sources of information. As a result, participants used the internet and other sources to fulfil their shortage of knowledge about this issue. Similar findings are reported in the PELICAN study (332) and in the systematic literature review, even when the diagnosis of thrombosis was suspected, inadequate and delayed information caused significant anxiety and patients filled the gap from potentially unhelpful and inaccurate sources. According to Wright (1996): when bad news is given by telephone the recipient may not fully understand what has been said and be unsure what is expected of them (453). This finding raises the question about the challenges of giving such diagnosis in this way and highlights an area of query and need for improvement.

It was clear that information is an essential part of the experience of living with cancer and i-PE as a means of handling the situation. The unmet information needs have gained a lot of interest worldwide (454). In recent years, evidence has shown that patients with cancer have substantial information needs both during and after treatment (455, 456) which often remain unmet. Longitudinal studies on information needs suggest that information needs rise soon after diagnosis and remain high overall (456). In a systematic review about information needs and sources of information among cancer patients, health professionals are the most frequently cited information source emphasizes the crucial role that physicians, nurses, and other health care professionals play in meeting patients' information needs (457). However, participants in this study have reported shortage and unavailable source of information, like brochures, and inability to see their consultant sooner. In the same context of previous research that reported insufficient information cancer patients received from health care professionals (458). It is recognised that physicians remain an important source of information during and post-treatment; however, nurses and other health care professionals become equally important sources of information during this time. In the study of Lavall and Costello most of the participants emphasized that they wanted to receive information about health from health-care professionals (459).

These results could be utilized to facilitate a less trouble trajectory course of illness for some patients. There is a need for health care professionals to acknowledge the dimension and the effects of uncertainty among cancer population who developed i-PE and cancer-associate thrombosis in general. That could help people to cope and go on with life. Uncertainty needs to be dealt with as a separate component of illness rather than as emotional outcomes of illness.

Overshadowed by cancer

Not only was the diagnosis of i-PE overshadowed by the diagnosis of cancer, the experience of i-PE once diagnosed was also overshadowed by cancer. Life with cancer and its treatments was full of difficulties, a series of a dramatic change, full of ups and downs. It is well documented that a diagnosis of cancer represents a life-threatening event that brings individuals into an uncertain situation and confusion that might trigger feelings of distress, including anxiety, fear, hope and despair (334, 335).

In this interview study, cancer overshadowed the patients' experience of the diagnosis of i-PE in the same way to that reported in the systematic literature review. Patients' main concern was the response of cancer to treatments, which was poor, leading to psychological distress. The diagnosis of i-PE was experienced in the context of living with cancer and other comorbidities, it was apparent that these patients perceive the i-PE diagnosis and therapy as having significant effects in a variety of physical and psychological areas, including response to the diagnosis, medical situations, and coping.

However, having advanced cancer and being on chemotherapy overshadowed the possible diagnosis of pulmonary embolism /deep vein thrombosis. Misattribution of symptoms in cancer patients not on in the clinical setting. Auer, reported that postoperative PE usually misdiagnosed because clinical symptoms and signs suggestive of PE, including (chest pain; shortness of breath; tachycardia; and oxygen desaturation), can be explained by the side effects of operations, such as incisional pain; hypovolemia; and atelectasis, or might be masked by analgesics (460).

The burden scale of i-PE was also significant (physically and psychologically) through the diagnosis process to the treatments and coping with in the everyday. The response to the diagnosis in both situations was profoundly associated with a high level of distress and uncertainty. The difficulty in accepting the diagnosis is associated with feelings of anger and sense of unfairness in the context as to previously reported (332, 461).

The management with self-injection of low molecular weight heparin was accepted among the patients in the interview study regardless of the side effects of the injections, similar to the findings in the SLR review. However, although the management of i-PE required the same immediate start of self-injected anticoagulation for a period that could be extended to six months, the patients had had no prior warning of this and had to adjust to this more quickly than those who's PE or DVT was not incidental. The uncertainty inherent in both i-PE diagnosis and long-term treatments again brought the importance of information into focus, as it allows patients to gain a sense of control and coping procedures to be made. Once adjusted, most were able to find ways to take the injections and adopt new routines to avoid complications.

However, the interview study analysis illustrates that coping with the daily injection treatments were challenging among patients with other illness relating to their cancer e.g. an intestinal ostomy and among those on hormonal injections. The need to use only one side of their abdomen for anticoagulation injections means a higher level of bruises and discomfort than other patients.

Strengths and limitations

At the time of writing this thesis, this is the first study to explore experiences of cancer patients living with i-PE specifically and provide data allowing comparison with previously reported experiences of patients with cancer-associated thrombosis.

The limitations are those inherent with all qualitative work – that is, the findings are not generalizable. However, qualitative research (see Chapter Three) does not aim to do so, rather recognising that individual experience is valid in its own right. All the included participants were recruited from a single dedicated service and were all managed as part of a developing nurse lead service, specializing in the management of cancer-related i-PE, and thus their experience once diagnosed, may represent a standard of care not available to cancer patients attending services which do not have cancer-associated thrombosis clinics. The main limitation of this study based in the small sample size, (usually anticipated that 15 to 20 participants are needed to achieve saturation of themes), however, unsurprisingly for such a focussed question, no new codes were arising from the latter interviews, suggesting data saturation was achieved.

6.9 Implications

The main issues arising from this study are the need for information carefully delivered in the light of the “out of the blue” nature of the diagnosis, diagnostic overshadowing and uncertainty. In the systematic literature review (37) we called for raising awareness and patients education about cancer-associated thrombosis. This study highlights the need to include the possibility of i-PE in patients’ education materials. Diagnostic overshadowing, even though it has only been described previously in mental health conditions, seems pertinent to the patients with i-PE in this study; the concept of diagnostic overshadowing could be usefully applied.

Future research needed to address overshadowing diagnosis to investigate how often other treatable co-morbidities are misattributed in patients with cancer and i-PE, to examine the impact of such errors and explore the related factors.

6.10 Conclusion

This chapter discussed the patients' experience of living with cancer and incidental diagnosis of pulmonary embolism. The issue of an unexpected, but serious, diagnosis and how this news of diagnosis is communicated to the patient especially if the phone is unavoidable. The uncertainty associated with such a life-changing diagnosis adds to the anxiety and timely and accurate information is important for those with i-PE as well as education about the risk, signs and symptoms of thrombosis. The issue of diagnostic overshadowing is a useful concept to emphasise the need to take care of a potential misattribution of thrombosis symptoms in all cancer patients.

The next chapter investigating the correlation between the level of thrombogenic biomarkers (tissue factor and protease receptor-2) and the risk of i-PE in cancer patients.

Chapter 7 A retrospective study of tissue factor and protease-activated receptor 2 mRNA in FFPE cancer tissues from patients with proximal incidental pulmonary embolism

7.1 Introduction

A key challenge in VTE management is the prediction of the likelihood of an event which enables effective prophylaxis (medicinal or otherwise) to reduce morbidity and mortality. The risk of thrombosis in cancer patients appears to be mainly driven by cancer-related factors, such as high tumour grade, advanced stage, antineoplastic therapies, and tumour type (462). Tumour types are often classified into high risk (pancreas and brain), moderate risk (colon and lung) and low risk (breast and prostate) risk groups (90).

The primary site of cancer is consistently identified as a risk factor for VTE across a variety of studies (251). Epidemiological studies suggest that haematological, lung, and gastrointestinal cancers, as broad diagnostic categories, are associated with a significant risk for VTE (11, 463). In a large population study included > 1.2 million, *Levitan* and colleagues reported Gastrointestinal (GI) cancers, (pancreas, stomach, liver, colon, rectum) were among the top 10 cancers with the highest rate of deep vein thrombosis (DVT) or pulmonary embolism (PE) out of 18 cancers reported (464). Chew et al, used a population-based inception registry cohort reported metastatic pancreatic cancer and colorectal cancer had a higher incidence VTE at 20.0 and 4.3 cases per 100 respectively (465). Lee. *et al* reported that patients with advanced gastric cancer (AGC) had a higher risk of developing VTE (24.4%) than those with lower-stage gastric cancer (0.5%-3.5%) (466). The risk of thrombosis increased in an advanced stage and with chemotherapy(467). *Lyman* and colloquies*, 2013* reported higher VTE risk among patients treating with chemotherapy for pancreas, stomach, and lung cancers (468), While Otten.et al reported a higher risk of VTE in a small group of colorectal cancer patients during chemotherapy(469).

Various mechanisms have been postulated that may support the pathophysiology of VTE in cancer patients. Tissue factor and circulating TF-bearing MPs were suggested to play a role in the pathogenesis of VTE in different cancer types (470, 471).

Tissue factor (TF) is the major initiator of coagulation which commonly expressed in a variety of malignancies (212, 472). It has been reported that TF expression is upregulated on both tumour and healthy cells in cancer patients (473). Various methods have been used to measure tissue factor, including studying the degree of TF expression in tumour cells by immunohistochemistry (159, 171), measuring systemic TF antigen levels (174), TF activity (174, 189) or quantification of TF-positive MPs in human plasma (174). However, may researchers demonstrated that released microvesicles are rapidly cleared by cellular uptake (474, 475), making the measuring of tumour-derived TF microvesicles at a perfect time challenging (210).

Increased levels of TF bearing microvesicles are often evident in cancer-related coagulopathy, however, there is no clear association with the incidence of thromboembolism (476). The correlation between the risk of thrombosis and the level of total and surface TF antigen, or cell-surface TF activity was varied (477-479). TF expression has been shown to correlate with the histological grade of malignancy of several types of cancer, being particularly associated with tumour dissemination and angiogenesis (159, 480-482).

Circulating TF-bearing MPs were suggested to play a role in the pathogenesis of VTE in different cancer types (471, 483). Tesselaar*. et al*. have demonstrated that MP-TF activity levels were significantly higher in cancer patients with acute VTE than in those without VTE including pancreatic cancer (484). In a similar study, Manly et al. found that cancer patients with acute VTE had significantly higher MP-TF activity levels compared to matched controls with no VTE (485). In the same manner, our research group has shown that the number of TF-bearing MP and the pro-coagulant activity of pancreatic cancer patients were significantly higher than the control. While immunohistochemically, Kakkar. *et al*. reported the expression of TF in pancreatic cancer tissues was strongly correlates with the degree of histological differentiation, suggesting that TF may be associated with tumour progression (159). In another small pilot study of patients with pancreatic cancer, elevated levels of systemic TF,

measured either in terms of antigen or activity levels, were predictive of VTE (174). In colorectal cancer, Hron et al reported higher levels of TF-MPs were in patients with VTE (190). It has been reported that TF is abnormally expressed on tumour cells in CRC and strongly correlates with clinicopathological factors including the advanced stage and hepatic metastasis (486). Furthermore, TF expression was also associated with poor prognosis with a 3-year survival of 39%, compared to 88% in TF-negative to positive TF CRC respectively ($p < 0.001$) (486).

Animal studies reported that TF-MPs are released from a variety of tumours in vivo (487, 488) and an increased circulating level of human TF protein in nude mice bearing human pancreatic tumours was associated with hypercoagulation (489). Tissue factor, not only associated with a high risk of developing thrombosis but it has been reported that it is associated with increased risk of reoccurrence as well. Certainly, in the CATCH study, TF measured in serum by ELISA was reported as having some utility in predicting recurrent VTE in the context of cancer (490). Patients with high serum TF levels had a significantly increased risk of recurrent VTE whilst on anticoagulation (19 vs. 6%, $p < 0.001$) (490).

It is known that upon activation cells may release microvesicles that may harbour TF depending on the stimuli (491-493). It has been reported that activation of protease-activated receptor 2 (PAR2) on the cell surface capable of inducing the release of TF as microvesicles (492, 493). The activation of PAR2 may occur through the proteolytic activity of coagulation factor Xa and TF-factor VIIa complex (228). Researchers have reported an increased expression of PAR-1 or PAR-2 in human cancers (206, 494). Therefore, exposure of cancer cells to these proteases, for example as a consequence of coming into contact with blood, may cause the release of TF-bearing microvesicles in high amount (210). These microvesicles are able to harbour the protein TF as well as containing phosphatidylserine, which is essential for the coagulation (495).

In this context, a previous study from our laboratory examined the ability of seventeen cancer cell lines spanning various tissues to release TF in response to PAR2 activation, reported that the level of microvesicles bearing TF (during the short-term

burst in TF release) was strongly correlated with TF-mRNA and PAR-2 mRNA expression more than to the TF protein level (210).

Therefore, the level of cellular TF protein stored within the cells may be a function of the turnover of TF, determined by both the expression of TF mRNA and TF protein release from the cells and hence, only partly correlates with TF mRNA expression.

Therefore, TF, and other coagulation proteins, may hold utility as biomarkers in GI cancers but methodology (type of assay) and source (tissue/blood other fluids) is far from standardised and there is no clarity on confounding factors generated by patient/sample selection but also as mentioned earlier by the dynamics of the TF pathway in the cancer cell.

In the context of these results, we hypothesized that analysis the TF and PAR-2 mRNA, as well as protein levels may provide valuable information to help in determine the risk of thrombosis

7.2 Hypothesis

Cancer tissues of patients with GI cancers who developed i-PE may have higher level of TF and PAR-2 –mRNAs and proteins that increase the potential to express TF into patients' blood.

Differences in incidence of i-PE between patients with the same cancer could be related to the ability of their cancer tissue to produce higher level of TF.

7.3 Aim

Is to determine any relationship between the level of TF/ PAR-2 mRNAs and proteins in cancer tissues and the diagnosis of i-PE in cancer population

7.4 Study design

This is a retrospective case control study. Cancer tissues of patients who developed i-PE identified by AM from the HEY i-PE data base at the Queens' Centre of Oncology at Castel Hill hospital, and two matched controls according to the Age, and cancer type / stage. The HYE i-PE database holds data from 2010. Tissues were retrieved anonymously from the pathology department at Hull Royal Infirmary. Every case had given an anonymous laboratory number. All samples were blindly analysed by NB. The results were then matched with identified cases by CE.

7.5 Ethical Approval

The study has a REC approval, IRAS project ID: 185389 REC reference: 16/NW/0378. (Appendix P)

The main ethical issue arising from the study is that it will be using patient samples without taking consent. This is because the study is retrospective and, due to the severity of i-PE and metastatic malignancy, many of these patients will unfortunately be deceased. A request for the tissue samples made from the pathology department once suitable patients have been identified from the HEY i-PE database. However, as is permitted by the HTA, these tissues released to the researchers NB in a non-identifiable form, coded by a laboratory accession number, and therefore patient confidentiality maintained. As the study is retrospective, there was no direct patient involvement and no planned interventions.

7.6 Materials and Methods

7.6.1 Materials / equipment

Table 7.1 Materials

Company	Materials	Cat No
Genflow Bioscience, Nottingham, UK	ProtoGel (acrylamide: bisacrylamide)	A2-0072
	ProtoGel resolving buffer	B9-0010
	ProtoGel staking buffer	B9-0014
QIAGEN® Ldt, UK	β Actin Primers, Hs_ACTB_2_SG	QT01680476
	PAR-2 Primers	QT00196966
	Qproteome FFPE Tissue extraction buffer (20) Kit	37623
	RNeasy FFPE Kit	73504
	TF- Primers, Hs_F3_Va.I_SG	QT02402344
National diagnostics, Georgia, USA	ND Protein Precipitation Kit	EC-888
	Histo-Clear	HS-200
Promega Corporation, UK	GOTag® 1-step RT-qPCR system	A6020
	Wizard Minipreps DNA purification system	A1330
Santa Cruz Biotechnology, Heidelberg, Germany	Anti-Tissue Factor Mouse mAb (TF9-10H10)	sc-80952
	m-IgGκ BP-B secondary antibody	sc-516142
	PAR-2 antibody (SAM11)	sc-13504
Sigma Aldrich	Bradford reagent	B6916

	Ethanol	459836-1L
	Laemmli's lysis-buffer (2X)	38733
	N,N,N',N'-tetramethylethylenediamine (TEMED)	411019
	Phosphate buffered saline (PBS)	P5493
	Sodium dodecyl sulfate	62862-1KG
	Sodium Chloride (NaCl)	C5533-5MG
	Tween 20	P1379

Table 7.2 Equipments

Equipment	Company
Axis-Shield Diagnostics Ltd,	Cuvettes 500 pieces
Bio-Rad, Hemel Hempstead, Hertfordshire, UK	iCycler real-time thermal cycle
	Nitrocellulose membrane
Heraeus centrifuge, Buckinghamshire, UK	Centrifuge
Hoefer, Inc, San Francisco, USA	TE 50X protein transfer tank

7.6.2 Methods

7.6.2.1 Bacterial cell culture and plasmid isolation

7.6.2.1.1 Preparation of LB broth and propagation of Escherichia coli TB-1

LB broth was prepared by dissolving LB broth powder (6.25 g) in de-ionised water (250 ml) and autoclaving. *E.coli* strain TB-1 was propagated overnight in 50 ml of LB broth at 37°C with gentle agitation.

7.6.2.1.2 Isolation of plasmid DNA

Plasmid DNA from bacterial cells was extracted and isolated using the Wizard Plus Miniprep DNA purification system as follows. 5 ml of bacterial cell suspension was centrifuged at 2,500 g for 10 min and the supernatant poured off. The bacteria were resuspended in 400 µl of Cell resuspension solution (50 mM Tris pH 7.5, 10 mM EDTA, 100 ug/ml RNase A). Cell lysis solution (0.2 M NaOH, 1 % SDS) (400 µl) was added and mixed by inverting the tube. 400 µl of Neutralisation solution (1.32 M potassium acetate pH 4.8) was added, mixed and the lysate centrifuged at 10,000 xg for 5 min in a microcentrifuge to pellet the cell debris. Plasmid purification; for each miniprep, a 3 ml syringe barrel was attached to a minicolumn connected to a vacuum manifold. 1 ml of DNA purification resin was pipetted to the column. The cell lysate was mixed with the resin and the solution cleared through, retaining the resin in the column. The column was washed with 2 ml of Column wash (80 mM potassium acetate, 8.3 mM Tris-HCl pH 7.5, 40 uM EDTA, 55 % ethanol) and cleared through under vacuum as before. The minicolumn was then placed into a 1.5 ml tube and residual ethanol removed by centrifugation at 10,000 xg for 2 min in a microcentrifuge. 50 µl of Nuclease free water was added to the minicolumn and incubated for 1 min. The plasmid DNA was then eluted from the column by centrifugation at 10,000 xg for 20 sec. The concentration of plasmid DNA in each sample was determined by measuring the absorption of a 1 in 10 dilutions of the DNA at 260 nm against a water blank. The concentration of DNA was then calculated as:

DNA concentration (µg/ml) = Absorbance (260 nm) x 50 x dilution factor

DNA purity was determined by measuring the 260:280 ratio, with a ratio above 1.3 indicating DNA of sufficient purity.

7.6.2.1.3 Ethanol precipitation of DNA

The DNA solution was mixed with (5 M) sodium acetate pH 5.2, and 100 % (v/v) ethanol at a ratio of 1:1:4 (v/v/v) and incubated at -20°C for 30 min. The sample was then centrifuged at 10,000 xg for 20 min in a microcentrifuge and the pellet washed with 200 µl of 75 % (v/v) ethanol. The sample was centrifuged at 12,000 rpm for 10 min and the ethanol removed. The pellet was dried and resuspended in DNase free water (50 µl).

7.6.2.1.4 Digestion of DNA using restriction enzymes

Using Promega® Protocol, samples for DNA digestion were prepared in a 0.2 ml tubes respectively according to Table**7.3**. Restriction enzymes used were (Hind III and ECOR I). To activate the restriction enzymes, samples were incubated at 37 °C and reaction was carried out for 60 min for complete DNA digestion.

Table7.3 Master Mix for DNA digestion

Reagent	**Volume (μl)**
DNA, 1 μg/μl	1.0 μl
Restriction Enzyme 10X Buffer	2
Acetylated BSA, 10 μg/μl	0.2
Restriction enzyme (10 U/μl)	0.5 μl
Sterile dH_2O	16.3 μl
Total	20 μl

7.6.2.1.5 DNA purifications after enzymatic digestion

Following DNA digestion, tissue factor DNA was purified of the enzymatic reaction using Illustra® purification system. The GFX column was placed into a collection tube. Capture buffer 2 (buffer solution contains chaotropic salts that denature proteins) (500 µl) was added to the samples (30 µl), mixed thoroughly and transferred onto a microspin column attached to collecting tube. The assembled column-collection tube was then centrifuged at 16,000 xg for 30 sec and the flow-through was discarded by emptying the collection tube. (500 µl) wash buffer type 1 (Tris-EDTA buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA, 80 % v/v ethanol) was then added to the column and centrifuged at 13,400 rpm for 30 sec. The column was transferred to a new collecting tube 1.5 ml DNase-free microcentrifuge tube. To elute DNA, elution buffer type 4 (10 mM Tris-HCl, pH 8.0) (50 µl) was added to the centre of the membrane in the column, incubated at room temperature for 1 min, and then centrifuged at 16,000 xg for 1 min. The concentration of digested plasmid tissue factor DNA was determined by measuring the absorption at 260 nm using UV spectrophotometer and calculated using the following equation:

$$\text{Concentration of plasmid } (\mu g/ml) = \lambda 260 \times 10 \times 50$$

Then samples were stored at -20 °C until required.

7.6.2.1.6 T7 DNA in vitro transcription

Following DNA purification from enzymatic reactions, DNA ligation was set up in 0.2 ml tubes. Samples were prepared according to Table.**7.4** and incubated for 30 min at 37 °C. Then the reaction product was precipitated as in 2.11.4 and measured as in 2.10.4. The product was analysed by agarose gel electrophoresis to confirm its length before used to construct the TF St curve in qPCR reactions.

Table.7.4 DNA purification protocol

component	volume
Nuclease free H_2O	Up to 20 μl
DNA template	1 μg
10 mM ATP	1 μl
10 mM CTP	1 μl
10 mM GTP	1 μl
10 mM UTP	1 μl
10X Transcription Buffer	2 μl
T7 Enzyme mix	2 μl

7.6.2.2 Deparaffinization and rehydration of FFPE Tissue Sections Cut Directly from a FFPE Sample Block

Histoclear was used for deparaffinization of the FFPE tissue sections. A 4 serial of 10–15 µm thick sections were cut from the same block and immediately placed in a 1.5 ml collection tube. Then a 1 ml of Histoclear pipetted into the tube, vortexed vigorously for 10 s and incubated for 10 min on the rotor. Then the tube was centrifuged in a microcentrifuge at full speed for 2 min and supernatant discarded. This was repeated twice.

Tissue rehydration; an 1 ml of 100% ethanol (100%) was pipetted into the tube containing the pellet and mixed by vortexing then incubated for 10 min before centrifuged at full speed for 2 min and supernatant discarded. This was repeated twice. Then 1 ml of (80%) ethanol was pipetted into the tube and mixed by vortexing and incubated for 10 min before centrifuged at full speed for 2 min and discard the supernatant. This step was repeated twice. Then an ml of 70% ethanol into the tube containing the pellet and mixed by vortexing. Incubated for 10 min then centrifuged at full speed for 2 min. This step was repeated twice. The pellet was used immediately for extraction of Total protein or total RNA.

7.6.2.3 Isolation of total RNA from FFPE cancer tissues

Qiagen® RNeasy FFPE Kit (catalogue no 73504) was used for PNA extraction as manufacture recommendations. Once tissue sections have been deparaffinised and rehydrated, 150µl of PKD reagent was added, mixed by vortexing then centrifuged at (10,000 rpm) for 1 min. Then a 10 µl proteinase K was added, mixed gently by pipetting up and down and the sample was incubated at 56°C for 15 min, then at 80°C for 15 min. sample then incubated on ice for three min, then centrifuged for 15 min at (13,500 rpm). The supernatant was transferred to a new tube. 16µl of DNase Booster Buffer added and 10 µl DNase I stock solution. Mixed by inverting the tube and incubated at room temperature for 15 min. to adjust binding condition a 320 µl of RBC Buffer was added to the lysate and mixed them thoroughly. 720 µl of ethanol (100%) was added to the sample, and mix well by pipetting. The sample then

transferred to RNeasy MiniElute spin column placed in a collecting tube and centrifuged at 10,000 rpm for 15 sec. repeated until the entire sample has passed through the RNAesy MiniElute spin column. A 500 µl RPE Buffer was added to the MiniElute spin column and centrifuged for 2 min at ≥ 10,000rpm and the supernatant discarded. To collect the RNA, the MiniElute was put in anew collecting tube and 30 µl of RNAes free water was added to the tube and centrifuged for 1 min at full speed. Stored at -20 ^{0}C until used.

7.6.2.4 Isolation of total RNA from cancer cells

10^6 of MDA-123 breast cancer cells were used to isolate total RNA using the same kit as in 2.10.2. The result RNA was used as positive control in qPCR assay.

7.6.2.5 Determination of RNA purity and concentration

The concentration of RNA isolated from tissue and cells samples was determined by measuring the absorption of each sample at 260 using a spectrophotometer. The spectrophotometer was calibrated using 54 µl of RNAes free water in a quartz micro-cuvette as a blank. 6 µl of RNA sample was then added to the cuvette and the sample mixed by pipetting. The absorption at 260 nm was measured and the concentration of RNA calculated as follows:

$$\text{RNA concentration (µg/ml)} = \text{Absorption (260 nm)} \times 40 \times \text{dilution factor}$$

The 260:280 ratio was used to determine the purity of RNA in each sample, with a ratio above 1.7 indicating RNA of sufficient purity for subsequent RT-PCR analysis.

7.6.2.6 Quantitative Polymerase Chain Reaction (q RT-PCR)

Isolated RNA was amplified with specific primers as well as with β-actin primers (housekeeping gene) as a reference. 1-Step Q-PCR protocol presented in Table **7.5** to convert mRNA to cDNA and then amplify the cDNA was used as illustrated. Pre optimised forward/ reverse primers mix from QuantiTect® were used for TF or PAR-2. GoTag Master Mix and reverse transcriptase enzyme mix. Every sample was run in triplicate as follows; for each RNA samples and St curve sample**,** GoTag Mater mix (12.5 µl/well), reverse transcriptase enzyme mix (0.25 µl/well), 10x QuantiTect Primer Assay (2.5 µl/well), Template RNA (10ng/well) and Nuclease free water were

added to the wells to make up the volume to 25 µl. Q-PCR was carried out using an iCycler thermal cycler RT-PCR machine at temperatures shown in (Table7.6).

Table 7.5 Reaction setup for one step Q-PCR

Component	Volume/reaction (96-well plate)	Final concentration
GoTag SYBR Green Master Mix	12.5 µl	1x
10x QuantiTec Primer Assay	2.5 µl	1x
GoTag Enzyme Mix	0.25 µl	-–
RNA template	Variable	≤ 10 ng/ reaction
RNase-free water	Variable	-–
Total volume	25 µl	-–

Table 7.6 Cycling conditions for one-step Q-PCR

Step	Time	Temperature	Additional comments
Reverse transcription	10 min	50 °C	
PCR initial activation step	5 min	95 °C	
2-step cycling			
Denaturation	10 s	95 °C	
Combined/annealing extension	30 s	60 °C	
Number of cycles	35-40		

7.6.2.7 Protein extraction from breast cancer cells MDA-231

(10^6 MDA-MB-231) cells were cultured in DMEM medium containing 10 % FCS. Cells were incubated at 37°C under 5 % CO_2 and supplemented every 3 days by replacing 3 ml of culture medium with fresh medium until 80 % confluence. Cells were washed, trypsinised, and pelleted as described in section 8.3.3. Cells then washed with PBS and centrifuged for 5min. 100 µl Extraction Buffer EXB Plus supplemented with β- mercaptoethanol was pipetted into the tube containing the cells pellet and mixed by vortexing and incubated on ice for 5 min then mixed again by vortexing. The tube then incubated for 20 min at 100 °C on a heating block. The tube then incubated in a water bath at 80C0 for 2hr with agitation. After that the tube incubated at 4 °C for 1 min then centrifuged for 15 min at 14,000 x g at 4 °C. The supernatant containing the extracted proteins was transferred to a new 1.5 ml. Protein samples were either used or stored at -70 °C until used.

7.6.2.8 Protein extraction from FFPE cancer tissues

A serial of 4 sections (5µm thick/ 100 cmm^3) was deparaffinised and rehydrated as illustrated in the section (7.6.2.1). Sections were pelleted and used for protein extraction. Qproteome FFPE Tissue Kit was used as manufacture required. A 100 µl Extraction Buffer EXB Plus supplemented with β- mercaptoethanol (6%) was pipetted into the tube containing the tissue pellet and mixed by vortexing and incubated on ice for 5 min then mixed again by vortexing.

Heat antigen retrieval

Formalin-fixation, the processing of the cancer tissues can cause damage and masking of the antigens by protein crosslinks (496).To retrieve the antigens, the tube contains the tissues incubated for 20 minutes at 100 °C on a heating block. The tube then incubated in a water bath at 80 °C for 2 hr with agitation. After that the tube incubated at 4 °C for 1 minute then centrifuged for 15 minutes at 14,000 x g at 4°C. The supernatant containing the extracted proteins was transferred to a new 1.5 ml.

7.6.2.9 Protein Precipitation

Protein samples were precipitated using Protein Precipitation Kit/ National diagnostics, (EC-888) before stored at -70 °C until used. In a centrifuge tube a 1/20 volume of reagent A was added and mixed well, then a 1/10 volume of reagent B was added to the sample. The sample then allowed to rest at room temperature for 20

minutes. The tube then centrifuged to remove supernatant. The pellet then dispersed in acetone to dissolve the reagent A:B complex. To remove salts and surfactants, pellet was washed with 70% ethanol. The sample was centrifuged on high speed to collect the protein, then pellet dissolved in BPS buffer.

