Resistance to Activated Protein C, Factor V Leiden and the Prothrombin G20210A Variant in Patients with Colorectal Cancer

Gregorios A. Paspatis\textsuperscript{a} Aikaterini Sfyridaki\textsuperscript{b} Nikolaos Papanikolaou\textsuperscript{a} Kostantinos Triantafyllou\textsuperscript{a} Aikaterini Livadiotaki\textsuperscript{b} Andreas Kapsoritakis\textsuperscript{a} Niki Lydataki\textsuperscript{a}

\textsuperscript{a}Department of Gastroenterology and \textsuperscript{b}Regional Blood Bank Center, Benizelion General Hospital, Heraklion, Greece

Key Words
Activated protein C · Factor V Leiden · Prothrombin G20210A variant · Colorectal cancer · Greece

Abstract
Objective: The aim of our study was to determine the frequency of resistance to activated protein C (APC), factor V Leiden (FVL) and the prothrombin G20210A variant in patients with colorectal cancer. Methods: 74 patients with colorectal cancer and 192 colonoscopically selected controls were prospectively investigated for the presence of APC resistance, FVL and the prothrombin G20210A variant. APC resistance was measured as the ratio of activated partial thromboplastin times with and without APC (APC sensitivity ratio, APC-SR). The FVL and prothrombin G20210A variant were detected by a polymerase-chain-reaction-based technique. Results: FVL was detected in the heterozygous form in 4 of 74 cancer patients (5.4%) and in 7 of 192 controls (3.6%; p > 0.5, odds ratio: 1.51). After excluding patients and controls with FVL, APC-SR was below 2 in 6 of 70 cancer patients (8.5%) and in 1 of 185 controls (0.5%; p > 0.05, odds ratio: 17.25), and the mean value of APC-SR was significantly lower in cancer patients than the respective level of controls (2.8 vs. 3.7, p < 0.001). The G20210A mutation in the prothrombin gene was found in the heterozygous form in 2 of 74 patients with colorectal cancer (2.7%) and in 5 of 192 colonoscopically control subjects (2.6%; p > 0.5, odds ratio: 1.03). Conclusions: These findings suggest that patients with colorectal cancer have a high frequency of resistance to APC but no significant differences in the frequency of the FVL or G20210A mutation of the prothrombin gene compared to colonoscopically selected controls.

Introduction

Thromboembolic episodes are a major complication in patients undergoing surgery. Moreover, patients undergoing colorectal surgery are at a significantly higher risk for the development of thromboembolic episodes than most other patients undergoing a general surgical procedure [1]. It has been reported that both coagulation and fibrinolysis...
may be disturbed in patients with colorectal cancer [2–4]. Genetic and circumstantial risk factors are involved in the risk for thrombosis.

In 1993, inherited resistance to activated protein C (APC), a new mechanism for thrombophilia, was reported [5]. The most common cause of APC resistance was found to be a point mutation in the factor V gene which predicts the synthesis of a factor V molecule (FVQ506 or factor V Leiden, FVL) that is not properly inactivated by APC [6]. A genetic polymorphism in the 3'-untranslated region of the prothrombin gene with a G to A transition at nucleotide 20210 (prothrombin G20210A variant) has been described [7]. The FVL and G20210A variant of the prothrombin gene seem to be risk factors for venous thrombosis [7–9].

Some studies have shown an increased frequency of resistance to APC in malignancies [10, 11]. However, in those studies colorectal cancer has not been extensively investigated. To the best of our knowledge, no data are available on the frequency of the prothrombin G20210A variant in patients with colorectal cancer.

The aim of our study was to thoroughly investigate the frequency of APC resistance, FVL and the prothrombin G20210A variant in patients with colorectal cancer.

