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Activated Protein C Resistance Is Uncommon in Sudden Death Due to Pulmonary Embolism*

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ABSTRACT: Activated protein C resistance (APC-R) is the most common inherited defect of the coagulation system known to date, affecting 3–5% of Americans. It is an autosomal dominant disorder associated with an increased risk of venous thrombosis and is reportedly found in 21% of individuals with deep venous thrombosis. Medical examiners are in a unique position to make the diagnosis since a fatal pulmonary embolism may be the first manifestation of the disorder. This study examines the prevalence of APC-R in individuals who die suddenly of pulmonary embolism to help medical examiners decide if routine testing is indicated. We examined 66 cases of sudden death due to pulmonary embolism seen at the Bexar County Forensic Science Center in San Antonio, Texas, from 1993–1997. The median age was 46 years with a range of 14 to 93 years. Fifty-three percent were Caucasian, 24% were African-American, and 23% were Hispanic. Twenty-seven percent had no known risk factors for pulmonary embolism. Whole blood was tested for the factor V codon 506Q mutation responsible for APC-R using polymerase chain reaction. The prevalence of APC-R was 4.5%, which is similar to the prevalence of APC-R in the general American population. These data imply that individuals with APC-R are not at increased risk for sudden death due to pulmonary embolism, or, conversely, that most fatal pulmonary emboli seen in the medical examiner setting are not induced by APC-R. Routine postmortem testing for the factor V 506Q mutation does not appear indicated at this time, given the low prevalence and high cost of testing.

KEYWORDS: forensic science, forensic pathology, activated protein C resistance, sudden death, pulmonary embolism, deep venous thrombosis

Activated protein C resistance (APC-R) is the most common inherited defect of the coagulation system identified to date (1). The mode of inheritance is autosomal dominant and it carries a 7-fold increased risk of venous thrombosis (2), thereby leading to significant morbidity. For example, APC-R is found in 21% of persons presenting with their first episode of a lower extremity deep venous thrombosis (2) and 30% of women with thrombosis associated with oral contraceptive use (3). Since deep venous thrombosis is the precursor for pulmonary embolism, medical examiners are in a unique position to diagnose APC-R when a fatal pulmonary embolism is the first manifestation of the disorder. Such was the case recently at the Bexar County Forensic Science Center in San Antonio, Texas, when a 14-year-old Hispanic boy who died of a pulmonary embolism was diagnosed with APC-R at autopsy. He had no other well-defined risk factors for pulmonary embolism.

Many medical examiners may not be familiar with APC-R because it was described only recently by Dahlbeck and colleagues (4). Protein C is a vitamin K-dependent zymogen to a serine protease that acts as an anticoagulant. Protein C is activated on the surface of endothelial cells by a complex of thrombin and thrombomodulin. Activated protein C (APC) normally downregulates the coagulation cascade by proteolytic cleavage of coagulation factors Va and VIIIa. Resistance to APC results from a point mutation of the factor V gene that alters its cleavage site (5). Individuals with the mutation are therefore hypercoagulatable and predisposed to thrombosis. The factor V mutation associated with APC-R is commonly called "factor V codon 506Q mutation" or "factor V Leiden" after Leiden, a town in the Netherlands where the mutation was discovered.

The prevalence of APC-R varies from 1–15% within the Caucasian race (1). It is highest in Sweden where approximately 15% of the population carries the mutation (1,6), while in the United States, the prevalence is about 3–5% (1). A recent study of 4047 Americans showed that the prevalence varies across racial groups, affecting 5.3% of Caucasian Americans, 2.2% of Hispanic Americans, 1.3% of Native Americans, 1.2% of African Americans, and 0.45% of Asian Americans (7). No significant difference in the carrier frequency was observed between men and women.

The laboratory diagnosis of APC-R is generally a two-step process in living patients (8). First, a functional assay for activated protein C resistance is performed on an anticoagulated blood sample. If the result is positive, borderline, or uninterpretable, then molecular testing is indicated to confirm the presence of the factor V codon 506Q mutation. Molecular testing can be performed on any nucleated cell sample, including clotted blood. In deceased individuals without a sample of antemortem anticoagulated blood, we must proceed directly to molecular testing of clotted blood. The commercial cost of the molecular assay typically is between $150 and $200. A forensic molecular laboratory may be able to provide the assay less expensively.