7.6.2.10 Estimation of protein concentration using the Bradford assay

Known protein concentrations of (0, 25, 50, 100, and 150 μg/ml) were used to obtain a standard curve. The know protein concentration samples were prepared by serial dilutions of lipid-free BSA in de-ionised water. 10 µl of each standard or sample was diluted in 90 µl de-ionised water. Diluted samples and standards (100 µl) were placed into individual 1 ml plastic cuvettes and 900 µl of diluted Bradford reagent (1:1 v/v) was added to each cuvette. Then both samples and standards were kept at room temperature in dark place for 10 minutes and the absorption of the samples and the standards were then recorded at 595 nm using a spectrophotometer. A standard curve was prepared using the absorption values of the standards against protein concentrations Figure **7.1**. The protein concentrations in the samples were then determined from the standard curve.

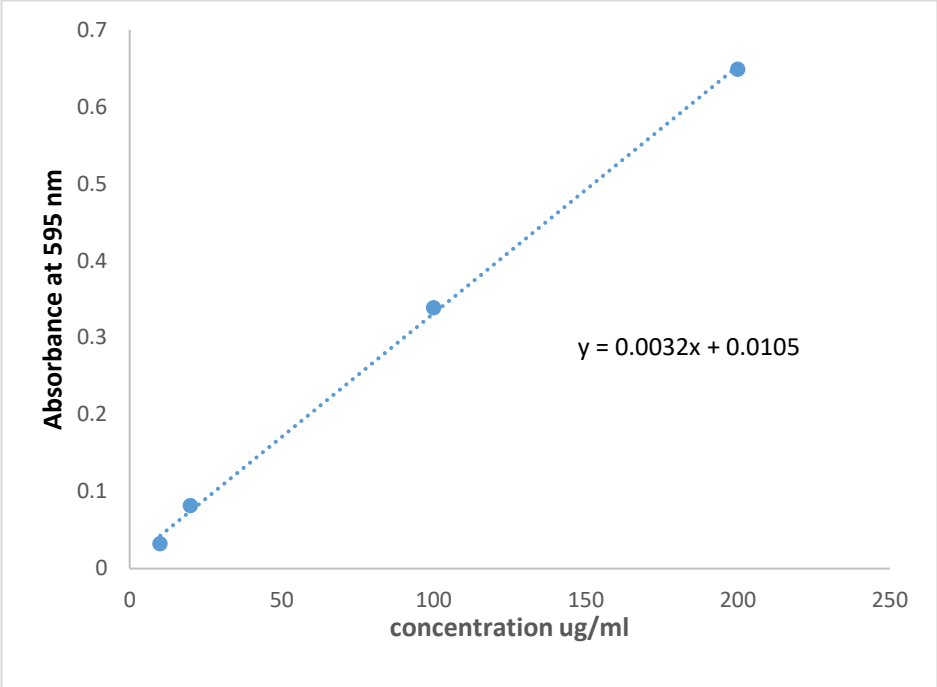


Figure 7.1 Bradford assay St Curve

A range of protein concentration standards (0-200 ug/ml) was prepared by serial dilutions of BSA in distilled water. 100 ul of each sample was placed in a separate plastic cuvette in duplicate and the diluted Bradford reagent (900 ul) added. Samples were allowed to develop for 10 min and the absorptions measured at 595 nm using a spectrophotometer. The data represents a typical standard curve ± SD that was prepared on each occasion.

7.6.2.11 SDS-polyacrylamide gel electrophoresis (SDS-PAGE)

To prepare the 12% (w/v) resolving gel; a 4 ml acrylamide solution [30 % (w/v) acrylamide, 0.8 % (w/v) bisacrylamide], 2.6 ml resolving buffer [1.5 M Tris-HCl, pH 8.8 0.4 % (w/v) SDS], 3.3 ml de-ionised water and 10 % (w/v) ammonium persulphate (100 µl) were mixed well to combine before adding 10 µl of N,N,N',N'-Tetramethylethylenediamine (TEMED) to begin polymerisation. The solution was poured in between the electrophoresis glass plates in a gel caster, then a layer of Butanol was added to ensure that the gel was level and allowed to set for at least 1 h. To prepare the 4 % (v/v) staking gel; a 0.65 ml of acrylamide solution, 1.3 ml staking buffer (0.5 M Tris-HCl pH 6.8, 0.4 % (w/v) SDS), 3 ml de-ionised water, and 100 µl ammonium persulphate (10 % w/v) were mixed thoroughly before adding 10 µl of TEMED. Once the resolving gel sets, the Butanol was poured off and the staking gel poured on top of it and a comb inserted and kept at room temperature to set for around 90 min. Then the comb was removed, the gel was removed from the gel caster and placed in the electrophoresis tank. Electrophoresis buffer (25 mM Tris-HCl pH 8.3, 192 mM glycine, 0.035 % (w/v) SDS) was poured into the electrophoresis tank and behind the gel. 5 µl of a set of molecular weight protein markers (10-260 kDa) was loaded into the first well, and the protein samples (20 µl) were loaded into the subsequent wells. Electrophoresis was carried out at for around 1.5 h at 100 V to separate the proteins until the Bromophenol blue bands reached approximately 1 cm from the end of the gel.

To transfer the separated protein onto nitrocellulose membrane; the gel was placed in between blotting paper and nitrocellulose membrane pre-soaked in transfer buffer made of (20 mM Tris-HCl pH 8.3, 150 mM glycine, 20 % (v/v) methanol), after removing the staking gel and placed in the transfer tank at 4 ^{0}C and transferred at 16 mA for 2 hrs.

7.6.2.12 Western blot analysis

After the transfer of proteins onto nitrocellulose membrane, the membrane was blocked with Tris-buffered Saline Tween 20 buffer (TBST) [20 mM Tris-HCl pH 8, 150 mM NaCl, 0.05 % (v/v) Tween 20] for 2 h and then incubated with primary antibodies diluted in TBST at final concentration recommended by the manufacture for 1.5 h at

room temperature. Then the membrane was washed twice in distilled water for 5 min each and incubated with phosphatase-conjugated secondary antibodies diluted in TBST at final concentration indicated by the manufacture for 1.5 hr at room temperature. The membrane was washed twice in distilled water for 5 minutes each and developed with Western Blue stabilised substrate for alkaline phosphatase.

Table 7.7 Primary and secondary antibody dilutions used during the western blot procedure

Primary antibody	Dilution	Secondary antibody	dilution
Mouse anti-human TF (10H10) anti body (monoclonal)	1:1000 µl	Goat anti-mouse alkaline phosphatase-conjugated antibody	1:5000 µl
Mouse anti- human PAR-2 (SAM11) antibody (monoclonal)	1:1000 µl		
Goat anti-GAPDH polyclonal IgG antibody	1:1000 µl	Donkey anti-goat alkaline phosphatase-conjugated antibody	1:5000µl

7.7 Statistical analysis methods

Data were analysed using SPSS 24, for comparison of data of various groups with the descriptive analysis and independent sample t test, all data are expressed as mean ± SD, statistical significance was considered when $p \leq 0.05$. The sensitivity of cancer tissues TF-mRNA level was determined with the receiver operating characteristic (ROC) curve analysis to differentiate between i-PE and non-PE samples. However, due to limitation of this this study and the small sample size, a nonparametric statistical analysis was applied as well.

7.8 Results

7.8.1 Ethical approval

The trial was approved by the NORTH west- Haydock Research Ethics Committee (REC reference: 16/NM/0378) and by the NHS Trust research and development (R&D) / NHS Trust organisation. (Appendix P: Ethical approval).

Once ethics approval was obtained the Hull data-base of cancer patients with i-PE in the Queens Centre of Oncology was searched by AM and HC to identify anonymised cancer patients diagnosed with i-PE (from 2010) according to the inclusion criteria.

Inclusion criteria

1. Adults ≥ 18 years
2. Active metastatic cancer (M1 stage). The patient can be on active treatment for the cancer Incidentally diagnosed with pulmonary embolism
3. Resection material or large biopsy available

1.1.1.1 Exclusion criteria

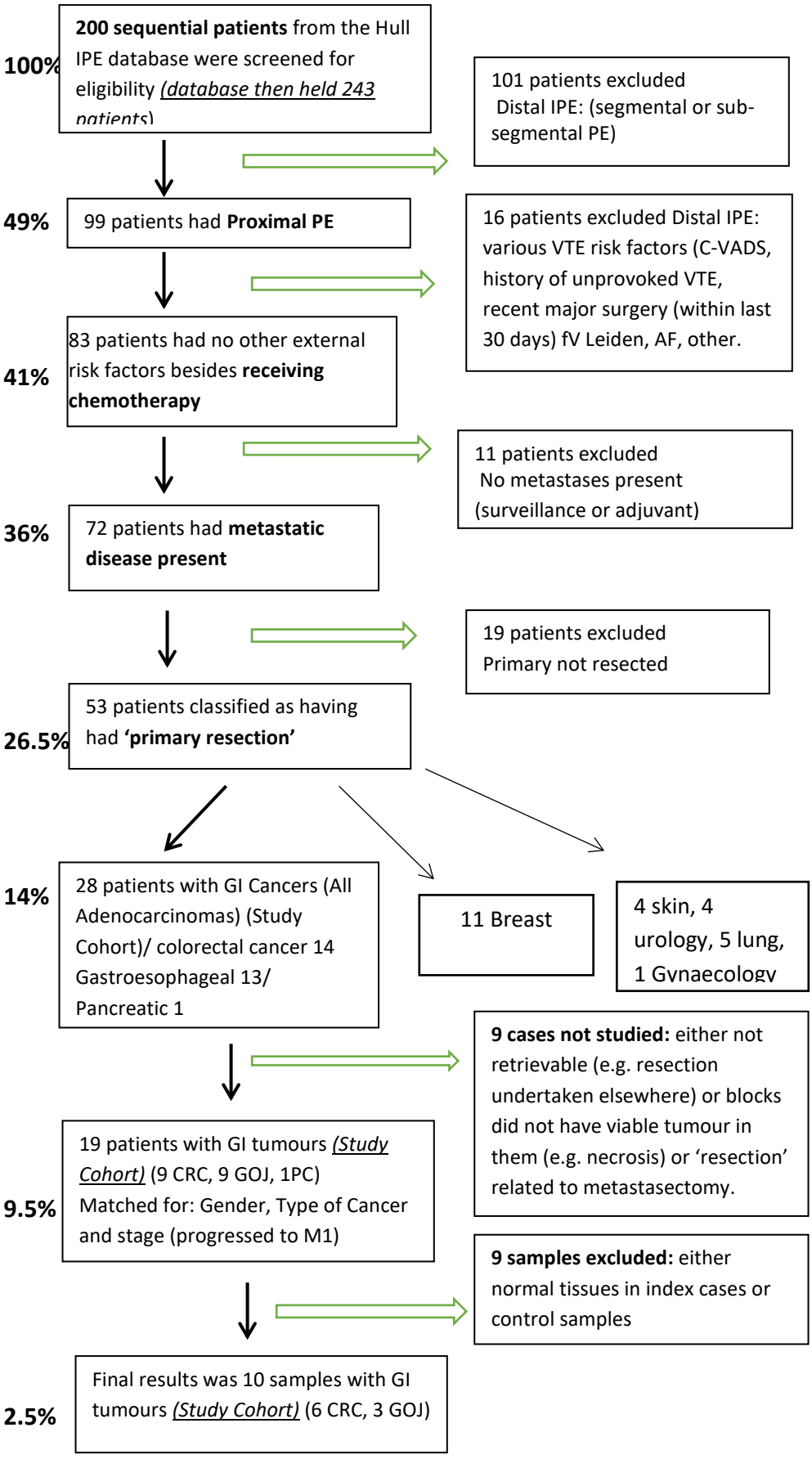
1. No cancer
2. No PE
3. History of previous unprovoked DVT/PE
4. Receiving treatment for procoagulant condition that pre-dated the cancer diagnosis
5. Other risk factors that may have provoked the PE - centrally inserted venous catheter, known coagulopathy (e.g. Factor V Leiden, Protein C or S deficiency, etc.), recent surgical procedure (within 2 months)

7.8.2 Identification of cancer tissues

200 sequential patients (at time of interrogation the database had complete data on 234 patients) screened for eligibility. Figure 7 is the flow chart detailing the patient selection process. 28 patients with GI adenocarcinoma (14 colorectal cancer, 13 gastroesophageal and 1 pancreatic) were identified to fulfil the entry criteria however, 18 patients were further excluded due to either tissues not retrievable, samples did not contain viable tumour or contained no tumour. These samples were matched with control patients for type of cancer, stage (M1) gender and existence of resected material by AM from the existing hospital database linked to the relevant MDTs. Once the paired samples were collected the cases were anonymised and then coded and delivered to the lab. The anonymised clinical data coded to the samples were kept at the Cancer trials unit. The operators in the lab (NBEH &CE) were blinded to the provenance of the tumour samples. Once all analyses were complete the data were locked and then the decoding took place for the clinical correlations to be generated and studied.

The final study sample stood at 10 patients with i-PE meeting the criteria, (7 colorectal and 3 gastroesophageal cancers) which represented (2.5 %) of the cohort.

Table 7.8 A flow chart of tissue samples identification



7.8.3 TF product and St Curve

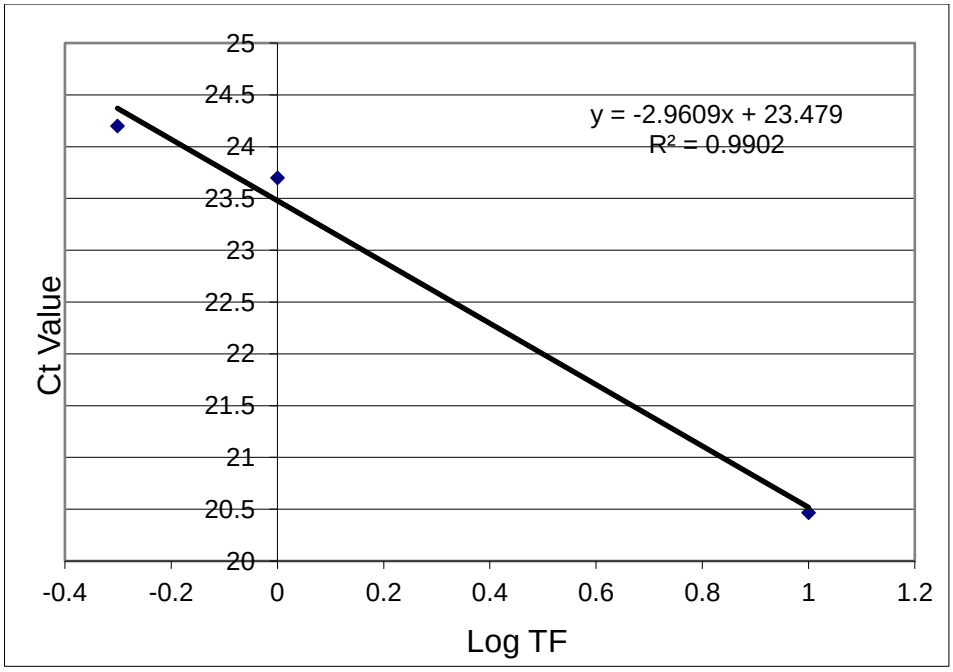


Figure 7.2 Standard curve for TF concentrations.

Serial dilutions of TF in vitro-transcribed TF mRNA ranging 0-10 ng were prepared in RNase free water. Single-step RT-PCR was performed on each dilution. The in vitro-transcribed TF mRNA concentrations were converted to log values and used to prepare a standard curve of TF mRNA concentrations against threshold cycle (Ct).

7.8.4 Examination of plasmid DNA by agarose gel electrophoresis

The plasmids for TF were extracted from bacteria E. coli strain TB-1 using a midi-prep kit and analysed by agarose gel electrophoresis Figure 7.3. The plasmid DNA was analysed by electrophoresis on a 0.5 % (w/v) agarose gel to ensure the purity of eluted DNA. A 0.5 % (w/v) gel was prepared by adding 0.25 g of agarose to 50 ml of TBE buffer (89 mM Tris, 89 mM boric acid, 2 mM EDTA, pH 8.3). The agarose-TBE mixture was heated in a microwave oven until dissolved and was then poured into a sealed gel tray. An appropriate comb was placed in the gel and it was allowed to solidify. The samples were prepared by adding 1 µl SYBR Green I to 10 µl of DNA sample and then mixed with 10 µl of loading buffer. Additionally, a DNA ladder was prepared by mixing 10 µl DNA marker and 1 µl of SYBR Green I. The samples along with the markers were then loaded into wells and electrophoresis was carried out at 100 V for approximately 1 h. The bands were visualized and inspected using a UV transilluminator.

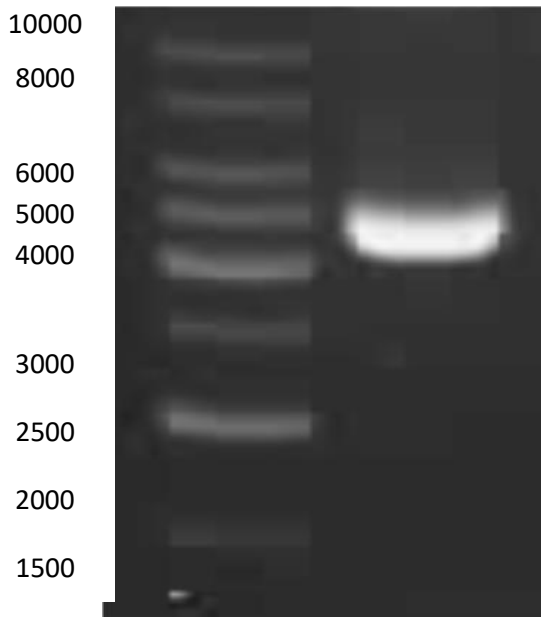


Figure 7.3 The plasmid DNA was extracted from E.

E coli strain TB-1 using a midi-prep kit and analysed by 0.5 % (w/v) agarose gel electrophoresis. A band representing pCMV-XL5-TF was observed at the expected size of approximately 4700 bp

7.8.5 Analysis of TF-mRNA expression GI cancer tissues of patients with i-PE

The TF mRNA and PAR-2 mRNA expression of a total of 20 human gastrointestinal cancer (10 i-PE including 7 CRC and 3 GITs) and 10 matched controls, were analysed using qRT-PCR technique against the TF St curve for mRNA expression of TF. The melting curves were derived from amplification of TF and β-actin. The peak indicates specific amplification of TF and β-actin. The results for FT-mRNA were normalized with B-actin gene. The amount of TF-mRNA/ng for both samples presented in Table **7.9** and Figure 7.5. The paired t-test was applied (Data are representative of 3 independent experiments and expressed as means ± sd, ($p<0.085$) 95% CI (80.5- -5.63)

7.8.6 Sensitivity and specificity of using RT qPCR test to measure TF mRNA

The amount of TF- mRNA was determined against the non i-PE sample using ROC curve. ROC analysis of TF-mRNA to differentiate between i-PE and no i-PE patients showed an area under the curve of 0.992 (Figure 7.6. Combined sensitivities and specificities were visualized in a receiver operating characteristic (ROC) curve. The area under the ROC curve (AUC) provides an estimate of overall discrimination and for evaluations of an appropriate cut-off value of the screening item. AUC of 0.5–0.7 indicates low accuracy, 0.7–0.9 indicates moderate accuracy and 0.9–1.0 indicates high accuracy.

The ROC curves (Figure 7.6) showed relatively high overall accuracies. The AUC was about 0.99 and $p< 0.05$ with 95% CI. For prediction of i-PE in gastrointestinal cancer, a cut-off point of 0.29 risk factors achieved a sensitivity of 100% and a specificity of 100% and a cut-off point of 0.445 risk factors achieved a sensitivity of 90% and a specificity of 10%. (Table **7.10**).

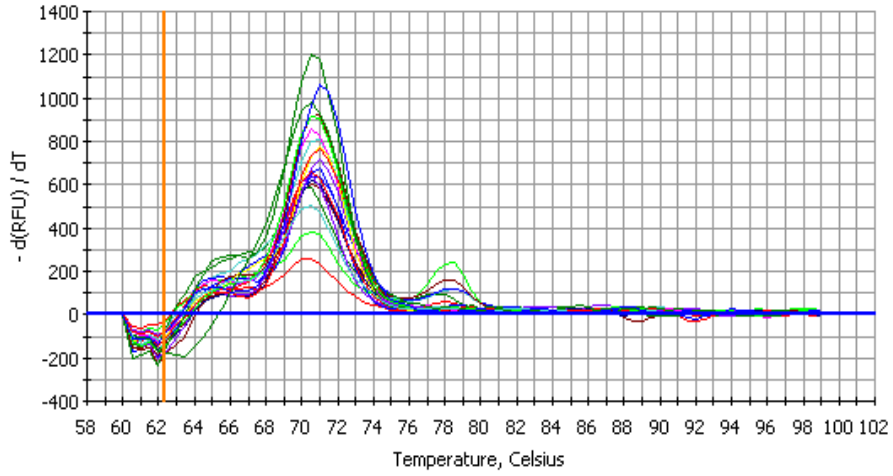


Figure 7.4 RT-PCR analysis of TF mRNA expression.

Total RNA was isolated from FFPE cancer tissues and the real time RT-PCR was carried out to amplify TF and β-actin. Single peak indicates specific amplification of TF and β-actin.

Table 7.9 TF mRNA levels/ ng in cancer tissues of i-PE and matched controls

I-PE Samples	TF mRNA/ng	Non-iPE Samples	TF mRNA/ng
i-PE 1	2.11	Non i-PE 1	0.11
i-PE 2	1.012	Non i-PE 2	0.12
i-PE 3	77.28	Non i-PE 3	0.19
i-PE 4	3.12	Non i-PE 4	0.00
i-PE 5	3.04	Non i-PE 5	0.50
i-PE 6	12.23	Non i-PE 6	0.11
i-PE 7	0.39	Non i-PE 7	0.18
i-PE 8	5.34	Non i-PE 8	0.05
i-PE 9	4.02	Non i-PE 9	0.14
i-PE 10	131.43	Non i-PE 10	0.00

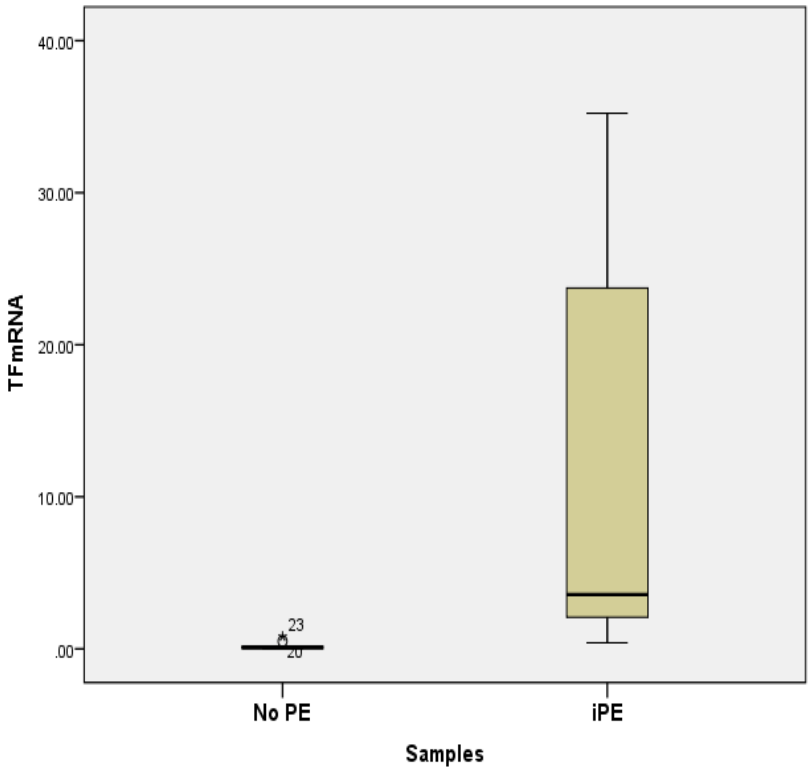


Figure 7.5 Differences in TF mRNA level between cancer tissues.

Examination of the difference in TF mRNA level between cancer tissue of patients with i-PE and matched controls with no thrombosis. 10 samples of i-PE and matched controls were analysed using the nonparametric independent sample median test. At the significance level of 0.05, the result shows a significant difference p= 0.003, rejecting the null hypothesis that "the median of TF mRNA are the same across the categories of groups.

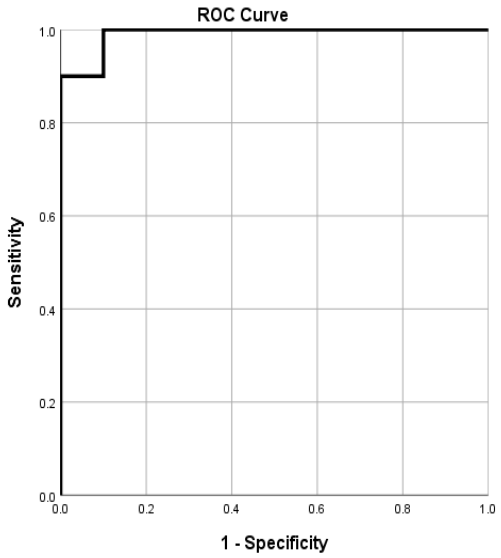


Figure 7.6 Roc Curve for TF mRNA/ ng.

The amount of TF- mRNA was determined against the non i-PE sample using ROC curve. The AUC was about 0.99 and $p < 0.05$ with 95% CI.

Table 7.10 Coordinates of the ROC curve

Positive if Greater Than or Equal Toa	Sensitivity	1 - Specificity
-1.000	1.000	1.000
.0250	1.000	0.900
.0115	1.000	0.800
.0340	.900	0.800
.0700	.900	0.700
.1000	.900	0.600
.1150	.900	0.400
.1300	.900	0.300
.1600	.900	0.200
.1850	.900	0.100
.2950	.900	0.000
1.0650	0.800	0.000
2.3900	0.700	0.000
3.5350	0.600	0.000
4.6800	0.500	0.000
7.7300	0.400	0.000
11.1800	0.300	0.000
44.7600	0.200	0.000
104.3550	.100	0.000
132.4300	.000	0.000

Table 7.11 Area under the ROC curve

			95% CI	
Area	St Err[a]	P*[b]	Lower	Upper
0.920	0.078	0.001	0.767	1.000

a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

7.8.7 Examination of the difference in PAR-2 mRNA level in FFPE cancer tissues of patients with i-PE and matched controls

RAP-2 m RNA extracted from cancer tissues (46 samples) of i-PE and matched controls was analysed using RT qPCR. Ct values detected in only 14 samples, showed no significant difference in the average of Ct values as presented in Table **7.12** and Figure 7.7.

Table 7.12 Ct value of RT qPCR for PAR-2 and β actin mRNA

i-PE	B actin Ct	PAR2 Ct	No i-PE	B actin Ct	PAR 2 Ct
12HXXXX	37.5	36	13HXXXX	24.6	36.9
12HXXX2	34	37.5	14HXX2B	24.4	32.7
13HXXXX	33.3	35.75	12HXXXX	32.7	35.1
12HXXX3	34.7	35.7	12HXX3B	35.4	37.9
14HXXXX	31.4	38.4	12HXXXX	35.7	33.8
11HXXXX	31.1	39.8	14HXXXX	36.2	37.6
12HXXX4	33.6	37.8	12HXX4B	35.4	35.6
Average		37.3			35.64

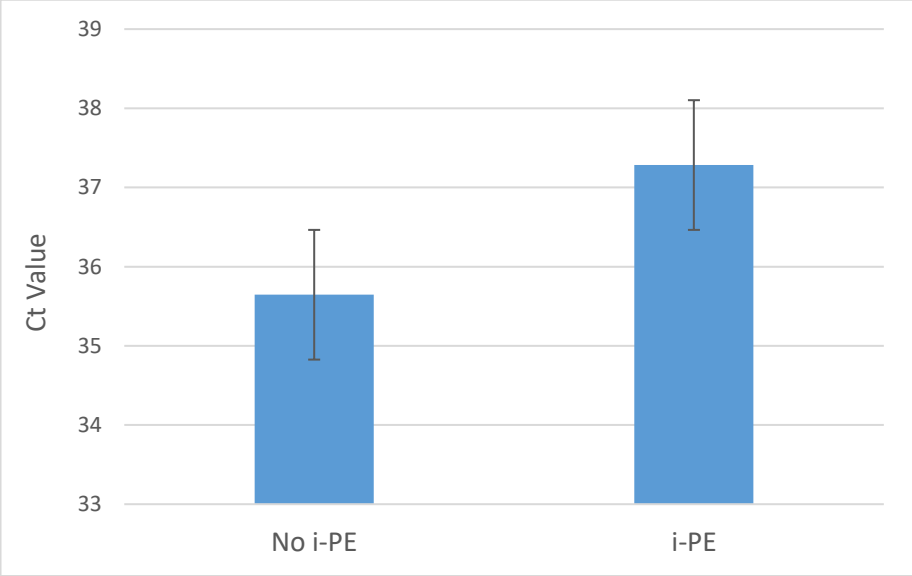


Figure 7.7 Differences of Ct values for PAR-2 between cancer tissues.

The figure shows no significant difference between the two groups. (Data are representative of 3 independent experiments and expressed as means ± SD, (IPE Sample vs no i-PE sample $p > 0.5$)

7.8.8 Examination of TF and PAR-2 protein level in FFPE cancer tissues

To analyse any correlation between the amount of mRNA and the protein level of tissue factor and PAR-2, protein extracts from the FFPE tissues were analysed using the Bradford assay. The concentration of total protein ranged from 0.17 to 0.3 µg/ µl. Ideally for SDS-PAGE analysis 20 µg load/well of protein is needed. It was unfeasible to load enough protein therefore I was unable to detect any signal of proteins under investigation.

For this reason, protein precipitation Kit/ National diagnostics, (EC-888) was used as described in section 7.6.2.8. However, the precipitated proteins could not be dissolved in the required buffer making it unfeasible to use.

