Clinical Value of Determination of Urokinase-type Plasminogen Activator Antigen in Plasma for Detection of Colorectal Cancer: Comparison with Circulating Tumor-associated Antigens CA 19-9 and Carcinoembryonic Antigen

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ABSTRACT

We determined urokinase-type plasminogen activator antigen (u-PA), gastrointestinal cancer-associated antigen (CA 19-9), and carcinoembryonic antigen (CEA) in the plasma of patients with colorectal cancer at the time of clinical tumor detection and in a group of patients with Crohn's disease and analyzed the specificity of these tumor markers. u-PA, CA 19-9, and CEA were indicative for colorectal cancer in 75.5%, 51.5%, and 51.5% of tumor patients, respectively, with a specificity of 79.3%, 94%, and 97.5%. Sensitivity increased when two or all three markers were determined in identical blood samples, whereby a combination of u-PA and CEA exhibited the highest sensitivity value (90.9%) as compared to the combinations of u-PA and CA 19-9 or CA 19-9 and CEA. The use of all 3 markers did not lead to further increased sensitivity. False negative results were obtained in 3 of 32 cancer patients (9.1%, using one of 3 markers as indicative for malignant disease). These results indicate the benefit of multiparametric tumor marker analyses including u-PA antigen for the diagnosis of colorectal cancer.

INTRODUCTION

Tumor-associated plasma antigens have been detected and described in a variety of human malignant diseases (1-10), and some of these have become clinically valuable tools as tumor markers for detection, clinical follow-up, and prognosis of malignant diseases (11-14).

Up to now, CEA (15) and the gastrointestinal cancer-associated antigen CA 19-9 are commonly used tumor-associated antigens in gastrointestinal malignancies (16, 17). Both antigens can be detected in tumor tissues and in blood samples from patients with gastrointestinal malignancies, e.g., colorectal or pancreatic carcinomas (18-27).

Although a combined use of different tumor-associated antigens might be of better clinical value for the detection and follow-up of various cancers (28, 29), it has been shown that a combined use of CEA and CA 19-9 does not lead to further increased sensitivity in colorectal cancer (15). Because of their low sensitivity of 50-70%, CEA and CA 19-9, both commonly used markers, seem to be unacceptable for screening for gastrointestinal malignancies (27). Therefore, there is a need for a search for additional tumor-related plasma antigens in gastrointestinal malignancies.

Tumor cells have been shown to produce and to release plasminogen activators, mainly of the urokinase type (30-42), which are thought to be involved in tumor invasion and metastasis (43-46). u-PA could be demonstrated immunohistochemically in various malignant tissues, e.g., colorectal cancer (47-52), breast cancer (53-55), lung cancer (56, 57), malignant melanoma (58), and cancer of the prostate (59, 60), and might therefore be useful as a diagnostic tool.

Preliminary studies have demonstrated an elevation of u-PA plasma levels in patients suffering from gastrointestinal malignancies (61-63) and renal cell carcinoma (64). These findings might be of clinical relevance but have not been proved in confirmatory studies. Furthermore, the clinical value of u-PA as a tumor marker in patients with malignancies of the gastrointestinal tract has also not been established until now. Only recently could we show that determination of u-PA plasma levels might be of clinical importance in patients suffering from liver cancer, especially in combination with the determination of α-fetoprotein (65). It was the aim of this study to prove a possible clinical relevance of plasma u-PA antigen levels for the detection of colorectal cancer as compared to and in combination with the established markers CEA and CA 19-9.

PATIENTS AND METHODS

Tumor Patients and Controls. Plasma samples were collected from 33 patients with colorectal cancer at the time of clinical tumor detection. In all patients diagnosis of adenocarcinoma was confirmed histologically. For all tumor patients extension of the disease was classified according to the postoperative tumor-node-metastasis classification (International Union Against Cancer, 1979).

For comparison we also investigated 53 patients with chronic inflammatory bowel disease (Crohn's disease as confirmed by clinical course and/or biopsy) who were further classified according to the CDAI (66) into groups of patients with quiescent disease (CDAI ≤ 150) and with active disease (CDAI ≥ 150). Clinical characteristics of tumor patients and controls are summarized in Table 1.

Blood Collection. Blood samples were collected from resting patients after a 12-h fast between 8:00 and 10:00 a.m. to avoid possible influences on the results of u-PA plasma levels due to known circadian variations (67, 68). Blood was drawn from a cubital vein with minimal venous occlusion directly into plastic tubes prepared with sodium citrate (0.11 M final concentration) as an anticoagulant. Plasma was obtained by centrifugation at 3000 × g for 15 min at 4°C, harvested, snap frozen, and stored at −70°C until use.

