Recurrence of venous thromboembolism in patients with gynaecological cancer – incidence, risk factors and impact on survival.

A thesis submitted in accordance with the requirements of
University of Dublin, Trinity College, for the degree of
Doctor of Medicine
Department of Obstetrics and Gynaecology, School of Medicine.

Zbigniew Marchocki
MD, MSc, DRCOG, MRCPI, MRCOG
16341234

Supervisors:
Dr. Noreen Gleeson
Dr. Lucy Norris

University of Dublin, Trinity College 2022
Statement of Original Authorship

I declare that this thesis has not been submitted as an exercise for a degree at this or any other University and is entirely my work carried out in collaboration with a team of researchers and supervisors who are duly acknowledged in the text of the thesis. I agree to deposit this thesis in the University’s open access institutional repository or allow the Library to do so on my behalf, subject to Irish Copyright Legislation and Trinity College Library conditions of use and acknowledgement. I consent to the examiner retaining a copy of the thesis beyond the examining period, should they so wish (EU GDPR May 2018).

Signed: 

Date: January 1st, 2022
Summary

Recurrence of venous thromboembolism in patients with gynaecological cancer – incidence, risk factors and impact on survival.

Patients with active cancer have a 4- to 6.5-fold higher risk of developing VTE when compared to the general population. Cancer-associated thrombosis has been linked to reduced short- and long-term survival and is the second leading cause of death in cancer patients after disease progression. Moreover, recurrent VTE adversely impacts the overall survival of cancer patients. Gynaecological malignancy is associated with one of the highest risks of VTE; however, the rate and the factors influencing the rate of recurrent VTE in patients with gynaecological malignancy are unknown.

One of the major risk factors for VTE in patients with gynaecological malignancies is surgery. While the incidence of post-operative VTE has been significantly reduced by the introduction of extended LMWH prophylaxis, the patients’ experience and compliance with extended LMWH prophylaxis following surgery for gynaecological malignancy have not been examined before.

The main focus of my research was to improve knowledge of recurrent VTE in Irish women with gynaecological cancer and search for predictive biomarkers of VTE recurrence in patients with gynaecological cancer in order to better their lives and increase the survival of our cancer patients.

The objectives of this thesis were:

(1) To define the incidence and risk factors for recurrent
VTE in patients with gynaecological cancer,

(2) To investigate the effect of VTE recurrence on overall and progression-free survival in patients with gynaecological cancer,

(3) To assess the role of common laboratory biomarkers (WCC, Hb, PLT, Urea, Creatinine, Albumin) as predictive biomarkers of VTE recurrence in patients with gynaecological cancer,

(4) To assess the role of plasma coagulation factors (fibrinogen, D-Dimer, factor V, VIII, free Protein S, and Calibrated Automated Thrombogram assay) as predictive biomarkers of VTE recurrence in patients with gynaecological cancer,

(5) To examine the patients’ experience and compliance with extended thromboprophylaxis following cancer surgery.

The first part of the study was subdivided into two:

Initially, I performed a retrospective cohort study of 124 patients with gynaecological malignancies treated in St. James’s University Hospital. Subsequently, I assessed the role of the common laboratory biomarkers as a predictor of VTE recurrence. My results showed that the incidence of recurrent VTE in patients with genital tract malignancies was 22%. Patients were at the highest risk of recurrent VTE if their first VTE had occurred before the commencement of primary cancer treatment. The highest number of VTE recurrences occurred within a year of the primary event (67% within six months of the first VTE and 81% within 12 months). Most of the patients (63%) were on a therapeutic dose of low
molecular weight heparin at the time of their recurrent VTE. There was no difference in progression-free and overall survival between patients who suffered a single VTE and those who had recurrent VTE. We did not find any differences in age, BMI, presence or absence of a second malignancy, smoking, menopausal status, type of surgery, or Charlson Comorbidity Index - between the recurrent VTE group and non-recurrent VTE group. Patients with recurrent VTE had significantly higher monocyte count compared to the non-recurrent VTE group.

In the second part, I examined pre-operative fibrinogen, D-Dimer, factor V, VIII, free Protein S, and Calibrated Automated Thrombogram assay prior to patients’ cancer surgery, radiotherapy, or chemotherapy and their first VTE event as predictive biomarkers of VTE recurrence in patients with gynaecological cancer. Patients with subsequent recurrence of VTE had higher fibrinogen, D-Dimer, and Factor V levels, although this did not reach statistical significance. A wide variation in factor VIIIc levels was found in our population; however, there was no difference between the groups. Lower levels of free protein S were found in patients with recurrent VTE but this did not reach statistical significance. We did not find significant changes in thrombin generation in our study.

In the final part of the study, I examined the patients’ experience and compliance with extended LMWH thromboprophylaxis following cancer surgery. We found that 62% of patients were compliant with a full 28-day course of LMWH. More than half of the patients (58%) were able to self-administer the injections. Although satisfaction was high (78%), most patients (86%) admitted they would much prefer an oral medication if available. The majority of patients (84%) reported at least one side effect during the extended prophylaxis, with bruising and pain being the most frequent. Older patients had an increased perception of pain related to the injection. None of our patients experienced major bleeding.
In conclusion, we found that the incidence of recurrent VTE in patients with genital tract malignancies was 22%. Patients who experienced their first VTE prior to any form of cancer treatment were more likely to experience recurrent VTE and patients remained at high-risk of recurrent venous thrombosis despite standard anticoagulation treatment. Our biomarker study did not identify any haemostatic biomarkers to identify patients at risk of recurrent VTE. However, our sample size was small. We showed good patients’ compliance with extended low molecular weight heparin prophylaxis following gynaecological oncology surgery. Satisfaction with the treatment regimen was high, despite the prevalence of minor side effects associated with the injections. Patients reported pain score was significantly lower when low molecular weight heparin was self-administered. The majority of patients would prefer oral prophylaxis if available, safe and effective.
Acknowledgments

I would like to thank Professor Noreen Gleeson for being my mentor, and a role model, for providing ongoing support, inspiration and expertise in the field of Gynaecologic Oncology, and for sharing with me passion for the research presented in this thesis.

I would like to thank Dr. Lucy Norris for all her time and support in every aspect of my work, guidance, and expertise in the thrombosis and laboratory fields, for her patience and constructive feedback, and for sharing the enthusiasm and dedication with me for the cancer-associated thrombosis field.

I would like to thank Dr. Feras Abu Saadeh for his guidance, practical advice, expertise, for being a role model, and for his constructive feedback.

I would like to thank all the patients and their families who took part in this study, without whom this research would not have been possible.

I would also like to thank all the staff from St. James’s Hospital Clinical Pathology Laboratory for analysing the routine patients’ blood work, which results were used in part of this thesis.

It has been a real honour and pleasure to work with all of you during this thesis.

And finally, I would like to express my gratitude to my wife Diana and my daughter Lilly for their understanding, encouragement, support, and love during this work which allowed me to complete this research.
Dedication

I would like to dedicate this work to all the patients who suffered from cancer-associated thrombosis and who did not have a chance to fight cancer because they died from cancer-associated thrombosis prematurely.
Publications from this thesis

Peer-reviewed


Published abstracts


3). Marchocki Z, Norris LA, O'Toole SA, Gleeson N, Abu Saadeh F.
Table of Contents

STATEMENT OF ORIGINAL AUTHORSHIP........................................... I

SUMMARY .......................................................................................... II

ACKNOWLEDGMENTS......................................................................... VII

DEDICATION....................................................................................... VIII

PUBLICATIONS FROM THIS THESIS............................................ VIII

PEER-REVIEWED ............................................................................... VIII

PUBLISHED ABSTRACTS.................................................................... VIII

TABLE OF CONTENTS ...................................................................... X

LIST OF TABLES................................................................................. XX

LIST OF FIGURES............................................................................... XXIII

LIST OF ABBREVIATIONS .................................................................. XXVIII

CHAPTER 1: GENERAL INTRODUCTION ......................................... 1

1.1 GYNAECOLOGICAL CANCER................................................... 2

1.1.1 INTRODUCTION ....................................................................... 2

1.1.2 UTERINE CANCER ............................................................... 4
1.2.3 Regulation of coagulation ........................................... 34
1.2.4 Treatment of VTE ...................................................... 36

1.3 Recurrence of VTE .......................................................... 38
1.3.1 Recurrence of VTE in general population ................. 38
1.3.2 Thrombosis related risk factors ................................. 42
1.3.3 Patient related risk factors .......................................... 43
1.3.3.1 Treatment effects .................................................. 47
1.3.4 Prediction of recurrent VTE ......................................... 48

1.4 VTE in cancer ................................................................. 50
1.4.1 Patient-related risk factors for Cancer Associated.
Thrombosis (CAT) ............................................................ 52
1.4.2 Cancer-related risk factors for CAT ......................... 53
1.4.3 Treatment-related risk factors for CAT ................. 54
1.4.4 Pathogenesis of CAT .................................................. 55
1.4.5 Prediction of VTE in cancer ........................................ 61

1.5 VTE treatment in CAT .................................................... 63
1.5.1 General recommendations ......................................... 63
1.5.2 The emerging role of DOACs in treatment of CAT .... 65

1.6 VTE prevention and prophylaxis in cancer
Patients ........................................................................... 69
1.6.1 VTE PROPHYLAXIS FOLLOWING SURGERY .................................................. 69
1.6.2 VTE PROPHYLAXIS DURING CHEMOTHERAPY ........................................ 72

1.7 VTE IN GYNAECOLOGICAL CANCER ........................................... 73

1.7.1 VTE IN OVARIAN CANCER ................................................................. 74
1.7.2 VTE IN CORPUS UTERI CANCER ........................................................... 75
1.7.3 VTE IN CERVICAL CANCER ................................................................. 76
1.7.4 VTE IN VULVAR CANCER ................................................................. 77
1.7.5 VTE IN VAGINAL CANCER ................................................................. 78
1.7.6 VTE FOLLOWING SURGERY FOR GYNAECOLOGICAL CANCER ...... 78
   1.7.6.1 VTE POST OPEN SURGERY .............................................................. 79
   1.7.6.2 VTE AFTER MINIMALLY INVASIVE SURGERY .................................... 80
   1.7.6.3 PREDICTION OF VTE IN GYNAECOLOGICAL CANCER .............. 81
   1.7.6.4 RECURRENCE OF VTE IN CANCER PATIENTS .................................. 82
   1.7.6.5 RECURRENCE OF VTE IN GYNAECOLOGICAL CANCER PATIENTS 84

1.8 PREDICTION OF RECURRENT VTE IN CANCER PATIENTS .................................... 84

1.8.1 BIOMARKERS AS PREDICTORS OF CAT RECURRENCE ...................... 87
   1.8.1.1 D-DIMER ...................................................................................... 87
   1.8.1.2 FIBRINOGEN ............................................................................... 88
   1.8.1.3 SOLUBLE P-SELECTIN ................................................................. 88
   1.8.1.4 MICROPARTICLES AND TF ......................................................... 89
   1.8.1.5 FACTOR V .................................................................................... 90
1.8.1.6 Factor VIII

1.8.1.7 Protein S

1.8.1.8 Thrombin Generation

1.9 Patients’ Experience of Thrombosis and Thrombosis Prophylaxis

1.10 Summary of Evidence Leading to This Research

1.11 Specific Aims of This Study

CHAPTER 2: Patients and Methods

2.1 Ethical Approval

2.2 Patients

2.2.1 Patients Included in the Clinical Study

2.2.2 Patients - Biomarker Study

2.2.2.1 Patients and Sampling

2.2.3 Trinity College Dublin Gynaecological Cancer Bioresource

2.2.4 Blood Sampling

2.3 Laboratory Methods

2.3.1 Fibrinogen and D-dimer
2.3.2 CALIBRATED AUTOMATED THROMBOGRAM (THROMBOSCOPE BV) ................................................................. 108

2.3.2.1 PRINCIPLE (FIGURE 16) ......................................................................................................................... 108

2.3.2.2 REAGENTS (THROMBOSCOPE™ SYNAPSE BV, MAASTRICHT, NETHERLANDS) .................................................. 110

2.3.2.3 ASSAY PROCEDURE................................................................................................................................. 111

2.3.3 FACTOR V (ZYMUTEST FACTOR V, # RK009A, HYPHEN BIMED, NEUVILLE SUR OISE, FRANCE) .............................................................. 112

2.3.3.1 PRINCIPLE .................................................................................................................................................. 112

2.3.3.2 REAGENTS AND MATERIALS REQUIRED ............................................................................................. 113

2.3.3.3 REAGENTS PREPARATION ..................................................................................................................... 113

2.3.3.4 ASSAY PROCEDURE................................................................................................................................. 114

2.3.3.4.1 PREPARATION OF STANDARDS ...................................................................................................... 114

2.3.3.4.2 PREPARATION OF SAMPLES............................................................................................................ 115

2.3.3.5 CALCULATION OF RESULTS ................................................................................................................ 115

2.3.4 FACTOR VIII (BIOPHEN FXII:C, HYPHEN BIMED, NEUVILLE SUR OISE, FRANCE) ......................................................... 116

2.3.4.1 PRINCIPLE ................................................................................................................................................ 116

2.3.4.2 REAGENTS REQUIRED ........................................................................................................................ 117

2.3.4.3 REAGENT PREPARATION ....................................................................................................................... 118

2.3.4.4 ASSAY PROCEDURE............................................................................................................................... 119

2.3.5 PROTEIN S (ZYMUTEST FREE PROTEIN S, #RK015A-RUO, HYPHEN BIMED, NEUVILLE SUR OISE, FRANCE) ......................... 120

2.3.5.1 PRINCIPLE ................................................................................................................................................. 121

2.3.5.2 REAGENTS REQUIRED ........................................................................................................................ 121
3.2.2.1 Characteristics of the first VTE ............................................. 135
3.2.2.2 Timing of the first VTE ......................................................... 136
3.2.2.3 Bleeding complications ....................................................... 138
3.2.3 Characteristics of recurrent VTE ........................................... 138
3.2.4 Khorana score for prediction of recurrent VTE .............. 141
3.2.5 Laboratory biomarkers in patients with recurrent and non-recurrent VTE .......................................................... 143
3.2.6 Survival analysis ................................................................. 145

3.3 Discussion .............................................................................. 146

CHAPTER 4: Coagulation biomarkers as predictors of venous thromboembolism recurrence in gynaecological cancer ................. 154

4.1 Introduction ........................................................................... 155

4.1.1 Clinicalopathological details of patients ....................... 156

4.2. Fibrinogen and D-dimer ......................................................... 159

4.3 Thrombin generation assay .................................................. 160

4.4 Factor V .................................................................................. 164

4.4 Factor VIIIc ............................................................................. 165

4.5 Free protein S ......................................................................... 166
CHAPTER 5: PATIENTS’ EXPERIENCE AND COMPLIANCE WITH EXTENDED LOW MOLECULAR WEIGHT HEPARIN PROPHYLAXIS POST-SURGERY FOR GYNAECOLOGICAL CANCER

5.1 INTRODUCTION

5.2 RESULTS

5.2.1 CLINICOPATHOLOGICAL DETAILS OF ALL PATIENTS

5.2.2 KNOWLEDGE AND COMPLIANCE

5.2.3 LMWH ADMINISTRATION

5.2.4 SIDE EFFECTS

5.2.5 PATIENT SATISFACTION

5.3 DISCUSSION

CHAPTER 6: GENERAL DISCUSSION AND CONCLUSION

6.1 GENERAL DISCUSSION AND CONCLUSION

6.1.1 RECOMMENDATIONS FOR FUTURE RESEARCH

REFERENCES

APPENDIX I PATIENTS’ LMWH INJECTIONS LOGBOOK
APPENDIX II PATIENTS’ LMWH INJECTIONS

QUESTIONNAIRE.................................................................295

APPENDIX III PATIENTS’ VTE LEAFLET .............................298
List of Tables

**TABLE 1** Incidence and mortality by cancer site in females in Ireland in 2018 (HTTP://HEALTH.GOV.IE, 2018) .......................................................... 3

**TABLE 2** The histological subtypes of uterine corpus malignancies (AMANT ET AL., 2018) ................................................................. 5

**TABLE 3** FIGO staging of cancer of the corpus uteri (AMANT ET AL., 2018). .................................................................................................................. 6

**TABLE 4** Surgical treatment of endometrial cancer (COLOMBO ET AL., 2016). .................................................................................................................. 7

**TABLE 5** FIGO staging of cancer of the ovary, Fallopian tube, and peritoneum (BEREK ET AL., 2018). ................................................................. 11

**TABLE 6** FIGO staging of cancer of the cervix uteri (BHATLA ET AL., 2018). .................................................................................................................. 19

**TABLE 7** FIGO staging of carcinoma of the vulva (ROGERS & CUELLO, 2018). .............................................................................................. 23

**TABLE 8** Survival by FIGO stage in carcinoma of the vulva (BELLER ET AL., 2006). .............................................................................................. 24

**TABLE 9** FIGO staging of cancer of the vagina (ADAMS & CUELLO, 2018). .............................................................................................. 26

**TABLE 10** Risk factors for VTE (DI NISIO ET AL., 2016). .......... 29

**TABLE 11** Risk score for predicting outpatient VTE in cancer patients (KHORANA ET AL., 2008). ................................................................. 61
TABLE 12 THE ORIGINAL AND MODIFIED OTTAWA SCORE, (LOUZADA ET AL., 2012)..................................................................................................................85
TABLE 13 SURGICAL CLASSIFICATION SHEET AND COMPLEXITY SCORE
GROUP – MODIFIED FROM ALETTI ET AL., 2007.........................103
TABLE 14 KHORANA SCORE (KORANA ET AL., 2008) USED IN THE
CURRENT STUDY.........................................................................................104
TABLE 15 CHARLSON COMORBIDITY INDEX SCORING SYSTEM
TABLE 16 STANDARD SOLUTIONS USED IN CALIBRATION OF PLASMA
FACTOR V ASSAY ..................................................................................114
TABLE 17 FACTOR VIIIc ASSAY (STANDARD DILUTIONS) ..................119
TABLE 18 STANDARD SOLUTIONS USED IN CALIBRATION OF PLASMA
PROTEIN S ASSAY ...............................................................................123
TABLE 19 CHARACTERISTICS OF THE FIRST VTE..............................136
TABLE 20 BASELINE LABORATORY BIOMARKERS IN PATIENTS WITH
GYNAECOLOGICAL CANCERS WITH RECURRENT AND NON-
RECURRENT VTE WHO HAD VENOUS BLOOD SAMPLES OBTAINED
BEFORE THEIR FIRST VTE AND BEFORE COMMENCEMENT OF ANY
CANCER TREATMENT: ...........................................................................144
TABLE 21 DEMOGRAPHICS AND BASELINE CHARACTERISTICS OF
PATIENTS WITH GYNAECOLOGICAL CANCERS WITH RECURRENT AND
NON-RECURRENT VTE WHO HAD VENOUS BLOOD SAMPLES
OBTAINED BEFORE FIRST VTE AND PRIOR TO COMMENCEMENT OF
CANCER TREATMENT (N=69).................................................................157
TABLE 22 STUDY POPULATION CHARACTERISTICS (N=106). ............176
TABLE 23 VARIABLES AFFECTING PATIENTS’ REPORTED COMPLIANCE$^1$.
......................................................................................................................................................... 178

TABLE 24 VARIABLES AFFECTING PATIENTS’ REPORTED PAIN$^1$........ 180

TABLE 25 VARIABLES AFFECTING PATIENTS’ REPORTED SATISFACTION$^1$.
.................................................................................................................................................................. 183
List of Figures

Figure 1 A modified version of Virchow’s triad including the effect of inflammation on the risk of VTE. Chronic low-level inflammation has little impact on VTE (unlike arterial thrombosis) as opposed to acute inflammation (Esmon, 2009). .................................................................30

Figure 2 The conventional coagulation cascade .................32

Figure 3 Cell based model of coagulation (Hoffman & Monroe, 2014). .................................................................33

Figure 4 Activated Protein C pathway (Semeraro & Colucci, 2000-2013). .................................................................35

Figure 5 The antithrombin pathway (Semeraro & Colucci, 2000-2013). .................................................................36

Figure 6 Provoked and unprovoked VTE according to the presence, type and magnitude of associated provoking factors for VTE (Kearon et al., 2016A).........................39

Figure 7 Risk factors for recurrence after a first VTE event (Palareti, 2012). .................................................................41

Figure 8 Risk of VTE over time in patients with cancer (Rao et al., 2008). .................................................................52

Figure 9 Virchow’s triad. .................................................................55

Figure 10 Direct effect of tumour cells in CAT (Abdol Razak et al., 2018). .................................................................57
FIGURE 11 INCREASED THROMBIN GENERATION IN THE CANCER SETTINGS (REDDEL ET AL., 2019) ................................................................. 58

FIGURE 12 INDIRECT EFFECT OF TUMOUR CELLS IN CAT (ABDOL RAZAK ET AL., 2018) ........................................................................................................ 59

FIGURE 13 THE PROTHROMBOTIC EFFECT OF CHEMOTHERAPY (HADDAD & GREENO, 2006) ................................................................. 60

FIGURE 14 PATIENT RISK STRATIFICATION ALGORITHM FOR THE TREATMENT OF CAT (CARRIER, BLAIS ET AL., 2018) .......... 67

FIGURE 15 TIME DISTRIBUTION OF VTE CASES AFTER SURGERY (PEEDICAYIL ET AL., 2011) ................................................................. 80

FIGURE 16 CALIBRATED AUTOMATED THROMBOGRAM ASSAY PRINCIPLE AND PARAMETERS OF THE THROMBIN GENERATION CURVE (CASTOLDI AND ROSING, 2011). .................................................... 110

FIGURE 17 FACTOR V STANDARD CALIBRATION CURVE .................... 116

FIGURE 18 PRINCIPLE OF THE FACTOR VIIIc ASSAY (BIOPHEN FVIII:C, HYPHEN BIOMED, NEUVILLE SUR OISE, FRANCE) ..................... 117

FIGURE 19 FACTOR VIII:C TYPICAL STANDARD CURVE ..................... 120

FIGURE 20 MICRO ELISA PLATE WITH PROTEIN S CALIBRATOR, TESTED SAMPLES AND PROTEIN S SAMPLE DILUENT IN BIOTEK® EL808. ............................................................................................................................. 124

FIGURE 21 FREE PROTEIN S STANDARD CALIBRATION CURVE ........ 125

FIGURE 22 FLOW DIAGRAM OF PATIENTS RECRUITED INTO THE STUDY (n=124). ............................................................................................................. 133

FIGURE 23 DISTRIBUTION OF THE FIRST VTE EVENTS (n=124) ACCORDING TO THE TIMING OF VTE OCCURRENCE ................................. 137

XXIV
FIGURE 33 Peak thrombin as determined by the thrombin generation assay in patients with recurrent VTE (n=7) compared with patients who did not have a recurrent VTE (n=23), (p=0.886)..............................................................162

FIGURE 34 Lag time as determined by the thrombin generation assay in patients with recurrent VTE (n=7) compared with patients who did not have a recurrent VTE (n=23), (p=0.701). ..............................................................163

FIGURE 35 Time to peak thrombin as determined by the thrombin generation assay in patients with recurrent VTE (n=7) compared with patients who did not have a recurrent VTE (n=23), (p=0.901)..............................................................164

FIGURE 36 Factor V in patients who did not have a recurrent VTE (n=18) compared with those who had recurrent VTE (n=7), (p=0.657). ..............................................................165

FIGURE 37 Factor VIIIc in patients who did not have a recurrent VTE (n=17) compared with those who had recurrent VTE (n=6) (p=0.865). ..............................................................166

FIGURE 38 Free protein S in patients who did not have a recurrent VTE (n=18) compared with those who had recurrent VTE (n=7), (p=0.244). ..............................................................167

FIGURE 39 Flow diagram of patients recruited into the study (n=117). ..............................................................175

FIGURE 40 Compliance with extended LMWH prophylaxis (n=106). ..............................................................177
FIGURE 41 SIDE EFFECTS AND COMPLICATIONS EXPERIENCED BY THE PATIENTS RECEIVING EXTENDED LMWH PROPHYLAXIS. ..........180

FIGURE 42 SATISFACTION WITH LMWH INJECTIONS. .........................182
### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACCP</td>
<td>American College of Chest Physicians</td>
</tr>
<tr>
<td>ACOG</td>
<td>American College of Obstetricians and Gynecologists</td>
</tr>
<tr>
<td>ADAM</td>
<td>Apixaban and Dalteparin in Active Malignancy</td>
</tr>
<tr>
<td>ADP</td>
<td>Adenosine Phosphatase</td>
</tr>
<tr>
<td>APC</td>
<td>Activated Protein C</td>
</tr>
<tr>
<td>APTT</td>
<td>Activated Partial Thromboplastin Time</td>
</tr>
<tr>
<td>ARID1A</td>
<td>AT-Rich Interaction Domain 1A</td>
</tr>
<tr>
<td>ASCO</td>
<td>American Society of Clinical Oncology</td>
</tr>
<tr>
<td>AVERT</td>
<td>Apixaban for the Prevention of Venous Thromboembolism in High-Risk Ambulatory Cancer Patients Trial</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>BRAF</td>
<td>v-raf murine sarcoma viral oncogene homolog B1</td>
</tr>
<tr>
<td>BRCA</td>
<td>Breast Cancer Gene</td>
</tr>
<tr>
<td>BSA</td>
<td>Bovine Serum Albumin</td>
</tr>
<tr>
<td>C</td>
<td>Concentration</td>
</tr>
<tr>
<td>CA 125</td>
<td>Cancer Antigen 125</td>
</tr>
<tr>
<td>Ca2+</td>
<td>Calcium</td>
</tr>
<tr>
<td>CARAVAGGIO</td>
<td>Apixaban for the Treatment of Venous Thromboembolism Associated Cancer</td>
</tr>
<tr>
<td>CASSINI</td>
<td>Rivaroxaban for Thromboprophylaxis in High-Risk Ambulatory Patients with Cancer</td>
</tr>
<tr>
<td>CAT</td>
<td>Cancer Associated Thrombosis</td>
</tr>
<tr>
<td>CATCH</td>
<td>Comparison of Acute Treatments in Cancer Haemostasis</td>
</tr>
<tr>
<td>CATS</td>
<td>The Vienna Cancer and Thrombosis Study</td>
</tr>
<tr>
<td>CCRT</td>
<td>Concurrent Platinum-based Chemoradiation</td>
</tr>
<tr>
<td>CHORUS</td>
<td>Primary Chemotherapy versus Primary Surgery for Newly Diagnosed Ovarian Cancer</td>
</tr>
<tr>
<td>CI</td>
<td>Common Iliac</td>
</tr>
<tr>
<td>CIN</td>
<td>Cervical Intraepithelial Neoplasia</td>
</tr>
<tr>
<td>CLEC</td>
<td>C-Type Lectin-like Receptor</td>
</tr>
<tr>
<td>COC</td>
<td>Combined Oral Contraceptive</td>
</tr>
<tr>
<td>COMPASS-CAT</td>
<td>Prospective Comparison of Methods for Thromboembolic Risk Assessment with Clinical Perceptions and Awareness in Real Life Patients-Cancer Associated Thrombosis Cancer Procoagulant</td>
</tr>
<tr>
<td>CP</td>
<td>Creatinine</td>
</tr>
<tr>
<td>Cr</td>
<td>Creatinine</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>CRNB</td>
<td>Clinically Relevant Nonmajor Bleeding</td>
</tr>
<tr>
<td>CT</td>
<td>Computed Tomography</td>
</tr>
<tr>
<td>CVC</td>
<td>Central Venous Catheters</td>
</tr>
<tr>
<td>DASH</td>
<td>D-Dimer, Age, Sex, Hormones</td>
</tr>
<tr>
<td>DDD</td>
<td>Double distilled and deionised water</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>DOACs</td>
<td>Direct Oral Anticoagulants</td>
</tr>
<tr>
<td>DVT</td>
<td>Deep Vein Thrombosis</td>
</tr>
<tr>
<td>ECOG</td>
<td>Eastern Cooperative Oncology Group</td>
</tr>
<tr>
<td>EI</td>
<td>External Iliac Region</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme-linked Immunosorbent Assay</td>
</tr>
<tr>
<td>ENOXACAN</td>
<td>Enoxaparin and Cancer Study</td>
</tr>
<tr>
<td>EOC</td>
<td>Epithelial Ovarian Cancer</td>
</tr>
<tr>
<td>EPCAM</td>
<td>Epithelial Cell Adhesion Molecule</td>
</tr>
<tr>
<td>ERAS</td>
<td>Enhanced Recovery After Surgery</td>
</tr>
<tr>
<td>EROTC</td>
<td>European Organization for Research and Treatment of Cancer</td>
</tr>
<tr>
<td>ESC</td>
<td>European Society of Cardiology</td>
</tr>
<tr>
<td>ESGO</td>
<td>European Society of Gynaecological Oncology</td>
</tr>
<tr>
<td>ETP</td>
<td>Endogenous Thrombin Potential</td>
</tr>
<tr>
<td>EV</td>
<td>Extracellular Vesicles</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>FDP</td>
<td>Fibrin Degradation Product</td>
</tr>
<tr>
<td>FDG-PET</td>
<td>Fludeoxyglucose Positron Emission Tomography</td>
</tr>
<tr>
<td>FIGO</td>
<td>The International Federation of Gynaecology and Obstetrics</td>
</tr>
<tr>
<td>FV</td>
<td>Factor V</td>
</tr>
<tr>
<td>FVIII:C</td>
<td>Factor VIII concentration measured by one stage assay</td>
</tr>
<tr>
<td>FVIII:Chr</td>
<td>Factor VIII concentration measured by chromogenic assay</td>
</tr>
<tr>
<td>G</td>
<td>Grade</td>
</tr>
<tr>
<td>G-CSF</td>
<td>Granulocyte-colony Stimulating Factor</td>
</tr>
<tr>
<td>GOG</td>
<td>Gynaecologic Oncology Group</td>
</tr>
<tr>
<td>Hb</td>
<td>Hemoglobin</td>
</tr>
<tr>
<td>HERDOO2</td>
<td>Hyperpigmentation, Edema, Redness, D-Dimer, Obesity, Older age</td>
</tr>
<tr>
<td>HGSC</td>
<td>High Grade Serous Cancer</td>
</tr>
<tr>
<td>HIC</td>
<td>High-Income Countries</td>
</tr>
<tr>
<td>HIPEC</td>
<td>Hyperthermic Intraperitoneal Chemotherapy</td>
</tr>
<tr>
<td>HIV</td>
<td>Human Immunodeficiency Virus</td>
</tr>
<tr>
<td>HMWK</td>
<td>High Molecular Weight Kininogen</td>
</tr>
<tr>
<td>HPV</td>
<td>Human Papillomavirus</td>
</tr>
<tr>
<td>HR</td>
<td>Hazard Ratio</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>HRP</td>
<td>Horse-Radish-Peroxidase</td>
</tr>
<tr>
<td>HRQOL</td>
<td>Health-related quality of life</td>
</tr>
<tr>
<td>HRT</td>
<td>Hormone Replacement Therapy</td>
</tr>
<tr>
<td>H2O2</td>
<td>Hydrogen peroxide</td>
</tr>
<tr>
<td>IDS</td>
<td>Interval Debulking Surgery</td>
</tr>
<tr>
<td>II</td>
<td>Internal Iliac</td>
</tr>
<tr>
<td>INR</td>
<td>International Normalized Ratio</td>
</tr>
<tr>
<td>ITAC-CME</td>
<td>International Initiative on Thrombosis and Cancer</td>
</tr>
<tr>
<td>IQR</td>
<td>Interquartile Range</td>
</tr>
<tr>
<td>IVC</td>
<td>Inferior Vena Cava</td>
</tr>
<tr>
<td>ISTH</td>
<td>International Society on Thrombosis and Haemostasis</td>
</tr>
<tr>
<td>JAK</td>
<td>Janus Kinase 2</td>
</tr>
<tr>
<td>KG</td>
<td>Kilogram</td>
</tr>
<tr>
<td>KRAS</td>
<td>Kirsten rat sarcoma viral oncogene homolog</td>
</tr>
<tr>
<td>LACC</td>
<td>Laparoscopic Approach to Cervical Cancer</td>
</tr>
<tr>
<td>LETS</td>
<td>Leiden Thrombophilia Study</td>
</tr>
<tr>
<td>LG</td>
<td>Lag Time</td>
</tr>
<tr>
<td>LGSC</td>
<td>Low grade serous cancer</td>
</tr>
<tr>
<td>LIC</td>
<td>Low Income Countries</td>
</tr>
<tr>
<td>LMIC</td>
<td>Low- and Middle- Income Countries</td>
</tr>
<tr>
<td>LMS</td>
<td>Leiomyosarcoma</td>
</tr>
<tr>
<td>LMWH</td>
<td>Low Molecular Weight Heparin</td>
</tr>
<tr>
<td>LN</td>
<td>Lymph Nodes</td>
</tr>
<tr>
<td>LVSI</td>
<td>Lymphovascular Space Invasion</td>
</tr>
<tr>
<td>M</td>
<td>Meter</td>
</tr>
<tr>
<td>MEGA</td>
<td>Multiple Environment and Genetic Assessment of Risk Factors for Venous Thrombosis</td>
</tr>
<tr>
<td>MLH</td>
<td>MutL Homolog 1</td>
</tr>
<tr>
<td>MIS</td>
<td>Minimally Invasive Surgery</td>
</tr>
<tr>
<td>MPs</td>
<td>Microparticles</td>
</tr>
<tr>
<td>MR</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>MSH</td>
<td>MutS Homolog</td>
</tr>
<tr>
<td>MSI</td>
<td>Microsatellite Instability</td>
</tr>
<tr>
<td>MSS</td>
<td>Microsatellite Stable</td>
</tr>
<tr>
<td>N</td>
<td>Number</td>
</tr>
<tr>
<td>NACT</td>
<td>Neo-adjuvant chemotherapy</td>
</tr>
<tr>
<td>NCCN</td>
<td>National Comprehensive Cancer Network</td>
</tr>
<tr>
<td>NCRI</td>
<td>National Cancer Registry Ireland</td>
</tr>
<tr>
<td>NETs</td>
<td>Neutrophil Extracellular Trap</td>
</tr>
<tr>
<td>NIMIS</td>
<td>National Integrated Medical Imaging System</td>
</tr>
<tr>
<td>OB</td>
<td>Obturator</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>ONKOTEV</td>
<td>The Preventing Venous Thromboembolism in Ambulatory Cancer Patients</td>
</tr>
<tr>
<td>OPTIMEV</td>
<td>Optimisation de l'interrogatoire dans l'évaluation du risque thrombo-embolique veineux</td>
</tr>
<tr>
<td>OR</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td>OS</td>
<td>Overall Survival</td>
</tr>
<tr>
<td>P53</td>
<td>Tumour Protein p53</td>
</tr>
<tr>
<td>PA</td>
<td>Para-aortic</td>
</tr>
<tr>
<td>PAI-1</td>
<td>Plasminogen Activator Inhibitor-1</td>
</tr>
<tr>
<td>PAR-1</td>
<td>Protease-Activated Receptors 1</td>
</tr>
<tr>
<td>PAR-4</td>
<td>Protease-Activated Receptors 4</td>
</tr>
<tr>
<td>PARP</td>
<td>Poly (ADP-ribose) polymerase</td>
</tr>
<tr>
<td>PDPN</td>
<td>Podoplanin</td>
</tr>
<tr>
<td>PE</td>
<td>Pulmonary Embolism</td>
</tr>
<tr>
<td>PEG</td>
<td>Pegylated</td>
</tr>
<tr>
<td>PELICAN</td>
<td>Patients’ Experiences of Living with CANcer associated Thrombosis</td>
</tr>
<tr>
<td>PELICANOS</td>
<td>Extension of PELICAN study into Spanish settings</td>
</tr>
<tr>
<td>PFS</td>
<td>Progression Free Survival</td>
</tr>
<tr>
<td>PI3K</td>
<td>Phosphoinositide 3-kinases</td>
</tr>
<tr>
<td>PLPs</td>
<td>Phospholipids</td>
</tr>
<tr>
<td>PLT</td>
<td>Platelet Count</td>
</tr>
<tr>
<td>PMS</td>
<td>PMS1 Homolog 2, Mismatch Repair System Component</td>
</tr>
<tr>
<td>pNA</td>
<td>Para-nitroaniline</td>
</tr>
<tr>
<td>PLPs</td>
<td>Phospholipids</td>
</tr>
<tr>
<td>POLE</td>
<td>Polymerase E</td>
</tr>
<tr>
<td>PPPr</td>
<td>Platelet poor plasma reagent</td>
</tr>
<tr>
<td>PREPIC</td>
<td>Prevention of Recurrent Pulmonary Embolism by Vena Cava Interruption</td>
</tr>
<tr>
<td>PROSPECT-CONKO</td>
<td>The Prospective, Randomised Trial of Simultaneous Pancreatic Cancer Treatment with Enoxaparin and Chemotherapy</td>
</tr>
<tr>
<td>PROTECHT</td>
<td>Prophylaxis of Thromboembolism during ChemoTherapy</td>
</tr>
<tr>
<td>PS</td>
<td>Pre-sacral</td>
</tr>
<tr>
<td>pTG</td>
<td>Peak Thrombin Generation</td>
</tr>
<tr>
<td>PT</td>
<td>Prothrombin Time</td>
</tr>
<tr>
<td>RAM</td>
<td>Risk Assessment Models</td>
</tr>
<tr>
<td>REVERSE</td>
<td>Risk of recurrent venous thromboembolism after a first oestrogen-associated episode</td>
</tr>
<tr>
<td>RCT</td>
<td>Randomised Controlled Trial</td>
</tr>
<tr>
<td>RIETE</td>
<td>Registro Informatizado Enfermedad TromboEmbólica</td>
</tr>
<tr>
<td>RFU</td>
<td>Relative Fluorescence Units</td>
</tr>
<tr>
<td>ROC</td>
<td>Receiver Operating Characteristic</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>ROS</td>
<td>Reactive Oxygen Species</td>
</tr>
<tr>
<td>RVT</td>
<td>Residual Vein Thrombosis</td>
</tr>
<tr>
<td>SAVE-ONKO</td>
<td>Semuloparin for Thromboprophylaxis in Patients Receiving Chemotherapy for Cancer</td>
</tr>
<tr>
<td>SCC</td>
<td>Squamous Cell Carcinoma</td>
</tr>
<tr>
<td>SELECT-D</td>
<td>The Anticoagulation Therapy in Selected Cancer Patients at Risk of Recurrence of Venous Thromboembolism</td>
</tr>
<tr>
<td>SLN</td>
<td>Sentinel Lymph Nodes</td>
</tr>
<tr>
<td>STI</td>
<td>Sexually Transmitted Infection</td>
</tr>
<tr>
<td>STIC</td>
<td>Serous Tubal Intraepithelial Carcinoma</td>
</tr>
<tr>
<td>TCD</td>
<td>Trinity College Dublin</td>
</tr>
<tr>
<td>TCGA</td>
<td>The Cancer Genome Atlas Research Network</td>
</tr>
<tr>
<td>TIC</td>
<td>Tubal intraepithelial carcinoma</td>
</tr>
<tr>
<td>TF</td>
<td>Tissue Factor</td>
</tr>
<tr>
<td>TFPI</td>
<td>Tissue Factor Pathway Inhibitor</td>
</tr>
<tr>
<td>TMB</td>
<td>Tetramethylbenzidine</td>
</tr>
<tr>
<td>TNF alpha</td>
<td>Tumour Necrosis Factor alpha</td>
</tr>
<tr>
<td>TTP</td>
<td>Time to Peak</td>
</tr>
<tr>
<td>µl</td>
<td>Microliter</td>
</tr>
<tr>
<td>VAIN</td>
<td>Vaginal Intraepithelial Neoplasia</td>
</tr>
<tr>
<td>VEGF</td>
<td>Vascular Endothelial Growth Factor</td>
</tr>
<tr>
<td>VIN</td>
<td>Vulvar Intraepithelial Neoplasia</td>
</tr>
<tr>
<td>VKA</td>
<td>Vitamin K Antagonists</td>
</tr>
<tr>
<td>VOL</td>
<td>Volume</td>
</tr>
<tr>
<td>VTE</td>
<td>Venous Thromboembolism</td>
</tr>
<tr>
<td>VQ</td>
<td>Ventilation-perfusion</td>
</tr>
<tr>
<td>VWF</td>
<td>Von Willebrand Factor</td>
</tr>
<tr>
<td>WCC</td>
<td>White Cell Count</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
<tr>
<td>Yr</td>
<td>Year</td>
</tr>
</tbody>
</table>
Chapter 1: General Introduction
1.1 Gynaecological cancer

1.1.1 Introduction

Cancer is the leading cause of mortality and morbidity worldwide. In 2018 an estimated 8.6 million new cancer cases in women were reported (Bray et al., 2018) with breast (24.2%), colorectal (9.5%) and lung (8.4%) cancers being the most commonly diagnosed. The female genital tract accounts for over 15% of cancers. They are cervical cancer (6.6%), corpus uteri cancer (4.4%), ovarian cancer (3.4%), and vulvar cancer (0.5%).

While the overall death rate from cancer in women worldwide in the same year reached 4.1 million (Bray et al. 2018), cervical cancer accounted for 7.5% of these deaths and was the fourth most common cause of death from cancer in women worldwide in 2018. Ovarian cancer was reported as the eighth most common cause of death (4.4%), corpus uteri cancer as the fourteenth (2.1%), and vulvar cancer as the twenty-second (0.3%).

