FACTOR V LEIDEN, PROTHROMBIN G20210A, and MTHFR C677T
POLYMORPHISMS IN CANCER PATIENTS WITH VENOUS
THROMBOEMBOLISM

by
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As members of the Practice Inquiry Committee, we certify that we have read the practice inquiry prepared by Lois Eileen Lattimore entitled FACTOR V LEIDEN, PROTHROMBIN G20210A, AND MTHFR C677T POLYMORPHISMS IN CANCER PATIENTS WITH VENOUS THROMBOEMBOLISM and recommend that it be accepted as fulfilling the practice inquiry requirement for the Degree of Doctor of Nursing Practice.

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Final approval and acceptance of this practice inquiry is contingent upon the candidate’s submission of the final copies of the practice inquiry to the Graduate College.

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SIGNED: Lois Eileen Lattimore
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DEDICATION

To my wonderful husband and loving, beautiful daughter. Thank you for your support, patience, and humor throughout this process. I love you, baby!

To my parents, who have always encouraged education, my sister’s and family for their constant support.

To Ted and Poufy – for your hours of patiently waiting for me to get off the computer.

It’s time to play ball!
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ABSTRACT

Intro/Aims: Venous thromboembolism (VTE) is a common complication in cancer patients. The role of thrombophilic polymorphisms in cancer related VTE remains poorly explored. Aim 1 of this study was to determine if Factor V Leiden (G1691A), Prothrombin (PT) G20210A or methylenetetrahydrofolate reductase (MTHFR) C677T are associated with the increased occurrence of VTE in adult oncology subjects compared to nononcology subjects. Aim 2 of this study was to determine if cancer patients with the MTHFR C677T polymorphism who are treated with antimetabolite therapy have an increased incidence of VTE compared to cancer patients who are treated with other chemotherapy.

Setting/Methods: A descriptive, comparative, retrospective chart analysis was utilized for this study in an outpatient hematology, oncology clinic in Southern Arizona. Enrolled were 100 adult subjects (age 18 – 85) with documented history of VTE (27 subjects with cancer and 73 noncancer). Subjects were evaluated for Factor V Leiden, PT G20210A, and MTHFR C677T prior to the study. Eleven subjects were treated with antimetabolite chemotherapy and 8 subjects were treated with other chemotherapy.

Results: The overall polymorphism frequency for Factor V Leiden was 21%, PT G20210A 4%, and MTHFR C677T 50%. Factor V Leiden was found in 11.1% of cancer subjects and 24.7% of noncancer subjects. Prothrombin G20210A was found in 3.7% of cancer subjects and 4.1% of noncancer subjects. MTHFR C677T was present in 25.9% of cancer subjects and 58.9% of noncancer subjects. No statistical significance was observed between subjects treated with an antimetabolite and positive for MTHFR.
C677T compared with those treated with other types of chemotherapy.

Conclusion: Analysis of the data collected in this study demonstrated overall higher rates than the expected frequencies of all polymorphism for both the cancer and noncancer patients with documented VTE. In this small retrospective study, the only significant finding was that the MTHFR C677T polymorphism was more prevalent in the noncancer group.

Currently, there are no specific guidelines for VTE prevention in the outpatient oncology setting. Identification of risk factors, including prothrombotic mutations may reduce risk of VTE and provide guidance for prophylactic treatment recommendations in the outpatient setting.
CHAPTER ONE

INTRODUCTION

Venous thromboembolism (VTE) is a common occurrence in cancer patients and the second leading cause of death in hospitalized cancer patients (Ambrus, Ambrus, Mink, & Pickren, 1975). The pathogenesis of VTE is multifactorial, and is not completely understood in cancer patients. A comprehensive understanding of the pathogenesis of VTE in cancer would allow for identification of those at increased risk for VTE who could potentially benefit from preventative measures.

Background

The pathogenesis of VTE, first described by Rudolf Virchow in 1856, is multifactorial. The theory known as Virchow’s triad involves hypercoagulability, vessel wall injury, and venous stasis (Lippi & Franchini, 2008). The association between cancer and thrombosis was first reported by Professor Armand Trousseau in 1865 (Levine, Lee, & Kakkar, 2003). The pathogenesis of thrombotic disorders in cancer follows Virchow’s theory. First, hypercoagulability is triggered by neoplastic cell activation of the clotting system. Tumor cells are able to directly activate the clotting system via thrombin production, or indirectly through the stimulation of mononuclear cells to express procoagulants. The best known tumor cell procoagulants are tissue factor and factor X activators (Levine, et al., 2003). Vascular endothelial cells may be activated by cytokines, in the presence of tumor cells, further producing tissue factor. Increased levels of hypercoagulability markers such as fibrinopeptide A, prothrombin fragment F1 + 2 and thrombin-antithrombin complexes, are evidence of enhanced clotting in cancer
patients. Secondly, the endothelium may be injured by direct vascular invasion of cancer cells, which causes a prothrombotic state. Neoplastic cells release vascular permeability factors that result in increased fibrinogen and other clotting factors at the tumor site. Finally, venous stasis allows accumulation of prothrombotic factors. Endothelial cell hypoxia, as a result of venous stasis, enhances production of additional prothrombotic molecules (Prandoni, Piccioli, & Girolami, 1999).

Risk factors for VTE can be congenital or acquired. The risk for VTE is further increased in cancer patients due to acquired factors such as: immobility, surgery, central venous catheter placement, or bulky tumor compression of the vasculature (Kakkar & Levine, 2004). Activated Protein C Resistance (APCR) is another major risk factor for venous thromboembolism, which may be congenital or acquired (Griffin, Evatt, Wideman, & Fernandez, 1993). The risk for VTE is higher in cancer patients with congenital thrombophilia. Several polymorphisms have been identified as a risk factor for VTE including: Factor V Leiden and Prothrombin G20210A mutation (Lippi & Franchini, 2008). The methylenetetrahydrofolate reductase (MTHFR) C677T mutation has been associated with VTE, however the literature is controversial (Donnelly & Rock, 1999).

*Factor V Leiden*

Factor V Leiden, discovered in the Dutch city Leiden, is the most common thrombophilic mutation (Bertina, et al., 1994). The Factor V Leiden polymorphism (G1691A) replaces the amino acid arginine (Arg) at codon 506 with the amino acid glutamine (Gln). This site is one of the activated protein C (APC) cleavage sites
(Thorelli, Kaufman, & Dahlback, 1999). This amino acid change decreases the rate of factor V inactivation by APC approximately 10-fold and results in increased thrombin production by the tenase complex (Heeb, Kojima, Greengard, & Griffin, 1995).

The minor allele of the Factor V Leiden polymorphism is present in approximately 5 percent of northern European normal populations. Significantly higher rates have been found in Greece (15 percent) and Sweden (10 percent) (Rees, Cox, & Clegg, 1995). Southern Italians were found to have an incidence similar to Greece, 15 percent (Sottilotta, et al., 2009). The highest reported frequency was among Israeli Arabs, 37 percent (Rosen, Renbaum, Heyd, & Levy-Lahad, 1999). Factor V Leiden is rarely found in non-Caucasian populations.

**Prothrombin G20210A**

Poort and colleagues (1996) first identified the second most common thrombophilic polymorphism, prothrombin G20210A. This polymorphism is located on the 3’ untranslated region of the prothrombin gene with a G to A transition at position 20210 (Lippi & Franchini, 2008). The mechanism of thrombosis related to this mutation remains unclear, however, increased levels of prothrombin have been associated with the prothrombin G20210A minor allele (Poort, Rosendaal, Reitsma, & Bertina, 1996).

The overall prevalence of prothrombin G20210A in healthy populations including Europe, the Middle East, and America is two percent. Depending on the specific geographic region evaluated, the prevalence was between one and four percent. The prothrombin variant is rare in the African and Asian population (Rosendaal, et al., 1998).
MTHFR Polymorphism

Methylenetetrahydrofolate reductase C677T is a missense mutation that results from the alteration of an alanine to valine amino acid (Hertzberg, 2005). This alteration is associated with a reduction in the enzyme activity that results in hyperhomocysteinemia and altered folate metabolism (Eroglu, Egin, Cam, & Akar, 2009).

The population frequencies of homozygous MTHFR C677T vary by ethnic group. In the United States, the reported minor allele frequency in Caucasians is 11.9%, African American 1.2%, and Hispanics 20.7%. The pooled analysis of minor allele frequency in the Japanese population was 11%. The heterozygous MTHFR C677T mutation is more common than the homozygous variant. In the United States, the pooled analysis allele frequency in Caucasians is 44.6%, African American 25.6%, and Hispanics 42% (Botto & Yang, 2000).

Antimetabolite Chemotherapy and MTHFR

Antimetabolite chemotherapy agents, such as fluorouracil, capecitabine, methotrexate, and gemcitabine, are commonly used in the treatment of solid tumors and hematologic malignancies. The activity of antimetabolite chemotherapy is dependent upon the folate cellular composition. Folate has a vital role in DNA synthesis and repair, and the enzymes involved in the folate metabolism pathway have been targeted for cancer therapy. The antimetabolites are cell cycle specific chemotherapeutic agents that interrupt normal cell metabolism, inhibit growth and ultimately induce apoptosis. The antimetabolites block the normal metabolism of folic acid into the metabolically active
form of circulating folate (Robien, 2005). Reduction in folate levels has been shown to increase homocysteine levels in cancer patients treated with antimetabolite chemotherapy. The effect of MTHFR C677T on toxicity and clinical outcomes has been studied in cancer patients treated with antimetabolite chemotherapy (DeMattia et al, 2009; Toffoli et al, 2003). Currently, however, no studies have evaluated VTE as an outcome in cancer patients treated with antimetabolite chemotherapy who have the MTHFR C677T polymorphism, which potentially further reduces folate levels and subsequently increases homocysteine levels. The elevated homocysteine levels increase the risk for venous thromboembolism.

Significance

In the United States, venous thromboembolism (VTE) is a significant health problem with more than 900,000 events annually. Over 200,000 cases of first incidence of VTE are reported in the US annually (Heit, Silverstein, et al., 2000). The incidence of VTE among Caucasians is 108 per 100,000 and 78 per 100,000 in the African-American population. Advancing age further increases the incidence to 114 per 100,000. A VTE diagnosis carries a significant mortality risk. The clinical presentation of nearly 25% of patients diagnosed with pulmonary embolism is sudden death (Heit, 2008). Approximately 25% of reported VTE patients die within 7 days of diagnosis (Heit, Silverstein, et al., 2000). The annual healthcare cost for one patient with a primary diagnosis of DVT requiring hospital admission is $10,804. The annual healthcare cost for primary diagnosis of PE hospital admission is even higher at $16,644. Readmission for recurrent DVT or PE increased the cost per event to $11,862 for DVT, but decreased the
cost to $14,722 for PE. The readmission rates for venous thromboembolism are 5.2% at 30 days and 8.3% at 90 days (Spyropoulos & Lin, 2007).