7.8.9 Optimisation of TF protein extraction and measurements

7.8.9.1 Optimisation of protein extraction from breast cancer cells (MDA-231)

Qproteome FFPE Tissue Kit was used according to the manufacture instructions. 10^6 MDA-MB-231 cells were cultured in DMEM medium containing 10 % FCS. Cells were incubated at 37°C under 5 % CO_2 and supplemented every 3 days by replacing 3 ml of culture medium with a fresh medium until 80 % confluence. Cells were washed, trypsinised, and pelleted as described in section 8.3.3. Cells then washed with PBS and centrifuged for 5min. 100 µl Extraction Buffer EXB Plus supplemented with β-mercaptoethanol was pipetted into the tube contains the cell pellet and mixed by vortexing and incubated on ice for 5 min then mixed again by vortexing. The tube then incubated for 20 min at 100 ^oC on a heating block. The tube was then incubated in a water bath at 80 ^oC for 2hr with agitation. The tube was then incubated at 4 ^oC for 1 min then centrifuged for 15 min at 14,000 x g at 4 °C. The supernatant containing the extracted proteins was transferred to a new 1.5 ml tube. Protein samples were either used or stored at -70 ^oC until used.

The protein extract was then separated by 12 % (w/v) SDS/PAGE as described in 7.6.2.10. After separation, the proteins were transferred onto nitrocellulose membrane, followed by western blotting as described in section 7.6.2.11.

The first result for the analysis of protein extracted from cells shows multiple bands on western blot suggesting protein degradation. However, in the second run, I was able to get a clear band (Figure 7.9 A/ B).

7.8.9.2 Optimisation of protein extraction from xenograft cancer tissues

Xenograft cancer tissue samples were used for protein extraction as described in section 7.6.2.7 and in section 7.6.2.7. These samples were either fresh stored in RNase free water, or recently fixed in formaldehyde or recently formaldehyde fixed and paraffin-embedded.

The amount of total protein generated varied according to the tissue fixation as measured by Bradford assay in section 7.6.2.9. The results show that that formaldehyde-fixed tissues and formaldehyde-fixed paraffin-embedded tissues generate the lowest protein amount (Table 7.12 and Figure 7.10).

Using the SDS gel separation and western blot analysis for tissue factor, as described in section 7.6.2.10 and 7.6.2.11 the results show that the bands generated from protein extracts from xenograft tissues were not clear compared to the positive control (Figure 7.11). Whereas, protein extracts from the study tissue samples (FFPE) show no bands (Figure 7.12), indicating that tissue fixation leads to diminishing the amount of protein extracted from these tissues.

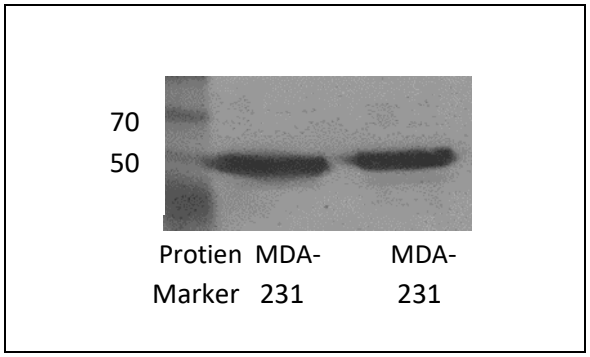


Figure 7.8 Examination of TF protein in Breast cancer cells MDA-231.

MDA-231 breast cancer cell line was cultured in DMEM medium containing 10 % FCS until 80 % confluence. The cells were then lysed using the Q proteome FFPE Tissue Kit and the protein extract was separated by 12 % (w/v) SDS/PAGE. After separation, Western blot analysis was carried out using rabbit anti-human TF antibody diluted in TBST at dilutions of 1:1000. Results show TF bands at a molecular weight of 50. (n=3 independent experiments)

Table 7.13 Protein level

Sample	Protein level as measured by Bradford assay µg/ml
Sample (1) Ovarian cancer tissue stored in 70% ethanol	115.1
Sample (2) Ovarian cancer tissue stored in RNase free water	131.1
Sample (3) Malignant melanoma cancer tissue stored in formaldehyde solution	90.2
Sample (4) formaldehyde fixed paraffin embedded Ovarian tissue, recently fixed	52.85
Sample (5) formaldehyde fixed paraffin embedded ovarian cancer tissue, recently fixed	83.72

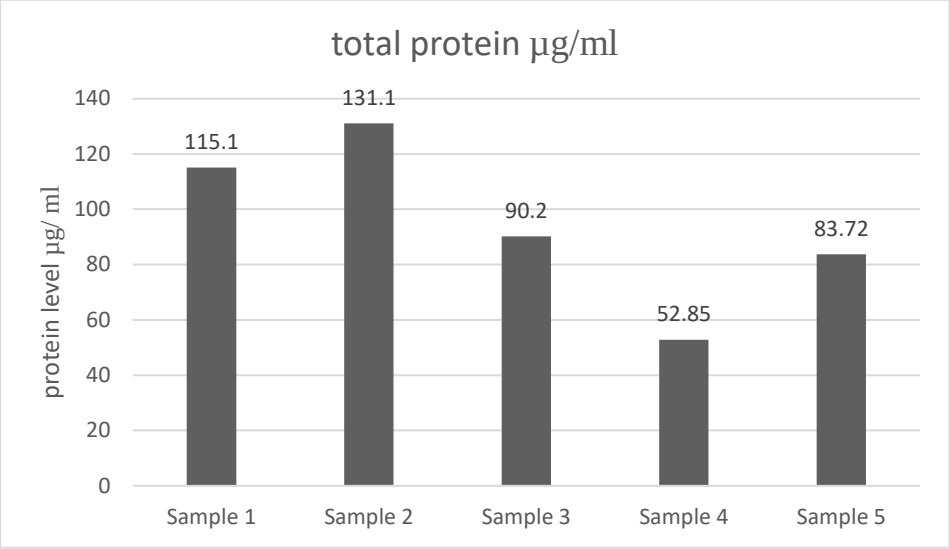


Figure 7.9. Total protein generated from tissue samples as measured by Bradford assay.

Xenograft cancer tissues either fresh in RNase free water or recently fixed in formaldehyde were lysed using the Q proteome FFPE Tissue Kit, and the protein extracts were analysed using Bradford assay. The figure shows that tissues which recently fixed generated the least amount of total protein compared to fresh tissue. Sample 1, Ovarian cancer in RNase free water, Sample 2; Ovarian cancer in 70% ethanol, Sample 3; Malignant melanoma in formaldehyde, Sample 4; Ovarian tissue FFPE, Sample 5; Ovarian tissue FFPE

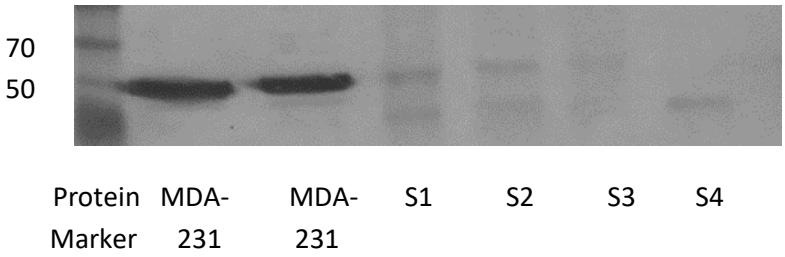


Figure 7.10. Examination of TF protein in xenograft cancer tissues

Protein extracts from Breast cancer MDA-231 (positive control) and fresh Ovarian xenograft cancer tissues (S1, S2) and FFPE ovarian xenograft cancer tissues (S3, S4) were separated by 12 % (w/v) SDS/PAGE. After separation, The samples were analysed by western blotting using rabbit anti-human TF antibody diluted in TBST at dilutions of 1:1000. Results show TF bands at a molecular weight of 50.

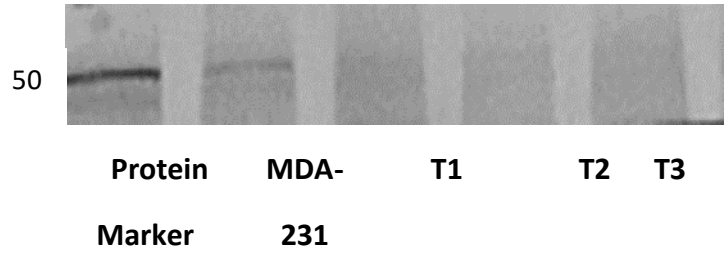


Figure 7.11. Examination of TF protein in FFPE cancer tissues

Protein extracts from MDA-231 (positive control) and from FFPE cancer tissues (T1,T2,T3) were separated by 12 % (w/v) SDS/PAGE. After separation, the proteins were transferred onto a nitrocellulose membrane. Western blot analysis was carried out using antibodies to determine TF protein using a rabbit anti-human TF antibody diluted in TBST at dilutions of 1:1000. TF band at a molecular weight of 50 for MDA-MB-231 and no bands detected for the tissue samples.

7.9 Discussion

In this retrospective study, I investigated the level mRNA and proteins of tissue factor and protease-activated recepter-2 in gastrointestinal cancer tissues (formaldehyde-fixed paraffin-embedded tissues) to determine their potential use in risk assessment for the diagnosis of i-PE. Although the sample size is small and only one cancer type was investigated, these initial data show a significant difference in the amount of TF-mRNA in the tissues of patients with cancer and i-PE comparing to matched controls.

In this retrospective study, the sample was retrieved from the Hull i-PE database. Two hundred sequential patients (100%) were screened for eligibility according to the inclusion criteria. Twenty-eight samples (14%) of gastrointestinal cancer were successfully matched and analysed.

Forty-six formaldehyde fixed paraffin embedded cancer gastrointestinal tissues samples of i-PE and non-i-PE cases were blindly analysed for TF/ PAR-2 mRNA and proteins level. After un-blinding I ended up with only 20 samples (10 i-PE and 10 non-PE matched controls) represents 2.5 % that met the inclusion criteria of the whole sample. Sample lost represent a drawback in this study and highlights the challenges in identifying tissue samples. 18 samples were excluded from the analysis due to the difficulties in the sampling procedure, as they were either, not retrievable, normal tissue, tumour amount not enough, no matching controls available. These difficulties will be addressed in the next section.

The results of this chapter show that the level of TF-mRNA in gastrointestinal cancer tissues from patients diagnosed with i-PE was significantly higher than matched controls and indicate that the development of i-PE in these selected patients may be influenced by the ability of cancer tissues to express tissue factor antigen.

Expression of TF in colorectal cancer has been analysed and reported previously immune-histologically (497). Recent clinical studies have mainly focused on the relations between TF expression, tumour progression, and histologic grade of

malignancy (486). TF has been shown to be involved in tumour growth and metastasis, in addition to its proposed role in cancer-associated thrombosis.

It is well documented that tissue factor is the principal initiator of blood coagulation, it is a transmembrane protein that can form a complex with coagulation factor VII (FVII). Accumulating evidence has shown that TF plays an important role in tumour growth, inflammation, angiogenesis, tumour invasion, and metastasis (155, 171, 480). GI cancers are among many types of cancer that express TF at a high level (including breast, glioma, lung cancer, and leukaemia) which are linked to the invasive potential, the progression of cancer, and may serve as a useful prognostic factor of tumour recurrence (480, 498). Furthermore, recent studies have revealed that the elevated risk of thrombosis in cancer is due to multiple risk factors (patients' related, cancer type related and therapeutic related) (251).

Recently marked progress has been made in determine laboratory parameters or biomarkers to predict the risk of cancer-associated thrombosis aiming to help stratify patients according to their thrombosis risk and facilitate the decisions of prophylaxis. The international guidelines recommend primary thromboprophylaxis in patients with cancer only in specific settings such as during hospitalization or after major cancer surgery (89, 499).

One way to identify patients at high risk of venous thrombosis by deploying risk scoring model using clinical and laboratory parameters. Available risk scoring includes; Khorana score, which incorporated the site of cancer, platelet count, haemoglobin, and/or use of erythropoiesis-stimulating agents, leukocyte count, and BMI, used for prediction of symptomatic VTE during chemotherapy (90), Vienna score which additionally includes two biomarkers (soluble P-selectin and D-Dimer) (114).

Furthermore, in cancer patients, a predictive potential of TF-bearing MPs was also reported in serially measured MP-associated TF activity levels before the occurrence of venous thrombosis (174). However, no significant association observed in patients with gastric, colorectal and brain cancers as reported in the (CATS) study (25). While there was a trend for predictive value of higher MP-TF

activity for VTE in pancreatic cancer patients (500). Most of the research previously has focused on measuring TF activity or MVs in patient's plasms, which represented a heterogeneous and in some cases controversial results (501, 502). However, at the time of writing these results, no study has reported on the association between the TF or PAR-2 mRNA levels in cancer tissues and the risk of thrombosis. The results of this study demonstrate that there is a relation between the level of TF-mRNA in cancer tissues and the risk of incidentally diagnosed PE in GI cancer, which may reflect the ability of cancer tissues to express TF.

In the post-genomic era, it is well acknowledged that protein profile changes are important reflectors of biological and clinical phenomena. Proteins are key effector molecules influencing pathological conditions, and the use of tissues are favourable on biological fluids, as investigating direct at the pathology site has several advantages, e.g., higher concentrations of disease-specific proteins. Although fresh or fresh frozen clinical specimens are ideal for proteomic analysis, the limited availability represents a serious drawback in research (503).

Although the study aimed to further examine the correlation between the TF/ PAR-2 mRNA and protein level in formaldehyde-fixed paraffin-embedded cancer tissues and the risk of thrombosis, I was not able to measure the TF/ PAR-2 protein level in these samples, which could be explained due technical issues and samples degradation which I will discuss in the next section. However, this limitation in the detection of proteins extracted from FFPE tissues was documented previously. Ahram et al. (2003) showed that the application of 2D gels to ethanol-fixed paraffin-embedded tissues was more successful than using tissues fixed with formaldehyde (504). Ono et al. (2009) did use 2D DIGE to compare the FFPE proteome of uterine cervix squamous cell carcinoma and healthy tissues, show a low-quality 2D map with smears (505). Donadio et al. (2011) as well applied 2D electrophoresis using FFPE material from patients with sporadic primary hyperparathyroidism and found a low-quality pattern of proteins with smears (506). However, the research groups of Alessandro Tanca and Maria Filippa Addis did manage to successfully separate proteins extracted from FFPE tissues using 2D gel electrophoresis (507, 508) but they report that identification of peptides decreases, as the fixation time increases (509).

The optimisation experiments revealed that the amount of total protein yielded from protein extraction was highly affected by the fixation method. Although the xenograft tissues were recently fixed in FFPE (only for few days), these tissues provided the least amount of total protein compared to fresh tissue samples stored in RNase free water. Formaldehyde fixation affected the protein yield and so affected the bands generated during western blotting. Furthermore, and due to the low amount of total protein, it was unfeasible to load enough protein in western blotting. These challenges were obvious when I started working on the study samples. It was clear that getting enough protein was unlikely, given the fact that these samples were too old and been fixed for long time.

Owing to its cross-linking effects, it is acknowledged that formalin fixation of tissues represents a barrier that negatively affects protein extraction and profiling (504). Furthermore, the sample quality and the amount of tumour contained within the tissue collected play a significant role in the amount of protein yielded. Acknowledging the side effects of long fixation time and protein cross-linking as discussed (section 7.10 challenges and limitations) this analysis was dismissed in this study.

The use of formalin as a fixative has been standard in the clinical routine for quite a long time (510). Fixation times in clinical routine may vary from tissue sample to tissue sample influenced by practical and organisational reasons. Owing to its cross-linking effects, it is currently acknowledged that formalin fixation of routinely processed tissues represents a barrier that negatively affects protein extraction and profiling (511). Furthermore, current research reported that protein cross-linking due to formalin fixation prevents protein profiling by western blot and protein microarrays (512, 513). Immunohistochemistry is a routinely used method for protein analysis in FFPE tissues but it is notoriously difficult to quantify. In addition, the analysis of membrane proteins was found to be difficult. This is an important point because a number of current treatments are directed against membrane proteins, e.g. a recombinant monoclonal antibody (514).

Furthermore, the correlation between mRNA and protein abundance depends on various biological and technical factors. The literature reports a poor correlation between mRNA and its relevant protein level and recommend to check both levels

(515). In research it is expected to see high protein level with high mRNA level, however, the protein concentration is affected by many steps in transcription and translation as well as degradation. One may find low mRNA expression and high levels of protein (if the protein directly influences its expression, negative feedback (downregulate mRNA expression) (516, 517).

7.10 Challenges and limitations:

7.10.1 Sample identification:

One of the major challenges in this study was sample identification of appropriate tumour tissue for the effectiveness of these assays. We opted for resected primary so that ample tissue would be available to allow extensive testing however the weakness of the quality of the target markers in these tissues which would have been best preserved if it were fresh and frozen, could not been mitigated. The nature of the IPE as an event risk makes this impossible. Assuming a 4-5 % risk of an I-PE one would have to store frozen tissue from 200 cancer patients to be able to study 10 I-PEs; furthermore, if one went down the route of attempting to exclude all other risk factors of VTE that could confound (or dilute) the biological drivers, one would need to store a few thousand frozen primary cancers to be able to replicate this study with frozen samples. Therefore, all the samples retrieved were formaldehyde fixed paraffin embedded, which may affect the material quality.

The specific 'quality effects' effects on this study from the sample drawbacks are as follows:

7.10.2 Tissue sample quality:

Many factors affect the tissue sample quality needed to be addressed. Factors including the tissue quality, the amount of tumour contained within the tissue collected as well as the yield and integrity of the genomic material extracted (518, 519). It has been advised that, to minimize the preanalytical variability, an appropriate method of sample handling and preservation needs to be defined and implanted up-front according to the type of downstream molecular profiling to be

performed. The other challenge was ensuring that the available samples are sufficient and able to provide high quality nucleic acids in sufficient amounts, to allow the analysis and interpretation of the genomic data (518, 520). However, this was not feasible in this study as it is a retrospective design, therefore, I had to depend on what already available at the histopathology department.

7.10.3 Tissue preservation

The use of formalin-fixed tissues instead of frozen tissues has many advantages and disadvantages. Its advantages manifested in: (a) it is a routine method of all Institutes of Pathology worldwide; (b) standardized technique; (c) broadly available; (d) linkage of molecular data with clinical information; (e) tissues of rare diseases are available; (f) cheap; (g) easy storage of tissues for many years or even decades (510). However, one of the disadvantages of formalin fixation presented in the difficulties in accessing the proteome locked within FFPE tissues which proved to be daunting task. It is well known that , formaldehyde fixation causing a molecular crosslinking that limits protein extraction efficiency, impairs immunoreactivity, and results in ambiguous identification of proteins by standard techniques (521).

7.10.4 Antigen retrieval

Crosslinking represents a major obstacle to be overcome for protein extraction. Current methods for antigen retrieval include proteinase predigestion (522) chemical pre-treatment (523) and heat-induced epitope retrieval (524) are used. However, heat-induced antigen retrieval technique represent a vapourable technique used by researchers (524). Nonetheless, extracting full-length protein, and specially membrane proteins found to be difficult (525) and yet is not as good as from fresh frozen tissues. Tissue factor and PAR-2 proteins are trans-membrane proteins, that they may be negatively affected by tissue manipulation and storage.

Extraction buffer and optimal extraction environment for FFPE tissue protein represent another challenge in the protein extraction process. Extraction buffer containing 2 % SDS has been in use for long time and proved to be effective in improving the quality and he quality of protein extracted (526). Many research groups produce their own extraction buffer to achieve better results, however, the

QProteome FFPE Tissue kit (Qiagen, Hilden, Germany) is commercially available. The QProteome FFPE Tissue Kit was released in 2006, and its use was described by Becker and co-workers, they were able to obtain full-length protein using this commercial extraction kit from tissues of different origins, and successfully subjected to WB and reverse-phase protein arrays (RPPA) (527). Although, extraction obtained was varied based on the tissue type (528).

7.10.5 Western blot

The next limitation of this study was applying the western blot assay for the protein extract. In order to obtain quantitative data from western blots, a rigorous methodology needed be applied (529). It is well known that western blot has been in use as a semi-quantitative measures for proteins for more than three decades (530). However, this multi-step procedure associated with many challenges. From sample preparation, SDS– PAGE gel loading, protein transfer, primary and secondary antibody selection, incubations, and washes, detection method selection to densitometric analysis.

Loading the right amount of protein was one of the challenges I faced during this study. Typically, 10-100 µg is needed to load per each well, however, I was unable to reach this required amount, which could be related to the extraction procedure from the start. Limitation in loading the right amount of protein may affected the entire procedure.

7.10.6 Other challenges

Tissue sample quality represent a major limitation in this study where I had to exclude a significant number of samples based on the tissue quality, some were normal tissues, others contain not enough cancer, and some were fat tissues. As a retrospective study this means that the results are highly dependent on what was already available in the tissue bank at that time, rather that collect the required tissues as needed prospectively sample prospectively verify the availability of the tissue before storing (frozen or paraffin).

Further limitation of the small sample size is that it might affect the results obtained. The lack of normal tissues make it difficult to make any comparison

between tissues from patients with cancer and i-PE and normal controls. To verify the relevance of these data, further study with large number of patients with GI cancer will be needed.

Finally, the population is IPE which is a subgroup of VTE therefore we cannot be sure that these findings will also relate to DVT.

7.10.7 Strengths of the is study

Despite the small sample the endpoint we found was a primary endpoint from a hypothesis generated by previous in vitro work and not the result of multiple testing. By its nature it is difficult to know how much of a VTE is attributable to the cancer state (surgery, hospitalization etc.) how much co-existing conventional risk factors (such as gender and age) and how much to the tumour biology. We think this is a major drawback of previous clinical correlative studies of TF markers (whether tissue staining, plasma, serum) to the VTE, where the risks of other factors that affect the cancer patient and may be contributing to the embolic event are not excluded. We went to enormous lengths to exclude these. We also studied only metastatic disease to make sure that the relevance of studying the primary lesion was not lost. Despite not having verification that the metastases in these patients had high TF mRNA we can say that this is likely to be the case as the histological variability between primary and secondary cancers in the GI tract is less than that seen for other malignancies (such as melanoma renal or lung). Additionally, we can be confident that these patients being ambulant were not impacted by other co-factors such as hospitalization, sepsis, surgery etc. With both groups of patients being metastatic one can assume that they also were receiving similar treatments- though data to this extent were not captured as not being part of the protocol. Finally, we studied only the patients with a proximal i-PE to avoid the controversy of the subsegmental /segmental group as outlined elsewhere and are confident that our data reflect the PE spectrum rather than only the IPE one.

7.11 Conclusion

In conclusion, these small data demonstrate a relation between TF-mRNA expression and clinical i-PE development in patients with GI cancer. This study highlighted the potential use of TF-mRNA as a predictive biomarker for cancer

thrombosis. These findings contribute to the available research aiming to identify cancer patients who are at high risk of thrombosis.

The next chapter an analysis of role of FXa in alteration of endothelial cells monolayer permeability by activating PAR-2.

Chapter 8 The Influence of Coagulation Protease factor Xa on Human Coronary Artery Endothelial cells permeability

8.1 Introduction:

In vivo, endothelial cells subjected to conditions of haemostasis or thrombosis is faced with cascade of coagulation proteases and not with single factors. However, very little is known of the human coronary artery endothelial cells (HCAECs) responses under coagulopathic conditions, when all these proteases are simultaneously generated. This chapter presents a single cell data, investigating the effects of Fxa on endothelial cell barrier and the role of Direct oral anticoagulation (DOACs / anti FXa).

The importance of the endothelium in cancer is well recognised and so is the fact that cancer is a hypercoagulable state(531). Furthermore, there is growing evidence that the two are linked, and that upon activation, endothelial cells can promote thrombosis and possibly angiogenesis which are the hallmarks of cancer progression, this link may also be causative in that changes to vascular biology in cancer lead to an increased risk of thrombosis(532).

Normally, non-activated, healthy endothelium forms a dynamic component of the barrier between blood and surrounding tissues and provides an antithrombotic and anticoagulant surface for flowing blood. Endothelial cells are able to produce and secret a wide variety of molecules and mediators involved in controlling thrombosis by sequestering thrombin and suppressing platelet activity; including von Willebrand factor, prostacyclin, nitric oxide, thrombomodulin and tissue factor inhibitors (533, 534). Unlike extravascular cells, endothelial cells normally do not express the primary trigger of the coagulation system namely tissue factor that acts as a receptor for Factor VII or thromboplastin(240).

In addition to its role in the regulation of haemostasis, ECs are involved in different tasks, such as maintaining tissue fluid balance, regulating permeability, host defence, regulation of vascular tone, angiogenesis and immunity through dynamically opening intercellular junctions which is performed either by all the endothelial cells in general or predominantly by endothelial cells in specific subsets

of organs or vascular beds (535-537). The unperturbed endothelial barrier has restrictive properties that are due primarily to closed IEJs.

As shown in figure 8.1 endothelial cells are connected to each other by a complex set of inter endothelial junctional proteins (IEJs) that comprise tight junctions (TJs), adherence junctions (AJs), and gap junctions (GJs). Whereas GJs form transmembrane channels between continuous cells, TJs and AJs form pericellular zipper like structures along the cell border through their transmembrane homophilic adhesion (537). In some cases like in inflammation, cancer or hypertension the endothelium cells become leaky and express thrombogenic molecules that promote clot formation. Loss of endothelial cell integrity and selective permeability as a result of vessel wall damage may facilitate of trans-endothelial migration of cancer cells during metastasis. (537, 538) In patients with cancer endothelial cells are significantly exposed to thrombogenic proteins secreted by tumours, e.g.; thrombin, VEGF, FXa, TF, and FVII.

The interactions between components of the haemostatic system and cancer cells are multifaceted. Strong evidence is available on the prothrombotic tendency of cancer patients, which is enhanced by anticancer therapy, such as surgery and chemotherapy. The mechanisms of thrombus promotion in malignancy include some general responses of the host to the tumour (acute phase, inflammation, angiogenesis) and specific interactions of tumour cells with the clotting/fibrinolysis systems and with blood (leukocytes, platelets) or vascular cells.(8)

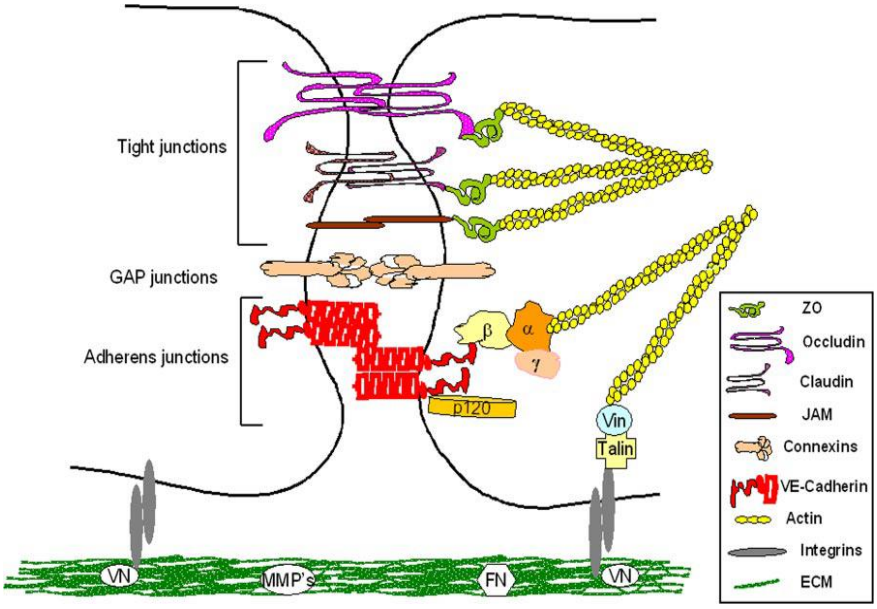


Figure 8.1 Structural organization of endothelial cell intercellular and matrix interactions.

The figure shows a typical arrangement of IEJs (comprising tight junctions, adherence junctions, and gap junctions) and integrin receptors by which endothelial cells adhere to each other and to ECM to maintain barrier function and intercellular communications. Figure adapted from Mechanisms regulating endothelial permeability (536)

Factor Xa (FXa) represents an important player in the coagulation cascade responsible for thrombin generation. More recently, FXa emerged as an essential player in cell biology via activation of protease-activated receptors (PAR)-1 and -2. Moreover, factor Xa forms a complex with TF–factor-VIIa complex that activates PAR 2 to initiate signalling responses in endothelial and other cell types.(9) This pleiotropic activity of FXa forms the basis for its potential contribution to the pathogenesis of several diseases.

Analysis of various types of human cancer tissues has demonstrated increased expression of both PAR-1 and PAR-2, compared to normal tissues (539, 540).

The mechanistic details of the specificity of PAR signalling by coagulation proteases are the subject of extensive investigation by many research groups worldwide. However, analysis of PAR signalling data in the literature has proved to be challenging since a single coagulation protease can elicit different signalling responses through activation of the same PAR receptor in endothelial cells. (539)

8.2 Aim

The aim of this chapter was to determine the following:

1- The effects of PAR2 activated by Fxa on HCAEC permeability
2- The effects of Fxa on HCAEC permeability at different doses
3- The effect of DOACs (Rivaroxaban/ Apixaban) on HCAEC permeability

8.3 Methods

8.3.1 Human coronary artery endothelial cells Culture and passaging

Cryopreserved human coronary artery endothelial cells (HCAEC) were cultured under sterile conditions in Promocell endothelial cell medium MV 2. All reagents were pre-warmed to 37°C prior to use. Complete HCAEC medium was prepared by mixing MV2 base medium with Promocell endothelial cell growth medium MV 2 supplement mix, with a final supplement concentration of (per ml): 5 % (v/v) foetal calf serum (FCS) and contained epidermal growth factor (recombinant human) (5 ng/ml), insulin-like growth factor (Long R3 IGF) (20 ng/ml), basic fibroblast growth factor (recombinant human) (10 ng/ml), vascular endothelial growth factor 165 (0.5 ng/ml), ascorbic acid (1 µg/ml), and hydrocortisone (0.2

µg/ml). The cells were cultured in T25 or T75 (25 cm²or 75 cm2) flasks with complete MV2 medium, incubated at 37°C under 5 % CO_2 and supplemented every 2 days by replacing of culture medium with fresh medium.