In Ireland cancer is the second most common cause of death after diseases of the circulatory system (http://health.gov.ie, 2018) (Table 1). Similar to the reports from other western world countries, cancer of the corpus uteri is the most common cancer of the female genital tract in Ireland. The high incidence of endometrial cancer is associated with an increased incidence of obesity. The prognosis for endometrial cancer is generally favourable due to its early presentation.

Ovarian cancer is associated with the highest mortality rate in female genital tract cancers in developed countries, with more than 75% of cases presenting at an advanced stage (Bankhead et al., 2005; Lataifeh et al., 2005).
Cervical cancer incidence has reduced in the western world countries in comparison to third world countries and this is mainly due to the cervical cancer screening programme.

Table 1 Incidence and mortality by cancer site in females in Ireland in 2018 (http://health.gov.ie, 2018).

<table>
<thead>
<tr>
<th>Cancer</th>
<th>New cases</th>
<th>Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Rank</td>
</tr>
<tr>
<td>All cancers</td>
<td>13272</td>
<td>4626</td>
</tr>
<tr>
<td>Breast</td>
<td>3334</td>
<td>1</td>
</tr>
<tr>
<td>Lung</td>
<td>1341</td>
<td>2</td>
</tr>
<tr>
<td>Colorectum</td>
<td>1216</td>
<td>3</td>
</tr>
<tr>
<td><strong>Corpus uteri</strong></td>
<td><strong>721</strong></td>
<td><strong>4</strong></td>
</tr>
<tr>
<td>Melanoma of skin</td>
<td>699</td>
<td>5</td>
</tr>
<tr>
<td><strong>Ovary</strong></td>
<td><strong>456</strong></td>
<td><strong>6</strong></td>
</tr>
<tr>
<td>Non-Hodgkin lymphoma</td>
<td>428</td>
<td>7</td>
</tr>
<tr>
<td>Thyroid</td>
<td>393</td>
<td>8</td>
</tr>
<tr>
<td><strong>Cervix uteri</strong></td>
<td><strong>340</strong></td>
<td><strong>9</strong></td>
</tr>
<tr>
<td>Kidney</td>
<td>288</td>
<td>10</td>
</tr>
<tr>
<td>Pancreas</td>
<td>284</td>
<td>11</td>
</tr>
<tr>
<td>Bladder</td>
<td>260</td>
<td>12</td>
</tr>
<tr>
<td>Leukaemia</td>
<td>247</td>
<td>13</td>
</tr>
<tr>
<td>Stomach</td>
<td>243</td>
<td>14</td>
</tr>
<tr>
<td>Brain, nervous system</td>
<td>192</td>
<td>15</td>
</tr>
<tr>
<td>Oesophagus</td>
<td>157</td>
<td>16</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>131</td>
<td>17</td>
</tr>
<tr>
<td>Gallbladder</td>
<td>111</td>
<td>18</td>
</tr>
<tr>
<td>Lip, oral cavity</td>
<td>105</td>
<td>19</td>
</tr>
<tr>
<td>Liver</td>
<td>88</td>
<td>20</td>
</tr>
<tr>
<td>Hodgkin lymphoma</td>
<td>63</td>
<td>21</td>
</tr>
<tr>
<td><strong>Vulva</strong></td>
<td><strong>56</strong></td>
<td><strong>22</strong></td>
</tr>
<tr>
<td>Larynx</td>
<td>25</td>
<td>23</td>
</tr>
<tr>
<td>Oropharynx</td>
<td>22</td>
<td>24</td>
</tr>
<tr>
<td>Salivary glands</td>
<td>19</td>
<td>25</td>
</tr>
<tr>
<td>Vagina</td>
<td>12</td>
<td>26</td>
</tr>
<tr>
<td>Hypopharynx</td>
<td>11</td>
<td>27</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>9</td>
<td>28</td>
</tr>
<tr>
<td>Nasopharynx</td>
<td>4</td>
<td>29</td>
</tr>
</tbody>
</table>
1.1.2 Uterine Cancer

1.1.2.1 Epidemiology

Anatomically the uterus consists of the cervix, isthmus, and corpus; however, the term uterine or endometrial cancer is referred to as cancer of the corpus uteri and excludes the cervix, which is a separate entity (Amant et al., 2018).

Worldwide, uterine cancer is the sixth most common female malignancy with 382,069 new cases diagnosed in 2018 (Bray et al., 2018). It is the most common gynaecological cancer in North America and Europe and the fourth most common site after breast, lung, and colorectal cancer (Siegel et al., 2015). Nearly 90% of uterine cancers in Ireland arise after menopause (www.ncri.ie, 2019). The risk of endometrial cancer up to the age of 75 years is estimated as 1.6% for high-income countries (HIC) and 0.7% for low-income countries (LIC) (Siegel et al., 2015). The higher prevalence of endometrial cancer in HIC is associated with an increased obesity rate and physical inactivity. As a result, the incidence of endometrial cancer has nearly doubled in the last twenty years.

This trend is expected to continue as the prevalence of obesity and life expectancy increases. Up to 5% of endometrial cancers are associated with Lynch syndrome type II (hereditary non-polyposis colorectal carcinoma syndrome) (Gruber & Thompson, 1996). This genetic condition caused by mutations in DNA mismatch repair genes (MLH 1, MSH 2, MSH 6, PMS 2, and EPCAM) predisposes to various types of cancer including colorectal, endometrial, stomach, breast, ovarian, small bowel, pancreatic, prostate, urinary tract, liver, kidney and bile duct. The lifetime risk of developing endometrial cancer in patients with Lynch syndrome is 30-60% (Aarnio et al., 1999).
1.1.2.2 Histology, grading, and staging

In the uterine corpus, there are three major categories of malignancy (Amant et al., 2018), (1) Epithelial, the most common uterine malignancy, (2) Mesenchymal or Sarcoma – which comprises 3-7% of all uterine malignancies (Major et al., 1993) and (3) Mixed epithelial and mesenchymal. The histological subtypes of each category are listed in Table 2 (Amant et al., 2018).

Table 2 The histological subtypes of uterine corpus malignancies (Amant et al., 2018).

| Epithelial (carcinomas) | Endometrioid carcinoma:  
| | ● adenocarcinoma  
| | ● adenocarcinoma-variants  
| | ▪ with squamous differentiation  
| | ▪ secretory variant  
| | ▪ villo-glandular variant  
| | ▪ ciliated cell variant  
| Mucinous adenocarcinoma  
| Serous adenocarcinoma  
| Clear cell adenocarcinoma  
| Undifferentiated carcinoma  
| Neuroendocrine tumours  
| Mixed carcinoma  
| ● carcinoma composed of more than one type, with at least 10% of each component  
| Mesenchymal (sarcomas) | Leiomyosarcoma (LMS)  
| | Endometrial stromal sarcoma  
| | Undifferentiated sarcomas  
| Mixed epithelial and mesenchymal | Adenosarcoma  
| | Carcinosarcoma  
| (recently reclassified based on its spreading pattern)  
| | dedifferentiated form of endometrial carcinoma  
| | metaplastic form of endometrial carcinoma  

Histopathological grades follow standard division into: well-differentiated (G1), moderately differentiated (G2), and poorly or undifferentiated (G3).

Based on exome sequence analysis, the Cancer Genome Atlas Research Network (TCGA) identified four molecular subgroups of endometrial cancer: Polymerase E (POLE) ultramutated group,
microsatellite instability (MSI)/hypermethylated group, copy number-low/microsatellite stable (MSS) group, and copy number-high/serous-like tumours group (Kandoth et al., 2013; Piulats et al., 2017). This molecular model seems to have prognostic value and could be useful in planning the extent of surgery and post-surgical adjuvant treatment. While the copy number-high group was shown to have the worse prognosis, POLE ultramutated group had the most favourable (Morice et al., 2016). However, it is yet to be determined how best to integrate this model into routine clinical practice.

The staging of uterine cancer is based on histopathology according to the International Federation of Gynecology and Obstetrics (FIGO) classification (Table 3) (Amant et al., 2018).

**Table 3 FIGO staging of cancer of the corpus uteri (Amant et al., 2018).**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Tumor confined to the corpus uteri</td>
</tr>
<tr>
<td>IA</td>
<td>No or less than half myometrial invasion</td>
</tr>
<tr>
<td>IB</td>
<td>Invasion equal to or more than half of the myometrium</td>
</tr>
<tr>
<td>II</td>
<td>Tumor invades cervical stroma but does not extend beyond the uterus</td>
</tr>
<tr>
<td>III</td>
<td>Local and/or regional spread of the tumor</td>
</tr>
<tr>
<td>IIIA</td>
<td>Tumor invades the serosa of the corpus uteri and/or adnexae</td>
</tr>
<tr>
<td>IIIB</td>
<td>Vaginal involvement and/or parametrial involvement</td>
</tr>
<tr>
<td>IIIC</td>
<td>Metastases to pelvic and/or para-aortic lymph nodes</td>
</tr>
<tr>
<td>IIIC1</td>
<td>Positive pelvic nodes</td>
</tr>
<tr>
<td>IIIC2</td>
<td>Positive para-aortic nodes with or without positive pelvic lymph nodes</td>
</tr>
<tr>
<td>IV</td>
<td>Tumor invades bladder and/or bowel mucosa, and/or distant metastases</td>
</tr>
<tr>
<td>IVA</td>
<td>Tumor invasion of bladder and/or bowel mucosa</td>
</tr>
<tr>
<td>IVB</td>
<td>Distant metastasis, including intra-abdominal metastases and/or inguinal nodes</td>
</tr>
</tbody>
</table>

Multiple factors have been identified for high-risk of recurrence in apparent early-stage disease: histological subtype other than endometrioid, grade 3 histology, myometrial invasion>50%,
lymphovascular space invasion (LVSI), lymph node metastases, and tumour diameter >2cm (Colombo et al., 2016). Clinicians subdivide stage I into three risk categories based on risk factors: a). Low risk including stage IA (G1 and G2) endometrioid types, b). Intermediate risk including stage IA, G3, and stage IB (G1 and G2) endometrioid types, c). High-risk including stage IB, G3 endometrioid, and all stages with non-endometrioid histologies.

The most common metastatic sites are the vagina, ovaries, and lungs.

1.1.2.3 Surgical management, treatment, and survival

The primary mode of treatment of endometrial cancer confined to the uterus is surgery including total hysterectomy, and bilateral salpingo-oophorectomy with or without lymphadenectomy (Table 4) (Colombo et al., 2016). Routine systemic pelvic lymphadenectomy in stage I endometrial cancer has been a subject of debate following reports of no benefit in disease-free and overall survival (Benedetti et al., 2008; ASTEC study group, 2009). Traditional open laparotomy and laparoscopy yield equivalent cancer outcomes (Tozzi et al., 2005; Walker et al., 2009).

Table 4 Surgical treatment of endometrial cancer (Colombo et al., 2016).

<table>
<thead>
<tr>
<th>Stage</th>
<th>Surgical treatment type</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>IA G1-G2</td>
</tr>
<tr>
<td></td>
<td>Hysterectomy with bilateral salpingo-oophorectomy</td>
</tr>
<tr>
<td></td>
<td>IA G3</td>
</tr>
<tr>
<td></td>
<td>Hysterectomy with bilateral salpingo-oophorectomy ± bilateral pelvic-para-aortic lymphadenectomy</td>
</tr>
<tr>
<td></td>
<td>IB G1-G3</td>
</tr>
<tr>
<td></td>
<td>Hysterectomy with bilateral salpingo-oophorectomy ± bilateral pelvic-para-aortic lymphadenectomy</td>
</tr>
<tr>
<td>II</td>
<td>Radical hysterectomy with bilateral salpingo-oophorectomy and bilateral pelvic-para-aortic lymphadenectomy</td>
</tr>
<tr>
<td>III</td>
<td>Maximal surgical cytoreduction with a good performance status</td>
</tr>
<tr>
<td>IV</td>
<td>IV A</td>
</tr>
<tr>
<td></td>
<td>Anterior and posterior pelvic exenteration</td>
</tr>
<tr>
<td></td>
<td>IV B</td>
</tr>
<tr>
<td></td>
<td>Systemic therapeutical approach with palliative surgery</td>
</tr>
</tbody>
</table>
Adjuvant therapy depends on the final stage, histological type, and presence of negative prognostic factors (G3 endometrioid, non-endometrioid histology, lymphvascular space invasion-LVSI, tumour size>2cm, etc.). For stage IA, G1-2 endometrioid uterine cancers, no adjuvant therapy is required, while stage IA, G3 and IB, G1-2 are usually offered vaginal vault brachytherapy. In stage IB, G3 pelvic radiotherapy is recommended. Patients with stage I disease and negative prognostic factors can be considered for pelvic radiotherapy or adjunctive chemotherapy. Patients with stage II require a combination of pelvic radiotherapy and vaginal brachytherapy. In the presence of negative prognostic factors chemotherapy can be added. In stages, III and IV adjuvant treatment consists of platinum-based chemotherapy, and external beam radiotherapy (Charo et al., 2019).

Primary radiation therapy is applied in medically inoperable patients (morbid obesity or severe cardiopulmonary disease) (Amant et al., 2018). Other treatment options include high-dose progestins (megestrol acetate or medroxyprogesterone acetate), tamoxifen, aromatase inhibitors, or uterine hormone-releasing device for endometrioid histology (Amant et al., 2018) and chemotherapy.

The 5-year survival rate depends on stage at diagnosis: stage IA - 88%; IB - 75%; II - 69%; IIIA - 58%; IIIB - 50%; IIIC - 47%; IVA - 17%; IVB - 15% (The AJCC Cancer Staging Manual, 2010).

1.1.3 Ovarian, Fallopian Tube and Primary Peritoneal Cancer

1.1.3.1 Epidemiology

Ovarian cancer is a relatively rare cancer with a lifetime risk of 1 in 70 and a peak in the 60-64 years age group (Siegel et al., 2011;
Berek et al., 2018). Although 23% of gynaecologic cancers are ovarian in origin, 47% of all deaths from female genital tract cancers are caused by ovarian cancer. The high mortality incidence ratio is related to delay in diagnosis, 70-80% of patients present in stage III or IV, (Bankhead et al., 2005; Lataifeh et al., 2005). To date, none of the screening protocols using Ca 125 measurement and transvaginal ultrasonography have been successful in reducing mortality or increasing survival rate from ovarian cancer (Henderson et al., 2018). Risk factors for ovarian cancer include age, early menarche, and late menopause, having first full-term pregnancy after the age of 35, nulliparity (two-fold increased risk), long term (at least 5 or 10 years) use of estrogen-only Hormone Replacement Therapy (HRT), endometriosis, polycystic ovarian syndrome, and family history of ovarian, breast or colorectal cancer. Approximately 15% of ovarian cancer is related to BRCA (BReast CAncer) gene mutation and 1% to Lynch syndrome (Lynch et al., 1993; Struewing et al., 1997; McLaughlin et al., 2007). Patients with an inherited BRCA1 mutation have about 72% risk of developing breast cancer and about 44% risk of developing ovarian cancer by the age of 80 (Kuchenbaecker et al., 2017). An inherited mutation in the BRCA2 gene is associated with about 69% and 17% risk of breast and ovarian cancer respectively by the age of 80. Women with hereditary ovarian cancer tend to develop the disease ~10 years earlier than women with non-hereditary ovarian cancer. The benefits of testing for BRCA mutations include the option of risk-reducing surgery by the age of 40-45 for relatives of the patient who tests positive (Pölcher et al., 2015). In addition, new treatments are emerging which are designed specifically for BRCA positive cancers patients (Moore et al., 2018). First pregnancy at an early age, breastfeeding, early menopause, use of oral contraceptives, tubal ligation, bilateral salpingo-oophorectomy and hysterectomy (even without oophorectomy) have been found to lower the risk of ovarian cancer (Negri et al., 1991; ACOG, Committee opinion no.620, 2015).
1.1.3.2 Histology, staging, and grading

Since 2014, ovarian, fallopian tube, and peritoneal cancer have been classified and staged (surgically) in the same system (Table 5).

The peritoneum, omentum, pelvic and abdominal viscera are the most common site for the spread of ovarian cancers (Berek et al., 2018). Histopathological and molecular analyses show that the most frequent high-grade serous histological subtype originates from the fimbrial end of the fallopian tube. P53 mutations and precancerous lesions serous tubal intraepithelial carcinoma (STIC) are found in the fimbriae adjacent to the ovary (Piek et al., 2001; Piek et al., 2004; Callahan et al., 2007; Crum et al., 2007; Kindelberger et al., 2007; Carlson et al., 2008).

Ovarian tumours are classified based on cell types, patterns of growth, and histogenesis (Meinhold-Heerlein et al., 2015).

There are 3 major categories of primary ovarian tumour:

1. Epithelial tumours (benign, borderline, or malignant/carcinomas):
   - Serous
   - Mucinous
   - Endometrioid
   - Clear cell
   - Brenner (transitional cell)
   - Undifferentiated carcinomas (epithelial structure but too poorly differentiated to be placed in any other group)
   - Mixed epithelial (composed of two or more of the five major cell types and minor component forms >10%)
Table 5 FIGO staging of cancer of the ovary, fallopian tube, and peritoneum (Berek et al., 2018).

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>IA</td>
<td>Tumor limited to 1 ovary (capsule intact) or fallopian tube; no tumor on ovarian or fallopian tube surface; no malignant cells in the ascites or peritoneal washings</td>
</tr>
<tr>
<td>IB</td>
<td>Tumor limited to both ovaries (capsules intact) or fallopian tubes; no tumor on ovarian or fallopian tube surface; no malignant cells in the ascites or peritoneal washings</td>
</tr>
<tr>
<td>IC</td>
<td>Tumor limited to 1 or both ovaries or fallopian tubes, with any of the following</td>
</tr>
<tr>
<td>IC1</td>
<td>Surgical spill</td>
</tr>
<tr>
<td>IC2</td>
<td>Capsule ruptured before surgery or tumor on the ovarian or fallopian tube surface</td>
</tr>
<tr>
<td>IC3</td>
<td>Malignant cells in the ascites or peritoneal washings</td>
</tr>
<tr>
<td>II</td>
<td>Tumor involves 1 or both ovaries or fallopian tubes with pelvic extension (below pelvic brim) or peritoneal cancer</td>
</tr>
<tr>
<td>IIA</td>
<td>Extension and/or implants on the uterus and/or fallopian tubes and/or ovaries</td>
</tr>
<tr>
<td>IIB</td>
<td>Extension to other pelvic intraperitoneal tissues</td>
</tr>
<tr>
<td>III</td>
<td>Tumor involves 1 or both ovaries or fallopian tubes, or peritoneal cancer, with cytologically or histologically confirmed spread to the peritoneum outside the pelvis and/or metastasis to the retroperitoneal lymph nodes</td>
</tr>
<tr>
<td>IIIA1</td>
<td>Positive retroperitoneal lymph nodes only (cytologically or histologically proven):</td>
</tr>
<tr>
<td>IIIA1(i)</td>
<td>Metastasis up to 10 mm in greatest dimension</td>
</tr>
<tr>
<td>IIIA1(ii)</td>
<td>Metastasis more than 10 mm in greatest dimension</td>
</tr>
<tr>
<td>IIIA2</td>
<td>Microscopic extrapelvic (above the pelvic brim) peritoneal involvement with or without positive retroperitoneal lymph nodes</td>
</tr>
<tr>
<td>IIIB</td>
<td>Macroscopic peritoneal metastasis beyond the pelvis up to 2 cm in greatest dimension, with or without metastasis to the retroperitoneal lymph nodes</td>
</tr>
<tr>
<td>IIIC</td>
<td>Macroscopic peritoneal metastasis beyond the pelvis more than 2 cm in greatest dimension, with or without metastasis to the retroperitoneal lymph nodes (includes an extension of tumor to a capsule of liver and spleen without parenchymal involvement of either organ)</td>
</tr>
<tr>
<td>IV</td>
<td>Distant metastasis excluding peritoneal metastases</td>
</tr>
<tr>
<td>IVA</td>
<td>Pleural effusion with positive cytology</td>
</tr>
<tr>
<td>IVB</td>
<td>Parenchymal metastases and metastases to extra-abdominal organs (including inguinal lymph nodes and lymph nodes outside of the abdominal cavity)</td>
</tr>
</tbody>
</table>

2. Sex cord-stromal tumours:
   a) Pure sex cord tumours:
      - Granulosa cell tumour (70% of sex-cord stromal tumours; produce estrogen, typically associated with late recurrence)
        - Juvenile (present with sexual precocity)
o Adult type (present with postmenopausal bleeding)
  • Sertoli cell tumour
b) Pure stromal tumours:
  • Fibroma
  • Thecoma
  • Fibrosarcoma
  • Leydig cell tumour
  • Steroid cell tumour
c) Mixed sex cord-stromal tumours:
  • Sertoli-Leydig cell tumour

3. Germ cell tumours:
  • Dysgerminoma (similar to seminoma in males)
  • Embryonal carcinoma
  • Polyembryoma
  • Teratoma
    o Immature
    o Mature
      o Mature with carcinoma (squamous cell, carcinoid, neuroectodermal, malignant struma, etc.)
  • Extraembryonal differentiation (choriocarcinoma, endodermal sinus tumour – yolk sac tumour)

More than 90% of ovarian malignancies are epithelial in origin. Serous carcinomas, accounting for up to 80% of advanced ovarian cancers, are divided into low and high grades (Bodurka et al., 2012). It has been recognised that they are two distinct disease entities. Tumours with mild to moderate cytologic atypia and low mitotic rates are classified as low-grade and account for 10% of serous cancers (Bodurka et al., 2012). The LGSC often contain wild-type TP53, and mutations in BRAF (v-raf murine sarcoma viral oncogene homolog B1) and KRAS (Kirsten rat sarcoma viral oncogene homolog) (Bodurka et al., 2012). Patients with severe
cytologic atypia and high mitotic rates are considered to have HGSC. The HGSC has a high frequency of mutations in TP53, present at an advanced stage, and responds well to platinum-based chemotherapy (70%). Women with LGSC tend to present at a younger age, have longer survival compared with women with high-grade tumours (Diaz-Padilla et al., 2012). They also do not respond to traditional chemotherapy regimens (Schmeler et al., 2008).

Mucinous ovarian cancer account for about 3% of Epithelial Ovarian Cancers (EOC) (Seidman et al., 2004). Around 65-80% of mucinous ovarian cancers are diagnosed in stage I making the prognosis more favourable compared to HGSC (Prat, 2015). This is mainly due to the fact that mucinous ovarian cancers are usually very large primary tumours (typically >15cm) causing symptoms while the disease is still confined to the ovary. Response of mucinous ovarian cancer to platinum-based chemotherapy is lower than for HGSC and is in a range of 20-60%.

Endometrioid histology accounts for approximately 10% of ovarian cancers, while clear cell accounts for up to 5% with a higher proportion in Japanese women (Nagase et al., 2019). Both ovarian cancer subtypes have been linked to endometriosis as a precursor lesion (Wiegand et al., 2010). Advanced clear cell cancer carries a worse prognosis than endometrioid or serous subtypes and tends to be resistant to standard platinum-based chemotherapy regimens (Sugiyama et al., 2000).

The dualistic classification of EOC based on clinic-pathological and molecular features has been proposed in the literature, dividing EOC into “type I” and “type II” (Shih & Kurman, 2004). Type I includes low-grade endometrioid, clear-cell, mucinous, and low-grade serous ovarian carcinoma. Type II tumours, accounting for the majority of all EOCs (about 90%), are generally high-grade serous, high-grade endometrioid, malignant mixed mesodermal tumours and undifferentiated tumours. However, this classification seems to be oversimplified and conflicts with recent molecular findings of the etiology of EOC. Molecular and cell of origin studies indicate that
while type II tumours could be classed together, type I tumours are not homogenous and can have varying clinical outcomes. It is likely that in the near future all subtypes of ovarian cancer will be considered as different diseases and treatment strategies will be based on the molecular characteristics of each tumour.

Sex cord-stromal tumours typically present in the first three decades of life, with an exemption of granulosa cell tumours which peak at age 50 to 55 years. Malignant forms, which usually present at an early stage, account for 1.2% of all primary ovarian malignancies (Quirk & Natarai, 2005). Apart from pain and pressure type symptoms, sex cord-stromal tumours often present with signs of hormonal production (estrogen and androgens) such as hirsutism and virilization, menstrual changes, or precocious puberty (Schneider et al., 2003).

Germ cell tumours are derived from primordial germ cells of the ovary and account for about 5% of ovarian cancers. They occur mainly between 10 and 20 years of age and can be broadly divided into those that differentiate toward embryo-like neoplasms (teratomas and their subtypes and dysgerminomas) and those that differentiate primarily toward extraembryonic foetal-derived (placenta-like) cell populations or a mixture of both (Tewari et al., 2000).

1.1.3.3 Surgical Management, Treatment, and Survival

Upfront debulking/cytoreductive surgery is the cornerstone of primary treatment in ovarian carcinoma which allows adequate resection and staging (Ledermann et al., 2018). Adequate, non-fertility-sparing surgery consists of peritoneal washings, bilateral salpingo-oophorectomy, hysterectomy, multiple peritoneal biopsies, at least infra-colic omentectomy, appendectomy in case of mucinous histology, and pelvic and para-aortic lymph node
dissection up to the renal veins. Intestinal resection, peritoneal stripping, diaphragmatic resection, removal of bulky para-aortic lymph nodes, and splenectomy are often necessary in order to achieve complete cytoreduction. The prognosis of epithelial ovarian cancer is independently affected by stage at diagnosis, histologic type and grade and most importantly the volume of residual disease after cytoreductive surgery (Hacker et al., 1983; Bristow et al., 2002).

Complete resection of all macroscopic visible disease has been shown to be associated with increased overall survival (OS) and progression-free survival (PFS) (van der Burg et al., 1995; du Bois et al., 2009; Vergote et al., 2010).

Patients with cytologically proven Stage IIIC and IV disease who are deemed not to be good surgical candidates may be given 3–4 cycles of neoadjuvant chemotherapy (NACT) followed by interval debulking surgery (IDS) and additional chemotherapy (Vergote et al., 2010; Kehoe et al., 2015).

The prognosis of patients with stage IA and IB, grade 1-2 epithelial cancers is very good and adjuvant chemotherapy is not indicated as it does not provide additional benefits. Patients with higher-grade tumours or stage IC or more should receive adjuvant platinum-based chemotherapy (Berek et al., 2018). The standard regime consists of 6 cycles of intravenous carboplatin or cisplatin and paclitaxel or docetaxel given every 3 weeks (Ozols et al., 2003; Bookman et al., 2009). Intraperitoneal chemotherapy is another option (Jaaback et al., 2011).

Recent randomised controlled trial (RCT) showed that the addition of hyperthermic intraperitoneal chemotherapy (HIPEC) to standard paclitaxel/carboplatin regime following interval cytoreductive surgery resulted in longer recurrence-free survival and overall survival and did not result in higher rates of adverse effects (van Driel et al., 2018).

Poly ADP-ribose polymerase (PARP) inhibitors are a group of pharmacological inhibitors of the enzyme poly adenosine
diphosphate-ribose (ADP) polymerase. There is evidence to support the role of PARP inhibitors as maintenance therapy following response to chemotherapy in patients with platinum-sensitive recurrent ovarian cancer, as well as monotherapy in selected patients with recurrent ovarian cancer (Coleman et al., 2017; Pujade-Lauraine et al., 2017). Interestingly patients with BRCA mutations (both germline and somatic) have the greatest benefit (Moore et al., 2018).

The 5-year survival for epithelial ovarian cancer is as follows: I - 78%, IA - 93%, IB - 91%, IC - 84%, II - 61%, IIA - 82%, IIB - 72%, IIC - 67%, III - 28%, IIIA - 63%, IIIB - 53%, IIIC - 41%, IV - 19% (The AJCC Cancer Staging Manual, 2010).

1.1.4 Cervical Cancer

1.1.4.1 Epidemiology

Cervical cancer is the fourth most common female malignancy worldwide, both in incidence and mortality (following breast, colorectal, and lung cancer) (Bray et al., 2018). There are approximately 527 600 new cases and 265 700 deaths annually (Torre et al., 2015). Cervical cancer is the second most commonly diagnosed female cancer and the third leading cause of cancer-related death in females in Low and Middle-Income Countries (LMIC) (Ferlay et al., 2015). Nearly 90 % of cervical cancer deaths occur in Low-Income Countries (LIC), with the highest incidence and mortality rates in sub-Saharan Africa, Southeast Asia, Latin America and the Caribbean, and Central and Eastern Europe.
Risk factors for cervical cancer include early onset of sexual activity, (twofold for younger than 18 years), multiple sexual partners (threefold for 6 or more partners), high-risk sexual partner (i.e. multiple previous partners), history of sexually transmitted infection (STI) (herpes, chlamydia), history of Vulvar Intraepithelial Neoplasia (VIN) or Vaginal Intraepithelial Neoplasia (VAIN) or cancers, immunosuppression (Human Immunodeficiency Virus-HIV), oral contraceptives, smoking (in squamous histology) (International Collaboration of Epidemiological Studies of Cervical Cancer, 2007). Human Papilloma Virus (HPV) is responsible for 99.7% of cervical cancer, with serotypes 16 and 18 responsible for 71%, and HPV types 31, 33, 45, 52, 58 for 19% of cases (Bosch et al., 2002; Schiffman et al., 2007).

The significant geographic variation in incidence and mortality is secondary to the availability of primary (HPV vaccination), secondary screening (cervical screening) prevention programmes and prevalence of human papillomavirus (HPV) infection. The prevalence of HPV infection varies from 5% in North America to 21% in Africa (Bruni et al., 2010). Another reason for a high incidence of cervical cancer in sub-Saharan Africa is a high prevalence of HIV infection, which has been shown to promote progression of pre- and cancerous cervical lesions (De Vuyst et al., 2013). The introduction of cervical screening programmes in HIC has led to a reduction in the incidence of cervical cancer by 4% annually and 75% overall (Bray et al., 2005; Gustafsson et al., 1997). In 2010 HPV vaccines against HPV 6, 11, 16, and 18 were introduced in Ireland. The new 9-valent HPV vaccine licensed by FDA in 2014 is expected to protect against 90% of cervical cancer (Herrero et al., 2015).

1.1.4.2 Histology, grading, and staging
The histopathologic types of cervical cancer include (Kurman et al., 2014):

- Squamous cell carcinoma – most common type accounting for 69% of cervical cancer cases (keratinizing; non-keratinizing; papillary, basaloid, warty, verrucous, squamotransitional, lymphoepithelioma-like)
- Adenocarcinoma – most are HPV related (18), few not HPV related, accounts for 20-25% of cervical cancer cases (endocervical; mucinous, villoglandular, endometrioid, arising from mesonephric remnants)
- Clear cell adenocarcinoma
- Serous carcinoma
- Adenosquamous carcinoma
- Glassy cell carcinoma
- Adenoid cystic carcinoma
- Adenoid basal carcinoma
- Small cell carcinoma (least frequent, highly aggressive, HPV related)
- Undifferentiated carcinoma

Histopathological grades are Grade 1 (well-differentiated), Grade 2 (moderately differentiated), and Grade 3 (poorly differentiated) (Bhatla et al., 2018).

In the past staging of cervical cancer was based on clinical examination with imaging of the chest and renal tract. However, in 2018 the FIGO Gynaecologic Oncology Committee updated the staging to include imaging and pathological findings in the staging (Table 6) (Bhatla et al., 2018). Cervical cancer spreads by direct extension into the parametrium, vagina, uterus, adjacent organs and along lymphatic channels to the regional lymph nodes. Distant metastases to lungs, liver and bones via haematogenous route occur late (Bhatla et al., 2018).
Table 6 FIGO staging of cancer of the cervix uteri (Bhatla et al., 2018).

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>The carcinoma is strictly confined to the cervix (extension to the uterine corpus should be disregarded)</td>
</tr>
<tr>
<td>IA</td>
<td>Invasive carcinoma that can be diagnosed only by microscopy, with a maximum depth of invasion &lt;5 mm</td>
</tr>
<tr>
<td>IA1</td>
<td>Measured stromal invasion &lt;3 mm in depth</td>
</tr>
<tr>
<td>IA2</td>
<td>Measured stromal invasion ≥3 mm and &lt;5 mm in depth</td>
</tr>
<tr>
<td>IB</td>
<td>Invasive carcinoma with measured deepest invasion ≥5 mm (greater than Stage IA), lesion limited to the cervix uteri</td>
</tr>
<tr>
<td>IB1</td>
<td>Invasive carcinoma ≥5 mm depth of stromal invasion, and &lt;2 cm in greatest dimension</td>
</tr>
<tr>
<td>IB2</td>
<td>Invasive carcinoma ≥2 cm and &lt;4 cm in greatest dimension</td>
</tr>
<tr>
<td>IB3</td>
<td>Invasive carcinoma ≥4 cm in greatest dimension</td>
</tr>
<tr>
<td>II</td>
<td>The carcinoma invades beyond the uterus, but has not extended onto the lower third of the vagina or to the pelvic wall</td>
</tr>
<tr>
<td>IIA</td>
<td>Involvement limited to the upper two-thirds of the vagina without parametrial involvement</td>
</tr>
<tr>
<td>IIA1</td>
<td>Invasive carcinoma &lt;4 cm in greatest dimension</td>
</tr>
<tr>
<td>IIA2</td>
<td>Invasive carcinoma ≥4 cm in greatest dimension</td>
</tr>
<tr>
<td>IIB</td>
<td>With parametrial involvement but not up to the pelvic wall</td>
</tr>
<tr>
<td>III</td>
<td>The carcinoma involves the lower third of the vagina and/or extends to the pelvic wall and/or causes hydronephrosis or non-functioning kidney and/or involves pelvic and/or para-aortic lymph nodes</td>
</tr>
<tr>
<td>IIIA</td>
<td>The carcinoma involves the lower third of the vagina, with no extension to the pelvic wall</td>
</tr>
<tr>
<td>IIIB</td>
<td>Extension to the pelvic wall and/or hydronephrosis or non-functioning kidney (unless known to be due to another cause)</td>
</tr>
<tr>
<td>IIIC</td>
<td>Involvement of pelvic and/or para-aortic lymph nodes, irrespective of tumor size and extent (with r and p notations)</td>
</tr>
<tr>
<td>IIIC1</td>
<td>Pelvic lymph node metastasis only</td>
</tr>
<tr>
<td>IIIC2</td>
<td>Para-aortic lymph node metastasis</td>
</tr>
<tr>
<td>IV</td>
<td>The carcinoma has extended beyond the true pelvis or has involved (biopsy-proven) the mucosa of the bladder or rectum. (A bullous edema, as such, does not permit a case to be allotted to Stage IV)</td>
</tr>
<tr>
<td>IVA</td>
<td>Spread to adjacent pelvic organs</td>
</tr>
<tr>
<td>IVB</td>
<td>Spread to distant organs</td>
</tr>
</tbody>
</table>

Lymph node (LN) metastasis is the most significant prognostic parameter for cervical cancer. Other adverse factors are LVSI, non-squamous histological type and deep stromal invasion (>two-thirds) (Hou et al., 2011).

1.1.4.3 Management, Treatment and Survival
Management of cervical cancer depends on the FIGO stage, age and fertility wishes (Bhatla et al., 2018). An excisional cervical cone biopsy is appropriate for stage IA. If lymphovascular space invasion is present, pelvic lymphadenectomy should be considered with a modified radical hysterectomy if a family is complete (Sevin et al., 1992; Elliott et al., 2000).

Stage IA2 is treated with type B radical hysterectomy and pelvic lymphadenectomy. Following the publication of the Laparoscopic Approach to Cervical Cancer trial (LACC) an open approach is recommended (Ramirez et al., 2018). Trachelectomy can be considered for patients desiring fertility in stages IA2-IB1 with tumour size less than 2cm, and node-negative on Magnetic Resonance (MR) or Computed Tomography (CT) imaging (Abu-Rustum et al., 2006).

For stages IB2 and IIA1, both surgery and radiotherapy can be considered as they have similar outcomes (Landoni et al., 1997). Efforts to avoid combined radical surgery and adjuvant radiotherapy are recommended as it is known to increase morbidity and compromise the quality of life (Yeo et al., 2011; Minig et al., 2014). Concurrent platinum-based chemoradiation (CCRT) is recommended for stages IB3 to IIA2 lesions and more. For disseminated disease (stage IVB) palliative systemic therapy may be considered depending on the Eastern Cooperative Oncology Group (ECOG) performance status (usually for patients with 0-2) (Bhatla et al., 2018).

Local radiation therapy may alleviate pain symptoms due to the local involvement of nerves. Due to short life expectancy (median duration of survival with stage IVB is approximately seven months), palliation radiotherapy should be given via larger fractions over shorter periods of time (Sharma et al., 2016).

1.1.5 Vulvar Cancer

1.1.5.1 Epidemiology

Vulvar carcinoma accounts for 4-5% of all gynaecological malignancies and it is diagnosed in two to five women per 100 000 every year (Siegel et al., 2015). Historically it was considered to be a disease of elderly women with peak occurrence in the 8th decade of life (Beller et al., 2006).

In Ireland, during 2010-2014 there were 38 cases of newly diagnosed vulvar cancer per year (https://www.ncrni.ie/news/article/hpv-associated-cancers-ireland-report-national-cancer-registry) as compared to 56 in 2018 (Bray et al., 2018). Over the last 50 years, the incidence of vulvar cancer has doubled and age-specific incidence rates increased among women aged ≤60 years (by 150% in the age group 0–39 years, 175% in age group 40–49 years and 68% in the age group 50–59 years) (Meltzer-Gunnes et al., 2017). This trend is thought to be mainly due to change in sexual behaviour and the increased prevalence of HPV infection.

1.1.5.2 Histology, grading, and staging

Squamous cell carcinoma (SCC) is the most common histological type constituting more than 90% of all cases (de Sanjosé et al., 2013). HPV is responsible for 63%-75% of SCC cases (6-7 in 10).
Most of the cases are attributable to HPV types 16 and 18 (cancer trends 33 website). Vulvar intraepithelial neoplasia (VIN) is a recognised precursor lesion in some SCC cases. There are two types of VIN: type 1, usual type (warty, basaloid, and mixed), which is HPV related and type 2, differentiated, seen mainly in older women, often associated with lichen sclerosus and/or squamous hyperplasia. Similar to cervical cancer, it is expected that the incidence of vulvar cancer will decrease with the introduction of HPV vaccination.

The histopathologic types of vulvar cancer include:

- Squamous cell carcinoma (90-92%)
- Melanoma (4-6%)
- Verrucous carcinoma
- Paget's disease of vulva
- Adenocarcinoma
- Basal cell carcinoma
- Bartholin gland carcinoma

Histopathological grades are Grade 1 (well-differentiated), Grade 2 (moderately differentiated) and Grade 3 (poorly differentiated) (Rogers & Cuello, 2018). The staging system for vulvar cancer is based on surgical findings and was last modified in 2009 by the FIGO Committee (Table 7) (Rogers & Cuello, 2018).

The pattern of metastases in vulvar cancer usually follows natural lymph node drainage from the vulval area (superficial inguinofemoral, deep inguinofemoral and femoral, and external iliac/pelvic nodes). Based on the poor outcome, involvement of pelvic lymph nodes is considered as stage IVB of the disease.
Table 7 FIGO staging of carcinoma of the vulva (Rogers & Cuello, 2018).

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Tumor confined to the vulva</td>
</tr>
<tr>
<td>IA</td>
<td>Lesions ≤2 cm in size, confined to the vulva or perineum and with stromal invasion ≤1.0 mm, no nodal metastasis</td>
</tr>
<tr>
<td>IB</td>
<td>Lesions &gt;2 cm in size or with stromal invasion &gt;1.0 mm, confined to the vulva or perineum, with negative nodes</td>
</tr>
<tr>
<td>II</td>
<td>Tumor of any size with extension to adjacent perineal structures (lower third of urethra, lower third of vagina, anus) with negative nodes</td>
</tr>
<tr>
<td>III</td>
<td>Tumor of any size with or without extension to adjacent perineal structures (lower third of urethra, lower third of vagina, anus) with positive inguinofemoral nodes</td>
</tr>
<tr>
<td>IIIA</td>
<td>1. With 1 lymph node metastasis (≥5 mm), or 2. With 1–2 lymph node metastasis(es) (&lt;5 mm)</td>
</tr>
<tr>
<td>IIIB</td>
<td>1. With 2 or more lymph node metastases (≥5 mm), or 2. With 3 or more lymph node metastases (&lt;5 mm)</td>
</tr>
<tr>
<td>IIIC</td>
<td>With positive nodes with extracapsular spread</td>
</tr>
<tr>
<td>IV</td>
<td>Tumor invades other regional (upper 2/3 urethra, upper 2/3 vagina), or distant structures</td>
</tr>
<tr>
<td>IVA</td>
<td>Tumor invades any of the following: 1. upper urethral and/or vaginal mucosa, bladder mucosa, rectal mucosa, or fixed to pelvic bone, or 2. fixed or ulcerated inguinofemoral lymph nodes</td>
</tr>
<tr>
<td>IVB</td>
<td>Any distant metastasis including pelvic lymph nodes</td>
</tr>
</tbody>
</table>

1.1.5.3 Management and Survival

All patients should have a careful colposcopic examination of the vagina, cervix, and anal region due to the risk of coexistent HPV lesions. Standard treatment of vulvar cancer is primarily surgical (Oonk et al., 2017). Depending on the location and size of the tumour, surgery involves radical local excision of the tumour. The aim of excision is to obtain tumour-free pathological margins of at least 1 cm. The risk of lymph node metastasis in stage IA is less than 1% vs. more than 8% in stage IB disease. Therefore, groin treatment should be performed in stages more than IA (Oonk et al., 2017).
For unifocal tumours less than 4 cm and no suspicious lymph nodes on clinical examination or imagining the sentinel lymph node procedure is recommended (Oonk et al., 2017). Positive SLN requires complete groin lymphadenectomy (Oonk et al., 2017). For tumours ≥ 4 cm and/or in case of multifocal invasive disease, inguinofemoral lymphadenectomy (including both superficial and deep lymphadenectomy) by separate incisions is recommended. In lateral tumours (medial border > 1 cm from midline), ipsilateral inguinofemoral lymphadenectomy is recommended. Contralateral inguinofemoral lymphadenectomy may be performed when ipsilateral nodes show metastatic disease (Oonk et al., 2017).