Incidence

Incidence of VTE in cancer patients

The true incidence of venous thromboembolism in cancer patients in the United States remains unknown because of the heterogeneity of the cancer population and challenges associated with conducting substantial epidemiologic studies (Lee, 2003). The annual incidence of a new VTE diagnosis in cancer patients was 48 in 100,000 or roughly 1 in 200, estimated in two population-based studies (Heit, Silverstein, et al., 2000; Lee & Levine, 2003; Silverstein, et al., 1998). The number of estimated new cancer diagnoses in 2009 was 1,479,350, excluding squamous and basal cell skin cancer (American Cancer Society, 2009). Based on the estimates of new cancer diagnoses, if 1 in 200 patients developed VTE, nearly 7400 new cases of VTE would be expected annually for this population. In comparison, another study estimated approximately 20% of the 201,000 reported new cases of VTE in the general population annually were associated with malignancy (Heit, et al., 2002). The expected annual incidence of new VTE in cancer patients would be more than 40,000 cases based on this 20% estimate. There is a great disparity in the literature regarding the estimated annual incidence of VTE in cancer patients when comparing the estimate of 7400 in one report versus 40,000 in another study, and the true incidence. The tumor type, cancer stage, treatment status, and other confounding factors also greatly influence the actual incidence and prevalence of VTE in cancer.
Prevalence

The true prevalence of VTE in cancer patients also remains uncertain. In the general population, 900,000 cases of VTE have been reported annually in the United States, however the specific prevalence in cancer patients has not been reported (Heit, 2008). Venous thromboembolism may be a first incidence or recurrence in cancer patients and is not always delineated in population studies (Lee, 2008). An estimated 30% of patients with VTE will have a recurrence within 10 years, with active cancer being a significant predictor (Heit, Mohr, et al., 2000). In 2005, the National Cancer Institute estimated 11.1 million people were living with cancer (American Cancer Society, 2009). The risk for VTE varies greatly by tumor type. Among hospitalized cancer patients, 4.3% of pancreatic cancer patients compared with 2% of any cancer type and 1% of nonmalignant patients had VTE documented on discharge (Stein, et al., 2006). The true prevalence of VTE in cancer patients is further complicated by lack of data from outpatient treatment and undiagnosed, asymptomatic VTE. The prevalence of asymptomatic VTE found in cancer patients on staging computerized tomography (CT) scans was 6.8%, in one retrospective study (Cronin, Lohan, Keane, Roche, & Murphy, 2007). Post mortem evaluation of cancer patients also notes a high incidence of undiagnosed pulmonary embolism (Svendsen & Karwinski, 1989). Cancer treatment type also affects the prevalence of VTE. A reported 11.9% of cancer patients developed VTE during treatment with the angiogenesis inhibitor, bevacizumab (Nalluri, Chu, Keresztes, Zhu, & Wu, 2008). Increased prevalence of VTE has also been reported in cancer patients treated with erythropoiesis-stimulating agents (ESA), thalidomide, hormonal
therapy, chemotherapy, radiation, and surgery (Bennett, et al., 2008; Dentali, et al., 2008; Jimenez-Zepeda & Dominguez-Martinez, 2006). Treatment modalities in cancer significantly impact the prevalence rates of VTE and contribute to the hypercoagulable state in cancer.

Statement of the Problem

Venous thromboembolism, including deep vein thrombosis (DVT) and pulmonary embolism (PE), is a common complication in cancer patients and is associated with significant morbidity and mortality (Akl, et al., 2008). The risk of death is 3 times higher in cancer patients with VTE compared with cancer patients without VTE and patients with VTE who are without cancer (Monreal & Trujillo-Santos, 2009). Malignancy alone is associated with a 4-fold increased risk for VTE compared with the general population. Cancer patients undergoing chemotherapy have a 6.5-fold increased risk for development of thrombosis (Heit, Silverstein, et al., 2000). Venous thromboembolism is also one of the leading causes of death in cancer patients receiving outpatient chemotherapy (Khorana, Francis, Culakova, Kuderer, & Lyman, 2007). The incidence of deep vein thrombosis can be as high as 28% in cancer patients with a central venous catheter (Verso & Agnelli, 2003). The cancer patient remains at high risk for VTE recurrence after initial diagnosis for as long as the cancer is active (Prandoni, et al., 1999). These genetic variants (Factor V Leiden, Prothrombin G20210A, and MTHFR C677T) may be associated with an increased risk of VTE in cancer patients.
Purpose

The purpose of this study was to determine if selected gene polymorphisms and if treatment with antimetabolite chemotherapy agents are associated with the increased occurrence of venous thromboembolism (VTE) in adult oncology patients compared to adult nononcology patients.

Specific Aims

Aim 1: To determine if the presence of polymorphisms Factor V Leiden, Prothrombin G20210A, and MTHFR C677T are associated with an increased occurrence of venous thromboembolism (VTE) in adult cancer patients compared with adult noncancer patients.

Aim 2: To determine if cancer patients with the MTHFR C677T polymorphism who are treated with antimetabolite therapy have an increased incidence of VTE compared to cancer patients who are treated with other chemotherapy.

Significance to Advanced Practice Nursing

Although VTE is a multifactorial disease, several hereditary risk factors for VTE have been established over the past 20 years (Blom, Doggen, Osanto, & Rosendaal, 2005). The contribution of these prothrombotic mutations to VTE risk in the cancer population is not well understood and requires further exploration. The reported event rate of VTE has remained fairly constant for over 25 years (Heit, Silverstein, et al., 2000). Difficulty in identifying patients at risk for VTE may contribute to this trend. Identifying risk factors, including prothrombotic mutations, in cancer patients may reduce the risk of VTE in this population. The
identification of factors that further increase a cancer patient’s risk for VTE may also
provide guidance for prophylactic treatment recommendations.

Venous thromboembolism is a significant problem in the cancer population and
the Doctor of Nursing Practice (DNP) degree prepares the advanced practice nurse for
multiple leadership roles to improve patient and healthcare outcomes (AACN, 2006).
First, the DNP is prepared for organizational and systems leadership for quality
improvement. For the cancer population, the DNP is in a position to influence
improvements in practice at the organizational and policy levels. In 2010, the National
Comprehensive Cancer Network (NCCN) established clinical practice guidelines for
venous thromboembolism in oncology. The panel consisted of multidisciplinary members
with nursing represented by one registered nurse. The DNP is the ideal candidate for
national guideline panels in their area of expertise. The DNP with extensive knowledge
of genomics, prothrombotic mutations, and evidence in the literature will contribute
significantly to a national panel for VTE guidelines. Interestingly, familial and acquired
hypercoagulability are briefly listed in the report as risk factors for venous
thromboembolism, but no further discussion or recommendations are mentioned.
Identifying increased risk factors for VTE in cancer patients and preventing recurrence
are significant safety and quality issues for the DNP. The literature in this area is limited
and the second leadership role the DNP provides is evidence-based practice through
translation and evaluation of current literature to improve health care practice and
outcomes (De Palma & McGuire, 2005). Third, the DNP is prepared to critically analyze
health policy proposals, and participate at all levels of policy making including task force
at the national level. Increasing numbers of US Preventative Task Force recommendations affecting cancer care have recently been instituted by Medicare and the DNP is prepared to influence policy makers through active participation on committees, boards or task forces. High-level decisions that ultimately affect cancer patients require interdisciplinary team collaboration. Fourth, interprofessional collaboration is necessary to accomplish the IOM (2003) mandate for safe, timely, effective, efficient, equitable and patient-centered care in healthcare. The DNP is prepared for a central leadership role within the interprofessional team to facilitate standards of care or practice changes. Finally, the DNP practices within an area of specialization in nursing. Essential nursing competencies in genetics and genomics have been established by the National Human Genome Research Institute (NIH, 2006). A current understanding of genomics in cancer care, and specifically as it relates to the risk of VTE, for the DNP is vital for optimal care of this specialized population.

Definitions

Polymorphism

A polymorphism is defined as a variation in the DNA sequence where the variation and the less common variant is present in at least 1% of the population tested. Single nucleotide changes in the DNA are the most common type of polymorphism. The single nucleotide polymorphism (SNP) is a single base pair change in a stretch of DNA. When an entire stretch of DNA contains short repeated units of nucleotide changes it is referred to as a short tandem repeat polymorphism (STRP) (Collins, 2010). Genes may have alleles, a variant of the gene. The allele is called common or wild-type when a
single prevailing allele is present in the majority of a population. When there has been a mutation, a permanent change to the DNA arrangement or nucleotide sequence, the allele is considered to be variant, mutant, or minor (Nussbaum, McInnes, & Willard, 2007).

*Factor V Leiden*

The factor V Leiden polymorphism is defined as the G1691A, which replaces the amino acid arginine (Arg) at codon 506 with a glutamine (Gln) at the activated protein C (APC) cleavage site.

*Prothrombin II*

The Prothrombin polymorphism is defined as the prothrombin G20210A, which is located on the 3’ untranslated region of the prothrombin gene with a G to A transition at position 20210.

*MTHFR*

The MTHFR polymorphism is defined as MTHFR C677T, a missense mutation that results from the alteration of an alanine to valine amino acid.

*Venous Thromboembolism*

Venous thromboembolism is defined as deep vein thrombosis or pulmonary embolism. Upper extremity thrombosis will include the superior vena cava, subclavian, innominate, and internal jugular veins in addition to deep arm veins. Confirmed portal or hepatic vein thrombosis will also be included.

*Extrinsic Pathway*

The extrinsic pathway is the portion of the coagulation cascade that is initiated by tissue factor, which enters into the blood from cells outside the blood vessel, leading to
formation of prothrombinase. It is the primary initiator of blood coagulation following tissue trauma.

Summary

Venous thromboembolism in cancer is multifactorial and incompletely understood. Clarification of the role of thrombophilic polymorphisms in cancer, cancer treatment, and VTE may improve identification of those patients at greatest risk and improve associated morbidity and mortality.
CHAPTER TWO

CONCEPTUAL FRAMEWORK AND REVIEW OF LITERATURE

Theoretical Foundation

A physiological framework was used to support this study (see Figure 1). The theoretical model involves the concept of gene polymorphisms interacting to potentially increase the incidence of VTE. The first variable, MTHFR C677T, decreases the amount of circulating folate available for homocysteine remethylation, resulting in elevated homocysteine levels. Antimetabolite chemotherapy further reduces the available folate and potentially increases the risk of hyperhomocysteinemia. Elevated homocysteine levels exert a toxic effect on the vascular endothelium which results in hemostatic changes (Falcon, Cattaneo, Panzeri, Martinelli, & Mannucci, 1994).

Hyperhomocysteinemia has been associated with VTE. Fermo, et al. (1995) noted the relative risk of venous thromboembolism in moderate homocysteinemia was 1.7 times greater than those with normal homocysteine levels.

The second variable, Factor V Leiden results in resistance of activated factor V (factor Va) to cleavage by APC and subsequent normal inactivation. The thrombogenic effect of factor Va is prolonged and potentially increases the risk of VTE. The risk of thrombosis with heterozygous Factor V Leiden is 3.5 to 8.1 times greater than the general population. The risk increases to 24 to 80 times the normal risk for homozygous Factor V Leiden (Cushman, et al., 2004; Rosendaal, Koster, Vandenbroucke, & Reitsma, 1995).

Third, Prothrombin G20210A increases the levels of prothrombin. Heterozygosity for this prothrombin polymorphism increases the risk of VTE development up to four-
fold and homozygosity, though rare, causes an even higher risk (Cushman, et al., 2004; Rosendaal, et al., 1995).

Finally, activated protein C resistance (APCR) is a major risk factor for development of venous thromboembolism (Dahlback, Carlsson, & Svensson, 1993). Acquired activated protein C resistance (aAPCR) is commonly found in cancer patients with VTE.