8.3.2 Culture of human breast cancer cell line MB231

The MDA-MB-231 breast cancer cell line (ATCC,Teddington, UK) was cultured in DMEM, containing 10% (v/v) foetal calf serum FCS in T75 (75 cm2) . Cells were incubated at 37°C under 5 % CO_2 and supplemented every 3 days by replacing culture medium with fresh medium.

8.3.3 Subculture, harvesting and counting of cells

Cells were grown in T75 flasks containing 25 ml fully-supplemented media and passaged approximately every 72-96 hours. Once the cells were 80- 90 % confluent, the medium was aspirated from the flask and the cells washed twice with 10 ml of warmed sterile phosphate buffered saline (PBS) (pH 7.2) to remove all trace of serum and to maintain the correct pH. The cells were then incubated at 37°C with 3 ml warm 0.25% w/v Trypsin-EDTA solution for 2-3 min to detach the cells. The flask was then tapped to dislodge the cells, and this was confirmed under a light microscope. The trypsin was neutralised with 3 ml fully-supplemented media. Cell suspension was then transferred to a 20 ml sterile tube and centrifuged at 400 g for 5 min at room temperature. The supernatant was carefully discarded without disturbing the pellet and the cells re-suspended in 3 ml of fresh medium and mixed to determine the cell density.

8.3.4 Cell counting

20 µl of cell suspension was loaded onto a haemocytometer and the number of cells in five 1mm2 squares counted. Then density of cells per ml was determined as the [average number of cells counted per mm² x dilution factor x 104]. This was taken to be the number of cells per ml and was then multiplied by the total volume of cell suspension to obtain the total number of cells.

8.3.5 Cryopreservation and recovery of cells

Cells were washed, trypsinised, and pelleted as described in section 2.3. The cell pellet was re-suspended in 3 ml of pre-warmed DMSO freeze medium. The cells

were mixed gently to ensure an even distribution and aliquoted into pre-labelled cryotubes at a density of 106 cells/tube. The tubes were placed in a freezing chamber surrounded with ethanol and frozen at -70°C overnight. The chamber allows the temperature to decrease gradually (-1°C/min) to retain optimal viability. Finally, the cryotubes were transferred to liquid nitrogen for long-term storage. To start new cultures from frozen cells, cryotubes containing the cells were thawed in a 37°C water bath, and immediately transferred to cell culture flasks containing pre-warmed medium at the required density.

8.3.6 Adaptation of HCAEC to serum-free medium

The complete culture medium contains FCS that allows rapid propagation of the cells. However, to prevent interaction between proteins under investigation and the supplements proteins cells were adapted to serum free medium for 2 hours before running the permeability assay.

8.3.7 Isolation of cancer cell-derived microparticles

The MDA-MB-231 breast cancer cell line known to express high levels of tissue factor, were used to obtain cell derived tissue factor-containing microparticles. Cells (~10^6 cells/well) were used. To obtain cell-derived microparticles, cells were washed twice with PBS then medium was replaced with serum free medium and incubated for a further 3 h. cells were treated with PAR2-agonist peptide (PAR2-AP) (20 µM) for around 90 min and the microparticles were isolated from the medium. The medium were then collected and centrifuged at 12,000 g for 20 min to pellet cell debris. The supernatant containing cell-derived microparticles was centrifuged at 100,000 g for 1 h at 20 °C to sediment the microparticles. Microparticles pellet were then resuspended in PBS (100 µl) and stored at -20 °C.

8.3.8 Determination of microparticles concentration using the Zymuphen microparticles assay kit

The kit contains reagent R1 (bovine FXa-FVa mixture, containing calcium), reagent R2 (purified human prothrombin), reagent R3 (thrombin specific chromogenic substrate) and microparticles calibrator. Microparticles samples were thawed and equilibrated at 37 ºC before the experiment. Samples of isolated microparticles (20

µl) were placed in the provided 96-well microplate. To create the calibration curve, the microparticles calibrator was diluted to a range covering 0-2.1 nM and equal volumes (20 µl) were placed in a 96-well microplate. R1 reagent (50 µl) was then added to the wells, followed by R2 reagent (25 µl) and allowed to incubate for 10 min at 37 °C. R3 reagent (25 µl) was then added to all the test samples and allowed to develop for exactly 5 min at 37 °C in the dark. The reaction was then terminated by adding 2 % (v/v) citric acid (25 µl) and the absorption was measured at 405 nm using a plate reader. Microparticles densities were then determined from the calibration curve.

8.3.9 Endothelial barrier permeability assay

An in vitro assay of endothelial barrier function was established as previously described (541, 542). Monolayer permeability was analysed in a dual-chamber system using dextran blue (MW 40x103). $6x10^4$ (HCAEC) human coronary artery endothelial cells were seeded on gelatine-coated transwell polycarbonate membranes of 3-µm pore size and 12-mm diameter (Costar®, Corning, NY). The upper and lower chambers were filled with 100 µl and 1000 µl growth medium, respectively. Cells were grown until confluence was obtained (4-6 days). Permeability was assayed using 1µg/mL dextran blue (Sigma, St Louis, MO) diluted in serum free growth medium. At the day of experiment, cells were washed twice with PBS before incubation with serum free medium for 120 minutes at 37 °C. For agonist treatment of the monolayers, medium of the upper chamber was replace with medium with dextran blue and agonists of interest then incubated at 37 C^o for time indicated then followed by analysis of permeability. After determined time points the optical density of the medium from the lower chamber were measured at 450 nm.

8.3.10 Analysing the effect of PAR 2 activation on EC monolayer permeability

HCAECs passage 4 were seeded on gelatine-coated transwell polycarbonate membranes at $6X10^4$ for 4 days till it reached a confluent monolayer with media changes every day. The cells then washed twice with PBS before incubated in Endothelium serum free medium for 2 hours. Blue dextran diluted in serum free

growth medium 1μg/mL was added to the upper chamber at a final, then the specific peptide agonist peptide PAR2-AP (SLIGKV-NH ; 20 μM or VEGF-A; 25 ng/ml) was added. Medium was harvested from the lower chamber after specific time points, and the permeability of Blue dextran was determined colorimetrically by absorbance at 450 nm. The mean from triplicate experimental wells was compared with the absorbance of wells of untreated cells (control).

8.3.11 Analysis of the influence of FXa on endothelial cell monolayer permeability at different doses and time points

HCAECs passage 4 were seeded on gelatine-coated transwell polycarbonate membranes at $6X10^4$ for 4 days till it reached a confluent monolayer with media changes every day were subject to FXa at two different concentrations (5nM and 10nM). The permeability to Blue dextran diluted in serum free growth medium 1μg/mL was measured at 450nm at specific time points (0, 30 and 60) minutes.

8.3.12 Determine the involvement of PAR 1 and PAR 2 receptor in FXa induced permeability of endothelial cell monolayer

HCAECs were grown in the same manner as described for the permeability assay in the section 8.3.9. Cells were incubated with serum free medium with PAR1 mouse monoclonal antibody (Thrombin R antibody, ATAP2/ 20 μg/ml)) or PAR 2 mouse monoclonal antibody (SAM 11/ 20 μg/ml)) for 30 minutes before activating with FXa (10 nM). Permeability assay was carried out as explained in section 8.3.9.

8.3.13 Examination of the effects of DOACs (Rivaroxaban and Apixaban) on FXa induced EC monolayer permeability

HCAECs were grown in the same manner as described for the permeability assay in the section 8.3.9. Cells then washed and incubated with FXa 10nM in the presence of Rivaroxaban (0.6μg/ml) or Apixaban (1μg/ml) at 37 °C for 30 and 60. The analysis of permeability was carried out as described in the permeability assay section 8.3.9.

8.3.14 Examination the effects of TF-MV on ECs permeability with and without fVIIa

Monolayers of HCAEC were prepared as indicated in section 8.3.9. Sets of cells were incubated with purified TF-containing microvesicles or with recombinant TF (1 U/ml), in the presence or absence of fVIIa (5 nM), and compared to treatment with fXa (10 nM) alone. The samples were incubated at 37 °C and examined at 60 or 120 min. the other sets of cells were incubated with purified TF-containing microvesicles or with recombinant TF (1 U/ml), in the presence of fVIIa (5 nM), and in the presence or absence of fXa (10 nM). The Blue dextran was added to a final concentration of 1 μg/ml to the upper chamber of each Transwell then the permeability assay then was carried out as in section 8.3.9

8.3.15 Examination the effects of Rivaroxaban on ECs permeability

Monolayers of HCAEC were prepared as indicated in section 8.3.9. A confluent monolayer was subject to Rivaroxaban treatment at concentration (0.06 μg/ml). The permeability to Blue dextran was measured at 30 and 60 minutes as indicated in section 8.3.9.

8.4 Statistical analysis

Data were collected from at least three wells are presented as mean ± standard error of the mean (S.E.M). Data were compared using one sample t-Test or one way ANOVA. A p-value <0.05 was considered to significant.

8.5 Results

8.5.1 Effects of activating PAR 2 on endothelial cell monolayer permeability comparing to the effect of VEGF-A

HCAECs monolayers formed a barrier which was, initially, virtually impermeable to the Blue dextran (MW 20X10^5) Sigma-Aldrich solution, with minimal flux through the intact monolayer detected during the first 30 minutes of incubation. Incubation of confluent HCAEC monolayers with VEGF-A (25 nmol/L) led to a progressive increase in monolayer permeability (n=3; P<0.05) similarly PAR2 agonist (SLIGKV-NH ; 20 nM) activation also led to increase in the permeability (n=3; P< 0.05) (Figure 8.2) and (Figure 8.3). The response was as early as within

15min, and it seems to continue beyond 45min. Activation of PAR2 using the agonist peptide (SLIGKV-NH; 20 μM) was capable of increasing the EC monolayer permeability to Blue dextran to a similar extent as that observed with the incubation with VEGF-A (25 ng/ml). The PAR2 agonist peptide used in this part of the investigation mimics the cleaved amino-terminus of the tethered ligands of PAR2 on ECs and promote its activation.

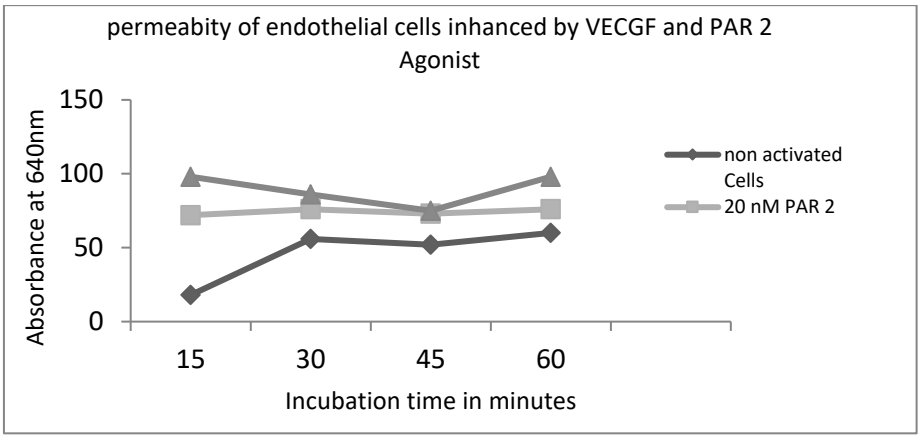


Figure 8.2 The Influence of PAR2 activation on the permeability of HCAEC monolayer.

HCAECs monolayer was activated with PAR2-AP (SLIGKV-NH; 20 µM) or VEGF-A (25 ng). Cells were incubated at 37°C for the required time. The transfer of Blue dextran was assessed by measuring the absorption at 450 nm at 0, 15, 30, 45, and 60 min. (n=3). Incubation of confluent HCAEC monolayers with VEGF-A/ PAR 2 agonist peptide led to increase in monolayer permeability

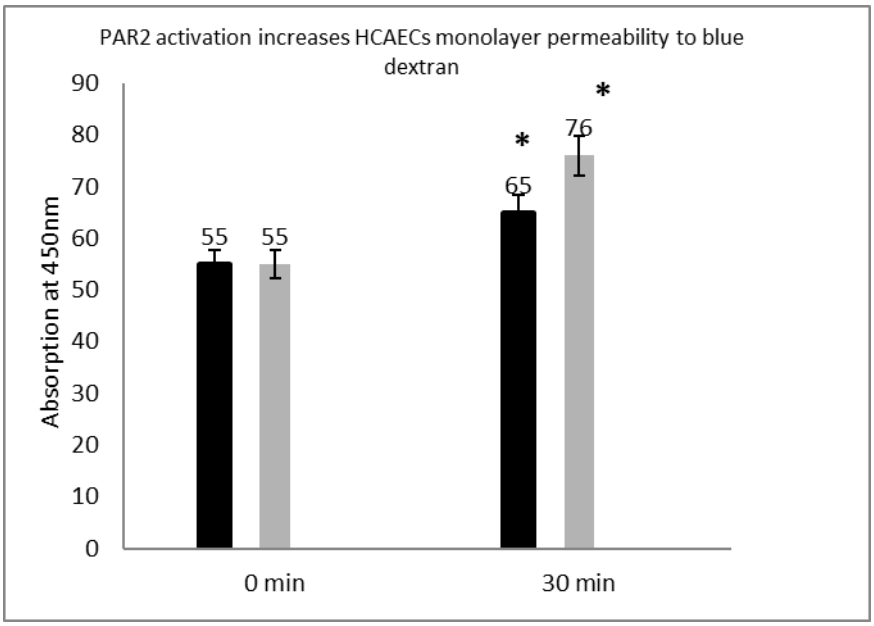


Figure 8.3 The influence of PAR-2 and VEGF activation on the permeability of HCAEC monolayer.

Monolayers of HCAEC were activated with PAR2-AP (SLIGKV-NH; 20 µM; Black bars) or VEGF-A (25 ng/ml; Grey bars) for 30 minutes. The transfer of Blue dextran was assessed by measuring the absorption at 450 nm at 0 and 30 min. Data were given as means (n=3; $P<0.05$).

8.5.2 Influence of FXa on endothelial cell monolayer permeability at different doses and two time points

The permeability of the endothelial cell layers was determined through the spectrophotometric measurement of the increase in absorbance in the sampled media (at 450 nm) in the lower chambers as a result of flux of the Blue dextran through the endothelial cell layer over 30 and 60 minutes. Incubation of HCAEC human coronary artery endothelial cells monolayer with FXa at concentration 10nM increased permeability to Blue dextran significantly. This was not seen at 5nM concentration (Figure 8.4). The effect on permeability at 10 nM of FXa had a comparable to PAR 2 peptide agonist. Remarkably, following a 30 min period of incubation with Fxa 10nM, the monolayers initially exhibited enhanced barrier activity with the degree of dextran blue flux observed to significantly reduced relative to that which was observed in the untreated monolayers (OD 450 nm at 30 minutes, 0.34±0.1; P<.01), suggesting that there is a differential, time dependent effect of FXa on basal endothelial barrier activity (Figure8.5).

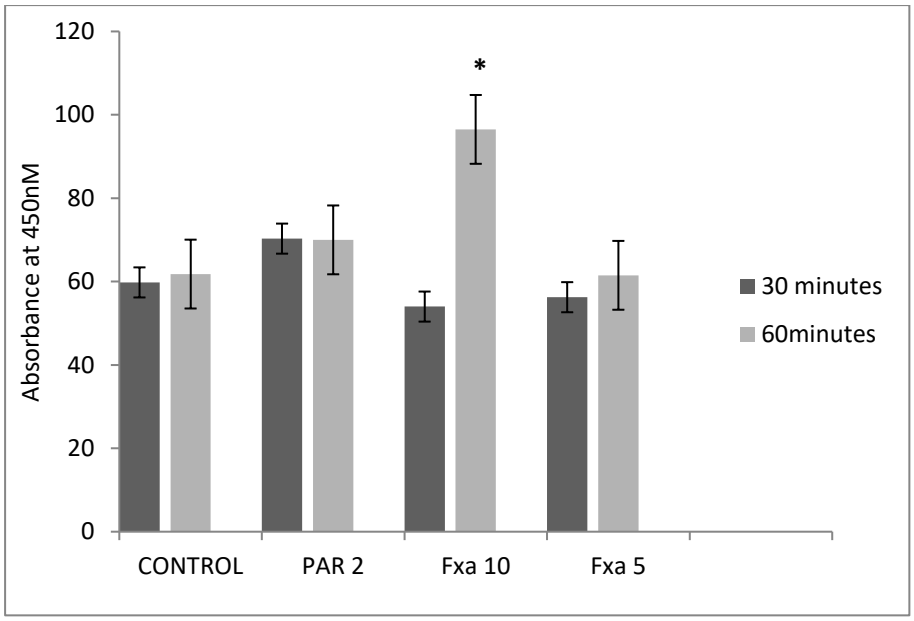


Figure 8.4 The influence of PAR-2 and Fxa activation on the permeability of HCAEC monolayer.

Monolayers of HCAEC were activated with PAR 2 peptide agonist (20 µM) or Fxa (5 or 10 nM). The transfer of dextran blue was assessed by measuring the absorption at 450 nm at 30 and 60 min. (n=3). Fxa at 10nM at 60 minutes has increased the permeability to dextran blue. * $P<0.05$

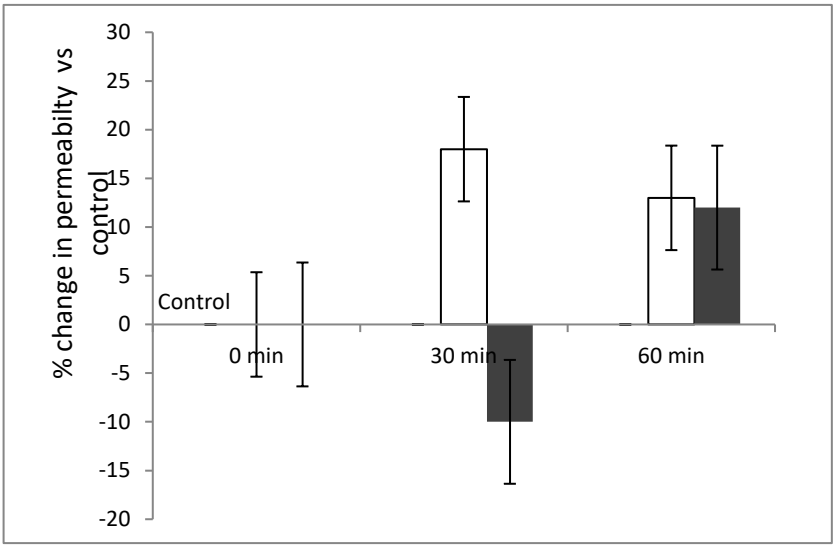


Figure 8.5 The influence of fXa on the permeability of HCAEC monolayer

Monolayers of HCAEC were activated with PAR2-AP (20 µM; white bars) or fXa (10 nM; grey bars). The transfer of Blue dextran was assessed by measuring the absorption at 450 nm at 30-60 min. The percentage change in permeability against the untreated samples was calculated. (n=3)

8.5.3 Examination of the involvement of PAR 1 and PAR 2 in FXa effect on permeability of endothelial cells monolayer

Fxa-mediated endothelial permeability is mainly PAR 2 dependent .Preincubation of HCAECs monolayer PAR2 antagonist (SAM11; 20 µg/mL) for 30 minutes before adding the FXa 10nM, significantly inhibited Fxa-induced permeability of ECs. While preincubation with PAR1antibody (ATAP2, thrombin receptor antibody; 20 µg/mL) did inhibit the effect of Fxa in the first 30 min. Confirming that Fxa-increased endothelial barrier permeability is entirely dependent on PAR-2 cleavage and the subsequent activation of PAR-2 intracellular signalling. (Figure 8.6).

8.5.4 Analysis of the effects of DOACs (Rivaroxaban and Apixaban) on FXa induced ECs monolayer permeability

8.5.4.1 Rivaroxaban

Rivaroxaban directly inhibits Fxa. Incubation of HCAECs monolayer with clinically relevant dose of Rivaroxaban significantly attenuated the Fxa-induced permeability to dextran blue. As expected Rivaroxaban has attenuated Fxa- enhanced EC permeability at both the 30 and 60 time points. (Figure 8.7) ($n=3$, $P<0.05$)

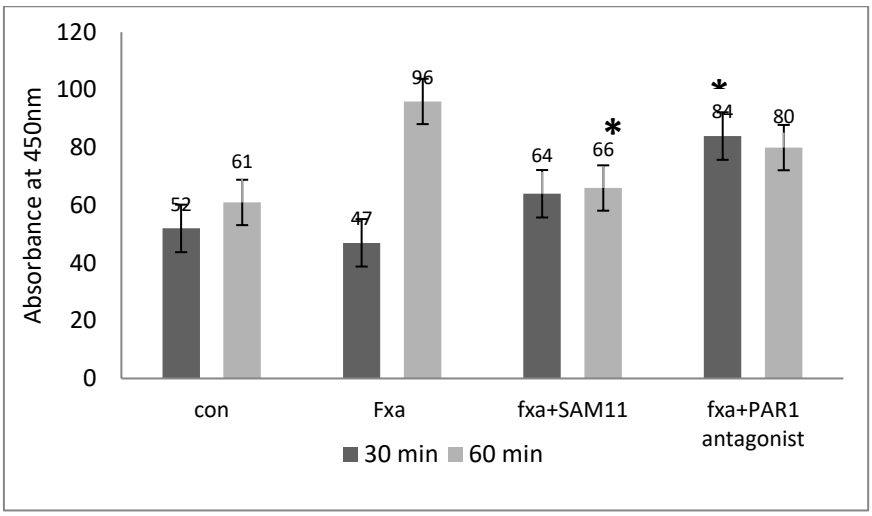


Figure 8.6 The effects of PAR 1/ 2 antagonist on the permeability of HCAEC monolayer

60X 103 ECS monolayer was preincubated with PAR2 antagonist (SAM 11; 20 µg/ml) or PAR1 antagonist (ATAP2, thrombin receptor antibody; 20 µg/mL) for 30 minutes before activation with Fxa (10nM). The transfer of Blue dextran was assessed by measuring the absorption at 450 nm at30 and 60 min. (The Fxa induced permeability has significantly inhibited with SAM11) (n=4; * = p = 0.05 vs. untreated sample)

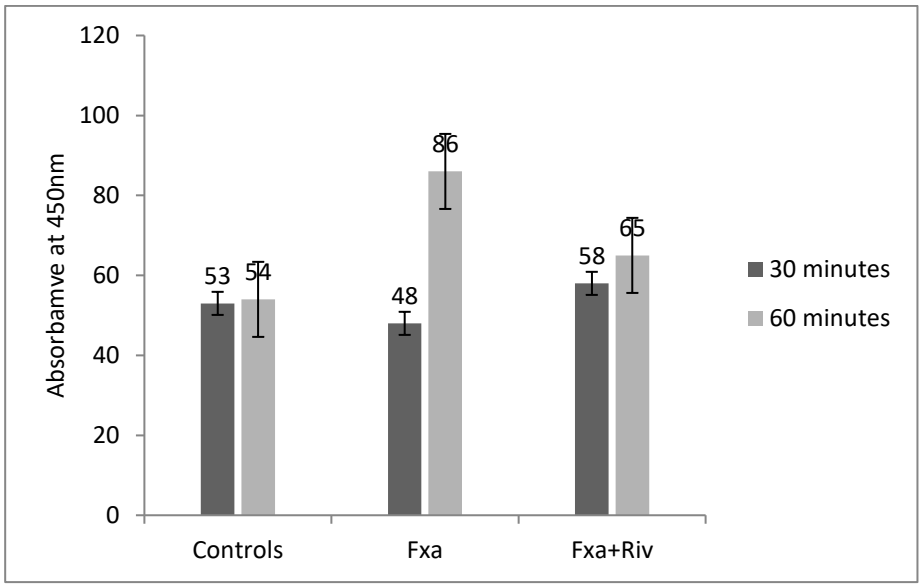


Figure 8.7 The influence of Rivaroxaban on fXa-mediated EC permeability

Monolayers of HCAEC were activated with fXa (10nM) alone, or fXa in the presence of Rivaroxaban (0.6 µg/ml). The transfer of dextran blue was assessed by measuring the absorption at 450 nm at 30 and 60 min. Data were presented in means ± SE (n=3; * = $p < 0.05$ vs. untreated sample)

8.5.4.2 Apixaban

To determine effects of Apixaban on the Fxa induced permeability across a functional EC monolayer, HCAECs were exposed to Apixaban at therapeutic dose in presence of Fxa. After 60 minutes the absorbance of dextran blue from the lower champers was measured in triplicate and compared to control. Apixaban has significantly inhibited the Fxa induced permeability but not completely inhibited. (Figure 8.8)

8.5.4.3 Examination of the effects of Rivaroxaban on ECs monolayer permeability

ECs monolayer was incubated with Rivaroxaban at therapeutic dose (0.6µg/ml). The permeability of the endothelial cell layers was determined through the spectrophotometric measurement of the increase in absorbance in the sampled media (at 450 nm) in the lower champers as a result of flux of the dextran blue through the endothelial cell layer over 60 minutes. Incubation of ECs with Rivaroxaban has significantly increased the ECs permeability to dextran blue. (Figure 8.8)

8.5.5 Influence of TF-MV on ECs permeability with and without VIIa

Since PAR 2 could be activated by TF-FVII complex. The ECs permeability was investigated in presence of TF-MV (10nM) with and without FVII (20nM). The permeability of the endothelial cell layers was determined through the spectrophotometric measurement of the increase in absorbance in the sampled media (at 450 nm) in the lower champers as a result of flux of the Blue dextran through the endothelial cell layer at 60 minutes. No significant effect has been detected at 60 min. (Figure 8.10)

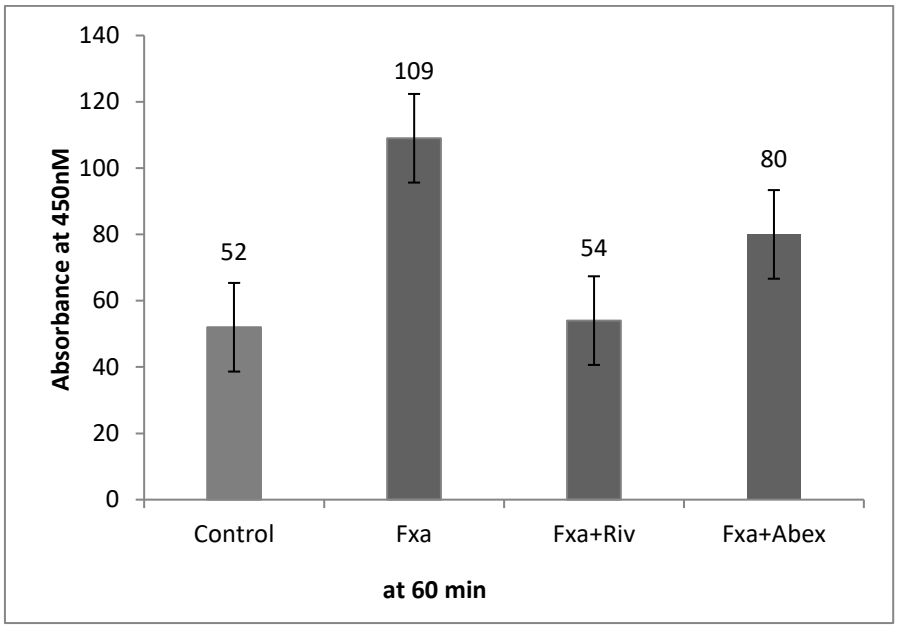


Figure 8.8 The influence of Apixaban on EC permeability

Monolayers of HCAEC were incubated with Apixaban (1 µg/ml). The transfer of dextran blue was assessed by measuring the absorption at 450 nm at 60 min. Data were presented in means (n=3; * = $p < 0.05$ vs. untreated sample)

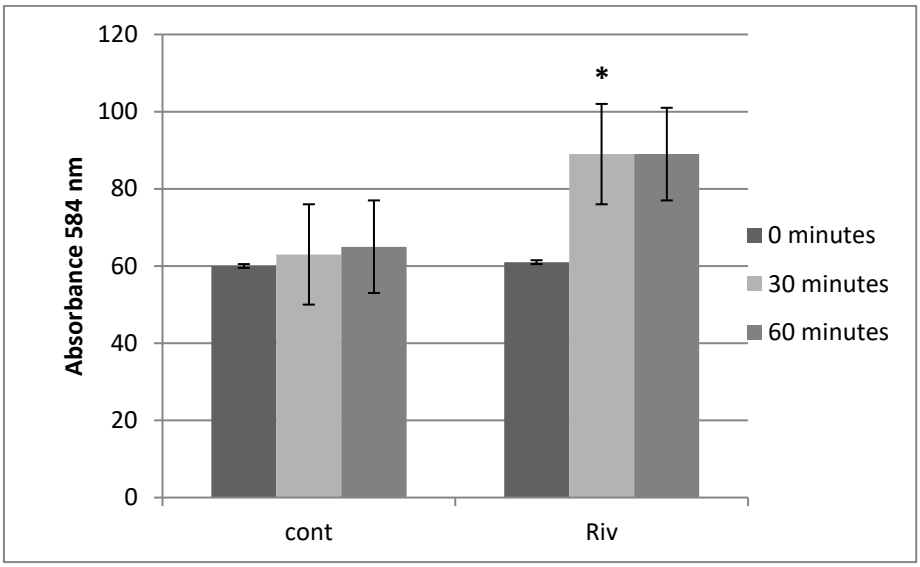


Figure 8.9 The influence of rivaroxaban on EC permeability

Monolayers of HCAEC were incubated with Rivaroxaban (0.6 µg/ml). The transfer of dextran blue was assessed by measuring the absorption at 450 nm at 60 min. Data were presented in means (n=3; * = $p < 0.05$ vs. untreated sample

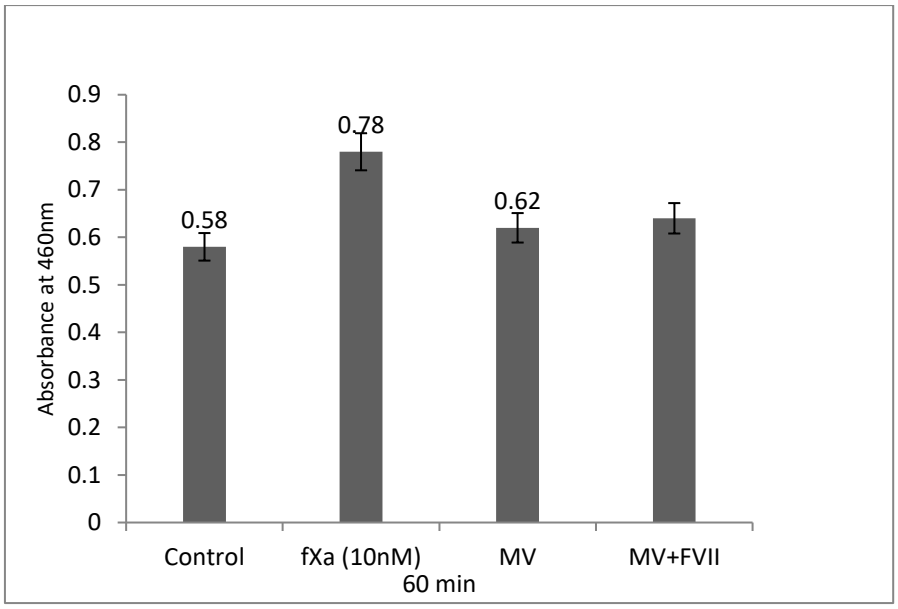


Figure 8.10 The influence of MVs with and with FVII on the permeability of HCAEC monolayer

Monolayers of HCAEC were activated with MVs (13nM) alone, or MV with FVII (20nM). The transfer of Blue dextran was assessed by measuring the absorption at 450 nm at 60 min. Data were presented in means (n=3; p=0.3)

8.6 Discussion

The results of this study demonstrate in vitro evidence suggesting that the exposure of endothelium cells to FXa leads to endothelial barrier disfunction and increase of permeability through activation of endothelial cells PAR2. The effect that significantly inhibited by DOACs.

