Factor V Leiden and Morbid Obesity in Fatal Postoperative Pulmonary Embolism

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Hypothesis: Currently, the risk for postoperative acute pulmonary embolism (APE) is assessed clinically. We hypothesize that the expensive screening for the most common genetic thrombophilic clotting defect (factor V Leiden; R$^{506}$Q) after exclusion of established clinical risk factors does not offer additional benefit to surgical patients.

Design: We reviewed protocols and histories from 8249 consecutive autopsies performed at the Mayo Clinic, Rochester, Minn. All patients who died of APE after routine surgery and who lacked any other clinical risk factors for APE were included and compared with matched controls. Genomic DNA was extracted from archival tissues and examined for R$^{506}$Q by polymerase chain reaction amplification, restriction enzyme digestion, and direct sequencing.

Results: Acute pulmonary embolism was the immediate cause of death in 454 patients (5.5%). Of those, 32 (7.0%) had undergone routine surgery. These patients represent less than 0.07% of all case-adjusted surgical procedures in the same period. The rate of postoperative death from APE was higher after neurosurgical procedures (0.3%) than all other procedures (0.04%). Sixteen patients (50.0%) were morbidly obese. Only 1 patient was heterozygous and none were homozygous for R$^{506}$Q.

Conclusions: (1) Fatal APE is uncommon in surgical patients lacking clinically apparent risk factors for venous thromboembolism. (2) Neurosurgical patients are at increased risk for postoperative APE. (3) Morbid obesity is a major independent risk factor in cases of sudden death from APE postoperatively. (4) Routine preoperative screening for R$^{506}$Q in the factor V gene does not appear to offer additional benefit in surgical patients without clinically recognizable thromboembolic risk factor(s).

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Venous thromboembolism is associated with more than 300,000 hospitalizations annually in the United States, and acute pulmonary embolism (APE) is directly responsible for 50,000 to 100,000 deaths each year. Because venous thromboembolism is rare in the general population, identification of individuals at risk and provision of attendant prophylaxis may offer a potential public health benefit. Many risk factors for APE have been convincingly demonstrated, including prior thromboembolism, major surgery, malignancy, multiple trauma and fractures, varicose veins, pregnancy and puerperium, cardiac and neurologic disease, advanced age, and estrogen therapy, with prolonged immobilization probably representing the predominant common denominator.

In developed countries, the prevalence of obesity has increased over the past 30 years, and it is clearly associated with an increased risk of many major diseases, approximating 8% of health care costs in the United States for obesity-related problems. Obesity was suggested as a risk factor for venous thrombosis 60 years ago, but only recently has it been demonstrated as an independent risk factor for death from APE.

In some instances, thrombosis seems to be hereditary, and several biochemical defects have been identified, including deficiencies of proteins C and S, antithrombin deficiency, hyperhomocysteinuria, dysfibrinogebulinemia, increased factor VIII levels, and resistance to activated protein C. Resistance to activated protein C, first described in 1993, leads to an ineffective down-regulation of the coagulation pathway. This defect, which is found in approximately one half of patients with venous thrombosis, is primarily caused by a single point mutation in the factor V gene of the coagulation cascade, predicting substitution of arginine at position 506 with glutamine (R$^{506}$Q). Resistance to activated protein C is far more common than the other
SUBJECTS, MATERIALS, AND METHODS