**Literature Review**

*Normal Blood Coagulation*

Platelet-dependent primary hemostasis and blood coagulation are significant mechanisms for prevention of bleeding. The first response to vascular injury is the formation of a platelet plug. Tissue factor (TF), when exposed to blood stimulates the coagulation system by binding to circulating Factor VIIa (FVIIa). The FVIIa-TF complex mediates the activation of Factor IX (FIX) and Factor X (FX). FIXa combines with cofactor FVIIIa in the tenase enzymatic complex which also activates FX. The prothrombinase complex FXa–Fva converts prothrombin to thrombin. The tenase and prothrombinase complexes increase the efficiency of the coagulation process resulting in the production of significant amounts of thrombin (Dahlback & Villoutreix, 2005). Thrombin has several procoagulant functions: activation of platelets, activation of FV and FVIII, conversion of fibrinogen to fibrin, and activation of FXIII that results in cross-linking of fibrin. The blood coagulation system is balanced by the anticoagulant protein C pathway.
Anticoagulant Protein C Pathway

Protein C is a vitamin K dependent, proteolytic enzyme that has anticoagulant, anti-inflammatory, and anti-apoptotic factor functions. Protein C is considered a key component in the anticoagulant protein C pathway. Protein C is activated by thrombin (T) when bound to an endothelial membrane protein, thrombomodulin (TM), which forms the T-TM complex. Activated protein C (APC) regulates the activity of coagulation cofactors FVα and FVIIIα, which downregulates the coagulation system (Dahlback & Villoutreix, 2005). There are several proteins involved in reactions of the protein C pathway. First, two cofactor proteins, Factor V and protein S, augment the anticoagulant effects of APC. Protein S has the ability to inactivate FVα and works synergistically with Factor V to regulate FVIIIα in the tenase complex. Protein S may be free form or plasma bound to the complement regulator C4b-binding protein (C4BP). The free form protein C functions as an APC cofactor. Second, endothelial protein C receptor (EPCR) stimulates the activation of protein C on the endothelial cell membrane in conjunction with the T-TM complex. Third, serine protease inhibitors have the ability to inhibit APC. Three protease inhibitors known to inhibit APC in plasma include: protein C inhibitor (PCI), α-1 antitrypsin, and α-2 macroglobulin (Dahlback, 2008).

Normally, the protein C anticoagulant pathway is significant in prevention of venous thromboembolism and microcirculation thrombosis (Nicolaes & Dahlback, 2003). Deficiencies in the protein C anticoagulant pathway lead to a prothrombotic state.
Activated Protein C Resistance and Factor V Leiden

Activated protein C resistance (APCR) is a term given for a condition of poor anticoagulant response to activated protein C and was first noted in several families in 1993 (Dahlback, et al., 1993). This condition creates a prothrombotic state that commonly results from inefficient degradation of factor V due to the mutation factor V Leiden (Ocal, Sadeghi, & Press, 1997). Factor V Leiden (G1691A) replaces Arg506 with a Gln and impairs the protein C anticoagulant pathway in two ways. First, the degradation of FVα by APC is impaired due to a reduction in the number of APC cleavage sites. Second, there is impaired degradation of FVIIIa, since the Arg506 site is not available for cleavage on the mutant Factor V. As a result, Factor V becomes an inefficient cofactor to APC and degradation of FVIIIa is impaired (Dahlback & Villoutreix, 2005). Svensson & Dahlback (1994) evaluated 104 Swedish patients with confirmed DVT and found a high prevalence of APCR in young patients when compared with healthy controls.

Prothrombin G20210A

Increased levels of prothrombin are associated with prothrombin G20210A, however the mechanism that leads these elevated levels to VTE development remains unclear. One potential mechanism is increased levels of prothrombin lead to higher rates of thrombin generation and subsequent excessive increase of fibrin clots (Poort, et al., 1996).
MTHFR C677T, Homocysteine, and Folate

MTHFR is the key enzyme involved in folate metabolism, which is essential for maintaining intracellular folate homeostasis and metabolism. MTHFR catalyzes the conversion of 5’, 10’ – methylenetetrahydrofolate to 5’-methyltetrahydrofolate (5’ – MTHF) – the circulating form of folate. This 5’-MTHF is the primary methyl donor involved in remethylation of homocysteine to methionine which is used for nucleic acid methylation (De Mattia & Toffoli, 2009). An inverse relationship exists between homocysteine and folate plasma concentrations (Kang, Wong, & Norusis, 1987).

Even a mild impairment in this remethylation pathway, seen in those with the thermolabile variant MTHFR C677T, can result in increased fasting plasma levels of homocysteine. (Kang, Zhou, Wong, Kowalisyn, & Strokosch, 1988). Kang and colleagues (1987) found that the majority of patients with homocysteinemia had decreased serum folate levels. Homocysteine is metabolized in two pathways, remethylation and transsulfuration. The remethylation pathway results in the formation of methionine and requires folate and vitamin B12 (Selhub, 1999). Folate supports nucleotide synthesis and methylation reactions. In the presence of low folate status, MTHFR C677T homozygous carriers had high homocysteine plasma concentrations (Jacques, et al., 1996). One study found that vascular complications associated with homocysteinemia are most likely a result of the toxic effect exerted on the vascular wall that cause hemostatic changes (Falcon, et al., 1994).
Relationship between MTHFR, VTE, and cancer patients

The pathogenesis of thrombosis in cancer patients is multifactorial and dependent on clinical, environmental and genetic factors (Decousus, et al., 2007). Cancer has an effect on each element of Virchow’s triad including hypercoagulability, vascular injury, and venous stasis. Hypercoagulability in cancer induced by procoagulant expression, cytokine release and interaction with endothelial cells, monocytes and platelets, has been previously discussed. A meta-analysis of studies on MTHFR, homocysteine and the risk of VTE demonstrated a modest association and support for causality (Den Heijer, Lewington, & Clarke, 2005). The role of genetic mutations, such as Factor V Leiden, Prothrombin G20210A, and MTHFR, in the occurrence of VTE in cancer patients, however is controversial and has not been widely evaluated (Eroglu, Egin, Cam, & Akar, 2009; Ramacciotti, et al., 2003). Methylenetetrahydrofolate reductase is an enzyme involved in the folate cycle required for remethylation of homocysteine to methionine (Eldibany & Caprini, 2007). A deficiency of folate has been associated with homocysteinemia (Kang, et al., 1987). The thermolabile variant MTHFR C677T is associated with a ten-fold increased risk of elevated homocysteine levels and altered folate metabolism with up to a 50% reduction of normal enzyme activity compared with wild-type MTHFR (D'Angelo, Mazzola, & Fermo, 2003; Lathrop Stern, et al., 2003). Elevated levels of homocysteine have major associations with vascular injury, increased TF expression, and inhibition of the activated protein C system, that promote thrombosis (Undas, Brozek, & Szczylik, 2005).
There are parallels between the prothrombotic properties of hyperhomocysteinemia and malignant cells, described previously (See Figure 2). Tissue factor expression by monocytes is increased in the presence of increased homocysteine concentration (Khajuria & Houston, 2000). Endothelial cells also increase TF activity by as much as 100% in response to slightly elevated homocysteine levels (Fryer, Wilson, Gubler, Fitzgerald, & Rodgers, 1993). Homocysteine has also been shown to decrease TM activity in endothelial cells, thereby inhibiting the activation of the protein C pathway (Hayashi, Honda, & Suzuki, 1992).

Vascular injury is associated with both hyperhomocysteinemia and malignancy, increasing the risk for VTE. The blood vessel wall may be injured by direct invasion of tumor cells, as well as solid tumor compression of vessels. Endothelial cell dysfunction and apoptosis have been implicated in homocysteine induced vascular wall injury as a major mechanism for thrombogenesis (Undas, et al., 2005). Endothelium dysfunction also impairs vessel dilation, most likely due to increased vascular oxidative stress (Weiss, 2005).

Hyperhomocysteinemia and cancer have been established as independent risk factors for VTE in the literature (Buller, et al., 2004). The data, however, has been inconsistent regarding the risk of VTE related to elevated homocysteine in combination with genetic thrombophilic factors (De Stefano, Casorelli, Rossi, Zappacosta, & Leone, 2000). The relationship between MTHFR, VTE and cancer remains poorly explored.
**Factor V Leiden, Prothrombin G20210A, MTHFR, Cancer, and VTE**

The prevalence of Factor V Leiden, Prothrombin G20210A, and MTHFR in cancer patients with VTE was evaluated in three studies. In a case control study that included the three polymorphisms, only Factor V Leiden was found to be significant between cancer patients with VTE (31.7%) and cancer patients without VTE (1.6%) (Eroglu, et al., 2009). In a prospective study, the MTHFR variant allele was found in 53.1% of cancer patients with VTE and 60.5% in cancer patients without VTE. Factor V Leiden (1.5% and 2.7%) and prothrombin G20210A (1.5% and 1.3%) for cancer patients with and without VTE, respectively (Ramacciotti, et al., 2003). Pihusch and colleagues (2002) demonstrated an increased incidence of Factor V Leiden in patients with gastrointestinal carcinoma and VTE (17.9%) versus without VTE (4.8%). Prothrombin G20210A rates were also higher in cancer patients with VTE than without VTE (10.7% and 4.8%, respectively). Only homozygous MTHFR C677T was evaluated, however, the prevalence was higher in cancer patients without VTE than with VTE (10.2% and 7.1%, respectively).

The prevalence of Factor V Leiden and Prothrombin G20210A was evaluated in several studies of cancer patients with VTE. In a case control study, only Factor V Leiden was found to be significantly increased in cancer patients with thrombosis compared with cancer patients without VTE (30.2% and 3.7%, respectively) (Eroglu, Ulu, Cam, Kurtman, & Akar, 2007). Both Factor V Leiden and the prothrombin G20210A mutations were found to increase the risk of VTE in cancer patients in another case control study (Blom, et al., 2005). Kennedy and colleagues (2005) associated Prothrombin G20210A
with increased risk of VTE in cancer patients but only a weak association for Factor V Leiden. A cohort study of breast cancer patients with subclavian vein thrombosis and a central venous catheter found 20% were heterozygous for Factor V Leiden compared with 4% without thrombosis. However, only one case of Prothrombin G20210A with thrombosis (4%) was identified versus controls without VTE (0%) (Mandala, et al., 2004). Abramson (2006) did not associate either Factor V Leiden or Prothrombin G20210A with VTE in breast cancer patients on Tamoxifen in the National Surgical Adjuvant Breast and Bowel Project’s Breast Cancer Prevention project.

Two studies evaluated the association of Factor V Leiden and VTE in cancer patients. A meta-analysis revealed Factor V Leiden has a higher prevalence in cancer patients with VTE (7.3%) compared with cancer patients without VTE (4.6%) (Eroglu, Sertkaya Karasoy, Eroglu, & Akar, 2008). Haim and colleagues (2001) found the presence of Factor V Leiden was not significantly different in cancer patients with VTE (2%), cancer patients without VTE (7%), and normal controls (4%). The presence of acquired activated protein C resistance, however, was common in cancer patients with VTE (54%), compared with cancer patients without VTE (17%), and VTE without cancer (19%). Most studies have evaluated gene polymorphisms individually, however, more could be learned by investigating the potential association between the presence of a combination of gene polymorphisms and VTE. (See table 10 in appendix for review of literature).
Antimetabolite Chemotherapy and MTHFR C677T

Antimetabolite chemotherapy is commonly given in cancer treatment regimens. The most common agents used are 5-fluorouracil, capecitabine, and methotrexate. In the presence of MTHFR C677T, further reduction of folate and therefore, increased homocysteine levels during antimetabolite therapy, have been noted in the literature. These studies focused on toxicity of the antimetabolites rather than clotting, but documented elevated homocysteine levels after therapy was initiated. One study noted, MTHFR C677T with reduced 5-methyl-tetrahydrofolate may have increased homocysteine levels which increases methotrexate toxicity (Gemmati, et al., 2007).

Another study found that ovarian cancer patients who were receiving methotrexate, and were homozygous for MTHFR C677T had significantly elevated homocysteine levels after 21 days of treatment and 77% had severe toxicity, grade 3 / 4 compared with other genotypes. The severe toxicity was associated with high homocysteine plasma levels, but the presence of VTE was not included in this study (Toffoli, et al., 2003).

