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Fatal Pulmonary Thromboembolism and Hereditary Thrombophilias

ABSTRACT: Pulmonary thromboembolism (PE) is found commonly in forensic pathology practice, as it typically causes sudden death. It is attributed to a wide variety of predominantly acquired etiologies. Although likely etiologically multifactorial, some common proximate causes include: surgery, pregnancy, injury, inactivity of any cause, cancer, obesity, or serum hyperviscosity. On occasion, no apparent predisposing condition is identified. In these instances, occult hereditary thrombophilias may play a causal role. Deaths referred to the Office of Chief Medical Examiner (OCME) of New York City between December, 2000 and September, 2003 and due to PE were retrospectively reviewed. Molecular analysis (FRET) was performed on selected cases for three common hereditary thrombophilias: mutations in factor V Leiden (FVL), prothrombin G20210A (PT), and methylenetetrahydrofolate reductase (MTHFR).During the study period, 124 of 15,280 deaths were primarily attributable to PE. Of those, 34 were selected for molecular analysis. One or more mutations were detected in 35% of those, five of which were clearly causally related to death. Given the potential benefits to surviving family members, our data indicate that postmortem molecular testing for the common hereditary thrombophilias is warranted in at least selected cases.

KEYWORDS: forensic science, pulmonary embolism, thrombophilia, hereditary, prothrombin, MTHFR, factor V Leiden

With recent advances in molecular pathology, interest in the etiologic and diagnostic issues related to vascular biology and hereditary thrombophilias has increased dramatically. Thrombophilias are diseases or conditions associated with an increased thrombotic risk. Heritable thrombophilias include mutations in factor V Leiden and prothrombin, as well as deficiencies of antithrombin (AT) and proteins C and S. A plethora of studies within the last five years has greatly contributed to our pathophysiologic understanding, diagnostic acumen, and clinical management of venous thromboembolism (VTE) (1–26). However, few reports have been published pertaining to the postmortem diagnosis and interpretation of hereditary thrombophilias, despite their potential relevance to the investigation of sudden, unexpected deaths (6,7,26).

The prevalence of certain thrombophilic heterozygous mutations is high. The most common vary from 1–7% in the Caucasian population and carry an independent risk of thromboembolic events (see Table 1). VTE is widely regarded as etiologically multifactorial and is the third most common cardiovascular disease in the United States, accounting for 100,000 deaths annually (10). Many of these deaths fall under the purview of the medicolegal death investigator, by virtue of their suddenness. As such, a forensic pathologist's recognition of common hereditary defects in persons dying from a pulmonary thromboembolism (PE) has potentially life-saving importance to surviving family members. This is the first large series investigating deaths due to VTE as they relate to hereditary thrombophilias. We discuss the role that these mutations, in concert with co-morbidities, play in these deaths, the implications for survivors, and our recommendations regarding postmortem diagnostic selection and testing.

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Methods

In New York City, statutes require that all sudden deaths of persons in apparent good health be reported to the Office of Chief Medical Examiner (OCME). Deaths caused primarily by PE between December 1, 2000 and September 30, 2003 were retrospectively reviewed. Of these, selected decedents meeting one or more of the clinically established criteria listed in Table 2 were tested for three hereditary thrombophilias: mutations in factor V Leiden (FVL), prothrombin G20210A (PT), and methylenetetrahydofolate reductase (MTHFR) (1,3,9,10). Serum was analyzed by the Invader assay using fluorescence resonance energy transfer (FRET) detection (27). The Invader assay is a signal amplification system able to accurately quantify DNA targets. Specificity is achieved by combining hybridization with enzyme recognition, which provides the ability to discriminate mutant from wild-type. The technology incorporates a homogeneous fluorescence readout and is used to analyze directly unamplified human genomic DNA to detect mutations and single-nucleotide polymorphisms associated with factor V Leiden, factor II, and MTHFR (27). Less common abnormalities involving antithrombin III, proteins C and S, plasminogen, dysfibrinogenemia, hyperhomocyteinemia, and antiphospholipid antibodies were not investigated, as functional and serologic diagnostic assays are ill-suited for postmortem blood.