A number of investigators have described several distinct mechanisms through which malignant tumors induce vascular permeability leading to enhanced metastasis. However, it was never related to thrombosis. It is well known that coagulation can be divided into three separate phases: 1) an initiation phase, in which low amounts of active coagulant factors are generated; 2) an amplification phase, in which the level of active coagulation factors is boosted; and 3) a propagation phase, in which coagulation factors bind to highly procoagulant membranes of activated platelets and fibrin clots are formed. Initiation phase, classically referred to as the extrinsic pathway of coagulation, starts when the vasculature is disrupted (in case of injury), and subendothelial cells like smooth muscle cells and fibroblasts become exposed to the bloodstream (543).

We therefore hypothesized that in resting endothelium, exposure of sub-endothelial cells TF to the blood stream may happen when endothelial cells get activated by FXa or PAR 2, that can increase endothelial barrier permeability. The results show that activating ECs PAR 2 with the agonist peptide (PAR 2-AP) results in increase cells monolayer permeability to dextran blue comparable to the effect of VEGF activation. It has been reported that both PAR2 and its-activating protenases, including tissue factor and trypsin, are aberrantly overexpressed in a variety of malignancies, and shown to be involved in many aspects of cancer biology including cell proliferation, angiogenesis, and metastasis (544). Accumulating evidence generated from recent in vitro and in vivo experimental studies strongly implicates an active role of PAR2 in tumour progression and development (545). Once activated, PARs can elicit a variety of cellular responses through multiple signalling cascade pathways(546) . However, the role of the other PARs in mediating increases in endothelial barrier permeability has not been fully elucidated.

Several distinct mechanisms through which malignant tumours induce vascular permeability have been described that upon inhibition in an animal model led to attenuate tumour-mediated vascular permeability and metastasis formation. Moreover, at the interface between the tumour cell and the vascular endothelium and the secretion of factors such as VEGF protein as well as the enhanced generation of thrombin also serves to induce loss of barrier integrity. Furthermore, it has been reported that the occupancy of endothelial protein C receptor (EPCR) by protein C was able to switch the signalling specificity of PAR2 from a barrier disruptive to a barrier protective effect in endothelial cells (547). This was evidenced by the observation that the PAR2 agonist peptide, SLIGKV, elicited a barrier disruptive response in HUVECs that could be effectively reversed to a protective response if cells were pre-treated with the protein C zymogen prior to stimulation by the PAR2 agonist peptide and pre-treatment of endothelial cells with either protein C or FX zymogen renders the signalling specificity of both PAR1 and PAR2 a protective one in endothelial cells (547).

Noteworthy, that *A. Dutra-Oliveira et al.2012* (6) reported PAR 2activation increased VEGF secretion in malignant glioma cell lines. It appears that all coagulation proteases capable of cleaving either PAR1 or PAR2 would elicit only protective signalling responses if cells express protein C and/or the putative factor X/Xa receptors and that the receptors are bound by their physiological ligands. Thus, this mechanism of co-receptor signalling plays a key role in determining the specificity of PAR1 and PAR2 signalling by coagulation proteases in endothelial cells. Furthermore, *Morris et al. (2006*) (4) has demonstrated that PAR-2 is essential for FXa-induced signalling, migration and invasion of breast cancer cells. These findings support the role of coagulant proteases like FXa in the development and progression of cancer in the tumour microenvironment.

However, analysis of PAR signalling data in the literature in Human Endothelial Cells has proved to be challenging since coagulation proteases can elicit paradoxical signalling responses through activation of the same PAR receptor in these cells. Based on these results, it has been advised that the physiological relevance of in vitro studies in cellular models, in particular those monitoring the signalling effects of PAR1 and PAR2 in the absence of co-receptor signalling, should

be interpreted with some caution.The results of this study show that FXa at 10nM induction of EC permeability is time dependent. At the first 30 minute time point Fxa shows enhancement of EC barrier, then at 60 minute time point the permeability of EC has increased significantly. The effect is reversed by PAR1 and 2 antagonists.

It is well known that FXa acting via PARs influences inflammation and thrombosis by activating a wide range of cell types including EC (548). Activated factor X (FXa) by TF expressed on tumor-derived MV could activate protease-activated receptors (PARs) on non-activated endothelial cells to induce a pro-adhesive and pro-inflammatory phenotype (549). Activation of endothelial cells by MV has been observed previously, resulting in increased vascular permeability, angiogenesis, and cancer metastasis (10–14); however, the mechanisms remain largely unknown(549). Endothelial responses induced by FXa have largely been ascribed to PAR-2 (550-552). Furthermore, FXa is a PAR-2 agonist (553) and PAR-2 activation has been linked to enhanced cell motility (545, 554). The role of FXa in signalling only recently attracted interest and a broad amount of data suggests that FXa mediates intracellular signalling via activation of PAR-1 and/or PAR-2. A picture emerges that FXa activates PAR-2 when engaged in the ternary TF/FVIIa/Fxa complex, whereas soluble FXa activates both PAR-1 and PAR-2. However, the actual receptor activated might be more complex and does not seem to rely solely on the fact whether FXa is in complex with TF/FVIIa or not. The cell type and the repertoire of receptors on individual cells seem of major importance for determining whether FXa signalling is PAR-1 or PAR-2 dependent (214).

However, in contrast to our results previouse reports demonstrate that FXa exerts a barrier protective effect in HUVECs in response to proinflammatory stimuli through activation of both PAR1 and PAR2 (246, 555). FXa elicits protective signalling responses in EC directly via PAR-2 and indirectly via endothelial protein C receptor (EPCR) dependent recruitment of PAR1 (555). And other studies have demonstrated that FXa can inhibit the barrier disruptive effect of proinflammatory cytokines in HUVECs through activation of PAR2 by a mechanism that was partly independent on the Gla-domain of the protease (552, 556). However, others have reported a proinflammatory role for FXa in similar cellular model systems (557-

559). Thus, the interesting feature is that different activators of the same receptor do not induce identical cellular responses (558, 560). Reasons of discrepancies in the results between different studies are not well understood.

In contrast to endothelial cell cultures, PAR1 appears to be the major mediator of Xa signalling in fibroblasts. Thus, different PARs appear to account for Xa signalling in different cell types, depending upon which PARs are expressed (19). Noteworthy, the molecular mechanism by which a receptor elicits distinct (and even opposite) cellular effects dependent on the ligand (or its concentration) is not completely understood and it is not the aim of this project, However, these contradictory results highlight the complexity of FXa signalling in the pathogenesis of cancer and will require future researches to explore the exact mechanisms of Fxa signalling functions in cancer biology.

As mentioned, TF is a key player in blood coagulation (3); we hypothesized that as a consequence of the disruption of the vessel wall (either by PAR2 directly or FXa) TF-expressing cells located in the underlying cell layers would be exposed to the bloodstream. Upon binding of activated factor VIIa (FVIIa), a coagulation factor circulating at low levels within the bloodstream, the so-formed TF/FVII complex initiates the extrinsic coagulation pathway; the TF/FVIIa complex proteolytically cleaves FX to FXa, which in turn converts prothrombin to thrombin. As a last step, thrombin will induce the formation of fibrin from fibrinogen thereby initiating the formation of a blood clot. A strong link between TF-dependent PAR2 signalling by FVIIa and tumour progression/metastasis has been established in several cellular and in vivo models(177, 561). FVIIa is the protease of the extrinsic pathway that binds to the cell surface receptor, tissue factor (TF), on negatively charged membrane phospholipids and activates factor X to FXa to initiate the clotting cascade (553, 562). The FVIIa-TF complex is also known to activate PAR2 to elicit intracellular signalling responses in various cell types independent of its procoagulant activity(553). It has been demonstrated that activation of PAR2 by FVIIa also modulates the pro-migratory properties of non-cancerous TF- expressing epithelial cells, suggesting a key role for TF-dependent PAR2 signalling by FVIIa in altering cellular functions.(246, 563)

DOACs are direct Factor Xa inhibitors, inhibit the activity of the prothrombinase complex as well as free and clot-bound Factor Xa (564, 565) at variance with coumarins, such as warfarin, which act as vitamin K antagonist and inhibit post-translational modification of several pro-factors in the coagulation pathway. Direct oral FXa inhibitors have been undergoing extensive clinical evaluations for the prevention and treatment of thromboembolic disorders but, to date, limited data are available on their potential effects beyond anticoagulation (566). We have shown that Rivaroxaban and Apixaban attenuate endothelial cells permeability induced by FXa, suggests that Rivaroxaban/ Apixaban may mediate a supportive effect on barrier function through a general mechanism involved in maintaining barrier integrity. Surprisingly, the results show that Rivaroxaban at therapeutic level has a potential to increase ECs monolayer permeability to Blue dextran. The result may be explained by the ability of Rivaroxaban to activate endothelial cells to produce VEGF as pointed by (*Tao-Cheng Wu 2015)* (567) he reported that Rivaroxaban has direct effects on endothelial progenitor cell to secret VEGF in vitro and promote neovascularization in diabetic mice in vivo. On the contrary, (*Robert Ploen 2014)*(568) has reported that Rivaroxaban does not increase BBB permeability after ischemia and thrombolysis in mice and rats.

8.7 Conclusion and future work

In conclusion, our results indicate that activation of endothelial cells PAR2 promotes disruption of the vessel wall leading to expose the TF-expressing cells located in the underlying cell layers would be to the blood stream could activate TF and promote thrombosis process locally. Experimental evidence strongly suggests that greater cellular expression of TF and PARs correlates with more aggressive tumour behaviour, for the direct targeting of these receptors with, for example, monoclonal antibodies or inactivating peptides, or the inhibition of activated coagulation proteases may, therefore, be developed as potentially useful therapeutic strategies (18). Finally, the role of Rivaroxaban in treatment of cancer thrombosis not only as Fxa inhibition but including some effect on EC barrier functions that worth further investigation

8.8 Limitation

As with any science method, the data obtained by this study method are not sufficient by themselves to explain the mechanisms and signalling pathway for Rivaroxaban mediated endothelial barrier protection remain to be fully characterized. furthermore, in vivo data supporting the role of Rivaroxaban in enhancing the endothelial barrier are currently lacking. Moreover, with regard to the clinical implications of our study, the interpretation of our findings is somewhat limited by the conflicting reports from other studies to date.

Chapter 9 Theses general discussion

9.1 Introduction

The overall aim of the theses was to undertake an investigation to inform about the extent of the effect of i-PE diagnosed in the cancer population in terms of patients' experience of living with cancer and i-PE and to explore clinical factors (e.g. symptoms) and thrombogenic biomarkers (TFMP/PAR2mRNA) with regard to predicting occurrence and outcome of i-PE in patients with cancer.

The previous chapters presented the findings from i) a systematic literature review and qualitative analyses of cancer patients' experience of living with cancer associated thrombosis, ii) a mixed method study (an observational cohort case control survey study with embedded qualitative semi-structured interviews with participants to investigate the impact of i-PE in cancer patients' life and QoL, iii) a retrospective case control study comparing the level of TF/ PAR 2 mRNA/ proteins in cancer tissues of patients with and without i-PE and investigate their role in predicting cancer patients who are at high risk of developing i-PE, and finally a laboratory analysis of the effects of FXa and direct factor Xa inhibitors on the permeability of endothelial cells monolayer and their role in thrombosis.

The results from the previous chapters illustrated a considerable gap in knowledge

In this discussion chapter a critical interpretive synthesis (263) of the findings from each methodological approach in relation to the research questions will be addressed.

9.2 Research questions

9.2.1 Overarching question

What is the is the impact of an i-PE diagnosis on cancer patients' outcomes and survival compared to matched patients with cancer and no thrombosis and what is the role of biological markers TF/PAR-2 in predicting patients at high risk of developing i-PE?

Research questions;

- Q1. What is the experience of people living with cancer associated thrombosis in regard to their response to the diagnosis, coping with the additional burden and the effects of long -anticoagulation on their daily life?
- Q2. What is impact of incidental pulmonary embolism on the clinical outcomes of cancer patients?
- Q3. What is the experience of people living with cancer and incidental pulmonary embolism?
- Q4. What is the relationship between thrombogenic proteins (TF, PAR2) level in cancer tissues and the risk of thrombosis?

9.3 Critical interpretive synthesis of the thesis findings

The theses key findings are summarised in **(**Table 9.1**)**, which provide the structure for the discussion.

Table 9.1 Theses summary

Research questions	Systematic literature review	CLOTS-QoL quantitative data	CLOTS-QoL Interview data	Laboratory work	Arguments
RQ1. What is the experience of people living with CAT in regard to their response to the diagnosis, coping with the additional burden and the effects of long-term anticoagulation on their daily life?	• Lack of knowledge among patients and care professionals • Associated with immense physical and psychological impact • Patients were able to cope with the new	No evidence reported on Impact of CAT on QoL in general (population of people with i-PE only)	• Lack of previous information among patients • Associated with Psychological impact • Patients were able to cope with the new diagnosis • LMWH was acceptable intervention with no added	Not applicable	• Lack of information about the risk of /and symptoms and signs of thrombosis in cancer. • Misattribution of symptoms from both patients and care professionals lead to delay diagnosis. • High level of uncertainty due to absence of enough information • LMWH was acceptable • Patients were able to cope with new diagnosis

	diagnosis within the context of living with cancer • LMWH was acceptable intervention with no added burden on patients' life		burden on patients' life		
RQ2. What is impact of incidental pulmonary embolism on the clinical outcomes (QoL/symptoms/treatment burden) of cancer patients?	• No published literature about the impact of diagnosis of i-PE in cancer on patient	• QoL is reduced compared to the norm and to matched controls at baseline and case control	• Reduced QoL related to presence of symptoms. • Improving in patients QoL after treatment with LMWH.	• No evidence found	• These preliminary data support that patient with cancer and i-PE have reduced QoL. • QoL improved with anticoagulation treatment and with

	report outcomes	• QoL improves after starting LMWH over time • Improvements in some key symptoms (fatigue, breathlessness) (at least initially) • Pain increases (in keeping with people with progressing disease • Treatment burden stable	• Knowledge about the reason for anticoagulation helped patients to continue and accept the treatment.		improvement in i-PE related symptoms • Treatment benefits outweigh burden (in general) • Knowledge about the reason of the anticoagulation helped patients to accept and continue on the treatment.

		• Treatment benefit increases • LMWH benefit scale outweigh the burden scale			
RQ3. What is the experience of people living with cancer and incidental pulmonary embolism in regard to their response to the diagnosis, coping with the additional burden and the effects of long term anticoagulation on their daily life?	• No published literature about the experience of people with i-PE specifically	• Breathlessness, fatigue, and pain were prominent symptoms •	• Diagnostic overshadowing with misattribution of PE related symptoms. • Lack of previous information among patients • Lack of timely information at diagnosis	• No evidence found	• Physical and Psychological impact of i-PE can be related to knowledge deficiency about the risk of i-PE, lack of suspicion and lack of timely information at diagnosis.. • Patients were able to cope with the new diagnosis and to integrate LMWH injections into their life.

			• Associated with Psychological impact especially when diagnosis perceived as “out of the blue” and over the phone • Living in the context of cancer. patients were able to cope with the new diagnosis • LMWH was acceptable intervention with no added		

			burden on patients' life		
RQ4. What is the relationship between thrombogenic proteins (TF, PAR2) level in cancer tissues and the risk of thrombosis?	• NO evidence found	• NO evidence found	• No evidence found	• Significant different level of TF mRNA between cancer tissues of patients with i-P E and matched controls with no thrombosis • Alteration in endothelial permeability	• Different levels of TF mRNA in cancer tissues may stratify patients at high risk of developing i-PE. • Anti factor Xa DOACs reduce endothelial cells monolayer permeability, suggesting a further potential beneficial effect to treat or prevent thrombosis.

				occurs in response to the activation of PAR2 by factor Xa but not directly by the TF-factor VIIa complex	
Summary findings	• Diagnostic overshadowing, misattribution of thrombosis symptoms by Patients and staff • Heightened distress due to unsuspected nature of the diagnosis of iPE. • Education and timely information communicated excellently – care with phone communication • People with i-PE are symptomatic, and do have reduced QoL and treatment is beneficial more than burden • Symptoms improved by treatment so likely related to the i-PE • The *in vitro* data suggest a potential additional mechanism through which DOACs may impact on thrombosis and confer potential biomarker status to tumour TF mRNA to help identify cancer patients at high risk of thrombosis to reduce the experience of an "out of the blue" distressing diagnosis **Implication**				

	If these findings are confirmed, patients with cancer need: 1. Patients education about the risk, in the context of risk stratification, what to look for, and who/when to seek help 2. Clinicians need to be educated about risk/signs, beware diagnostic overshadowing and provide excellent and timely communication for those with i-PE 3. However, symptoms are still non-specific, and the finding of this biomarker has therefore useful potential in helping the early diagnosis of CAT and to prevent incidentally found CAT by which time patients have become symptomatic.

9.3.1 Research question one;

What is the experience of people living with cancer associated thrombosis in regard to their response to the diagnosis, coping with the additional burden and the effects of long-term anticoagulation on their daily life?

The systematic review and qualitative analysis demonstrated the significant burden effect of cancer-associated thrombosis (CAT) on patients' lives. It was a diagnosis that patients did not have enough knowledge about it, and this lack of knowledge shaped their response and coping. Education for patients and clinicians should therefore be part of routine care and further work to address this patient priority. These finding are similar to the results of a recent qualitative study by (*A.Hutchinson. at al. 2018*) (569) exploring cancer patients' understanding and their experiences of cancer associated thrombosis and DOACs. Researchers reported that only 18.9% of included participants were aware about the increased risk of cancer associated thrombosis, and again patients tent to misattribute their symptoms to cancer or its treatments which lead in many cases to delayed diagnosis (569)

This lack of knowledge was also a prominent finding in the qualitative study. Furthermore, none of the included participants in the qualitative study reported receiving any information regarding symptoms or signs of CAT. Consequently, when patients developed new symptoms before the diagnosis of PE they and their clinicians tended to misattribute them to the side effects of cancer or its treatments resulting in delayed diagnosis and treatment of the PE ; the possibility of co-morbid CAT being overshadowed by the diagnosis of cancer. Participants in the i-PE interview study had the additional burden of no warning of the diagnosis of CAT no clinician warned them that they were looking for CAT as a possible explanation for their symptoms because the clinician had not suspected it either.

The findings in the qualitative study add that even when patients receive information about cancer associated thrombosis they were un aware of the possibility of an incidental diagnosis of thrombosis.

In contrast to other cancer associated complication, such as post chemotherapy neutropenia where guidelines are routinely applied for patients and carers, awareness about cancer associated thrombosis is far behind. Education programs on cancer associated pain, fatigue, and side effects of chemotherapy have shown to decrease side effects of treatment, improve self-care abilities, and improve patients' quality of life (570-572).

Timely, accurate and compassionately delivered information helps patients to gain control on their own lives, by gaining understanding of the consequences of the disease and treatment, and helps them deal with fear (454). Inadequate information-giving is an ongoing issue and is not new, driving patients to inappropriate and sometimes inaccurate sources of information which may aggravate anxiety. However, the findings from both SLR and qualitative part of the mixed method study, in this thesis show that patients were clear about what they want to know and about the amount of information they need and the timeframe within which they need it. The information needs as reported previously is varied between patients depending on; age, gender, and education (454). In this study information need varied from participants who did not want to know anything as long as cancer is responding to those who reported the need to know the course of i-PE, the effectiveness of the treatment and the complication of the new diagnosis. Others have reported disappointment regarding the lack of information.

9.3.2 Research question two;

What is the impact of incidental pulmonary embolism on the clinical outcomes of cancer patients?

The aim of this part of the theses was to investigate the effects of i-PE on cancer patients PS, QoL, and other key clinical outcomes such as VTE (re)occurrence, haemorrhage, and days in hospital compared to matched controls with no thrombosis.

However, due to time constraints, design shortcomings and difficulties to recruit as discussed, not enough participants could be recruited, hence this thesis can only present a summary of preliminary and partial data.

Quality of life among patients with cancer and i-PE was impaired, especially in the physical component summary as measured with SF-12. High levels of pain, breathlessness and tiredness were prominent findings in both the survey and during the interviews. These findings are consistent with the literature review findings reporting higher level of fatigue and breathlessness in patients with i-PE compared to patients with no thrombosis (14). This thesis however demonstrates that there is also a substantial symptom burden outside the symptoms one would commonly associate with PE. It is worth noting that, most of the participants had advanced stage of cancer which may further complicate interpretation of symptoms. Fatigue is the most frequently reported symptom in cancer patients, around 80% to 96% of cancer patients on chemotherapy report fatigue (19). Many studies have demonstrate the negative effect of fatigue on cancer patients' life physically, emotionally and socially (573). In this study the aim was to compare patient reported outcomes between patients with i-PE and matched controls. The included controls reported no substantial impact on QoL, and tiredness, pain, depression, however, conclusions cannot be drawn with this small sample.

In the survey study, pain increased over the study period consistent with patients with advanced and progressing cancer. Cancer patients may suffer from pain requiring opioid analgesia (574). A systematic literature review of 122 articles, provided a pain prevalence ranging from 39.3% after curative treatment, to 66.4% in advanced cancer (575). However, interview study participants did not mention pain. This could be because cancer patients may see pain as inevitable with cancer, or that it was controlled in this group. However, cancer pain remains grossly undertreated throughout the world (576). Many barriers have been reported to explain poor pain management, one of them being poor communication between patients and care professionals in general (577).

Data about the survival rate among cancer patients with i-PE is inconsistent, many researchers reported low survival rate among cancer patients incidentally diagnose with VTE compared to those with no thrombosis or to those with symptomatic VTE, while others reported no difference (50, 51, 578). The presence of symptoms even with the smallest PE (sub-segmental PE) in cancer patients is associated with poor survival (79). Furthermore, the presence of symptoms in cancer patients with i-PE have

also been correlated with worse outcome (51, 579). This has been confirmed by our research group where the presence of new or worsening of old symptoms and lower performance status was found to correlate with worse survival irrespective of the anatomical distribution of the PE (47). Due to the low sample size, I cannot comment on the effects of i-PE on cancer patients' survival.

However, these preliminary data show that these reported symptoms improved over the study time after starting anticoagulation treatments (low molecular weight heparin). This highlights the positive effect of low molecular weight heparin in improving patients' symptoms and subsequently their quality of life measures. These findings are consistent with previous research that low molecular weight heparin benefits outweigh its complications (437).

Regarding the low molecular heparin treatments, the benefit score outweighs the burden score indicating patients' acceptability of the treatment, these findings confirmed by interview data. These findings are consistent with previous research by Noble and colleagues in parallel with other recent research (345, 575). Improved symptoms with low molecular weight heparin and the higher scores reported by patients themselves is consistent with patients' satisfaction with low molecular weight heparin in treating cancer-associated i-PE. The observation of high benefit scores over the three-month study period suggests that the low molecular heparin treatment regimen did not have a net negative impact on QoL. This finding is consistent with recent research the QUAVITEC study (437). This prospective, longitudinal, multicentre study recruited 400 adult cancer patients diagnosed with CAT. The group showed that low molecular heparin did not negatively affect QoL in cancer patients who survived to 6-month follow-up and who exhibited improvement in their overall health. However, it may be anticipated that long-term-(qualify of life this –Beyond 6 months) low molecular heparin treatment can affect QoL, but to date, this has not been studied in cancer patients with i-PE. Moreover, with the advent of DOACs, it can be anticipated that these patients may be switched to oral agents, with evidence that these may be a preferable choice and appropriate for particular patients (107)

9.3.3 Research question three;

What is the experience of people living with cancer and incidental pulmonary embolism in regard to their response to the diagnosis, coping with the additional burden and the effects of long term anticoagulation on their daily life?

The systematic literature review presented in Chapter Four did not directly report on i-PEs, however, the findings of the interview study showed some similarities and some additional findings. The diagnosis of i-PE occurs during cancer follow up when participants were waiting to find out about the response of cancer to treatments, e.g. results of re-staging CT scan. The investigations were not planned to look for CAT. Patients were therefore totally unprepared for such a diagnosis and had no previous information, whether or not associated with acute symptoms, the diagnosis came as a great shock, associated with distress and fear. These findings were consistent with recent research exploring cancer patients' understanding and awareness regarding CAT, unawareness, shock and worries were the main findings (569), but heightened by the "out of the blue" nature of the diagnosis.

Similar to the systematic literature review in chapter five (36), patients reported high levels of uncertainty associated with the unexpected diagnosis, and lack of information even after diagnosis. Uncertainty about the cause, the outcome of the new diagnosis, and about the effects of the treatments were major factors and was amplified by the need to have immediate information due to the lack of the previous suspicion. Uncertainty management theory as previously reported is one theoretical framework to help understand how patients encounter, appraise, levels of danger, and information-seeking behaviour (427). Consequently, some patients appraised the diagnosis of i-PE in the context as vague and dangerous that required more explanation, whereas, others were less worried about it as long as the cancer was responding to treatment. These findings indicate that information plays an important role in reducing certain patients' distress and anxiety.

Despite previously reported concern about the therapeutic burden of low molecular weight heparin injections on patients with cancer (342, 580), in this study the included participants reported that low molecular weight heparin injections to acceptable as reported by previous research (341, 569, 581).

However this acceptance was not without unpleasant side effects including; bruising, pain, and lumps at the site of the injection, but these were accepted as a trade-off daily, similar to have been reported by previous research (569). Participants were able to integrate the injection into their daily schedule that enabled them to maintain some normality. Consistent with previous research participants were able to find ways to cope and to minimise the side effects of the injections (332). Participants reported symptoms improvement after starting anticoagulation, which I noticed from the survey study Chapter Five as well, these improvements of patients' QoL and symptoms after starting the low molecular weight heparin injections may have enhanced participants' acceptance of the injections regardless the side effects.

9.3.4 Research question four;

What is the relationship between thrombogenic proteins (TF, PAR2) level in cancer tissues, thrombosis in general i-PE in particular?

This line of investigation was prompted by previous *in vitro* data from our group demonstrating a substantial correlation of TF and PAR2 expression in cancer cells and their ability to release microparticles showed on the one hand in a retrospective case-control study, that substantial differences existed in tissue factor mRNA level between cancer patients with i-PE and matched controls with no thrombosis. The results from the i*n vitro* permeability work illustrated a novel PAR – stimulated mechanism of endothelial barrier permeability through activation of endothelial cells. The rapid initial decrease in permeability requires PAR2 and PAR1 which may act to constrain bleeding however the longer-term response is mediated by PAR2 resulting in increased permeability and sub-endothelial exposure, suggesting a further mechanism that propagates and sustains thrombosis in the region of damaged/activated endothelium. It was demonstrated that the exposure of endothelial cells to FXa lead to endothelial barrier dysfunction and an increase in dextran blue leakage across the endothelial monolayer. It has been reported that both PAR2 and its-activating proteases, including tissue factor and trypsin, are aberrantly overexpressed in a variety of malignancies, and shown to be involved in many aspects of cancer biology including cell proliferation, angiogenesis, and metastasis (544). A strong link between TF-dependent PAR2 signalling by FVIIa and tumour progression/metastasis has been established in several cellular and in vivo models (177, 561). A number of investigators have

described several distinct mechanisms through which malignant tumours induce vascular permeability leading to enhanced metastasis (582, 583). However, to date, this has not been studied as a mechanism that promotes/underpins thrombosis. The present results support the hypothesis that activation of endothelial cell-PAR2 promotes disruption of the vessel wall leading to exposure of the TF-expressing cells located in the underlying cell layers to the bloodstream could activate TF and promote thrombosis process locally.

The diagnosis of i-PE in cancer population represents a compelling challenge, adds more burden on patients' life, decreases QoL, and can delay cancer treatments, and as shown by other studies, is associated with a shock when unsuspected. Using validated, easily measurable biomarkers may help identifying patients who are at higher risk of thrombosis and for whom thromboprophylaxis may help minimise these challenges. Identification of a group of patients who need particular education and warning may help prevent significant psychological disease, and ensure patients seek early advice and help protect clinicians from diagnostic overshadowing.