Acquired Activated Protein C Resistance and VTE

Activated protein C resistance is considered a major risk factor for development of venous thromboembolism (Dahlback, et al., 1993). However, acquired activated protein C resistance (aAPCR), noted in the absence of the factor V Leiden mutation is also a risk factor for venous thromboembolism (Griffin, et al., 1993). One study showed 52% to 64% of patients with VTE demonstrated APC resistance (Griffin, et al., 1993) Another study, showed acquired activated protein C resistance was more prevalent in cancer patients with VTE than nonmalignant
patients with VTE (54% versus 19%) (Haim, et al., 2001). One possible mechanism for increased expression of aAPCR in cancer patients, is an interaction with cancer procoagulants and tissue factor (Haim, et al., 2001). Increased levels of factor V and factor VIII were identified in one study as potential mechanisms for aAPCR in cancer patients (Sarig, Michaeli, Lanir, Brenner, & Haim, 2005). In another study, APCR was associated with increased levels of factor VIII and fibrinogen in cancer patients with thrombosis (Green, Maliekel, Sushko, Akhtar, & Soff, 1997).

Risk of VTE with more than one polymorphism

The combined effects of thrombophilic polymorphisms and VTE have been evaluated in the noncancer population. One combined study revealed 62% of patients with idiopathic VTE had one or more prothrombotic polymorphisms, including Factor V Leiden, Prothrombin G20210A, and MTHFR C677T, compared with 22% of controls. Each of the polymorphisms was found to be an independent risk factor for idiopathic VTE, with factor V Leiden the highest risk and homozygous MTHFR the lowest. More than 16% of the patients had two or more of the polymorphisms versus 0.9% of controls. The significance of the results in this study may be limited by the population studied, however, since 75% of the women in the study were pregnant, postpartum or using oral contraceptives (Salomon, et al., 1999). A pooled analysis of mainly European case control studies found the risk of VTE significantly higher in patients with double heterozygosity of factor V Leiden and Prothrombin G20210A and a first episode of VTE at a younger age (Emmerich, et al., 2001). Patients with APCR and hyperhomocysteinemia had a 10-fold risk of VTE development when compared with
patients with neither of these abnormalities (Selhub & D'Angelo, 1998). Cattaneo et al., (1997) found patients with APCR, found in factor V Leiden, mild hyperhomocysteinemia and MTHFR C677T are at a higher risk for DVT than for either factor alone. A synergistic effect between the two polymorphisms was predominant in patients with spontaneous DVT compared to those with DVT related to surgery or trauma.

Limitations of Current Research

Reliable assessment of the risk of VTE in cancer patients related to single or combined prothrombotic polymorphisms is difficult as many of the studies had small samples. No studies evaluating the combination of Factor V Leiden, Prothrombin G20210A, MTHFR C677T polymorphisms, and aAPCR, in cancer or noncancer patients with VTE are documented in the literature.

Gaps in Knowledge

Both the antimetabolite chemotherapy and the MTHFR C677T polymorphism have documented decreased folate levels and increased homocysteine levels, which increases the risk of VTE. There are no studies currently in the literature evaluating the relationship of cancer patients, treated with antimetabolite chemotherapy, with VTE and MTHFR C677T polymorphisms.

Summary

The precise role of prothrombotic polymorphisms in cancer patients with VTE remains controversial. All three polymorphisms included in this study have potential direct or indirect impact on the extrinsic, common or APC pathways, thereby increasing the risk of thrombosis.
CHAPTER THREE
RESEARCH METHOD

Study Design

*Descriptive comparative design*

A descriptive comparative, retrospective design was used to determine if the polymorphisms of interest were associated with the occurrence of VTE in adult oncology patients. In this study, cancer and noncancer patients with a history of VTE were evaluated for Factor V Leiden, Prothrombin G20210A, MTHFR C677T, and fasting homocysteine level. The two groups were compared for the presence of Factor V Leiden, Prothrombin G20210A, and MTHFR C677T to determine if there was a significant increase in the polymorphisms for the cancer group with VTE. In addition, the incidence of VTE was compared between cancer patients treated with antimetabolite therapy and other chemotherapy, who were positive for the MTHFR C677T polymorphism.

Setting

The setting for this study was an outpatient hematology oncology clinic in southern Arizona.

Sample

The study sample of adults age 18 - 85, were identified by ICD-9 codes for cancer and venous thromboembolism and anticoagulation flowsheet patient lists in an outpatient hematology oncology clinic in Southern Arizona for the years 2004-2009. The sample included 100 total subjects, 27 cancer and 73 noncancer. There were 11 subjects with
VTE treated with antimetabolite chemotherapy and 8 subjects treated with other chemotherapy.

Inclusion criteria:

1. Adult cancer and noncancer patient with a documented history of venous thromboembolism in an outpatient hematology oncology clinic.


Exclusion criteria:

1. History of venous thromboembolism without confirmation with venous Doppler or CT scan verification of thrombosis.

Protection of Human Subjects

Approval for this study was obtained from the Human Subjects Protection Program Internal Review Board prior to data collection. Approval was also obtained from the Site Review Authority at the University Medical Center and Arizona Cancer Center. Human subject information collected during the chart review was protected by identification numbers and a password protected file.

Data Collection Procedures

Retrospective chart analysis included: demographics, past medical history, cancer type, cancer stage, cancer treatment, chemotherapy administration, antimetabolite chemotherapy administration, central venous access device, treatment dates in relation to VTE, history of recurrent VTE, and documentation of confirmed DVT (upper extremity,
lower extremity, or other site) or PE. Confidentiality of subjects was maintained in their records and in the results.

Data were collected from each subject’s medical record for gene polymorphisms, homocysteine levels and antimetabolite therapy. Four laboratory tests were previously collected and measured at a standardized laboratory prior to the study:

**Factor V Leiden:** Positive for heterozygous, homozygous, or wild type variant

**Prothrombin G20210A:** Positive for heterozygous, homozygous, or wild type

**MTHFR C677T:** Positive for heterozygous, homozygous, or wild type

**Homocysteine level:** Fasting level of homocysteine was classified as:

- Normal: 4-15 microcmoles per liter (µmol/L)
- Mild – moderate hyperhomocysteinemia: 16 – 30 µmol/L
- Moderate – severe hyperhomocysteinemia: 31-100 or greater µmol/L

**Data Analysis**

The data were analyzed with SPSS statistical software package version 16.0. Descriptive statistics were used to describe the distribution of the gene polymorphisms.

**Aim 1:** To determine if the presence of polymorphisms Factor V Leiden, Prothrombin G20210A, and MTHFR C677T are associated with an increased incidence of venous thromboembolism (VTE) in adult cancer patients compared with adult noncancer patients, categorical variables were tested with Chi-square. Descriptive statistics were used to report the frequency of the polymorphisms.

**Aim 2:** To investigate if cancer patients treated with antimetabolite therapy and have the polymorphism MTHFR C677T have an increased incidence of VTE
compared with those without the MTHFR C677T polymorphism. Chi square analysis was used to compare the 2 groups. The sample size was not adequate to support association so the gene polymorphism distribution was described with descriptive statistics.

Limitations of this Study

There were several limitations of this study. The relatively small sample size reduced statistical power. There were many confounding factors in cancer patients that may affect VTE risk including: multiple cancer types, cancer stages, and treatment status. Finally, APCR status, whether congenital or acquired, was not measured in this study.

Summary

This retrospective, descriptive comparative study investigated the association of prothrombotic polymorphisms with the risk of VTE in cancer and noncancer patients at an outpatient hematology oncology clinic in Southern Arizona. Data analysis described individual and combined polymorphism risk for VTE and effect of antimetabolite therapy for MTHFR C677T positive cancer patients and VTE risk.
CHAPTER FOUR

RESULTS

This chapter describes characteristics of the sample and addresses the research questions. The Statistical Package for the Social Sciences (SPSS) 16.0 was used to analyze the collected data. Summary statistics, chi-square analysis and Fisher’s exact test were included in the data analysis.

Sample Characteristics

The data were collected for 100 subjects (27 had a cancer diagnosis and 73 did not have cancer) with documented VTE, in this study. The mean age was 56.9, age range of 18-85, and 48 percent of total subjects were over the age of 60. The cancer patients in this study were all over the age of 48 years. Increased age was noted in the cancer patients with 88.8% over the age of 60 and more than 51% were over the age of 70 years. The noncancer patients included significantly younger patients with 46.5% under the age of 50 and 28.7% under the age of 40 years.

The total sample included 27 males and 73 females. Eighty seven (87%) of total subjects were Caucasian, 2 (2%) African American, 10 (10%) Hispanic, and 1 (1%) Asian. For the overall sample, pulmonary embolism was documented for 12 (12%) subjects, DVT included 44 (44%), DVT and PE 30 (30%), and other sites noted in 14 (14%) subjects. Frequencies for positive family history of VTE were observed for 14 (14%), negative family history for 73 (73%), and unknown family history for 13 (13%). Forty of 100 subjects (40%) had a history of recurrent VTE, 12 of 27 (44.4%) cancer patients and 28 of 73 (38.4%) noncancer patients. The past medical history for the overall
sample included: hypertension 33%, hyperlipidemia 19%, myocardial infarction or coronary artery disease 11%, cerebrovascular accident (CVA) 16%, and diabetes mellitus 13%. Eleven subjects (40.7%) with a diagnosis of cancer were treated with an antimetabolite chemotherapy agent. Variable frequencies are detailed in table 1.

Table 1. Demographic and Variable Frequencies

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total Sample (n = 100)</th>
<th>Cancer (n = 27)</th>
<th>Noncancer (n = 73)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Mean = 56.9</td>
<td>Mean = 69.4</td>
<td>Mean = 51.5</td>
</tr>
<tr>
<td></td>
<td>Range 18 – 85</td>
<td>Range 48 - 85</td>
<td>Range 18 - 85</td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>27 (27%)</td>
<td>6 (22.2%)</td>
<td>21 (28.8%)</td>
</tr>
<tr>
<td>Female</td>
<td>73 (73%)</td>
<td>21 (77.8%)</td>
<td>52 (71.2%)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>87 (87%)</td>
<td>25 (92.6%)</td>
<td>62 (84.9%)</td>
</tr>
<tr>
<td>African Am.</td>
<td>2 (2%)</td>
<td>0 (0%)</td>
<td>2 (2.7%)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>10 (10%)</td>
<td>1 (3.7%)</td>
<td>9 (12.3%)</td>
</tr>
<tr>
<td>Asian</td>
<td>1 (1%)</td>
<td>1 (3.7%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>VTE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PE</td>
<td>12 (12%)</td>
<td>3 (11.1%)</td>
<td>9 (12.3%)</td>
</tr>
<tr>
<td>DVT</td>
<td>44 (44%)</td>
<td>11 (40.7%)</td>
<td>33 (45.2%)</td>
</tr>
<tr>
<td>DVT/PE</td>
<td>30 (30%)</td>
<td>9 (33.3%)</td>
<td>21 (28.8%)</td>
</tr>
<tr>
<td>Other site</td>
<td>14 (14%)</td>
<td>4 (14.8%)</td>
<td>10 (13.7%)</td>
</tr>
<tr>
<td>Fam hx VTE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>14 (14%)</td>
<td>0 (0%)</td>
<td>14 (19.2%)</td>
</tr>
<tr>
<td>No</td>
<td>73 (73%)</td>
<td>17 (63%)</td>
<td>56 (76.7%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>13 (13%)</td>
<td>10 (37%)</td>
<td>3 (13%)</td>
</tr>
<tr>
<td>Recurrent VTE</td>
<td>40 (40%)</td>
<td>12 (44.4%)</td>
<td>28 (38.4%)</td>
</tr>
<tr>
<td>Medical History</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>33 (33%)</td>
<td>12 (44.4%)</td>
<td>21 (28.8%)</td>
</tr>
<tr>
<td>Lipid elevation</td>
<td>19%</td>
<td>6 (22.2%)</td>
<td>13 (17.8%)</td>
</tr>
<tr>
<td>MI/CAD</td>
<td>11%</td>
<td>0 (0%)</td>
<td>11 (15.1%)</td>
</tr>
<tr>
<td>CVA</td>
<td>16%</td>
<td>5 (18.5%)</td>
<td>11 (15.1%)</td>
</tr>
<tr>
<td>DM</td>
<td>13%</td>
<td>3 (11.1%)</td>
<td>10 (13.7%)</td>
</tr>
<tr>
<td>Antimetabolite</td>
<td>11 (40.7%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Aim 1

Chi-square analysis and Fisher’s Exact Test were used to determine if the presence of polymorphisms Factor V Leiden, Prothrombin G20210A, and MTHFR C677T are associated with an increased occurrence of venous thromboembolism (VTE) in adult cancer patients compared with adult noncancer patients. All three polymorphisms were more common in the noncancer group compared with the cancer group, in this study, and were higher than the general population frequencies. Factor V Leiden and prothrombin G20210A frequencies (11.1% and 3.7%, respectively) were higher than the general population expected frequencies in the cancer group, however, MTHFR C677T was lower (23.9%) than the expected frequency.