### Patients and Methods

Seventy-four patients with newly diagnosed untreated colorectal cancer and 192 colonoscopically selected controls matched for age and sex were prospectively investigated for the presence of APC resistance, FVL and G20210A mutation in the prothrombin gene. Protein C, protein S, antithrombin III, fibrinogen, D-dimer, prothrombin fragments 1 + 2, plasminogen activity, α2-antiplasmin, plasminogen activator inhibitor type 1 (PAI-1), prothrombin activity and tissue plasminogen activator (t-PA) were also measured for the estimation of hemostatic balance. All patients were also tested for lupus anticoagulants. None of the subjects had a history of thromboembolic episodes or malignancy, or clinical or laboratory evidence of serious hemorrhage or thrombosis. Controls underwent colonoscopy for alterations in bowel habits or abdominal pain, but no colorectal abnormality was detected. Therefore, the control group included subjects who were colonoscopically free of colorectal neoplasia (including adenomas). Among the cancer patients, colorectal cancer staging (Dukes classification, Astler-Coller modification) included 2 patients with Dukes A, 9 with Dukes B1, 20 with Dukes B2, 17 with Dukes C1, 13 with Dukes C2 and 13 with metastatic disease. Patients and controls with presence of lupus anticoagulant, use of anticoagulants (coumarin or heparin), contraceptive pills or hormone replacement therapy, or pregnant women were excluded. Demographic data concerning the study groups are presented in table 1.

Venous blood samples were collected and centrifuged at 2,000 × g for 10 min at 4 °C. Plasma was removed, recentrifuged and stored at −70 °C until assayed. Plasma fibrinogen concentration was measured by the Clauss method, using bovine thrombin (Brownes) and Qwen’s buffered saline. Protein C, antithrombin III, plasminogen, PAI-1, and α2-antiplasmin were measured using a chromogenic substrate kit (Dade-Behring, Marburg, Germany) in a BCT (Behring Coagulation Timer) analyzer. Prothrombin fragments 1 + 2 (Dade-Behring) and D-dimer, protein S and t-PA were determined by enzyme-linked
APC resistance was measured as the ratio of activated partial thromboplastin time with and without APC (APC sensitivity ratio, APC-SR, Dade-Behring). Normal values of APC-SR were considered to be 2–5. All the cases were further studied using genetic testing. Genomic DNA isolation from EDTA blood, polymerase chain reaction and detection of the FVL and G20210A mutation in the prothrombin gene were done using the factor V gene mutation assay and prothrombin gene mutation assay, respectively (Vienna Lab, Vienna, Austria).

**Statistical Analysis**

Continuous data were compared with unpaired Student’s t or Mann–Whitney tests as appropriate. Categorical variables were tested using corrected $\chi^2$ or two-sided Fisher’s exact tests for univariate comparisons, as appropriate. To show the strength of the associations, we have included p values, odds ratios and the 95% confidence intervals for the odds ratios in the text. Statistical significance was determined as $p < 0.05$.

**Results**

Significant comorbidity in patients and controls included: 25 and 75 with a history of coronary artery disease, 5 and 15 with chronic obstructive pulmonary disease, 30 and 80 with high blood pressure and 12 and 38 with diabetes mellitus, respectively. Comedications for patients and control subjects were pertinent to the above-mentioned comorbid conditions, and in no patient treatment with the drugs mentioned in the exclusion criteria was administered. As far as the comorbidity or the comodication are concerned, no significant differences between the two study groups were observed (table 1). The pre- to post-menopausal ratio in female controls and in females with colorectal cancer were similar (6/66 and 3/27, respectively).