Results

Of 15,280 persons autopsied during the study period, 124 deaths were primarily caused by PE. Of those, 34 selected blood samples (27%) were analyzed for three heterozygous and homozygous mutations. One or more mutations were detected in 12/34 decedents (35% of those selected) involving FVL (1 case), PT (3 cases), and MTHFR (9 cases). With the exception of one homozygous mutation for MTHFR, all detected defects were heterozygous (Table 3). Of these, five deaths were interpreted as causally related to one or more of these mutations and are described in greater detail

TABLE 1—*Genetics, biology, risk, and prevalence of hereditary thrombophilias (7,38–40,56).*

Genetics		Biology	Independent Risk for VTE	Population Prevalence of Heterozygous Defect			
				Cauc	AA	Hisp	Asian
Prothrombin (20210A)	Autosomal dominant mutation in prothrombin gene	Increases prothrombin concentrations and venous thrombosis	3.7-fold (heterozygous) Unknown Risk (homozygous)	1–2%	0.2%	UK	UK
Factor V Leiden	Mutation in Factor V	Causes resistance to activated Protein C, leading to excess thrombin formation and venous thrombosis	7-fold (heterozygous) 100–fold (homozygous)	3–7%	1.2%	2.2%	0.45%
MTHFR	Mutation in Enzyme involved in metabolism of homocysteine	Can result in hyperhomocysteinemia causing venous and arterial thrombosis	Dependent upon serum folate and B12 concentrations	40–50%	UK	UK	UK

Cauc: Caucasian, AA: African–American, Hisp: Hispanic, UK = Unknown.

TABLE 2—*Selection criteria for postmortem hereditary thrombophilia testing.*

Age under 45 years old
Pregnancy–related death
History of recurrent or unexplained stillbirths (especially in the second or third trimesters)
Oral contraceptive pill use (OCP)
Hormone replacement therapy (HRT)
Chemotherapy (especially methotrexate or tamoxifen), particularly if cancer–free at autopsy
Personal or family history of venous thromboembolism (VTE)
VTE in an unusual anatomic site
"Soft"/unconvincing risk factors (e.g., slight to moderate obesity, antecedent long car ride)
Deep venous thrombosis of inapparent cause (i.e., "unprovoked" VTE at any age)

below. Possible causal relationships for the remaining deaths are also discussed.

Cases

Case 1: Oral Contraceptive Use and a Remote Long Plane Ride (Heterozygous Prothrombin G20210A Mutation)

A 34-year-old Black woman with no prior medical problems was prescribed albuterol for a one-week history of shortness of breath. One evening, light-headedness and dizziness were followed by syncope. In transit to the hospital, she had a seizure and became asystolic. She took oral contraceptive pills (OCP's), but had no history of smoking. She also had taken a cross country airplane trip four months earlier (28,29). Her father had a history of thrombophlebitis at age 52.

At autopsy, she was a 5′9″, 146 lb. woman with bilateral proximal and distal pulmonary thromboemboli, an acute to well-organized right lower extremity deep venous thrombus (DVT), and no evidence of asthma.

Molecular diagnostic studies revealed a heterozygous prothrombin G20210A mutation (4,27,30).

Case 2: Probable Viral Illness and Remote Injury (Heterozygous Factor V Leiden Mutation)

A 24-year-old Black man without prior medical problems had a one-week history of malaise, weakness, diarrhea, and low-grade fever (100.8 F). He presented to the emergency department following mental status changes and an episode of syncope/seizure activity. He was tachycardic, tachypneic, hypotensive, and complained of shortness of breath, after which he suddenly became asystolic. A lumbar puncture and chest radiograph were negative. Electrolyte values (in mEq/L) in the emergency department were: sodium 141, potassium 4.3, chloride 103, BUN 8, creatinine 1.5, CO2 12, and a trace of acetone.

He had been a driver in a minor motor vehicle collision approximately 50 days before his death. He had complained of pain in his left knee and lower back, but had not been hospitalized. He was undergoing physical therapy and acupuncture treatments, and was last seen by his physician approximately one week before his death. He was ambulating well and had no complaints of chest pain or dyspnea.

At autopsy, he was a 6′3″, 203 lb. man with bilateral proximal and distal pulmonary thromboemboli and acute and organized left lower extremity DVT.

Molecular diagnostic studies revealed a heterozygous factor V Leiden mutation (3,27,30).