This study was approved by the Mayo Clinic Institutional Review Board. Clinical histories and autopsy protocols from 8249 consecutive autopsies performed between 1985 and 1998 at the Mayo Clinic were reviewed, yielding 454 patients with APE as the immediate cause of death. Of those, all patients with prior thromboembolism, malignant disease, immobilizing cardiac or neurologic disease, multiple trauma, prolonged immobilization, parturition, estrogen therapy, and smoking were excluded. Thirty-two deaths from APE were identified in which no clinical or environmental risk factors were present, except for a routine surgical procedure performed 30 or fewer days before death (low- and moderate-risk patients, as classified by Clagett et al.2) For each of these 32 patients (group A), age- and sex-matched controls were randomly assigned in 2 groups (groups B and C) according to the following criteria. Group B: (1) sudden, unexpected death from APE; (2) no identifiable clinical or environmental risk factor for venous thromboembolism on history review; (3) age-matched plus or minus 3 years; and (4) year of death matched plus or minus 2 years. Group C: (1) sudden, unexpected death; (2) no identifiable acute or chronic thromboembolic event at autopsy and on history review; (3) age-matched plus or minus 3 years; and (4) year of death matched plus or minus 2 years. For each case and control, the postmortem body mass index (BMI) (calculated as weight in kilograms divided by the square of height in meters) was calculated. Arithmetic means and SDs were calculated, and a paired t test was used to compare the BMI values in both groups.

Genomic DNA was extracted from archival paraffin-embedded autopsy tissue,4,9 and a segment of the factor V gene containing R506Q was amplified in all 96 patient samples, using a half-nested polymerase chain reaction (PCR) approach, as described previously.8 In brief, an initial 189-base pair segment encompassing R506Q was amplified, and a second round of PCR was subsequently performed, yielding a 147-base pair final PCR product. Primer sequences have been described previously.8 Negative controls consisted of tubes with digest buffer left open during the DNA extraction process and amplified along with the patient samples. Positive controls used genomic DNA from individuals with known wild-type, heterozygous, and homozgyous R506Q status. All PCR products were subjected to an R506Q-specific endonuclease digestion with MnlI, and the fragments were analyzed by agarose gel electrophoresis. All samples indicative of either a heterozygous or a homozygous R506Q status were reamplified and redigested. In addition, the presence of the mutation was confirmed by semiautomated dRhodamine terminator cycle sequencing using an automated sequencer (ABI Prism Automated Sequencer 377XL; ABI, Foster City, Calif).

RESULTS

Of 8249 consecutive autopsies performed between 1985 and 1998 at the Mayo Clinic, APE was listed as the immediate cause of death in 454 (5.5%). Most of these patients exhibited one or more previously recognized clinical or environmental risk factor or factors on review of autopsy protocols and clinical histories. Thirty-two patients were identified with sudden postoperative death (median, 6 days; range, 1-26 days) from a pulmonary thromboembolic event and in whom none of the other established risk factors were present. This group accounts for 7.0% of deaths from APE. Sixteen of these patients (50%) were morbidly obese, as defined by a BMI greater than 30. The surgical procedures performed in these patients are given in Table 1. The rates of death forms of hereditary thrombophilia,4,9 with an estimated allelic prevalence of R506Q among white patients of 3% to 15%.11

Despite strong associations between R506Q and risks for thromboembolism, decisions on whether to screen for this inherited defect are complex and vary in different clinical settings.3 Screening before surgery is controversial and is generally not thought to be warranted, particularly because adequate thromboprophylaxis in the postoperative period reduces risks in all patients, regardless of R506Q status.3,12 However, comprehensive data evaluating the association of R506Q with other risk factors for APE in surgical patients are relatively scarce and often limited to only selected patient cohorts.3,13

We show that screening for R506Q would not have been effective in a large surgical patient population to prevent postoperative death from APE. Instead, morbid obesity seems to be a major risk factor in this population after correcting for other established clinical and molecular risk factors.

Table 1. Procedure-Adjusted Rates of Fatal Postoperative Acute Pulmonary Embolism in Patients Without Clinically Recognizable Risk Factors*