The results of global routine tests (prothrombin and activated partial thromboplastin time and the international normalized ratio) were not significantly different between both groups. FVL was detected in the heterozygous form in 4 of the 74 cancer patients (5.4%) and in 7 of the 192 controls (3.6%; $p > 0.5$, odds ratio: 1.51; the 95% confidence interval for the odds ratio is from 0.43 to 5.18). After excluding patients and controls with FVL, APC-SR was below 2 in 6 of the 70 cancer patients (8.5%) and in 1 of the 185 controls (0.5%; $p < 0.01$, odds ratio: 17.25; the 95% confidence interval for the odds ratio is from 2.04 to 140.45), and the mean value of APC-SR was significantly lower in cancer patients than the respective level of controls ($2.8 \pm 0.5$ vs. $3.7 \pm 0.9$, $p < 0.001$; fig. 1). Among the 18 patients and controls with APC-SR <2, FVL was detected in 11 cases. Among the 6 cancer
patients with APC-SR <2 in the absence of FVL, Dukes stage C1 was detected in 1 patient and metastatic disease in the remaining 5 (fig. 2).

The G20210A mutation in the prothrombin gene was found in the heterozygous form in 2 of 74 patients with colorectal cancer (2.7%) and in 5 of 192 control subjects (2.6%; \(p > 0.5\), odds ratio: 1.03; the 95% confidence interval for the odds ratio is from 0.19 to 5.33). A combination of FVL and the G20210A variant of the prothrombin gene in the same subject was not detected. In the 2 cancer patients with the G20210A variant, the APC-SR >2 and staging revealed Dukes stage B1 and C1.

The mean values of D-dimer, prothrombin fragments 1 + 2, fibrinogen and PAI-1 levels were significantly higher in cancer patients than in the controls (\(p < 0.01\)). t-PA activity was significantly reduced in cancer patients compared to controls (\(p < 0.01\)). Table 2 shows the hemostatic parameters in patients with colorectal cancer and controls.

### Discussion

Thrombosis is the most common complication and the second cause of death in patients with overt cancer [12]. In 1992, Prandoni et al. [13] reported a significant association between idiopathic venous thrombosis and the subsequent development of clinically overt cancer. The frequency of thromboembolic events after colorectal cancer surgery is similar to or somewhat higher than that found after other types of major abdominal operations [14]. Biologically plausible mechanisms for thrombosis in cancer patients include the capacity of tumor cells and their products to interact with platelets, clotting and fibrinolytic systems [12].

In most cases, APC resistance is caused by a single mutation in the factor V gene [15]. A transition (G to A) at nucleotide 1,691 in exon 10 results in the synthesis of a variant factor V molecule (FVL) with the substitution Arg→Gln at amino acid position 506 [6]. Resistance to APC caused by FVL is the most common genetic risk factor that leads to a state of hypercoagulation [8, 16, 17].

There are very few data in previous studies evaluating the APC resistance and FVL in patients with colorectal cancer. In the present study, we have more extensively investigated the frequency of both APC resistance and FVL in patients with colorectal cancer compared to previous studies in this area. The mean levels of APC-SR are lower in patients with colorectal cancer compared to controls in a statistically significant level. Previous studies in cancer patients have reported roughly similar results regarding the mean level of APC-SR [10, 11].

Similarly to previous studies in our study group, FVL was detected in the cases with the lowest values of APC-SR [10]. Dahlback [18] has calculated that a low APC-SR predicts the presence of FVL in about 69%. In contrast, the predictive value of normal APC-SR for the absence of FVL is 99%. In two pertinent studies on APC resistance in cancer patients [10, 11], a limited number of patients (6 and 12, respectively) with metastatic colorectal cancer were included. Both studies show a high frequency of APC resistance in cancer patients but a low frequency of