Case 3: No Apparent Cause (Homozygous MTHFR and Heterozygous Prothrombin G20210A Mutations)

A 49-year-old White male chiropractor had a several day history of malaise, back pain, and indigestion of unknown etiology. He consulted his internist after having a minor episode of hemoptysis. He had a past medical history of hypertension and smoking (2–3 packs per day for many years). A chest radiograph revealed a right hilar fullness. He had no recent leg pain or injuries and there was no family history of thrombophilia. He was found dead two days later in the kitchen of his apartment.

At autopsy, he was a 6′0″, 248 lb. man with acute and organizing bilateral proximal pulmonary thromboemboli and a DVT involving the right femoral and popliteal veins. There was slight coronary artery disease and moderate pulmonary emphysema. There was no malignancy.

Molecular diagnostic studies revealed homozygous MTHFR and heterozygous prothrombin G20210A mutations (4,5,27,30).

Case 4: Status Post Cesarean Section with Myomectomies (Heterozygous Prothrombin G20210A Mutation)

A 34-year-old Black woman without prior medical problems presented to the hospital with preterm labor at 26 weeks gestation. The fetus was in breech position and a Cesarean-section was performed. Uterine leiomyomata were removed following the delivery in order to surgically close the uterus. The following day, she arose from bed to go to the bathroom, felt faint, and suddenly became unresponsive upon returning to her bed. Resuscitative efforts were unsuccessful.

TABLE 3—*Decedents tested for hereditary thrombophilias.*

	Age (yr) Race Sex	History/Risk Factors	Hereditary Thrombophilia		
			Factor V	Prothrombin	MTHFR
1	34 BF	Case 1: OCP use and remote airplane travel	Negative	Heterozygousus	Negative
2	24 BM	Case 2: Remote injury	Heterozygous	Negative	Negative
3	49 WM	Case 3: Obesity	Negative	Heterozygous	Homozygous
4	34 BF	Case 4: C–section and myomatous uterus	Negative	Heterozygous	Negative
5	62 WM	Case 5: Stage I Breast Cancer and chemotherapy	Negative	Negative	Heterozygous
6	19 WM	Obesity, Inactivity	Negative	Negative	Heterozygous
7	26 WM	Femur Fracture	Negative	Negative	Heterozygous
8	64 WF	Unclear/"soft" risk factors	Negative	Negative	Heterozygous
9	2 WF	Nephrotic syndrome, minimal change disease	Negative	Negative	Heterozygous
10	45 HM	Schizophrenic on multiple sedatives	Negative	Negative	Heterozygous
11	25 BF	5 months postpartum (c-section)	Negative	Negative	Heterozygous
12	23 WM	Dilated cardiomyopathy, unknown etiology	Negative	Negative	Heterozygous
13	56 BF	Unknown/undetected risk factors	Negative	Negative	Negative
14	29 BM	Breast Cancer: Chemotherapy* & Surgery	Negative	Negative	Negative
15	27 WF	Smoker, OCPs (polycystic ovaries), depression	Negative	Negative	Negative
16	43 BM	Schizophrenic (not catatonic)	Negative	Negative	Negative
17	26 BM	Hypertension, Sickle trait	Negative	Negative	Negative
18	35 BF	Inactivity due to remote stroke (unknown etiology)	Negative	Negative	Negative
19	36 WM	HIV, estrogen therapy for trans–gender process	Negative	Negative	Negative
20	39 WF	Inactivity following vertebral injury, obesity	Negative	Negative	Negative
21	52 BF	Hypertension, substance abuse/In apparent risk	Negative	Negative	Negative
22	37 BM	AIDS, peritoneal lymphadenopathy, Kaposi	Negative	Negative	Negative
23	29 HF	Unknown/undetected risk factors	Negative	Negative	Negative
24	63 BM	Unknown/undetected risk factors	Negative	Negative	Negative
25	28 WF	Inactivity due to renal colic, obesity	Negative	Negative	Negative
26	41 BM	Myocardial infarct, obesity, schizophrenia	Negative	Negative	Negative
27	33 WF	OCP use, obesity	Negative	Negative	Negative
28	17 BM	Unknown/undetected risk factors	Negative	Negative	Negative
29	28 BM	Atrial septal defect, asthma	Negative	Negative	Negative
30	39 WM	Idiopathic dilated cardiomyopathy	Negative	Negative	Negative
31	54 HF	Unknown/undetected risk factors	Negative	Negative	Negative
32	25 BF	Inactivity due to status epilepticus, obesity	Negative	Negative	Negative
33	29 BF	1 month post–partum (c–section)	Negative	Negative	Negative
34	29 BF	8 weeks pregnant & myomatous uterus (1400 gm)	Negative	Negative	Negative

* Adriamycin and cytoxan.