The result for noncancer subjects with VTE and MTHFR C677T was statistically significant using Fisher’s Exact one-tailed test (p = .003) compared with cancer subjects positive for MTHFR C677T. No statistical significance was observed for the Factor V Leiden or prothrombin G20210A polymorphisms in cancer and noncancer groups (p > .05).

Table 2. Polymorphism Frequency

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Cancer (n = 27)</th>
<th>Noncancer (n = 73)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVL</td>
<td>3 (11.1%)</td>
<td>18 (24.7%)</td>
<td>.112</td>
</tr>
<tr>
<td>PT G20210A</td>
<td>1 (3.7%)</td>
<td>3 (4.1%)</td>
<td>.706</td>
</tr>
<tr>
<td>MTHFR C677T</td>
<td>7 (25.9%)</td>
<td>43 (58.9%)</td>
<td>.003</td>
</tr>
</tbody>
</table>

**Homzygous Polymorphisms**

Three of 21 subjects (14%) positive for factor V Leiden were homozygous for
FVL and 4 of 50 (8%) subjects were homozygous for MTHFR C677T.

Table 3. Homozygous Polymorphism

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Overall (n = 100)</th>
<th>Cancer (n = 27)</th>
<th>Noncancer (n = 73)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homozygous FVL</td>
<td>3 (3%)</td>
<td>1 (3.7%)</td>
<td>2 (2.7%)</td>
</tr>
<tr>
<td>Homozygous MTHFR</td>
<td>4 (4%)</td>
<td>1 (3.7%)</td>
<td>3 (4.1%)</td>
</tr>
</tbody>
</table>

Aim 2

To determine if cancer subjects with the MTHFR C677T polymorphism who were treated with antimetabolite therapy have an increased incidence of VTE compared to cancer subjects who were treated with other types of chemotherapy, Fisher’s exact test was utilized due to the small sample. No statistical significance was observed between MTHFR C677T positive subjects who were treated with an antimetabolite compared to other types of chemotherapy (p = .337).

A total of 11 subjects (40.7%) were treated with antimetabolite chemotherapy. Five of 11 subjects (45.5%) treated with antimetabolite therapy were positive for the MTHFR C677T polymorphism. Three of 11 subjects (27.3%) were treated with more than one antimetabolite and these subjects were also positive for the MTHFR C677T mutation. A total of 8 patients were treated with chemotherapy that did not include an antimetabolite. Two of the eight chemotherapy patients (25%) were also positive for MTHFR C677T.

Table 4. Antimetabolite Therapy

<table>
<thead>
<tr>
<th>MTHFR C677T</th>
<th>Antimetabolite (n = 11)</th>
<th>Chemotherapy (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>5 (45.5%)</td>
<td>2 (25%)</td>
</tr>
<tr>
<td>Negative</td>
<td>6 (54.5%)</td>
<td>6 (75%)</td>
</tr>
</tbody>
</table>
Cancer Patients

The 27 cancer patients included 6 males (22.2%) and 21 females (77.8%). The age range for these subjects was 48 to 85. A known family history of VTE was not observed in any of the cancer patients (0%). There were 12 patients (44.4%) with a history of recurrent VTE. The most common cancer types in this study were breast (29.6%), gastrointestinal (11.1%), and lymphoma (11.1%). Cancers listed as other included; appendiceal, renal, adrenal, sarcoma, endometrial, and head and neck. Thirty seven percent of cancer patients had received chemotherapy within 3 years of the VTE diagnosis. Metastatic disease was present in 11 cancer patients (40.7%). The association of antimetabolite chemotherapy and MTHFR status is further described by cancer type in Table 5. Three of eight (37.5%) breast cancer subjects were positive for both the MTHFR C677T polymorphism and treatment with an antimetabolite. One of 2 (50%) subjects diagnosed with pancreatic cancer was MTHFR C677T positive and received antimetabolite treatment.

Table 5. Cancer Type

<table>
<thead>
<tr>
<th>Cancer Diagnosis</th>
<th>(n = 27)</th>
<th>Antimetabolite (n = 11)</th>
<th>MTHFR (n = 50)</th>
<th>MTHFR and Antimetabolite</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>8 (29.6%)</td>
<td>5 (62.5%)</td>
<td>4 (50%)</td>
<td>3 (37.5%)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>3 (11.1%)</td>
<td>1 (33.3%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>3 (11.1%)</td>
<td>0</td>
<td>2 (66.6%)</td>
<td>0</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>2 (7.4%)</td>
<td>1 (50%)</td>
<td>1 (50%)</td>
<td>1 (50%)</td>
</tr>
<tr>
<td>Prostate</td>
<td>2 (7.4%)</td>
<td>0</td>
<td>1 (50%)</td>
<td>0</td>
</tr>
<tr>
<td>Lung</td>
<td>1 (3.7%)</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Leukemia</td>
<td>1 (3.7%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Myeloma</td>
<td>1 (3.7%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ovarian</td>
<td>1 (3.7%)</td>
<td>1 (100%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>6 (22.2%)</td>
<td>3 (50%)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
**Noncancer Patients**

The noncancer diagnosis group included 73 subjects (21 males and 52 females) and 27.3% were under the age of 40. The VTE was associated with oral contraceptive use or pregnancy in 10% of noncancer subjects. Twenty-eight (38.4%) of the 73 noncancer subjects had a history of recurrent VTE and 14 (19.2%) had a family history of VTE.

Table 6. Oral Contraceptive and Pregnancy Related VTE

<table>
<thead>
<tr>
<th>Variable</th>
<th>Oral Contraceptive</th>
<th>Pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noncancer (n = 73)</td>
<td>5 (6.8%)</td>
<td>3 (4.1%)</td>
</tr>
<tr>
<td>Age Range for VTE event</td>
<td>30 – 46</td>
<td>30 - 45</td>
</tr>
<tr>
<td>Factor V Leiden</td>
<td>1 (20%)</td>
<td>1 (33.3%)</td>
</tr>
<tr>
<td>Prothrombin G20210A</td>
<td>0</td>
<td>1 (33.3%)</td>
</tr>
<tr>
<td>MTHFR C677T</td>
<td>3 (60%)</td>
<td>3 (100%)</td>
</tr>
</tbody>
</table>

*Expected Polymorphism Frequencies*

Factor V Leiden is present in approximately 5 percent of northern European normal populations (Rees, et al., 1995). Notably higher rates of FVL were observed in this study with 21% of overall subjects (18% heterozygous and 3% homozygous). The overall prevalence of prothrombin G20210A in healthy populations in America is 2% (Rosendaal, et al., 1998). Similar results for the frequency of prothrombin G20210A were observed in this study, 4% overall. In the United States, the pooled analysis allele frequency for heterozygous MTHFR C677T in Caucasians is 44.6% (Botto & Yang, 2000). A higher frequency for the MTHFR C677T polymorphism was observed in the study population, 50% overall (46% heterozygous and 4% homozygous).
Table 7. Polymorphism Frequencies

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>General Population Expected Frequency</th>
<th>Study Population n = 100 Observed Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVL</td>
<td>5%</td>
<td>21%</td>
</tr>
<tr>
<td></td>
<td>Heterozygous</td>
<td>18%</td>
</tr>
<tr>
<td></td>
<td>Homozygous</td>
<td>3%</td>
</tr>
<tr>
<td>PT G20210A</td>
<td>2%</td>
<td>4%</td>
</tr>
<tr>
<td>MTHFR C677T</td>
<td>44.6%</td>
<td>50%</td>
</tr>
<tr>
<td></td>
<td>Heterozygous</td>
<td>46%</td>
</tr>
<tr>
<td></td>
<td>Homozygous</td>
<td>4%</td>
</tr>
</tbody>
</table>

Additional Results

Hyperhomocysteinemia is the proposed mechanism for increased risk of VTE formation in MTHFR C677T positive subjects. The thermolabile variant MTHFR C677T is associated with increased homocysteine levels, and homozygous carriers potentially have high homocysteine in the presence of low folate (Jacques, et al., 1996).

*Homocysteine*

Homocysteine levels were normal for 95 of 100 (95%) of subjects, in this study. The homocysteine levels were normal for all cancer patients and moderately to severely elevated in 5 of 73 (6.8%) noncancer subjects. Four subjects of the total sample were homozygous for MTHFR C677T patients. Three of these 4 subjects (75%) had a normal homocysteine level and one subject (25%) had a high homocysteine level. The one subject homozygous for MTHFR C677T with a high homocysteine level was in the noncancer group. One of four (25%) subjects homozygous for MTHFR C677T was in the cancer group, and the homocysteine level was normal.
Table 8. Homocysteine

<table>
<thead>
<tr>
<th>Homocysteine</th>
<th>Overall (n = 100)</th>
<th>Cancer Patients (n = 27)</th>
<th>MTHFR positive (n = 50)</th>
<th>Homozygous MTHFR (n = 4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>95 (95%)</td>
<td>27 (100%)</td>
<td>47 (94%)</td>
<td>3 (75%)</td>
</tr>
<tr>
<td>Moderate</td>
<td>2 (2%)</td>
<td>0</td>
<td>2 (4%)</td>
<td>0</td>
</tr>
<tr>
<td>High</td>
<td>3 (3%)</td>
<td>0</td>
<td>1 (2%)</td>
<td>1 (25%)</td>
</tr>
</tbody>
</table>

*Combined Polymorphisms*

A combination of polymorphisms was observed in this study. Factor V Leiden was present with another polymorphism in 11 of 21 subjects (52.4%). Of the Factor V Leiden positive subjects, 9 of 21 (42.9%) also were MTHFR C677T positive. All 4 subjects with the PT G20210A polymorphism also had a second genetic variant, 2 (50%) were Factor V Leiden positive and 2 (50%) were MTHFR C677T positive. Twenty two percent of the MTHFR C677T carried an additional polymorphism. The presence of more than one polymorphism in this study was found in 12 of these 13 subjects in the noncancer group (92.3%) compared with one of these 13 in the cancer group (7.7%).