<table>
<thead>
<tr>
<th>Surgical Procedure</th>
<th>Total No. of Procedures 1985-1998</th>
<th>Postoperative Deaths From APE (Group A), No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Major joint arthroplasty</td>
<td>9768</td>
<td>4 (0.04)</td>
</tr>
<tr>
<td>Open reduction of distal extremity fracture</td>
<td>1509</td>
<td>3 (0.20)</td>
</tr>
<tr>
<td>Abdominal hernia repair</td>
<td>15 343</td>
<td>2 (0.01)</td>
</tr>
<tr>
<td>Gastrointestinal tract resection, nonmalignant</td>
<td>NA</td>
<td>2 (&lt;0.01)†</td>
</tr>
<tr>
<td>Vaginal hysterectomy</td>
<td>6 410</td>
<td>2 (0.03)</td>
</tr>
<tr>
<td>Aortic valve replacement</td>
<td>4 393</td>
<td>1 (0.02)</td>
</tr>
<tr>
<td>Aortic aneurysm repair</td>
<td>3 794</td>
<td>1 (0.03)</td>
</tr>
<tr>
<td>Popliteal artery bypass</td>
<td>NA</td>
<td>1 (&lt;0.01)†</td>
</tr>
<tr>
<td>Coronary artery bypass graft</td>
<td>13 925</td>
<td>4 (0.03)</td>
</tr>
<tr>
<td>Retinal detachment repair</td>
<td>NA</td>
<td>1 (&lt;0.01)†</td>
</tr>
<tr>
<td>Vascular/aneurysm neurosurgery</td>
<td>1 684</td>
<td>4 (0.23)</td>
</tr>
<tr>
<td>Resection of meningeval meningioma</td>
<td>9 09</td>
<td>5 (0.55)‡</td>
</tr>
<tr>
<td>Resection of acoustic neuroma</td>
<td>7 58</td>
<td>2 (0.26)</td>
</tr>
</tbody>
</table>

*APE indicates acute pulmonary embolism; NA, not available.
†Estimated.
‡One patient heterozygous for point mutation R506Q.
from APE in surgical patients who lacked clinical risk factors for thromboembolism are exceedingly small when adjusted for all procedures performed in the same period (Table 1). The procedure-adjusted rates of death from APE in neurosurgical patients were approximately 10 times greater than for nonneurosurgical patients.

The individuals in control group B died suddenly of an APE without having had surgery. Twenty-four patients (75.0%) were morbidly obese (BMI > 30). None of the individuals in the control group C (sudden death) showed evidence of an acute or previous thromboembolic event at autopsy or on history review. Only 4 patients (12.5%) had a BMI greater than 30; all of these patients died a sudden cardiac death.

The distribution of BMI values and their paired differences in cases and controls were symmetric, with no evidence of outliers or nonnormality (Figure and Table 2). The BMI values were significantly greater in patients who had postoperative APE deaths than in patients who died suddenly from causes other than APE (P < .001). Patients with sudden death from APE without prior surgery had significantly higher BMIs than patients who had postoperative deaths from APE (P = .03) and sudden deaths not from APE (P < .001).

A segment of the factor V gene containing R506 was successfully amplified from archival paraffin-embedded autopsy tissue in all 96 patients studied. Subsequent molecular analyses revealed 1 patient in the postoperative group and 3 individuals in each control group with at least one R506Q mutant allele (Table 2). Each of 7 patients with R506Q had a BMI less than 30. Further statistical analyses also revealed significantly different BMI values in cases than in control groups B and C (P = .01 and .002, respectively) when all patients positive for R506Q were excluded or in case-control pairs in which the cases or controls positive for R506Q were excluded.

The aim of the present study was to investigate whether preoperative screening for R506Q would provide additional benefit in surgical patients with no clinically recognizable risk factor. A rigorous exclusion of all high-risk and very high-risk patients, as classified by Clagett et al,2 identified 7% of all deaths from APE lacking those accepted risk factors. Selection bias is of concern in any study based on autopsy data because autopsy rates at all major US medical centers are steadily declining.15 The present study, however, included only patients with a sudden and unexpected death, with an autopsy rate of virtually 100% at our institution. We were able to amplify and analyze the gene region of interest from routinely processed autopsy tissue in all 96 patients studied, some of which had been in paraffin-embedded, archival storage for more than 15 years. This attests to the extraordinary value of meticulously maintained autopsy tissue registries for tackling current clinical problems with sophisticated, evolving technology.