At autopsy, she was a 5′3″, 147 lb. woman with bilateral pulmonary thromboemboli and an acute DVT in the right femoral vein. The 900 g uterus and endometrial cavity were distorted by numerous mural leiomyomata, the largest measuring 7.5 cm in diameter.

Molecular diagnostic studies revealed a heterozygous prothrombin G20210A mutation (4,27,30).

Case 5: Cancer-free, Post-methotrexate Chemotherapy (Heterozygous MTHFR Mutation)

A 62-year-old White woman had been diagnosed with lymph node negative (Stage I) left breast cancer several months prior to her death, for which she had undergone lumpectomy and seven cycles of adjuvant chemotherapy (cytoxan, 5-fluorouracil, and methotrexate) over 3–4 months. She had completed her final cycle seven days prior to her death and was scheduled to start on tamoxifen therapy. Other medical history included hypertension. She quit smoking more than 10 years prior to her death and was described as "very active," although more recently slightly fatigued as a result of chemotherapy. There was neither history of lower extremity trauma, vomiting or dehydration associated with the chemotherapy, nor any personal or family history of VTE. She was not taking hormone replacement therapy (HRT).

On the day of her death, she suddenly became severely dyspneic, feeling that she was "going to die." Shortly thereafter, she collapsed and died.

At autopsy, she was a 5′7″, 175 lb. woman without cancer, but with a saddle and distal pulmonary thromboemboli and bilateral lower extremity DVT's, ranging from acute to organizing with recanalization. There was no thrombophlebitis. She had slight, nondilated cardiac hypertrophy and finely granular kidneys. Vitreous electrolytes did not reveal dehydration.

Molecular diagnostic studies revealed a heterozygous MTHFR mutation (5,27).

Discussion

Thrombophilias, both acquired and genetic, are more common than bleeding diatheses (18). The incidence of venous thrombosis increases from 1 in 100,000 among children to 1 in 100 in the elderly (31). Clinical medicine has focused on stasis and hypercoagulability in the development of treatments to prevent DVT and its life-threatening sequela, the so-called "massive" PE. For years, clinical diagnostic tests for heritable thrombophilias were primarily limited to the rare antithrombin (AT) and protein C and S deficiencies, together comprising only 5–10% of all patients with thromboses, having a frequency of only 1 in 500 to 1 in 300 in the general population (8). Since 1994 and 1996, respectively, the more prevalent and potent factor V Leiden (FVL) and prothrombin G20210A (PT) single nucleotide mutations have been added to the list, marking a new era in molecular diagnostic testing and clinical relevance (Table 1) (3,4,32). In contrast to the heritable AT and

protein C and S defects, FVL and PT have respective frequencies of 1 in 14 and 1 in 28 in the general population (8); heterozygous forms of each confer independent thrombophilic risk. A probe for the highly prevalent methylenetetrahydrofolate reductase (MTHFR) mutation is also available (18), although its clinical significance is in question, as it is not believed to confer independent thrombophilic risk in either its heterozygous or homozygous forms. The benefits of screening for these defects and the parameters of patient selection continue to be debated, and prophylactic treatment is controversial (1).

Taken together, the cases described in this series underscore the importance of molecular testing when the cause of a fatal thromboembolic event is inadequately explained. They also illustrate the multifactorial pathogenesis of VTE (10). The majority of typical "thrombophilic" individuals, defined as those meeting one or more of the selection criteria in Table 2, will have at least one of the five major inherited defects: AT, Protein C, Protein S, FVL, or PT. Together, FVL and PT account for more than half of all patients with inherited thrombophilias, while the remaining three defects account for fewer than 10% of all patients with VTE (10).