Table 9. Combined Polymorphisms

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Overall (n = 100)</th>
<th>FVL</th>
<th>PT G20210A</th>
<th>MTHFR C677T</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVL</td>
<td>21 (21%)</td>
<td>2   (9.5%)</td>
<td>9 (42.9%)</td>
<td></td>
</tr>
<tr>
<td>PT G20210A</td>
<td>4 (4%)</td>
<td>2   (50%)</td>
<td>2 (50%)</td>
<td></td>
</tr>
<tr>
<td>MTHFR C677T</td>
<td>50 (50%)</td>
<td>9   (18%)</td>
<td>2 (4%)</td>
<td></td>
</tr>
</tbody>
</table>

*Homozygous Factor V Leiden*

Three subjects in this study were homozygous for Factor V Leiden and all were female. Two subjects had recurrent VTE and one subject carried a second polymorphism,
MTHFR C677T. One of these 3 subjects homozygous for Factor V Leiden, was a 48 year old female with recurrent VTE, diagnosed with metastatic breast cancer. This subject’s cancer treatment included multiple therapies that increase the risk for VTE: hormonal therapy, erythropoietin stimulating agent, chemotherapy, surgery, radiation, and central venous catheter.

**Recurrent VTE**

Venous thromboembolism recurrence was noted in 40 (40%) of total subjects. In this subset of 40 subjects, 30% were male, 70% female, and 30% were diagnosed with cancer. The polymorphism frequency for Factor V Leiden was 30% with 5% homozygous, PT G20210A 5%, and MTHFR C677T 47.5% with 7.5% homozygous for the variant allele. Homocysteine levels were high in 2.5%, and moderately elevated in 5% of subjects. Past medical history was noted for coronary artery disease in 7.5%, hyperlipidemia 10%, cerebrovascular accident 12.5%, and diabetes mellitus in 15%.

**Summary**

Analysis of the data collected in this study demonstrated overall higher rates than the expected frequencies of all polymorphism for both the cancer and noncancer patients with documented VTE. Statistical significance was only found for the MTHFR C677T polymorphism in the noncancer group.
CHAPTER FIVE

DISCUSSION

The primary purpose of this study was to compare the Factor V Leiden, prothrombin G20210A, and MTHFR C677T polymorphism frequencies in cancer and noncancer subjects with documented VTE in the ambulatory setting. The secondary purpose of this study was to determine if cancer subjects with the MTHFR C677T polymorphism who were treated with antimetabolite chemotherapy had an increased risk of VTE compared with subjects treated with other types of chemotherapy. A physiologic framework was utilized to support this study. The findings in this study, comparison with previous research studies and the implications for nursing are discussed in this chapter.

Aim 1

The first aim of this study was to determine if the presence of polymorphisms Factor V Leiden, Prothrombin G20210A, and MTHFR C677T were associated with an increased occurrence of venous thromboembolism (VTE) in adult cancer patients compared with adult noncancer patients.

Factor V Leiden

Factor V Leiden, the most common thrombophilic mutation, is present in approximately 5% of the general population. The overall, cancer patient, and noncancer patient frequencies for Factor V Leiden in this study were notably higher than the general population expected frequencies (21%, 11%, and 24%, respectively). Eroglu and colleagues (2009) found higher rates for FVL in cancer patients with VTE (31%) compared with the findings in this study (11%). Eroglu et al. (2007) demonstrated an
increased incidence of FVL in cancer patients with VTE (30%) and noncancer patients with VTE (17%). The findings for Factor V Leiden in the noncancer group with VTE were notably higher (24%) in this study. Pihusch and colleagues (2002) also demonstrated increased incidence for the polymorphism in patients with gastrointestinal cancer (17%). Significantly lower rates of FVL in cancer patients with VTE (1.5%) were documented in a prospective study (Ramacciotti, et al., 2003). Finally, a meta-analysis demonstrated a 7.3% (range 1.5% - 30%) incidence of Factor V Leiden in cancer patients with VTE, which is lower than the results of this study for cancer patients (11%) (Eroglu et al., 2008). Despite the increased incidence of FVL in both cancer and noncancer groups with VTE, no statistical significance between the groups was obtained in this study. However, the sample size (n = 100) is a limitation, in this study.

PT G20210A

The second most common thrombophilic polymorphism, PT G20210A, has an expected general population frequency of 2%. The overall incidence (4%) of prothrombin G20210A for this study was quite similar (3.7% of cancer and 4.1% of noncancer patients). Higher rates (5% - 10%) of PT G20210A in cancer patients with VTE were found in several studies (Blom, et al., 2005; Kennedy, et al., 2005; Pihusch, et al., 2002). Eroglu and colleagues (2007) reported 0% of cancer patients and 3% of noncancer patients with VTE carried the PT G20210A polymorphism. No statistical significance between cancer and noncancer groups was found for PT G20210A in this study.
**MTHFR C677T**

The United States, Caucasian, general population expected frequency of heterozygous MTHFR C677T is 44.6% and homozygous is 11.9% (Botto & Yang, 2000). Slightly higher rates for the MTHFR C677T polymorphism were found in this study, 50% of the total sample. However, the frequencies of subjects who were positive for MTHFR were significantly higher among the noncancer subjects compared with cancer subjects (58.9% vs. 25.9%, respectively). The group differences were statistically significant for this polymorphism (p = .003). In this study, four subjects (4%) were homozygous for MTHFR C677T, slightly lower than the expected frequency of 11.9%. Of the four subjects homozygous for MTHFR C677T, one had cancer and the other three were in the noncancer group.

**Homocysteine**

A normal homocysteine level was reported for 95% of subjects in this study. The subjects in the cancer group all had normal homocysteine levels in this study (100%). This finding differs from a study by Gatt and colleagues (2007) that found 39% of advanced breast cancer patients and 22.6% of those with early breast cancer had elevated homocysteine levels. The two subjects (2%) with reported moderately elevated homocysteine levels were also positive for MTHFR C677T, in this study. High homocysteine levels were documented for 3 (3%) of subjects and 1 of those 3 subjects was positive for homozygous MTHFR C677T. Although half of the total subjects (50%) were positive for MTHFR C677T, only a small percentage (5%) of patients in this study had elevated homocysteine levels.
Jacques and colleagues (1996) and Kang et al. (1998) reported high homocysteine levels in homozygous MTHFR C677T in the presence of low folate. This study did not measure folate levels so the question remains whether the one homozygous MTHFR C677T patient with high homocysteine levels also had an altered folate level. The majority of homozygous MTHFR C677T patients (75%) had normal homocysteine levels. The combined MTHFR C677T and hyperhomocysteinemia numbers are too small for further meaningful analysis in this study.

**Aim 2**

To determine if subjects with cancer and the MTHFR C677T polymorphism who were treated with antimetabolite therapy had an increased incidence of VTE they were compared to cancer subjects who were treated with other chemotherapy, in this study.

*Antimetabolite Chemotherapy*

In the literature, studies involving MTHFR and antimetabolite therapy have focused on toxicity and homocysteine levels. No studies to date have included VTE as an endpoint. In this study, 11 subjects with cancer received an antimetabolite and 8 subjects received other chemotherapy. Of the 11 subjects treated with antimetabolites, 5 (45%) were also positive for MTHFR C677T. Three of these 11 subjects (27.3%) received more than one antimetabolite. All 3 of these subjects were positive for the MTHFR C677T SNP. There was no significant difference in the number of subjects with MTHFR C677T between the antimetabolite and other chemotherapy groups using Fisher’s exact test. Although Fisher’s exact test is based on the hypergeometric function, there remains concern about the limited sample size and it’s distribution across cells for this question.
Cancer Diagnosis

There were 27 subjects with cancer included in this study and breast cancer comprised the largest group (29.6%). Gastrointestinal cancer and lymphoma subjects each represented 11.1% of the sample, and pancreatic and prostate cancer each accounted for 7.4% of all cancer subjects. Paneesha and colleagues (2010) reported similar findings for DVT in cancer and noncancer outpatients. The most frequently described cancer-associated DVT were noted in common cancers including; breast, prostate, colorectal, and lung (Blom, et al., 2005; Eroglu, Ulu, Cam, Kurtman, & Akar, 2007). In the literature, pancreatic cancer was the most common cancer diagnosis associated with VTE among hospitalized patients (4.3%) compared with all other cancer types (2%) (Stein, et al., 2006). Of the 8 breast cancer patients four (50%) were also positive for MTHFR C677T, in this study.

Combined Polymorphisms

Seventy-five of 100 subjects (75%) in this study had at least one polymorphism, and 13 of these (17.3%) had two polymorphisms present. Similar overall results (16%) were found in one study, which evaluated the presence of more than one polymorphism and VTE risk in the noncancer population (Salomon, et al., 1999). The combined effect of thrombophilic polymorphisms and VTE has not been evaluated in the cancer population, however, several studies in the noncancer population have reported increased risks for VTE with two or more polymorphisms (Cattaneo, et al., 1997; Emmerich, et al., 2001; Salomon, et al., 1999). The synergistic effect in the literature was more predominant in patients who developed spontaneous VTE. Subjects with cancer
potentially have more risk factors for VTE development, such as hypercoagulability in the cancer state, associated treatments, surgeries, hospitalizations or immobility, compared with noncancer patients. The presence of more than one polymorphism, found in 13 (13%) of overall subjects in this study, was more commonly found in the noncancer group, 12 of 13 subjects (92.3%) compared with the cancer group, 1 of 13 subjects (7.7%). This study is the first, to date in the literature, to report frequencies of more than one thrombophilic polymorphism in both noncancer subjects and cancer subjects with VTE. In this study, 52.4% of subjects positive for Factor V Leiden also carried another polymorphism (9.5% prothrombin G20210A and 42.9% MTHFR C677T). All four subjects positive for prothrombin G20210A (100%) had a second polymorphism present (50% Factor V Leiden and 50% MTHFR C677T). Twenty-two percent of subjects positive for MTHFR C677T were also positive for another polymorphism (18% Factor V Leiden and 4% prothrombin G20210A).

Demographics

The literature describes an increased incidence of VTE in advancing age (Heit, 2008). In this study, the cancer subjects were all over the age of 48 and the mean age was 69.41. The mean age in this study is similar to the mean age reported in a demographic study (69.41 versus 66.43, respectively) (Paneesha, et al., 2010). Increased age was noted for the cancer patients in this study (88.8% over the age of 60). The noncancer group comprised a younger population with 46.5% under the age of 50 and 28.7% under the age of 40. The mean age for noncancer subjects in this study was 51.58, lower than the mean of the cancer group. Paneesha and colleagues (2010) similarly reported a younger age
(mean 58.85) for noncancer-associated DVT and VTE was uncommon for cancer patients younger than 31.

The female population in this study was notably higher overall and in both cancer and noncancer groups compared with males. In the literature, cancer-associated thrombosis studies reported more equality in gender groups (Blom, et al., 2005; Eroglu, et al., 2007; Kennedy, et al., 2005).

**VTE Recurrence**

Twelve of 21 subjects (57%) positive for factor V Leiden had VTE recurrence in this study. Segal and colleagues (2009) reported an increased risk for recurrent VTE for patients with factor V Leiden in a systematic review. Subject’s who were homozygous for MTHFR C677T, in this study, had a notably higher rate of recurrent VTE compared with those heterozygous for MTHFR C677T (3 of 4 patients (75%) versus 19 of 50 patients (38%), respectively).

**Oral Contraceptive Use**

In this study, 5 noncancer subjects with DVT were concurrently taking oral contraceptives. Three of these 5 subjects (60%) were heterozygous for MTHFR C677T and 1 (20%) heterozygous for Factor V Leiden. Although oral contraception associated with increased VTE risk has been documented in the literature, thrombophilic polymorphism frequency reports in this group are uncommon. One case report in the literature described a 22-year old female who developed bilateral DVT with PE 2 months after oral contraceptive use and was positive for factor V Leiden, homozygous MTHFR C677T and prothrombin G20210A (Charafeddine, et al., 2010).
Pregnancy

Three noncancer subjects had pregnancy related DVT and all 3 subjects (100%) had at least one polymorphism that included MTHFR and 2 (66.6%) of those subjects had two polymorphisms (factor V Leiden + MTHFR and prothrombin G20210A + MTHFR). A case report in the literature described a 26-year old female with VTE during pregnancy and was also a carrier for both factor V Leiden and prothrombin G20210A (Couto, Nomura, Barini, & Pinto e Silva, 2005).