Validated, easily measurable biomarkers can facilitate clinical trials and aid in risk assessment, diagnosis, monitoring of treatment response, and assessment of prognosis for cancer-associated thrombosis. However, biomarker development for cancer-associated thrombosis has proven to be challenging, due to patients' heterogeneity, difficulty in measuring candidate markers in the circulation reproducibly (such as MPs), and the transient nature of some biomarkers, affected by the different applications of cancer treatment and the induction (or otherwise) of cancer remission. Tissue factor despite its central role in the induction of thrombosis in solid malignancies such as the gut-related ones studied in this thesis, lacks standardised or accepted biomarker methodology. Available assays including; immune-histochemical TF staining or tumours, enzyme-linked immunosorbent assay of serum or plasma, flow cytometry-based methods for the plasma poor samples, or measurement of TF-MPs activity (584) have failed to address this gap. Most studies investigating for, example TF-MPs, as predictive biomarkers for cancer-associated thrombosis have reported inconsistent results and none of these biomarkers have been studied in the context of predicting solely cancer associated i-PE. In advanced colorectal cancer, for example, researchers reported TF-MPs in the systemic circulation

associated with increased risk of venous thromboembolism by two-fold when compared with healthy controls (190); we have also shown elevated TF-MPs circulating preoperatively in pancreatic cancer patients drop significantly after resection of the primary (24). Other cancer-associated thrombosis biomarkers or predictive tools of marker combinations also have several challenges. Once again the main one is heterogeneity within patients, as no cohort studied and then validated is alike. The risk of cancer-associated thrombosis is not only dependant on tumour-related factors but non- cancer related factors play a role including patient age (585, 586), BMI, gender (585), concurrent infection (585), and anticancer but also supportive treatments such as growth factors and high dose steroids (483, 587-589).

Despite these challenges, several candidate biomarkers have been investigated. One of the commonly used/available biomarkers is D- dimer. Several studies considered D-dimer as a predictive biomarker for cancer-associated thrombosis. It has been reported to be associated with an increase in risk for VTE, and in was a significant predictor of mortality among cancer patients (590). Elevated plasma levels of D-dimer are associated with a 3-fold increase in risk for VTE re-occurrence, and in the observational Vienna Cancer and Thrombosis Study (Vienna CATS) sub-study of patients with cancer, elevated D-dimer was a significant predictor of cancer-associated thrombosis. However, in the past years, stratifying tools designed to identify high risk patients and to guide clinical decision have been proposed. Among them, is the Khorana score (90) that successfully being used in risk assessment models to stratify patients with cancer into low, intermediate and high-risk categories for the occurrence of VTE (90, 114). Recently, two large randomised studies (CASSINI and AVERT) have demonstrated that the Khorana score can 'enrich' the study population for VTE risk by at least 2.5 fold (from a baseline of around 3.8% seen in the PROTECHT and SAVE-ONVO studies over 3- 4 months to around 9-11% over 6 months (591, 592). Vienna CATS Score (114) added Soluble P-selectin (sP-selectin) and D-dimer, were found to be independent risk factors for VTE in cancer patients but makes the score more unwieldy and incorporates the use of non-standard tests. All these tools were generally applicable for the total spectrum of CAT and not specific to predicting the risk of i-PE in cancer patients.

Despite these challenges, the results presented in this thesis add to the literature the possibility of using tumour level TF-mRNA measurement to identify patients who are at high risk of thrombosis. This study was in i-PE patients which were however selected for an increased volume of the clot (proximal only) and could translate to cancer-associated thrombosis risk overall but the relevant study would need to be done. The use of combined strategies including both clinical assessment/symptoms assessment/ and tumour level TF-mRNA measurement may improve diagnosis with acceptable cost efficiency.

9.4 Theses summary

The aim of this theses was to investigate the impact of an incidentally diagnosed pulmonary embolism on cancer population' outcome and to explore their experience of living with cancer and i-PE. The second aim was to explore the role of thrombogenic biomarkers (tissue factor and protease-activated receptor-2) as predictive biomarkers of thrombosis.

The data from the systematic literature review demonstrated that cancer-associated thrombosis negatively affects patients' life. It is a diagnosis that is associated with physical, psychological and social limitation. The results highlighted the gap in knowledge about the increased risk of thrombosis and i-PE in particular among cancer patients and care professionals as well. The absence of clear guidance and information about cancer associated thrombosis and the possibility that cancer-associated thrombosis can be a spurious diagnosis (e.g. i-PE) represent a major issue that needs intervention. The same finding was raised from the qualitative study synthesis, where none of the included participants reported receiving any information about cancer-associated thrombosis before their diagnosis. The concept of diagnostic overshadowing may be helpful in raising awareness of misattribution and is applied to cancer and thrombosis for the first time in this theses.

The observational data presented useful preliminary data that the diagnosis of i-PE in cancer patients negatively affects their QoL. Patients' QoL was below the population average, patients reported PE related symptoms that in most cases were misattributed by patients to cancer or to its treatments as seen in the literature review in CAT in general. The presence of these symptoms further impact patient quality of life as

participants tend to experience the new diagnosis in a context of cancer, which in most cases represents a new burden added to their life.

The qualitative analysis if the interviews show that symptoms tend to improve after starting anticoagulation, indicating the positive effects of anticoagulation. Participants in this qualitative study were committed to complete the treatment course and have shown an ability to incorporate the treatments within their life schedule, similar to those reported in the systematic literature review. However, most of the included participants reported on anticoagulation injections side effects, including bruising and fibrosis, non-have developed major bleeding.

The laboratory analysis presented in this thesis, suggest the hypothesis that tumour tissue analysis of TF-mRNA could be useful in predicting i-PE in cancer patients. Validation of these biomarkers as surrogate markers that can reliably predict i-PE in the cancer population is needed. Finally, this work has also uncovered a further thrombosis promoting mechanism that seems to be mediated by the PSR proteins through the activation of endothelial cells, where FXa may play a significant role in cancer thrombosis. Although FXa inhibition is known to be a property of the LMWHs (particularly fondaparinux) this finding raises the possibility that the new anti-Xa inhibitors (such as Apixaban and rivaroxaban) may be more useful clinically in protecting patients from thrombosis. These findings encourage further research to highlight the role of increased endothelial cells monolayer permeability in cancer thrombosis.

9.5 Strengths

A strength of the presented data in this thesis is the prospective nature of the mixed-method study. The case-control study using patients reported outcome measures that help to measure patients' outcome as they see it and not from a professional point of view. I was able to record changes prospectively over time. Using both general and disease-specific measures allowed me to see the full picture. It is well reported that patients reported outcome measures can provide real-world data on the benefits of treatments, to evaluate health care provider performance, to assess changes in patient functional status over time, and to assess the effectiveness of treatments (593). Recent initiatives, such as the United Kingdom's policy encouraged the implementation of patient-reported outcomes to facilitate patient–clinician communication and evaluate

the quality of National Health Service care (594, 595). The qualitative study further explores the research question by including the same cohort of patients and further strengthening the findings. The main strength of the interview study is that it enables interviewees to “speak in their own voice and express their own thoughts and feelings” (596).

9.6 Limitations

Risk of bias can occur at any phase of research, including study design or data collection, as well as in the process of data analysis (597). Further, as with any clinical study, this research has faced a lot of challenges. Identification and recruitment of enough participants during the allocated time for my PhD proved impossible. Obtaining the ethical approval proved a challenging process. The nature of the illness under investigation represents a major challenge as well, especially in recruiting the control patients for cancers like biliary tree, stomach oesophageal and pancreas where most of the patients sought to be the matching controls had either performance status deterioration or were deceased. Hence I was unable to compare a significant enough number of patients with and without i-PE to reach any robust conclusions although they provide useful “pointers” for future study. The tissue analysis study weaknesses reside in the type of tissue obtained (I was not able to make the protein quantification work in FFPE) and the fact that through the triage **(Table** 7.8**),** I ended up with a very small cohort of patients to study (2.5 % of the samples). Furthermore, the on occasion low or no cancer tissue in case or control samples was a challenge.

9.7 Implications for research and clinical practice

The data generated from this thesis further improve our understanding of the broad clinical and human effects of the diagnosis of i-PE on a cancer population including their experience of living with cancer and thrombosis in general. Information and awareness of patients, as well as care professionals, regarding the high risk of thrombosis among cancer population represent an urgent need including the i-PE, since none of the available tools (not even the Video that Thrombosis UK promotes report anything about chance/spurious diagnosis of clots). It was clear that patients experience the diagnosis of CAT in context of living with cancer, associated with decreased QoL, so providing enough information and support would ease some of the burdens of the new diagnosis, increase patients’ efficacy and improve their life quality.

Risk assessment tools to predict patients at increased risk of thrombosis would be of value and help target education and reduce the risk of diagnostic overshadowing. Patients who are diagnosed with i-PE have had PE related symptoms that had been misattributed to cancer by patient and physician. Regular symptom assessments, especially for those on chemotherapy, could help detect this complication earlier provided clinicians had sufficient awareness that CAT may not present with classical signs and symptoms of DVT or PE.

Future research is needed to validate the interesting findings of the potential of tumour TF mRNA as a thrombogenic biomarker and the impact of the (TF/ PAR-2) axis on endothelial permeability and contribution to CAT.

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Appendices

Appendix A Search terms used in the systematic literature review

EMBASE	Neoplasm OR neoplasms OR cancer OR cancers OR tumor OR tumors OR Tumour OR Tumours OR carcinoma PR carcinomas OR neoplas* OR cancer* OR carcinom* OR Oncol* OR oncology OR oncologic OR carcinogenesis OR glioma OR sarcoma OR leukemia OR lymphoma OR gliomas OR sarcomas OR leukemias OR lymphomas OR malignancy OR malignancies OR malignant AND Venous thromboembolism OR venous thrombosis OR vein thrombosis OR Thromboembolism OR deep vein thrombosis OR Lung embolism OR Pulmonary embolism OR DVT OR PE OR symptomatic pulmonary embolism OR a symptomatic pulmonary embolism OR incidental venous thrombo* OR Splenic thrombosis AND Quality of life OR health-related quality of OR QoL OR Life style OR experience OR questionnaire OR questionnaires OR interview OR interviews OR structured intervie* OR semi-structured intervie* OR VEINES-QOL OR qualitative OR impact on OR clinical burden OR PEmb-QoL OR Karnofsky performance status.
MEDLINE	Neoplasm OR neoplasms OR cancer OR cancers OR tumor OR tumors OR Tumour OR Tumours OR carcinoma PR carcinomas OR neoplasm$ OR cancer$ OR carcinoma$ OR Oncol$ OR oncology OR oncologic OR carcinogenesis OR glioma OR sarcoma OR leukemia OR lymphoma OR gliomas OR sarcomas OR leukemias OR lymphomas OR malignancy OR malignancies OR malignant AND Venous thromboembolism OR venous thrombosis OR vein thrombosis OR Thromboembolism OR deep vein thrombosis OR Lung embolism OR Pulmonary embolism OR DVT OR PE OR symptomatic pulmonary embolism OR a symptomatic pulmonary embolism OR incidental venous thrombo$ OR Splenic thrombosis AND Quality of life OR health-related quality of OR QoL OR Life style OR experience OR questionnaire OR questionnaires OR interview OR interviews OR structured intervie$ OR semi-structured intervie$ OR VEINES-QOL OR qualitative OR

	impact on OR clinical burden OR PEmb-QoL OR Karnofsky performance status.
CINHAL	Neoplasm OR neoplasms OR cancer OR cancers OR tumor OR tumors OR Tumour OR Tumours OR carcinoma PR carcinomas OR carcinomas OR oncology OR oncologic OR carcinogenesis OR glioma OR sarcoma OR leukemia OR lymphoma OR gliomas OR sarcomas OR leukemias OR lymphomas OR malignancy OR malignancies OR malignant AND Venous thromboembolism OR venous thrombosis OR vein thrombosis OR Thromboembolism OR deep vein thrombosis OR Lung embolism OR Pulmonary embolism OR DVT OR PE OR symptomatic pulmonary embolism OR a symptomatic pulmonary embolism OR incidental venous thromboembolism OR Splenic thrombosis AND Quality of life OR health-related quality of OR QoL OR Life style OR experience OR questionnaire OR questionnaires OR interview OR interviews OR structured interview OR semi-structured interview OR VEINES-QOL OR qualitative OR impact on OR clinical burden OR PEmb-QoL OR Karnofsky performance status.
PsychINFO	Neoplasm OR neoplasms OR cancer OR cancers OR tumor OR tumors OR Tumour OR Tumours OR carcinoma PR carcinomas OR carcinomas OR oncology OR oncologic OR carcinogenesis OR glioma OR sarcoma OR leukemia OR leukaemia or lymphoma OR gliomas OR sarcomas OR leukemias OR lymphomas OR malignancy OR malignancies OR malignant AND Venous thromboembolism OR venous thrombosis OR vein thrombosis OR Thromboembolism OR deep vein thrombosis OR Lung embolism OR Pulmonary embolism OR DVT OR PE OR symptomatic pulmonary embolism OR a symptomatic pulmonary embolism OR incidental venous thromboembolism OR Splenic thrombosis AND Quality of life OR health-related quality of OR QoL OR Life style OR experience OR questionnaire OR questionnaires OR interview OR interviews OR structured interview OR semi-structured interview OR

	VEINES-QOL OR qualitative OR impact on OR clinical burden OR PEmb-QoL OR Karnofsky performance status.

Appendix B Inclusion and exclusion criteria used in the systematic literature review

Inclusion Criteria

- Articles in English language
- Primary qualitative research or mixed methods where qualitative data is available to extract
- Studies in adult cancer patients with venous thromboembolism with or without treatments
- Studies addressing patients experience or their quality of life of living with cancer and venous thromboembolism

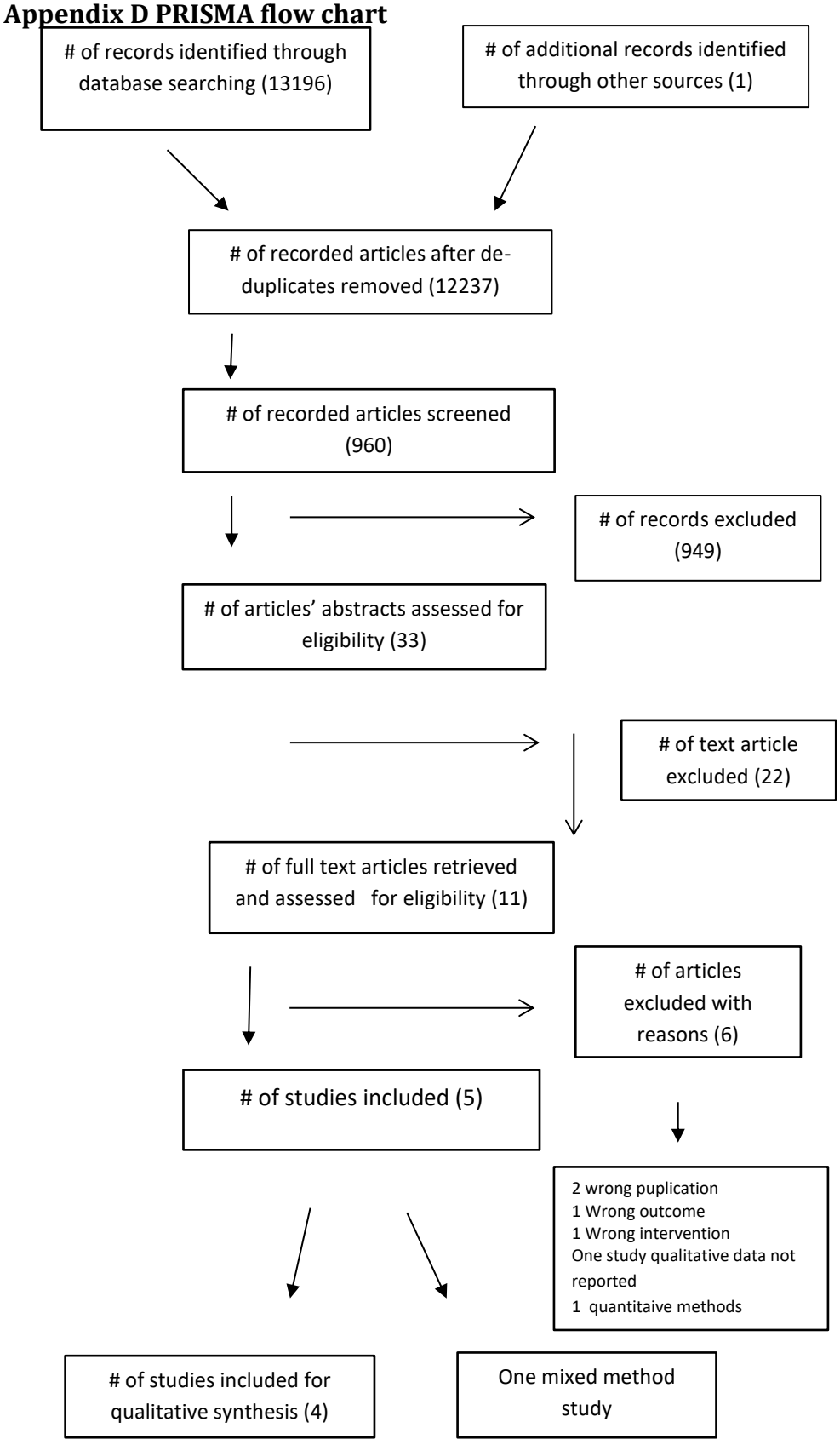
Exclusion criteria

- Quantitative research studies
- Studies in non-English language

Appendix C CASP Critical Appraisal Skills Programme tool for appraising qualitative research

Study/ Question	Yes	No	Not clear
Q1. Was there a clear statement of the aims of the research?			
Q2. Is a qualitative methodology appropriate?			
Q3. Was the research design appropriate to address the aims of the research?			
Q4. Was the recruitment strategy appropriate to the aims of the research?			
Q5. Was the data collected in a way that addressed the research issue?			
Q6. Has the relationship between researcher and participants been adequately considered?			
Q7. Have ethical issues been taken into consideration?			
Q8. Was the data analysis sufficiently rigorous?			
Q9. Is there a clear statement of findings?			
Q10. How valuable is the research?			

Appendix D PRISMA flow chart



Appendix E Characteristics of included studies

Study	Design	Methods & Sitting	Aim	Participants characteristics	Analysis	Principal findings
Noble. S and Finlay. I.G. 2005. (342)	Qualitative	Semi-structured interview. Palliative care patients, both in the community and in-patient units. Cardiff. UK	Assessing the appropriateness of low molecular weight heparin in palliative care patients and the extent of daily injection burden	N =40 (18 male and 22 female) Age: 32-76 years Advanced cancer, receiving low molecular weight heparin for confirmed cancer-associated thrombosis. In total 33 patients had initially received Warfarin then changed to low molecular weight heparin due to poor control.	Thematic analysis	• Acceptability: all patients understood why they are on LMWH and considered it acceptable. • Simplicity: the majority found that daily injection of LMWH simpler than the frequent INR needed for warfarin. • Freedom: many patients expressed a feeling of freedom from hospitals, from being restricted to their home. • Optimism: the feeling that something active being done. • Bruising: a total of 11 patients described bruising as a negative aspect of low molecular weight heparin.
Mockler. A, et al. 2012. (340)	Qualitative	Semi-structured interview. Inpatients and outpatients of a large urban university-affiliated hospital.	Exploring the experiences of patients with cancer who developed cancer-associated thrombosis	N = 10 (4 women and 6 men) Age: 35-78 years Various cancer types diagnosed 2-18 months prior to the interview Various stages from early with active treatment to advanced stage	Thematic analysis	Coping with venous thromboembolism : • Prior knowledge of cancer- associated thrombosis risk and symptoms (or lack of knowledge) determined reaction to cancer- associated thrombosis symptoms • For some, cancer-related concerns overshadowed those due to cancer-associated thrombosis cancer-associated thrombosis as a setback in cancer care: • cancer-associated thrombosis symptoms preventing a return to normal life after cancer treatment

		Montreal. Canada				• Cancer-associated thrombosis treatment interfering with their cancer care. Attitudes about venous thromboembolism treatments: • Positive for some participants however associated with a sense of obligation. • Many show acceptance of self-injection of low molecular weight heparin especially among those with previous experience with warfarin
Seaman. S et al. 2014. (341)	Qualitative	Semi-structured interview. Palliative care and cancer-associated thrombosis unit. Cardiff. UK	Exploring the acceptability of long term low molecular weight heparin for the treatment of cancer-associated thrombosis in the contexts of living with cancer and quality of life	N = 14 (8 women and 6 men) Age: 52-84 years Receiving low molecular weight heparin for confirmed cancer-associated thrombosis (PE n=8/ DVT n=6), 8 patients were on Warfarin then changed to low molecular weight heparin	Thematic analysis	Impact of venous thromboembolism: • Symptom burden of cancer-associated thrombosis, • cancer-associated thrombosis in context of cancer, • Impact on activities of daily living. Acceptability of low molecular weight heparin: • Necessary inconvenience, • Systematic approach to injection. Hypothetical views on New Oral Anticoagulants: • Efficacy paramount, • Willing to engage in clinical trials.
Noble.S, et al. 2015. PELICAN (332)	Qualitative	Semi-structured interview. Cancer-associated thrombosis clinic within regional	Exploring the patients experiences of cancer-associated thrombosis within the context of cancer journey	N = 20 patients (10 women and 10 men) Age: 53-81 years Different primary cancers receiving low molecular weight heparin for (2-20 months)	Thematic analysis	Diagnosis and treatment of cancer-associated thrombosis , : • Lack of knowledge of venous thromboembolism in the context of cancer, patients unaware of risks of thrombosis or symptoms to look out for, • Limited awareness among health professionals.

		cancer centre and district general hospital. Cardiff. UK				• Symptoms of cancer-associated thrombosis attributed to cancer or chemotherapy and therefore delayed presentation to hospital • Initial reaction is shock, little information. Living with cancer-associated thrombosis : • Treatment helps get over the initial shock, i getting on with life, ritualization of new routines.
Noble.S, et al. 2015. ALICAT (343)	Embeded Qualitative study iwithin a RCT	Focus groups with clinicians. Semistructured interviews with patients and their relatives	Explore clinician' attitudes / patients' and their relatives' experiences towards the RCT of ongoing low molecular weight heparin treatment for cancer-associated thrombosis versus cessation at 6 months in patients with locally advanced or metastatic cancer. Patients' perception of cancer-associated thrombosis and Anticoagulation	N of clinician= 3-11/ group (3 focus group) Oncology, Heamatology and Primary care. N of patients=8 (4 women and 4 men) Different primary cancers	Framework analysis	• The study adds further information on cancer patients' decisions to continue or stop low molecular weight heparin treatment is highly influenced by their experience of symptomatic venous thromboembolism versus a symptomatic • Patients with experience of symptomatic cancer-associated thrombosis were willing and keen to continue on low molecular weight heparin injection as long s it takes. • Patients who had a symptomatic cancer-associated thrombosis were keen to stop low molecular weight heparin injection as soon as possible aiming to have some normality back

Appendix F CLOTS-QoL study ethical approval document

NHS
Health Research Authority
East Midlands - Nottingham 1 Research Ethics Committee
The Old Chapel
Royal Standard Place
Nottingham
NG1 6FS

Please note: This is an acknowledgement letter from the REC only and does not allow you to start your study at NHS sites in England until you receive HRA Approval

16 November 2016

Professor Miriam Johnson
Professor of Palliative Medicine
Hull York Medical School, University of Hull
Hertford Building
University of Hull, Cottingham Road
Hull
HU6 7RX

Dear Professor Johnson

Study title:	**CLinical OuTcomes, Symptoms and Quality of Life in cancer patients with i-PE**
REC reference:	**16/EM/0474**
IRAS project ID:	**216188**

Thank you for your letter of 16/11/2016. I can confirm the REC has received the documents listed below and that these comply with the approval conditions detailed in our letter dated 08 November 2016

Documents received

The documents received were as follows:

Approved documents

The final list of approved documentation for the study is therefore as follows:

Document	*Version*	*Date*
Evidence of Sponsor insurance or indemnity (non NHS Sponsors only) [University of Hull Insurance Letter 1]	1	07 September 2016

Appendix G CLOTS-QoL study protocol

Hull and East Yorkshire Hospitals NHS
NHS Trust

Queen's Centre for Oncology & Haematology
Castle Hill Hospital Castle Road
Cottingham
HU16 5JQ

CLinical OuTcomes, Symptoms and Quality of Life in cancer patients with i-PE

Acronym: CLOTS-QoL study

Protocol Version 1.0

September 2016

Principal Investigator: Miriam Johnson
Professor of Palliative Medicine
Hull York Medical School
Hertford Building
University of Hull HU6 7RX

Signature …… Date ……

Sponsor: Hull and East Yorkshire Hospitals NHS Trust, R&D department
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Study Summary:

Cancer patients undergo thoracic multidetector computed tomography (CT) scanning for clinical evaluation at greater frequency that non-cancer patients; this has led to an increased likelihood of the unsuspected, incidental detection of a pulmonary embolism (PE). While a few studies have evaluated the risk factors and the natural history of incidentally-found pulmonary embolism (i-PE) in cancer patients, little is known about the impact of these clots on the survival, performance status (PS) and quality of life (QoL) of these patients.

Aims: The overarching aim of the study is to investigate the relationship between i-PE in cancer patients and PS, symptoms, QoL and other key clinical outcomes such as venous thromboembolism (VTE) o(re)currence and haemorrhagic complications. A secondary aim is to identify clinical and demographic predictors of VTE o(re)currence, haemorrhage, QoL and PS.

Design: This is a mixed-methods observational, non-interventional, prospective case-control study which will recruit patients with incidentally diagnosed PE (Study Arm). Quantitatively, the study aims to evaluate the clinical outcomes of cancer patients with i-PE compared with matched cancer patient controls without PE or other VTE with regard to clinical outcomes including survival, symptoms, PS and QoL. The qualitative component will consist of semi-structured interviews with a sample of participants willing to be interviewed about their experience, to explore the true impact of an i-PE diagnosis in the context of a patient's cancer journey.

Cancer patients who are referred from the CT scan department to the i-PE clinic at the Queen's
Centre for Oncology and Haematology (QCOH) at Castle Hill Hospital (CHH), Hull and East Yorkshire Hospitals NHS Trust (HEYHT), will be approached by a member of the clinical team and asked to participate. Routine assessments performed at the first clinic visit will provide baseline data, after which at specified intervals (+7 days, +30 days, +90 days and +180 days) consenting participants will complete QoL and symptom assessment questionnaires and PS will be noted. Control patients will be identified once clinical variables of the i-PE patient have been obtained at baseline.

Lay Summary:

Advances in medical technology have resulted in an increase in the detection of unexpected blood clots in the lung of patients with cancer. We are looking to see the effects these blood clots may have on the survival and quality of life of these patients, when compared to cancer patients without blood clots. It is hoped that we can find a

way to identify more quickly those patients who are deteriorating and are at greater risk of developing further clots.

Background

Incidental pulmonary embolism

VTE, presenting as deep vein thrombosis (DVT) and/or pulmonary embolism (PE), is a common complication of malignant disease and represents one of the leading causes of morbidity and mortality among cancer patients [1]. The advent of multidetector CT (MDCT) scanning, with its higher sensitivity and resolution, has led to an increased rate of detection of 'incidental' PE (i-PE) in cancer patients [2, 3]. Cancer patients undergo routine imaging much more frequently than those without cancer, either for staging purposes, treatment response evaluation or metastasis screening, which has led to an increased opportunity to identify i-PE in cancer patients [4]. In fact, about half of all PE diagnosed in oncology centres are incidental [3, 5-8].

The significance of these unsuspected or incidental events is becoming increasingly recognised [9]. They now represent a prevalent clinical problem, with reported incidences ranging from 2% in the general population to 4.5% in cancer patients [2]. Indeed, as with symptomatic VTE, the presence of cancer is associated with a significantly increased risk of i-PE. In a recent meta-analysis of 12 studies including over 10,000 patients, the weighted mean prevalence of i-PE in cancer patients was higher than in non-cancer patients (3.1% vs 2.5% respectively) [10], and this is likely to be even higher in high-risk patients [11].

Incidental PE refers to PE that is clinically unsuspected at the time of diagnosis, but there is some consensus to recommend it be referred to as 'unrecognised', 'unsuspected' or 'incidental' rather than truly asymptomatic [3, 9]. Indeed these cancer patients may actually be found to have symptoms on closer review [2, 3], while others may genuinely have no PE-related symptoms or signs. Clinicians however may often erroneously attribute these non-specific symptoms to the underlying malignancy or to side effects of cancer therapy, rather than the PE, which may contribute to the missed or delayed diagnosis [3].

Possible signs and symptoms, as for symptomatic PE, may include fatigue, chest pain, persistent tachycardia, shortness of breath, and limb pain or swelling [12]. Indeed in a retrospective chart review of 59 patients with i-PE [12], O'Connell *et al*. showed patients with PE were significantly more likely than control patients to experience fatigue (54% vs 20%; P=0.0002) and breathlessness (22% vs 8%; P=0.02). Their own later study also showed that, compared to patients without i-PE, shortness of breath, cough and fatigue were significantly more prevalent among cases of i-PE than controls [13]. Additionally, in our own recent audit of data from a prospectively recorded dataset of 234 consecutive ambulatory cancer patients with i-PE, a variety of

symptoms were observed on diagnosis, notably dyspnoea (51.7%), fatigue (77.4%), chest pain (11.1%), lower limb oedema (33.3%) and haemoptysis (3.4%) [14]. In addition, the presence of i-PE will result in an increased patient engagement with the health services (treatment of the condition for either 6 months or indefinitely) and could potentially disrupt or delay anti-cancer treatments. Moreover, i-PE carries a significant risk of VTE current and major bleeding, and may be responsible for an increase in a patient's symptoms and a reduction in QoL for the cancer patient.

Current management of i-PE

The appropriate treatment and management of i-PE in cancer patients has been poorly investigated. In the absence of sufficient convincing evidence to withhold anticoagulant treatment in these patients, current international clinical guidelines suggest or recommend the same initial and longterm anticoagulation as for comparable patients with symptomatic PE [15-17].