Homozygous individuals often present little diagnostic challenge, clinically or forensically. Typically, a personal or family history of VTE is easily elicited. Heterozygous defects, on the other hand, require a careful, systematic approach and high index of suspicion. Classically, individuals will present with their first thrombotic event before the age of 45, and earlier if they are a homozygote or have more than one inherited thrombophilia (2). Carriers of AT deficiency have the highest annual risk of VTE, while those of FVL have the lowest (2). Because of the variable expressivity and incomplete penetrance characteristic of these mutations, however, not all carriers (heterozygotes) of the same defect will present at the same time in life or with the same severity. In fact, the vast majority of FVL and PT heterozygotes will not develop a symptomatic VTE in their lifetimes (10). Moreover, in greater than half of those cases in which a symptomatic VTE does develop, it is brought on by surgery, advancing age, pregnancy, or the use of OCP's or HRT (2). Therefore, what becomes critical in predicting, diagnosing, and later explaining a VTE is the myriad of environmental risk factors that may compound and unmask an otherwise silent genetic mutation, the so-called "multiple-hit" theory. The cases in this series exemplify the multiple-hit phenomenon, wherein the environmental factors alone are weak and poorly substantiate the mechanism of death (PE).

Independent risk factors for VTE in all persons include older age (continuous risk), male gender, confinement to a hospital or nursing home, recent surgery requiring anesthesia, trauma sufficient to require hospitalization, cancer (with or without chemotherapy), neurologic disease with chronic extremity paresis, superficial vein thrombosis, and prior central venous catheter or transvenous pacemaker. Women who are pregnant, or taking OCP's, HRT, tamoxifen, or raloxifene are at additional risk (33,34). Moreover, "morbid" obesity, any condition increasing serum viscosity (e.g., severe dehydration, polycythemia vera, leukemia), congestive heart failure, and protein-losing nephropathies are widely viewed as risk factors. Advanced liver disease decreases this risk by as much as 90% (10,19).

Factor V Leiden (FVL)

FVL is the most common inherited cause of thrombophilia, manifesting most commonly as DVT, with or without PE, as a result of a resistance to activated protein C. The hereditary nature of this defect has been confirmed by its identification in at least one first-degree relative of patients diagnosed with FVL (35). The heterozygous form is found in approximately 12–20% of all patients with clinically diagnosed VTE, and in approximately 40–50% of all those with recurrent or familial VTE (24,30,36,37). Approximately 3–7% of white populations of northern European or Scandinavian descent, 2.2% of Hispanic Americans, 1.2% of African-Americans, 1.2% of Native Americans, and 0.45% of Asian Americans are FVL carriers. Despite these data, there is no evidence linking FVL heterozygosity to increased mortality, per se (38).

In Case 2, the youngest of the described decedents had a heterozygous FVL mutation. His age at the time of a fatal VTE (24 years) made him a likely candidate for the existence of a hereditary thrombophila, although his race mitigated somewhat against it. Recent physical and metabolic changes that would constitute sufficient acute environmental risks were sought. Possible recent relative inactivity due to a knee injury and even more recent diarrhea and fever together were viewed as possible "soft" risk factors that may have aggravated an underlying genetic thrombophilia. Given his young age, however, it is also possible that his genetic thrombophilia alone was responsible for the fatal PE.

Prothrombin G20210A (PT)

The 20210A mutation of the PT gene was discovered in 1996 and is less common than FVL (4). The overall prevalence of the heterozygous form of this mutation in the United States ranges between 1% and 2%, although it is overwhelmingly found in Caucasians, particularly of southern European descent (11). It is distinctly uncommon in African-Americans (approximately 0.2%), Asians, and Native Americans (39). Interestingly, two of the three described decedents testing positive for this heterozygous defect were African-Americans, a disproportionate representation perhaps reflecting a study population bias (40). In terms of VTE risk, both the heterozygous and homozygous mutations for this gene are considered far weaker than analogous mutations for FVL or protein C and S (22). The relative risk of VTE for a heterozygote is thought to be two to three times that of the general population (4,11), as compared to an up to 7-fold risk for a FVL heterozygote (10).

In Case 1, the decedent's week long history of dyspnea combined with the microscopic appearance of the DVT indicated that the thrombosis was at least, in part, of a chronic nature. It is unknown whether her OCP use was of recent onset. OCP's alone confer an increased risk of VTE, ranging from 3- to 9-fold, depending upon whether a second or third generation formulation is used, the latter conferring twice the risk of the former (9,20,21,41–43). By contrast, HRT confers up to a 2- to 4-fold risk, which probably disappears after the first year of treatment (2, 25). These risks exist independent of smoking or genetic predisposition, although the existence of either steeply increases the likelihood of VTE. Despite these statistics, however, she was tested for a more tenable genetic explanation given her young age, the rarity with which OCP's lead to a fatal VTE in the absence of smoking, and the unlikelihood that a remote plane ride would have resulted in an acute PE (28,29). A heterozygous prothrombin mutation (PT) was detected.