Limitations

There are several limitations in this study. The retrospective, descriptive design contributes lower levels of evidence to the literature in an area that is understudied. The subject population was restricted to those with available electronic health records after recent transition to an electronic system, which may introduce sample bias. This study did not include a control group, which may have strengthened the results. This study did not include age and sex matched groups. The female to male ratio was 2:1 in this study, making comparison with other studies with even ratios more challenging. The total sample and cancer group size was small, precluding more meaningful statistical analysis. The small cancer sample in this study included multiple cancer types, multiple cancer stages, and varied treatment levels. The data were not sufficient to determine tumor type, stage or treatment impact on VTE risk. The small sample size for the second aim, cancer patients treated with chemotherapy who carried the MTHFR C677T, was not sufficient to provide insight to the potential role of MTHFR in this subgroup.
Implications

Future Research

There are several future research recommendations for the role of thrombophilic polymorphisms in cancer and noncancer associated VTE. First, a large prospective design with control groups that include cancer subjects without a history of VTE and healthy controls is recommended. The outpatient cancer cohort would be matched for age, sex, tumor type, stage, and treatment status to contribute meaningful clinical findings. Further study to evaluate the effects of MTHFR C677T, homocysteine levels at baseline and during treatment and a combined effect of more than one antimetabolite for this population is also needed. Future studies to clarify the potential combined effect of polymorphisms and cancer associated thrombosis, are also recommended. Finally, studies to further the understanding on the effect of more than one polymorphism and oral contraceptive or pregnancy associated VTE are needed. Future studies are needed that contribute to the development of risk assessment models for VTE prevention in the outpatient cancer setting.

Clinical Practice for DNP

The DNP, in multiple practice settings, needs additional information to clarify the role of polymorphisms and VTE risk assessment for outpatients. Risk assessment and prophylaxis for VTE has been described for the hospitalized cancer population but is lacking in the outpatient setting. Current recommendations from the American College of Physicians focus on VTE risk assessment and prophylaxis for surgical and hospital patients. Future recommendations for risk assessment and prophylaxis in the outpatient
setting will require improved levels of evidence in the literature. Multidisciplinary teams, including the DNP, will be needed to develop clinical practice guidelines for this population in the future. The DNP must have knowledge of the potential risk factors for VTE development and recurrence to properly care for those in both the cancer and noncancer population. Results from this study demonstrated increased age was more common with cancer related thrombosis and younger age groups were noted for noncancer associated VTE. The DNP in primary care, obstetric, and gynecology practice needs outpatient assessment tools to calculate for oral contraceptive and pregnancy associated risk for young adult females.

Summary

The primary purpose of this study was to compare the Factor V Leiden, prothrombin G20210A, and MTHFR C677T polymorphism frequencies in cancer- and noncancer-associated VTE in the outpatient setting. A statistically significant difference in this study was noted only for the MTHFR C677T in the noncancer group compared to the cancer group. While the factor V Leiden frequency was notably higher than the expected frequency, no significant differences between the cancer and noncancer groups were detected. Prothrombin G20210A frequencies were similar to the expected frequencies and not statistically significant between groups, in this study. A significant portion of total subjects (75%) in this study had at least one polymorphism and 13% had more than one polymorphism.

The secondary purpose of this study was to determine the if subjects with cancer and the MTHFR C677T polymorphism who were treated with antimetabolite...
chemotherapy had an increased risk of VTE compared with those who received other
types of chemotherapy. No significant differences were found between the two groups,
however, the sample size was too small to contribute meaningful results. A physiologic
framework was utilized to support this study. Homocysteine elevation was the proposed
mechanism for increased risk for thrombosis associated with MTHFR C677T. The
homocysteine levels were normal in 95% of the total sample and 100% of subjects with
cancer. Measurement of the homocysteine level at baseline and during active treatment
for those with cancer may provide more accurate data. Further exploration of
polymorphisms and VTE risk in cancer and noncancer cohorts in the outpatient setting is
needed for the development of clinical practice guidelines.
TABLE 10
Table 10. Literature Review

<table>
<thead>
<tr>
<th>AUTHOR/DATE/DESIGN</th>
<th>PURPOSE/AIMS/HYPOTHESIS</th>
<th>SAMPLE</th>
<th>METHODS</th>
<th>RESULTS STRENGTHS/WEAKNESSES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eroglu et al. 2009</td>
<td>Purpose: Prevalence of MTHFR C677T, FVL, PT G20210A and dihydrofolate reductase (DHFR) in cancer patients with and without VTE</td>
<td>2 groups: -63 cancer pt with VTE -124 CA pt without VTE evaluated for VTE during cancer tx (chemo, XRT, surgery, hormono-therapy)</td>
<td>PCR for MTHFR, FVL, PT G20210A Categorical variables-Chi square or 2 sided Fisher exact Logistic regression for analysis of more than 1 risk factor and univariate analysis. P&lt;0.05 significant SPSS</td>
<td>Group 1 MTHFR C677T similar in both groups- no significant differences between groups. Logistic regression with multivariate showed only Factor V Leiden significantly associated with risk of VTE in Cancer. Strengths: Weakness: Wide CI Small sample Homocysteine level not drawn Ethnic population- turkey</td>
</tr>
<tr>
<td>Racacciotti et al. 2003</td>
<td>Purpose: to evaluate prevalence and clinical significance of FVL, FII G20210A, FXIII Val34Leu, and MTHFR C677T in cancer patients with and without venous thrombosis</td>
<td>Sample: 211 patients at 2 university oncology clinics in Brazil divided into groups of VTE vs no VTE</td>
<td>Methods: Cancer pts enrolled prospectively and evaluated for sx of DVT/PE. Verified VTE were the cases and non VTE were controls.</td>
<td>Results: They found no significant association between the 4 polymorphisms and VTE in cancer patients. MTHFR C677T in 53.1% vs 60.1% of cancer pt ‘s with and without VTE (OR 0.8</td>
</tr>
<tr>
<td>Study</td>
<td>Design and Setting</td>
<td>Participants</td>
<td>Methods</td>
<td>Findings</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-----------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------</td>
<td>--------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Pihusch et al. 2002</td>
<td>Prevalence of FVL, PT G20210A and MTHFR C677T homozygous in GI cancer patients with active malignancy with and without VTE</td>
<td>175 patients with gastrointestinal adenocarcinoma admitted to cancer rehabilitation hospital – University of Munich 2 groups: -147 without VTE associated with malignancy -28 with VTE in setting of malignancy</td>
<td>PCR analysis for FVL, PT G20210A and MTHFR C677T homozygous all patients -PCR analysis for FVL, PT G20210A and MTHFR C677T homozygous all patients - Compared 2 groups with Mann-Whitney U test, Fisher exact, and Pearson's chi-square for categorical variables - RR with 95% CI</td>
<td>FVL with VTE: 5/28 (17.9%) RR 4.4 (1.3 – 14.9) FVL without VTE: 7/147 (4.8%) PT with VTE: 3/28 (10.7%) RR 2.4 (0.6 – 9.9) PT without VTE: 7/147 (4.8%) MTHFR with VTE: 2/28 (7.1%) MTHFR without VTE: 15/147 (10.2%) Strengths: VTE after diagnosis of CA Weaknesses: 44% immobilized at time of admission</td>
</tr>
<tr>
<td>Study</td>
<td>Design</td>
<td>Aim: evaluate the prevalence of FVL and PT G20210A in cancer patients with and without VTE compared with nonmalignant VTE and healthy controls</td>
<td>4 groups: -43 ca pt. developed VTE during cancer tx; -81 CA pt without VTE (in hospital); -100 VTE pt without CA; -100 healthy controls Random selection group 2 Exclusion criteria: personal or family Hx VTE</td>
<td>PCR – FVL and PTG20210A polymorphisms Chi square OR P&lt;0.05 significant</td>
</tr>
<tr>
<td>------------------------------</td>
<td>-----------------------------</td>
<td>-----------------------------------------------------------------------------------</td>
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<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Eroglu et al. 2007 Turkey    Case control</td>
<td></td>
<td></td>
<td>FVL significantly increased in CA pts with VTE (30.2% vs. 3.7%, 18%, 8% in groups 1-4)(p&lt;0.0001) OR for group 1 8.1 (95%CI 2.4-27.1) No significant dif. In prevalence of PT G20210A among the groups (p&lt;0.625) Strength: Weakness: Wide CI Slightly higher rates of FVL in Turkey Study suggests eval for FVL in CA pt with VTE</td>
<td></td>
</tr>
<tr>
<td>Blom et al. 2005 Netherlands Population-based, case-control, multicenter in 6 clinics in Netherlands</td>
<td>Purpose: Identify pt. with cancer with increased risk of VTE by evaluating different tumor sites, hx of metastasis, and carrier status of FVL and PT G20210A</td>
<td>Sample: 3220 patients age 18-70 at anticoag clinics with a first DVT –LE or PE 2131 control patients (partners of pt.) Hx of malignancy factored out for each group for subgroup analysis Exclusion:</td>
<td>Methods: Case sample recruited from 6 anticoag clinics Blood drawn for FVL and PT at 3 month visit or 1 year for those who were on lifelong Coumadin Telephone interview if unable to come to clinic- buccal swab sent to patient Questionnaire on</td>
<td>Results: FVL and Prothrombin G20210A have a higher risk for VTE in cancer patients Strengths: large study with case control Weakness: buccal swab – potential source of reliability or validity issues Ethnic study</td>
</tr>
<tr>
<td>Study</td>
<td>Purpose</td>
<td>Methods</td>
<td>Findings</td>
<td></td>
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<tr>
<td>-----------------------</td>
<td>-------------------------------------------------------------------------</td>
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<td>--------------------------------------------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Kennedy et al. 2005</td>
<td>Determine risk association of FVL and PT G20210A and VTE in non-hematologic cancer patients</td>
<td>101 cancer patients with VTE compared with 101 cancer patients without VTE in two general oncology practices</td>
<td>Case control analysis; All subjects tested for VTE and PT G20210A via whole blood analyzed by PCR</td>
<td></td>
</tr>
<tr>
<td>Vermont, USA</td>
<td></td>
<td></td>
<td>FVL with VTE 5/101 (5%) FVL w/o VTE 3/101 (3%) OR = 1.7 (0.3 – 10.7)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>PT with VTE 5/101 (5%) PT w/o VTE 0/101 (0%) OR = 6.7 (0.9 - ∞)</td>
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<td>Strengths: Case matched for gender, age, cancer type and stage</td>
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<td>Weaknesses: Insufficient power Exclusion of hematologic cancers</td>
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<td>Mandala et al. 2004</td>
<td>Analyze influence of FVL and PT G20210A on risk of first catheter related DVT in breast CA patients treated with chemotherapy</td>
<td>Cohort of 300 locally advanced or metastatic breast CA patients treated with 5-FU based chemotherapy through an</td>
<td>Nested case control analysis; 25 cases with catheter related VTE and 50 controls without VTE</td>
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<td>Italy</td>
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<td>25 women developed CRT (8.3%)</td>
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<td>FVL with VTE 5/25 (20%) FVL w/o VTE 2/50 (4%)</td>
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<td>PT with VTE 1/25 (4%) PT w/o VTE 0/50 (0%)</td>
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<td>Eroglu et al. 2008</td>
<td>Purpose: To determine the effect of FVL on the development of VTE in cancer patients</td>
<td>Pooled analysis of 9 studies included 397 cancer patients with VTE and 687 cancer patients without VTE</td>
<td>Meta-analysis Fixed and random effects models. OR calculated with Fisher's exact</td>
<td>FVL in cancer patients with VTE (7.3%) FVL in cancer pt w/o VTE (4.6%) Conclusion: FVL is associated with cancer related thrombosis</td>
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<td>Turkey US Israel Germany Brazil Italy Meta-analysis</td>
<td>implanted port at a single institution</td>
<td>some retested with RFLP Fisher’s exact compared groups Mann-Whitney U for continuous variables Logistic regression for age adjusted OR for risk of VTE with mutations</td>
<td>Age adjusted OR for either mutation 7.6 (1.4-41.0) Strengths: Specific cancer population with highly matched controls Weaknesses: Small study</td>
<td></td>
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<tr>
<td>Haim et al. 2001</td>
<td>Purpose: Evaluate activated protein C resistance and FVL in cancer patients with and without VTE</td>
<td>4 groups: 55 Cancer with VTE 58 Cancer w/o VTE 54 VTE w/o cancer 56 Healthy volunteers w/o cancer or VTE</td>
<td>FVL determined by PCR Activated protein C resistance – sensitivity ratio (ratio of APTT times with and without APC) Mean values compared with t-test</td>
<td>Acquired APC resistance common in cancer with VTE patients (not due to FVL) FVL in cancer pt with VTE 1/55 (1.8%) FVL in cancer without VTE 4/58 (6.8%) FVL in VTE without CA 18/54 (33%) FVL in normal control 2/56 (3.5%) Acquired APC resistance : CA pt with VTE 29/54 (54%) CA without VTE 9/54 (17%) VTE without CA 7/36 (19%) Normal control 0/54 (0%) Strengths: Comparison groups Weaknesses: Rates of FVL in most groups lower than average for a normal</td>
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<td>population and unusually high for the VTE without CA group</td>
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FIGURE 1
Figure 1. Theoretical Model of Risk Factors for Venous Thromboembolism