For the general population, the standard treatment for acute VTE consists of initial therapy with a low-molecular-weight heparin (LMWH), followed by longer term treatment (3 to 6 months) with an oral vitamin K antagonist (VKA) [18]. However, due to the substantial and continuing risk of VTE recurrence and haemorrhagic complications in patients with cancer [19, 20], LMWH monotherapy for 3 to 6 months (and possibly indefinitely) is currently recommended and commonly used for the treatment of cancer-associated VTE [21], demonstrating clinical benefit over VKAs in the secondary prevention of VTE [22-25].

The clinical outcomes of cancer patients with i-PE

Incidental-PE appears to be a clinically relevant phenomenon, potentially associated with unfavourable outcome [18]. Indeed, the clinical outcomes of cancer patients with i-PE do not seem to differ significantly from those associated with symptomatic VTE in terms of 1-year VTE recurrence risk, risk of major bleeding complications and mortality rate [4], while survival appears worse compared to matched controls without PE [13]. However major variables that determine survival in cancer patients such as symptoms and PS were not matched in this study, and are also not reported in similar registry cohort studies [26].

Furthermore it appears that there is variation in outcome amongst i-PE patients, and it now appears that while some i-PEs are low risk for recurrence and death, others may be progressive, and become symptomatic and life-threatening. O'Connell and colleagues found that patients with more proximal i-PE have significantly higher death rate compared to matched controls (hazard ratio [HR] for death 1.51; 95% CI 1.01-2.27) [13], while for patients with isolated subsegmental PE (SSPE), survival was not

significantly different from matched controls (HR 1.04; 95% CI 0.44-2.39, P=0.92). Similarly, a recent meta-analysis by van der Hulle *et al*. found that the weighted pooled 6-month mortality was higher in patients with a centrally located i-PE than in those with a peripherally located i-PE: 42% (95% CI 33-52%) versus 30% (95% CI 25-36%) with a HR of 1.5 (95% CI 1.1-2.0) adjusted for age, sex, type of cancer, cancer stage and management [27]. However, van der Hulle *et al*. argue against different management for SSPE and more proximally located i-PE due to a comparable VTE recurrence risk (HR 0.65; 95% CI 0.22-1.9 adjusted for age, sex, type of cancer, cancer stage and management) [27].

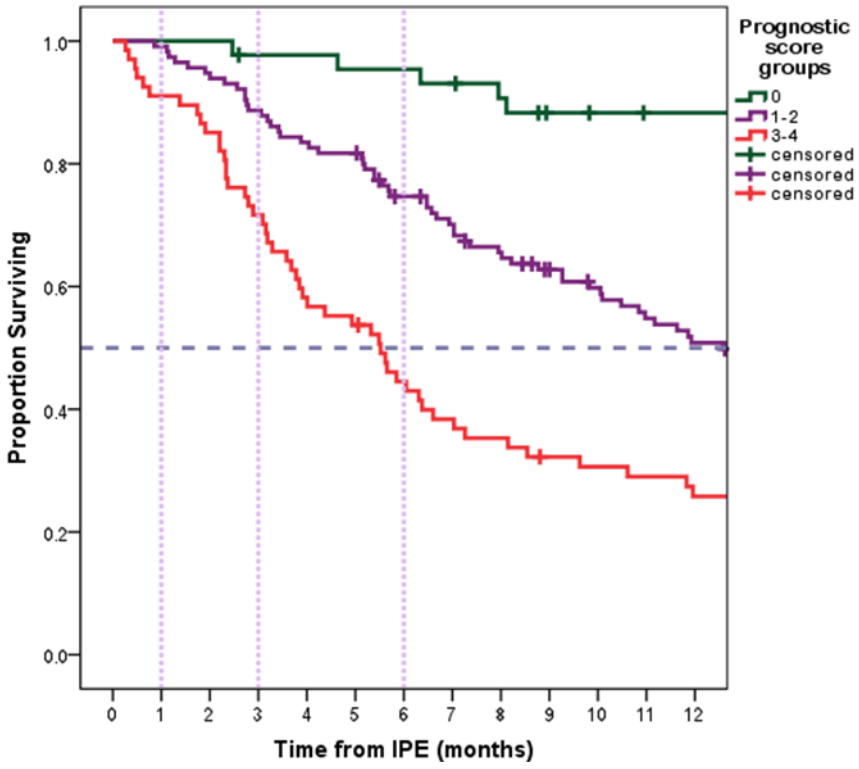
Further uncertainties arise from the recent meta-analysis by van der Hulle *et al*. where only a very small survival deficit for patients with i-PE that did not receive anticoagulation versus the ones that did was found [27]. The weighted pooled 6-month mortality was 37% (95% CI 29-44%) in patients treated with LMWH compared to 47% (95% CI 28-66%) in patients who did not receive anticoagulation; admittedly the numbers of untreated patients were low, and the reasons for no treatment were not documented. However, the 6-month risk of symptomatic recurrent VTE in patients with cancer-associated i-PE receiving LMWH was almost halved in comparison to those who did not receive anticoagulant treatment: 6.2% (95% CI 3.5-9.6%) versus 12% (95% CI 4.7-23%).

Interestingly, the presence of symptoms in cancer patients with i-PE have also been correlated with worse outcome [13]. Our prospective audit also found the presence of new symptoms, along with PS, to be the most consistent predictors of survival, with 52% patients reporting new or worsening pre-existing symptoms [14]. As a result, the Hull Prognostic Score was proposed, asking patients two key questions at assessment:

1) Have you any new symptoms above the usual symptoms related to your cancer or the treatment?
2) Have any of your usual symptoms suddenly worsened?

The Hull Score is obtained by assigning 0 points for PS = 0, 2 points for PS = 1-2, and 3 points for PS = 3-4, and scoring 0 points for no new and no worsening symptoms and 1 point for any new or any worsening symptoms, or both. This has enabled stratification of patients into 3 groups: with a maximum score of 4, low risk (score = 0); intermediate risk (score = 1-2); and high risk (score = 3-4), with excellent survival separation (Figure 1).

Figure 1 [14]: Survival curves as demonstrated by the Hull Score prognostic model, stratified into low (0), intermediate (1-2) and high (3-4) risk groups for the first 12 months of follow-up, with indicators for the 30-day, 3-month and 6-month cut-offs and the median for survival.



The differences in the symptom burden in cancer patients with i-PE versus those without, evolution of symptoms and changing PS are also factors that have not been studied sufficiently. Our data

suggest that these are equally, if not more important to the anatomical location, or the anticoagulant treatment of the i-PE. Furthermore, a diagnosis of i-PE accompanied by new or worsening symptoms, will invariably impact upon the QoL of the patient, additional to the existing burden of the cancer itself, as would any recurrent VTE or significant bleeding that occurred as a consequence. A diagnosis of VTE is generally distressing in itself, yet in the context of the cancer journey, is likely to have further compounding effects on QoL. Qualitative studies conducted with patients receiving long-term treatment for symptomatic VTE suggest the VTE produces a significant symptomatic and psychological burden for these patients [28, 29], with a significant additional impact on health-related QoL [30]. To date, the possible impact of an incidentally-diagnosed PE on a patient's psychological and emotional wellbeing has also received little or no attention.

In conclusion therefore, the need for more robust, prospectively gathered data regarding the impact of an i-PE on a patient's overall outcome is necessary to guide evidence-based management. A randomised trial of treatment versus non-treatment is not feasible as there is questionable equipoise, but understanding the survival and QoL deficit of a PE that is treated would be useful. Therefore, gathering the prospectively relevant clinical data (symptom severity, complexity and grade), PS and QoL data could facilitate a more stratified and individually tailored approach to the treatment of i-PE in cancer patients.

We therefore propose a prospective matched case-controlled study that takes into consideration the major parameters that define outcome in the cancer patient.

Research Questions

1. For people with i-PE, compared with those without i-PE:
    - What is the symptom-burden and QoL at baseline?
    - What is the symptom-burden, QoL, PS, survival, occurrence of VTE and haemorrhage over time?
2. For the study group as a whole:
    - Does the presence of i-PE or other clinical and demographic factors predict symptomburden, QoL, PS, survival, occurrence of VTE, haemorrhage and/or days in hospital?
3. For people with i-PE:
    - What is the experience of people with i-PE of being diagnosed and their management?

Aims of the Research

The primary aim of the study is to compare PS, symptoms, QoL, survival and other key clinical outcomes such as VTE o(re)currence, haemorrhage, days in hospital, i-PE treatment-related complications where relevant and disease progression/recurrence between people with cancer with and without i-PE.

A secondary aim is to identify clinical and demographic predictors of symptom burden, VTE o(re)currence, haemorrhage, QoL and PS.

Our initial work [31] used a simple symptom burden tool based on the work of O'Connell *et al.* [12].
In this study we aim to refine and expand on the capture of QoL and symptom burden by using validated tools: the short form (SF-12®) Health Survey [32] (Appendix A); the Edmonton scale for symptom assessment (ESAS) [33] (Appendix B); and the Anti-Clot Treatment Scale (ACTS) [34] (Appendix C). We also aim for the first time to capture evolution of symptoms through the longitudinal assessment of these patients over 6 months.

The qualitative component, consisting of semi-structured interviews with a sample of participants willing to be interviewed about their experience, aims to explore the true impact of an i-PE diagnosis in the context of a patient's cancer journey.

Research Methods

Participants and setting

Participants will be recruited from a single regional oncology tertiary centre. This is an observational, non-interventional, prospective case-control cohort study, which will recruit a maximum of 77 patients with incidentally diagnosed PE (Study Arm), and 154 controls with no thrombosis (Control Arm) who will be matched to the i-PE patients according to the below predetermined matching criteria:

- Age (+/- 5 years)
- Gender
- Tumour type
- Tumour stage: patients will be matched according to the stage of the tumour at the time of i-PE diagnosis. Survival will be calculated from study entry for control participants, and the date of i-PE diagnosis for Study Arm participants.

- Performance score: patients will fall into one of two groups, ECOG PS = 0-1 and PS ≥2

It is acknowledged that a range of age and PS may need to be implemented as absolute matching might not be possible. While every effort will be made to match as closely as possible, it is possible that some discrepancy may exist (e.g. a patient with PS of 1 may be matched to controls with PS of 1 and 0, or even two 0s). No active screening to exclude i-PE in these patients will be undertaken. The basis of 'non i-PE' will be that of the results of existing investigations (CT scans related to regular management) that will be screened to make sure that the matching patients do not have a reported i-PE.

Study arm; identification

The i-PE pathway used in HEYHT has been implemented since 2010 [35]. Patients are most commonly diagnosed either at the time of scanning by the radiographer/radiologist (the so-called 'first pass' patients) or at the time or reporting ('second pass' patients), and are then referred to the i-PE clinic.

The patient information sheet (PIS) will be provided to all potential participants when they attend for their first clinic appointment by a member of their clinical team. All interested patients will be offered the opportunity to discuss the study with a trained member of the study team and to ask any questions.

Inclusion criteria

1) Adults ≥ 18 years
2) Active cancer
3) Incidentally diagnosed with pulmonary embolism
4) Able to provide written informed consent
5) Able to complete study assessments

Exclusion criteria

1) No current cancer
2) Known other current thrombosis (either receiving treatment or within the last 12 months)
3) Inability to provide informed consent or complete study assessments

Control arm

Two matched controls will be identified through the HEY cancer multidisciplinary team (MDT) database for each cancer patient with i-PE recruited (154 controls in total). These will be identified in an ongoing fashion as soon as the characteristics of the patient with the i-PE become available. Identified patients will be contacted to participate in the study at their next regular oncology outpatient's appointment or sent a PIS through the post with an invitation letter from their usual consultant or nurse specialist.

Control patients who agree to participate will be provided with the PIS, and will have a chance to ask any questions.

Inclusion criteria matched by specified criteria to index study group participant

1) Adults ≥ 18 years
2) Active cancer
3) Able to provide written informed consent
4) Able to complete study assessments

Exclusion criteria

1) No current cancer
2) Known other current thrombosis (either receiving treatment or within the last 12 months)
3) Inability to provide informed consent or complete study assessments

Informed consent

Before enrolment into the study, participants will be fully informed of the nature of the study and all relevant aspects of the study procedures. Each subject will be provided with the PIS and Informed Consent Form (ICF), and will have ample opportunity to ask questions. They will be reassured that their participation is voluntary and will be informed about their right to withdraw from the study at any time without giving reason and without any disadvantage to their future medical care.

Consent will be taken either by the PhD student or another member of the study team; all those taking consent will have up-to-date Good Clinical Practice (GCP) training. The ICF will be signed and dated by the appropriate parties and, once complete, the original ICF will be retained by the

Investigator, a copy will be filed in the patient's medical notes, and a copy will be provided for the patient to keep.

In view of the purely observational nature of the study, and the potential burden involved in returning to the clinic for further consenting procedures, especially for those who live a distance away, although a usual "thinking time" of 24 hours will be given, those who are keen to participate straight away may provide written consent at their first clinic visit. All patients will be informed that participation is voluntary, that they can withdraw at any time without giving a reason and without their care being affected.

Qualitative sub-study

For the interview component of the study, the sample will be derived from those with i-PE who have already consented to the main study. The main PIS will include information about the qualitative sub-study. The consent form to the main study will include an option to be approached for interview if needed. A purposive sample (according to age, gender and PS) will be invited during study assessment visits, until the recruitment target (15-20 patients) is met. Participants meeting the purposive sampling requirements will be contacted and verbal confirmation obtained that the participant is still happy to participate in an interview and to discuss any questions the participant may have.

Consenting participants will have two interviews; the first within 1 month of study baseline, and a second between 3 and 6 months post baseline.

If the participant is happy to proceed, an arrangement will be made for a face to face interview with the researcher at a time and place convenient for the patient. In practice this is likely to be at the next study assessment visit, or at the patient's home.

Prior to the interview, participants will be asked to sign a separate consent form which will give permission to participate in an audio-recorded interview and to used anonymised quotes in any publication. A topic guide will be used, developed from the literature and expertise of the research team. Interviews will be audio-recorded and verbatim transcribed. Thematic framework analysis will be used to analyse the data including familiarisation, and line by line coding to form a coding framework. A summary of each transcript will also be recorded as a memo along with any additional contemporaneous field comments from those conducting the interview and analysing the transcript. This is important to allow constant

comparison between transcripts and within each transcript. Constant comparison of these initial codes will lead to the development of categories which will form the main themes.

Study Assessments

Table 1: Study Procedures for Study Arm patients

Assessment	Baseline (from clinical record after consent)	Visit 1 (+7 days from baseline*)	Visit 2 (+30 ±3 days from baseline*)	Visit 3 (+90 ±3 days from baseline*)
SF-12	✓	✓	✓	✓
ESAS	✓	✓	✓	✓
ACTS		✓	✓	✓

*N.B. This may be earlier than 7 days from consent.

Table 2: Study Procedures for Control Arm patients

Assessment	Baseline	Visit 1 (+7 days)	Visit 2 (+30 ±3 days)	Visit 3 (+90 ±3 days)
SF-12	✓	✓	✓	✓
ESAS	✓	✓	✓	✓

Baseline measures

Study arm patient baseline data will be sourced from the clinical record of the first i-PE clinic visit; there these are routinely recorded as part of clinical care. Access to the clinical record for these data for the purposes of the study will only occur following patient consent.

Control arm patient baseline data will be collected following consent.

1. Clinico-demographic data
    a. Demographic characteristics (age, sex, ethnicity)
    b. Symptom burden and QoL
        i. SF-12; a short-form multidimensional generic tool designed to provide a measure of a patient's health-related QoL [32].
        ii. Edmonton Symptom Assessment System (ESAS); a commonly used symptom assessment tool for advanced cancer and palliative care patients, which can be used to provide a clinical profile of the

severity of nine commonly encountered symptoms over time [33, 36].

c. Disease-related factors (stage, site)
d. Cormorbidities (Charlson Comorbidity)
e. Australian-modified Karnofsky Performance Status (AKPS) (Control Arm only; Study

Arm patients will have a calculated AKPS from their routinely collected World Health Organisation [WHO] PS [37]); the AKPS is a palliative care modified Karnofsky Performance Scale [38] which can detect a change in status in 10% gradations and is thus likely to be more sensitive to change in this population who may include people with WHO 3 or 4.

f. WHO performance scale for matching purposes

2. Routinely assessed thrombosis risk (Study Arm only)
    a. The Pulmonary Embolism Severity Index, or PESI score, is a simple tool used to estimate the risk of 30-day mortality in patients with acute PE, consisting of 11 objective clinical variables which are combined to produce a risk classification (classes I-V) [39, 40].
    b. Hull Score tool (Appendix D).
3. Routinely collected blood test data (where present in the clinical record)
    a. Most recent D-dimer (if within 1 month of entry)
    b. Most recent C-Reactive Protein (CRP) (if within 1 month of entry)
    c. Medications

Table 3: Data collected at study visits

Data collected	Baseline (from clinical record after consent)	Visit 1 (+7 days)	Visit 2 (+30 ±3 days)	Visit 3 (+90 ±3 days)	Visit 4 (+180 ±3 days)	Visit 5 (+ 1 year ±3 days)
Demographic data	✓					
Diseaserelated factors	✓					

Comorbidities	✓					
AKPS	✓					
WHO PS	✓					
PESI Score	✓					
Hull Score	✓					
D-dimer	✓					
CRP	✓					
Medications	✓					
SF-12	✓	✓	✓	✓		
ESAS	✓	✓	✓	✓		
ACTS (Study Arm only)	✓	✓	✓	✓		
Survival		✓	✓	✓	✓	✓
O(re)currence of VTE		✓	✓	✓	✓	✓
Haemorrhage		✓	✓	✓	✓	✓
Days in hospital		✓	✓	✓	✓	✓
Cancer progression		✓	✓	✓	✓	✓

Follow up

Follow up assessments will be taken at +7, +30, +90, +180 days and one year post baseline measures.

Patient report measures will be requested at follow up time points to +90 days. In view of likely attrition and mindful of participant burden, clinical record data only will be needed for the +180 day and 1 year follow up.

Study Arm patients will complete the SF-12, ESAS and Anti-Clot Treatment Scale (ACTS) questionnaires at each appointment, and the control patients will complete the SF-12 and ESAS questionnaires only. Filling out the questionnaires should take approximately 15 minutes (SF-12 and ESAS only) and 30 minutes if ACTS is included. Participants will have the option of completing these in clinic or over the phone assisted by the research, or by postal return according to preference.

In the Study Arm, the first clinic measures will be counted as the baseline data. In the control arm, the baseline data will be the date baseline study measures are collected following consent.

Outcome measures

Patient report measures

- SF-12
- ESAS
- ACTS (Study Arm patients on anticoagulation only); an anticoagulation-specific, 15-item patient-reported instrument to delineate the burdens and benefits associated with anticoagulation therapy and thereby to measure overall patient satisfaction with their treatment [34, 41].

Clinician/researcher-rated and clinical record measures

- AKPS
- Survival
- O(re)currence of VTE
- Haemorrhage
- Days in hospital
- Cancer progression
- Medications

Arrangements for patients with special communication needs

For participants who need help understanding the written information, the PhD member of the research team taking consent will read and explain the information provided in the study information sheet to the participant, and there will be a large print version of the PIS available for those who are visually impaired.

Withdrawal of subjects

Both patients and controls have the right to withdraw from the study at any time for any reason or, if necessary, the PI may withdraw a subject from the study to protect the subject's health. The type and timing of the withdrawal will be fully recorded on the case report form (CRF). Data collected up to that point will be used in the analysis, but no further data will be collected. Their management will not be affected in any way. They should continue on regular follow-up with their care team.

Primary and secondary endpoints

The primary endpoint is patient QoL at +90 days.

The secondary endpoints are:

i) Survival, AKPS, o(re)currence of VTE; cancer progression; number of days in hospital; episodes of documented haemorrhage at +180 days and one year, and

ii) Symptom burden and anticoagulant-related QoL (where relevant) at +90 days.

Data Management

Source data

As a minimum, the following information will be recorded in the patient's clinical record for study visits or telephone contacts:

- Clearly written date of visit or contact, brief study title/acronym and visit number
- Date patient given PIS
- Date consent form signed
- Date of screening
- Medical history, concomitant diseases and medication
- Any other relevant information

Participant data protection

The use and control of all data will comply at all times with the requirements of the Data Protection Act 1998. The PI will be responsible for data collection, recording and quality. Data will be collected and collated using a specifically designed database. Access will be via password protected log-in on hospital servers only and will be limited to members of the research team. The file itself will have password protected opening.

Participants will be informed that their data are held on file, and provide consent that these data may be viewed by the Sponsor and by external auditors on behalf of either the sponsor or regulatory agencies. They will be similarly informed that this data and a report of the study will be submitted to the Sponsor and may also be submitted to government agencies and for publication, but that they will not be individually identifiable in any report. The investigators undertake to hold all personal information in confidence and in compliance with the Data Protection Act 1998.

IT Services Department has a backup procedure approved by auditors for disaster recovery. Servers are backed up to disk media each night. The disks run on a 4 week cycle. Files stay on the server unless deleted by accident or

deliberately. Anything deleted more than 4 weeks previously is therefore lost. Additional 'archive' backups are taken for archived data, so data should not be lost from this type of system, e.g. File Vision, which stores Medical Records. Disks are stored in a fireproof safe. Study documents (paper and electronic) will be retained in a secure (kept locked when not in use) location during the trial. Access to stored records is strictly controlled.

Statistical Considerations

Statistical advice has been sought from the Statistical Consultancy Service (HYMS, University of York) in preparing this protocol.

Descriptive statistics will be used to describe the baseline characteristics of the group as a whole and then by study and control groups.

Question 1

Data will be analysed as intention to treat, that is, by study or control group at baseline, even if control patients subsequently develop i-PE and then attend the i-PE clinic.

The primary aim is to compare the two groups with regard to QoL at +90 days. ANCOVA will be used to compare QoL at +90 days, adjusting for baseline QoL. A further generalised linear model will allow adjustment for confounding variables, including baseline QoL, age, sex, disease stage, comorbidities and AKPS.

Secondary aims: to compare the other outcomes by group:

6.1.1. In terms of survival analysis, this will be measured from the first diagnosis of the tumour stage within which the i-PE occurred, rather than from the event itself, so as to avoid lead time bias. A log rank test will be used to compare overall survival between the cancer patients with i-PE and cancer patients without i-PE. Survival at 6 months will also be reported as a surrogate VTE-related outcome. Cox regression will be used to measure the effects of other variables (confounders). A Kaplan-Meier curve will be drawn.

6.1.2. The study is also designed to collect data for specified secondary endpoints (clinically significant events), such as the recurrence of PE/VTE; cancer progression or recurrence; number of days in hospital; haemorrhage; and anticoagulant-related QoL; of the i-PE cancer patients compared to cancer patients without VTE. Log rank tests, chi-square tests and t-tests will be used as

appropriate to compare the secondary outcomes between the cancer patients with i-PE and cancer patients without i-PE.

Question 2

A secondary analysis of data from the group as a whole will be analysed to investigate which clinicdemographic factors are predictive of QoL, symptoms, days in hospital and survival. In addition, and events such as VTE o(re)currence or haemorrhage which reach sufficient events to be treated as a dependent variable will also be investigated.

Each clinical outcome variable will be modelled separately with multilevel modelling techniques. Multilevel models consider repeated observations to be 'nested' within participants and accommodate data in which the number of observations vary among participants (Baseline, +7 days, +30 days, +90 days, +180 days and +1 year). To explore if there are changes in the clinical outcome variables over time, a basic pattern of change over time will be modelled. If the fixed slope estimate is statistically significant then there will be evidence of an increase or decrease over time. Individual multilevel models will then be undertaken for each covariate to explore the association with each clinical outcome variable. Summary statistics will be presented as mean (standard deviation, SD) or n (%). The models will be further controlled for covariates which showed a significant univariate association with the clinical outcome measures ($p<0.10$), along with age and gender (as these are biologically plausible). The statistical significance was tested with a t-test (estimate/standard error) and a p-value of <0.05. All analyses were undertaken on Stata/SE (v14.0).

The Stata (v14.0) computer package will be used with a *p*-value of <0.05 taken as the level of significance.

Sample size calculation

The calculation of the sample size required was overseen by Victoria Allgar (statistician).

For the SF-12, to detect a minimal clinically important difference (MCID) of 5 between the i-PE group and the control group at 90 days, for PCS (Physical Component Summary) we require 55/110 (165) and for MCS (Mental Component Summary) we require 42/84 (126) patients using scores from data in an Australian study https://health.adelaide.edu.au/pros/docs/reports/general/qol_quality_of_l

ife_sf_12.pdf, based on 80% power and 5% significance (calculated using Stata/SE 14.0).

Sample calculation is based on the survival difference seen in a small retrospective study by Dentali and colleagues [26]; based on a log-rank test with a 5% significance level, this will have 80% power to detect the difference between an expected survival rate of 45% in the i-PE group and one of 30% in the control group at 6 months when the sample sizes are 77 and 154, respectively (a total sample size of 231) (calculated using State/SE 14.0).

Hence to maximise the power the total sample size will be 231.

Monitoring and Quality Assurance

The study may be monitored in accordance with HEY R&D department's standard operating procedures to ensure compliance with ICH GCP and the Research Governance Framework 2005. All study-related documents will be made available upon request for monitoring by R&D monitors.

Administrative Procedures

Study Approvals

The study will be conducted subject to Research Ethics Committee (REC) favourable opinion, Health Research Authority (HRA) approval, and confirmation of local R&D capacity and capability. In the event that a protocol amendment needs to be made that requires REC and HRA approval, the changes in the protocol will not be implemented until the amendment and revised study documentation have been reviewed and received a favourable opinion/approval.

Investigator indemnification

This is an NHS-sponsored research study. If there is negligent harm during the clinical trial when the NHS body owes a duty of care to the person harmed, NHS indemnity covers NHS staff and medical academic staff with honorary contracts only when the trial has been approved by the Trust R&D department. NHS indemnity does not offer no-fault compensation and is unable to agree in advance to pay compensation for non-negligent harm.

Financial Aspect

The cost of the study will be covered by the Academic Oncology's HYMS Donation Account.

Reporting and Dissemination

Data arising from the research will be made available to the scientific community in a timely and responsible manner. A detailed, scientific report will be submitted to a widely accessible scientific journal. All authors will comply with internationally agreed requirements for authorship and will approve the final manuscript prior to submission.

Quality Control and Quality Assurance

This study will be conducted in accordance with the International Conference for Harmonisation of Good Clinical Practice (ICH GCP) guidelines; and the Research Governance Framework for Health and Social Care 2005.

Archiving: Retention of Study Records

Data will be collected and retained in accordance with the Data Protection Act 1998. Study documents (paper and electronic) will be retained in a secure (kept locked when not in use) location during and after the trial has finished. All essential documents including source documents will be retained for a minimum period of 5 years after study completion. A sticker stating the date after which the documents can be destroyed will be placed on the inside front cover of the clinical record of trial participants. Access to stored records is strictly controlled.

Overall Timescale for the Study

Recruitment will begin as soon as all necessary approvals have been obtained (REC, HRA, local R&D capacity and capability confirmation). Recruitment will run for approximately 24 months. Individual participants are involved in the trial for 180 days (+/- 7 days).

Study Completion

Study completion refers to the date of final data collection from the last patient. Paper records from the study will be stored for 5 years from study end.

The Chief Investigator will notify the REC in writing that the research has ended within 90 days from completion of the study, and within 15 days if the study is discontinued prematurely. A summary of the final report on the research will be submitted to the REC within 12 months of the conclusion of the study.
The final report shall include the data from all of the investigations and a summary of the statistical data. The final report will be generated by the PI.

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Appendix H CLOTS-QoL study patient information sheet (PIS)

Queen's Centre for Oncology and Haematology
Castle Hill Hospital
Castle Road
Cottingham
HU16 5JQ

Participant Information Sheet for patients with incidentally found Pulmonary Embolism

Short Title: CLOTS-QoL Study

CLinical OuTcomes, Symptoms and Quality of Life in cancer patients with i-PE

Department: Queen's Centre for Oncology and Haematology, Castle Hill Hospital
Principal Investigator: Professor Miriam Johnson

Introduction

You are being invited to take part in a research study. We are studying the effects of blood clots that are found unexpectedly (incidentally) in the lung of patients with cancer, compared with patients who do not have blood clots (controls). These are known as i-PE.

Before you decide whether or not to take part, it is important for you to understand why this study is being carried out and what it will involve if you decide to take part. This study will not alter your care in any way. Please take time to read the following information carefully, and please feel free to discuss it with friends, relatives and your GP if you wish.

Part 1 explains the purpose of the study, and what will happen to you if you decide to take part.

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

Please ask us if there is anything that is not clear, or if you would like more information. Please take time to decide whether or not you wish to take part in the study.

Part 1

What is the purpose of the study?

The purpose of the study is to try to understand the effects of an incidentally diagnosed blood clot in the lung (i-PE) on cancer patients' quality of life, symptoms and survival from the patients' point of view.

Why have I been invited?

Your recent CT scan arranged by your doctor as part of your cancer treatment plan has identified an unexpected blood clot in the lung (i-PE). You have therefore been referred by the CT scan department to the i-PE clinic for assessment of this clot and to start treatment. We are inviting all patients aged 18 years and over who have been referred to the i-PE clinic as part of routine care to participate in this study.

Do I have to take part?

No. It is entirely up to you to decide whether or not to take part. If you do decide to take part, you will be asked to sign a consent form indicating your willingness to participate in the study. If you later change your mind about participating, you are free to withdraw from the study at any time and without giving a reason.

If you decide not to take part, or after joining the study you decide to withdraw, the standard of care you receive will not be affected in any way.

What will happen to me if I take part?

Agreeing to take part in the study will not affect your treatment or care in any way. If you do agree to take part, you will be asked to complete a consent form, and then complete the study questionnaires (3 questionnaires) 7 days after your first clinic appointment. You will already have completed 2 of these questionnaires as routine at that first clinic appointment, but we need your permission to use the information from these in our study.