---

**Table 2. Plasma levels of hemostatic parameters in patients with colorectal cancer and controls**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Patients with colorectal cancer</th>
<th>Controls</th>
<th>p</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fibrinogen, mg/dl</td>
<td>450.5±121.1</td>
<td>306.3±78.2</td>
<td>&lt;0.01</td>
<td>200–400</td>
</tr>
<tr>
<td>Prothrombin fragments 1 + 2, nmol/l</td>
<td>0.9±0.7</td>
<td>0.6±0.1</td>
<td>&lt;0.01</td>
<td>0.4–1.1</td>
</tr>
<tr>
<td>D-dimer, µg/l</td>
<td>410.6±195.3</td>
<td>251.0±93.6</td>
<td>&lt;0.01</td>
<td>&lt;400</td>
</tr>
<tr>
<td>Antithrombin III, %</td>
<td>96.7±18.4</td>
<td>98.7±18.0</td>
<td>NS</td>
<td>85–120</td>
</tr>
<tr>
<td>Protein C, %</td>
<td>105.5±33.3</td>
<td>102.5±16.7</td>
<td>NS</td>
<td>80–120</td>
</tr>
<tr>
<td>Protein S, %</td>
<td>95.1±19.7</td>
<td>99.4±17.3</td>
<td>NS</td>
<td>70–140</td>
</tr>
<tr>
<td>Plasminogen activity, %</td>
<td>98.0±10.9</td>
<td>96.7±11.3</td>
<td>NS</td>
<td>80–120</td>
</tr>
<tr>
<td>Prothrombin activity, %</td>
<td>93.7±8.2</td>
<td>92.6±11.3</td>
<td>NS</td>
<td>60–120</td>
</tr>
<tr>
<td>α2-Antiplasmin, %</td>
<td>96.4±9.6</td>
<td>98.5±10.8</td>
<td>NS</td>
<td>80–120</td>
</tr>
<tr>
<td>PAI-1, IU/ml</td>
<td>1.8±1.2</td>
<td>1.3±0.8</td>
<td>&lt;0.01</td>
<td>0.1–3.35</td>
</tr>
<tr>
<td>t-PA, ng/ml</td>
<td>5.0±2.9</td>
<td>6.7±2.8</td>
<td>&lt;0.01</td>
<td>1–12</td>
</tr>
</tbody>
</table>

Results are expressed as means ± SD. NS = Not significant.
FVL suggesting that, most likely, an acquired defect is responsible for the APC resistance. In our study, a higher proportion of cancer patients and controls with low APC-SR were detected to have FVL. More specifically, 87.5% of the controls with low APC-SR were detected to have FVL as compared to 40% of the patients with colorectal cancer, and this enforces the hypothesis that acquired rather than genetic factors are more likely to be responsible for the resistance to APC in patients with colorectal cancer. Moreover, most of the cancer patients with APC resistance without FVL had advanced colorectal cancer (5 of 6). We speculate that cancer cells synthesize molecules interfering with APC resistance, and therefore the increased tumor burden of patients with advanced disease may explain the increased frequency of APC resistance without FVL we observed. Hille et al. [19] have previously reported that there was no significant risk of death from malignancy in 171 patients with FVL. The discrepancy with the previous studies [10, 11] regarding the frequency of FVL in patients with low APC-SR might be due to the small number of patients with colorectal cancer included in those studies, or it may reflect genetic differences between the study populations regarding the different ethnic background.

We must emphasize that according to a previous study of 50 Swedish APC-resistant families, individuals with the APC resistance phenotype but without FVL are also at increased risk for thrombosis [15]. Recent studies have identified other mutations which may affect a small percentage of patients with colorectal cancer, we preferred to study a group of controls in whom different populations determined by multiplex allele-specific PCR. The scientific basis and the underlying etiological factors of these observations need further investigation.

In conclusion, our findings further enforce the hypothesis that patients with colorectal cancer have a disturbance in the coagulation/fibrinolysis balance. Patients with colorectal cancer have a high frequency of resistance to APC but no significant differences in the frequency of FVL or G20210A mutation of the prothrombin gene compared to the controls. The scientific basis and the underlying etiological factors of these observations need further investigation.

Acknowledgments

Part of this work was presented at the Meeting of the British Society of Gastroenterology, Birmingham, March 21–23, 2000. The authors are grateful to Dr. Dimitrios Mavroudis for review of the manuscript.

References