3-year recurrence-free survival ranges from 75% (in node-negative patients) and 26% (in node-positive patients) (Mahner et al., 2015). Survival according to the FIGO staging is shown in Table 8 (Beller et al., 2006).

Table 8 Survival by FIGO stage in carcinoma of the vulva (Beller et al., 2006).

<table>
<thead>
<tr>
<th>Stage</th>
<th>Overall survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 year</td>
</tr>
<tr>
<td>I</td>
<td>96.4</td>
</tr>
<tr>
<td>II</td>
<td>87.6</td>
</tr>
<tr>
<td>III</td>
<td>74.7</td>
</tr>
<tr>
<td>IV</td>
<td>35.3</td>
</tr>
</tbody>
</table>

1.1.5.4 Treatment

Postoperative radiotherapy to the groin is recommended for cases with > 1 metastatic lymph node and/or the presence of extracapsular lymph node involvement. Treatment includes the ipsilateral groin area and the distal part of the iliac nodes with an upper limit at the level of the bifurcation of the common iliac artery. The addition of radio-sensitising chemotherapy (weekly cisplatin) is
considered based on the evidence from other SCC (cervix, head and neck, anal). Chemoradiation is the treatment of choice in patients with the unresectable disease (Oonk et al., 2017).

1.1.6 Vaginal Cancer

1.1.6.1 Epidemiology

Vaginal cancer accounts for only 2% of malignant neoplasms of the female genital tract with 0.7 new cases per 100,000 a year (Siegel et al., 2013; Hacker et al., 2015). Approximately 50% of cases present in women older than 70 (Creasman et al., 1998).

In Ireland, during 2010-2014 there were 10 cases of primary vaginal cancer a year (https://www.ncr.ie/news/article/hpv-associated-cancers-ireland-report-national-cancer-registry-last accessed April, 2020) and 12 in 2018 (Bray et al., 2018). Vagina is a common site of metastases from either gynaecological (vulval, cervical, endometrial cancer or gestational trophoblastic disease) or non-gynaecological tumours (bladder, urethra, periurethral glands, rectum, rarely the breast, lung, or other sites).

1.1.6.2 Histology, grading and staging

The majority of the vaginal tumours (80%) are secondary tumours representing metastasis from other sites. The squamous cell carcinomas (90%) and adenocarcinomas (10%) are the two most common types of primary vaginal cancers. Precursor of vaginal cancer, vaginal intra-epithelial neoplasia (VAIN) has been associated with HPV infection in more than 92% of cases (Smith et al., 2009). Up to 75% of primary vaginal cancers are HPV-related, with type 16 (55%) and 33 (18%) being the most common (Sinno et
Histopathologic grades are well-differentiated (G1), moderately differentiated (G2) and poorly or undifferentiated (G3). The staging of vaginal cancer is based on clinical evaluation of the tumour (Table 9) (Adams & Cuello, 2018). In developed countries, clinical evaluation is often supplemented by various imaging techniques (Fluorine-18 fludeoxyglucose-positron emission tomography -18F-FDG-PET and MRI) to improve detection of lymphadenopathy and to evaluate the local spread of the disease.

Table 9 FIGO staging of cancer of the vagina (Adams & Cuello, 2018).

<table>
<thead>
<tr>
<th>Stage</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>The carcinoma is limited to the vaginal wall</td>
</tr>
<tr>
<td>II</td>
<td>The carcinoma has involved the sub-vaginal tissue but has not extended to the pelvic wall</td>
</tr>
<tr>
<td>III</td>
<td>The carcinoma has extended to the pelvic wall</td>
</tr>
<tr>
<td>IV</td>
<td>The carcinoma has extended beyond the true pelvis or has involved the mucosa of the bladder or rectum; bullous edema as such does not permit a case to be allotted to Stage IV</td>
</tr>
<tr>
<td>IVA</td>
<td>Tumor invades bladder and/or rectal mucosa and/or direct extension beyond the true pelvis</td>
</tr>
<tr>
<td>IVB</td>
<td>Spread to distant organs</td>
</tr>
</tbody>
</table>

1.1.6.3 Management and Survival

Surgical management is challenging due to the proximity of bladder and rectum and is reserved for early-stage cancer (Hoffman et al., 1992). Occasionally pelvic exenteration might have a role in patients with stage IVA, especially with rectovaginal or vesico-vaginal fistula or in patients with a central recurrence after radiation therapy.

More commonly treatment strategies are based on a combination of external beam radiotherapy and brachytherapy with concurrent chemotherapy (van Dam et al., 2004; Dalrymple et al., 2004). The advantage of this treatment is the conservation of the vagina.
Overall five-year survival for vaginal cancer is about 52% (Hacker, 2015). A study of 193 patients reported five-year disease-specific survival rates of 85% for 50 patients with Stage I disease, 78% for 97 patients with Stage II, and 58% for 46 patients with Stages III–IVA (Frank et al., 2005).

1.2 Venous thromboembolism

Venous thromboembolism (VTE) is a term describing the formation of a “clot” or thrombus in a vein, which clinically manifests as deep vein thrombosis (DVT) or pulmonary embolism (PE). DVT refers to clot formation in deep veins, occurring most commonly in the legs. PE is a partial or complete blockage of pulmonary arteries by a clot that has moved from elsewhere in the body through the bloodstream and often is a sequel of DVT. Development of VTE in arms, splanchnic or cerebral veins occurs less frequently (Heil et al., 2017; Devasagayam et al., 2016).

VTE is the third most common cardiovascular disease after myocardial infarction (Di Nisio et al., 2016) and stroke (Ellekjaer et al., 1997). The incidence of VTE in the general population varies between 1 and 2 per 1000 person-years (Anderson et al., 1991; Nordström et al., 1992; Hansson et al., 1997; Silverstein et al., 1998; Strekerud et al., 1998; Oger, 2000; Cushman et al., 2004). The reported incidence of PE (with or without DVT) ranges from 29 to 78 and for DVT (without PE) 45 to 117 per 100,000 person-years (Spencer et al., 2006; Naess et al., 2007; Spencer et al., 2009; Tagalakis et al., 2013; Huang et al. 2014). VTE is a cause of significant morbidity and mortality. Post-thrombotic syndrome occurs in 20-50% of patients with DVT and chronic thromboembolic pulmonary hypertension occurs in 0.1-4% of patients with PE (Hoeper et al., 2014; Kahn et al., 2014). More recently Klok et al., emphasised the importance of non-fatal consequences of VTE,
which are more varied and common than it was previously thought. These consequences are collectively termed the “post-VTE syndrome” and include exercise intolerance, anxiety, and functional limitations. These symptoms have a major impact on functional outcomes (increased unemployment), quality of life, and health care costs (Klok et al., 2019). More serious implications of PE include haemodynamic instability and death. Twenty percent of patients with PE die before or shortly after the diagnosis is made (Goldhaber et al., 1999). Approximately 30% of patients with VTE experience recurrence within 10 years (Kearon, 2003; Iorio et al., 2010; Heit et al., 2015). Up to 200,000 hospital deaths in the United States are related to PE every year (Heit, 2015). This accounts for 5 to 10% of hospital deaths annually, which makes PE, the most common preventable cause of in-hospital mortality (Geerts et al., 2004). There are around 370,000 VTE-related deaths per annum in European countries with 300 million inhabitants (Cohen et al., 2007).

A third to a half of VTE episodes have no identifiable trigger and are therefore classified as unprovoked (Kearon et al., 2016A). The remainder is caused by a transient or persistent factor that induces hypercoagulability, stasis, or vascular wall damage or dysfunction (Table 10) (Rosendaal, 1999; Heit, 2015; Di Nisio et al., 2016).
### Table 10 Risk factors for VTE (Di Nisio et al., 2016).

<table>
<thead>
<tr>
<th>Clinical and environmental risk factors</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hypercoagulability</strong></td>
</tr>
<tr>
<td>• Older age</td>
</tr>
<tr>
<td>• Active cancer</td>
</tr>
<tr>
<td>• Antiphospholipid syndrome</td>
</tr>
<tr>
<td>• Oestrogen therapy</td>
</tr>
<tr>
<td>• Pregnancy or puerperium</td>
</tr>
<tr>
<td>• Personal or family history of venous thromboembolism</td>
</tr>
<tr>
<td>• Obesity</td>
</tr>
<tr>
<td>• Autoimmune and chronic inflammatory diseases (eg, inflammatory bowel disease)</td>
</tr>
<tr>
<td>• Heparin-induced thrombocytopenia</td>
</tr>
<tr>
<td><strong>Vascular damage</strong></td>
</tr>
<tr>
<td>• Surgery</td>
</tr>
<tr>
<td>• Trauma or fracture</td>
</tr>
<tr>
<td>• Central venous catheter or pacemaker</td>
</tr>
<tr>
<td><strong>Venous stasis or immobilisation</strong></td>
</tr>
<tr>
<td>• Hospitalisation for acute medical illness</td>
</tr>
<tr>
<td>• Nursing-home residence</td>
</tr>
<tr>
<td>• Long-haul travel for more than 4 h</td>
</tr>
<tr>
<td>• Paresis or paralysis</td>
</tr>
<tr>
<td><strong>Heritable risk factors</strong></td>
</tr>
<tr>
<td>• Factor V Leiden</td>
</tr>
<tr>
<td>• Prothrombin 20210G→A mutation</td>
</tr>
<tr>
<td>• Antithrombin deficiency</td>
</tr>
<tr>
<td>• Protein C deficiency</td>
</tr>
<tr>
<td>• Protein S deficiency</td>
</tr>
<tr>
<td>• Non-0 blood group</td>
</tr>
</tbody>
</table>

In most cases there is more than one underlying pathophysiologic process contributing to VTE and risk factors are often additive. Surgery, immobilization and cancer are among the major risk factors for VTE. Major orthopaedic surgery is associated with 1% risk of post-operative VTE despite Low Molecular Weight Heparin (LMWH) thromboprophylaxis (Januel et al., 2012). Immobilization and cancer account for 15% and 20% of VTE cases respectively (Timp et al., 2013; Jensvoll et al., 2015). With regard to heritable risk factors, non-0 blood group is the most common followed by factor V Leiden and prothrombin gene mutation (Di Nisio et al., 2016).
1.2.1 Pathogenesis of VTE

The pathogenesis of venous thromboembolism is centred on three key factors known as Virchow’s triad, (a) the hypercoagulability of circulating blood, (b) changes in the vessel wall such as endothelial injury and (c) stasis causing abnormal blood flow (Fig. 1).

Figure 1 A modified version of Virchow’s triad including the effect of inflammation on the risk of VTE. Chronic low-level inflammation has little impact on VTE (unlike arterial thrombosis) as opposed to acute inflammation (Esmon, 2009).

Stasis is thought to be more of a permissive factor and blood constituents, including inflammatory mediators and changes in the vascular endothelium are considered more important (Esmon, 2009). Platelets are the core component of arterial thrombosis, whereas fibrin is the main constituent in venous thrombosis facilitating the attachment of the thrombus to the vessel wall. Acute inflammation and stasis play a key role in thrombus formation. The release of inflammatory mediators at the site of endothelial damage can down-regulate anticoagulant pathways and promote thrombus
formation. Reduced blood flow in venous stasis causes an accumulation of prothrombotic components such as thrombin (López & Chen, 2009).

### 1.2.2 Coagulation

The primary function of the coagulation system is to stop bleeding through the formation of a fibrin clot. Coagulation is triggered by injury to the blood vessel wall and thrombus formation which occurs in three steps: vasoconstriction of the blood vessel, formation of a platelet plug, and blood coagulation/secondary haemostasis. Activation of the haemostatic system occurs as a result of the cascade in which inactive zymogens and cofactors are sequentially activated by proteolytic cleavage. Historically this was considered to occur through two separate pathways, the extrinsic and intrinsic pathways, which join together to form a final common pathway (Fig. 2).

The extrinsic coagulation pathway (or tissue factor pathway) is initiated by vascular injury, which results in exposure of tissue factor (TF). The intrinsic pathway (or contact factor pathway) is initiated by collagen and prekallikrein and kallikrein, which results in the activation of FXI. The intrinsic and extrinsic pathways merge when FX is activated to form the common pathway. The procoagulant factors are then activated sequentially resulting in the formation of a fibrin clot. This pathway requires the presence of calcium (Ca2+) ions and negatively charged phospholipids. Coagulation is down-regulated through a series of negative feedback loops (Modell et al., 2015).
Figure 2 The conventional coagulation cascade.

**Intrinsic pathway (APTT)**

contact with the damaged surface, kininogen, kallikrein

XII → XIIa

XI → XIa

**Extrinsic pathway (PT)**

IX → IXa

VIIa → tissue damage

(VIII, Ca2+, PL)

X → Xa

(TF, PL, Ca2+)

(Va, PL, Ca2+)

prothrombin (II) → thrombin (IIa) (serine protease)

XIII

XIIIa

fibrinogen (I) → fibrin (Ia) → stable fibrin clot

However, this model of the coagulation cascade did not fully explain many clinical and experimental findings. It has been replaced by the cell-based model, which proposes that clotting occurs not as a cascade, but in three overlapping phases: initiation, priming/amplification and propagation (Hoffman & Monroe, 2001) (Fig. 3). All require the presence of negatively charged phospholipids and calcium ions (Monroe et al., 2002; Monroe & Hoffman, 2006).
The processes involved in the initiation phase take place on the surface of Tissue Factor (TF) - bearing cells. TF is the initiator of the haemostatic process. It is present in smooth muscle cells, fibroblasts, in the subendothelial layer of blood vessels, monocytes, macrophages, endothelial cells and platelets (Lawson et al., 1997; Mann et al., 2006; Versteeg et al., 2013). Following endothelial injury, exposed TF activates FVII present in the plasma. The TF-VIIa complex then activates FIX and FX. FXa activates the conversion of prothrombin to thrombin, which is produced in small amounts and controlled by circulating inhibiting factors. FXa is inhibited by the binding of endothelium derived tissue factor pathway inhibitor (TFPI) and by antithrombin III (ATIII) present on normal endothelium (Hoffman & Monroe, 2001). The TFPI/Xa complex also inhibits the VIIa/TF complex. The small amount of
thrombin produced during the initiation phase is sufficient to initiate the amplification phase.

The amplification process occurs on the surface of platelets adherent to the injured vasculature. Thrombin formed in the initiation phase activates platelets, which change shape to increase surface area in the coagulation process and start expressing receptors and binding sites for clotting factors. Thrombin from the initiation phase activates FV, FVIII and FXI present on the platelet surface. The activated platelets and FVa, FVIIIa and FXIa on the platelet surface set the stage for large production of thrombin in the propagation phase. The activated FIXa combines with FVIIIa forming the tenase complex (VIIIa/IIXa), which converts the inactive FX to activated FXa. Activated FXa now forms a complex with FVa to form the prothrombinase complex (Xa/Va), which converts prothrombin into large amounts of thrombin. This process is referred to as “burst of thrombin” (Wolberg et al., 2008).

Thrombin leads to the conversion of fibrinogen, a plasma protein synthesized in the liver, to fibrin monomers. These fibrin monomers, in the presence of factor XIIIa, interact with each other and platelets to form a stable fibrin plug, able to seal the wound and stop the bleeding (Wolberg et al., 2008).

The cell-based model of coagulation suggests that the coagulation reactions are localised to the area of injury. Antithrombotic mechanisms present on a healthy endothelium prevent inappropriate activation of the coagulation cascade (van Hinsbergh, 2012).

1.2.3 Regulation of coagulation

The coagulation system is regulated at many levels to ensure the clotting process is limited to the site of injury. This prevents
inappropriate activation and spread of coagulation cascade throughout the vasculature. TFPI binds to the TF/FVIIa complex and inhibits the action of FXa (Fig. 2). Through negative feedback loops, thrombin in complex with thrombomodulin activates the anticoagulant protein C to activated protein C (aPC) (Fig. 4). aPC binds to its cofactor protein S and limits further thrombin formation by inactivation of FVa and FVIIIa. This limits the formation of prothrombinase and tenase complexes (Hoffman, 2003). Dysfunction of the aPC pathway, either genetic (Factor V Leiden) or acquired may be associated with an increased risk of VTE (Shaheen et al., 2012). Pathophysiology of acquired aPC resistance is not entirely understood. Previous studies suggested that changes in the levels of coagulation factors (V, VII, IX) as well as anticoagulation factors (protein S and protein C) can change the aPC function leading to a hypercoagulable state (Sedano- Balbás et al., 2011).

**Figure 4 Activated Protein C pathway (Semeraro & Colucci, 2000-2013)*.**

*Figure in a public domain, available from Madame Curie Bioscience Database (https://www.ncbi.nlm.nih.gov/books/NBK6509/).
Antithrombin III is a serine protease inhibitor expressed in the liver. It inactivates thrombin, FIX, FX, FXI, FXII (Fig. 5). That antithrombin potential is augmented by heparin sulphate, which is the basis for its anticoagulant action. Plasminogen activation in the presence of fibrin leads to conversion to plasmin and degradation of a fibrin clot in a process known as fibrinolysis (Chapin & Hajjar, 2015).

**Figure 5 The antithrombin pathway (Semeraro & Colucci, 2000-2013)**.*

*Figure in a public domain, available from Madame Curie Bioscience Database (https://www.ncbi.nlm.nih.gov/books/NBK6509/).

### 1.2.4 Treatment of VTE

Traditionally following diagnosis of VTE, patients were started on the Vitamin K antagonist (VKA), warfarin and bridging low molecular weight heparin (LMWH). LMWH was continued together with warfarin until the international normalised ratio (INR) was greater than 2.0 for two consecutive days. The availability of direct oral anticoagulant (DOAC) has simplified treatment of VTE due to more
predictable pharmacokinetics, with no need for regular monitoring, no known food interaction, and fewer drug interactions. The direct Factor Xa inhibitors Apixaban and Rivaroxaban have been shown to be as effective as warfarin in preventing recurrent VTE (EINSTEIN–PE Investigators, 2012; Agnelli et al., 2013; Makam et al, 2018). The duration of treatment depends on provoking factors and individual patients’ assessments. The risk of recurrent VTE is relatively low following major surgery or trauma (3% recurrence rate at 5 years) therefore treatment is recommended for 3 months unless there are persisting risk factors (Iorio et al., 2010; Kearon et al., 2016B; Mazzolai et al., 2018; Konstantinides et al., 2019; Ortel et al., 2020). The risk of VTE recurrence following VTE provoked by a minor transient risk factor (travel>8hours, oestrogen therapy etc.) is intermediate (15% at 5 years) (Iorio et al., 2010). Extension of anticoagulation beyond 3 months should be considered in patients with minor transient or reversible risk factors and non-malignant persistent risk factors (eg Inflammatory bowel disease) (Mazzolai et al., 2018; Konstantinides et al., 2019). However, the authors suggest that in this case, treatment duration should be individualized (based on the risk of recurrence if the anticoagulation is stopped and the risk of bleeding if anticoagulation is extended) taking into consideration the patient's preference. Due to the risk of recurrent VTE in patients without identifiable risk factor being up to 30% by 5 years (Iorio et al., 2010), extended oral anticoagulation of indefinite duration should be considered in these patients (Mazzolai et al., 2018; Konstantinides et al., 2019).
1.3 Recurrence of VTE

1.3.1 Recurrence of VTE in the general population

Recurrent VTE is defined “as venous thrombosis of a site that was either previously uninvolved or had interval documentation of incident DVT or PE resolution” (Heit et al., 2011). Recurrent VTE also occasionally called a breakthrough event can be difficult to diagnose. The clinical symptoms of recurrent VTE are very common in the natural course of primary VTE event, therefore the diagnosis of the recurrent VTE must be based on objective assessment (ultrasound Doppler, CT pulmonary angiogram or Ventilation-Perfusion (VQ) scan) (Labropoulos et al., 2010).

Current evidence shows that the VTE recurrence risk is not significantly influenced by the duration of initial treatment with VKA (Boutitie et al., 2011). Warfarin is associated with a lower risk of recurrent VTE compared to LMWH in the general population (Kearon et al., 2012). However, in patients with cancer, LMWH is associated with lower risk of recurrent VTE compared with VKA therapy (Kearon et al., 2012). DOACs (apixaban and rivaroxaban) were shown to be as efficacious as warfarin in preventing recurrent VTE in the general population (EINSTEIN–PE Investigators, 2012; Agnelli et al., 2013).

One of the main predictors of recurrent VTE is the persistence of the primary provoking factor (Kearon&Akl, 2014). The annual risk of recurrent VTE in patients with VTE provoked by a major transient risk factor (i.e. VTE 1 week after major elective surgery) is around 1% (Fig. 6) (Kearon et al., 2016B; Kearon et al., 2016A).
Figure 6 Provoked and unprovoked VTE according to the presence, type and magnitude of associated provoking factors for VTE (Kearon et al., 2016A).

Note: Extreme left represents a major transient risk factor such as major elective surgery; extreme right of the figure represents a major and progressive persistent irreversible risk factor such as progressive metastatic cancer.

*N*Reprinted with permission from John Wiley and Sons (License Number 5166810563254).

Nearly half of VTE cases are unprovoked (no identifiable major or minor risk factor) (Heit, 2015; Di Nisio et al., 2016), with a risk of VTE recurrence after cessation of anticoagulation treatment around 5-10% after 1 year, and 30% after 5 years (Kearon et al., 2016B). Therefore, it has been recommended that VTE treatment should be continued indefinitely in patients with unprovoked VTE as secondary prevention (Kearon et al., 2016B; Ortel et al., 2020). Although the strength of this recommendation is based on the moderate certainty in the evidence of effects, patients should be reassessed regularly to review the risks and benefits of continued anticoagulant therapy (Ortel et al., 2020). In addition, the indefinite anticoagulation has to be balanced against the risk of bleeding, quoted at 1-3% per year depending on the type of anticoagulation (Wolberg et al., 2015).
A systematic review showed that case-fatality rates of recurrent VTE and major bleeding events among patients treated for VTE were similar during the initial 6-month period of VTE treatment with an anticoagulant (the rate of recurrent VTE was 0.4% with a case-fatality rate of 11.3% and the rate of fatal major bleeding events was 0.2% with a case-fatality rate of 11.3%) (Carrier et al., 2010). However, after completion of 3 to 6 months of anticoagulation the case-fatality rate of recurrent VTE was lowered to 3.6%). Martinez et al. noted a higher risk of VTE recurrence in the first few months following a primary event and showed that the incidence rate for recurrent VTE peaked in the first six months at 11 per 100 person years (Martinez et al., 2014). Because the risk of VTE recurrence is greatest in the first 6–12 months following the initial event and progressively decreases afterward (Heit et al., 2000A), the benefit of extended anticoagulation may be exceeded by the risk of clinically important bleeding. In this context it would be very useful to know which patients would benefit most from long-term anticoagulation. Bleeding assessment tools such as ACCP risk table (Kearon et al., 2016B), VTE-BLEED (Klok et al., 2016), RIETE (Ruíz-Giménez et al., 2008), and HAS-BLED (Pisters et al., 2010) have been developed to stratify the risk of major bleeding in patients with VTE.

Several studies have identified risk factors for recurrent VTE, which are thrombosis or patient-related (Fig. 7) (Palareti, 2012).
1.3.2 Thrombosis related risk factors

The most important factors associated with risk of VTE recurrence after cessation of anticoagulation treatment are the presence of reversible risk factor associated with primary VTE event (secondary or provoked event); absence of obvious clinical risk factor (unprovoked or idiopathic) and presence of persistent risk factor (active cancer or antiphospholipid syndrome). Many studies have shown that the risk of VTE recurrence is much higher after an apparent unprovoked event compared to a secondary event (Heit et al., 2000A; Palareti et al., 2002; Baglin et al., 2003).

The cumulative risk of VTE recurrence after 10 years of follow-up in patients with an unprovoked event was 52.6% compared to 22.5% in patients with an identifiable triggering event in a large cohort of patients (Prandoni et al., 2007).

Distal DVT (limited to deep calf veins) is associated with a lower risk of recurrence than proximal DVT or PE (Schulman et al., 1995; Douketis et al., 2001; Pinede et al., 2001; Eichinger et al., 2010). In
a large meta-analysis of seven prospective studies, Baglin et al. estimated that the rate of recurrent VTE was 4.8 times higher in patients with proximal versus isolated distal DVT (Baglin et al., 2010). The same study also showed also that patients presenting with the first episode of PE are at the same risk of recurrent VTE as patients presenting with the first episode of proximal DVT alone. However, they are 3-fold more likely to suffer PE than DVT as a recurrence. Given that the risk of fatal PE is two to four times greater in patients with symptomatic PE, the mode of initial presentation is an important factor in determining the duration of anticoagulant therapy in individual patients after the first episode of VTE. Although PE tends to recur more often as another PE (2.4-fold more likely than as DVT) (Arshad et al., 2017, Konstantinides et al., 2019), this alone does not determine the duration of the anticoagulation treatment. As elegantly outlined in the 2020 ASH guidelines (Ortel et al., 2020) and 2019 ESC guidelines on acute PE (Konstantinides et al., 2019), treatment duration is more related to the circumstances of the first PE. Furthermore, a three-year follow-up of patients included in the OPTIMEV registry demonstrated that while the patients with an initial isolated distal DVT have a significantly lower VTE recurrence risk than those with isolated proximal DVT (2.7% v 5.2% per annum, p=0.02), the incidence of recurrent PE was similar (0.9% v 1.0%, p=0.83). Predictors of recurrence included age > 50 years, an unprovoked event, and involvement of multiple distal veins (Galanaud et al., 2014).

The role of residual vein thrombosis (RVT) as a predictor of recurrent VTE was evaluated by Mazetto et al. (Mazetto et al., 2018). The absence of RVT in the patient’s history of DVT on at least 3 months of oral anticoagulation was a protective marker for recurrence with a negative predictive value of 100%. Also, the presence of hypoechoic RVT, determined by the greyscale median of the images (with values below 24) on what imaging, positively predicted 75% of DVT recurrences (Mazetto et al., 2018).
1.3.3 Patient-related risk factors

Several studies have confirmed that the male gender significantly increases the risk of VTE recurrence. A large meta-analysis of 15 studies enrolling a total of 5416 patients showed that the relative risk of recurrent VTE for men compared with for women was 1.6 (McRae et al., 2006). Similar findings were confirmed in a meta-analysis of seven prospective studies mentioned earlier, which calculated a three-year incidence of recurrence of 9.1% in women and 19.7% in men (Baglin et al, 2010).

There is conflicting evidence regarding older age as a risk factor for VTE recurrence. In some studies older age has been found to be a risk factor for VTE recurrence (Vučković et al., 2017) but not in others (Eischer et al., 2009; Christiansen et al., 2010). Tosetto et al. found that younger age < 50 years increases the risk of VTE recurrence (Tosetto et al., 2012).

In a population of older patients (≥65yrs), 3-year cumulative VTE recurrence was 14.8% with a high case-fatality in the cohort with recurrence of 20.5% higher in patients with unprovoked VTE (23%) and Cancer-Associated Thrombosis (CAT) (29%) (Lauber et al., 2018). Unprovoked VTE and proximal DVT were the only factors associated with recurrent VTE in that study.

Personal history of thrombophilia may be a risk factor for primary VTE but its impact on the risk of recurrent VTE is less clear. In a retrospective review of patients with recurrent VTE by De Stefano et al. deficiency of antithrombin was found to be an independent risk factor for recurrence (De Stefano et al., 2006). Protein C or Protein S deficiency only marginally increased recurrent VTE risk (De Stefano et al., 2006).

Carriers of heterozygous factor V Leiden or prothrombin mutation were found to be at higher risk of VTE recurrence in a prospective
study by Miles et al., and a systematic review by Ho et al. (Miles et al., 2001; Ho et al., 2006). However, this finding was not replicated by other authors (De Stefano et al., 2001; Eichinger et al., 2002; Christiansen et al., 2005).

Other hematologic conditions such as polycythemia vera and essential thrombocythemia (especially with homozygous Janus kinase 2 (JAK2) V617F mutation) have been also found to be risk factors for recurrent VTE (De Stefano et al., 2008; De Stefano et al., 2010).

Compared to women with unprovoked VTE, women with pregnancy-associated VTE have a significantly lower long-term risk of recurrent VTE but a higher risk of recurrent VTE during a subsequent pregnancy (White et al., 2008).

Studies evaluating the impact of hormone use on VTE recurrence risk have reported conflicting results (Áinle & Kevane, 2020). While some studies found that women with hormone-related VTE (combined oral contraceptives and/or hormone replacement therapy) were at similar risk of recurrent VTE as women with unprovoked VTE (Kyrle et al., 2004), hormone use is categorized as an intermediate provoking factor by ESC guidelines (3-8% recurrence risk) (Konstantinides et al., 2019) and as a minor (yet important) transient risk factor (with a 50% predicted recurrence risk compared with unprovoked VTE) by ISTH (Kearon et al., 2016A). Absolute reported recurrence risks in contraceptive users also vary between studies, from approximately <1-4% per annum. This discrepancy is likely due to the heterogeneity in study design, population studied, age-matched comparisons, and study power.

Similar findings were demonstrated in an analysis of the Risk of Recurrent Venous Thromboembolism After a First Oestrogen-Associated Episode (REVERSE) cohort study (Le Gal et al., 2010). REVERSE suggested that women with hormone-related VTE
should be considered as having an “unprovoked” VTE and that other risk factors should be taken into consideration when deciding on the length of anticoagulation treatment.

While obesity has been shown to be a major risk factor for first VTE (Stier et al., 2020), its influence on the risk of recurrent VTE remains elusive. A linear relationship between the increased BMI and VTE recurrence was described in patients with first unprovoked VTE following the discontinuation of treatment with an anticoagulant (Eichinger et al., 2008). Increased BMI was also reported as an independent predictor of recurrent VTE (Heit et al., 2000B). However, this association was not confirmed in the most recent studies (Mueller et al., 2017; Vučković et al., 2017; Giorgi-Pierfranceschi et al., 2020).

Swiss venous Thromboembolism COhort (SWITCO65+) study (Mueller et al., 2017) showed that there was no difference in a 3-year cumulative incidence of recurrent VTE in patients over the age of 65 based on their BMI. Moreover, the results from the MEGA follow-up study showed no association between the excess BMI and recurrent VTE (Vučković et al., 2017). In addition data from the RIETE (Registro Informatizado Enfermedad TromboEmbólica) registry demonstrated that the risk for VTE recurrences or major bleeding did not differ in patients with or without morbid obesity (Giorgi-Pierfranceschi et al., 2020).

Patients with a history of VTE are at increased risk of VTE recurrence following surgery (Heit et al., 2015; Nemeth et al., 2019). A population-based case-cohort study found that a new hospitalization (for surgery or acute medical illness) was an independent predictor of a VTE recurrence compared to no hospitalization (Heit et al., 2015). The magnitude of risk was highest while in the hospital the increased risk persisted to a lesser extent for the next 92 days following hospital discharge. Similar findings were demonstrated in the observational study by Nemeth et al.
The cumulative incidence of recurrent VTE post-surgery was 2.1% at 1 month, 3.3% at 3 months, and 4.6% at 6 months, compared with an incidence of 0.8% at 3 months in patients who did not undergo surgery. Interestingly all patients received recommended postoperative prophylaxis. Gastrointestinal, major orthopaedic or cancer-related surgery, male gender and Factor V Leiden mutation were associated with the highest cumulative incidence of VTE by six months (Nemeth et al., 2019).

D-Dimer (or D Dimer) is a fibrin degradation product (FDP) and as such acts as a global biomarker of coagulation activation and fibrinolysis (Adam et al., 2009). D-Dimers are considered a sensitive tool and are commonly used in clinical algorithms for the diagnostic work-up of patients with suspected VTE as well as in predicting the risk of CAT (Wells et al., 2003; Righini et al., 2008; Pabinger et al., 2018). However, despite its high sensitivity, D-Dimer lacks specificity when used to rule out VTE and is affected by many conditions including infection, surgery, and cardiovascular disorders (Lippi et al., 2014). D-Dimer levels increase with age and it has been suggested that an age-adjusted cut-off value should be used in patients aged > 50 years (Lippi et al., 2014; Righini et al., 2014).

In a recent study by Avnery et al., patients with raised D-Dimer levels after discontinuing anticoagulant therapy for VTE provoked by a minor transient risk factor were at an increased risk for VTE recurrences (Avnery et al., 2020). In patients who developed VTE secondary to major transient risk factors (postoperative period), the recurrence rate of VTE following the discontinuation of anticoagulation was 5.74 events per 100 patient-years in patients with raised D-Dimer levels and 2.68 in those with normal levels (Avnery et al., 2020). In patients with minor risk factors (pregnancy, oestrogen use, immobilisation, recent travel), the rates were 7.79
and 3.34 respectively. The role of D-Dimers in the prediction of CAT recurrence is discussed in more detail in Chapter 1.8.1.1.

1.3.3.1 Treatment effects

As mentioned in Chapter 1.2.4 treatment of the primary VTE event is one of the determinants of VTE recurrence (EINSTEIN–PE Investigators, 2012; Kearon et al., 2012; Agnelli et al., 2013). Warfarin has been associated with a lower risk of VTE recurrence in the general population compared to LMWH, but a higher risk in the cancer population (Kearon et al., 2012). DOACs have similar efficacy as warfarin in preventing recurrent VTE in the general population (EINSTEIN–PE Investigators, 2012; Agnelli et al., 2013), and some studies have shown that they may be more efficacious than LMWH in a cancer population (McBane et al., 2020).

Inferior vena cava filters are inserted when there is a contraindication to or failure of anticoagulation but raise the concern of provoking thrombosis distal to the filter. The Prevention of Recurrent Pulmonary Embolism by Vena Cava Interruption (PREPIC) study at 8 years found that vena cava filters reduced the risk of PE but increased that of DVT and had no effect on survival (PREPIC Study Group, 2005). In a population based retrospective analysis insertion of a vena cava filter was not associated with a significant reduction in the 1-year incidence of rehospitalization for PE (White et al., 2000). The use of a filter was associated with a higher incidence of rehospitalization for DVT in patients who initially manifested PE.

Patients with cancer have a significantly higher risk of developing recurrent VTE, this will be discussed in more detail in chapter 1.7.6.4.
1.3.4 Prediction of recurrent VTE

Prevention of recurrent VTE poses a great challenge, as there is a balance between the risk of recurrent VTE if anticoagulation treatment is stopped and the risk of bleeding if it is continued. Attempts have been made to quantify VTE recurrence risk at an individual level in order to predict it. The three best known predictive models for recurrent VTE following unprovoked VTE are Hyperpigmentation, Edema, Redness, D-Dimer, Obesity, Older age (HERD002) score, Vienna Prediction Model and the D-Dimer, Age, Sex, Hormonal therapy (DASH) score (Rodger et al., 2008; Eichinger et al., 2010; Tosetto et al., 2012). More recently The Leiden Thrombosis Recurrence Risk Prediction model (L-TRRiP) was published (Timp et al., 2019).

The HERDOO2 score was created from the results of a prospective multicentre cohort study of 646 participants with a first, unprovoked VTE (Rodger et al., 2008). The following factors were identified as predictors of VTE recurrence: post-thrombotic findings after 5-7 months of oral anticoagulant therapy (hyperpigmentation, edema or redness of either leg); D-Dimer ≥ 250 μg/L while taking warfarin; BMI ≥ 30 kg/m²; or age ≥ 65 years. Women with 0 or 1 characteristics had an annual risk of recurrent VTE of 1.6%. Women who had 2 or more of these findings had an annual risk of 14.1%. The authors concluded that women with a first unprovoked VTE and none or one of the HERDOO2 criteria could safely discontinue anticoagulants after completing 6 months of anticoagulation treatment, while men and women with ≥ 2 should continue anticoagulation indefinitely. The HERDOO2 model was prospectively validated in 2785 patients with the first unprovoked VTE (Rodger et al., 2017). The results were consistent with the original findings. In low-risk women who discontinued anticoagulation, VTE recurrence per patient-year was 3% while in
high-risk women and men who discontinued anticoagulation it was 8.1%. High-risk women who discontinued anticoagulation had a 7.4% VTE recurrence rate per patient-year. In high-risk women and men who continued anticoagulation the recurrence rate was 1.6%. The original Vienna Prediction Model was derived in 929 patients with a first unprovoked VTE (Eichinger et al., 2010 and 2014). It was based on 3 parameters, which were associated with the highest risk of VTE recurrence: sex (male > female), location of VTE (PE/proximal DVT > distal DVT), and a quantitative D-Dimer level at 3 weeks after discontinuation of anticoagulation. A nomogram was developed to calculate risk scores and expected cumulative recurrence rates in individual patients at 12 and 60 months. However, the model has been subsequently updated to allow the prediction of recurrent VTE at different time points after stopping anticoagulation and to allow for the competing risk of VTE recurrence and death (Eichinger et al., 2014).

The original and updated Vienna Prediction Model showed an ability to identify patients at high and low risk of recurrent VTE in the derivation data set. However, when validated in the external population the ability to identify patients at high-risk of recurrent VTE was weaker (Marcucci et al., 2015) or non-existent (Tritschler et al., 2015).

Abnormal D-Dimer after stopping anticoagulation (+2 score), age <50 years (+1 score), male sex (+1 score) and VTE not associated with hormonal therapy (hormone use in women, -2 score) were the main predictors of recurrence and were used to derive a DASH score showing a satisfactory predictive capability (Receiver Operating Characteristic (ROC) area =0.71) (Tosetto et al., 2012). Using DASH, the annualized recurrence risk was 3.1% for a score ≤ 1, 6.4% for a score=2 and 12.3% for a score ≥ 3. Tosetto et al. suggested that patients with a combined DASH score ≤1 could potentially stop anticoagulation therapy while patients with a DASH score >1 should continue it. The DASH model was validated in an
independent cohort of 827 patients (Tosetto et al., 2017). The results confirmed validity, particularly in young subjects (<65 years old).

Both Vienna and DASH models were recently externally validated in the Multiple Environment and Genetic Assessment of Risk Factors for Venous Thrombosis (MEGA) follow-up study (Timp et al., 2019). This validation study showed that predicting the risk of unprovoked recurrent VTE is possible to some extent with the currently available models, but that their predictive performance is lower than in the original studies. Predictive ability was also dependent on the definition of the unprovoked first event, which varied slightly between the studies (Timp et al., 2019). The Vienna model remains most studied and validated as outlined by the recent ESC (Konstantinides et al., 2019) and ASH guidelines (Ortel et al., 2020).

1.4 VTE in cancer

Armand Trousseau first described the link between VTE and malignancy in the 1860s (Trousseau et al., 1867). He found an association between recurrent or migratory thrombophlebitis and malignancy (mainly with pancreatic, gastric and lung cancer). This phenomenon was later called Trousseau’s syndrome. Approximately one in five of all VTE events are associated with cancer (Brose & Lee, 2008). Patients with active cancer have 4 to 6.5-fold higher risk of developing VTE compared with the general population (Heit et al., 2000B; Walker et al., 2013; Heit et al., 2015). It is well recognised that VTE can be the presenting sign of an occult malignancy. A systematic review of studies screening for occult cancer in patients with unprovoked VTE, showed 5.2% cancer prevalence in the 12-month period following VTE diagnosis (van Es et al., 2017). Subsequent analysis of the individual patient data
showed a similar prevalence of occult cancer in patients with DVT only, PE only, or both (Jara-Palomares et al., 2019). Most cancers were located in the abdomen and there was no relationship between VTE type and location of occult cancer. To date, the Scientific and Standardized Committee from the International Society of Thrombosis and Haemostasis suggests that patients with unprovoked VTE should only undergo a limited cancer screening including thorough medical history and physical examination, basic laboratory investigations, chest X-ray as well as age- and gender-specific cancer screening according to national guidelines (Carrier et al., 2015; Robin & Carrier, 2018).

VTE is the most common cardiovascular event in cancer patients and is linked to a reduction in short- and long-term survival (Khorana, 2010). The risk of VTE varies and peaks around the cancer diagnosis, hospitalization and treatment and reduces during remission. It increases again with cancer relapse and palliative end of life phase (Fig. 8) (Rao et al., 2008). It is the leading cause of death in cancer patients receiving chemotherapy after cancer progression (Khorana et al., 2007B; Khorana et al., 2013). This makes it a high priority clinical problem.

Cancer patients are subjected to many radiological investigations for staging and to assess disease progression or to exclude cancer recurrence. As a result, 3.6% of cancer patients are diagnosed with incidental or asymptomatic PE (Klok & Huisman, 2017). Some studies found that as many as half of CAT are diagnosed incidentally (Moore et al., 2011). Studies have shown that incidental finding of PE did not differ in regards to VTE recurrence and survival compared to symptomatic VTE (Shinagare et al., 2012). A Korean study evaluated an effect of anticoagulation treatment in patients with symptomatic and incidental PE (Sun et al., 2010). Overall survival was reduced in patients who did not receive anticoagulation for incidental PE. Guidelines recommend that incidental VTE should be treated in the same manner as symptomatic VTE (Lyman et al., 2015; Farge et al., 2019).
In patients with cancer, VTE is considered a clinical manifestation of a multifactorial systemic disease and is related to a number of associated risk factors, which can be broadly divided into 3 groups: patient-, cancer- and treatment-related (Agnelli et al., 2006; Kröger et al., 2006; Khorana et al., 2007A; Khorana et al., 2007B; Khorana & Connolly, 2009; Khorana, 2012; Gerotziafas & Elalamy, 2016; Falanga et al., 2017).