The questionnaires will include questions about your general health and any other symptoms that you might have experienced. This should take about 20 minutes.

Over the next 3 months, you will need to see the research team at 2 additional visits (after 1 month and after 3 months). Each time you will be asked to complete the same 3 questionnaires. We will try to coincide these appointments with regular appointments you will have for your management, although this may not always be possible. The researcher will be able to help you complete the questionnaires if you wish. The questionnaires can be filled out during a visit with the researcher, or over the phone, or you can fill them in yourself and post them back to the researcher, whichever is most convenient to you. A stamped addressed envelope will be provided if you wish to post them back.

Interview Study

In addition, we would like to talk to some participants in more detail about their experience of receiving a diagnosis of i-PE and its treatment to get a more in-depth understanding.

If you are interested in sharing your experience with us, please indicate this on the consent form for the study. You may be invited to participate in two one-to-one interviews with one of the researchers, one within a month of your i-PE and a second one within 3 to 6 months. Please note, not all participants will be needed for an interview. Interviews would be arranged for a time and place that is convenient for you, for example, if it is easier, this could be in your own home.

During the interviews, the researcher will ask you questions about your experience of your i-PE and the treatment you have received for it. The questions will be broad to enable you to talk about anything in relation to the i-PE that is important to you.

Each interview would last for between 30 – 60 minutes, and will be audio recorded by the researcher. If you take part in one interview and decide you do not wish to be interviewed a second time, you do not have to give a reason and this will not affect your care in any way.

What will happen to the recording?

The audio recording will be securely stored electronically. This means that only the research team will have access to it.

The researcher will listen to the recording of the interview and type it out word for word. This transcript will be anonymous (people will not be able to identify you from it) and securely stored. The information from all the interview records in the study will be analysed and summaries collected.

Will other people know what I have said?

During the interview, you may speak about things which you do not want others to know, e.g. family, nurses. Everything you discuss in the interview will remain anonymous and confidential. Information that identifies you (e.g. signed consent forms and your personal information) will only be accessible to the researchers or authorised people responsible for checking the study has been run correctly.

What are the potential disadvantages and risks of taking part?

This study does not change the care you receive; however, some of the questionnaires may bring to mind situations or symptoms which you find difficult. If this should happen, your usual care team will be available if extra support is needed.

What are the possible benefits of taking part?

Although there are no expected direct benefits to you personally, we hope that the information we will gain from this study will help us to understand the effects of i-PE diagnosis on the life of cancer patients, and to use this to find ways of improving the experience of cancer patients in the future.

Will taking part in the study cost me anything, and will I be paid?

Participation in the study will not cost you anything and there will not be any payment for taking part in the study.

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 have suffered, will be addressed. More detailed information on this will be given in Part 2.

Will my taking part in this study be kept confidential?

Yes. All the information about your participation in the study will be kept confidential. The details are included in Part 2.

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.

<u>Part 2</u>

What if there is a problem?

This study does not change your treatment in any way. In case of any problems with your cancer or its treatment you should contact your usual clinical team to seek advice. You will have been given contact numbers for regular hours and out-of-hours. Please use these accordingly.

Complaints:

If you have a concern about any aspects of the way you have been approached and treated whilst taking part in this research study, you should ask to speak to the researchers who will do their best to answer your questions:

Contact Prof Miriam Johnson on 01482 463482

Independent information about participants' rights, research-related questions and research-related injury may be obtained from either:

Patient Advice and Liaison Service (PALS)
Contact Telephone Number: 01482 623065
Address: Hull Royal Infirmary, Anlaby Road, Hull, HU3 2JZ
Contact Email Address: pals.hey@hey.nhs.uk

Or:

Healthwatch Kingston upon Hull
Contact Telephone Number: 01482 332999
Address: The Strand, 75 Beverley Road, Hull, HU3 1XL
Contact Email Address: enquiries@healthwatchkingstonuponhull.co.uk
Website: www.healthwatchkingstonuponhull.co.uk

Harm:

It is extremely unlikely that any harm can come to any participant in this study. In the event that something does go wrong, no special compensation arrangements exist. However, if you are harmed during the research due to someone's negligence, then you may have grounds for legal action against the NHS Trust, but you may have to pay your own legal costs.

Will information about me be kept confidential?

Yes. We will follow ethical and legal practice, and all information about you will be kept strictly confidential in accordance with the 1998 Data Protection Act. You will be given a unique identification code number by the research team to put on the questionnaires, so no names will be used and, when results are reported, only this code will be used. All files will be kept in locked filing cabinets at the Clinical Trials Office at the Queen's Centre for Oncology and Haematology, which is only accessible to the research team.

The Principal Investigator will act as custodian of the data and will regulate access. Authorised members of the research team will have access to your medical records in order to collect the information needed for the study, and they will be responsible for ensuring this data is anonymised. Also, authorised persons (e.g. representatives from the Sponsor of the study or regulatory authorities) may need to look at information collected about you, including your medical notes, for monitoring or inspection purposes.

Information about you will be kept on paper records kept in secure offices, and on electronic databases stored on secure computers, which will only be accessible by the research team. We will keep our research records for up to 5 years so that we can

answer any questions about our scientific methods which may be asked by other doctors and scientists in the future.

With your permission, we will send a brief letter to your GP to inform them you are taking part in the study.

What will happen to the results of the research study?

The results of the study will be analysed and the findings may be presented at scientific conferences and published in a scientific journal over the next few years, so others can read about and learn from the research. We will also provide a summary report to groups involved with supporting or caring for people with cancer and i-PE. You will not be identified in person in any report or publication arising out of this study.

We would be happy to provide a simplified summary of the main results at the end of the study for your information. If you wish to receive a copy of this, please let us know.

After the study has been reported, anonymised information from this study may be requested by authorised researchers studying other relevant projects.

Who has reviewed the study?

The study has been reviewed and given a favourable ethical opinion for conduct in the NHS by East Midland- Nottingham 1 Research Ethics Committee. REC reference:

16/EM/0474

What should I do if I wish to take part in the study?

If you wish to participate in the study, you will be asked to sign a consent form after discussion with the research nurse. You will also be given a copy of this information sheet and a signed copy of the consent form to keep. A copy of the consent form will be filed in your patient notes, and one will be filed with the study records.

If you decide that you do not want to take part in the study, it will have no effect whatsoever on any treatment or care that you will receive.

Contact for further information:

If you have any problems or further questions, or would like any further information about this study, please contact:

Prof Miriam Johnson (via her secretary at the University of Hull on 01482 463482).

Thank you for taking the time to read this information sheet and considering this study.

Appendix I: i-PE Patients consent form

Queen's Centre for Oncology and Haematology
Castle Hill Hospital
Castle Road
Cottingham
HU16 5JQ

PATIENT CONSENT FORM

Title of Study: CLinical OuTcomes, Symptoms and Quality of Life in cancer patients with i-PE (CLOTS-QoL Study)

Name of Researcher: Dr Naima Benelhaj

Principal Investigator: Professor Miriam Johnson

Participant Study Number:

Please INITIAL boxes:

1. I confirm that I have read and understood the information sheet *(V2-16/11/16*) for the above study. ☐

2. I confirm that I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

3. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my medical care or legal rights being affected. ☐

4. I agree that information needed for the study collected at my first i-PE clinic appointment, including the routine questionnaires, may be used for the purposes of the study. ☐

5. I understand to enable the study to be properly monitored and regulated, relevant sections of my medical notes and ☐

data collected during the study, may be looked at by members of the research team, and individuals from the sponsor (Hull and East Yorkshire Hospitals NHS Trust) or from regulatory authorities. I give permission for these individuals to have access to my medical records to view sections that are relevant to my taking part in this study.

6. I understand that the information will be used for medical research only and that I will not be identified in any way in the analysis and reporting of the results. ☐

7. I agree that all information collected about me as part of the study can be stored and analysed by the research team at the University of Hull. ☐

8. I understand that the information collected about me will be used to support other research in the future, and may be shared anonymously with other authorised researchers. ☐

9. I agree to my GP being informed of my participation in the study. ☐

10. I agree to take part in the above study. ☐

Interview Study

11. I agree to being contacted to take part in an interview with the researcher to talk about my experiences of receiving a diagnosis of i-PE.

_____	_____	_____
Name of patient	Signature	Date
_____	_____	_____
Name of person taking consent (*if different from researcher*)	Signature	Date

Appendix J: CLOTS-QoL study matched controls PIS and consent form

Queen's Centre for Oncology and Haematology
Castle Hill Hospital
Castle Road
Cottingham
HU16 5JQ

Participant Information Sheet for patients without incidentally found Pulmonary Embolism (Controls)

Short Title: CLOTS-QoL Study

CLinical OuTcomes, Symptoms and Quality of Life in cancer patients with i-PE

Department: Queen's Centre for Oncology and Haematology, Castle Hill Hospital
Principal Investigator: Professor Miriam Johnson

Introduction

You are being invited to take part in a research study. We are studying the effects of blood clots that are found unexpectedly (incidentally) in the lung of patients with cancer, compared with patients who do not have blood clots (controls). These are known as i-PE. You are being approached as a control patient that does **not** have an i-PE.

Before you decide whether or not to take part, it is important for you to understand why this study is being carried out and what it will involve if you decide to take part. This study will not alter your care in any way. Please take time to read the following information carefully, and please feel free to discuss it with friends, relatives and your GP if you wish.

Part 1 explains the purpose of the study, and what will happen to you if you decide to take part.

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

Please ask us if there is anything that is not clear, or if you would like more information. Please take time to decide whether or not you wish to take part in the study.

Part 1

What is the purpose of the study?

The purpose of the study is to try to understand the effects of an incidentally diagnosed blood clot in the lung (i-PE) on cancer patients' quality of life, symptoms and survival from the patients' point of view.

You have not been diagnosed with an i-PE and would be a control patient for this study.

Who are matched patients (controls)?

These are cancer patients who have a similar age range, gender, type of cancer, length of diagnosis and performance status (a measure of fitness or overall wellbeing and ability to perform daily activities) to patients who have been diagnosed with an i-PE.

Why have I been invited?

You have been chosen because you are a cancer patient who has not been diagnosed with an i-PE and you fit the matching criteria above.

Do I have to take part?

No. It is entirely up to you to decide whether or not to take part. If you do decide to take part, you will be asked to sign a consent form indicating your willingness to participate in the study. If you later change your mind about participating, you are free to withdraw from the study at any time and without giving a reason.

If you decide not to take part, or after joining the study you decide to withdraw, the standard of care you receive will not be affected in any way.

What will happen to me if I take part?

Agreeing to take part in the study will not affect your treatment or care in any way. If you do agree to take part, you will be asked to complete a consent form, and then complete the study questionnaires (2 questionnaires).

The questionnaires will include questions about your general health and any other symptoms that you might have experienced. This should take about 10-15 minutes.

Over the next 3 months, you will need to see the research team at 3 additional visits (after 7 days, after 1 month and after 3 months). Each time you will be asked to complete the same 2 questionnaires. We will try to coincide these appointments with regular appointments you will have for your management, although this may not always be possible. The researcher will be able to help you complete the questionnaires if you wish. The questionnaires can be filled out during a visit with the

researcher, or over the phone, or you can fill them in yourself and post them back to the researcher, whichever is most convenient to you. A stamped addressed envelope will be provided if you wish to post them back.

What are the potential disadvantages and risks of taking part?

This study does not change the care you receive; however, some of the questionnaires may bring to mind situations or symptoms which you find difficult. If this should happen, your usual care team will be available if extra support is needed.

What are the possible benefits of taking part?

We hope that the information we will gain from this study will help us to understand the effects of i-PE diagnosis on the life of cancer patients, and to use this to find ways of improving the experience of cancer patients in the future. As a control patient, there will be no direct benefit to you from this research.

Will taking part in the study cost me anything, and will I be paid?

Participation in the study will not cost you anything and there will not be any payment for taking part in the study.

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 have suffered, will be addressed. More detailed information on this will be given in Part 2.

Will my taking part in this study be kept confidential?

Yes. All the information about your participation in the study will be kept confidential. The details are included in Part 2.

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.

Part 2

What if there is a problem?

This study does not change your treatment in any way. In case of any problems with your cancer or its treatment you should contact your usual clinical team to seek advice. You will have been given contact numbers for regular hours and out-of-hours. Please use these accordingly.

Complaints:

If you have a concern about any aspects of the way you have been approached and treated whilst taking part in this research study, you should ask to speak to the researchers who will do their best to answer your questions:

Contact Prof Miriam Johnson on 01482 463482

Independent information about participants' rights, research-related questions and research-related injury may be obtained from either:

Patient Advice and Liaison Service (PALS)
Contact Telephone Number: 01482 623065
Address: Hull Royal Infirmary, Anlaby Road, Hull, HU3 2JZ
Contact Email Address: pals.hey@hey.nhs.uk

Or:

Healthwatch Kingston upon Hull
Contact Telephone Number: 01482 332999
Address: The Strand, 75 Beverley Road, Hull, HU3 1XL
Contact Email Address: enquiries@healthwatchkingstonuponhull.co.uk
Website: www.healthwatchkingstonuponhull.co.uk

Harm:

It is extremely unlikely that any harm can come to any participant in this study. In the event that something does go wrong, no special compensation arrangements exist. However, if you are harmed during the research due to someone's negligence, then you may have grounds for legal action against the NHS Trust, but you may have to pay your own legal costs.

Will information about me be kept confidential?

Yes. We will follow ethical and legal practice, and all information about you will be kept strictly confidential in accordance with the 1998 Data Protection Act. You will be given a unique identification code number by the research team to put on the questionnaires, so no names will be used and, when results are reported, only this code will be used. All files will be kept in locked filing cabinets at the Clinical Trials Office at the Queen's Centre for Oncology and Haematology, which is only accessible to the research team.

The Principal Investigator will act as custodian of the data and will regulate access. Authorised members of the research team will have access to your medical records in order to collect the information needed for the study, and they will be responsible for ensuring this data is anonymised. Also, authorised persons (e.g. representatives from the Sponsor of the study or regulatory authorities) may need to look at information

collected about you, including your medical notes, for monitoring or inspection purposes.

Information about you will be kept on paper records kept in secure offices, and on electronic databases stored on secure computers, which will only be accessible by the research team. We will keep our research records for up to 5 years so that we can answer any questions about our scientific methods which may be asked by other doctors and scientists in the future.

With your permission, we will send a brief letter to your GP to inform them you are taking part in the study.

What will happen to the results of the research study?

The results of the study will be analysed and the findings may be presented at scientific conferences and published in a scientific journal over the next few years, so others can read about and learn from the research. We will also provide a summary report to groups involved with supporting or caring for people with cancer and i-PE. You will not be identified in person in any report or publication arising out of this study.

We would be happy to provide a simplified summary of the main results at the end of the study for your information. If you wish to receive a copy of this, please let us know.

After the study has been reported, anonymised information from this study may be requested by authorised researchers studying other relevant projects.

Who has reviewed the study?

The study has been reviewed and given a favourable ethical opinion for conduct in the NHS by East Midland – Nottingham 1 Research Ethics Committee. REC Reference 16/EM/0474.

What should I do if I wish to take part in the study?

If you wish to participate in the study, you will be asked to sign a consent form after discussion with the research nurse. You will also be given a copy of this information sheet and a signed copy of the consent form to keep. A copy of the consent form will be filed in your patient notes, and one will be filed with the study records.

If you decide that you do not want to take part in the study, it will have no effect whatsoever on any treatment or care that you will receive.

Contact for further information:

If you have any problems or further questions, or would like any further information about this study, please contact:

Prof Miriam Johnson (via her secretary at the University of Hull on 01482 463482).

Thank you for taking the time to read this information sheet and considering this study.

Queen's Centre for Oncology and Haematology
Castle Hill Hospital
Castle Road
Cottingham
HU16 5JQ

PATIENT CONSENT FORM

Title of Study: CLinical OuTcomes, Symptoms and Quality of Life in cancer patients with i-PE (CLOTS-QoL Study)

Name of Researcher: Naima Benelhaj

Principal Investigator: Professor Miriam Johnson

Participant Study Number:

Please INITIAL boxes:

12. I confirm that I have read and understood the information sheet (*V2. 16/11/16*) for the above study. ☐

13. I confirm that I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

14. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, and without my medical care or legal rights being affected. ☐

15. I understand to enable the study to be properly monitored and regulated, relevant sections of my medical notes and data collected during the study, may be looked at by members of the research team, and individuals from the sponsor (Hull and East Yorkshire Hospitals NHS Trust) or from regulatory authorities. I give permission for these individuals to have access ☐

to my medical records to view sections that are relevant to my taking part in this study.

16. I understand that the information will be used for medical research only and that I will not be identified in any way in the analysis and reporting of the results. ☐

17. I agree that all information collected about me as part of the study can be stored and analysed by the research team at the University of Hull. ☐

18. I understand that the information collected about me will be used to support other research in the future, and may be shared anonymously with other authorised researchers. ☐

19. I agree to my GP being informed of my participation in the study. ☐

20. I agree to take part in the above study. ☐

_____	_____	_____
Name of patient	Signature	Date

_____	_____	_____
Name of person taking consent (*if different from researcher*)	Signature	Date

When completed: 1 copy for patient, 1 copy to be kept in medical notes, 1 original for researcher site file.

Appendix K: SF-12: Quality of life questionnaire

CLOTS-QOL Questionnaire NO ☐ Participant NO ☐
Study arm

SF-12: Quality of life questionnaire

SF-12 v2 Health and Well-Being

This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities

For each of the following questions, please circle or mark an X in the one box that best describes your answer.

1.) In general, would you say your health is:

Excellent	Very Good	Good	Fair	Poor
•	•	•	•	•

2.) The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

	Yes, Limited A Lot	Yes, Limited A little	No, Not Limited At All
• Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	•	•	•
• Climbing several flights of stairs	•	•	•

3.) During the past week, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
• Accomplished less then you would like	•	•	•	•	•
• Were limited in the kind of work or other activities	•	•	•	•	•

Appendix N: CLOTS-QoL study (Interview topic guide)

Queen's Centre for Oncology and Haematology
Castle Hill Hospital
Castle Road
Cottingham
HU16 5JQ

CLinical OuTcomes, Symptoms and Quality of Life in cancer patients with i-PE (CLOTS-QoL Study)

Interview Topic Guide

Introduction:

Researcher introduces self and thanks participant for willingness to take part in the interview.

Check of consent:

Researcher will check that the participant has read the original information sheet, understands the information about the interview study and addresses any questions. The researcher will then confirm that the participant is happy to proceed and will take written consent for the interview part of the study.

Explanation:

The researcher will make sure that the participant is aware of the audio-recorder and know when it is switched on, and off. The recorder will be switched on.

Interview:

The researcher will ask questions in the following areas:

1. Please tell me a bit about how life was for you before you found out about the i-PE?
2. How did you find out that you had an i-PE?
3. Can you tell me how getting an i-PE has affected you?
4. How has having an i-PE affected your cancer?
5. Have you been started on treatment for the i-PE? If so, what is your experience of it; if not, how do you feel about not getting treatment? (researcher will ask the appropriate question)
6. Is there anything else you would like to tell me about your i-PE or the cancer?

Thanks and conclusions:

The researcher will switch off the recorder and thank the participant. Permission will be sought to contact the participant in 2 – 5 (3mon) months for a repeat interview. The participant will be given an opportunity to ask the researcher any questions. The researcher will check whether the patient is concerned by any of the issues raised, and if so, offer to contact the clinical team at the next available opportunity.

Appendix L: Edmonton Symptom Assessment Scale ESAS

Symptoms, quality of life and place of care study Version 1.0 19/090/2014
ESAS-r

Please circle the number that best describes how you feel NOW:

No Pain	0	1	2	3	4	5	6	7	8	9	10	Worst Possible Pain
No Tiredness *(Tiredness = lack of energy)*	0	1	2	3	4	5	6	7	8	9	10	Worst Possible Tiredness
No Drowsiness *(Drowsiness = feeling sleepy)*	0	1	2	3	4	5	6	7	8	9	10	Worst Possible Drowsiness
No Nausea	0	1	2	3	4	5	6	7	8	9	10	Worst Possible Nausea
No Lack of Appetite	0	1	2	3	4	5	6	7	8	9	10	Worst Possible Lack of Appetite
No Shortness of Breath	0	1	2	3	4	5	6	7	8	9	10	Worst Possible Shortness of Breath
No Depression *(Depression = feeling sad)*	0	1	2	3	4	5	6	7	8	9	10	Worst Possible Depression
No Anxiety *(Anxiety = feeling nervous)*	0	1	2	3	4	5	6	7	8	9	10	Worst Possible Anxiety
Best Wellbeing *(Wellbeing = how you feel overall)*	0	1	2	3	4	5	6	7	8	9	10	Worst Possible Wellbeing
No _____ Other Problem *(for example constipation)*	0	1	2	3	4	5	6	7	8	9	10	Worst Possible

Appendix M: The Anti-Clot Treatment Scale (ACTS) questionnaire

Instructions: We are interested in your experiences of anti-clot treatment during the past 7/ 30/ 90 days. Please circle the number in the box that best describes your views

During the past 4 weeks…	**Not at all**	**A little**	**Moderately**	**Quite a bit**	**Extremely**
1. How much does the possibility of bleeding as a result of anti-clot treatment limit you from taking part in vigorous physical activities? (e.g. exercise, sports, dancing, etc.).	1	2	3	4	5
2. How much does the possibility of bleeding as a result of anti-clot treatment limit you from taking part in your usual activities? (e.g. work, shopping, housework, etc.).	1	2	3	4	5
3. How bothered are you by the possibility of bruising as a result of anti-clot treatment?	1	2	3	4	5
4. How bothered are you by having to avoid other medicines (e.g. aspirin) as a result of anti-clot treatment?	1	2	3	4	5
5. How much does anti-clot treatment limit your diet? (e.g. food or drink, including alcohol).	1	2	3	4	5
6. How much of a hassle (inconvenience) are the daily aspects of anti-clot treatment? (e.g. remembering to take your medicine at a certain time, taking the correct dose of	1	2	3	4	5

your medicine, following a diet, limiting alcohol, etc.).					
7. How much of a hassle (inconvenience) are the occasional aspects of anti-clot treatment? (e.g. the need for blood tests, going to or contacting the clinic/doctor, making arrangements for treatment while travelling, etc.).	1	2	3	4	5

Now I want to ask you about daily and occasional aspects of your anticoagulation therapy during the past 7/ 30/ 90 days.

During the past 4 weeks…	**Not at all**	**A little**	**Moderately**	**Quite a bit**	**Extremely**
8. How difficult is it to follow your anti-clot treatment?	1	2	3	4	5
9. How time-consuming is your anti-clot treatment?	1	2	3	4	5
10. How much do you worry about your anti-clot treatment?	1	2	3	4	5
11. How frustrating is your anti-clot treatment?	1	2	3	4	5
12. How much of a burden is your anti-clot treatment?	1	2	3	4	5
13. **Overall**, how much of a negative impact has your anti-clot treatment had on your life?	1 5	2 4	3 3	4 2	5 1
14. How confident are you that your anti-	1	2	3	4	5

clot treatment will protect your health? (e.g. prevent blood clots, stroke, heart attack, DVT, embolism).					
15. How reassured do you feel because of your anti-clot treatment?	1	2	3	4	5
16. How satisfied are you with your anti-clot treatment?	1	2	3	4	5
17. **Overall**, how much of a positive impact has your anti-clot treatment had on your life?	1	2	3	4	5

_____ _____ _____

Name of patient Signature Date

Thank you very much for your cooperation

Appendix O: Summary of themes, quotes and codes from the interview study

Theme	Subtheme	Codes	Quotes examples
i-PE in the context of living with a disease	Life with cancer	Cancer journey, ups and down, difficult, hard, worries.	*Cancer was doing fine until the blood clot came a long". (P01)* *"I think the cancer worried me than the blood clot". (P01)* *"Well it was upsetting to find out it was growing again before Christmas and so I am still waiting to find out results of the cancer search, and then to find out I got this It was in a big shock". (P02)* *"I've been living under concurrent radiotherapy, chemotherapy which hit me quite hard". (P03)* *"I have reacted very very badly throughout three cycles I have ticked off every side effect unfortunately". (P07)*
	CAT in context of cancer	did not know, un aware, lack of knowledge, out of a blue, symptom misattribution	*"I have heard of it, but I did not know it is associated with cancer treatment". (P02)* *"No,no I wasn't aware". (P03)* *"No point during my chemotherapy has anybody ever mentioned the possibility that it could cause a blood clot". (P05)* *"No, I never heard about it, in fact I didn't thought about it be quite honest". (P09)*

			"If I get a pain I immediately related to cancer, if I am a bit fluctuant or wind in my stomach I put it down to cancer". (P01)
		Diagnosis in the context of cancer between acceptance and shock.	*"My attitude at that point was Ok fine!, because, having been told you've had advanced progressive cancer and the prognosis that you've got 5 years". (P05)* *"after the cancer don't worry really we decide to tackle everything else as it comes just get it move".(P08)*
	i-PE in context of comorbidity	context of comorbidities, symptom misattribution,	*"So one presumes it could be happened with cold, I've no idea". (P01)* *"I have a lot of problem before I had a stroke, and a heart attack". (P02)* *"But also she got Lupus as well, before, and your breathing could be bad because of lupus" P08)*
Diagnosis of i-PE	Delayed diagnosis as a consequence of theme 1	symptom misattribution, recognition of significance of symptoms (lack of knowledge), misattribution by clinician	*"After the first session of chemotherapy, I got really bad chest pain, so I was referred to the cardiology department to check on my heart so the chemotherapy continued and every session get progressively worse. I didn't do anything about it for a week, I wasn't , I should have done properly , it was a week before I went to my GP ". (P05)* *"I woke up in the morning with a top of my calf, red a bit sore and feeling hot, she said it more likely cellulitis, so put me on a course of antibiotics but then of course 2 or 3 weeks" (P06)*

	Incidental does not mean asymptomatic	New symptoms, cough, breathlessness, wheezing, symptom misattribution,	*"the only symptom I had a cold a heavy cough, and I was rather short of breath, and I was wheezing, which I have never wheezed in my life, but I put it down to the cold"(P01)* *"I said I am breathless, he said this is due to the clot".(P10)*
	Response to i-PE diagnosis	Angry, Chock, double worry, uncertainty,	*"But I kept saying to my wife I am wheezing (angrily) because I used to it with my wife because she suffered from asthma". (P01)* *"It was a big shock. Well, I think I was such a chock I did not really take things in". (P02)* *"I was worried in case the clot moved as in case the clot moved to my brain and have a stroke". (P03)* *"It was a big shock. Blood clot, I'm not great with the body and medical, but when you hear a blood clot you so I think there's a quite a big thing".(P07)*
	Need for information	Internet as source of information, relatives. Access information with no support.	*"And I did something that never normally do I did look at the internet" (P01)* *"I went on the internet to look up Pulmonary embolism to find out about it, and my sister who is now lived in Suffolk for many years, her daughter is pharmacy assistant in hospital in Great Yarmouth, and she said Ah it is quite common".(P02)*

			"So I did what you shouldn't do and as soon as I get home I went on Google and I found out all about the symptoms and what it could be". (P07)
Living with i-PE treatments	Impact of injection	Hate it, do not like it, avoidance, reluctant, stigma. bruises, bleeding, Other injections breathlessness improved, find time, family engagement,	*"Because no pleasure in sticking needle in myself".(P01)* *"well ,I hate having to do it (the injection) I found myself messing round with things. I feel a bit fear of it like I am injecting Heroin". (P02)* *"It is bruise all over, I am on hormone Zoladex, that is why I am trying to leave a space on my stomach". (P02)* *"She got bruises, few ones here, and we did one in her leg, she got them on one side because she got the colostomy bag on the other side". (P08)*
			"after two day of injection the pain disappeared it just went really quickly actually".(P07) *"Since I've been taking the injections, I am feeling better" (P10)* *"I think the breathlessness has improved since I've been on fragmin."(P10)*
	Coping, more broadly with i-PE and treatment	Learning to cop. Being positive, previous experience.	*"but I am trying to live normally as I can". (P02)* *"I have given injections to other people in the past so injecting myself is in away easier". (P06)* *"I am quite positive person". (P07)*

Overarching theme: Lack of knowledge and uncertainty	Information needs. Symptoms did not know, what if? , how can I know?		*"They've just told that there is a small clot, but once again I have no pain, and I asked how I will find out if the clot been reduced"(P01)* *"I asked how I will find out if the clot been reduced. And the only way that they can tell me is by a scan which is in three months' time, so one worry is what happen in during three months' time?" (P02)* *"I mean I know there are the normal side effects of having chemotherapy, loss of appetite, and bad mouth and that sort of things. But nothing about the blood clot". (P05)* *"No, one mentioned to you the possibility of developing a blood clot".* *"I was not told". (P04)*

Appendix P: Ethical approval for the *in vitro* study analysis

NHS
Health Research Authority

North West - Haydock Research Ethics Committee
3rd Floor - Barlow House
4 Minshull Street
Manchester
M1 3DZ

Telephone: 0207 104 8012

11 May 2016

Professor Anthony Maraveyas
Hull and East Yorkshire Hospitals NHS Trust
Department of Academic Oncology, Queen's Centre for Oncology and Haematology
Castle Hill Hospital, Castle Road
Cottingham
HU16 5JQ

Dear Professor Maraveyas

Study title:	**A retrospective study of the tumour levels of tissue factor and protease-activated receptor 2 in patients with incidental pulmonary embolism**
REC reference:	**16/NW/0378**
IRAS project ID:	**185389**

The Proportionate Review Sub-committee of the North West - Haydock Research Ethics Committee reviewed the above application on 10 May 2016.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this favourable opinion letter. The expectation is that this information will be published for all studies that receive an ethical opinion but should you wish to provide a substitute contact point, wish to make a request to defer, or require further information, please contact the REC Manager Ms Rachel Katzenellenbogen, nrescommittee.northwest-haydock@nhs.net. Under very limited circumstances (e.g. for student research which has received an unfavourable opinion), it may be possible to grant an exemption to the publication of the study.

Favourable opinion

On behalf of the Committee, the sub-committee gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the