Determination of Tumor Markers and u-PAs. CA 19-9 (Centocor carbohydrate antigen 19-9 radioimmunoassay; Oris Industrie, Gif-sur-Yvette, France; cutoff limit, 37 units/ml) and CEA (CEA-M-K immunoradiometric assay; Sorin Biomedica, Saluggia, Italy; cutoff limit, 10 ng/ml) were determined by the use of commercially available test kits and monoclonal antibodies. Cutoff limits were taken as recommended by the manufacturers. Urokinase antigen in plasma was determined by means of a competitive radioimmunoassay for high-molecular-weight urokinase, as described and published by us earlier (69), using a monospecific polyclonal anti-human high-molecular-weight urokinase antibody. Using this test system, age- and sex-dependent variations of u-PA plasma levels have been described (70) whereby the normal range of u-PA plasma levels for patients of both genders up to 80 years of age has been shown with this assay to be between 5.5 and 7.5 ng/ml. A cutoff limit of 8.5 ng/ml (>110% of the upper normal limit) was arbitrarily chosen to differentiate between normal and elevated u-PA plasma levels. As demonstrated earlier, less than 2% of healthy control individuals, most of them older than 70 years, exhibit u-PA levels above this cutoff limit using the described assay.
significant differences could be demonstrated between the tumor group and the Crohn's disease group with Crohn's disease who had concentrations of the tumor markers above the respective cutoff limits. Specificity was calculated as the percentage of individuals who showed plasma concentrations of the tumor markers within the normal range. Associations between u-PA, CEA, and CA 19-9 were calculated by means of the paired t test. No significant differences could be calculated between patients with localized malignant disease (M₀) as compared to patients with metastasizing disease (M₁) for all three tumor markers, using the assay system described. Normal u-PA plasma levels in age- and sex-matched healthy controls were 7.3 ± 0.5 ng/ml (±SE) for the age group of 30-50 years, which was the same as in patients with Crohn’s disease and 8.1 ± 0.4 ng/ml for the age group of 51-80 years, which was the same as in tumor patients (mean age and SD) (70).

### Statistical Methods

Significance in differences of tumor marker levels between the different groups of tumor patients and Crohn’s disease were calculated by analysis of variance. Significance of differences of the tumor markers between patients with quiescent or active Crohn’s disease or between patients with (M₁) or without (M₀) evidence of metastases in the tumor group was calculated by means of the paired t test.

### RESULTS

#### u-PA Plasma Levels in Tumor Patients and Controls

In Fig. 1, individual u-PA plasma levels of the patients with Crohn’s disease and of patients with colorectal cancer are shown. As can be seen, the majority of patients with Crohn’s disease exhibited u-PA plasma levels below the cutoff limit of 8.5 ng/ml. In 11 of 53 patients (20.7%) with Crohn’s disease slightly elevated u-PA levels above the cutoff limit could be demonstrated; however, elevation of u-PA antigen levels did not depend on the presence of active disease. In contrast, the majority of patients with colorectal cancer exhibited elevated u-PA plasma levels which were similarly distributed in patients with localized disease (M₀) as compared to patients with metastases (M₁).

Mean plasma levels (±SE) of u-PA antigen are summarized in Table 2. Within the tumor group M₁, patients exhibited a higher mean u-PA plasma level than M₀ patients which was, however, not statistically significant. The Crohn’s disease group exhibited significantly lower mean u-PA plasma levels as compared to the tumor group (P < 0.0001).

### Plasma Levels of CEA and CA 19-9 in Tumor Patients and Patients with Crohn’s Disease

Mean plasma levels of CEA were elevated significantly in patients with colorectal cancer as compared to the patients with Crohn’s disease (P < 0.001). Within the cancer group, patients with advanced colorectal cancer (M₁) exhibited about a 50-fold increase as compared to localized disease (M₀), which, however, was not significant. Patients with colorectal cancer also exhibited significantly higher CA 19-9 levels as compared to patients with Crohn’s disease (P < 0.05). Within the tumor group, those with advanced disease (M₁) exhibited a 10-fold increase of mean CA 19-9 levels as compared to those with localized disease (M₀) which was not significantly different.