**1.4.1 Patient-related risk factors for Cancer Associated Thrombosis (CAT)**

Similar to the general population, increasing age, (ie >65 years) is a risk factor for VTE in cancer patients (Khorana et al., 2007B). Females are at greater risk of developing VTE, while males are at higher risk of arterial thrombosis (Khorana et al., 2006). Black patients have the highest rates of VTE (5.1%), followed by whites and Hispanic (4%) and Asian/Pacific Islander patients (3.3%).

*Reproduced with permission from Rao MV, Francis CW, Khorana AA.
(Chew et al., 2006; Khorana et al., 2007B). Many medical comorbidities such as renal failure, respiratory disease, heart disease, obesity, and acute infection (one of the strongest risk factors) have been found to be associated with an increased risk of CAT (Khorana et al., 2007B; Connolly & Francis, 2013). Poor performance status, which could be a marker of reduced mobility and bed rest for longer than 3 days are risk factors for CAT (Khorana et al. 2005; Agnelli et al., 2006; Al Diab, 2010). Cancer patients with a previous history of VTE have a 6- to 7-fold increased risk of recurrent VTE compared to cancer patients without prior VTE (Agnelli et al., 2006; Connolly & Khorana, 2010).

1.4.2 Cancer-related risk factors for CAT

Incidence of VTE varies depending on the primary site of cancer, stage of disease, histology and time from diagnosis (Khorana & Connolly, 2009; Horsted et al., 2012). Patients with the brain (glioblastoma), pancreas, kidney, stomach, lung, gynaecologic (specifically ovarian cancer), and haematological malignancies have the highest rates of VTE (Blom et al., 2006; Haddad & Greeno, 2006). Adenocarcinoma is particularly thrombogenic (Haddad & Greeno, 2006). Patients with high-grade tumours (G3) have a 2-fold increased risk of developing VTE compared with those with low-grade tumours (G1 and 2) (Ahlbrecht et al., 2012). In a Danish population-based cohort study, the risk of VTE increased with cancer stage, with adjusted relative risks for stage I, II, III, and IV of 2.9, 2.9, 7.5, and 17.1 respectively (Cronin-Fenton et al., 2010). Similar findings were confirmed by the Vienna Cancer and Thrombosis Study (CATS) (Dickmann et al., 2013). The initial 3-6 months following a cancer diagnosis is linked to the highest risk of VTE (Fig. 8) (Rao et al., 2008; Fuentes et al., 2016). Many
therapeutic interventions including surgery, chemotherapy and radiotherapy probably contribute to the risk in this time frame.

1.4.3 Treatment-related risk factors for CAT

VTE risk in patients with malignancy can be exacerbated by cancer therapy. Surgery for cancer increases the risk of DVT 2-fold and fatal PE 3-fold in the postoperative period compared with similar procedures performed in non-cancer patients (Agnelli et al., 2006). Pelvic and abdominal surgeries carry the highest risk of VTE. Open surgery is associated with a higher risk of post-operative VTE compared to Minimally Invasive Surgery (MIS) (Barber et al., 2016). Analysis of 17,284 patients with cancer showed that 12.6% of patients developed VTE after initiation of chemotherapy and the highest rates were associated with stomach and pancreatic cancer (Khorana et al., 2013). Cisplatin and bevacizumab (bevacizumab was administered to 10.3% of patients across all tumour types) were associated with the highest risk of VTE (OR 1.36 and 1.43 respectively) (Khorana et al., 2013). Combination chemotherapy with epirubicin, cisplatin and fluorouracil was associated with a 2-fold increased risk of VTE compared to a combination of epirubicin, oxaliplatin (another platinum-based agent) and fluorouracil (Cunningham et al., 2008).

Bevacizumab is an anti-angiogenic monoclonal antibody targeting circulating vascular endothelial growth factor (VEGF) released by cancer cells. It has been shown to increase the risk of both arterial and venous thrombosis (Scappaticci et al., 2007; Schutz et al., 2011). Adjuvant hormonal therapy with tamoxifen also increases the risk for VTE (Saphner et al., 1991). In a study on 2600 patients with breast cancer, the frequency of venous and arterial thrombosis was significantly higher among patients who underwent adjuvant chemotherapy patients compared with observation-only patients.
Radiation therapy is an independent risk factor for both primary and recurrent CAT (Cherkashin & Berezina, 2017; Guy et al., 2017). Supportive cancer therapies such as granulocyte colony-stimulating factors (G-CSF), erythropoietin-stimulating agents and blood transfusion also increase the risk of VTE (Bohlius et al., 2006; Bennett et al., 2008; Douros et al., 2016). Central venous catheters (CVC), used for administration of intravenous medications, and blood sampling, are associated with a 5 to 30% risk of VTE (DeLoughery, 2015).

1.4.4 Pathogenesis of CAT

Malignancy contributes to all three components of Virchow’s triad of stasis, endothelial injury and hypercoagulability in the pathogenesis of VTE (Fig. 9).

**Figure 9 Virchow’s triad.**

External pressure from the tumour mass or endoluminal tumour growth causes vascular stasis. This slowdown in blood flow rate leads to the accumulation of prothrombotic components (mainly
thrombin), (López & Chen, 2009). Prolonged bed rest and dehydration contribute to the effect of stasis.

Slower blood flow also leads to the desaturation of haemoglobin and stimulation of hypoxic responses in leucocytes, platelets and endothelial cells and subsequent endothelial injury (López & Chen, 2009). Since the endothelium covering the valve cusps of veins depends on luminal oxygen, it is very susceptible to hypoxia. Hypoxia induces expression of P-selectin and secretion of VWF (from endothelial cells) which binds platelets, leucocytes, erythrocytes and fibrin (Wick et al., 1987; Closse et al., 1997; Arya et al., 2002; Bernardo et al., 2005) leading to thrombus formation. Stasis induced hypoxia also induces TF synthesis from monocytes (Lawson et al., 1997).

Tumour cells themselves are major contributors to the hypercoagulable state arising in cancer patients. This effect is expressed through direct and indirect mechanisms (Fig. 10, Fig. 12) (Abdol Razak et al., 2018).

Multiple factors including Tissue Factor (TF) expressed on or released from cancer cells activate coagulation and platelets directly. TF is expressed on the surface of malignant cells and is present in microparticles (MP) released by the cancer cells.
The effect of tumour TF expression on the risk of VTE has been described especially in pancreatic and ovarian cancer (Khorana et al., 2007A; Uno et al., 2007; Abu Saadeh, Norris, O’Toole, Mohamed et al., 2013). Studies on patients with glioma revealed the link between podoplanin and venous thromboembolism (Riedl at al., 2017). Podoplanin (PDPN) is a protein expressed by cancer-associated fibroblasts, which can induce platelet activation and aggregation through the C-type lectin-like receptor 2 (CLEC-2) on platelets (Suzuki-Inoue et al., 2007; Shindo et al., 2013).

Tumour cells increase the potential for thrombin generation (Fig. 10).
both directly, through the expression and release of procoagulant factors in host cells (endothelial cells, monocytes) (Falanga et al., 2009), and indirectly, through signals that activate other cells types and components including platelets, leukocytes, erythrocytes, extracellular vesicles (EVs) and neutrophil extracellular traps (NETs) (Fig. 12) (Abdol Razak et al., 2018; Reddel et al., 2019).

**Figure 11 Increased thrombin generation in the cancer settings (Reddel et al., 2019)*.**

NET - neutrophil extracellular traps, EV - extracellular vesicles.

*This figure is from an open access article distributed under the Creative Commons Attribution License which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited (http://creativecommons.org/licenses/by/4.0/).
Tumour necrosis factor-alpha (TNF alpha) and interleukin 1 (IL-1) were found to induce expression of TF and von Willebrand Factor (VWF) on vascular endothelial cells, downregulate the expression of thrombomodulin, suppress the release of nitric oxide and prostacyclin and stimulate the production of PAI-1 (Bevilacqua et al., 1986; Nawroth & Stern et al., 1987; Moore et al., 1989; Kanno et al., 1994; Johnson et al., 1994).

Cancer cells stimulate neutrophils to release neutrophil extracellular traps (NETs) (Leal et al., 2017). NETs are networks of extracellular fibres, primarily composed of DNA from neutrophils, which can entrap and activate platelets with subsequent clot formation. Increased citrullinated histone H3, a biomarker for NET formation, was associated with an increased risk of VTE in cancer patients (Mauracher et al., 2018). Adhesion molecules expressed on cancer cells’ surface such as E-selectin, vascular cell adhesion molecule-
1 (VCAM-1), allow for direct interaction and attachment with the host cells. This leads to disturbed blood flow, localised clotting activation and thrombus formation (Giavazzi et al., 1993). Chemotherapy can exacerbate the procoagulant effects of cancer. One of the prothrombotic effects of chemotherapy involves direct endothelial and vascular toxicity, which exposes TF (Fig. 13) (Gobel et al., 1983; Licciarello et al., 1985; Cwikiel et al., 1996; Oner et al., 1999).

**Figure 13 The Prothrombotic Effect of Chemotherapy (Haddad & Greeno, 2006)**

![Diagram of the prothrombotic effect of chemotherapy](image)

TF-Tissue Factor, TNF-Tumour Necrosis Factor, VEGF-Vascular Endothelial Growth Factor, ATIII-Antithrombin III.

*Reprinted with permission from Elsevier (License Number 5166831498670).

Expression of TF is also increased on vascular endothelial cells and monocytes/macrophages, which induces a procoagulant response from the host cells. It has been reported that cisplatin induces endothelial cell apoptosis, which causes a release of procoagulant endothelial microparticles that generate thrombin independently of TF (Lechner et al., 2007). Chemotherapeutic agents cause platelet activation and aggregation and stimulate the secretion of immunomodulatory and proangiogenic cytokines from tumour cells (Haddad & Greeno,
Chemotherapy alters the regulatory pathways associated with the coagulation process by reducing the expression of thrombomodulin, endothelial protein C receptor and plasma levels of naturally occurring anticoagulants such as protein S, protein C and antithrombin (Kinhult et al., 2001; Key et al., 2009; Previtali et al., 2011). reflecting direct hepatotoxicity.

1.4.5 Prediction of VTE in cancer

Several models have been developed to identify cancer patients at high-risk of VTE (Riondino et al., 2019). The most widely used and externally validated of these, the Khorana risk score (Table 11), was developed to identify patients undergoing chemotherapy who are at increased risk of VTE using 5 easily available clinical and laboratory parameters (Khorana et al., 2008).

**Table 11 Risk Score for Predicting Outpatient VTE in Cancer Patients (Khorana et al., 2008).**

<table>
<thead>
<tr>
<th>Patient Characteristics</th>
<th>Risk Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site of cancer</td>
<td></td>
</tr>
<tr>
<td>Very high risk (stomach, pancreas)</td>
<td>2</td>
</tr>
<tr>
<td>High risk (lung, lymphoma, gynecologic, bladder, testicular)</td>
<td>1</td>
</tr>
<tr>
<td>Prechemotherapy platelet count 350000/mm³ or more</td>
<td>1</td>
</tr>
<tr>
<td>Hemoglobin level less than 10g/dL or use of red cell growth factors</td>
<td>1</td>
</tr>
<tr>
<td>Prechemotherapy leukocyte count more than 11000/mm³</td>
<td>1</td>
</tr>
<tr>
<td>Body mass index 35kg/m² or more</td>
<td>1</td>
</tr>
</tbody>
</table>

High-risk score ≥ 3, Intermediate risk score =1-2, Low-risk score =0.

In the Khorana’s high-risk group, 6-7% of patients suffered a VTE compared with 0.3-0.8% of patients in the low risk group (Khorana et al., 2008). Although this score has been validated by several groups in mixed cancer populations (Ay et al., 2010; Sharma et al.,
(2012; Patell et al., 2016; Posch et al., 2016) it lacks stratification power among cohorts of a single cancer type, as has been shown in studies of lung cancer patients (Mansfield et al., 2016; Kuderer et al., 2018). Recent systematic review and meta-analysis of the Khorana score for prediction of CAT enrolling 34,555 ambulatory cancer patients demonstrated that although the Khorana score can be used to select ambulatory cancer patients at high-risk of VTE for thromboprophylaxis, most events occur outside this high-risk group (Mulder et al., 2019A).

Several investigators have attempted to improve the original Khorana score either by adding chemotherapy agents, as in the case of the PROTECHT score (Verso et al., 2012) or coagulation based biomarkers (Ay et al., 2010). The addition of metastatic disease, history of previous VTE and vascular/lymphatic macroscopic compression to the original Khorana score in The Preventing Venous Thromboembolism in Ambulatory Cancer Patients (ONKOTEV) study significantly improved its predictive value (Cella et al., 2017). Although these approaches greatly improve predictive power, few have been validated.

The COMPASS-CAT risk assessment model included the patient’s co-morbidities (presence of cardiovascular risk factors; recent hospitalization for acute medical illness; personal history of VTE; platelet count), cancer-related (time since cancer diagnosis; stage of cancer) and treatment-related factors (anthracycline or anti-hormonal therapy; central venous catheter) (Gerotziafas et al., 2017). At 6 months, patients stratified at low/intermediate risk and high-risk groups had VTE rates of 1.7% and 13.3% respectively). However, the majority were on active treatment when assessed and a recent large external validation of the model showed that discrimination was moderate and calibration was poor (Spyropoulos et al., 2020). The addition of biomarkers of hypercoagulability to the COMPASS-CAT model improved
predictive power in a small group of lung cancer patients (Syrigos et al., 2018)

The Tic-Onco risk score model used patients’ clinical and genetic risk factors for thrombosis (Muñoz Martín et al., 2018). Although this model has the powerful predictive ability, its common use is limited by the cost and availability of genetic testing. The most recent score from the Vienna CATS study attempted to simplify risk prediction by developing a nomogram focussing only on tumour-site and D-Dimer levels (Pabinger et al., 2018) However, as with the Khorana score this model is heavily dependent on tumour site which limits its stratification power in cohorts of a single cancer type.

1.5 VTE treatment in CAT

1.5.1 General recommendations

The management of anticoagulant therapy for the treatment of CAT is complex and there is a fine balance between an increased risk of recurrent VTE and major bleeding compared to the general population. There are five published clinical guidelines on the treatment of CAT:

1. American College of Chest Physicians (ACCP) (Kearon et al., 2016B).
3. International Society on Thrombosis and Haemostasis guidelines (ISTH) (Khorana et al., 2018).
4. International clinical practice guidelines (ITAC) (Farge et al., 2019).
5. American Society of Clinical Oncology (ASCO) (Key et al., 2020).

Traditionally LMWH was considered the standard and first line treatment of CAT (level or evidence and recommendation: grade 1B) for at least 3 to 6 months (grade 1A). It is associated with a lower rate of VTE recurrence as compared to VKA (Lee et al., 2015). The treatment should be continued longer in the presence of active cancer or if the risk factors for recurrent VTE persist (metastatic disease, chemotherapy, etc.). There is a lack of evidence on the optimal duration of VTE treatment and the decision is often made on an individual basis balancing the risk of VTE recurrence and major bleeding. Other factors such as the impact on patient’s quality of life, patient values and the point in their cancer journey, also play a role in decision making (Lee, 2017).

Certain clinical situations make the treatment of CAT more challenging. Patients on chemotherapy are at increased risk of major bleeding (Elting et al., 2001). Up to 24% of patients with solid tumours treated with chemotherapy develop clinically significant thrombocytopenia (Hitron et al., 2011). In general, guidelines recommend full anticoagulant dose if platelet count > 50 x 10^9 L. In patients with platelet counts between 25 and 50 x 10^9 L, platelet transfusion should be considered to allow full-dose of anticoagulation or a 50% dose reduction. In patients with platelet count <20 x 10^9/L (ASCO) or 25 x 10^9/L (ISTH) anticoagulation is contraindicated. Patients with primary or metastatic brain tumours are at increased risk of bleeding and although (Navi et al., 2010) anticoagulation treatment is not absolutely contraindicated ISTH guidance states that outpatient pharmacological thromboprophylaxis is not recommended in patients with a diagnosis of primary brain tumour (Khorana et al., 2014). CAT in the peri-operative period is sometimes managed with unfractionated
heparin, due to its shorter half-life (1-2 h vs. 4h for LMWH) and potential for reversal with protamine. Although the use of an inferior vena cava filter in peri-operative period would not be a usual practice, it should be considered in patients with a recent thrombosis (within 4 weeks prior to surgery).

Recently emerging data from clinical practice experience (Mantha et al., 2017; Ross et al., 2017; Streiff et al., 2018) and new RCTs on the use of DOACs specifically for cancer patients (Agnelli et al., 2020; Raskob et al., 2018; Young et al., 2018; McBane et al., 2020) have altered the guideline recommendations (Khorana et al., 2018; Streiff et al., 2018; Farge et al., 2019; Key et al., 2020) and will be discussed below.

1.5.2 The emerging role of DOACs in treatment of CAT

There is growing evidence on the efficacy and safety of DOACs in the cancer population, which have been already used as the first line treatment of VTE in non-cancer patients (Mazzolai et al., 2018; Konstantinides et al., 2019).

Two classes of DOACs are currently available and licenced for use:
- oral direct factor Xa inhibitors (apixaban, rivaroxaban, edoxaban, betrixaban),
- oral direct thrombin inhibitor (dabigatran).

The Anticoagulation Therapy in Selected Cancer Patients at Risk of Recurrence of Venous Thromboembolism (SELECT-D) was a randomized 2-phase, open-label pilot study that compared rivaroxaban with dalataparin for treatment of CAT (Young et al., 2018). The second phase of the trial (to evaluate duration of treatment) was closed due to poor enrolment (Marshall et al., 2020).
In the first phase, the 6-months cumulative recurrent VTE rate was 11% in the dalteparin group and 4% in the rivaroxaban group (HR 0.43; 95% CI, 0.19 to 0.99). Major bleeding was similar in both groups at 4–6% but clinically relevant non major bleeding (CRNMB) was significantly higher with rivaroxaban (13%) than dalteparin (4%). Most of CRNMBs were gastrointestinal or urologic. Patients with gynaecologic malignancies accounted for 12% of patients in the dalteparin group and 9% of patients in the rivaroxaban group.

The SELECT-D:12m study assesses 12-month outcomes of the placebo versus rivaroxaban randomization of the SELECT-D Trial (Marshall et al., 2020). After 6 months of planned VTE treatment in the SELECT-D Trial, patients were randomized to a further 6 months of either rivaroxaban or placebo. The primary outcome was VTE recurrence at 12 months. There was no statistical difference in VTE recurrence rate at 12-months, however, the study was underpowered and closed prematurely due to low recruitment.

The Hokusai trial showed treatment with edoxaban for up to 12 months to be noninferior to treatment with dalteparin in the composite outcome of recurrent VTE or major bleeding in patients with CAT (Raskob et al., 2018). The risk of major bleeding in the Hokusai study was significantly higher with edoxaban (6.9% vs 4.0%), while there was a nonsignificant reduction in recurrent VTE with edoxaban compared with dalteparin (7.9% vs 11.3%)(Raskob et al., 2018). The difference in the rate of major bleeding was due to the higher rate of upper gastrointestinal bleeding with edoxaban. Patients with gynaecological malignancies accounted for 9% of the edoxaban group and 12% of the dalteparin group.

The median duration of drug exposure was shorter for dalteparin than edoxaban (184 days vs 211 days), largely due to patient preference.

A treatment algorithm for CAT has been suggested based on the evidence from the published trials and the ISTH recommendations (Fig. 14) (Carrier, Blais et al., 2018).
In the Apixaban and Dalteparin in Active Malignancy (ADAM) VTE trial, (McBane et al., 2020) three hundred cancer patients with an ECOG performance status ≤2 with acute symptomatic or incidental VTE were randomized to receive apixaban or dalteparin for 6 months. The rate of major bleeding and of clinically relevant non-major bleeding was similar in the two treatment groups and the VTE recurrence rate was significantly lower with apixaban compared to dalteparin (3.4% vs. 14.1%).

Figure 14 Patient risk stratification algorithm for the treatment of CAT (Carrier, Blais et al., 2018)*.

* Copyright 2018 Multimed Inc. This figure is from an open access article distributed under the Creative Commons Attribution License.
Among cancer patients included in the ADAM-VTE trial, 65.5% had metastatic disease and 74% received systemic anticancer therapy. In the apixaban group there were 9.5% of patients with gynaecological malignancies, compared to 10.1% in the dalteparin group. The study was powered to detect a decrease in the 6-month major bleeding rate of 6% with dalteparin (as observed in the LMWH arm of landmark studies) to 1.4% with apixaban. However, no major bleeding occurred in patients treated with apixaban, and the rate of major bleeding in the dalteparin group was lower than predicted (2.1%).

The difference in risk of recurrent VTE for DOAC over LMWH treatment in the ADAM-VTE trial (−10.7%) was higher than those observed in the Hokusai VTE Cancer (−3.4%) and SELECT-D trials (−7.0%), whereas the rate of recurrent VTE in the LMWH arm of ADAM-VTE (14.1%) was higher than those observed in Hokusai VTE Cancer (11.3%) and SELECT-D (11%). Despite the small sample size and the limitations of the ADAM-VTE trial, these results suggest a favourable risk–benefit ratio for apixaban in the treatment of acute VTE in cancer patients (McBane et al., 2020).

More recently results of the Apixaban for the Treatment of Venous Thromboembolism Associated Cancer (Caravaggio) Trial were published (Agnelli et al., 2020). Patients were randomly assigned to oral apixaban or subcutaneous dalteparin for 6 months. Oral apixaban was not inferior to subcutaneous dalteparin and did not increase the risk of major bleeding. Patients with gynaecological malignancy accounted for 10.4% of the apixaban group and 10.2% of the dalteparin group.

In the recent systematic review and meta-analysis by Mulder et al. (2020), the risk of recurrent VTE in patients with CAT was non-significantly lower with DOACs compared to LMWHs (RR, 0.68; 95% CI, 0.39-1.17). Patients treated with DOACs had a higher risk of major bleeding and clinically relevant nonmajor bleeding, although this difference was not statistically significant. The
mortality rate was similar in both groups of patients (Mulder et al., 2020). According to the ITAC and ASCO the choice of anticoagulant agent (LMWH vs. DOAC) in the treatment of primary CAT depends on clinical circumstances. In the absence of strong drug-drug interactions, gastrointestinal absorption impairment, high-risk of gastrointestinal or genitourinary bleeding apixaban, rivaroxaban or edoxaban should be used (Grade 1A) (Farge et al., 2019).

**1.6 VTE prevention and prophylaxis in cancer patients**

Surgery is a major risk factor for VTE especially in gynaecological cancer patients (see Chapter 1.4.6). Standard of care VTE prevention measures include: attention to hydration, intermittent compression boots during and after surgery, early mobilisation and physiotherapy (Gould et al., 2012; Lyman et al., 2015; Nelson et al., 2019). In addition, four weeks of anticoagulation has been recommended since 2012 (Gould et al., 2012). The type of anticoagulation, the dose and duration are still a matter for debate. The benefits of prophylaxis must be balanced against the risk of bleeding and other health impairments including quality of life.

**1.6.1 VTE prophylaxis following surgery**

Traditionally LMWH prophylaxis was given to patients during the hospital stay, until multiple studies showed a reduction in postoperative VTE rate in patients receiving extended prophylaxis (defined as 4 weeks) (Bergqvist et al., 2002; Rasmussen et al., 2006). This evidence referred to patients undergoing either an open surgery for abdominal or pelvic cancer (Bergqvist et al., 2002), or major abdominal surgery (in gastric tract, biliary system, pancreas,
intestine or exploratory laparotomy with the duration of the procedure longer than an hour (Rasmussen et al., 2006). The incidence of VTE within 30 days of laparotomy for gynaecological cancer was decreased from 2.7% to 0.6% following implementation of extended LMWH prophylaxis (Schmeler et al., 2013). However, the rate of VTE at 90 days was not different between the groups and the authors concluded that extended prophylaxis delayed rather than prevented VTE (Schmeler et al., 2013).

The Enoxaparin and Cancer (ENOXACAN) II study found that 4 weeks of anticoagulation vs. 7 days decreased the VTE rate from 12% to 4.8% in patients undergoing abdominal, gynaecological or urological cancer surgery (Bergqvist et al., 2002). This effect was still present at 3 months post-surgery. Extended LMWH prophylaxis has been shown to reduce post-discharge VTE incidence without increasing the risk of bleeding (Rasmussen et al., 2009).

For patients with cancer undergoing major surgical procedures, existing guidelines recommend 28 days of post-operative LMWH for VTE prophylaxis (Gould et al., 2012; Lyman et al., 2015; Farge et al., 2019; Key et al., 2020). There is a lack of evidence on the role of extended LMWH prophylaxis following minimally invasive surgery and the guidelines are less clear in this regard (Bouchard-Fortier et al., 2014, Barber & Clarke-Pearson et al., 2017).

Extended LMWH prophylaxis is also incorporated into the Enhanced Recovery After Surgery (ERAS) protocol in gynaecologic oncology. The recommendation includes two types of VTE prophylaxis following surgery: sequential compression devices (to be placed prior to induction of anaesthesia) and low molecular weight heparin started on post-operative day 1 and continued for 28 days for all patients undergoing laparotomy for cancer (Nelson et al., 2019).
A recent systematic review of published and unpublished evidence indicated a significant reduction in rates of VTE with no increased bleeding or treatment-related deaths for extended compared with standard LMWH thromboprophylaxis following abdominopelvic cancer surgery (Carrier, Altman et al., 2018). A recent randomised study compared oral apixaban to subcutaneous enoxaparin for thromboprophylaxis in 400 women undergoing surgery for suspected gynaecological cancer (Guntupalli et al., 2020). Seventy-nine percent of surgeries were open laparotomies. Two major bleeding events occurred on treatment: 1/205 in the apixaban arm and 1/195 in the enoxaparin arm (OR=1.04; 95%CI: 0.07–16.76; P>0.99). Five VTE events occurred: 2/205 in the apixaban arm and 3/195 in the enoxaparin arm (OR=1.57; 95%CI: 0.26–9.50; P=0.68). Women receiving enoxaparin were more likely to report pain (OR=9.20; 95%CI, 2.67-31.82; P<0.001) compared to apixaban, while women receiving apixaban were less likely to report difficulty administering treatment compared to enoxaparin (OR=0.06; 95%CI, 0.01-0.25; P<0.001).

Despite all available evidence, there has been a wide variation in clinicians’ compliance with the recommendation for extended post-operative thromboprophylaxis. Previous studies demonstrated that only 21.6% - 39% of surgical patients received prophylaxis (Schleyer et al., 2011; Vázquez et al., 2015). Samama and colleagues have shown that 57.4% of patients received four to six weeks of venous thromboembolism prophylaxis following all major abdominal or pelvic surgeries for cancer (Samama et al., 2014). We have previously shown that among European Gynaecological-oncology society (ESGO) members, only 65% of cancer surgeons prescribed extended low molecular weight heparin prophylaxis for at least four weeks (Petch et al., 2016).
1.6.2 VTE prophylaxis during chemotherapy

Chemotherapy is associated with an increased risk of VTE; therefore, it has been suggested that VTE prophylaxis may be of benefit in this group of patients. The Prospective, Randomised Trial of Simultaneous Pancreatic Cancer Treatment with Enoxaparin and Chemotherapy (PROSPECT-CONKO 004) randomised patients with advanced pancreatic cancer to receive first-line chemotherapy in an outpatient setting with or without enoxaparin (Pelzer et al., 2015). The risk of symptomatic VTE within the first 3 months was significantly reduced by enoxaparin (HR 0.12; 95% CI, 0.03 to 0.52, p=0.001) with no increase in the risk of major bleeding (Pelzer et al., 2015). Similar findings were demonstrated in the Semuloparin for Thromboprophylaxis in Patients Receiving Chemotherapy for Cancer (SAVE-ONCO) Trial and PROphylaxis of ThromboEmbolism during CHemoTherapy (PROTECHT) trial (Agnelli et al., 2009; Agnelli et al., 2012). Semuloparin (an ultra-LMWH) arm had a lower VTE risk compared with placebo arm with no difference in major or clinically relevant non-major bleeding. However, semuloparin is not commercially available. In the PROTECHT trial, nadroparin was also associated with reduced incidence of VTE versus placebo in ambulatory patients receiving chemotherapy for metastatic or locally advanced cancer. However; for all studies event rates were low and therefore guidelines could not recommend routine thromboprophylaxis in ambulatory patients receiving chemotherapy based on this evidence (Khorana et al., 2018; Streiff et al., 2018). More recently, two randomised trials have used risk stratification to identify intermediate/high-risk ambulatory patients for randomisation (Carrier et al., 2019; Khorana et al., 2019).

The Apixaban for the Prevention of Venous Thromboembolism in High-Risk Ambulatory Cancer Patients (AVERT) trial showed that Apixaban arm significantly reduced the rate of VTE (HR 0.41, 95%
CI 0.26 to 0.65; p<0.001) but increased major bleeding episodes (HR 2.0, 95% CI 1.01 to 3.95, p=0.046).

In the Rivaroxaban for Thromboembolism in High-Risk Ambulatory Patients with Cancer (CASSINI) trial, treatment with rivaroxaban did not result in a significantly lower incidence of VTE or death due to VTE dose in the 180-day trial period. However, during the intervention period (after exclusion of patients who discontinued the trial regimen), rivaroxaban led to a substantially lower incidence of VTE, with a low incidence of major bleeding (Khorana et al., 2019). Data from these two studies suggest that DOACs may play a role in CAT primary prophylaxis in high-risk VTE patients (defined as Khorana score ≥2). The number needed to treat was 17 (number needed to harm 59) in the AVERT trial (Carrier et al., 2019) and 36 (number needed to harm 100) in the CASSINI trial (Khorana et al., 2019).

1.7 VTE in gynaecological cancer

There is a wide disparity in the reported incidence of VTE in literature in patients with gynaecological malignancies, ranging from 3% to 25% (Santoso et al., 2009; Cohen et al., 2017). As mentioned in Chapter 1.4 multiple factors such as the site of the tumour, histological subtype, stage and the type of treatment influence the VTE incidence in cancer patients. Study design, including diagnosis (clinical vs. radiological) and duration of follow-up, symptomatic versus asymptomatic CAT also significantly influences results (Wun & White, 2009).
1.7.1 VTE in ovarian cancer

Ovarian cancer carries the highest risk of VTE within the group of genital tract cancers with reported rates varying from 1.4% in early stage disease to 42% in clear cell cancer patients (Rodriguez et al., 2007; Duska et al., 2010). VTE is associated with significantly higher mortality rates in patients with ovarian cancer. A retrospective cohort study of ovarian cancer patients showed that the mean overall survival time of those with VTE was 34.8 months compared to 55.8 months for those patients without VTE (Stålberg et al., 2014). In a large registry study of patients with ovarian cancer (n=13,031), 5.2% of patients were diagnosed with VTE within 24 months of cancer diagnosis (Rodriguez et al., 2007). Advancing age, an increasing number of chronic comorbid conditions, more advanced cancer stage, invasive histology were found to be significant risk factors for VTE in that study. Among women with all stages of ovarian cancer, only advanced stage and age over 75 were stronger predictors of death than the occurrence of VTE (Rodriguez et al., 2007).

Tateo et al. reported an overall incidence of VTE in ovarian cancer at 16.6% (Tateo et al., 2005). Risk factors associated with VTE were history of deep vein thrombosis, chemotherapy, older age, high BMI, FIGO stage IIC-IV and presence of residual tumour after surgery (Tateo et al., 2005).

Satoh et al. found a 25% incidence of VTE in patients with ovarian cancer prior to initiation of cancer treatment (Satoh et al., 2007). Massive ascites and clear cell histology were found to be independent risk factors for VTE. In the extension of that study including larger sample size, Tasaka et al. found a 27% incidence of VTE in patients with ovarian cancer prior to commencing cancer treatment (Tasaka et al., 2020). Age greater than or equal to 60 years, clear cell carcinoma and massive ascites were found to be significant risk factors for VTE in ovarian cancer patients (Tasaka...
et al., 2020).
Abu Saadeh et al. reported an overall incidence of VTE in patients with ovarian cancer of 9.7% (Abu Saadeh et al., 2013A). Risk factors for VTE in that cohort of patients were BMI $\geq$ 30, clear cell carcinoma, advanced stage, high grade and CA125 $> 500$ IU/ml. The diagnosis of VTE was also associated with decreased overall survival time.

Salinaro et al. examined the incidence of VTE among patients receiving neoadjuvant chemotherapy for advanced epithelial ovarian cancer (Salinaro et al., 2020). The overall incidence of VTE was 27% with distribution as follows: 9.6% at diagnosis, 7.7% during neoadjuvant chemotherapy, 3.3% post-operatively, 3% during adjuvant chemotherapy and 12% during treatment for recurrence. Patients experiencing venous thromboembolism during neoadjuvant chemotherapy had a longer mean time to interval debulking and were less likely to undergo optimal cytoreduction (50% vs 80.2%).

1.7.2 VTE in corpus uteri cancer

The 2-year cumulative incidence of VTE among 18,440 patients with uterine cancer from the California Cancer Registry was 2.7% (Rodriguez et al., 2011). The incidence varied between 1.5% in localised to 10.5% in advanced disease. Major surgery, presence of long-term comorbidities, black race and sarcoma histology were found to be risk factors for VTE within 1 year in localised disease. A high 2-year VTE incidence of 18% was noted in young women (aged $<$ 45 years) with advanced disease. Development of VTE within 2 years was a significant predictor of decreased survival for all stages of uterine cancer in this study with the highest risk for patients with localised disease (Rodriguez et al., 2011).
A Japanese study reported an incidence of pre-treatment DVT and DVT+PE of 9.9% and 4.7% respectively in patients with endometrial cancer (Satoh et al., 2008). Extrauterine spread and non-endometrioid histology were independent risk factors for pre-treatment VTE in this group of patients (Satoh et al., 2008). In a study by Tasaka et al., the incidence of pre-treatment VTE in patients with endometrial cancer was 11.5% (Tasaka et al., 2020). Age over 60 years, stage III/IV, clear cell carcinoma and tumour long diameter greater than or equal to 60 mm were found to be independent risk factors for VTE (Tasaka et al., 2020).

In a retrospective analysis of 516 patients with endometrial cancer, Matsuo et al. found an 8.1% incidence of VTE following cancer diagnosis (Matsuo et al., 2013). Multivariate analysis in this study identified four independent risk factors for VTE: elevated CA-125, extrauterine disease, thrombocytosis, and high-risk histology (serous and clear cell). Diagnosis of VTE was associated with decreased progression-free survival and overall survival and the author concluded that VTE was a surrogate for aggressive disease. In a study of 906 patients, the overall incidence of VTE was 7.9% with 1-, 2-, and 5-year cumulative incidences of 5.1%, 7.3%, and 10.2%, respectively. Increased risk of developing VTE was associated with older age, non-Asian race, large body habitus, residual disease at surgery, tumour size ≥5cm, and stage IV disease. Patients with VTE had significantly decreased progression-free survival (Matsuo et al., 2018).

### 1.7.3 VTE in cervical cancer

The 5-year cumulative incidence of VTE in patients with cervical cancer was 3.3% in a nationwide study in Taiwan (Tsai et al., 2012). CAT was associated with shorter survival in cervical cancer patients.
Pre-treatment incidence of VTE (all DVTs, n=13) in cervical cancer was 4.8% (Satoh et al., 2013). He found that tumour size (>50 mm) and stage IV of disease were independent risk factors for VTE. Interestingly, out of 13 DVTs diagnosed, only one was symptomatic, which supports previous observations which suggest that the incidence of CAT is underestimated. In a similar study, the pre-treatment incidence of VTE in cervical cancer was reported as 7.3% (Tasaka et al., 2020). Age greater than or equal to 60 years and tumour long diameter greater than or equal to 40mm were risk factors for VTE (Tasaka et al., 2020).

Jacobson et al. (Jacobson et al., 2009), found an 11.7% incidence of VTE in 436 patients with cervical cancer. Advanced stage, chemotherapy, brachytherapy, and radiation therapy were amongst the risk factors for VTE in his cohort.

In a retrospective study of 798 patients with cervical cancer the incidence of VTE was 12.3%, with 1-, 2-, and 5-year cumulative incidence of 8.4%, 11.3%, and 18.7% respectively (Matsuo et al., 2016). High WBC and platelet counts and low haemoglobin and albumin levels were associated with VTE. Affected patients were also more likely to have a locally-advanced stage and distant metastasis compared to cervical cancer patients without VTE. Both PFS (5-year rates, 22.3% versus 68.7%) and OS (5-year rates, 55.1% versus 90.0%) were significantly decreased in cervical cancer patients with VTE (Matsuo et al., 2016).

1.7.4 VTE in vulvar cancer

In a 10-year retrospective review on the incidence, timing and distribution of VTE in Chinese patients with all gynaecological cancers (n=7562), the overall VTE rate was 2% with a 3.7% VTE rate in vulvar cancer (Ye et al., 2015). Graul et al. reported a 1.2%,
30-day incidence of VTE in patients post-surgery for vulvar cancer (Graul et al., 2017).

1.7.5 VTE in vaginal cancer

Data related to the VTE incidence in patients with vaginal cancer is very scarce. In a study by Ye et al. only one patient with vaginal cancer was included, giving a VTE incidence rate of 0.7% in that study of 141 patients (Ye et al., 2015).

1.7.6 VTE following surgery for gynaecological cancer

Treatment of gynaecological cancers involves complex surgery and often chemotherapy and/or radiotherapy. The surgery itself is a risk factor for VTE (Heit et al., 2000B), and the risk is amplified in patients following surgery for cancer (Sivanesaratnam et al., 1993; Barber et al., 2015).

In the absence of post-operative VTE prophylaxis, VTE rates of 15-40% have been reported in gynaecological cancer (Clarke-Pearson et al., 1984). Agnelli et al. reported 30-day incidence of VTE following surgery for gynaecological cancer to be 2% when 30% of 450 patients received extended (28 days) VTE prophylaxis (Agnelli et al., 2006). The overall death rate post-surgery was 1.7% and 46.3% of deaths were attributed to VTE, making it the most common post-operative cause of death in that cohort. PE is the main cause of mortality in postoperative period in patients with gynaecological cancer (Clarke-Pearson et al., 1983). Ye et al. found that the incidence of VTE in gynaecological cancer was 2%, with a peak in preoperative (29.1%) and post-operative (35.1%) periods (Ye et al., 2015). In a study of 400 ovarian cancer patients, one-third of
ovarian cancer-associated VTE was diagnosed within 28 days of surgery or during chemotherapy (Abu Saadeh et al., 2013A). Graul et al., compared all gynaecologic cancer surgeries, and found ovarian cancer patients are 1.5 times more likely to develop post-operative VTE compared with other gynaecological cancer types (Graul et al., 2017). The incidence of post-operative VTE largely depends on the surgical approach.

1.7.6.1 VTE post open surgery

Open surgery is associated with a higher risk of post-operative VTE compared to minimally invasive surgery. Patients undergoing open hysterectomy for endometrial cancer had a 2.8% incidence of postoperative VTE as compared to 0.7% in the MIS group (Barber et al., 2016). This difference persisted even after adjustment for age, race, operative time, surgical complexity, and patient comorbidities (Barber et al., 2016).

The incidence of clinically symptomatic VTE in the first 28 days after open surgery for ovarian cancer in our centre was 3.2% (Abu Saadeh et al., 2013A).

The risk of VTE remains high for several weeks to months following surgery. The 90 days incidence of VTE after major gynaecologic cancer surgery was reported as 4% (Fig. 15) (Peedicayil et al., 2011). Most of these VTE (76%) cases occurred after discharge from the hospital (24% between day 1 and 7, 40% between day 8 and 28, 36% between day 29 and 90). Only 9% of patients, who developed VTE post-surgery, received either UFH or LMWH prophylaxis. Notably, the duration of heparin prophylaxis was not specified in the paper (Peedicayil et al., 2011).
The incidence of VTE post-cytoreductive surgery (defined as the time from initial surgery to last follow-up) for patients with ovarian cancer was 13.2% in a study by Gunderson et al. (Gunderson et al., 2014). Patients with VTE had significantly shorter PFS and OS.

1.7.6.2 VTE after Minimally Invasive Surgery

The incidence of VTE following minimally invasive surgery (MIS) is reported to be lower compared to open surgery for gynaecological cancer (Sandadi et al., 2012; Kumar et al., 2013). The 30-day rate of VTE following MIS for endometrial, cervical and ovarian cancer without thromboprophylaxis was reported at 0.57% (Bouchard-Fortier et al., 2014) to 0.7% (Mahdi et al., 2016). There was no difference in VTE rate between sites of cancer, however there was a trend towards higher risk of VTE among patients with disseminated cancer compared with those with early cancers (3.6% vs 0.6%). Gynaecological cancer patients with a diagnosis of VTE had significantly increased 30-day post-operative mortality (Mahdi et al., 2016), however, data on extended thromboprophylaxis was
not recorded. The risk of VTE following MIS varies with the complexity of the surgery.

The six-week rate of VTE following laparoscopic gynaecologic surgery without extended LMWH thromboprophylaxis was 0.7% (Nick et al., 2010). However 2.8% of patients who had high-complexity MIS for gynaecological cancer (radical hysterectomies, pelvic, paraaortic lymphadenectomy, splenectomy or bowel surgery) experienced a VTE.