This study was designed to examine the prevalence of APC-R in a group of individuals with sudden death due to pulmonary embolism in order to help medical examiners decide whether routine testing is indicated. While a postmortem diagnosis of APC-R has important implications for surviving family members, the cost of

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routine testing of all cases of pulmonary embolism in the medical examiner setting can be justified only if the prevalence in this group exceeds that of the general population.

Methods

We examined all cases of sudden death due to pulmonary embolism seen at the Bexar County Forensic Science Center in San Antonio, Texas, from 1993 to 1997. Of the 73 cases identified, seven were excluded from the study for the following reasons: five did not have autopsy-proven pulmonary embolism as the diagnosis was based on an external examination and clinical information; one was embalmed prior to autopsy so blood was not available; and one blood sample did not contain amplifiable DNA. In total, the study group contained 66 cases. The diagnosis of pulmonary embolism was based on gross findings alone in 73% of the cases; 27% of the cases included microscopic examination of the thromboembolism.

Autopsy reports and available medical records were reviewed for the following information: sex, age, race, height, weight, and risk factors for thrombosis. Risk factors for thrombosis included obesity, immobilization, trauma, oral contraceptive use, pregnancy, a peripartum state, prior thrombotic events, pelvic obstruction, and malignancy. Obesity was defined as a body mass index greater than 30 kg/m^2 (9).

Frozen and refrigerated EDTA-preserved whole blood samples were analyzed in the Molecular Diagnostics Laboratory at the University of Texas Health Sciences Center at San Antonio. To test for the factor V codon 506Q mutation, DNA was first isolated from blood mononuclear cells using the Puregene DNA Isolation kit (Gentra Systems Inc., Minneapolis, MN). Polymerase chain reaction was used to amplify a 267 bp region of the factor V gene surrounding codon 506. After enzyme digestion with Mnl 1, the product was sized by electrophoresis in agarose gels stained with ethidium bromide. The band pattern was analyzed on a UV Polaroid photograph to identify specific genotypes. Individuals heterozygous for the mutation exhibit 4 bands of sizes 200, 163, 67, and 37 bp. Individuals homozygous for the mutation have only 2 bands of sizes 200 and 67 bp. The wild type has 3 bands of 163, 67, and 37 bp. Included in each experiment were the following three controls: normal, heterozygous mutant, and a blank containing reagents without DNA.

Results in the experimental study population were compared to those in the general population as defined by a large study of over 4000 Americans (7).

The next of kin were notified when a mutation was detected and provided a copy of the test result with information about APC-R. They were encouraged to follow up with their physicians for screening. Subsequent follow up of the screening results of relatives was not performed.

Results

Fifty-four percent of individuals included in the study were male and 44% were female. The median age was 46 years with a range from 14 to 93 years. Seventy-seven percent of the individuals were younger than 60 and 18% were less than 30 years old. Ethnicity was distributed as follows: 53% non-Hispanic Caucasian, 24% African American, and 23% Hispanic.

Seventy-three percent of the individuals in the study had one or more known risk factors. Specifically, 39% were obese; 38% were relatively immobile; 6% were on oral contraceptives or depoprovera; 6% had an underlying malignancy; 5% had prior thrombotic events; 2% had a recent natural spontaneous vaginal delivery; and 2% had pelvic obstruction due to large benign ovarian masses. Immobilization includes those individuals who were wheelchair-bound, on bedrest, on crutches, in a persistent vegetative state and those with decreased mobility following surgery.

Twenty-seven percent of the individuals in the study had no known risk factor for thrombosis. Of this group, 61% were non-Hispanic Caucasian, 22% were African American, and 17% were Hispanic. The median age among those with no known risk factor was 48 years with a range of 14 to 93 years; 66% were between the ages of 40 and 60 years and 44% were between 40 and 48 years old.

Three of the 66 cases (4.5%) were heterozygous for the factor V 506Q mutation and the remaining 63 showed no evidence of the mutation. No homozygotes were identified. The three heterozygotes included the 14-year-old Hispanic boy mentioned earlier who had been at home several days with an upper respiratory illness, a 58-year-old obese Caucasian woman, and a 56-year-old Caucasian man with severe idiopathic pulmonary fibrosis and a long history of severe varicose veins. The 14-year-old boy had no known risk factors for thrombosis and the latter two individuals were considered to have risk factors, namely, obesity and severe varicose veins, respectively.

