Fatal Pulmonary Embolism: A Study of Genetic and Acquired Factors

KATHLEEN J. SLOVACEK, MD, AUDREY F. HARRIS, BA, CLSp (MB), JOHN F. GREENE, JR, MD, ARUNDHATI RAO, MD, PhD

Temple, Texas

Background: Investigators speculate that hereditary thrombotic disease coupled with acquired factors account for a large number of pulmonary thromboemboli. Clinical correlation between genetic and acquired factors with fatal pulmonary thromboemboli has not been extensively studied.

Methods: Archival autopsy material was obtained from patients who died of or with pulmonary emboli for whom confirmed autopsy results were available. Polymerase chain reaction–restriction fragment length polymorphism analysis was performed for factor V Leiden and factor II/20210A allele. Retrospective chart review was performed to determine the presence or absence of acquired factors that can predispose to pulmonary thromboemboli.

Results: Two of 36 patients (5.5%) were heterozygous for factor V Leiden. No patients had detected abnormalities for factor II/20210A allele. Eight patients (22.2%) had a malignancy present, one of whom was heterozygous for factor V Leiden. Fourteen patients (38.8%) had recent major surgery or were immobilized.

Conclusions: The incidence of factor V Leiden and factor II/20210A allele in patients with fatal pulmonary thromboemboli is not greater than published results of the incidence of these factors in the general population. Recognized acquired factors such as malignancy, recent surgery, and immobilization are frequently present in these patients. Our results suggest that genetic profiling of thrombotic disease will not replace clinical vigilance in reducing the risk for death from pulmonary thromboemboli.

Key words: PCR–restriction fragment length polymorphism, factor V Leiden, factor II/20210A, thromboemboli.
nancy, major surgery, orthopedic surgery, and oral contraceptive use, as well as antiphospholipid syndrome [1].

The interplay of genetic and acquired factors in the manifestation of fatal pulmonary emboli has not been extensively evaluated. Some investigators have studied the association of factor V Leiden with the development of pulmonary emboli. One group found that the prevalence of factor V Leiden was high in a population of patients with nonfatal pulmonary embolism and deep vein thrombosis [3]. Other investigators evaluated patients with fatal pulmonary embolism and found that the factor V Leiden mutation did not appear to influence the development of emboli in the face of severe illness [4]. This investigation is designed to examine the hypothesis that the incidence of hereditary thrombotic disease is greater in patients with fatal pulmonary thromboemboli and also to evaluate the frequency of acquired factors in this patient population.

Methods

Autopsy records from January 1994 through June 1998 were reviewed (330 autopsies). From this initial population, 36 patients were found to have pulmonary emboli diagnosed at autopsy by the pathologist. In 29 cases, the pathologist determined that the pulmonary embolism caused the patient’s immediate demise. In seven cases, pulmonary emboli were listed at autopsy as a diagnostic finding contributing to the patient’s death. All 36 patients, as determined by autopsy record review, were included in this study.

DNA Extraction

For each of the 36 cases, DNA was extracted from formalin-fixed, paraffin-embedded spleen or lymph node sections. DNA from autopsy blocks was slightly more difficult to amplify than standard surgical blocks; therefore, standard paraffin extraction methods were combined with the GlassMAX DNA Isolation Spin Cartridge System (Life Technologies/Gibco BRL, Gaithersburg, MD)[5].

For DNA preparation and extraction, four to five tissue sections of 8 μm were collected in a sterile 1.5-mL microcentrifuge tube. Sections were dewaxed by two steps of soaking in 1 mL xylenes (commercially available mixture of ortho-, meta-, and paraisomers with some ethyl benzene) at 65°C, vortexed, spun down, and the xylenes removed. Sections were washed in 1 mL 100% ethanol, spun down, and the ethanol removed, then 1 mL 70% ethanol, spun down, the ethanol removed, and the samples air dried. Samples were then incubated overnight in 200 μL of a solution containing 50 mM Tris-Cl, pH 8.5; 1 mM EDTA; 0.5% Tween 20 (polyoxyethylene(20) sorbitan monolaurate; Mallinckrodt OR, Paris, KY); 200 μg/mL protease K; and 0.1% sodium dodecyl sulfate at 65°C. The following day, 100 μL of a 1:1 Chelex 100 slurry (Chelex® 100 resin; Bio-Rad Laboratories, Hercules, CA) was added, and samples were boiled for 10 minutes. After samples were centrifuged for 5 minutes at maximum speed, the supernatant was purified using the GlassMAX Spin Cartridge System according to the manufacturer’s instructions. First, a chaotrope, sodium iodide (provided in the kit), was added to the sample at a ratio of 4.5 volumes binding solution per volume DNA solution. This DNA/sodium iodide solution was added to the GlassMAX Spin Cartridge, and the DNA binds to a silica-based membrane. After centrifugation, three washes with a cold buffer (provided in the kit) removed impurities and the sodium iodide. The spin cartridge was transferred to a clean recovery tube, and DNA was eluted in 65°C Tris-EDTA buffer (1.0 M Tris-HCl, 0.1 M EDTA).

Factor V Leiden: Polymerase Chain Reaction—Restriction Fragment Length Polymorphism Analysis

The region surrounding nucleotide 1,691 of exon 10 in the factor V gene was amplified to screen for the Leiden mutation (G→A substitution) that results in the loss of a recognition site for the restriction endonuclease MnlI [6]. Each 100 μL polymerase chain reaction (PCR) contained a final concentration of: 1X Extaq PCR buffer (Panvera, Madison, WI), 0.2 mM each dNTP, 0.6 μM primer PR-6697 (5’-TGCCCAGTGCTTAACAAGACCA-3’), 0.6 μM primer PR-990 (5’-TGTTATCACACTGGTAA-3’), 2.5 U ExTaQ (Panvera), and 6.0 μL DNA extract. Cycling conditions on a Perkin Elmer (Norwalk, CT) 9600 were: initial denaturation of 94°C for 5 minutes, followed by 35 cycles of 94°C for 1 minute, 58°C for 1 minute, 72°C for 1 minute, and a final extension of 72°C for 7 minutes. After thermocycling, a 25.0-μL aliquot PCR product was digested overnight with 24 U MnlI enzyme, 1X