In a study by Graul et al., patients who had MIS were 64% less likely to have a VTE regardless of malignancy site, although if they had disseminated disease, they remained at higher risk of VTE (Graul et al., 2017). This emphasizes the likelihood that it is not the surgical approach on its own that influences the risk of VTE, but the extent of disease and amount of surgical dissection undertaken.

### 1.7.6.3 Prediction of VTE in Gynaecological cancer

As described in section 1.4.5, several models exist for the prediction of VTE in cancer in mixed patient cohorts, however in the majority of these studies gynaecological cancer patients are poorly represented which limits their applicability in gynaecological oncology care. In addition, scores that are dependent on tumour site (including the Khorana score) have limited stratification in cohorts of a single type. The Caprini score is recommended to assess VTE risk post-operatively (Caprini et al., 2001). Points are allocated to a range of risk factors for VTE and based on the total score the patients are categorised as having a very low (score of 0, VTE incidence <0.5%), low (score of 1-2, VTE incidence of 1.5%), moderate (score 3-4, VTE incidence 3%), and high-risk (score of 5 and above, VTE incidence of 6%) of VTE if no VTE prophylaxis is given (Barber & Clarke-Peterson 2016 and 2017). When applied to gynaecological cancer patients, the score places >90% of patients in the high-risk category, and hence lacks the power to accurately
stratify patients (it has a high specificity, but very low sensitivity) (Barber & Clarke-Peterson 2017). The Thrombogyn score is a recently developed risk model for post-operative gynaecological patients (Norris et al., 2020) BMI>30, Hemoglobin <11.5 and chemotherapy treatment form the 3 point score. 17.6% of patients identified as high-risk developed VTE compared with 1.3% in the low-risk group. Addition of coagulation biomarkers (ETP and D-Dimer) to form the extended Thrombogyn score greatly improved the predictive power of the score. Using the extended score, the high-risk group has a 16.8 fold increased risk of VTE after surgery despite extended (28 days) LMWH prophylaxis.

1.7.6.4 Recurrence of VTE in cancer patients

The risk of recurrent VTE and anticoagulation-related bleeding is much higher in patients with cancer. Prandoni et al. found that the 12-month cumulative incidence of recurrent thromboembolism and major bleeding in cancer patients was 20.7% and 12.4% compared to 6.8% and 4.9% respectively in patients without cancer (Prandoni et al., 2002). Recurrent VTE and major bleeding events were more likely to occur during the first month of anticoagulation treatment and in patients with advanced cancer stages. A similar VTE recurrence rate (19.4%) was reported in patients with leukaemia (Luong et al., 2017). Chee et al. reported an adjusted 10-year cumulative VTE recurrence rate of 28.6% and a 90-day incidence of major bleeding on anticoagulation of 1.9% in patients with active cancer (Chee et al., 2014). In a multivariate model, brain, lung, pancreatic, ovarian cancer, myeloproliferative or myelodysplastic disorders, stage IV, cancer progression and leg paresis were associated with an increased risk of recurrent VTE (Chee et al. 2014). Patients who suffered VTE within a year prior to diagnosis of cancer (occult cancer) were more likely to suffer from recurrent VTE.
compared to patients who experienced first VTE within 2 years after cancer diagnosis (38.6% vs. 15.5% respectively) (Gran et al., 2017). As nearly half (45%) of the patients with an occult cancer-related VTE died within 6 months after cancer diagnosis, it is likely that the increased risk of VTE recurrence in patients with occult cancer was related to the advanced cancer stage in these patients.

In a recent study by Cohen et al., the overall incidence of recurrent VTE in mixed cancers was 9.6 per 100 person-years (Cohen et al., 2017). The peak at 22.1 per 100 person-years was noted in the first six months, which fell to 7.9 between six and 12 months. The author did not specify the rate of VTE recurrence for different cancer types.

The risk of VTE recurrence in patients with glioblastoma multiforme was 26.9% (Edwin et al., 2016). Lack of long-term anticoagulation (HR 11.2) and the presence of a second primary malignancy (HR 3.69), were found to be significant contributors to VTE recurrence (Edwin et al., 2016).

However, in a cohort of 212 cancer patients with recurrent VTE, 70% were on LMWH and 27% on a VKA at the time of the recurrent VTE event (Schulman et al., 2015) suggesting that factors other than the lack of long term anticoagulation contribute more to the risk of VTE recurrence in cancer patients.

Cancer patients with recurrent VTE have a significantly worse survival (threefold increased hazard of death in mixed cancer population) than patients with a single VTE episode, and that effect is even more pronounced in patients with recurrent PE +/- DVT as compared to DVT alone (Chee et al., 2014, Weber et al., 2010). Given that the incident PE is an independent predictor of reduced survival among cancer patients (Heit et al., 1999), preventing PE recurrence in the cancer population could potentially improve their survival.
1.7.6.5 Recurrence of VTE in gynaecological cancer patients

Although gynaecological cancers are associated with one of the highest rates of CAT, there are no studies of VTE recurrence exclusively in this group of patients. There are a few studies on recurrent VTE in mixed cancer populations, which contained small sub-groups of patients with gynaecological malignancies.

In a prospective cohort study on 117 patients with a variety of active cancers, (van Es et al., 2018) the incidence of VTE recurrence was 9.4%. Eight patients had ovarian cancer, one of whom developed recurrent VTE. In a mixed cancer population, ovarian cancer was found to be an independent predictor of VTE recurrence (Chee et al., 2014).

In the study by Gran et al., described in Chapter 1.5.2, there were 13 patients with gynaecologic cancer (13/110) who developed VTE within 2 years after cancer diagnosis and 3 developed recurrent VTE, while of the 3 patients (3/40) who developed VTE within the year prior to cancer diagnosis of which one developed recurrent VTE (Gran et al., 2017).

1.8 Prediction of recurrent VTE in cancer patients

The Ottawa score model has been proposed to stratify the risk of recurrent VTE during the first 6 months of anticoagulation therapy in patients with CAT (Louzada et al., 2012). Two scores were derived: the original and modified score (Table 12).

In the original score (Table 12), patients were divided into low (score ≤ 0) or high-risk (score ≥ 1) for VTE recurrence. Among patients with a score ≤0, 4.5% had VTE recurrence compared to 19% in patients with a score >1 (Louzada et al., 2012).
The modified score (Table 12) classified patients into low (score \( \leq -1 \)), intermediate (score = 0) and high-risk (score \( \geq 1 \)) for VTE recurrence. Among patients with a score <0, 5.1% had VTE recurrence, in patients with score of 0, 9.8% had a recurrence and in patients with a score \( \geq 1 \), 15.8% had VTE recurrence.

**Table 12 The Original and Modified Ottawa Score, (Louzada et al., 2012).**

<table>
<thead>
<tr>
<th>Factors</th>
<th>Original Ottawa Score</th>
<th>Modified Ottawa Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female sex</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Prior history of VTE</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>-1</td>
<td>-1</td>
</tr>
<tr>
<td>Cancer stage I</td>
<td>-2</td>
<td>Not applicable</td>
</tr>
<tr>
<td>Cancer stage I+II</td>
<td>Not applicable</td>
<td>-1</td>
</tr>
<tr>
<td>Low risk</td>
<td>( \leq 0 )</td>
<td>( \leq -1 )</td>
</tr>
<tr>
<td>Intermediate</td>
<td>Not applicable</td>
<td>0</td>
</tr>
<tr>
<td>High</td>
<td>( \geq 1 )</td>
<td>( \geq 1 )</td>
</tr>
</tbody>
</table>

While this score appeared to perform well in two retrospective cohort studies (den Exter et al., 2013; Astruc et al., 2016), it did not hold up in a sub-analysis from the Comparison of Acute Treatments in Cancer Haemostasis (CATCH) study evaluating 900 patients (Khorana et al., 2015). The recurrent VTE rates were 3.4% in low, 9.7% in intermediate and 8.2% high-risk groups (Khorana et al., 2015). In addition, the Ottawa score does not perform well in cancer patients with incidental PE (Mulder et al., 2019B).

However, recent systematic review and meta-analysis of studies validating either the Ottawa score in its original or modified version showed promising results similar to the original study (Delluc et al., 2019). Nine eligible studies were identified including 14,963 patients with mixed cancers of which 5.7% to 22.6% were
The rate of recurrent VTE was 18.6% in high-risk patients and 7.4% in low-risk patients in the original score. Using the modified score 2.2% of patients from the low-risk group experienced recurrent VTE compared to 10.2% in the high-risk group (Delluc et al., 2019).

RIETE-VTE (Computerized Registry of Patients with Venous Thromboembolism database) has been recently designed to predict 30-day mortality in cancer patients with VTE (Fuentes et al., 2019). Six predictors of 30-day mortality were identified as follows: leukocytosis ≥11.5x10⁹/L; PLT count ≤160x10⁹/L, metastasis, recent immobility, initial presentation as PE and BMI<18.5. Based on the score the patients could be categorised as low (score 0-3), moderate (score 4-6) and high (score ≥7) risk groups. By using a cut off ≥7 points, the negative predictive value of the score was 94.4%, a positive predictive value of 23.1%, a sensitivity of 73.3%, and a specificity of 64.6% (Fuentes et al., 2019).

More recently Pfaundler et al., validated the RIETE-VTE score and compared its prognostic performance with the modified Ottawa score (Pfaundler et al., 2020). Authors enrolled 178 patients with a median age of 74 (IQR 69-80) with CAT and assessed 30-day all-cause mortality, 90-day overall complications (mortality, major bleeding or VTE recurrence), and 6-month VTE recurrence. 3.93% of patients had cervical and ovarian cancer. Patients were stratified into RIETE-VTE and modified Ottawa score risk classes (low, intermediate, high). Fifteen patients (8.4%) died within 30 days, 42 (23.6%) experienced an overall complication by day 90, and 6 (3.4%) had recurrent VTE within 6 months. The RIETE-VTE and the modified Ottawa score classified similar proportions of patients as low risk (35.4% versus 31.5%; P = 0.37). No low-risk patient died within 30 days. Low-risk patients identified by the RIETE-VTE and modified Ottawa score had similar rates of overall complications (7.9% versus 8.9%) and VTE recurrence (1.6% versus 1.8%). The modified Ottawa score and the RIETE-VTE score had similar areas
under the ROC curve for predicting all-cause mortality (0.84 versus 0.75; \( P = 0.21 \)), overall complications (0.74 versus 0.68; \( P = 0.26 \)), and VTE recurrence (0.67 versus 0.64; \( P = 0.78 \)).

1.8.1 Biomarkers as predictors of CAT recurrence.

Many of the scores described above use plasma and serum biomarkers as part of their predictive score. The majority of these biomarkers are coagulation-based plasma markers, which are described in more detail below.

1.8.1.1 D-Dimer

Elevated D-Dimer levels measured during or after cessation of anticoagulant therapy are associated with increased risk of VTE recurrence in the general population (Baglin et al., 2008; Bruinstroop et al., 2009; Douketis et al., 2010; Bjøri et al., 2017). Similar findings arise in cancer. Elevated levels of D-Dimer (>600 ng/mL) at 21 days following cessation of anticoagulation treatment have been reported to predict VTE recurrence among patients with CAT (Jara-Palomares et al., 2018). The authors concluded that approximately 56% of patients could safely stop anticoagulation treatment based on their 21-day D-Dimer values.

In patients with active cancer and VTE, treated with the LMWH - Tinzaparin, baseline D-Dimer levels above the median value (2759 ng/mL) were predictive of VTE recurrence at 6 months (Piatek et al., 2016).

In contrast, D-Dimer levels were not found to predict the risk of recurrent VTE in the Recurrent VTE Biomarkers (REMARK) study (van Es et al., 2018).
1.8.1.2 Fibrinogen

Fibrinogen (Factor I) is a soluble glycoprotein, which during tissue or vascular injury is converted enzymatically by thrombin to fibrin and subsequently to a fibrin-based blood clot. It has been postulated that increased levels of fibrinogen may lead to increased thrombus size, the formation of tight and rigid network structures and impaired fibrinolysis due to interference with the binding of plasminogen to its receptor (Koenig, 2003).

Elevated fibrinogen levels have been also associated with increased risk of VTE in the general population (Koster et al., 1994; Kamphuisen et al., 1999; van Hylckama et al., 2003).

In the Leiden Thrombophilia Study (LETS) patients with a fibrinogen level >4 g/l had a more than a 2-fold increased risk of VTE compared with those with lower levels (Koster et al., 1994). Fibrinogen levels of 5 g/l were associated with an even higher risk. In a recent study fibrinogen levels ≥ 5.0 g/l were associated with an increased risk of VTE in the general population compared to controls (Rietveld et al., 2019). The role of elevated fibrinogen in VTE recurrence is unknown.

1.8.1.3 Soluble P-Selectin

Soluble P-selectin (sPsl) is a plasma marker of platelet activation and endothelial cell activation. Elevated levels of sPsl were associated with a 2.6-fold increased risk of cancer-associated VTE in 687 patients with various types of cancer (Ay et al., 2008).

Higher baseline (at diagnosis of primary VTE) sPsl levels have been reported in association with mixed cancer diagnoses in patients who developed recurrent VTE (median 163ng/mL and 95 ng/mL; P=0.01) (van Es et al., 2018).
However, P-selectin levels were found not to be predictive of recurrent VTE in a secondary analysis of 482 patients enrolled in the CATCH (Comparison of Acute Treatments in Cancer Hemostasis) trial (Khorana et al., 2017).

1.8.1.4 Microparticles and TF

Microparticles are heterogeneous negatively charged cell-derived membrane vesicles ranging in size from 0.1 to 1.0 µm, and primarily originating from platelets (Barteneva et al., 2013). Tissue factor microparticle activity level is higher in cancer patients with VTE compared to cancer patients without VTE (Amin et al., 2008; Ay et al., 2009; Bucciarelli et al., 2012). Although many studies linked elevated levels of MPs with the future occurrence of thrombosis in cancer patients (Zwicker et al., 2009; van Doormaal et al., 2012), others failed to demonstrate their role as predictive biomarkers (Tesselaar et al., 2007; Thaler et al., 2011). Much of the variation in MP data results from the variation in analytical methods and poor standardisation of pre-analytical variables detection (Lee et al., 2012).

Tissue Factor (TF) is expressed in many tumour types with expression linked to histologic grade (Geddings et al., 2016). TF expression in ovarian tumours is significantly higher in patients who subsequently develop VTE (Abu Saadeh et al., 2013B). TF expression was highest in clear cell and endometrioid subtypes, which may explain the higher risk of VTE in these subgroups.

In the CATCH trial circulating TF was strongly associated with recurrent VTE in patients with cancer who were on anticoagulation treatment (Khorana et al., 2017). This study included mixed types of cancers, of which 22.6% were gynaecological.
1.8.1.5 Factor V

Factor V is Vitamin K dependent serine protease which functions as a cofactor for the Factor X dependent conversion of prothrombin to thrombin (Kane & Majerus, 1981; Monkovic & Tracy, 1990). In addition to its procoagulant function, FV has also been reported to function as an anticoagulant by enhancing the activity of the activated Protein C (APC)-mediated inactivation of FVIII. Approximately 5% of the population carry Factor V Leiden (Arg506Gln) mutation and are less sensitive to inhibition by activated protein C (APC) (Bertina et al., 1994). This mutation may be associated with an increased risk of thrombosis (Bertina et al., 1994). The dual function of Factor V as both pro- and anti-coagulant complicates the assessment of its role as a predictor of VTE with both low levels and high levels possibly contributing to the increased VTE risk (Kamphuisen et al., 2000; Suehisa et al., 2010; Rietveld et al., 2018). Although studies suggested that Factor V Leiden may be also associated with an increased risk of cancer associated thrombosis (Pabinger et al., 2015; Heraudeau et al., 2018), there have been few reports of the role of Factor V levels in CAT. A recent study has shown that tumour and plasma levels of FV are increased in gynaecological cancers however this was not associated with an increased risk of VTE (Martin et al, 2015). The role of Factor V in VTE recurrence is unknown.

1.8.1.6 Factor VIII

The role of elevated levels of Factor VIII as a risk factor for VTE was first described by The Leiden Thrombophilia Study (LETS) in the general population and was later confirmed in multiple studies (Koster et al., 1995; Rosendaal, 2000; Tsai et al., 2002; Jenkins et
A recent study showed that raised FVIII and VWF levels were the major contributors to venous thrombosis (Rietveld et al., 2019).

Factor VIII is independently associated with an increased risk of VTE in patients with hematologic malignancies and mixed solid tumours (Vormittag et al., 2009). The study showed that increased risk conferred by Factor VIII was dose-dependent. The association between Factor VIII and VTE was strongest in younger patients and declined with age (Vormittag et al., 2009).

Tafur et al., (2015) found patients with mixed solid organ tumours and a high pre-chemotherapy baseline Factor VIII level were three times as likely to develop VTE (HR 3.16; 95% CI 1.4–7). High plasma Factor VIII levels have been found to be an independent risk factor for recurrent VTE in the general population (the risk of recurrent VTE associated with elevated Factor VIII was also shown to be dose-dependent (Kyrle et al., 2000).

Kraaijenhagen et al. calculated that for each 10 iu/dl increment in plasma Factor VIII level, the risk for a single and recurrent episode of VTE increased by 10% and 24% respectively. Furthermore, for patients with Factor VIII levels above 200 iu/dl, the odds ratio for a recurrent VTE was markedly elevated at 45 (Kraaijenhagen et al., 2000).

Cristina et al. reported that in patients suffering a first idiopathic VTE, the risk of recurrence was more than 5-fold higher in patients with Factor VIII levels exceeding the 90th percentile (Cristina et al., 2004).

Evidence on factor VIII as a predictor of recurrent VTE in cancer patients is limited. In the CATCH trial, Khorana et al., (2017) no difference in factor VIII levels was found between patients with
recurrent VTE compared to patients who did not experience VTE recurrence.

1.8.1.7 Protein S

Protein S is a vitamin K-dependent glycoprotein, which circulates in two forms, free and bound. Free protein S is active and serves as a cofactor for Activated Protein C (APC), which inactivates Factors V and VIII and reduces thrombin generation as discussed in section 1.2.2.

Hereditary protein S deficiency is an autosomal dominant disorder that has been associated with a risk of recurrent VTE (Comp & Esmon, 1984; Comp et al., 1984). In a large population-based case-control study, low protein S (free and total) concentration was not associated with an increased risk of VTE (Pintao et al, 2013).

Few studies have investigated the role of protein S in cancer-associated VTE. Protein S mRNA expression was significantly down-regulated in malignant gynaecological tumours compared with benign controls; this was not associated with VTE (Martin et al., 2016). These changes may contribute to local thrombin production and increased thrombotic potential of patients with gynaecological cancer.

1.8.1.8 Thrombin generation

Thrombin is the serine protease responsible for the activation of platelets and the conversion of fibrinogen to fibrin as explained in Chapter 1.2.1.

The potential for thrombin generation in patient plasma can be measured ex vivo by using a thrombin-specific substrate to measure the thrombin burst generated after exposure of plasma to
an exogenous stimulant (usually tissue factor). The Calibrated Automated Thrombogram or thrombin generation test can be used as a global test for hyper- and hypo-coagulability in a variety of settings (Hemker et al., 2002). Several studies have shown that increased thrombin generation as measured by this assay is associated with an increased VTE risk in multiple cancer types (Ay et al., 2011; Falanga et al, 2015; Leiba et al, 2017)

Cho et al. (2017) found that high endogenous thrombin potential (ETP) at the beginning of systemic therapy was also a predictive biomarker for VTE in patients with pancreatic and lung cancer. In agreement with these findings, Ward et al. showed that increased ETP was observed in patients who suffered a VTE after cancer surgery. This increase was observed when thrombin generation was stimulated with both low (1pm) and high (5pm) TF concentrations (Ward et al., 2018). Patients with clear cell cancer of the ovary and patients with endometrial cancer had higher ETP and peak thrombin compared with patients with benign disease (Abu Saadeh et al., 2016). The addition of ETP values above the 75th centile in a large study of gynaecological cancer patients, significantly increased the predictive power of the Thrombogyn risk score for VTE in gynae cancer (Norris et al., 2020).

ETP levels above the 50th centile were predictive of recurrent VTE in patients with the first unprovoked VTE in the cohort of 188 patients examined by Besser et al. (Besser et al., 2008).

Patients with high ETP following discontinuation of anticoagulation treatment for spontaneous VTE had a significantly higher risk of VTE recurrence (HR 1.6) (Eichinger et al., 2008). Similarly, high ETP and peak thrombin levels were associated with increased risk of recurrent VTE one month after discontinuation of anticoagulant treatment in 254 patients with first unprovoked VTE (Tripodi et al., 2008).
In the Longitudinal Investigation of Thromboembolism Etiology (LITE) study, a peak thrombin level above the median was predictive of a high-risk of recurrent VTE (Lutsey et al., 2009). The Leiden Thrombophilia Study (LETS) found that high ETP was associated with the first event of idiopathic DVT but failed to demonstrate an association with recurrent thrombotic events (Tappenden et al., 2007).

1.9 Patients’ experience of thrombosis and thrombosis prophylaxis

Qualitative research in the non-cancer population has shown that VTE has a significant negative psychological impact. Patients described symptomatic PE as a life-changing distressing event leading to behaviour modification and in some, post-traumatic stress disorder (Noble et al., 2014). The impact of CAT is often considered by patients as more distressing than cancer itself (Noble et al., 2014; Seaman et al., 2014).

The Patients’ Experiences of Living with CANcer associated thrombosis (PELICAN) study addressed experiences of patients living with CAT (Noble et al., 2015A). Participants reported that the CAT journey was very distressing, with limited support and information, which was in was in contrast to the supports available during their cancer treatment.

Noble et al. concluded that regardless of the strong evidence supporting the diagnosis and treatment of CAT, without an insight into the patient’s experience, we are unlikely to deliver optimal patient-centric treatment. A dedicated CAT service has been shown to improve overall standards of care and was viewed positively by patients and clinicians alike in a subsequent study by Noble et al. (Noble et al., 2016). A study of 221 US patients demonstrated that a centralized approach to the care of CAT reduces treatment
variation and appears to reduce VTE-related hospitalizations (Rabinovich et al., 2016). This subsequently leads to cost savings through reduction in recurrent VTE, bleeding and hospital admissions.

Extension of PELICAN study into Spanish settings (PELICANOS) showed that the distress experienced by patients with CAT is not limited to patients in the United Kingdom (Font et al., 2018). The study showed that one of the key drivers in patients’ distress was the unexpected nature of the CAT and the fact that it occurred so soon after their cancer diagnosis, which was perceived as a further threat to life. Patients were keen to understand the natural history of their treated CAT and its effect on their cancer treatment and overall prognosis. The study highlighted that the CAT has also a profound impact on the patient’s family. Patients were very mindful that their illness was affecting those around them. Some patients would try to minimise distress on the family by not sharing their concerns with them (Font et al., 2018). This was an important finding as close family members often witness the impact of CAT and are involved in its treatment, for example by administering the daily LMWH injections.

Studies examining patients who received LMWH for CAT have shown that LMWH remains an acceptable intervention for the treatment of CAT, if only as an acceptable compromise against their strongly negative experiences of symptomatic VTE (Seaman et al., 2014).

Sub-analysis of the data from the CATCH trial looked at the impact of recurrent VTE and bleeding events on health-related quality of life (HRQOL) (Lloyd et al., 2018). Recurrent VTE had a significant detrimental impact on quality of life, measured as a decline in five-dimensional questionnaire (EQ-5D) scores. Bleeding events had a smaller and non-statistically significant impact on EQ-5D scores. These findings highlighted the considerable impact that treatment failure (defined as recurrent VTE) has on patients’ HRQOL. The HRQOL impact from recurrent VTE indicates the potential
patient/quality-of-life benefit related to prophylaxis- based treatment strategy (Lloyd et al., 2018). However, patients might be less likely to accept the side effects during extended LMWH prophylaxis after surgery, especially if they are not aware of the potential consequences of not taking it. In patients undergoing major orthopaedic or major cancer surgery, lack of education and a negative opinion toward injection has negatively influenced compliance (Kalka et al., 2009). In a study examining patients’ adherence and experience with extended LMWH prophylaxis following pancreas and liver resection, more than 81% of patients complied with their LMWH regime (Lemke et al., 2016). These findings suggest that inadequate prescribing as described by Petch et al. (2016) rather than patient adherence is the major contributor to poor compliance with VTE prophylaxis guidelines. Patients’ experience and compliance with LMWH extended prophylaxis in the setting of gynaecological cancer is largely unknown. An understanding of the patients’ experience with extended LMWH prophylaxis and their understanding of their risk of thrombosis may offer insight into factors that influence compliance.

1.10 Summary of evidence leading to this research

The presence of VTE in patients with cancer is associated with significant implications including a need for chronic anticoagulation, potential delay in cancer treatment (surgery, chemotherapy, radiotherapy), risk of recurrent VTE, reduced quality of life and consumption of health care resources (Prandoni et al., 2002; Elting et al., 2004). Moreover, the mortality rate is increased two-fold in cancer patients with VTE compared with cancer patients without VTE even after adjusting for stage (Sørensen et al., 2000; Chew et al., 2006) and
thromboembolism is a leading non-cancer cause of death in cancer patients (Khorana et al., 2007B).

There is a fine balance between the risk of VTE recurrence and major bleeding in patients with cancer receiving anticoagulation therapy. Since the optimal LMWH regimen for the treatment of CAT is uncertain, risk stratification tools are needed to help clinicians identify patients at high-risk of recurrent VTE. This is particularly important given the emerging role of new oral anticoagulants in CAT treatment and prophylaxis. Clinical risk factors and serum biomarkers may identify patients with gynaecological malignancy at high-risk of VTE recurrence and help to plan the most appropriate treatment.

There is an evident knowledge gap in the field of patients’ experience and compliance with LMWH extended prophylaxis in the setting of gynaecological cancer. Exploring this further might help derive strategies to improve understanding of cancer-associated thrombosis risk and overcome the obstacles in patients’ compliance. Ultimately this could potentially reduce the risk of VTE following gynaecological cancer surgery.
1.11 Specific aims of this study


2. Investigate the effect of VTE recurrence on overall and progression-free survival in gynaecological cancers.

3. Assess the role of common laboratory biomarkers (WCC, Hb, PLT, Urea, Creatinine, Albumin) as predictive biomarkers of VTE recurrence in patients with gynaecological cancer.


5. Examine the patients’ experience and compliance with extended thromboprophylaxis following gynaecological cancer surgery.

The overall aim is to identify risk factors for recurrent VTE. We hypothesise that recurrent VTE is associated with reduced overall and progression-free survival in patients with gynaecological cancers.
Chapter 2: Patients and methods
2.1 Ethical approval

All three studies described below have been granted approval from the Clinical Research Ethics Committee of the St James’s Hospital/ Adelaide and Meath Hospital ethical committee, amendments to Original Study 2009/29/01 (2014-10 List (11) and 2017-06 List 21(14)). All patients have given full and informed written consent to participate in the studies.

2.2 Patients

2.2.1 Patients included in the clinical study

Data from all patients diagnosed and treated for gynaecological malignancy in St. James’s Hospital Gynaecological Cancer Centre, a tertiary referral centre for gynaecological cancer patients in Ireland, between May 2006 and June 2017 were extracted from hospital records. Patients with the first episode of cancer-associated thrombosis (CAT) were identified and the accuracy of records was verified through the general practice/community records. A detailed questionnaire inquiring about patients’ history of VTE was sent out to general practitioners. Where the answers were not clear or if the questionnaires were not returned the send out was followed up by a telephone call. CAT was defined either as venous thromboembolism (VTE) diagnosed within six months prior to, or at any time following the cancer diagnosis, during chemotherapy or radiotherapy treatment while the malignancy was active or following cancer related surgery. VTE was defined as any symptomatic or incidentally detected proximal deep-vein thrombosis of the lower or upper limbs, any nonfatal symptomatic or incidental pulmonary
embolism diagnosed on staging CT. Asymptomatic subsegmental PEs were included, however, routine ultrasonographic testing was not performed.

The diagnosis of VTE was based on documented objective testing including compression ultrasonography, venography, computed tomography, and pulmonary angiogram in case of a pulmonary embolism (PE). The time frame for diagnosis of postoperative VTE was 6 weeks.

Inclusion criteria:

- All patients treated in St James’s Hospital between January 2006 and March 2017 for gynaecological cancer who were diagnosed with CAT.

Exclusion criteria:

- Previous history of VTE (more than 6 months prior to the cancer diagnosis),
- Personal history of VTE unrelated to cancer,
- Documented thrombophilia.

Recurrent (second episode) of VTE was defined according to the international standards (Heit et al., 2011). This defines recurrent VTE as “thrombosis of a site that was either previously uninvolved or had interval documentation of incident DVT or PE resolution” (See Section 1.3 in the Introduction). Patients with the first VTE episode were followed up until death or the end of the study period (2017). Progression-free survival and overall survival were calculated. In order to minimise the risk of missing the recurrent VTE events (e.g., patients presenting to a different hospital), radiology records available on National Integrated Medical Imagining System (NIMIS) were reviewed for all patients with CAT.
included in our study.

The following baseline data were recorded at cancer diagnosis from hospital records:

- age,
- Body Mass Index (BMI),
- medical comorbidities,
- menopausal status,
- smoking status,
- cancer grade and FIGO stage,
- histological subtype,
- hormonal therapy,
- chemotherapy treatment (neoadjuvant/adjuvant and number of cycles given and type of the agent used),
- occurrence of another primary malignancy,
- surgical approach,
- surgical complexity (see Table 13),
- duration of a hospital stay,
- type and duration of anticoagulation treatment (Low Molecular Weight Heparin (LMWH)/Unfractionated Heparin, warfarin, direct oral anticoagulant (DOAC)),
- type of primary and recurrent VTE (DVT/location, PE, cerebral sinus thrombosis), the timing of recurrence from the initial VTE episode,
- whether the patient was symptomatic or not,
- circumstances surrounding the recurrence of VTE.

The data were recorded in a spreadsheet using the Microsoft Excel software (Version 16.37).
Table 13 Surgical classification sheet and complexity score group – modified from Aletti et al., 2007.

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hysterectomy +/-salpingo-oophorectomy</td>
<td>1</td>
</tr>
<tr>
<td>Radical Hysterectomy</td>
<td>3</td>
</tr>
<tr>
<td>Omentectomy</td>
<td>1</td>
</tr>
<tr>
<td>Pelvic lymphadenectomy</td>
<td>1</td>
</tr>
<tr>
<td>Para-aortic lymphadenectomy</td>
<td>2</td>
</tr>
<tr>
<td>Pelvic &amp;/or abdominal peritoneal stripping</td>
<td>1</td>
</tr>
<tr>
<td>Recto sigmoid resection with anastomosis</td>
<td>3</td>
</tr>
<tr>
<td>Large bowel resection</td>
<td>2</td>
</tr>
<tr>
<td>Small bowel resection</td>
<td>1</td>
</tr>
<tr>
<td>Diaphragmatic stripping</td>
<td>2</td>
</tr>
<tr>
<td>Splenectomy</td>
<td>2</td>
</tr>
<tr>
<td>Liver resection</td>
<td>2</td>
</tr>
<tr>
<td>Radical Vulvectomy</td>
<td>2</td>
</tr>
<tr>
<td>Groin dissection</td>
<td>2</td>
</tr>
<tr>
<td>Simple vulvectomy</td>
<td>1</td>
</tr>
<tr>
<td>Vaginal Hysterectomy</td>
<td>1</td>
</tr>
<tr>
<td>Trachelectomy</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Complexity score group</th>
<th>Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>≤ 3</td>
</tr>
<tr>
<td>Intermediate</td>
<td>4-7</td>
</tr>
<tr>
<td>High</td>
<td>≥ 8</td>
</tr>
</tbody>
</table>

Extended prophylaxis was introduced into routine practice at St. James’s Hospital Gynaecologic Oncology Centre in May 2012. Data relating to the dose of LMWH and duration of prophylaxis was collected as part of this study. The Khorana score (Khorana et al., 2008) was calculated for all patients retrospectively (Table 14).
Table 14 Khorana score (Korana et al., 2008) used in the current study.

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Risk score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Risk depending on the site of cancer</td>
<td></td>
</tr>
<tr>
<td>● Very high risk</td>
<td>Stomach, pancreas</td>
</tr>
<tr>
<td>● High risk</td>
<td>Lung, lymphoma, gynaecologic, bladder, testicular</td>
</tr>
<tr>
<td>Pre-chemotherapy platelet count</td>
<td>≥ 350x109/L</td>
</tr>
<tr>
<td>Haemoglobin or use of red cell growth factors</td>
<td>&lt;10 g/L</td>
</tr>
<tr>
<td>Pre-chemotherapy leucocyte count</td>
<td>≥ 11x109/L</td>
</tr>
<tr>
<td>BMI</td>
<td>≥ 35kg/m2</td>
</tr>
</tbody>
</table>

Patient co-morbidities were quantified and calculated according to the Charlson Comorbidity Index (Table 15, Charlson et al., 1987, 1994).
Table 15 Charlson Comorbidity Index Scoring System (Charlson et al., 1987, 1994).

<table>
<thead>
<tr>
<th>Score</th>
<th>Condition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Myocardial infarction (history, not ECG changes only)</td>
</tr>
<tr>
<td></td>
<td>Congestive heart failure</td>
</tr>
<tr>
<td></td>
<td>Peripheral vascular disease (includes aortic aneurysm ≥6 cm)</td>
</tr>
<tr>
<td></td>
<td>Cerebrovascular disease: CVA with mild or no residua or TIA</td>
</tr>
<tr>
<td></td>
<td>Dementia</td>
</tr>
<tr>
<td></td>
<td>Chronic pulmonary disease</td>
</tr>
<tr>
<td></td>
<td>Corrective tissue disease</td>
</tr>
<tr>
<td></td>
<td>Peptic ulcer disease</td>
</tr>
<tr>
<td></td>
<td>Mild liver disease (without portal hypertension, includes chronic hepatitis)</td>
</tr>
<tr>
<td></td>
<td>Diabetes without end-organ damage (excludes diet-controlled alone)</td>
</tr>
<tr>
<td>2</td>
<td>Hemiplegia</td>
</tr>
<tr>
<td></td>
<td>Moderate or severe renal disease</td>
</tr>
<tr>
<td></td>
<td>Diabetes with end-organ damage (retinopathy, neuropathy, nephropathy, or brittle diabetes)</td>
</tr>
<tr>
<td></td>
<td>Tumor without metastases (exclude if ≥5 y from diagnosis)</td>
</tr>
<tr>
<td></td>
<td>Leukemia (acute or chronic)</td>
</tr>
<tr>
<td></td>
<td>Lymphoma</td>
</tr>
<tr>
<td>3</td>
<td>Moderate or severe liver disease</td>
</tr>
<tr>
<td>6</td>
<td>Metastatic solid tumor</td>
</tr>
<tr>
<td></td>
<td>AIDS (not just HIV positive)</td>
</tr>
</tbody>
</table>

NOTE: For each decade > 40 years of age, a score of 1 is added to the above score.
Abbreviations: ECG, electrocardiogram; CVA, cerebrovascular accident; TIA, transient ischemic attack; AIDS, acquired immunodeficiency syndrome; HIV, human immunodeficiency virus.

2.2.2 Patients - biomarker study

2.2.2.1 Patients and sampling

The following biomarkers: Endogenous Thrombin Potential (ETP), Factor V, Factor VIII and Protein S were analysed on patients' plasma samples stored in the Trinity College Gynaecology cancer bioresource. The analysis was performed by the author (Zbigniew Marchocki) and Dr. Lucy Norris. The samples were obtained prior to cancer surgery, radiotherapy or chemotherapy and the first VTE event.
2.2.3 Trinity College Dublin Gynaecological cancer bioresource

The Trinity College Dublin Gynaecological cancer bioresource is an extensive biobank of tissue, serum and plasma from gynaecological malignancies. This bioresource has been running for 15 years and is linked to a dedicated database, which provides full follow-up data on each patient.

Having given full and informed consent, new patients attending the Gynaecological Oncology Service and scheduled for surgery at St James’s Hospital are invited to donate blood samples. They are typically obtained 24-48 hours prior to surgery. The cancer bioresource has the approval of the Hospital Ethics committee and the TCD School of Medicine Ethics committee. Patients from the bioresource were included in the study if they met the following criteria.

Inclusion criteria:

- Patients with postoperative VTE.

Exclusion criteria:

- Patients with pre-operative VTE,
- Patients on anticoagulation at the time of sampling,
- Patients with known thrombophilia.
- Patients treated with primary chemotherapy or chemo-radiation therapy.
2.2.4 Blood sampling

Plasma samples were obtained from the TCD gynaecological cancer bioresource (see 2.2.3). All blood samples were performed by the hospital’s phlebotomist and obtained prior to surgery for resection/staging of the tumour and prior to the first VTE event. Venous blood samples were obtained with the minimum venous stasis from the antecubital fossa into tubes containing trisodium citrate (0.015M)(1:9 vol/vol) and transferred to the laboratory within 2 hours of venipuncture. Whole blood was centrifuged at 2000 g for 20 minutes, platelet poor plasma was aliquoted and snap frozen in liquid nitrogen and stored at -80 until assay.

Patients, also had white cell count (WCC), hemoglobin (Hb), platelet count (PLT), creatinine (Cr), albumin, fibrinogen and D-Dimer levels measured by the St. James’s Hospital Clinical Pathology Laboratory prior to cancer treatment, but only samples obtained prior to the first VTE event and any cancer treatment were used for the purpose of this study.

2.3 Laboratory methods

2.3.1 Fibrinogen and D-Dimer

Standard laboratory methods were used to analyse fibrinogen (The Clauss fibrinogen assay) and D-Dimer levels (VIDAS D-Dimer test). Fibrinogen and D-Dimer assays are accredited tests as per the ISO15189 standard. Both tests were performed in the St. James’s Hospital Clinical Pathology Laboratory.
The Clauss fibrinogen assay is a measure of fibrinogen clotting activity. It is performed on a dilution of test plasma to reduce or eliminate interference by substances such as heparin and fibrin degradation products and is measured using clotting time following the addition of excess fibrin. The reference interval for fibrinogen as measured by the Clauss method at the time assayed was 1.9-3.5 g/L (Roshal, 2013).

VIDAS D-Dimer (bioMérieux) is a quantitative ELISA for D-Dimer determination designed for the VIDAS automated system. The two-step capture/tag test relies on two complementary monoclonal anti-D-Dimer antibodies, the second one being labelled with alkaline phosphatase. The normal range for adults (>16 yrs of age) is <500 ng/ml (Goldhaber et al., 1993).

### 2.3.2 Calibrated Automated Thrombogram

(Thrombinsoscope BV)

#### 2.3.2.1 Principle (Figure 16)

The Calibrated Automated Thrombogram assay required two fluorescent measurements in the same plasma. In one well (the measurement well) TF and synthetic phospholipids vesicles were added to plasma to initiate coagulation and induce thrombin formation. In a second well (the calibration well), a known amount of substrate-converting activity (the ‘thrombin calibrator’) was added to the plasma without activating coagulation. The thrombin calibrator consists of thrombin bound to α2-macroglobulin (α2M-thrombin), a form of thrombin that cannot be inhibited by plasma protease inhibitors. A mixture of CaCl2 and fluorogenic substrate was subsequently dispensed in both wells and the developing
fluorescence was recorded in real time by a fluorimeter. The thrombin generation curve was obtained by taking the first derivative fluorescence recorded in the measurement well after i) correction for inner filter effects and substrate consumption (based on the fluorescence measured in the calibration well) and ii) subtraction of the fluorescence signal deriving from α2M-thrombin (based on a mathematical algorithm). The thrombin generation curve can be described in terms of lag time, time to peak, peak height and area under the curve (endogenous thrombin potential, ETP). A typical thrombin generation curve is shown in Figure 16.
Figure 16 Calibrated Automated Thrombogram assay principle and parameters of the thrombin generation curve (Castoldi and Rosing, 2011)*.

TF - tissue factor, PL - phospholipids, RFU - raw fluorescence unit, ETP - endogenous thrombin potential.

* Reprinted with permission from Elsevier (License Number 5176680261905).

2.3.2.2 Reagents (Thrombinscope ™ Synapse BV, Maastricht, Netherlands)

Thrombin calibrator: Reconstituted with 1ml of DDD water without
vortex.

- Platelet-poor plasma reagent (PPPr) (Tissue Factor 5pM) is reconstituted with 1ml DDD water, shaken carefully.
- Fluca substrate and Fluca buffer: The Fluca substrate is stored at 4°C until opened. The Fluca substrate was incubated at 37°C for 2 minutes and vortexed. On the day of assay, 40μls of Fluca was added to the vial of buffer (1600 μls, diluted 40:1). The diluted reagent is stable for 1 hour at room temperature.
- Normal reference plasma: (Biophen Cat No 223201) lyophilised normal control plasma reconstituted with 1ml of distilled water.
- Nunc 96 well round bottom plates (Nunc. Cat no MPA-510-010V).

2.3.2.3 Assay Procedure

- Plasma samples, fluca buffer, and reference plasma were incubated at 37°C for 10-15 minutes prior to assay.
- 80 μls of plasma was pipetted in triplicate into wells. For each sample, an additional 80 μl plasma sample was added to an adjacent well (t-cal well) for thrombin calibration. The same procedure was repeated for the reference plasma.
- 20 μls of PPPr was added to the test samples only and not to the t-cal wells.
- Thrombin calibrator (20 μls) was then added to the t-cal well only.
- The dispenser was cleaned as prompted using DDD water. Upon completion of the cleaning cycle, the DDD water in the dispenser reservoir was replaced with Fluca.
- The plate was placed into a fluorimeter (Flouroscan, Thermofisher UK) with dedicated thrombinscope™
software, the dispenser was enabled.