- **Factor V Leiden**
- **PT G20210A**
- **MTHFR C677T**
- **Antimetabolite Treatment (5-FU, Methotrexate)**
- **APCR**
- **Increased Prothrombin**
- **Decreased Folate**
- **Increased Homocysteine**
- **Venous Thromboembolism**

The diagram illustrates the relationships between various risk factors and the development of venous thromboembolism.
FIGURE 2
Figure 2 Polymorphisms, Cancer Cells and Homocysteine effect on coagulation
APPENDIX A: INSTITUTIONAL REVIEW BOARD APPROVALS
HSPP Correspondence Form

Date: 07/30/10
Investigator: Lois Lattimore, MSN, Doctoral Student
Department: Nursing
Project No./Title: 10-0549-04 Factor V Leiden, Prothrombin G20210A, and MTHFR C677T Polymorphisms in Cancer Patients with Venous Thromboembolism
Current Period of Approval: 07/30/10 – 07/29/11

IRB Committee Information
IRB4 – IRB00005448 Expedited Review – New Project
FWA Number: FWA00004218

<table>
<thead>
<tr>
<th>Documents Reviewed Concurrently</th>
<th>Status</th>
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<tbody>
<tr>
<td>Project Approval Form – Chart Review Form (signed 07/09/10)</td>
<td>Appr</td>
</tr>
<tr>
<td>VOTF (version 07/09/10)</td>
<td>Appr</td>
</tr>
<tr>
<td>Data Collection Instrument</td>
<td>Appr</td>
</tr>
<tr>
<td>Other (define): CV for PI Lois Lattimore</td>
<td>Ack</td>
</tr>
<tr>
<td>AZCC Scientific Review Committee Outcome Report (dated 07/16/10)</td>
<td>Ack</td>
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</tbody>
</table>

Determination
Approved as submitted effective 07/30/10

Requirements
• UMC Approval Requirement: Prior to initiation of research at University Medical Center, approval from the UMC Site Review Authority must be obtained. For more information, please refer to the SRA website at http://www.umcarizona.org/body.cfm?id=22 or email Anita Crockett, RN, PhD, or Valerie Evans, Co-Chairs of the SRA, at sra@umcarizona.edu. A copy of the approval letter must be forwarded to the UA HSPP office for file completion.

Regulatory Determination(s)
• Criteria for Approval has been met (45 CFR 46.111): The criteria for approval listed in 45 CFR 46.111 have been met (or if previous met, have not changed) in that (1) Risks to subjects are minimized; (2) By using procedures which are consistent with sound research design and which do not unnecessarily expose subjects to risk, and (iii) whenever appropriate, by using procedures already being performed on the subjects for diagnostic or treatment purposes. (2) Risks to subjects are reasonable in relation to anticipated benefits, if any, to subjects, and the importance of the knowledge that may reasonably be expected to result. In evaluating risks and benefits, the IRB should consider only those risks and benefits that may result from the research (as distinguished from risks and benefits of therapies subjects would receive even if not participating in the research). The IRB should not consider possible long-range effects of applying knowledge gained in the research (for example, the possible effects of the research on public policy) as among those research risks that fall within the purview of its responsibility. (3) Selection of subjects is equitable. In making this assessment the IRB should take into account the purposes of the research and the setting in which the research will be conducted and should be particularly cognizant of the special problems of research involving vulnerable populations, such as children, prisoners, pregnant women, mentally disabled persons, or economically or educationally disadvantaged persons. (4) Informed consent will be sought from each prospective subject or the subject’s legally authorized representative, in accordance with, and to the extent required by 46.116. (5) Informed consent will be appropriately documented, in accordance with, and to the extent required by §46.117. (6) When appropriate, the research plan makes adequate provision for monitoring the data collected to ensure the safety of subjects. (7) When appropriate, there are adequate provisions to protect the privacy of subjects and to maintain the confidentiality of data. (b) When some or all of the subjects are likely to be vulnerable to coercion or undue influence, such as children, prisoners, pregnant women, mentally disabled persons, or economically or educationally disadvantaged persons, additional safeguards have been included in the study to protect the rights and welfare of these subjects.

Reminders: Continuing Review materials should be submitted 30–45 days prior to the expiration date to obtain project re-approval
• Projects may be concluded or withdrawn at any time using the forms available at http://umcarizona.org.
• No changes to a project may be made prior to IRB approval except to eliminate apparent immediate hazard to subjects.
• Original signed consent forms must be stored in the designated departmental location determined by the Department Head.
• Expedite Approval (45 CFR 46.110 Category 5): Research involving materials (data, documents, records, or specimens) that have been collected, or will be collected solely for nonresearch purposes (such as medical treatment or diagnosis).

• Waiver of Informed Consent (45 CFR 46.116(d)): the research involves no more than minimal risk to subjects (The potential risks are minimal for this study. The electronic records will be reviewed by the PI and de-identified information will be shared with the Doctoral Committee, UA College of Nursing. The polymorphism results documented in the patient records were previously evaluated by an outside laboratory. Breach of confidentiality is protected by password protected files and de-identification); the waiver or alteration will not adversely affect the rights and welfare of the subjects (the information will be de-identified and results of the study will not impact the patients involved); the research could not practically be carried out without the waiver or alteration (some patients appropriate for this study may now be deceased); and whenever appropriate, the subjects will be provided with additional pertinent information after participation (Patients for this study are already aware of their polymorphism testing results and the implications of the results).

• Waiver of PHI Authorization (45 CFR 164.512(i)(2)(ii)): the use or disclosure of protected health information involves no more than minimal risk to the individuals (The potential risks are minimal for this study. The electronic records will be reviewed by the PI and de-identified information will be shared with the Doctoral Committee, UA College of Nursing. The polymorphism results documented in the patient records were previously evaluated by an outside laboratory. Breach of confidentiality is protected by password protected files and de-identification); the alteration or waiver will not adversely affect the privacy rights and the welfare of the individuals (the information will be de-identified and results of the study will not impact the patients involved); the research could not practicably be conducted without the alteration or waiver (some patients appropriate for this study may now be deceased); the research could not practicably be conducted without access to and use of the protected health information (Information for this study is contained in the patient record); the privacy risks to individuals whose protected health information is to be used or disclosed are reasonable in relation to the anticipated benefits if any to the individuals, and the importance of the knowledge that may reasonably be expected to result from the research (This study will not provide direct benefits to the subject, however, adding to the knowledge that can potentially reduce the morbidity and mortality of VTE in cancer patients will improve outcomes and reduce healthcare costs); there is an adequate plan to protect the identifiers from improper use and disclosure (the data collected from the electronic health record will be de-identified by a code sheet and data collection sheets will not contain personal identifying data); there is an adequate plan to destroy the identifiers at the earliest opportunity consistent with conduct of the research, unless there is a health or research justification for retaining the identifiers, or such retention is otherwise required by law (Code sheets will be destroyed after data collection and analysis with the conclusion of the study); and there are adequate written assurances that the protected health information will not be reused or disclosed to any other person or entity, except as required by law, for authorized oversight of the research project, or for other research for which the use or disclosure of protected health information would be permitted by this subpart (The protected health information will be shared only with committee members at University of Arizona overseeing this project).

Brenda J. Wittman, M.D.  
Co-Chair, IRB4 Committee  
UA Institutional Review Board  
BJW/dcg  
cc: Departmental/College Review Committee

07/30/10  

Reminders: Continuing Review materials should be submitted 30-45 days prior to the expiration date to obtain project re-approval  
• Projects may be concluded or withdrawn at any time using the forms available at https://versv.rpr.arizona.edu/lbh.  
• No changes to a project may be made prior to IRB approval except to eliminate apparent immediate hazard to subjects.  
• Original signed consent forms must be stored in the designated departmental location determined by the Department Head.
APPENDIX B: SITE AUTHORIZATION LETTERS
AZCC SCIENTIFIC REVIEW COMMITTEE
OUTCOME REPORT

Protocol title: Factor V Leiden, PT G20210A, and MTHFR C677T polymorphisms in cancer patients with venous thromboembolism

Protocol number: 20814

Principal investigator: Lois Lattimore, B.S.N., M.S.N.

Sponsor: Arizona Cancer Center

Date: July 16, 2010

Statistical review (i.e., approval, pending, etc.): N/A

Findings/determination: EXEMPT

Comments: This study is exempt from full SRC review because it is a retrospective chart review.

This trial will be initiated at the following clinical sites:

X Arizona Cancer Center, Tucson

Premiere Oncology of Arizona, Scottsdale

SAVAHCS, Tucson

July 16, 2010

Date

Steven P. Stratton, Ph.D.
Chair, AZCC Scientific Review Committee
August 3, 2010

Lois Lattimore, DNP(c), MSN
UA College of Nursing
1305 N. Martin
PO Box 210203
Tucson, AZ 85721-0203

RE: UMC Final Site Approval for Research

No/Title: 127-10 Lattimore 10-0549-04/ FACTOR V LEIDEN, PROTHROMBIN G20210A, and MTHFR C677T POLYMORPHISMS IN CANCER PATIENTS WITH VENOUS THROMBOEMBOLISM

Dear Ms. Lattimore:

You are hereby notified that your project has been granted final approval by the UMC Site Review Authority. You are now authorized to initiate your research project and may use UMC as your research site during the following timeframe:

From: August 3, 2010 To: July 29, 2011

We wish you well in your research endeavors! Should you have any questions or concerns please feel free to contact us at your convenience.

Valerie Evans
Valerie Evans, MLS, SH (ASCP)CM
Corporate Compliance Officer / Privacy Officer
Co-Chair, UMC Site Review Authority

Anita B. Crockett, PhD
Director of Research
Co-Chair, UMC Site Review Authority
References

AACN (2006). *The Essentials of Doctoral Education for Advanced Nursing Practice*