Interestingly, because of the negative effect that OCP's have on factor V, it is the FVL mutation rather than that of PT which results in a multiplicative increase in risk when found in women taking OCP's (35-fold for heterozygotes and 100-fold for homozygotes) (10,44). Regardless, the additive independent risks of OCP's and a heterozygous PT mutation served as competent combined etiologic explanations for this decedent's fatal PE, although the PT mutation alone could have been sufficient.

In Case 3, the decedent was tested because of no apparent proximate cause. Unconvincing risk factors included his age (49 years) and his weight (6′, 248 lb.), neither of which had caused prior symptomatic VTE, despite his underlying genetic predisposition (heterozygous PT and homozygous MTHFR). The only acute physical/lifestyle change consisted of "malaise, back pain, and indigestion," the cause of which was not clearly elucidated by history or autopsy. Arguably, his advancing age combined with the relative inactivity of a nonspecific illness or prodrome may have been enough to aggravate and ultimately unmask his previously silent genetic thrombophilia. The significance of the homozygous MTHFR mutation is less clear and discussed below.

By contrast, three significant environmental/physical risk factors were identified in the young woman described in Case 4: the peripartum period, surgery, and a myomatous uterus. Despite this, she was tested because of her young age (34 years), relatively low body mass, and the setting of a gestational VTE. A heterozygous PT mutation was detected.

Pregnancy is a transient hypercoagulable state, conferring a 3 to 4-fold risk during the entire gestational period and extending into the first few postpartum months (9). Endogenous hormonal and chemical changes drive the system into one of procoagulation, only to be mechanically aggravated by the third trimester's rapidly enlarging uterus. Superimposed large leiomyomata further increase the risk. Despite these acquired risks, however, the majority of women with gestational VTE, if carefully evaluated, are found to have a superimposed inherited thrombophilia. These include: FVL in 30%–60%, PT in 10%–20%, antiphosolipid antibodies in 10%–20%, and some combination of AT, protein C, and protein S in another 10% (9). The absolute risk of a gestational VTE in PT carriers is 1 in 200, greater than in FVL carriers (1 in 500) (23). This case is an excellent example of the need to look further after the etiologic bottom line is thought to have been reached, and underscores the importance of testing all women dying of a PE during or just after pregnancy. Certifying this death, without molecular testing, as due to the combined effects of postpartum hypercoagulability, surgery, and the venous compressive effects of a myomatous uterus would have been reasonable, but the opportunity to diagnose a hereditary thrombophilia would have been missed.

Methylenetetrahydofolate Reductase (MTHFR)

The C677T (thermolabile) variant of the MTHFR mutation is extremely prevalent, particularly among Caucasians, in the general population: 10–13% for homozygotes and 40–45% for heterozygotes (16,45). Despite this high prevalence, far exceeding the other two mutations, it's clinical relevance is debated, and neither heterozygous nor homozygous forms are thought to represent independent risk for venous thrombosis. The mechanism by which homozygous or heterozygous MTHFR mutations might favor thrombophilia, however, is through impairment of the methionine-homocysteine metabolic pathway, one that is dependent upon activated forms of folate and vitamin B12. Hyperhomocysteinemia as an independent arterial and venous thrombotic risk factor has received much attention in the clinical and scientific literature (13,16,45). The extent to which MTHFR mutations contribute to hyperhomocyteinemia is less clear, but it is widely viewed as being correlated with the extent to which serum folate is depleted or antagonized (13,15,46–49).

It is with this mechanism in mind that the unique clinical characteristics of the decedent described in Case 5 offer a compelling argument for the likely relevance of her heterozygous MTHFR mutation. She had just completed her seventh and final cycle of methotrexate, cytoxan, and 5-fluorouracil (MTX, CTX, and 5-FU) one week prior to her death. Patients undergoing this specific adjuvant triple chemotherapeutic regimen have a statistically significant increase in the risk of VTE relative to the general population, despite their low stage disease and normal ambulatory lifestyles (50–52). Although the mechanism underlying this risk is likely complex, markedly decreased serum protein C and S concentrations in all patients undergoing therapy have been observed and, therefore, hypothesized as contributory (52). What is confounding is the seeming arbitrariness with which clinically manifest VTE occur in these patients. All patients experience similar reductions in their serum protein C and S concentrations, but fewer than 10% experience VTE severe enough to require medical attention or result in death. Patients older than 50 years have a slightly increased risk (51), but, after tumor burden and all other independent environmental thrombophilic risk factors are controlled for, clinical features predictive of severe VTE outcomes in this population of patients have not been identified.