- Thrombin generation was initiated by the automated dispensing of fluca (20 µls) to each well.
- The fluorescence signal generated as the reaction proceeded was recorded and using the dedicated software (Thrombinoscope™ Synapse BV, Maastricht, Netherlands) was converted into nM of thrombin generated.
- Peak thrombin, time to peak, lag time and endogenous thrombin potential (ETP) were all recorded for each patient.

2.3.3 Factor V (Zymutest Factor V, # RK009A, Hyphen BioMed, Neuville sur Oise, France)

2.3.3.1 Principle

ZYMUTEST Factor V is a sandwich enzyme-linked immunosorbent assay (ELISA) specific for human Factor V antigen. The diluted tested plasma or biological fluid is introduced into a microwell coated with a monoclonal antibody specific for human Factor V. When present, this protein is captured onto the solid phase. Following a washing step, the immunoconjugate, which is a horse polyclonal antibody coupled to horse-radish-peroxidase (HRP), is introduced, and binds to the free epitopes of immobilized Factor V. Following a new washing step, the peroxidase substrate, Tetramethylbenzidine (TMB) in presence of Hydrogen Peroxide (H₂O₂), is introduced and a blue colour develops. The colour turns yellow when the reaction is stopped with sulfuric acid and the absorbance is read on a spectrophotometer. The amount of colour developed is directly proportional to the concentration of human Factor V in the tested sample and can be calculated from the known amount of Factor V in the standard.
2.3.3.2 Reagents and Materials Required

- Micro ELISA plate, containing 12 strips of 8 wells, coated with a mouse monoclonal antibody specific for human Factor V.
- 50 mL of Factor V Sample Diluent.
- 3 vials of Plasma Factor V calibrator (normal human plasma calibrated with a reference plasma pool), lyophilised, prediluted. When reconstituted, with 2 mls of diluent, the calibrator contains a known amount of factor V diluted 1/50. The exact concentration is unique to each batch of reagents.
- Factor V Control I (high), human plasma lyophilized containing a high concentration of Factor V.
- Factor V Control II (low), human plasma lyophilized containing a low concentration of Factor V.
- 3 vials of Anti-(h)-Factor V-HRP immunoconjugate, a horse polyclonal antibody coupled to HRP, lyophilised.
- 25 mL of Factor V Conjugate Diluent, ready to use.
- 50 mL of 20 fold concentrated Wash Solution.
- 25 mL peroxidase substrate: 3,3',5,5' – Tetramethylbenzidine containing hydrogen peroxide.
- 6 mL of 0.45 M Sulfuric Acid (Stop Solution).

2.3.3.3 Reagents Preparation

- Plasma Factor V calibrator: each vial was restored with 2 mL of Factor V Sample Diluent in order to obtain the calibrator plasma, already diluted 50 fold.
- Plasma Factor V Control I (high): was restored with 0.5 mL
distilled water.
- Plasma Factor V Control II (low): was restored with 0.5 mL distilled water.
- Anti-(h)-Factor V-HRP immunoconjugate: each vial was restored with 7.5 mL of Factor V Conjugate Diluent. The pellet was left to completely dissolve before use and shaken gently in order to homogenize the content.
- Wash Solution: the vial was incubated for 15-30 minutes in a water bath at 37°C until the complete dissolution of solids. The vial was shaken, and the amount required was diluted to 1:20 in distilled water (the 50 mL contained in the vial allow preparing 1 litre of Wash Solution). It contained 0.05% Kathon CG.

2.3.3.4 Assay Procedure

2.3.3.4.1 Preparation of standards

Using the Plasma Factor V calibrator provided in the kit (2 mL of calibrator, already prediluted 1:50, and with a Factor V concentration "C" indicated on the flyer provided in the kit, for each lot of reagents), the standard solutions were prepared as indicated in Table 16. Samples were mixed gently for complete homogenisation.

Table 16 Standard solutions used in the calibration of plasma Factor V assay.
Vol=volume, C=concentration.

2.3.3.4.2 Preparation of samples

Each citrated sample and the control samples were diluted 1/100 with sample diluent (10 µls sample + 990 µls sample diluent).

200 µls of Plasma Factor V calibrator, controls, tested samples and sample diluent (blank) were added in duplicate to the microELISA plate. Following incubation for 2 hours at 37°C, each well was filled with 300 µls of Wash Solution and removed manually by inverting the plate to shake out the contents into the sink and the wells were sharply struck onto absorbent paper to remove any residual buffer. This was repeated 4 times, for a total of 5 washes. 200 µls of the Anti-(h)-Factor V-HRP immunoconjugate was then introduced in each well. Following further incubation for 2 hours at 37°C, 5 successive washings using 300 µls of wash solution were performed as described earlier. Immediately after the washing, 200 µls of TMB/H2O2 substrate was introduced into the wells. The substrate distribution was done accurately, row by row at exact time intervals. Samples were then incubated for exactly 5 minutes at room temperature (18-25°C). Colour development was stopped by introducing 50 µls of 0.45M sulfuric acid following exactly at the same time intervals as for the addition of substrate. The colour was allowed to stabilize for 10 minutes and absorbance was measured at 450 nm on BioTek® EL808 spectrophotometer.

2.3.3.5 Calculation of results

The duplicate readings for each standard, control, and sample were averaged and subtracted from the average zero standard optical density.
A standard curve was constructed by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis; a best-fit curve was drawn (Fig. 17). Factor V values for each sample value were interpolated from the curve.

**Figure 17 Factor V standard calibration curve.**

![Factor V standard calibration curve](image)

2.3.4 Factor VIII (Biophen FVIII:C, Hyphen BioMed, Neuville sur Oise, France)

2.3.4.1 Principle

BIOPHEN FVIII:Chr (6) kit is a chromogenic assay for measuring the Factor VIII:Chr activity in human plasma or in Factor VIII:Chr concentrates, using a chromogenic method. When activated by thrombin, Factor VIII:Chr forms an enzymatic complex with Factor IXa, phospholipids and Calcium, which activates Factor X to Factor Xa (Fig. 18).
Figure 18 Principle of the Factor VIII:Chr assay (Biophen FVIII:C, Hyphen BioMed, Neuville sur Oise, France).

BIOPHEN Factor VIII:Chr is a chromogenic assay for testing the cofactor activity of Factor VIII:Chr. In the presence of a constant amount of Factor IXa, Phospholipids (PLPs) and Calcium, thrombin activated Factor VIII:Chr forms an enzymatic complex, which activates Factor X, supplied in the assay at a constant concentration and in excess, to Factor Xa. This activity is directly related to the amount of Factor VIII:Chr, which is the limiting factor in presence of an excess amount of Factor IXa. Generated Factor Xa is then exactly measured by its activity on a specific Factor Xa chromogenic substrate (SXa-11). Factor Xa cleaves the substrate and releases pNA. The amount of pNA generated is directly proportional to the Factor Xa activity. Finally, there is a direct relationship between the amount of Factor VIII:Chr in the assayed sample and the Factor Xa activity generated, measured by the amount of pNA released, determined by colour development at 405 nm.

2.3.4.2 Reagents Required

- Human Factor X lyophilized in presence of a fibrin polymerization inhibitor.
- Factor IXa (human), at a constant and optimized concentration, contains human thrombin, calcium and synthetic phospholipids SXa-11.
- Chromogenic substrate, specific for Factor Xa (SXa-11), lyophilized. 2 vials containing 36 mg of SXa-11 with a thrombin inhibitor.
- Tris-BSA Buffer. Contains 1% BSA, PEG, FVIII:Chr Stabilizer and sodium azide (0.9 g/L).
- Acetic Acid (20%).
- Plasma Calibrator (BIOPHEN Plasma Calibrator Ref 222101).
- Normal or Abnormal Control Plasmas (BIOPHEN Normal Control Plasma Ref 223201, and BIOPHEN Abnormal Control Plasma Ref 223301).

2.3.4.3 Reagent preparation

- Human Factor X and fibrin polymerization inhibitor - each vial was reconstituted with exactly 6.0 mL of distilled water and shaken until complete dissolution of the content.
- Factor IXa, with thrombin, phospholipids and Calcium - each vial was reconstituted with exactly 6.0 mL of distilled water and shaken until complete dissolution of the content. Each vial was allowed to stand for 30 minutes at room temperature with gentle agitation.
- Factor Xa specific Chromogenic substrate (SXa-11): each vial was reconstituted with exactly 6.0 mL of distilled water and shaken thoroughly. Each vial was allowed to stand for 30 minutes at room temperature with gentle agitation Plasma calibrator and controls were reconstituted with 1ml of distilled water before use.
2.3.4.4 Assay Procedure

Preparation of standard curve

The standard curve was prepared using prepared plasma calibrator with a known amount of Factor VIII:Chr. Following reconstitution, the calibrator is diluted 1/20 (0.5 mls of calibrator + 9.5 mls of buffer). A series of dilutions is prepared as displayed in Table 17.

Table 17 Factor VIII:Chr assay (Standard dilutions).

<table>
<thead>
<tr>
<th>Factor VIII:Chr (%)</th>
<th>Calibrator plasma (µls)</th>
<th>Tris Buffer (µls)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>500</td>
</tr>
<tr>
<td>25</td>
<td>62.5</td>
<td>437.5</td>
</tr>
<tr>
<td>50</td>
<td>125</td>
<td>375</td>
</tr>
<tr>
<td>100</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>125</td>
<td>325</td>
<td>175</td>
</tr>
<tr>
<td>150</td>
<td>375</td>
<td>125</td>
</tr>
<tr>
<td>200</td>
<td>500</td>
<td>0</td>
</tr>
</tbody>
</table>

Each plasma and control sample is diluted 1:40 with Tris-BSA buffer. 50 µls diluted standards/controls and samples are added to the plate in triplicate and pre-incubated at 37°C for 5 mins.

- 50 µls of preincubated Factor X (Reagent 1) was introduced in each well followed by 50 µls of preincubated Factor IX (Reagent 2). The plate was agitated to ensure mixing of reagents and incubated for 5 minutes at 37°C.
- 50 µls of preincubated chromogenic substrate (Reagent 3) was added into each duplicate well (the triplicate well is left as a blank), agitated and incubated for exactly 5 minutes at 37°C.
The reaction was stopped by introducing 50 µls of 20% acetic acid into each well. The absorbance was measured at 405 nm against the sample blank using BioTek® EL808 spectrophotometer.

The duplicate optical density reading for each standard, control, and sample were averaged and subtracted from the blank value (triplicate well).

A standard curve was constructed by plotting the mean absorbance for each standard on the y-axis against the FVIII:Chr concentration on the x-axis. A typical standard curve is shown in Figure 19.

Factor VIII:Chr values for each sample values were interpolated from the curve.

Figure 19 Factor VIII:C typical standard curve.

2.3.5 Protein S (Zymutest Free Protein S, #RK015A-RUO, Hyphen BioMed, Neuville sur Oise, France)
2.3.5.1 Principle

The ZYMUTEST Total Protein S kit is a one step, two-site immunoassay, for measuring human Total Protein S in plasma.

First, the immunoconjugate, which is a monoclonal antibody specific for Free Protein S coupled to horseradish peroxidase (HRP), is introduced into the microwells coated with another monoclonal antibody specific for Free Protein S. Then, the diluted tested plasma or biological fluid is immediately introduced, and the immunological reaction starts. When present, the Free Protein S binds onto the monoclonal antibody-coated solid phase through one epitope, and fixes the second monoclonal antibody coupled to HRP by another epitope. Only Free Protein S is bound while Protein S-C4b-BP (C4b Binding protein) complexes are not reactive in the assay.

Following a washing step, the peroxidase substrate, 3,3′,5,5′ – TMB, in presence of hydrogen peroxide (H2O2), is introduced and a blue colour develops. When the reaction is stopped with sulfuric acid, a yellow colour is obtained. The amount of colour developed is directly proportional to the concentration of human Total Protein S in the tested sample.

2.3.5.2 Reagents required

- Micro ELISA plate, containing 12 strips of 8 wells, coated with a mouse monoclonal antibody specific for the two forms of human Protein S, then stabilized.
- 2 vials containing 50 ml of Protein S-Sample Diluent, ready to use (contains calcium).
- Plasma Protein S calibrator, (normal plasma calibrated with a reference plasma pool), lyophilised, prediluted 1:50. The exact Total Protein S concentration varies for each batch and
is indicated on the flyer provided in the kit.

- 0.5 ml of lyophilised Protein S Control I, (Plasma, high). Control plasma containing a known high Protein S concentration.
- 0.5 ml of lyophilised Protein S Control II, (Plasma, low). Control plasma containing a known low Protein S concentration.
- Anti-(h)-free-Protein S-HRP immunoconjugate, a mouse monoclonal antibody coupled to HRP, lyophilised.
- 15 ml of Protein S Conjugate Diluent.
- 50 ml of 20 fold concentrated Protein S Wash Solution.
- 25 ml of peroxidase substrate: 3,3',5,5' – TMB, containing hydrogen peroxide.
- 0.45M Sulfuric Acid.

**2.3.5.3 Reagent Preparation**

- Plasma Protein S calibrator: each vial was restored with 2 ml of Protein S-Sample Diluent, in order to obtain the calibrator plasma, containing the Total PS concentration “C%”, already diluted 50-fold.
- Protein S Control I (human plasma, high) was restored with 0.5 ml distilled water.
- Protein S Control II (human plasma, low) was restored with 0.5 ml distilled water.
- Anti-(h)-free-Protein S-HRP immunoconjugate: each vial was restored with 4 ml of Protein S Conjugate Diluent. The lyophilised powder was allowed to completely dissolve before use, and shaken gently in order to homogenize the content.
- Protein S Wash Solution: the vial was incubated for 15-30 minutes in a water bath at 37°C until complete dissolution of
solids occurred. The vial was shaken, and the amount required was diluted to 1:20 in distilled water (the 50 ml contained in the vial allowed preparing 1 litre of Wash Solution).

2.3.5.4 Assay Procedure

Preparation of the standard curve

Using the Protein S calibrator provided in the kit (2 ml of plasma calibrator already prediluted 1:50 and with a Total Protein S concentration "C" indicated, for each lot of reagents, on the flyer provided in the kit) the standard solutions were prepared as indicated in Table 18. Samples were mixed gently for complete homogenisation.

Table 18 Standard solutions used in the calibration of Plasma Protein S assay.

<table>
<thead>
<tr>
<th>Total Protein S concentration (%)</th>
<th>C</th>
<th>C/2</th>
<th>C/4</th>
<th>C/10</th>
<th>C/20</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vol. of Protein S calibrator</td>
<td>1 ml</td>
<td>0.5 ml</td>
<td>0.25 ml</td>
<td>0.1 ml</td>
<td>0.05 ml</td>
<td>0 ml</td>
</tr>
<tr>
<td>Vol. of Protein S-Sample Diluent</td>
<td>0 ml</td>
<td>0.5 ml</td>
<td>0.75 ml</td>
<td>0.9 ml</td>
<td>0.95 ml</td>
<td>1 ml</td>
</tr>
</tbody>
</table>

- Samples and controls were diluted 1/50 with protein S sample diluent 490 µls of sample diluent + 10 µls of Protein S calibrator.
- 100 µls of the Anti-(h)-free Protein S-HRP immunoconjugate was added to each well on the plate.
- 100 µls of the diluted standards, controls were added in duplicate to the ELISA plate.
- The plate was mixed gently and incubated for 1 hour at room
temperature.
- Each well was filled with 300 µls of Protein S Wash Solution and removed manually by inverting the plate to shake out the contents into the sink and the wells were sharply struck onto absorbent paper to remove any residual buffer. This was repeated 4 times, for a total of 5 washes.
- Immediately after the washing, 200 µls of TMB/H2O2 substrate was introduced into each well accurately and at exact time intervals. The plate was incubated for exactly 5 minutes at room temperature (18-25°C).
- Following exactly the same time intervals as for the addition of substrate, the colour development was stopped by introducing 50 µls of 0.45 M Sulfuric Acid into each well. After 10 minutes absorbance was measured at 450 nm on BioTek® EL808 spectrophotometer (Fig. 20).

Figure 20 Micro ELISA plate with Protein S calibrator, tested samples and Protein S Sample Diluent in BioTek® EL808.
2.3.5.5 Calculations

The duplicate reading for each standard, control, and sample were averaged and subtracted from the average zero standard optical density.

A standard curve was constructed by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis a best-fit curve was drawn (Fig. 21). Sample values were interpolated from the curve.

Figure 21 Free Protein S Standard Calibration Curve.

2.4 Patient experience survey

2.4.1 Patients

All patients undergoing a major surgery for confirmed or suspected gynaecological cancer in St. James’s Hospital between July 2017 and March 2018 and who were given extended LMWH prophylaxis were invited to participate.
Inclusion criteria:

- Postoperative patients who were receiving extended low molecular weight heparin prophylaxis following gynaecological cancer surgery in St. James’s University Hospital who gave full and informed consent.

Exclusion criteria:

- Patients receiving long-term therapeutic anticoagulation.
- Patients who had a recent diagnosis of venous thromboembolism.

All participants received weight-adjusted LMWH within 24 hours of surgery (4500 IU of Tinzaparin once daily for Body Mass Index < 40 kg/m\(^2\); and 75 IU/kg for patients with Body Mass Index >40 kg/m\(^2\)). Prior to discharge from the hospital, patients or their carers were trained in the administration of low molecular weight heparin.

Following informed and written consent, the participants received:

- a low molecular weight heparin prophylaxis logbook to record the injections and any observed side effects (Appendix I),
- a questionnaire to be completed at the end of the extended low molecular weight heparin prophylaxis (Appendix II),
- an information leaflet on signs and symptoms of venous thromboembolism (Appendix III).

The reason and rationale for extended LMWH prophylaxis based on evidence and international recommendations were explained to all patients again prior to discharge. The purpose of the study was explained thoroughly, and it was made clear that the study was designed to investigate compliance and patients’ experience with
extended LMWH prophylaxis and not the effectiveness of LMWH in the prevention of post-operative VTE. Patients were also educated on simple VTE preventive measures and early signs and symptoms of VTE.

2.4.2 Patient logbook methodology

The following data was recorded in the patients' logbook:

- date of LMWH administration,
- reason for missed injections,
- site of the administration,
- the person who administered the injection,
- pain score and side effects (pain score relating to LMWH injection was assessed using a Visual Analogue Scale (Appendix I)).

2.4.3 Patient Follow-up

Following 28 days of low molecular weight heparin prophylaxis (4 weeks), the patients were asked to complete the questionnaire in order to assess their knowledge and experience with the extended venous thromboembolism prophylaxis (Appendix II). All participants were followed up at 30 days post-surgery by a telephone interview and medical records were reviewed at 6 months to check whether any patient developed VTE within that period of time, and circumstances surrounding the event.

2.5 Statistical analysis

Descriptive statistics were used to describe patient demographics. Median and interquartile ranges were calculated for continuous variables. Categorical variables were expressed as counts and
percentages. Normal Probability Plot and Shapiro-Wilk Test were used to assess the distribution of data for normality. Data, which were not normally distributed, were log-transformed prior to analysis. All data was analyzed using the SPSS v.23 programme.

2.5.1 Chi-square test

Differences in categorical variables between the patients with and without recurrent VTE were assessed using the chi-square test. In all cases $p < 0.05$ was considered statistically significant.

2.5.2 Student’s t-test

The means between two groups of independent, continuous variables were compared using Student’s t-test in cases of parametric data. A paired Student t-test was used in cases where a group of non-independent parametric data was involved. In all cases $p < 0.05$ was considered statistically significant. The Bonferroni correction was applied when several dependent or independent statistical tests were being performed simultaneously.

2.5.3 Mann-Whitney U test

In cases of nonparametric data, the differences between two groups of independent continuous variables were assessed with Mann-Whitney U Test. In all cases $p < 0.05$ was considered statistically significant.

2.5.4 Kaplan-Meier method and log-rank test
Progression-free and overall survival were estimated with the Kaplan-Meier method and compared using log-rank test $p < 0.05$ was considered significant.

**2.5.5 Cox regression analysis**

The cumulative incidence of VTE recurrence was analysed using Cox regression analysis. In all cases $p < 0.05$ was considered statistically significant.
Chapter 3:
Recurrence of venous thromboembolism in patients with gynaecological malignancies: incidence, risk factors and impact on survival.
3.1 Introduction

Gynaecological cancer patients have a high-risk of VTE. As discussed in the Introduction, the risk is particularly high in ovarian cancer (Abu Saadeh et al., 2013A) and less pronounced in cervical and vulval cancers (Tsai et al., 2012; Ye et al., 2015). Treatment of gynaecological cancers includes complex surgery, chemotherapy and/or radiotherapy all of which further increase VTE risk. Although VTE prophylaxis with LMWH is effective at reducing the incidence of VTE postoperatively, VTE and recurrent VTE still occur in high-risk patients (Barber & Clarke-Pearson, 2017). Recently published clinical practice guidelines suggest that cancer-associated thrombosis should be treated with LMWH or DOAC (rivaroxaban or edoxaban in patients with low risk of bleeding) for a minimum of six months (Farge et al., 2019). Chee et al. reported an adjusted ten-year cumulative VTE recurrence rate of 28.6% in patients with active cancer (Chee et al., 2014). Survival is significantly reduced in cancer patients who experience VTE recurrence (Weber et al., 2010; Chee et al., 2014). In the recent systematic review and meta-analysis of RCTs Moik et al. demonstrated that DOACs significantly reduced recurrent VTEs compared to LMWHs (5.2% vs 8.2%), but were associated with a nonsignificant increase in major bleedings (4.3% vs 3.3%). Major bleeding was more frequent in patients with GI cancer treated with DOACs (RR, 2.30 [95% CI, 1.08-4.88]). Discontinuation of treatment was less common with DOACs (RR, 0.88 [95% CI, 0.81-0.96]) (Moik et al., 2020).

Although gynaecological cancers are associated with one of the highest rates of cancer-associated VTE, no studies have specifically investigated VTE recurrence and its effect in these women. In addition, the factors which influence VTE recurrence in
gyneaeological cancer patients have not been documented. In this study we performed a retrospective cohort study of patients with gynaecological malignancies who underwent surgical treatment in a tertiary gynaecological cancer centre between 2006 and 2017. Primary and recurrent cancer related VTE in the population were recorded (with a minimum follow-up period of one year). Demographic data, including histology, cancer stage, surgery, comorbidities and VTE treatment and prophylaxis were analyzed to determine their role in VTE recurrence. Hb, WCC (Neutrophils, Lymphocytes, Monocytes, Eosinophils, Basophils), platelets, urea, creatinine and albumin levels had been measured prior to the first VTE event, and prior to any cancer treatment. The Khorana score was calculated for all patients. Overall survival and progression-free survival were also recorded.

The aims of this study were:

- To investigate the rate of recurrence of VTE in the gynaecological cancer population,
- To investigate the factors that influence the recurrence of VTE,
- To investigate the impact of recurrent VTE on patient survival.

### 3.2 Results

#### 3.2.1 Clinicopathological details of all patients

One hundred and twenty-four gynaecological cancer patients diagnosed with gynaecological cancer-associated VTE met the
inclusion criteria (Fig. 22). The median (IQR) age of the patients was 60 (54-68) years. BMI was higher than 30 in 49 (40%) patients. Most women were post-menopausal (n=100, 81%) and non-smokers (n=103, 82%).

The tumour sites were ovary (n=62, 50%), corpus uteri (n=39, 31%), cervix (n=13, 11%), vulva (n=3, 2%), and more than one site (n=7, 6%). The histological subtypes were: high-grade serous (n=54, 43%), endometrioid (n=32, 26%), and squamous (n=13, 10%).

**Figure 22 Flow diagram of patients recruited into the study (n=124).**

The majority of the cancers were an advanced stage. Twenty-two patients had another primary malignancy more than six months
prior to their current gynaecological malignancy (breast n=9, skin n=4, colorectal n=2, lung n=2, cervical n=2, vulval n=1, gallbladder n=1, adrenal n=1). Two patients had synchronous primary malignancies (one patient had high-grade serous endometrial cancer and clear cell renal cancer; another had endometrioid endometrial cancer with co-existent neuroendocrine appendiceal tumour).

Open surgery was performed in 96 (77%) patients. The complexity of surgeries as assessed by a modification of the scale developed by Aletti et al. (see Table 13 in Methods) (Aletti et al., 2007) was: low (n=24, 21%), intermediate (n=80, 71%) and high (n=9, 8%). Chemotherapy and radiotherapy were administered in eighty-five (69%) and forty-seven (38%) patients respectively. Seventy-six patients (63%) experienced recurrence or progression of cancer.

The median (IQR) duration of surgical hospital stay was 10 (8-18) days, and the median (IQR) Charlson Comorbidity Index was 8 (6-10).

3.2.2 Incidence and risk factors of recurrent VTE

Twenty-seven (22%) patients had VTE recurrence in our cohort. Age, BMI, smoking, menopausal status, presence of prior malignancy, cancer characteristics (site, histology, stage, recurrence/progression), duration of hospital stay, and Charlson Co-morbidity Index were similar in both non-recurrent and recurrent VTE groups. Although there was no significant difference between the recurrent and non-recurrent groups with respect to the surgical approach (open vs. laparoscopic), the complexity of surgeries was significantly higher in patients with recurrent VTE (p=0.027).
3.2.2.1 Characteristics of the first VTE

One hundred and twenty-four first VTEs are described in Table 19. The median follow-up time was 25 (range 1–120) months. First VTE events were PE (n=46, 37%), DVT (n=58, 47%), PE+DVT (n=14, 11%), and other (n=6, 5%). In patients with first PE+DVT, 43% (n=6) experienced recurrent VTE.

Anticoagulants were prescribed for the first VTE for a median (IQR) time of six months (IQR 3-8).

Ninety-three (75%) patients were diagnosed with first VTE while ambulatory and not on any form of anticoagulation. In patients receiving prophylaxis at the time of first VTE, approximately 19% (n=6) had a recurrent VTE. The first VTE in the majority of patients was treated with LMWH and 19% had a recurrence. Eleven were treated with oral VKA which was associated with a higher rate of recurrence (36%) however this did not reach statistical significance.
### Table 19 Characteristics of the first VTE.

<table>
<thead>
<tr>
<th>Variable</th>
<th>All patients, N=124</th>
<th>Recurrent VTE, N=27</th>
<th>No recurrent VTE, N=97</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>First VTE site (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.125</td>
</tr>
<tr>
<td>PE</td>
<td>46 (37%)</td>
<td>11 (41%)</td>
<td>35 (36%)</td>
<td></td>
</tr>
<tr>
<td>DVT</td>
<td>58 (47%)</td>
<td>10 (37%)</td>
<td>48 (50%)</td>
<td></td>
</tr>
<tr>
<td>PE+DVT</td>
<td>14 (11%)</td>
<td>6 (22%)</td>
<td>8 (8%)</td>
<td></td>
</tr>
<tr>
<td>Other*</td>
<td>6 (5%)</td>
<td>0</td>
<td>6 (6%)</td>
<td></td>
</tr>
<tr>
<td>Inpatient at the time of first VTE (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.900</td>
</tr>
<tr>
<td>Yes</td>
<td>31 (25%)</td>
<td>7 (26%)</td>
<td>24 (25%)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>93 (75%)</td>
<td>20 (74%)</td>
<td>73 (75%)</td>
<td></td>
</tr>
<tr>
<td>Prophylactic anticoagulation at the time of index VTE event (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.706</td>
</tr>
<tr>
<td>Yes</td>
<td>31 (25%)</td>
<td>6 (22%)</td>
<td>25 (26%)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>93 (75%)</td>
<td>21 (78%)</td>
<td>72 (74%)</td>
<td></td>
</tr>
<tr>
<td>Treatment of the index VTE</td>
<td></td>
<td></td>
<td></td>
<td>0.175</td>
</tr>
<tr>
<td>LMWH</td>
<td>99 (82%)</td>
<td>19 (70%)</td>
<td>80 (85%)</td>
<td></td>
</tr>
<tr>
<td>Oral VKA</td>
<td>11 (9%)</td>
<td>4 (15%)</td>
<td>7 (7%)</td>
<td></td>
</tr>
<tr>
<td>IVC filter+LMWH</td>
<td>9 (7%)</td>
<td>3 (11%)</td>
<td>6 (6%)</td>
<td></td>
</tr>
<tr>
<td>IVC filter+oral VKA</td>
<td>1 (1%)</td>
<td>1 (4%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>DOAC</td>
<td>1 (1%)</td>
<td>0</td>
<td>1 (1%)</td>
<td></td>
</tr>
</tbody>
</table>

*Other (n=6): catheter related thrombosis (n=5), inferior vena cava thrombosis (n=1).

#### 3.2.2.2 Timing of the first VTE

Thirty-three (27%) patients were diagnosed with the first VTE prior to any form of cancer treatment (surgery, chemotherapy or radiotherapy) (Fig. 23).
Within this group, 18 (15%) patients presented with symptomatic VTE and occult gynaecological malignancy, nine (7%) had an incidental finding of VTE at cancer diagnosis, and six (5%) developed VTE in the interval between cancer diagnosis and treatment.

Thirty-eight (31%) patients developed VTE within six weeks of surgery; 21 while in hospital and 22 on VTE prophylaxis prior to the initiation of chemotherapy or radiotherapy. Twenty-six (21%) patients developed VTE during chemotherapy or radiotherapy, six (5%) patients developed VTE following completion of cancer treatment, and 21 (17%) at the time of cancer recurrence or progression. In 15 (12%) patients the first VTE was the herald sign of cancer recurrence.
3.2.2.3 Bleeding complications

There was one bleeding associated mortality in our cohort of patients; a 73-year-old-woman with high-grade serous ovarian cancer and above knee DVT treated with LMWH. During her second cycle of neo-adjuvant chemotherapy (single-agent carboplatin) she had a fatal upper gastro-intestinal bleed.

3.2.3 Characteristics of recurrent VTE

Recurrent VTE were: PE (n=12, 10%), DVT (n=12, 10%), PE and DVT (n=2, 2%) and central venous catheter related VTE (n=1). Eleven (24%) patients with the first PE experienced recurrent VTE, as PE (six), DVT (four), and catheter related VTE (one). Ten (17%) patients with the first DVT experienced recurrent VTE, as PE (three), DVT (six), PE, and DVT (one). Six (43%) patients with first PE and DVT had recurrent VTE, as PE (three), DVT (two), PE, and DVT (one). Patients with the first catheter-related VTE or vena cava VTE did not experience recurrence of VTE.

The tumour sites in patients with recurrent VTE were ovary (n=14, 52%), corpus uteri (n=7, 26%), cervix (n=3, 11%), vulva (n=1, 4%), and more than one site (n=2, 7%).

The cumulative incidence of recurrent VTE was 21% (95% CI, 12.6%-28.5%) after 1 year, 22% (95% CI, 13.6%-30%) after 3 years, 26% (95% CI, 16.2%-35.6%) after 5 years, and 38% (95% CI, 17.8%-54.4%) after 10 years of follow-up (Fig. 24)

The median time to VTE recurrence was four (range: 1–90) months following diagnosis of their first VTE. Fourteen 14/27 patients had recurrent VTE within three months of their first event, 18/27 within six months, and 22/27 within one year following their first VTE.
Recurrent VTE events occurred within seven days after surgery in five (18%) patients, despite LMWH treatment (Fig. 25). Recurrent VTE occurred during chemotherapy or radiotherapy (n=8, 30%), at the time of cancer recurrence or progression (n=8, 30%), following completion of cancer treatment (n=3, 11%) and prior to cancer treatment (n=3, 11%). In two of these cases, recurrent VTE was the herald sign of cancer; a third patient developed recurrent VTE between cancer diagnosis and treatment.
Figure 25 Distribution of recurrent VTE events (n=27) according to the stage of treatment*.

*N Legend: percentages refer to the total number of recurrent VTE events.

Nineteen (70%) patients with a recurrent VTE event were diagnosed in the community setting, 15 were symptomatic, and four were asymptomatic for VTE. Twenty-one (78%) patients were receiving anticoagulation when recurrent VTE occurred. Seventeen patients (63%) were on a full therapeutic dose of LMWH, one patient was on oral VKA, and three were on prophylactic LMWH. Ten patients had inferior vena cava (IVC) filter inserted following their first VTE and left in situ (n=4) or removed (n=6). Four (40%) of these patients experienced recurrent VTE, three of whom developed recurrent VTE (all DVT) with IVC filter still in situ. Two of these patients had additional VTE risk factors at the time of recurrent VTE (either progression of disease, or sepsis and interruption in anticoagulation treatment).

Four patients (15%) had a third VTE within 12 months of the second VTE (PE, DVT, IVC thrombosis, cerebral venous sinus thrombosis).
Twelve (36%) of 33 patients who experienced first VTE before their primary treatment for gynaecological cancer experienced VTE recurrence (Fig. 26). Patients who experienced a first VTE pre-treatment were more likely to have a recurrence than those who had a first VTE post-surgery or chemotherapy (OR 2.2, 95% CI 1.1-4.2).

**Figure 26** Recurrent VTE (n=27, red) in relation to the timing of the first VTE (n=124, blue)*.

*Legend: percentages refer to the total number of recurrent VTE events in the group of patients with first VTE at different stages of cancer treatment (for instance: 12 (36%) patients out of 33 who experienced first VTE prior to cancer treatment experienced recurrent VTE later on at any stage of their cancer treatment journey).

### 3.2.4 Khorana score for prediction of recurrent VTE

Data for calculation of the Khorana score was available for 112 (90%) patients (Khorana et al., 2008). The Khorana score was
calculated to predict the first VTE. The results were as follows: score 1 (n=48, 43%), score 2 (n=32, 29%), score 3 (n=23, 20%), score 4 (n=6, 5%), and score 5 (n=3, 3%). 28% of patients who scored 3 or more suffered a recurrent VTE compared with 17% of patients who scored 1, there was no statistical difference between the groups. The cumulative incidence of recurrent VTE following the index VTE according to the Khorana score is demonstrated in Figure 27. Patients who scored 3 or more had a greater cumulative incidence of recurrent VTE compared with those who scored 2 or less but this was not significant (HR = 2.2, 95% CI 0.84-5.84).

**Figure 27 Cumulative incidence of recurrent VTE (n=25) in the first 5 years following index VTE according to the Khorana score.**
3.2.5 Laboratory biomarkers in patients with recurrent and non-recurrent VTE

Ninety-six patients had had venous blood samples obtained before their first VTE and before the commencement of any cancer treatment (including 79 patients in the non-recurrent VTE group and 17 patients in the recurrent VTE group) (Table 20).

Patients with recurrent VTE had significantly higher pre-cancer treatment monocyte count ($p = 0.047$) compared to the non-recurrent group (Table 20). However, the difference did not hold the Bonferroni correction ($p=0.0045 –$ Bonferroni-corrected p-value – a threshold that needs to be reached for a single test to be classified as significant). There was no difference in other routinely tested pre cancer treatment blood parameters.
Table 20 Baseline laboratory biomarkers in patients with gynaecological cancers with recurrent and non-recurrent VTE who had venous blood samples obtained before their first VTE and before the commencement of any cancer treatment.

<table>
<thead>
<tr>
<th>Variable, median (IQR)</th>
<th>Normal values</th>
<th>All patients</th>
<th>Recurrent VTE</th>
<th>Non-recurrent VTE</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WCC</td>
<td>4.0-11.0 (10^9/L)</td>
<td>7.7 (6.2-9) n=96</td>
<td>8.3 (6.8-10.8) n=17</td>
<td>7.7 (6.1-8.9) n=79</td>
<td>0.21</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>2.0-7.5 (10^9/L)</td>
<td>5.1 (3.9-6.8) n=96</td>
<td>5.4 (4.5-7.3) n=17</td>
<td>4.8 (3.6-6.8) n=79</td>
<td>0.215</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>1.5-3.5 (10^9/L)</td>
<td>1.6 (1.1-2.1) n=96</td>
<td>1.5 (1.1-2.2) n=17</td>
<td>1.6 (1.1-2.1) n=79</td>
<td>0.916</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0.2-0.8 (10^9/L)</td>
<td>0.6 (0.5-0.7) n=96</td>
<td>0.7 (0.5-0.9) n=17</td>
<td>0.6 (0.5-0.7) n=79</td>
<td>0.047</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>0-0.4 (10^9/L)</td>
<td>0.1 (0.1-0.2) n=96</td>
<td>0.2 (0.1-0.3) n=17</td>
<td>0.1 (0.1-0.2) n=79</td>
<td>0.101</td>
</tr>
<tr>
<td>Basophils</td>
<td>0-0.1 (10^9/L)</td>
<td>0 n=96</td>
<td>0 n=17</td>
<td>0 n=79</td>
<td>0.723</td>
</tr>
<tr>
<td>Hb</td>
<td>11.5-16.4 (g/dL)</td>
<td>12.3 (11.1-13.5) n=96</td>
<td>12.3 (11-13.3) n=17</td>
<td>12.2 (11.1-13.7) n=79</td>
<td>0.859</td>
</tr>
<tr>
<td>PLT</td>
<td>140-450 (10^9/L)</td>
<td>330 (264-423) n=96</td>
<td>395 (269-475) n=17</td>
<td>326 (264-411) n=79</td>
<td>0.393</td>
</tr>
<tr>
<td>Urea</td>
<td>3.0-7.0 (mmol/L)</td>
<td>5.1 (3.9-6.4) n=95</td>
<td>4.5 (3.5-5.6) n=17</td>
<td>5.1 (3.9-6.4) n=78</td>
<td>0.214</td>
</tr>
<tr>
<td>Creatinine</td>
<td>44-80 (µmol/L)</td>
<td>69 (60-80) n=95</td>
<td>60 (53-78) n=17</td>
<td>70.5 (62-81.2) n=78</td>
<td>0.117</td>
</tr>
<tr>
<td>Albumin</td>
<td>35-50 (g/L)</td>
<td>41 (37-44) n=95</td>
<td>41 (36.5-43) n=17</td>
<td>40.5 (36.7-44.2) n=78</td>
<td>0.563</td>
</tr>
</tbody>
</table>
3.2.6 Survival analysis

Sixty-three (51%) patients in the study died between January 2006 and September 2017. Seventeen patients in the recurrent VTE group (63%) and forty-six in the non-recurrent VTE group (47%) \((p=0.153)\). VTE was not the labelled direct cause of death in any patient within this cohort. One patient died due to severe upper gastro-intestinal bleeding while on a therapeutic dose of LMWH. The median five-year progression-free survival was 25 months for recurrent and 30 months for the non-recurrent VTE group (Fig. 28).

**Figure 28** Kaplan-Meier cancer progression free survival curve for women with recurrent VTE versus women without recurrent VTE.
The median five-year overall survival was 40 months for those with recurrent VTE and 60 months for those without recurrent VTE; this difference did not reach statistical significance (Fig. 29).

Figure 29 Kaplan-Meier overall survival curve for women with recurrent VTE versus women without recurrent VTE.

3.3 Discussion

Venous thromboembolism is a common complication in cancer patients and has a high impact on morbidity and mortality (Khorana, 2010). Understanding the risk factors associated with VTE recurrence is important and can influence decisions regarding the dose and duration of anticoagulation.

In this study the incidence of recurrent VTE in patients with genital tract malignancies was 22%. This is similar to findings from previous
studies on recurrent VTE in mixed cancer populations. Prandoni et al. found that the 12-month cumulative incidence of recurrent venous thromboembolism in cancer patients was 20.7% (95% CI, 15.6%-25.8%) vs. 6.8% (95% CI, 3.9%-9.7%) in patients without cancer (Prandoni et al., 2002). A similar VTE recurrence rate (19.4%) was reported in patients with leukaemia (Luong et al., 2017). In a recent study, the overall incidence rate for recurrent VTE in mixed cancers was 9.6 per 100 person-years (Cohen et al., 2017). The peak at 22.1 per 100 person-years was noted in the first six months, with a reduction to 7.9 between six and 12 months. Similarly, Martinez et al. (2014) showed that the incidence rate for recurrent VTE peaked in the first six months at 11 per 100 person years (Martinez et al., 2014). The present findings are in agreement with both of these studies. Most recurrent VTE events in this cohort occurred within six months of first VTE (67%) and the majority within 12 (81%) months. These findings confirm that the highest risk of VTE recurrence is early in the cancer treatment pathway and support the recommendation that anticoagulation should be continued for at least six months. The antithrombotic benefit of LMWH must be balanced against the risk of bleeding in cancer patients. The 12-month cumulative incidence of major bleeding was 12.4% (95% CI, 6.5%-18.2%) in patients with cancer compared to 4.9% (95% CI, 2.5%-7.4%) in patients without cancer in previous studies (Prandoni et al., 2002). In the present cohort one bleeding associated mortality was recorded.

It is however disconcerting that the majority of patients were receiving anticoagulation at the time of their recurrent VTE event (63% therapeutic and 11% prophylactic dose of LMWH). This has been reported by previous researchers. In Schulman et al.’s cohort of 212 patients with recurrent VTE, 70% were on LMWH and 27% on a VKA at the time of the recurrent VTE event (Schulman et al., 2015). This raises questions regarding the efficacy, dosage, and compliance with current standard anticoagulation regimes.
Surgery and chemotherapy are known to exacerbate the risk of VTE in cancer patients, however, in the present study, the highest incidence of VTE recurrence occurred in patients who had suffered their first VTE before primary treatment for cancer. In addition, patients who suffered recurrent VTE had more complex surgery than those who did not. These findings are unlikely to be independent of the biology of the tumours. The more thrombogenic tumours would likely have more systemic impact and associated metastases. This reflects the highly thrombogenic nature of the primary tumour. Cancer cells interact with the haemostatic system through several pathways (Falanga et al., 2017). These include expression of haemostatic factors [TF, cancer procoagulant], microparticles, inflammatory cytokines, and proangiogenic factor production. Individuals who experience VTE prior to any cancer treatment may be more susceptible to the thrombotic effect of the malignancy and therefore remain at high-risk of further VTE. Previous studies conducted in our institution showed that expression of TF in clear cell and endometrioid type ovarian cancer is significantly higher in patients who develop VTE (Abu Saadeh et al., 2013B). Further work is required to determine whether tumour derived TF plays a role in the risk of recurrent VTE in cancer patients.