All cases (group A) and the controls from group B had a well-documented postoperative APE as the immediate cause of death, whereas no patient from control group C showed signs of acute or chronic venous thromboembolism of any type at autopsy. All controls in group C with a BMI greater than 30 (n = 4) died a sudden cardiac death, consistent with the known association of morbid obesity and sudden cardiac death.16

The use of BMI as a measure of obesity for many studies is widely accepted because it is easily measured and remembered and predicts morbidity and mortality in most studied populations.5,6 Morbid obesity should be defined as a condition of excess adipose tissue associated with adverse health outcomes7 because data linking mild overweight (ie, a BMI of 25-29) and death are limited, fragmentary, and often ambiguous.18 In contrast, a BMI

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**Table 2. Clinical and Laboratory Characteristics of Cases and Controls**

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Age, y. Mean ± SD</th>
<th>Male-Female Ratio</th>
<th>BMI, kg/m², Mean ± SD</th>
<th>Individuals With BMI &gt; 30, No.</th>
<th>Individuals With R506Q</th>
</tr>
</thead>
<tbody>
<tr>
<td>A, Postoperative APE (n = 32)</td>
<td>66.0 ± 15.3</td>
<td>0.68</td>
<td>29.7 ± 7.1</td>
<td>16</td>
<td>1 Homozygous 0</td>
</tr>
<tr>
<td>B, † “Spontaneous” APE (n = 32)</td>
<td>68.0 ± 14.0</td>
<td>0.68</td>
<td>34.7 ± 7.5</td>
<td>24</td>
<td>3 Homozygous 0</td>
</tr>
<tr>
<td>C, † Sudden death, not APE (n = 32)</td>
<td>68.5 ± 14.7</td>
<td>0.68</td>
<td>25.7 ± 4.8</td>
<td>4</td>
<td>1 Homozygous 0</td>
</tr>
</tbody>
</table>

*APE indicates acute pulmonary embolism.† Data from Blaszyk et al.*

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greater than 30 is clearly correlated with serious health problems and carries an important mortality risk.\textsuperscript{3,6} Therefore, this threshold was used to define morbid obesity in the present study.

The presence of R\textsuperscript{506}Q as the cause for resistance to activated protein C is recognized as one of several genetic risk factors for thrombosis, but it is far more common than other forms of hereditary thrombophilia.\textsuperscript{4,9,10} The exclusion of patients with a mutant R\textsuperscript{506}Q status did not change the results of our statistical analysis. None of 96 studied individuals carrying R\textsuperscript{506}Q were morbidly obese.

Few studies have addressed the relationship between the factor V Leiden genotype and occurrence of venous thromboembolism after operation, and the available data are conflicting.\textsuperscript{3,12,13} Generally, it is believed that preoperative screening would not provide additional benefit, but the data are limited and were obtained by studying only select surgical patients. Moreover, the cost of routine screening far outweighs potential benefits.\textsuperscript{12}

We have shown that the exclusion of patients at high or very high risk for postoperative APE\textsuperscript{2} on clinical grounds alone in a large patient population left only a few patients who died in spite of such risk factors not being present (low- and moderate-risk patients, as classified by Clagett et al\textsuperscript{2}). In this group, knowledge of the R\textsuperscript{506}Q status would not have improved the potential prevention of death. The observed approximately 10-fold higher incidence of death from APE in neurosurgical patients (Table 1) attests to the increased risk in this population because routine anticoagulation is generally not used.\textsuperscript{20} Preoperative screening for R\textsuperscript{506}Q may prove effective among selected patient cohorts,\textsuperscript{21,22} recognizing the interaction of clinical, environmental, and genetic risk factors for APE. This process requires much additional knowledge about the combined effect of all possible combinations of risk factors and the dynamics of their interplay.\textsuperscript{4}

Our data strongly support the idea that morbid obesity is an independent risk factor for sudden death from postoperative APE. Because obesity is common in developed countries and because the prevalence of morbid obesity is increasing,\textsuperscript{5,6} synergy with other risk factors becomes likely and could account for an even greater number of postoperative deaths from pulmonary thromboembolic events. Measures to prevent morbid obesity remain the public health approach of choice because a progressive fattening of the population is not inevitable.\textsuperscript{28}

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