### Evaluation of Specificity and Sensitivity

When u-PA was used as a single marker, 79.3% of the patients with Crohn’s disease exhibited
UROKINASE-TYPE PLASMINOGEN ACTIVATOR IN COLORECTAL CANCER

Table 3  Sensitivity values of the different tumor markers in colorectal cancer

<table>
<thead>
<tr>
<th>Tumor Marker</th>
<th>Colorectal cancer (ng/ml)</th>
<th>M0</th>
<th>M1</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>u-PA</td>
<td>(cutoff limit &gt;8.5 ng/ml)</td>
<td>81.8</td>
<td>72.7</td>
<td>75.5</td>
</tr>
<tr>
<td>CA 19-9</td>
<td>(cutoff limit 37 U/ml)</td>
<td>27.2</td>
<td>63.6</td>
<td>51.5</td>
</tr>
<tr>
<td>CEA</td>
<td>(cutoff limit &gt;10 ng/ml)</td>
<td>18.8</td>
<td>68.1</td>
<td>51.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Combination</th>
<th>Both</th>
<th>Either</th>
</tr>
</thead>
<tbody>
<tr>
<td>u-PA + CA 19-9</td>
<td>42.4</td>
<td>84.8</td>
</tr>
<tr>
<td>u-PA + CEA</td>
<td>33.3</td>
<td>90.9</td>
</tr>
<tr>
<td>CA 19-9 + CEA</td>
<td>42.4</td>
<td>60.6</td>
</tr>
<tr>
<td>u-PA + CA 19-9 + CEA</td>
<td>33.3</td>
<td>90.9</td>
</tr>
</tbody>
</table>

A correct negative plasma level of <8.5 ng/ml. Specificity values for CA 19-9 and CEA were 94% (CA 19-9) and 97.6% (CEA), respectively.

Sensitivity values for the three different tumor markers are given in Table 3. As can be demonstrated, sensitivity of u-PA as a single marker was superior to that of the established markers (75.5% versus 51.5% of both CEA and CA 19-9). Furthermore, sensitivity values for u-PA were higher in patients with localized cancer (M0). Both CA 19-9 and CEA exhibited significantly higher sensitivity values when metastases were clinically evident. When two or three markers were determined in identical blood samples, combined sensitivity values disclosed the superiority of the combination of u-PA together with CEA for the detection of colorectal cancer (both markers correctly positive in 33.3%, one of two markers correctly positive in 90.9%) as compared to the combinations of u-PA with CA 19-9 or CA 19-9 with CEA, respectively. The combined use of all three markers did not lead to a further increase in sensitivity.

Fig. 2 illustrates associations between u-PA and CEA (Fig. 2a) or u-PA and CA 19-9 (Fig. 2b) determined in identical blood samples obtained from Crohn’s disease and tumor patients. No significant correlations could be shown between u-PA and CEA or u-PA and CA 19-9 for tumor patients or patients with Crohn’s disease (Table 4). In Table 4 correlations between CA 19-9 and CEA are also given, which show a significant correlation of the markers only in the group with Crohn’s disease. False negative results using all three markers were obtained in 3 of 32 patients with colorectal cancer (9.1%).

The clinical relevance of the different tumor markers is demonstrated by means of sensitivity-specificity diagrams. In cancer patients, the ROC curve of u-PA was located closer to the theoretical 100% sensitivity and specificity value than the ROC curves of CA 19-9 or of CEA (Fig. 3).

DISCUSSION

We could demonstrate that a majority of patients with colorectal cancer exhibit elevated plasma levels of u-PA as compared to patients with Crohn’s disease; however, elevation of u-PA levels does not depend on tumor stage. This might be explained by a mechanism in which a chronic inflammatory process induced by the tumor mediates u-PA production and release by monocytes, macrophages, or stromal cells of the tumor infiltrate (73-77). In contrast to u-PA, CEA and CA 19-9 are thought to be produced mainly by tumor cells. Therefore,
increasing tumor mass reflected by tumor stage might lead to an increase of mean CEA or CA 19-9 plasma levels and to an increased sensitivity for these markers, as has been described also by others (18-24, 27). The lack of a significant difference in CEA or CA 19-9 plasma levels between our patients with M0 and A/ tumor stages (18-24, 27). The lack of a significant difference in CEA or CA 19-9. Furthermore, u-PA seems to be more sensitive in localized patients was superior compared to both established markers CEA and CA 19-9 with CEA. Furthermore, the combined use of CA 19-9 and CEA might be of greater relevance as prognostic markers (22).

In conclusion, our data provide evidence for the possible clinical relevance of the determination of u-PA antigen in addition to the determination of CEA and/or CA 19-9 in patients with colorectal cancer or diseases known to be at risk for the development of colorectal malignancy, e.g., chronic inflammatory bowel disease (83).

REFERENCES


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