Methotrexate is a folate antagonist. Among a population of women receiving methotrexate for low-stage breast cancer, the prevalence of the MTHFR mutation should be similar to that of the general population (40–45% for heterozygotes; 10–13% for homozygotes). Therefore, it is possible that an as yet clinically unidentified thrombophilic characteristic in patients experiencing severe VTE is a MTHFR mutation, suddenly becoming clinically manifest in the setting of iatrogenic folate antagonism (MTX), combined with known protein C and S reductions. The extent to which folate is depleted during therapy is unknown, as neither it nor homocysteine are routinely monitored. It is not known to what extent severe folate depletion may increase the clinical risk of a previously silent heterozygous MTHFR mutation. But, given the high prevalence of this mutation in the general population and that of women undergoing adjuvant chemotherapy for low-stage breast cancer, a prospective study of these patients measuring VTE outcomes against the presence of this mutation could lead to clinically relevant information. It is conceivable that the simple monitoring of homocysteine during therapy could significantly reduce morbidity and mortality.

The decedent described in Case 5 died cancer-free and without convincing environmental thrombophilic risk factors. She was survived by similarly-aged sisters who, given the familial nature of breast cancer, are at an increased risk of finding themselves faced with the prospect of receiving methotrexate for low-stage disease. The existence of a MTHFR mutation in their sister might have important bearing on treatment decisions for these survivors and their physicians, possibly averting a fatal outcome. Her death poses a strong argument in favor of postmortem molecular testing for thrombophilic risk in this context, despite the fact that heterozygous MTHFR has not yet been clinically proven to independently increase venous thrombotic risk.

The relevance of the homozygous MTHFR defect in the decedent described in Case 3 is unclear. The coexistence of two or more hereditary thrombophilias may have a multiplicative effect (53,54). Because of the indeterminate risk conferred by a homozygous MTHFR mutation, however, its significance in this death is not clear. It may have played a synergistic role in combination with the heterozygous PT mutation.

Heterozygous MTHFR mutations alone were found in 7 of the 29 remaining decedents in whom molecular testing was performed; the ages, races, and identifiable environmental risk factors of all tested individuals are summarized in Table 3. The overall prevalence of the heterozygous form of this mutation in the mixed ethnicity study

population was 24% (8/34), lower than that published for Caucasian populations at large (45%). Interestingly, that prevalence increases to 54% (7/13) when African-American and Hispanic individuals are excluded from the sample. Still, the relevance of the MTHFR mutations in these decedents is unclear without knowing their serum homocysteine and/or folate concentrations at the time of death. If, however, the prevalence of the heterozygous MTHFR defect were known in all persons reported to our office and dying as a result of PE during the same time period, then it could be compared with that of heterozygous MTHFR in the general population. Only with this denominator can a causal relationship between a MTHFR carrier state and thrombophilic risk be postulated. To date, no such data exist.

The most common indication for case selection in this series was age less than 45 years (27/34, or 79%), irrespective of co-existent risk factors. Of the remaining decedents, "soft" or inapparent risk factors were the primary determinant. Of the total selected for testing, four were intra- or postpartum at the time of their deaths. Of those, two had detectable heterozygous mutations, one of which was MTHFR. Also of interest was the slightly disproportionate racial composition, with African-Americans being unexpectedly overrepresented both in those selected and among those with mutations thought to be causal in their deaths (53% and 60%, respectively), a phenomenon that may reflect a selection bias. There are few studies that have specifically measured the prevalence of these mutations in African populations, against which the data of this study could be compared (40). By contrast, of all those who tested positive for the MTHFR mutation, 77% were Caucasian, a prevalence more reflective of established epidemiologic data. Also interesting were the prevalences of FVL and PT mutations in African-Americans within this select population: 5.5% and 11%, respectively, much higher than expected when compared to general population-based prevalence statistics (Table 1). Although compelling, the small sample size makes interpretation of such data difficult.