We did not find any differences in age, BMI, presence of secondary malignancy, smoking, menopausal status, type of surgical approach, Charlson Comorbidity Index or tumour site – between the recurrent VTE group and non-recurrent VTE group. However, the surgical complexity was higher in the patients who experienced recurrent VTE. Open surgery is known to be associated with a higher risk of post-operative VTE compared to MIS (Barber et al., 2016). Patients with gynaecological malignancies (ovarian cancer in particular) often require lymph node dissection, bowel surgeries and multivisceral resection resulting in prolonged operative time (Aletti et al., 2006). The duration of the surgery, which is often a marker of surgical complexity has been found to increase the risk.
of postoperative VTE (Kim et al., 2015; Qiu et al., 2018; Zhang et al., 2018). In patients with ovarian cancer, surgery time greater than 150 min was an independent risk factor for post-operative VTE in a study by Zhang et al. (2018). Patients undergoing longer and complex surgical procedures are more likely to experience blood stasis, vascular trauma with endothelial damage and inflammation (Turpie et al., 2002). All these factors lead to exposure of vascular endothelial cells, factor III (thromboplastin) leak, the release of cytokines and activation of coagulation process as described in Chapter 1.2.2.

Charlson Comorbidity Index was higher among our study population patients compared to the similar population of patients without VTE (Di Donato et al., 2019; Asthana&Modi, 2020). However, there was no difference regarding Charlson Comorbidity Index between patients with single and recurrent VTE. Although endometrial cancer is the most common gynaecological cancer in the general population (Siegel et al., 2015), ovarian cancer is associated with the highest rate of VTE (Rodriguez et al., 2007; Duska et al., 2010). In line with this evidence, patients with ovarian cancer accounted for a majority of patients with single and recurrent VTE in our study. High BMI, and comorbidities are all risk factors for VTE, however these did not appear to play a role in VTE recurrence in this study. In agreement with this, Chee et al. found that only cancer type (ovarian, pancreatic, brain, lung), myeloproliferative or myelodysplastic disorders, stage 4 disease, and leg paresis were predictive of recurrence of VTE in a cancer population (Chee et al., 2014). The adjusted ten-year cumulative VTE recurrence rate in active cancer in their population was 28.6%. Lack of long-term anticoagulation and the presence of a second primary malignancy were significant contributors to VTE recurrence in patients with glioblastoma multiforme (Edwin et al., 2016). These risk factors were non-contributory in the present study of gynaecological related VTE recurrence.

The Khorana risk score is a prediction model used to assess
cancer-associated thrombosis before initiation of chemotherapy (Khorana et al., 2008). Although it is not designed to predict recurrence of VTE or VTE in surgical patients, we evaluated it to classify the patients into low (1), intermediate (2), and high (≥ 3) risk groups. There was no difference between the groups with regards to the VTE recurrence. However, there was a trend towards higher cumulative VTE recurrence with Khorana scores of 3-5, but our numbers of recurrent VTE patients were small and larger numbers would be needed to confirm this. In addition, as mentioned earlier, the Khorana score was not designed for patients in the immediate post-surgical phase. Therefore it does not take into account other risk factors specific to the surgical patients (such as the extent and duration of surgery, blood loss, immobilization, and the length of hospital stay). These factors can limit its use and lower its accuracy in cancer patients undergoing major surgery.

The site of a recurrent VTE in the non-cancer population usually follows the site of the first event (Baglin et al., 2010). A patient-level meta-analysis showed that in patients with first proximal DVT, the 5-year DVT recurrence rate was 26.4%, and recurrence with PE was 3.6% (Baglin et al., 2010). In the same study the risk of recurrence as PE was 3.1-fold greater in patients presenting with symptomatic PE than in patients with proximal DVT. Cancer patients with the first DVT experience recurrent VTE as DVT in 64% (Schulman et al, 2015). Similarly in our study, 60% of recurrent VTE events were DVT in patients with the first DVT and 55% of recurrent events were PE in patients with first PE. Additionally, the site of the first VTE has been shown to impact the risk of VTE recurrence in a non-cancer population (Prandoni et al., 2007; Kovacs et al., 2010). Patients with unprovoked combined first DVT + PE were 2.3 times more likely to experience VTE recurrence than patients with a first episode of unprovoked isolated PE (17.7% vs. 7.7%) in a study by Kovacs et al. (Kovacs et al., 2010). The analysis of the RIETE Registry showed that the presence of co-
existent DVT in patients with the first episode of acute PE was an independent predictor of death within 3 months following VTE diagnosis (Jiménez et al., 2010). In mixed cancer patients, the VTE recurrence rate was similar for patients with the first episode of DVT (8.8%) and PE (10.5%) (Cohen et al., 2017). The author did not comment on the rate of VTE recurrence in patients with combined first DVT + PE and this information was also not recorded in other studies on recurrent VTE in cancer patients (Prandoni et al., 2002; Chee et al., 2014). Although the site of the first VTE did not influence the risk of recurrence in our study, 43% of patients with first PE+DVT experienced recurrent VTE compared to 24% of patients with first PE and 17% with the first DVT. There was no VTE recurrence in patients with first central venous catheter-related VTE or vena cava VTE in our cohort, which is consistent with published literature (Tran et al., 2010).

IVC filters have been shown to reduce the risk of PE but increase the risk of DVT with no effect on survival (PREPIC Study Group, 2005). In the Worcester population-based VTE observational study there was no statistically significant difference in the rate of PE but the incidence of recurrent DVT was 21% for patients with an IVC filter and 14.9% for patients treated without a filter (Spencer et al., 2010). In our cohort three patients out of four who had an IVC filter left in situ experienced recurrent VTE, all in form of DVT. However other risk factors present in these patients (progression of the disease, sepsis, and interruption in anticoagulation treatment) might have also contributed to the development of their recurrent VTE.

Monocytes count obtained prior to the first VTE event and any cancer treatment was numerically higher in the recurrent VTE group but the values remained within the normal clinical range. Monocytes are known to express tissue factor and to significantly contribute to procoagulant activity (Niemetz et al., 1974; Lorenzet et al., 1983; Semeraro et al., 1983). Vieira et al. showed that the
levels of monocyte bound TF are significantly elevated in patients with VTE and suggested their use as a marker for DVT detection (Vieira et al., 2007). Monocytosis defined as monocyte counts greater than 930 per microliter (≥ 0.93 x 10⁹/L) has been associated with the risk of DVT in the general population (Maldonado-Peña et al., 2016). The role of the monocyte in the initiation and propagation of thrombosis has been demonstrated in mice in vivo (von Brühl et al., 2012). Together with neutrophils they were the predominant leukocyte subsets that actively accumulated at the vessel lining during thrombus formation in veins. A trend towards increased monocyte count in patients with recurrent VTE is an interesting finding but requires further study as their values fell within the normal clinical range. In addition, when accounting for the Bonferroni correction the difference became non-significant, suggesting spurious positive results. We should also note that the laboratory markers were measured prior to the first VTE event. Therefore any difference in these markers would reflect the circumstances at the time of the first VTE rather than the VTE recurrence. This should be regarded as one of the limitations of this finding.

Although the progression and overall survival were shorter in our cohort of patients with recurrent VTE as compared to those with a single VTE event, the difference did not reach statistical significance (small numbers of recurrent VTE resulted in our study being underpowered). Despite the fact that multiple other studies on larger mixed cancer populations demonstrated the adverse effect of recurrent VTE on survival (Weber et al., 2010; Chee et al., 2014), this association may be more complex and mirror the aggressiveness of the tumours. Activation of coagulation cascade by the malignant tumour growth creates a prothrombotic environment that manifests not only as thrombosis but also has a profound effect on tumour cell behaviour including invasion, metastasis, and angiogenesis (Rak et al., 2006) that impact on cancer survival.
The main weakness of this study is that it is not adequately powered to identify significant associations and risk factors. Such a study would require a national and potentially even an international collaborative effort given a relatively small population size in Ireland. Additionally, in our statistical analysis, we did account for death as a competing risk. The occurrence of death in patients with CAT may prevent the recurrent VTE event from being observed. This could have introduced bias and altered the true incidence of recurrent VTE rate as well as its effect on survival. However, as shown by Parpia et al., when the distribution of competing risks is similar within each group of interest (single VTE and recurrent VTE group in our study), the standard and competing risk methods yield comparable results (Parpia et al., 2011). Thus it is unlikely that our results would have been significantly different from those demonstrated. In conclusion, the current study found that the incidence of recurrent VTE in patients with genital tract malignancies was 22%. There were no significant differences between recurrent and non-recurrent VTE groups in terms of identifiable risk factors, demographics, and survival, but this may be due to the retrospective nature of the study and the low number of events. In addition, the routine post-mortem examination on patients with cancer at the study site is not performed and hence the rate of recurrent VTE may be underestimated. Patients who experienced their first VTE prior to any form of cancer treatment were more likely to experience recurrent VTE. Patients with gynaecological cancer treated for VTE remain at high-risk of recurrent venous thrombosis despite standard anticoagulation treatment. We need to improve the prediction of and prophylaxis for recurrent VTE in gynaecological cancer care.
Chapter 4:
Coagulation biomarkers as predictors of venous thromboembolism recurrence in gynaecological cancer
4.1 Introduction

Although several studies have reported the role of coagulation biomarkers in cancer-associated thrombosis, few have investigated the role of these biomarkers in VTE recurrence. In the non-cancer population coagulation biomarkers have been reported as useful predictors of VTE recurrence. Elevated levels of D-Dimers, FVIII and high ETP and peak thrombin level one month after completion of anticoagulation following the first VTE event were found to be predictive of VTE recurrence following first idiopathic VTE (Cristina et al., 2004; Tripodi et al., 2008). Similar findings were reported in a study of mixed cancer types (Piatek et al., 2016). Resumption of anticoagulation is considered for those with high D-Dimers to reduce the risk of recurrent VTE. A robust biomarker at baseline (prior to first VTE and any cancer treatment) that would predict a higher risk of recurrence would avoid the risk of disruption of anticoagulation.

In gynaecological cancers, pretreatment ETP and D-Dimer levels are predictive of first VTE following gynaecological cancer surgery (Ward et al., 2018; Norris et al., 2020). However, the role of coagulation biomarkers in predicting VTE recurrence in gynaecological cancer patients is not known. In this prospective cohort study, patients with single and recurrent CAT who were treated for gynaecological malignancies in St. James’s University Hospital between 2011 and 2017 were identified from the TCD Gynaecological cancer bioresource (see methods section 2.2.3). Venous blood samples were obtained 24-48 hours prior to cancer surgery in a cohort of patients who had not received prior anticoagulants. Thrombin generation was measured using a fluorogenic assay following stimulation with 5pm TF. Lag time, peak thrombin and area under the thrombin generation curve (ETP) were determined and reported for each sample. The levels of Factor V
(FV), and free protein S antigen were measured by ELISA and factor VIIIc was measured using a chromogenic assay. D-Dimer and fibrinogen levels were measured in the hospital laboratory using standard techniques.

The aims of this study were:

- To compare thrombin generation, FV, FVIII, free protein S, fibrinogen and D-Dimer levels before surgery and their first VTE event in gynaecological cancer patients who subsequently developed recurrent VTE compared with those who had a single VTE event.

**4.1.1 Clinicopathological details of patients**

Sixty-nine patients had venous blood samples available from the biobank that had been obtained prior to surgery and their first VTE event (Fig. 22, Table 21). Fibrinogen levels were available for 51 patients and D-Dimer levels were available for 50 patients. Valid ETP data were obtained for 30 samples. Factor V and free Protein S was assessed in 25 patients. Factor VIIIc levels were available in 23 patients. The median (IQR) age of patients was 63 (54-70) years. Seventeen patients (25%) experienced recurrent VTE.
Table 21 Demographics and baseline characteristics of patients with gynaecological cancers with recurrent and non-recurrent VTE who had venous blood samples obtained before first VTE and prior to commencement of cancer treatment (n=69).

<table>
<thead>
<tr>
<th>Variable</th>
<th>All patients</th>
<th>Recurrent VTE</th>
<th>Non-recurrent VTE</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients (%)</td>
<td>69</td>
<td>17 (25%)</td>
<td>52 (75%)</td>
<td></td>
</tr>
<tr>
<td>Fibrinogen D-Dimer</td>
<td>51</td>
<td>12 (24%)</td>
<td>39 (76%)</td>
<td></td>
</tr>
<tr>
<td>ETP</td>
<td>50</td>
<td>12 (24%)</td>
<td>38 (76%)</td>
<td></td>
</tr>
<tr>
<td>FV, FVIII:Chr and Protein S</td>
<td>30</td>
<td>7 (23%)</td>
<td>23 (77%)</td>
<td></td>
</tr>
<tr>
<td>Age, median (IQR)</td>
<td>63 (54-70)</td>
<td>62 (54-66)</td>
<td>63 (56-71)</td>
<td>0.349</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td></td>
<td></td>
<td></td>
<td>0.561</td>
</tr>
<tr>
<td>&lt;30</td>
<td>34 (60%)</td>
<td>8 (53%)</td>
<td>26 (62%)</td>
<td></td>
</tr>
<tr>
<td>&gt;30</td>
<td>23 (40%)</td>
<td>7 (47%)</td>
<td>16 (38%)</td>
<td></td>
</tr>
<tr>
<td>Cancer Site (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.665</td>
</tr>
<tr>
<td>Ovary</td>
<td>34 (52%)</td>
<td>10 (62%)</td>
<td>24 (40%)</td>
<td></td>
</tr>
<tr>
<td>Corpus uteri</td>
<td>19 (29%)</td>
<td>3 (19%)</td>
<td>16 (33%)</td>
<td></td>
</tr>
<tr>
<td>Cervix</td>
<td>6 (9%)</td>
<td>1 (6%)</td>
<td>5 (10%)</td>
<td></td>
</tr>
<tr>
<td>Vulva</td>
<td>1 (1%)</td>
<td>0</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>More than one site*</td>
<td>5 (8%)</td>
<td>2 (12%)</td>
<td>3 (6%)</td>
<td></td>
</tr>
<tr>
<td>Histology (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.380</td>
</tr>
<tr>
<td>High Grade Serous</td>
<td>27 (41%)</td>
<td>8 (50%)</td>
<td>19 (39%)</td>
<td></td>
</tr>
<tr>
<td>Clear Cell</td>
<td>6 (9%)</td>
<td>2 (12%)</td>
<td>4 (8%)</td>
<td></td>
</tr>
<tr>
<td>Endometrioid</td>
<td>18 (28%)</td>
<td>2 (12%)</td>
<td>16 (33%)</td>
<td></td>
</tr>
<tr>
<td>Mucinous</td>
<td>1 (1%)</td>
<td>1 (6%)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Squamous</td>
<td>6 (9%)</td>
<td>1 (6%)</td>
<td>5 (10%)</td>
<td></td>
</tr>
<tr>
<td>Sarcoma</td>
<td>2 (3%)</td>
<td>1 (6%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Other**</td>
<td>5 (8%)</td>
<td>1 (6%)</td>
<td>4 (8%)</td>
<td></td>
</tr>
<tr>
<td>Stage (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.336</td>
</tr>
<tr>
<td>I</td>
<td>19 (29%)</td>
<td>2 (12%)</td>
<td>17 (35%)</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>5 (8%)</td>
<td>1 (6%)</td>
<td>4 (8%)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>27 (41%)</td>
<td>8 (50%)</td>
<td>19 (30%)</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>14 (21%)</td>
<td>5 (31%)</td>
<td>9 (18%)</td>
<td></td>
</tr>
<tr>
<td>Surgery (%)</td>
<td></td>
<td></td>
<td></td>
<td>0.314</td>
</tr>
<tr>
<td>Open abdominopelvic</td>
<td>51 (81%)</td>
<td>15 (94%)</td>
<td>36 (77%)</td>
<td></td>
</tr>
<tr>
<td>Laparoscopic</td>
<td>11 (17%)</td>
<td>1 (6%)</td>
<td>10 (21%)</td>
<td></td>
</tr>
<tr>
<td>Vulvectomy</td>
<td>1 (2%)</td>
<td>0</td>
<td>2 (4%)</td>
<td></td>
</tr>
<tr>
<td>Surgery complexity score</td>
<td></td>
<td></td>
<td></td>
<td>0.110</td>
</tr>
<tr>
<td>Low (≤3)</td>
<td>9 (14%)</td>
<td>1 (6%)</td>
<td>8 (17%)</td>
<td></td>
</tr>
<tr>
<td>Intermediate (4-7)</td>
<td>47 (75%)</td>
<td>15 (94%)</td>
<td>32 (68%)</td>
<td></td>
</tr>
<tr>
<td>High (≥8)</td>
<td>7 (11%)</td>
<td>0</td>
<td>7 (15%)</td>
<td></td>
</tr>
<tr>
<td>Duration of hospital stay</td>
<td></td>
<td></td>
<td></td>
<td>0.058</td>
</tr>
<tr>
<td>Median (IQR)</td>
<td>10 (7-16)</td>
<td>13 (8-23)</td>
<td>9 (7-15)</td>
<td></td>
</tr>
<tr>
<td>Chemotherapy</td>
<td></td>
<td></td>
<td></td>
<td>0.556</td>
</tr>
<tr>
<td>Adjuvant</td>
<td>20 (48%)</td>
<td>7 (64%)</td>
<td>13 (42%)</td>
<td></td>
</tr>
<tr>
<td>Neo-adjuvant</td>
<td>18 (43%)</td>
<td>3 (27%)</td>
<td>15 (48%)</td>
<td></td>
</tr>
<tr>
<td>Palliative</td>
<td>3 (7%)</td>
<td>1 (9%)</td>
<td>2 (6%)</td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>1 (2%)</td>
<td>0</td>
<td>1 (3%)</td>
<td></td>
</tr>
<tr>
<td>Radiotherapy</td>
<td></td>
<td></td>
<td></td>
<td>0.230</td>
</tr>
<tr>
<td>Yes</td>
<td>20 (31%)</td>
<td>3 (19%)</td>
<td>17 (35%)</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>45 (69%)</td>
<td>13 (81%)</td>
<td>32 (65%)</td>
<td></td>
</tr>
<tr>
<td>Cancer</td>
<td></td>
<td></td>
<td></td>
<td>0.378</td>
</tr>
</tbody>
</table>
Cancer sites included: ovary (n=34, 52%), corpus uteri (n=19, 29%), cervix (n=6, 9%), vulva (n=1, 1%), and more than one site (n=5, 8%). The most common histological subtypes were: high-grade serous (n=27, 41%) and endometrioid (n=18, 28%). The majority of the cancers were advanced stage.

Open abdominopelvic surgery was performed in 51 (81%) patients, with the remainder laparoscopic (n=11, 17%) and vulvectomy (n=1). Complexity of surgeries was: low (n=9, 14%), intermediate (n=47, 75%) and high (n=7, 11%). Chemotherapy and radiotherapy were administered in forty-two (61%) and twenty (31%) patients respectively. Thirty-eight patients (59%) experienced recurrence or progression of cancer.

The median (IQR) duration of hospital stay was 10 (7-16) days. Patients with recurrent VTE had a longer inpatient stay compared to the non-recurrent VTE group (p=0.05). Median (IQR) Charlson Comorbidity Index was 8 (6-10). The majority of patients were post-menopausal (n=57, 88%) and non-smokers (n=51, 85%). There were significantly more smokers in the recurrent VTE group (p=0.022).
4.2. Fibrinogen and D-Dimer

Fibrinogen levels were available on 51 and D-Dimer levels on 50 patients. Patients with recurrent VTE had higher fibrinogen (median 4.8g/L vs. 4.0g/L, p=0.074) and D-Dimer levels (median 4872 ng/ml vs. 2065 ng/ml, p=0.180), but these did not reach statistical significance (Fig. 30 and Fig. 31).

Figure 30 Fibrinogen levels in patients with recurrent VTE (n=12) compared with patients who did not have a recurrent VTE (n=39), (p=0.074)*.

* Samples were taken prior to surgery and first VTE. Individual values and medians are depicted in the graph.
Figure 31 D-Dimer levels in patients with recurrent VTE (n=12) compared with patients who did not have a recurrent VTE (n=38), (p=0.180)*.

* Samples were taken prior to surgery and first VTE. Individual values and medians are depicted in the graph.

4.3 Thrombin generation assay

Thrombin generation assay was performed on 30 patients prior to surgery for gynaecological cancer. Samples were taken prior to the initial VTE.

There was no significant difference in ETP, lag time and time to peak between the recurrent and non-recurrent VTE groups (Fig. 32-35). Peak thrombin was slightly lower in the recurrent group but this was not statistically significant (Fig. 33).
Figure 32 Endogenous thrombin potential (ETP) as determined by the thrombin generation assay in patients with recurrent VTE (n=7) compared with patients who did not have a recurrent VTE (n=23), (p=0.564)*.

* Individual values and medians are depicted in the graph.
Figure 33 Peak thrombin as determined by the thrombin generation assay in patients with recurrent VTE (n=7) compared with patients who did not have a recurrent VTE (n=23), (p=0.886)*.

* Individual values and medians are depicted in the graph.
Figure 34 Lag time as determined by the thrombin generation assay in patients with recurrent VTE (n=7) compared with patients who did not have a recurrent VTE (n=23), (p=0.701)*.

*Individual values and medians are depicted in the graph.
Figure 35 Time to peak thrombin as determined by the thrombin generation assay in patients with recurrent VTE (n=7) compared with patients who did not have a recurrent VTE (n=23), (p=0.901)*.

* Individual values and medians are depicted in the graph.

4.3 Factor V

Median Factor V levels were slightly higher in the recurrent VTE group but this did not reach statistical significance (Fig. 36).
Figure 36 Factor V in patients who did not have a recurrent VTE (n=18) compared with those who had recurrent VTE (n=7), (p=0.657)*.

* Individual values and medians are depicted in the graph.

4.4 Factor VIII:Chr

A wide variation in Factor VIII:Chr levels was observed in both groups (Fig. 37). There was no significant difference in Factor VIII:Chr levels between the groups.
Figure 37 Factor VIII:Chr in patients who did not have a recurrent VTE (n=17) compared with those who had recurrent VTE (n=6) (p=0.865)*.

*Individual values and medians are depicted in the graph.

4.5 Free Protein S

Lower levels of free Protein S were found in the recurrent VTE group but this did not reach statistical significance (Fig. 38).
Figure 38 Free protein S in patients who did not have a recurrent VTE (n=18) compared with those who had recurrent VTE (n=7), (p=0.244)*.

*Individual values and medians are depicted in the graph.

4.6 Discussion

Gynaecological malignancies are associated with one of the highest rates of VTE. This has been linked with increased morbidity and mortality. In line with the findings of our study and those reported in the literature, recurrent VTE is common despite compliance with anticoagulation regimes. Biomarkers for recurrent VTE would be useful at identifying those at higher risk of recurrent VTE and in guiding the duration and perhaps the type of anticoagulation treatment.

Fibrinogen and D-Dimer levels were available in a subgroup of patients in the current study prior to their first diagnosed thrombotic event and the start of cancer treatment. However, there was no
statistical difference in levels between the recurrent and non-recurrent VTE groups. Previous studies showed high fibrinogen levels to be associated with arterial thrombosis and VTE in non-cancer patients (Koster et al., 1994; Kamphuisen et al., 1999; van Hylckama et al., 2003; Danesh et al., 2005). The Leiden Thrombophilia Study (LETS) found a positive association between the plasma fibrinogen level of >4 g/l and thrombotic risk (Koster et al., 1994). Similar findings were confirmed in a more recent study where fibrinogen levels ≥ 5.0 g/l were associated with increased risk of VTE in the general population compared to controls (Rietveld et al., 2019). There is limited information on fibrinogen in primary or recurrent CAT. The median fibrinogen levels in patients with recurrent VTE in the current study was 4.8 g/l compared to 4g/l in patients who did not experience recurrent VTE, but this was not statistically significant (p=0.07). Sample numbers in both groups were small and larger studies are required to determine whether a true difference in fibrinogen exists between the groups.

D-Dimer has been identified as a strong predictive marker for cancer-associated VTE (Pabinger et al., 2018). The D-Dimer level above 2759 ng/mL at first VTE event in patients with active cancer treated with Tinzaparin was shown to be useful in predicting the risk of recurrent VTE (Piatek et al., 2016). Similarly, elevated levels of D-Dimer 21 days after cessation of anticoagulation treatment have been reported to predict VTE recurrence among patients with CAT (Jara-Palomares et al., 2018). There was a trend towards higher D-Dimer levels at presentation with their cancer in our group for those who subsequently had recurrent VTE compared to single event VTE but that difference did not reach statistical significance. Ovarian cancer is a rich source of tissue factor that promoted thrombosis and elevated D-Dimer level has even been proposed as a diagnostic marker in ovarian cancer hence the specificity of D-Dimer as a biomarker for VTE in this population is low and may explain the wide variation in D-Dimer levels observed. A larger
sample may be required to assess the ability of D-Dimer to predict those at risk of recurrent VTE in a gynaecological cancer population.

Elevated thrombin generation was associated with an increased risk of VTE in The Vienna Cancer and Thrombosis Study and in the Hypercan Study (Ay et al., 2011; Falanga et al., 2015). Increased thrombin generation was associated with an increased risk of VTE following surgery in a gynaecologic cancer population (Abu Saadeh et al., 2016; Ward et al., 2018; Norris et al., 2020). High ETP and peak thrombin levels were found to be associated with increased risk of recurrent VTE in the general population (Eichinger et al. 2008; Tripodi et al., 2008; Lutsey et al., 2009). No significant changes in thrombin generation were observed in the present study; however, the sample size was small and given the variability of thrombin generation assays the study may not have had the required statistical power to detect a significant difference. The role of thrombin generation assay in the prediction of recurrent CAT remains to be determined.

A small increase in Factor V was found in the recurrent VTE group but this did not reach statistical significance likely due to the small sample size. A recent study has shown that tumour and plasma levels of FV are increased in gynaecological cancers however this was not associated with an increased risk of VTE (Martin et al, 2015). In the general population both high levels and low levels of factor V (in the absence of the factor V Leiden mutation) have been implicated in VTE risk (Kamphuisen et al., 2000; Suehisa et al., 2010; Rietveld et al., 2018) however data on the role of factor V in CAT is scarce. Patients with Factor V Leiden mutation are at increased risk of VTE in the cancer population (Pabinger et al., 2015; Heraudeau et al., 2018). The role of Factor V in VTE recurrence in gynaecological cancer remains uncertain.
Elevated levels of Factor VIII:C have been shown to be associated with increased risk of VTE in both general and cancer populations (Rosendaal, 2000; Vormittag et al., 2009; Rietveld et al, 2019). High plasma Factor VIII levels have also been found to be an independent risk factor for recurrent VTE in the general population (Kraaijenhagen et al., 2000; Kyrle et al., 2000). However, the evidence on Factor VIII as a biomarker in VTE recurrence in the cancer population is limited. The CATCH trial failed to show a role for Factor VIIIc in the prediction of recurrent VTE with cancer (Khorana et al., 2017). Our study showed a wide variation in factor VIIIc levels with no difference between the recurrent and single episode VTE groups. It is unlikely that Factor VIIIc will be of value in predicting recurrence in gynaecological cancer.

Lower levels of free protein S were found in patients with recurrent VTE in the present study; however, this did not reach statistical significance. The association between the low levels of protein S and thrombogenic effect is plausible but research studies performed to date have failed to prove its value (Pintao et al, 2013). Few studies have investigated the role of protein S in cancer-associated VTE. In gynaecological cancer, protein S mRNA expression was significantly down-regulated in malignant tumours compared with benign controls and this was not associated with VTE (Martin et al., 2015). These changes may contribute to local thrombin production and increased thrombotic potential of patients with gynaecological cancer.

However, several limitations of this study require consideration. First, only a selected group of patients that had plasma samples available prior to surgery and the first VTE event were included. In addition, the sample size was small, and the statistical power of the study was low. It is also worth pointing out that the biomarkers were not collected prior to the recurrent VTE event as in previous studies (Jara-Palomares et al., 2018) or at the time of VTE recurrence, but rather before the first VTE event. Although it would be very useful
to know well in advance who is at an increased risk of recurrent VTE, the timing of biomarkers assessment and other contributing factors present at the time of sample collection (active cancer, etc.) will ultimately limit their interpretation and therefore its clinical application. In conclusion, this small study did not show that haemostatic biomarkers prior to first VTE and before cancer treatment were useful in identifying gynaecological cancer patients who went on to develop recurrent VTE. This was not surprising because apart from the inherent study limitations discussed above, resolution of the first VTE event and subsequent cancer status would be expected to be the main determinants of further thrombosis risk.

Baseline fibrinogen and D-Dimer showed the strongest trends for predicting this high-risk group but larger studies are required to determine the role of these biomarkers at first presentation in predicting recurrent VTE. Elevated plasma fibrinogen and D-Dimer may also have value in predicting metastases and prognosis in cancer (Luo et al., 2015; Luo et al., 2017) so further investigation of these parameters is recommended on that account as well.
Chapter 5:
Patients’ experience and compliance with extended low molecular weight heparin prophylaxis post-surgery for gynaecological cancer.
5.1 Introduction

As previously discussed in Chapter 1.7, the risk of VTE in patients with gynaecological malignancies is high particularly following major surgery (Timp et al., 2013; Abu Saadeh et al., 2016; Barber et al., 2016). The implementation of extended LMWH prophylaxis following laparotomy for gynaecological cancer significantly reduced the incidence of post-operative VTE (Schmeler et al., 2013).

The International Initiative on Thrombosis and Cancer (ITAC-CME) guidelines recommend 28 days of post-operative LMWH for VTE prophylaxis for patients undergoing major cancer surgery (Farge et al., 2019). Despite level I evidence, there has been a wide variation in clinicians’ adherence to these guidelines (Samama et al., 2014). Reluctance to prescribe post-operative VTE prophylaxis could be due to concern about bleeding risk, financial cost or patient acceptability and compliance. However, a study looking at patients’ adherence to extended LMWH prophylaxis following pancreas and liver resection, found that most patients complied with their LMWH regime (Lemke et al., 2016). This showed that patient adherence was not a major contributor to poor compliance with VTE prophylaxis guidelines. Our group has previously shown prescriber inertia is an important contributor to non-compliance with VTE prophylaxis in gynaecological cancer care (Petch et al., 2016). With this study we wanted to address the paucity of data evaluating patients’ experience and compliance with LMWH extended prophylaxis in the setting of gynaecological cancer.

In this prospective observational study, patient compliance, satisfaction and experience with the extended low molecular weight heparin prophylaxis after major surgery for gynaecological cancer was assessed between July 2017-March 2018. All participants
received a log book to record all injections, side effects, and a questionnaire to be completed at the end of the study (see description in Chapter 2.4). A sample patient log book and questionnaire are attached in the Appendix I and II. In addition, patients received printed educational materials on the importance of early recognition, signs and symptoms of VTE (Appendix III).

The aims of this study were:

- To investigate patient compliance and experience with extended LMWH prophylaxis following major surgery for gynaecological cancer.

5.2 Results

5.2.1 Clinicopathological details of all patients

A total of 117 consecutive patients who were deemed eligible for extended 28 days of VTE prophylaxis were recruited to the study over the 9-month period (Fig. 39). One patient was excluded due to the development of PE two days after interval cytoreductive surgery for stage IV ovarian cancer, despite the administration of standard VTE prophylaxis. A further 10 patients were excluded from the final analysis because they did not return the logbook and questionnaire. A total of 106 (91%) patients who returned the completed logbook and questionnaire were included.
Figure 39 Flow diagram of patients recruited into the study (n=117).

Assessed for eligibility (n=117)

Excluded (n=1)
- PE two days after surgery

Prescribed extended LMWH prophylaxis (n=116)

Did not return the pack (n=10)

Analysed (n=106)

The patients’ characteristics are detailed in Table 22. The median age of the patients was 57 years (range 29–84) and the length of the hospital stay was 6.5 days (range 2–53). Seven patients spent more than 28 days in the hospital following primary surgery. Sixty-five (61%) patients had open surgery and ovarian cancer was the most common cancer site (n=41, 46%). Eighty-nine (84%) patients had malignant pathology and seventeen (16%) had borderline or benign pathology.

Three patients had a VTE event - one PE two days after surgery and two patients developed VTE following discharge from the hospital. One patient was diagnosed with PE 3 months following laparoscopic radical hysterectomy for cervical cancer, while receiving chemotherapy; it is unclear whether she completed an extended thromboprophylaxis course, as she did not return her logbook or questionnaire. The second patient had ovarian cancer and developed PE and DVT 6 weeks following laparotomy for a small bowel obstruction, having completed a course of extended thromboprophylaxis.
Table 22 Study population characteristics (n=106).

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (range)</td>
<td>57 (29-84)</td>
</tr>
<tr>
<td>Duration of hospital stay, median (range)</td>
<td>6.5 (2-53)</td>
</tr>
<tr>
<td>Surgical Approach (%)</td>
<td></td>
</tr>
<tr>
<td>Open</td>
<td>65 (61%)</td>
</tr>
<tr>
<td>Laparoscopic</td>
<td>31 (29%)</td>
</tr>
<tr>
<td>Vulvectomy</td>
<td>9 (9%)</td>
</tr>
<tr>
<td>Radical Vaginectomy</td>
<td>1 (1%)</td>
</tr>
<tr>
<td>Pathology (%)</td>
<td></td>
</tr>
<tr>
<td>Malignant</td>
<td>89 (84%)</td>
</tr>
<tr>
<td>Borderline¹</td>
<td>10 (9%)</td>
</tr>
<tr>
<td>Benign²</td>
<td>7 (7%)</td>
</tr>
<tr>
<td>Cancer site (%)</td>
<td></td>
</tr>
<tr>
<td>Ovary</td>
<td>41 (46%)</td>
</tr>
<tr>
<td>Corpus uteri</td>
<td>31 (35%)</td>
</tr>
<tr>
<td>Cervix</td>
<td>7 (8%)</td>
</tr>
<tr>
<td>Vulva</td>
<td>9 (10%)</td>
</tr>
<tr>
<td>Vagina</td>
<td>1 (1%)</td>
</tr>
</tbody>
</table>

¹ All ovarian in origin.
² 6 ovarian and 1 uterine in origin.

5.2.2 Knowledge and compliance

Eighty-five (80%) patients understood the reason for extended prophylaxis while 21 (20%) did not. The majority of patients heard about the importance of prophylaxis for the first time after their surgery (n=74, 70%). The remaining patients were informed about the need for prophylaxis before surgery, either in the outpatient clinic (n=21, 20%) or after admission to the ward (n=8, 7%). Three patients (3%) knew about extended prophylaxis from their previous experience.

The median number of days of injections received was 28 (range 12–28). There were 2823 injections in total received by 106
patients. Sixty-six (62%) patients received prophylaxis for the full 28 days (Fig. 40). Twenty-five (24%) patients completed 26 to 27 days and 15 (14%) completed 12 to 25 days. Reasons for missed injections in this group included: forgetfulness (n=12), medical procedures (n=6), inadequate prescription (n=4), pain (n=2), patient’s choice not to take the injection on a particular day (n=2), non-availability of the person administering the injections (n=1), unknown (n=5).

**Figure 40 Compliance with extended LMWH prophylaxis (n=106).**

Eight (8%) patients stopped low molecular weight heparin within 23 days of surgery due to pain (n=3), cost (n=2), physician’s request (n=2) or because they believed it was not necessary (n=1). The injection site, age, hospital stay, surgical approach or person administering the injections did not significantly influence compliance. Patients with borderline mucinous and borderline serous tumours were less likely to complete 28 days of injections compared with patients with cancer (OR 5.33, 95% CI 1.23 to 23.07; p<0.025) (Table 23).
Table 23 Variables affecting patients’ reported compliance\textsuperscript{1}.

<table>
<thead>
<tr>
<th>Injection site</th>
<th>28 injections n=66</th>
<th>≤27 injections n=40</th>
<th>OR (95% CI)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arms</td>
<td>16</td>
<td>10</td>
<td>Reference</td>
<td>1.46 (0.72-2.96)</td>
</tr>
<tr>
<td>Thighs</td>
<td>43</td>
<td>21</td>
<td>Reference</td>
<td>2.68 (0.92-7.78)</td>
</tr>
<tr>
<td>Abdomen</td>
<td>5</td>
<td>8</td>
<td>Reference</td>
<td>1.26 (0.23-6.80)</td>
</tr>
<tr>
<td>Buttocks</td>
<td>2</td>
<td>1</td>
<td>Reference</td>
<td>0.286</td>
</tr>
</tbody>
</table>

| Age            | 0.99 (0.96-1.02) | 0.741 |

| Hospital Stay  | 1.01 (0.97-1.06) | 0.42 |

<table>
<thead>
<tr>
<th>Surgical approach</th>
<th>Open</th>
<th>Laparoscopic</th>
<th>Vulvectomy</th>
<th>Radical vaginectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>39</td>
<td>21</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>26</td>
<td>10</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Reference</td>
<td>0.91 (0.33-2.50)</td>
<td>1.79 (0.41-7.80)</td>
<td>0.869</td>
<td>0.433</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pathology</th>
<th>Malignant</th>
<th>Borderline</th>
<th>Benign</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>57</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Reference</td>
<td>5.33 (1.23-23.07)</td>
<td>0.62 (0.10-3.66)</td>
<td>0.025</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Administration</th>
<th>Self</th>
<th>Somebody else</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>38</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>17</td>
</tr>
<tr>
<td>Reference</td>
<td>1.56 (0.77-3.18)</td>
<td>0.213</td>
</tr>
</tbody>
</table>

\textsuperscript{1}Note: The outcome measure in this model was compliance, which was represented in the model as a binary variable (28 injections vs. 27 or fewer injections). n=number of patients (106).

At a 30-day telephone follow-up, many patients provided feedback that tracking the progress and number of remaining injections in a study logbook played an important role in maintaining their compliance.

5.2.3 LMWH administration

All patients or caregivers were educated on how to perform injections by nursing staff, either before (n=2, 2\%) or following surgery (n=104, 98\%). Sixty-one (58\%) patients were able to administer the injections themselves, the remainder were helped by a family member (n=28, 26\%) or a community nurse (n=17, 16\%). Despite the prior experience with self-administration of injection in
26 (25%) patients, only 16 of these patients self-administered the injection on this occasion.

Within the group of patients who self-administered injections, 15 (14%) declared it was “very easy”, 30 (28%) “easy”, 14 (13%) “slightly difficult”, 1 (1%) “very difficult”, and 1 (1%) “impossible”. Patients who did not self-administer the injections were either not confident enough to proceed, were too afraid, found it too difficult to inject themselves (n=31, 29%), or were afraid of needles (n=5, 5%). Below are some of the patients’ comments regarding the self-administration of LMWH:

“I couldn’t face doing it myself”

“I was too nervous…”

“I only tried to inject myself once and it was not very successful.”

The sites of the injections were thighs (n=64, 60%), arms (n=26, 25%), abdomen (n=13, 12%), and buttocks (n=3, 3%). Following completion of the extended prophylaxis, 44 (42%) patients returned syringes to their local pharmacy, in 27 (25%) cases a community nurse collected them, 25 (24%) returned them to the hospital, and six (6%) discarded them in domestic waste.

5.2.4 Side effects

Eighty-nine (84%) patients experienced some form of side effect during the extended prophylaxis, with pain and bruising being the most common (Fig. 41).
Figure 41 Side effects and complications experienced by the patients receiving extended LMWH prophylaxis.

*Small, non-significant and self-limited bleeding from the injection site experienced by 6 patients.

Fifty-four (51%) patients described no pain at injection. Fifty-eight percent of injections (n=1635) were associated with pain at the time of injection, which in 49% of patients persisted for some time afterward. Injections into thighs were more likely to cause pain than those administered into arms (OR 2.49, 95% CI 1.21 to 5.11; p<0.013) (Table 24).

Table 24 Variables affecting patients’ reported pain.

<table>
<thead>
<tr>
<th>Site</th>
<th>No pain n=1188</th>
<th>Any pain n=1635</th>
<th>OR (95% CI)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arms</td>
<td>321</td>
<td>459</td>
<td>Reference</td>
<td>0.013</td>
</tr>
<tr>
<td>Thighs</td>
<td>674</td>
<td>1027</td>
<td>2.49 (1.21-5.11)</td>
<td>0.806</td>
</tr>
<tr>
<td>Abdomen</td>
<td>177</td>
<td>108</td>
<td>1.11 (0.47-2.59)</td>
<td>0.382</td>
</tr>
<tr>
<td>Buttocks</td>
<td>16</td>
<td>41</td>
<td>2.31 (0.35-5.17)</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td>0.94 (0.91-0.96)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Hospital Stay</td>
<td></td>
<td></td>
<td>1 (0.97-1.03)</td>
<td>0.831</td>
</tr>
</tbody>
</table>
### Surgical approach

<table>
<thead>
<tr>
<th></th>
<th>Open Laparoscopic Vulvectomy</th>
<th>Laparoscopic Radical vaginectomy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>0.79 (0.38-1.65)</td>
<td>2.32 (0.48-1.01)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.03 (0.01-0.06)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

### Pathology

<table>
<thead>
<tr>
<th>Pathology</th>
<th>Malignant Borderline</th>
<th>Benign</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>1.80 (0.56-5.75)</td>
<td>3 (0.65-13.87)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.321</td>
<td>0.158</td>
</tr>
</tbody>
</table>

### Administration

<table>
<thead>
<tr>
<th>Administration</th>
<th>Self Somebody else</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td>2.81 (1.42-5.56)</td>
</tr>
<tr>
<td>P-value</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Note: The outcome measure in this model was pain associated with low molecular weight injection, which was represented as a binary variable (no pain - 0 vs. any pain 1-10). n=number of injections (2823).