Discussion

This study of 66 individuals with sudden death due to pulmonary embolism revealed a prevalence of APC-R of 3/66 or 4.5% (95% confidence interval = 0–9.5%). This is much lower than the rate of 21% reported for persons with venous thrombosis (2) and it does not significantly differ from the rate of 3–5% reported in the general American population (1,7). If the race-specific prevalences cited in the literature (7) are applied to the racial mix in our study, one can predict a prevalence of 3.6% which is not significantly different from the measured value of 4.5%. These data imply that individuals with APC-R are not at greatly increased risk for sudden death due to pulmonary embolism, or, conversely, that most fatal pulmonary emboli seen in the medical examiner setting are not induced by APC-R.

To our knowledge, this is the first study that examines the prevalence of APC-R in individuals who die suddenly of a pulmonary embolism. However, there are several European studies of the prevalence of APC-R in individuals with non-fatal pulmonary embolism. A Swiss study of 145 patients showed a prevalence of 5.5% based on a functional assay; this was only slightly higher than the prevalence of 4.0% in patients in whom a pulmonary embolism was ruled out (10). It was suggested that the low prevalence of the Swiss study reflected limitations of the functional assay (11), however subsequent studies have confirmed a relatively low incidence of the factor V 506Q mutation in living patients with pulmonary embolism. In a study from the Netherlands, 4 of 45 patients (9%) with proven pulmonary embolism and no clinical signs or symptoms of deep venous thrombosis were diagnosed with APC-R based on a molecular assay for the factor V 506Q mutation (12). Martinelli et al. (13) used the molecular assay to study 106 Italians who presented with a first episode of symptomatic pulmonary embolism, confirmed by ventilation-perfusion lung scans, and found a prevalence of 12.3% (13/106) compared with a prevalence of 2.8% in 212 healthy controls.

Our data and the results of the three European studies mentioned above reveal a surprisingly low prevalence of APC-R associated with pulmonary emboli considering the often-quoted prevalence of 21% in individuals with deep venous thrombosis. This figure of 21% comes from a study of 301 patients from Leiden, Amsterdam, and Rotterdam with a first episode of confirmed deep venous thrombosis, without underlying malignancy or anticoagulation therapy (2). The patients were matched by age and sex to 301

healthy controls. A functional assay was used to show a prevalence of APC-R of 21% in the patient group and 5% in the control group. In fact, when one examines the literature, the reported prevalence of APC-R in various groups of thrombosis patients varies from 17.5 to 64% with associated prevalences in control populations varying from 2 to 7% (14). In all likelihood, much of the range reported in the literature is due to patient selection criteria and geographic variation in allelic prevalence. If, as our study suggests, the prevalence of APC-R is truly lower in those with pulmonary embolism than in those with venous thrombosis alone, then further research is required to understand the underlying pathophysiologic mechanisms responsible for the discrepancy. It is conceivable that venous thrombi are less likely to embolize in persons with the factor V 506Q mutation compared with persons having other predisposing factors for thrombus formation.

It is interesting to note that of the 66 individuals in our study, only one was an adolescent, and he was found to carry the factor V 506Q mutation. All of the other individuals were 19-years-old or older. For unknown reasons, the risk of a first thrombotic event increases sharply at 14 years of age in persons with hereditary thrombotic disorders such as APC-R, protein C deficiency, protein S deficiency, and anti-thrombin III deficiency (8). Perhaps the biologic events triggered at adolescence also induce the clinical expression of APC-R and other hereditary thrombotic disorders. At present, routine testing for protein C deficiency, protein S deficiency and anti-thrombin III deficiency is not available for clotted, postmortem specimens. Although our study included few adolescents, we believe the factor V gene mutation test should be considered in adolescents who die of pulmonary embolism.

In summary, our results suggest that routine postmortem testing for the factor V mutation in the medical examiner setting is not indicated in all individuals with pulmonary embolism, given the low prevalence and high cost of the test. However, testing for APC-R should be considered if there is a history that suggests the possibility of a hereditary thrombotic disorder, such as recurrent venous thrombosis or a family history of venous thrombosis, and if thrombosis presents in adolescence.

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