It is important to recognize the technical limitations of diagnosing hereditary thrombophilias in postmortem samples. Despite conscientious molecular diagnostic testing, a certain percentage of deaths without clearly identifiable acquired or heritable risk factors will remain undetermined. These may represent those persons with as yet undiscovered heritable defects, or less common defects that require functional or serologic assays that are ill-suited for postmortem blood (55). The latter include protein C and S and antithrombin deficiencies, dysfibrinogenemia, coagulation factor polymorphisms, hyperhomocyteinemia, antiphospholipid antibodies, and plasminogen abnormalities. One or more of these abnormalities also may co-exist with each other and/or with one of the other three tested for, further increasing unidentifiable risk. This is particularly true of the FVL mutation because of its interplay with protein C and S.

Conventional wisdom and common forensic practice dictate that if one or more of the "classic" environmental thrombophilic risk factors is identified in an individual dying of a PE, it constitutes the proximate cause of death. Given the prevalence of the common hereditary thrombophilias, however, it is illogical to assume that heterozygous defects and "classic" environmental risk factors are mutually exclusive. Acquired thrombophilic risk factors far exceed that of the morbidity and mortality attributable to PE. Therefore, one must ask what enhances the susceptibility of those who suffer a fatal VTE? It is possible, if not likely, that some of those persons who are immobilized, obese, postoperative, pregnant, taking OCP's or HRT, aged, leukemic, and/or have cancer and die as a result of a PE do so because they also carry a heterozygous mutation. Particularly since greater than 50% of those patients with a hereditary thrombophilia experience VTE's in the setting of one or more acquired factors known to impart "independent" risk. The death of the postpartum woman described in Case 4 is illustrative of this phenomenon. Conversely, the vast majority of those with the same acquired risk factors never experience a clinically manifest VTE, perhaps, for many, because they are not carriers of a heterozygous mutation. For these reasons, ideally all PE deaths, not just those meeting specific selection criteria, should be tested for the three most common hereditary thrombophilias: FVL, PT, and MTHFR. Only then can one ascertain the significance of the highly prevalent MTHFR mutation and lay the groundwork for possible clinical application and family counseling. As it stands, of those autopsied by the OCME during the study period, 0.8% (124/15,280) died from a PE. Thirty-four (0.2% of all autopsied and 27% of those dying from PE) met the criteria for further molecular studies. Of those, one or more mutations of some type were detected in 35% (12/34).

Discussions between immediate family members and pathologists regarding detected defects should be general in terms of survivors' potential risk, but a clear recommendation that they discuss the results with their primary physician is singularly important. As a result of counseling and testing by their primary physician, affected first degree relatives might be treated differently postoperatively, choose to take prophylaxis during or after pregnancy, take frequent walks or use compression stockings during long car or plane rides, or decide against HRT or OCP therapies.

This retrospective analysis of hereditary thrombophilias as they relate to mortality is the first of its kind. The scope is limited by case selection criteria. Also, the lack of epidemiologic prevalence data in populations that are race-matched to that of an urban medical examiner's office make biostatistical comparisons less meaningful. Consequently, conclusive statements regarding fatal thrombotic risk, particularly with MTHFR, would be premature. Our data do suggest, however, that further clinical investigation may be warranted in the monitoring of low stage breast cancer patients undergoing adjuvant chemotherapy. In addition, a history of prior methotrexate therapy in persons dying from PE, particularly without cancer or any other apparent cause, should warrant postmortem testing for MTHFR.

At this juncture, the extent to which forensic pathologists miss important molecular diagnoses is largely unknown. The cases described in this series underscore the need for postmortem testing in at least selected cases (Table 2), insofar as testing facilities and budgetary constraints allow. However, the fact that an individual dies as a result of a PE already puts him in a select risk category, arguably making moot clinically established selection criteria. In other words, death, the most adverse of outcomes, may be the single risk factor necessary for case selection. Ideally, initial postmortem testing could include all persons dying from PE, as a means of data collection and guidance in the future development of appropriate selection criteria. Until that is possible, however, any and all data generated by increased postmortem testing could bring illuminating information to the medical literature, allowing forensic practice the ability to keep pace with this important and rapidly developing field, and potentially contribute to the reduction of morbidity and mortality and the enhancement of public health.

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