Increasing age was associated with an increased perception of pain (p<0.0001).

Patients who had the injections administered by somebody else were more likely to experience pain compared with those who self-administered (OR 2.81, 95% CI 1.42 to 5.56; p=0.003). Interestingly, patients commented that as they gained experience in the injection technique the amount of pain was reduced. Some of the patients’ comments in that regard are listed below:

“Once I learned how to do my own injection I did it really carefully and it was less painful with experience and practice”.

“I refined my technique with each one and had no pain afterwards”.

“I found that if the injection is given very slowly there is no pain and very little stinging afterward”

In five cases, the pain was responsible for the premature cessation of prophylaxis, all other patients who experienced pain or other side effects admitted they still continued with the injections, as they believed it was important to do so.
Increased risk of bruising was not affected by injection site, age, hospital stay, surgical approach, pathology or person administering the injections. Six patients experienced mild and self-limited bleeding from the injection site. There were no serious complications arising from the injections.

5.2.5 Patient satisfaction

Most patients (n=83, 78%) were either satisfied or highly satisfied with injections (Fig. 42).

Figure 42 Satisfaction with LMWH injections.

However, 91 (86%) patients admitted that they would prefer an oral medication if available and as effective as the injections. Patient satisfaction was not affected by the site of injection, age, hospital stay, surgical approach or whether injections were self-administered or not (Table 25).
### Table 25 Variables affecting patients’ reported satisfaction\(^1\).

<table>
<thead>
<tr>
<th></th>
<th>Group 1 n=83</th>
<th>Group 2 n=9</th>
<th>OR (95% CI)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Site</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arms</td>
<td>24</td>
<td>2</td>
<td>Reference 0.77</td>
<td>0.78</td>
</tr>
<tr>
<td>Thighs</td>
<td>53</td>
<td>5</td>
<td>(0.12-4.79)</td>
<td></td>
</tr>
<tr>
<td>Abdomen</td>
<td>4</td>
<td>1</td>
<td>1.85 (0.16-0.36)</td>
<td>0.613</td>
</tr>
<tr>
<td>Buttocks</td>
<td>2</td>
<td>1</td>
<td>3.55 (0.25-9.72)</td>
<td>0.346</td>
</tr>
<tr>
<td><strong>Age</strong></td>
<td></td>
<td></td>
<td>0.97 (0.93-1.02)</td>
<td>0.3</td>
</tr>
<tr>
<td><strong>Hospital Stay</strong></td>
<td></td>
<td></td>
<td>1.01 (0.89-1.14)</td>
<td>0.823</td>
</tr>
<tr>
<td><strong>Surgical approach</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Open</td>
<td>52</td>
<td>2</td>
<td>Reference 4.94</td>
<td>0.166</td>
</tr>
<tr>
<td>Laparoscopic</td>
<td>25</td>
<td>3</td>
<td>(0.51-7.62)</td>
<td></td>
</tr>
<tr>
<td>Vulvectomy</td>
<td>6</td>
<td>3</td>
<td>15.78 (0.43-569.93)</td>
<td>0.132</td>
</tr>
<tr>
<td>Radical vaginectomy</td>
<td>0</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pathology</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Malignant</td>
<td>72</td>
<td>7</td>
<td>Reference 18.17</td>
<td>0.027</td>
</tr>
<tr>
<td>Borderline</td>
<td>6</td>
<td>2</td>
<td>(1.39-236.98)</td>
<td></td>
</tr>
<tr>
<td>Benign</td>
<td>5</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Administration</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Self</td>
<td>48</td>
<td>4</td>
<td>Reference 0.67</td>
<td>0.624</td>
</tr>
<tr>
<td>Somebody else</td>
<td>35</td>
<td>5</td>
<td>(0.14-3.21)</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)Note: The outcome measure in this model was patient satisfaction, which was represented as a binary variable (highly satisfied or satisfied – Group 1 vs. partially satisfied, not satisfied or not at all satisfied Group 2). n=number of patients.

Patients with borderline mucinous and borderline serous tumours were more likely to be partially satisfied, not satisfied or not at all satisfied compared with patients with cancer (p=0.027). When patients were asked what was most difficult about the experience, 37 (35%) answered that it was the injection itself, 25 (24%) thought it was the side effects (pain and bruising), nine (8%) listed compliance difficulty, and five (5%) stated dependence on somebody else for administration. Thirty (28%) patients did not report any problems.
Some of the patients’ comments in regards to the satisfaction with LMWH prophylaxis are listed below:

“The injection if it was given in pill form it would be fantastic.”

“A very painful and uncomfortable procedure, I am sure a lot of people would have given up sooner…I feel I would have stopped doing the injections earlier only I knew the importance of them.”

“If injections have kept me safely covered, I will be happy enough.”

5.3 Discussion

Surgery is a major risk factor for VTE in patients with gynaecological malignancies (Agnelli et al., 2006). The incidence of post-operative VTE has been significantly reduced following the introduction of extended LMWH prophylaxis (Schmeler et al., 2013). However, previous studies have proven the uptake of extended prophylaxis to be inadequate (Samama et al., 2014; Petch et al., 2016). Assessing the patients’ experience and compliance with extended post-operative LMWH prophylaxis could help to overcome these barriers and improve clinical practice.

In the current study, ten patients out of 116 failed to return their questionnaires. Sixty-six (62%) patients received a full 28-day course of LMWH rendering full compliance adequate. More than half of the patients (58%) were able to self-administer the injections, which was associated with less pain. Although satisfaction was high (78%), most patients (86%) admitted they would much prefer a tablet version.
It is disappointing that very few patients received any instruction on LMWH administration prior to admission. The empowerment of patients through preoperative education is a key element in enhancing their performance in the surgical pathway and after discharge. Models and instruction leaflets on injection techniques are available. Our ambulatory services are poorly resourced. Cancer nurse specialist to patient ratios are below average. In addition many patients are admitted through the emergency services and the interval to surgery can be short. Although one could argue that the most effective way (in regard to retaining information and having practical experience of self-injection) to train people is at the time of giving the injection themselves.

Extended prophylaxis is an additional burden on the cancer patient who is responsible for purchasing, and in many cases, self-injecting the medication. Furthermore, the educational and support services are also lacking in the community and many patients experience side effects (Seaman et al., 2014; Noble et al., 2015B). The results of the current study show that the overall patient-reported compliance with extended prophylaxis was good, with 86% of patients receiving injections for >26 days. This compares favourably with a similar study where 81% of patients reported full adherence to the extended prophylaxis following pancreas and liver resection (Lemke et al., 2016). The results alleviate concerns regarding patient compliance and correspond with previous studies, demonstrating that the problem with adherence to the guidelines is mainly the resistance of the prescribing clinician rather than patient compliance (Kalka et al., 2009). One of the main factors influencing the decision to prescribe extended prophylaxis is the concern regarding bleeding risk. In a study of advanced cancer patients undergoing treatment for cancer-associated thrombosis, patients reported that a low major bleeding rate was an important feature in their anticoagulant preference (Noble et al., 2015B). Previous studies have demonstrated that there was no significant increase in
haemorrhagic complications with the extended low molecular weight heparin prophylaxis (Bergqvist et al., 2002; Rasmussen et al., 2006). In agreement with these findings, none of the patients in the present study experienced any major bleeding. It is important to emphasize that only 12% of patients in the study injected into the abdomen, which has been known to be associated with increased risk of rectus sheath hematoma (Sullivan et al., 2014).

More than half of the patients in our study were able to self-administer the injections and this was associated with less pain, although this statistically significant difference in pain perception might not be clinically significant. Previous studies suggest that the slower administration of subcutaneous heparin is associated with less pain and bruising (Chan, 2001; Mohammady et al., 2017). This could be true in patients who self-administer the injection, as they can adjust the speed of injection according to the pain intensity. However, self-administration had no impact on the bruising reported by patients.

Previous studies reported that using a specific injection technique, needle-oriented bevel-down parallel to the skin, helps to achieve a painless injection (Zelickson et al., 2017). Self-administration minimizes dependency on family members and healthcare staff, and potentially increases compliance with extended prophylaxis. A study of German haematologists/oncologists has suggested that practical issues like the need for injections and the availability of a partner to perform injections are important in their choice of anticoagulant (Matzdorff et al., 2016). However, studies have shown that injections are not a barrier to good compliance and should not therefore influence the choice of an anticoagulant (Bergqvist et al., 2012; Noble et al., 2015B). This suggests that the capability of cancer patients to accept injectable anticoagulant treatment is probably underestimated. The majority of patients (84%) reported at least one side effect associated with the extended prophylaxis injections, with bruising and pain being the most
frequent. Increased perception of pain related to injection in older patients in our study could be explained by the biology of aging (Devor, 1991; Edwards et al., 2003). Advancing age is associated with the degeneration of endogenous inhibitory systems and increasing cell death that may increase susceptibility to pain. We found that injections into thighs were more likely to be associated with pain compared with arms. Previous studies found arms to be more favourable injection sites, with fewer blood vessels, painful sensations and less discomfort for the patients (Lister & Sarpal, 2004). Thighs on the other hand have been associated with more pain and bleeding by the patients (King, 2003). Despite the high rate of minor side effects, the overall satisfaction with injections was high. In the present study, patients who experienced pain or other side effects admitted they still continued with injections, as they believed it was important to do so. This is in agreement with the studies of patients receiving low molecular weight heparin following cancer-associated thrombosis where patients were willing to persevere with the treatment in order to avoid further thrombosis (Seaman et al., 2014).

Ninety-one (86%) patients acknowledged they would much prefer an oral form if available and as effective as low molecular weight heparin. Oral direct inhibitors of thrombin and/or Factor Xa are emerging as suitable for the treatment and prevention of VTE in cancer patients. Thromboprophylaxis with apixaban in ambulatory cancer patients starting chemotherapy (Khorana score ≥2) resulted in a significantly lower rate of venous thromboembolism (4.2%) than did placebo (10.2%) in a study by Carrier et al. (Carrier et al., 2019). However, the rate of major bleeding episodes was higher with apixaban than with placebo. Following the discharge, DOACs did not show a net benefit compared to enoxaparin for 6 to 14 days regarding the reduction of the rate of VTE in high-risk medical patients (Dalen et al., 2020). The evidence for use of direct oral anticoagulants in the context of post-surgical prophylaxis in the
cancer setting is still awaited and the risk of bleeding associated with direct oral anticoagulants in these patients is still a cause for concern (Brunetti et al., 2017). It has been shown that patient preference for oral administration over injection was only of moderate importance and patients favoured efficacy and safety over the convenience of the route of administration (Noble et al., 2015B).

Given that tracking the progress and number of remaining injections in a study logbook proved useful to our patients, there might be a role for an App, which could be used on a smart device for this purpose. However, this would be associated with an additional cost required to develop such an App. In addition, older patients might find it harder to use a smart device/phone than a hard copy logbook limiting its functionality.

There are a few limitations of our study. The non-return of questionnaires has been addressed. The self-reporting method we used to assess patient compliance could be considered a weakness of the current study. Although there is no gold standard method for assessing adherence to medications in clinical practice, self-reporting methods are one of the most common indirect methods used in the outpatient setting (Osterberg & Blaschke, 2005; Garfield et al., 2011). Indirect methods including self-report are considered less reliable compared to direct methods. Direct observation of the patient taking the injection or measuring anti-factor Xa levels (Wei & Ward, 2015) would certainly be more reliable; however, they would also be invasive, expensive and impractical in the outpatient setting than self-reported measures. There is a possibility that the patient adherence rate in the current study is overestimated. Patients could document the injections in the logbook even if not taken, in order to appear to be following the regimen. Ten patients who did not return the pack could have done so because they were not compliant with treatment. There is also the possibility that the patient experience was not fully captured since we only used
quantitative methods of assessment (questionnaire) as opposed to mixed methods (combining both qualitative and quantitative methods). Although there is no standard method or ideal instrument for measuring the experiences of patients, the current study attempted to increase the chances of doing so by using prospective records of daily injections and a questionnaire with a mix of open and closed questions. The questionnaire used had not been evaluated by the National Adult Literary Agency or patient representatives, neither it was previously validated in patients with gynaecological cancer, which could be regarded as another limitation. To the author’s knowledge, this is the first study to examine patient perspectives on extended prophylaxis in a gynaecological cancer setting. The logbook and questionnaire return rate was high (91%), which is a major strength of this study. Patient compliance and experience with extended prophylaxis were good. These findings should be applicable to other countries and settings in which extended thromboprophylaxis is recommended and might encourage physicians to prescribe it more often. Future strategies should focus on the education of healthcare providers on the importance of extended post-surgical venous thromboembolism prophylaxis in cancer settings, as this seems to be the major obstacle rather than patient compliance.

In conclusion, the current study showed good patient compliance with extended low molecular weight heparin prophylaxis following gynaecological oncology surgery. Satisfaction with the treatment regimen was high, despite the prevalence of minor side effects associated with the injection. Patients reported pain scores to be significantly lower when low molecular weight heparin was self-administered. The majority of patients would prefer oral prophylaxis if available, safe and effective.
Chapter 6:
General discussion
and
conclusion
6.1 General discussion and conclusion

Patients with active cancer have a 4- to 6.5-fold higher risk of developing VTE compared to the general population and up to 25% of patients with gynaecological malignancies experience VTE (Heit et al., 2000B; Santoso et al., 2009; Walker et al., 2013; Cohen et al., 2017). The incidence of VTE is probably underestimated since patients are not routinely imaged for VTE. In addition, reports suggest that VTE can be found in up to 50% of cancer patients on autopsy (Thompson & Rogers, 1952). Cancer-associated thrombosis has been linked to reduced short- and long-term survival and is the second leading cause of death in cancer patients after disease progression (Khorana et al., 2007B). Recurrent VTE affects one in five patients with CAT and disconcertingly not infrequently arises while on therapeutic anticoagulation treatment, this is a significant clinical challenge (Prandoni et al., 2002). Overall survival of cancer patients is worse if they experience recurrent VTE (Chee et al., 2014). I sought to measure the frequency of recurrent VTE in women with gynaecological cancer and to identify biomarkers prior to the first VTE event that might herald a higher risk of recurrence.

The incidence of recurrent VTE in patients with genital tract malignancies in the current study was 22%. This is in line with findings from other studies, where the incidence of recurrent VTE in mixed cancer patients was in the region of 20% (Prandoni et al., 2002; Luong et al., 2017). Patients who had VTE diagnosed prior to any form of cancer treatment were more likely to experience VTE recurrence. In agreement with the present study Gran et al., demonstrated that patients who suffered VTE within a year prior to diagnosis of cancer (occult cancer) were more likely to suffer recurrent VTE compared to patients who experienced first VTE within the two years after their cancer diagnosis (Gran et al., 2017).
The highest number of VTE recurrences occurred within a year of the primary event, 67% happened within six months of the first VTE and 81% within 12 months. Similarly, other investigators found the peak of VTE recurrence occurred within 6 months of the primary event in the general population and mixed cancer patients (Cohen et al., 2017). ITAC and recent ASCO guidelines recommend a minimum of 6 months of anticoagulation to treat the first CAT event (Farge et al., 2019; Key et al., 2020). Since most of the recurrent VTE events in the present study occurred within 12 months of primary VTE, this data would suggest that treatment for more than 6 months may be warranted. However, the outcome of the SELECT-D randomised trial that compared 12-month rivaroxaban versus placebo failed to show benefit in extending treatment beyond 6 months (Marshall et al., 2020). That study was underpowered to detect a statistically significant reduction in recurrent VTE with extended anticoagulation. Therefore, the question of the duration of anticoagulation treatment remains unanswered.

DOACs were introduced about a decade ago and rapidly transformed the treatment of patients with acute VTE outside of the cancer setting (Kearon et al., 2016B). In comparison to warfarin, DOACs are more convenient to use, are given in fixed doses, do not require laboratory monitoring, have similar efficacy and lower risk of intracranial haemorrhage (Makam et al., 2018). In cancer patients LMWH is known to be superior to warfarin due to lower rates of recurrent VTE and serious bleeding (Lee et al., 2015). More recently, DOACs have been suggested for the treatment and prevention of cancer-associated thrombosis (Farge et al., 2019; Key et al., 2020). According to the ITAC guidelines, the choice of anticoagulant agent (LMWH vs. DOAC) in the treatment of primary CAT depends on clinical circumstances. In the absence of strong drug-drug interactions, gastrointestinal absorption impairment,
high-risk of gastrointestinal or genitourinary bleeding, rivaroxaban or edoxaban should be used (Farge et al., 2019). Recent studies however represent a paradigm shift in the treatment of CAT with four trials reporting the effects of DOACs in the treatment of CAT. Hokusai VTE Cancer trial (edoxaban), SELECT-D trial (rivaroxaban), ADAM VTE and Caravaggio trial (apixaban) (Raskob et al., 2018; Young et al., 2018; Agnelli et al., 2020; McBane et al., 2020). All four studies showed either noninferiority of DOACs to LMWH or a lower rate of VTE recurrence in patients treated with DOACs. In Hokusai and SELECT-D trials, DOACs were associated with a higher rate of major bleeding or clinically relevant non-major bleeding respectively, mainly upper gastrointestinal bleeding in patients with gastrointestinal cancer. Gynaecological cancer patients accounted only for about 10% of the study population in all four trials, therefore it is difficult to draw any definitive conclusion on DOACs in gynaecologic cancer population based on this evidence alone. More recently, Lee et al. performed a retrospective review of 162 gynaecologic cancer patients treated for VTE with dalteparin (n=60) or rivaroxaban (n=102) (Lee et al., 2020). Although patients treated with rivaroxaban had a higher rate of gastrointestinal and urinary tract bleeding, the difference was not statistically significant. The rate of recurrent VTE was similar in both groups. Although this study is limited by the retrospective design, caution is needed in using DOACs in gynaecological cancer patients with gastrointestinal or urologic involvement and large-scale trials are required to determine whether DOACS demonstrate an improvement in recurrent VTE rate in the setting of gynaecological cancer.

Patients in the current study were treated from 2006-2017, prior to the emerging DOAC evidence and new guidelines (Farge et al., 2019; Key et al., 2020). Therefore, the majority of patients in our cohort were treated with LMWH and only one patient with DOAC. Although not all patients may be candidates for treatment with DOACs, the rate of recurrent VTE in patients with gynaecological
malignancy may be lower in the future with the more widespread use of DOACs.

Most of the patients who experienced recurrent VTE (63%) in the current study were receiving a therapeutic dose of LMWH at the time of the recurrent VTE event. Similar findings were reported in a mixed cancer type population (Schulman et al., 2015). This highlights the complexity of treating patients with CAT, putting in question the efficacy of anticoagulation agents, appropriate dosage and patients’ compliance. Although many recommendations have been made (Farge et al., 2019; Key et al., 2020) there is a lack of evidence to guide practice in this particular situation and further research is required. From personal clinical experience I know there is a common misconception amongst physicians that new VTE cannot occur in patients who are already on therapeutic anticoagulation. The findings of the current study highlight the fact that patients remain at high risk of recurrent venous thrombosis despite standard anticoagulation treatment.

In our study patients who experienced recurrent VTE underwent more complex surgical procedures. Open surgery and longer operative time are known to be associated with a higher risk of post-operative VTE compared to MIS (Barber et al., 2016; Zhang et al., 2018). The risk of post-operative VTE in patients with gynaecological malignancies undergoing MIS is low (Bouchard-Fortier et al., 2014). Our findings suggest that the increased risk of VTE is related to the increased complexity of surgical procedures rather than the surgical approach (open versus laparoscopic) per se. Since more complex surgery is required for biologically more aggressive cancers such as high-grade ovarian cancer and these may be more thrombogenic it is not possible to determine cause or effect in that matter. The peri-operative VTE risk assessment and anticoagulation planning should take into account the complexity of the surgery.
High BMI and medical comorbidities are both risk factors for VTE (Agnelli et al., 2006; Khorana et al., 2007B; Connolly & Francis, 2013), but we did not find any differences in that regard between the recurrent and single episode VTE groups. Similarly, despite the existing evidence of worse survival in patients with recurrent VTE (Chee et al., 2014) we found no difference in overall and progression-free survival in patients with recurrent VTE compared to the non-recurrent VTE group. Monocytes count measured prior to cancer treatment and first VTE event was higher in the recurrent VTE group, but was still within the normal clinical range. In addition, when accounting for the Bonferroni correction the difference became non-significant, suggesting likely spurious positive results. Monocytes are known to express tissue factor and to significantly contribute to procoagulant activity (Niemetz et al., 1974; Lorenzet et al., 1983; Semeraro et al., 1983). Previous studies showed that the expression of tumour TF in clear cell and endometrioid type ovarian cancer is significantly higher in patients who develop VTE (Abu Saadeh et al., 2013A). Plasma TF has been found to be a predictor of recurrent VTE in a mixed cancer population (Khorana et al., 2017). Enhanced procoagulant activity as measured by thrombin generation and D-Dimer was previously found to be a predictor of VTE in a gynaecological cancer population (Ward et al., 2018; Norris et al., 2020). In the mixed cancer population, elevated thrombin generation was also associated with increased risk of CAT (Ay et al., 2011; Falanga et al., 2015; Abu Saadeh et al., 2016) and recurrent VTE in the general population (Eichinger et al. 2008; Tripodi et al., 2008; Lutsey et al., 2009). However, we did not find a significant association between D-Dimer or thrombin generation and recurrent VTE in our study. Similarly Factor VIII:C, V fibrinogen and protein S were not associated with recurrent VTE in our gynaecological cancer population. This may be due to the small number of samples available for analysis in an anticoagulation naive population. Larger studies would be required to determine if
these biomarkers could predict the likelihood of recurrent VTE in patients with gynaecological cancer at all or whether they would be better applied during the cancer treatment pathway when the impact of disease progression and treatments would be incorporated.

As discussed earlier, effective prevention of both primary and recurrent VTE is dependent on a number of factors, including patient compliance. In our centre, patients undergoing major surgery for gynaecological cancer are prescribed extended post-operative LMWH prophylaxis for 28 days following surgery in accordance with international guidelines (Farge et al., 2019; Key et al., 2020). Extended prophylaxis is a burden to the patient. The current study assessed the patient experience of extended LMWH prophylaxis in the setting of gynaecologic cancer and found good patient compliance, with 86% of patients receiving injections for >26 days. This compares favourably with a similar study in hepatobiliary settings and alleviates concerns regarding poor patient compliance (Lemke et al., 2016). Preadmission education was largely lacking but despite this more than half of the patients (58%) were able to self-administer the injections. Patients with cancer are a highly motivated group of patients. Although satisfaction with LMWH injections was high (78%), most patients (86%) admitted they would much prefer an oral medication if available and as effective as LMWH. DOACs have been used in prophylaxis after orthopaedic surgery for nearly a decade. Apixaban has been shown to be more effective than enoxaparin without an increased rate of bleeding following hip and knee replacement (Raskob et al., 2012). Evidence on DOACs in VTE prophylaxis in cancer settings is emerging (Carrier et al., 2019; Khorana et al., 2019), however rates of major bleeding are still a cause for concern. In a multi-institutional RCT published recently, apixaban was found to be a safe and effective alternative to enoxaparin for thromboprophylaxis following gynaecologic cancer surgery (Guntupalli et al., 2020). As expected,
women randomised to apixaban had less pain and difficulty administering treatment (Guntupalli et al., 2020). Apixaban is almost three times cheaper than LMWH as well. Two patients in the current study discontinued LMWH injections shortly after discharge from the hospital as they could not afford to pay for it, so financial cost is important. This is certainly an area that should be discussed with policymakers moving forward. In addition, our findings should be taken into consideration in any future reimbursement decisions for patients whose medications are not covered by a medical card.

One of the main factors influencing the physicians’ decision to prescribe extended prophylaxis is the concern regarding bleeding risks. More information and larger studies will be needed on the safety profile of DOACs in the context of VTE prophylaxis following major gynaecological cancer surgeries before their routine introduction to clinical practice. However, considering patients’ preference for oral thromboprophylaxis and trends in the use of DOACs it seems that the introduction of DOACs in post-operative VTE prophylaxis is imminent.

The use of the chart review in this study may seem an old-fashioned, laborious and time-consuming method, but it was feasible in this small group and supplemented by telephone calls to patients and primary care providers in order to get the complete set of data. However, this would not be feasible in a larger scale study. In addition, our hospital’s cancer database does not include morbidities arising from cancer. This and the small sample size as well as the lack of statistical significance of some of our results (presented in Chapters 3 and 4) highlight the importance of the development of the national and international collaborations that would lead to the increased power of the study and maximise its impact. There is an urgent need for a comprehensive national database to provide complete information on all cancer related outcomes and to facilitate epidemiological research in gynaecologic cancer in Ireland. The establishment of biobanks and prospectively
maintained databases requires a collective effort but they are essential for meaningful large-scale research studies and clinical trials, especially if a similar study was being conducted in Ireland in the future. There is also a need for clinical research assistants, who could help to maintain such databases and help in collecting longitudinal blood samples following the first VTE event. This could perhaps allow to better discriminate who is at increased risk of recurrent VTE, rather than using the samples from prior to the first VTE event.

In conclusion, the current study showed that the incidence of recurrent VTE in patients with genital tract malignancies was 22%. To the author’s knowledge, it is the first study to look at the recurrent VTE rate exclusively in a gynaecological cancer population. There were no significant differences between recurrent and non-recurrent VTE groups in terms of potential risk factors, demographics, and survival. Both fibrinogen and D-Dimer levels prior to the first VTE event showed trends towards higher levels with recurrent VTE, but the biomarker study was not adequately powered to detect significant differences between the groups. The study showed good patient compliance and gained valuable insight into patients’ experience with extended post-operative LMWH prophylaxis. Satisfaction with the treatment regimen was high, despite the prevalence of minor side effects associated with the injections. Patients reported pain score was significantly lower when low molecular weight heparin was self-administered. The majority of patients would prefer oral prophylaxis if available, safe and effective.
6.1.1 Recommendations for future research:

- The present study showed a high rate of recurrent VTE in patients with gynaecological malignancy despite being prescribed standard anticoagulation treatments. Future studies should aim to better identify those at risk and work towards improved strategies for prevention.

- Although data is emerging on the use of DOACS in mixed cancer patients, data is lacking in gynaecological cancer patients. Randomised controlled trials are required to assess the effectiveness and safety profile of DOACs as primary treatment and primary and secondary prophylaxis for CAT in patients with gynaecological malignancy. This should include studies to measure patients’ acceptability and compliance.

- The study found a trend towards higher monocyte counts in the recurrent VTE group, the significance of these changes is unclear. Further investigation of the role and clinical significance of monocytes in recurrent VTE in cancer settings is warranted.

- This study observed a non-significant increase in fibrinogen and D-Dimer prior to the first clinical thrombotic event in patients with recurrent VTE compared to those with a single VTE event. Sequential measurements of those markers might be a better predictor of recurrent VTE. They are also markers of aggressiveness of disease and prognosis in gynaecological cancers. Their inclusion in baseline and serial follow-up blood tests warrants assessment.
References:


ASTEC study group, Kitchener H, Swart AM, Qian Q, Amos C, Parmar MK. Efficacy of systematic pelvic lymphadenectomy in endometrial cancer (MRC ASTEC trial): a randomised study.


Biophen FVIII,
https://www.aniara.com/mm5/PDFs/IFU/IFU_A221402-RUO.pdf


Carrier M, Altman AD, Blais N, Diamantouros A, McLeod D, Moodley U, Nguyen C, Young S, Schwenter F. Extended thromboprophylaxis with low-molecular weight heparin (LMWH)


Charo LM, Plaxe SC. Recent advances in endometrial cancer: a review of key clinical trials from 2015 to 2019. F1000Res. 2019 Jun 12;8:F1000 Faculty Rev-849. doi:


Christiansen SC, Lijfering WM, Helmerhorst FM, Rosendaal FR, Cannegieter SC. Sex difference in risk of recurrent venous thrombosis and the risk profile for a second event. J Thromb


De Stefano V, Martinelli I, Mannucci PM, Paciaroni K, Rossi E, Chiusolo P, Casorelli I, Leone G. The risk of recurrent venous thromboembolism among heterozygous carriers of the G20210A


Delluc A, Miranda S, Exter PD, Louzada M, Alatri A, Ahn S, Monreal M, Khorana A, Huisman MV, Wells PS, Carrier M. Accuracy of the


Eichinger S, Hron G, Bialonczyk C, Hirschl M, Minar E, Wagner O,


227


Ho WK, Hankey GJ, Quinlan DJ, Eikelboom JW. Risk of recurrent venous thromboembolism in patients with common thrombophilia:


AM. Primary chemotherapy versus primary surgery for newly
diagnosed advanced ovarian cancer (CHORUS): an open-label,
randomised, controlled, non-inferiority trial. Lancet. 2015 Jul

Key N, Makris M, O'Shaughnessy D, Lillicrap D. Practical
Hemostasis and Thrombosis, 2nd ed.; Wiley-Blackwell: Oxford, UK,
2009.

Key NS, Khorana AA, Kuderer NM, Bohlke K, Lee AYY, Arcelus JL,
Wong SL, Balaban EP, Flowers CR, Francis CW, Gates LE, Kakkar
AK, Levine MN, Liebman HA, Tempero MA, Lyman GH, Falanga A.
Venous Thromboembolism Prophylaxis and Treatment in Patients

Khorana AA, Connolly GC. Assessing risk of venous

Khorana AA, Francis CW, Culakova E, Lyman GH. Risk factors for
chemotherapy-associated venous thromboembolism in a
prospective observational study. Cancer. 2005 Dec

Khorana AA, Francis CW, Culakova E, Fisher RI, Kuderer NM,
Lyman GH. Thromboembolism in hospitalized neutropenic cancer
patients. J Clin Oncol. 2006 Jan 20;24(3):484-490. doi:
10.1200/JCO.2005.03.8877.

Khorana AA, Ahrendt SA, Ryan CK, Francis CW, Hruban RH, Hu
YC, Hostetter G, Harvey J, Taubman MB. Tissue factor expression,
angiogenesis, and thrombosis in pancreatic cancer. Clin Cancer


Khorana AA, Otten HM, Zwicker JI, Connolly GC, Bancel DF, Pabinger I; Subcommittee on Haemostasis and Malignancy of the Scientific and Standardization Committee of the International


King L. Subcutaneous insulin injection technique. Nurs Stand. 2003 May 7-13;17(34):45-52; quiz 54-5. doi: 10.7748/ns2003.05.17.34.45.c3388.


Lemke M, Beyfuss K, Hallet J, Coburn NG, Law CH, Karanicolas PJ. Patient Adherence and Experience with Extended Use of Prophylactic Low-Molecular-Weight Heparin Following Pancreas


Monroe DM, Hoffman M. What does it take to make the perfect clot?


Mulder FI, Bosch FTM, Young AM, Marshall A, McBane RD, Zemla TJ, Carrier M, Kamphuisen PW, Bossuyt PMM, Büller HR, Weitz JI,


Schleyer AM, Schreuder AB, Jarman KM, Logerfo JP, Goss JR. Adherence to guideline-directed venous thromboembolism


Semeraro N, Colucci M. Endothelial Cell Perturbation and Disseminated Intravascular Coagulation. In: Madame Curie Bioscience Database [Internet]. Austin (TX): Landes Bioscience;


Tesselaar ME, Romijn FP, Van Der Linden IK, Prins FA, Bertina RM, Osanto S. Microparticle-associated tissue factor activity: a link


van Es N, Louzada M, Carrier M, Tagalakis V, Gross PL, Shivakumar S, Rodger MA, Wells PS. Predicting the risk of recurrent venous thromboembolism in patients with cancer: A


Versteeg HH, Heemskerk JW, Levi M, Reitsma PH.


White RH, Zhou H, Kim J, Romano PS. A population-based study of the effectiveness of inferior vena cava filter use among patients


Appendix I
Patients’ LMWH injections logbook

Dear Patient,

Thank you very much for filling in the injection logbook.

People with cancer have a greater risk of blood clots than people without cancer and an operation can increase this risk even more. To minimise the risk of getting a blood clot you are given a blood thinning medication for 28 days following the operation. This will be given by injection under the skin.

We would like to assess your experience with the injections.

In the attached leaflet you will find more information on the risk of blood clots in patients with cancer.

You are prescribed one injection per day. In the table provided, please record the following every day:
- whether you took the injection or not
- if not, explain what was the reason for not taking it
- what was the site of the injection (Right thigh, Left thigh, Abdomen, Right arm, Left arm)
- whether you experienced any pain (picture below will help you score the level of your pain):
<table>
<thead>
<tr>
<th>Day</th>
<th>Did you take the injection Yes/No</th>
<th>If &quot;no&quot;, why not</th>
<th>Site of injection (see front page)</th>
<th>Pain (1-10) (see front page)</th>
<th>Bruising</th>
<th>Who gave the injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
- whether there was any bruising after taking the injection
- and who gave you the injection (yourself, family member, friend, public health nurse, GP)

We are also enclosing a short questionnaire to find out how happy you were with the injection course.

Date of first injection:

More comments: .................................................................

...........................................................................................................

..

Please complete and return at your convenience. Thank you.
Appendix II
Patients’ LMWH injections questionnaire

Patient’s questionnaire on anti-clotting injection following your operation

Please complete this when you finish the course of injections or after 28 days

Please return it in the self-addressed stamped envelope

1. Do you know why you were asked to take these injections?
   A. Yes ☐
   B. No ☐

   If yes please state the reason:........................................................................................................
   ......................................................................................................................................................
   ......................................................................................................................................................

2. When were you first told about the injections?
   A. On a clinic visit before you were admitted ☐
   B. On the ward before your surgery ☐
   C. On the ward after your surgery ☐
   D. Some other time ..................................................... ☐
      (Please state when)..........................................................
3. Who showed you how to give the injection?
   A. Nurse in clinic
   B. Nurse on ward
   C. Somebody else
   (Please state who showed you)……………………………………………………………

4. If you injected yourself, how easy was it to inject:
   A. Very easy
   B. Easy
   C. Slightly difficult
   D. Very difficult
   E. Impossible

5. If you did not self-inject, why did you need somebody else to do it…………………………………………………………………………………

6. Had you ever given yourself injections before?
   A. Yes
   B. No

7. If you stopped the injection sooner than recommended what was the reason for stopping?
   …………………………………………………………………………………………………

8. Did you experience any discomfort from the injections (please tick):
   A. Pain after injection Yes☐ No☐
   B. Bruising Yes☐ No☐
   C. Itching Yes☐ No☐
   D. Other
   …………………………………………………………………………………………………

9. How did you dispose of the syringes when you finished the treatment?
   A. Returned to Pharmacy Yes☐ No☐
B. Returned to Hospital ☐ Yes ☐ No

C. Other ☐ Yes ☐ No

If other please specify………………………………………………………………………………

10. How satisfied were you with the anti-clotting injections (please circle):

<table>
<thead>
<tr>
<th>Highly Satisfied</th>
<th>Satisfied</th>
<th>Partially Satisfied</th>
<th>Not Satisfied</th>
<th>Not at all Satisfied</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>0.75</td>
<td>0.50</td>
<td>0.25</td>
<td>0.00</td>
</tr>
</tbody>
</table>

11. What did you find most difficult about LMWH injection?

……………………………………………………………………………………
……………………………………………………………………………………

12. If a tablet was available instead and was as effective as the injection, would you prefer to take a tablet?

A. Yes ☐

B. No ☐

Comments

……………………………………………………………………………………
……………………………………………………………………………………

Thank you for completing this form

If you have any questions please do not hesitate to contact me
Dr Zibi Marchocki, Research Registrar, Department of Gynaecology,
St James’s Hospital
Dublin 8
Tel 01 453 18 88, zibimarchocki@dubgyn
What Can be Done to Prevent DVT/PE?

Before your surgery your doctor will assess your risk.

You should stay as active as you can up to the day of surgery. You should drink plenty of fluids until the midnight before your surgery to encourage your urine. You may put on long (thigh-length) compression stockings that are attached to a pump in order to massage your legs to keep the blood flowing' faster during and after surgery. Simple elastic (anti-embolism) stockings can also be worn. You may continue to wear the elastic stockings after discharge from hospital.

Your doctor may give you blood thinning drugs, called anticoagulants, to help prevent blood clots. This is given by injection under the skin. Depending on the type of surgery you have, you may be given an injection each day while you stay in hospital. You may be advised to continue to have this treatment for at least 4 weeks after your operation when you are at home. Your medical team will let your patients health nurse and GP know. They will advise you how long to take your treatment for and who to contact if you develop any symptoms or if your treatment causes any problems.

After your surgery you will be given plenty of fluids using an intravenous drip until you start to drink again. Your nurses and physiotherapists will get you out of bed and moving around as soon as possible.

Other Tips for Preventing DVT

- Try and stay as mobile as possible
- If you can’t move around much, do simple leg exercises every hour, such as bending and straightening your knees
- Drink plenty of fluids
- Report any symptoms to your doctor or nurse straight away

How is DVT/PE treated?

If you are diagnosed with a DVT or PE, you will be treated with a daily or twice daily injection of an anticoagulant called Low Molecular Weight Heparin. This is a blood thinning drug which helps to prevent the condition. Treatment is usually given for at least 6 months.

Gynaecological Cancer and the Risk of Blood Clots

This leaflet was produced by:

Dr. Lucy McNeice, Congregation Research Laboratory, TCD Department of Obstetrics and Gynaecology. Templemore Centre for Health Sciences, St. James’s Hospital, Dublin 8.

The assistance of the following healthcare professionals in developing this leaflet is greatly acknowledged:

Dr. Niamh Gloneen, Consultant Gynaecological Oncologist, and Reina: Surgery, St. James’s Hospital, Dublin 8.

Dr. Niamh O’Connor, Consultant Haematologist, National Centre for Hematological Disorders, St. James’s Hospital, Dublin 8.

Dr. Deirdre O’Donnell, Consultant Medical Oncologist, St. James’s Hospital, Dublin 8.

Information for Patients

Funded by the Health Research Board Knowledge Translation and Dissemination Scheme (KTD-2019-1329)
Introduction

People with cancer have a greater risk of blood clots than people without cancer. Certain types of cancer carry a higher risk and gynaecological cancer is one of these. About 50% of patients with gynaecological cancer get clots during their cancer journey but you are at particular risk following surgery. A clot in the veins is most likely to form in the thigh, lower leg or in the area between the knee and foot (the calf). A blood clot can block the normal flow of blood through the veins. If it moves to the lungs it is more serious. These symptoms can be medically treated so it is important that you report any symptoms to your doctor or nurse immediately.

There are two main types, Deep Vein Thrombosis (DVT) and Pulmonary Embolism (PE).

Warning Signs and Symptoms

Deep Vein Thrombosis (DVT)
- Pain or tenderness, in the leg
- Swelling of leg, ankle or foot
- Redness or discoloration
- Warmth

Pulmonary Embolism (PE)
- Unexplained shortness of breath
- Chest pain
- Rapid heart rate
- Light headedness or passing out
- Coughing blood mixed with phlegm

What is a DVT?
A deep vein thrombosis (DVT) is a blood clot in a deep vein. It usually affects a vein in the leg or pelvis, but sometimes can affect the arm. A DVT can block the normal flow of blood through the veins.

What is a PE?
Pulmonary embolism (PE) is a blood clot in the lungs. It often occurs when a DVT breaks free from the vein wall, travels to the lungs and blocks the blood supply to the lung. However, PE can also occur without an obvious DVT. A large PE which blocks a lot of the blood supply to the lung and obstructs the heart can be fatal.

Incidental DVT/PE
Some people are diagnosed with DVT/PE based on a scan done for other reasons and may not have any symptoms.

Why Does Having Gynaecological Cancer Increase my Risk of a Clot?

- Changes in your blood clotting system: Gynaecological cancer cells, particularly cancer cells in your body’s blood clotting system, cause changes in your blood clotting system, changes that make the blood more likely to clot by itself.
- Being less active: Sometimes your cancer or treatment can make you feel too tired or weak to move around as much as usual. Staying still increases the risk of clotting because you are not moving your legs, which normally helps keep blood moving.
- Surgery to remove your tumour: Surgery in the abdominal and pelvic area to remove your tumour causes changes in the walls of blood vessels. You will also be less mobile in the days following surgery. This will increase your risk of developing a blood clot.
- Chemotherapy: When chemotherapy kills cancer cells, the cells can release substances that cause an increase in blood clotting. Some types of chemotherapy can themselves make the blood more likely to clot. Your doctor will explain to you if the drugs you are having increase your risk of getting a blood clot.

What About Other Risk Factors for DVT/PE?

If you have had a DVT/PE in the past or have a family history of DVT/PE you are more at risk. The risk is also greater in the over 75s, in smokers and in those who are overweight. If you have a central venous catheter you are also at risk. Long journeys such as long haul flights can also increase your risk of DVT/PE.

If you are experiencing any of these symptoms it is very important that you contact your doctor or nearest emergency department IMMEDIATELY.