PREOPERATIVE EVALUATION AND POSTOPERATIVE SURVEILLANCE FOR PATIENTS WITH COLON/RECTAL CARCINOMA: ROLE OF PROLACTIN AND CARCINOEMBRYONIC ANTIGEN

INTRODUCTION

Carcinoma of the colon/rectum is the seventh prevalent cancer in Southern Asia. At The Gujarat Cancer and Research Institute, Ahmedabad, the incidence of this malignancy for the year 1989 was 2.9%.

More complete understanding of the genetic alterations that initiate colorectal carcinogenesis and identification of nutritional and environmental factors that promote the process, should ultimately provide the opportunity to reduce substantially the incidence of this disease and to improve its prognosis. The development of colon/rectal tumor is obviously affected by many factors in the luminal environment (foods, vitamins, minerals, bile salts), as well as in the milieu of the vascular and extravascular spaces (hormonal influences, Conteas et al. 1988).

Colorectal carcinoma is a heterogenous disease in which the prognosis varies, not only with the extent of the
disease but also with its biological behaviour. The goals of preoperative assessment of patients with carcinoma of the colon/rectum are to define operative risk factors, to exclude the presence of synchronous lesions, to stage the disease and identify low-risk and high-risk subgroups of patients, so that high-risk patients can be followed up more intensely and treated accordingly (Vignati and Roberts 1993).

The goal of post-operative surveillance after curative resection for colon/rectal carcinoma is to detect recurrent tumor at a stage when it is still curable (Ho et al. 1988). Intensive follow-up studies must be carried out because when a recurrent tumor is detected, most often, it is unresectable, and majority of the patients will die of the disease within two years.

Therefore, in this study the merits of circulating prolactin and carcinoembryonic antigen in patients with colon/rectal carcinoma was compared. This study addresses the usefulness of prolactin and carcinoembryonic antigen:

1) to differentiate low-risk and high-risk subgroups,
2) to identify a better prognosticator,
3) to detect early recurrence(s)/metastasis by serial measurement,
4) to compare the tumor draining venous blood marker levels with that of peripheral blood and
5) to investigate whether prolactin is produced ectopically by colon/rectal tumors.

STUDY DESIGN

Patients
In this study, a total of 62 male patients with Dukes B or C colon/rectal carcinoma, registered at The Gujarat Cancer and Research Institute, Ahmedabad, between January 1989 and December 1990 were enrolled.

The details of clinical history were noted from the case files maintained at the Medical Record Department of the Institute and recorded at the Division of Molecular Endocrinology. The clinical history included anatomic site of the lesion (colon/rectum), operative findings; Dukes stage, histologic type and grade of the tumor, treatment offered, appearance of clinical recurrence/metastasis and survival time.

The reference group consisted of 30 healthy (nonsmokers
and smokers) age-matched controls.

Disease was staged according to modified Dukes classification (Dukes and Bussey 1958). The histologic characteristics were assessed independently by two histopathologists.

Blood collection

(a) Venous blood

Blood samples were collected in ethylenediamine tetraacetic acid, disodium salt (1-2 mg/ml) coated tubes, strictly between 9:00 and 11:00 AM to avoid diurnal variation.

Samples were collected:

(i) preoperatively to obtain baseline value of individual patients,
(ii) on 10th postoperative day and 
(iii) monthly/bimonthly for first two years and every three months thereafter.

(b) Tumor draining venous blood

Tumor draining venous blood samples (N=17) were collected at the time of surgery. The blood was collected from the main draining vein of the lesion which was ligated proximally and then tumor venous blood was collected by insertion of venous catheter.
Both the blood samples were separated within two hours, plasma and serum were aliquoted and preserved at 0 to -70°C till the analysis of prolactin and carcinoembryonic antigen.

Follow-up of patients
All patients were followed for a period of 3 years or their death within 3 years. Relapse free survival period was calculated only for patients with Dukes B disease. Dukes C is an advanced disease and therefore, the relapse free survival period was not calculated.

Marker assay
Plasma prolactin (PRL) was assayed using immunoradiometric assay (IRMA) kits procured from Binax, USA, which used WHO Third International Standard 84/500. Carcinoembryonic antigen (CEA) assay was performed using double antibody radioimmunoassay (RIA) kits obtained from Diagnostic Products Corporation, UK. Radioactivity in the pellet was counted using COBRA, Packard Gamma Counter, USA. The assays were performed in duplicate with an intra- and inter-assay coefficient of variation (CV) of 3 to 8% along with internal quality controls. The sensitivity of the assay kit was: (i) PRL - <0.3
ng/ml plasma (ii) CEA - approximately 0.9 ng/ml plasma. The normal range of PRL was up to 20.0 ng/ml plasma and that of CEA was up to 5.0 ng/ml plasma.

Assessment of disease activity
Routine preoperative assessment of disease activity was done using standard methods viz., sigmoidoscopy, barium enema, abdomino-pelvic ultrasonography, chest X-ray and biochemical tests for liver and renal functions. Computed tomography scan was done whenever necessary.

During follow-up, the patients underwent routine investigations, complemented if necessary, with radiologic investigations and fine needle aspiration cytology (FNAC).

Treatment
The primary treatment offered to the patients was surgery (N=62), followed by radiotherapy (N=5) or chemotherapy (N=23), or radiotherapy followed by chemotherapy (N=16). Surgical procedures were performed by the Surgical Oncology Unit and radiotherapy and chemotherapy were instituted by the Radiotherapy and Medical Oncology Units, respectively.
Criteria for positive test
Based on the published data in patients with colorectal cancer (Bhatavdekar et al. 1992), a criteria for positive predictive value has been fixed for PRL: a continual rise in PRL level after an initial fall or persistent high level of PRL is an indicator of relapse and/or no response to treatment.

Prognostic value
To determine the prognostic value of PRL and CEA, the patients were subgrouped according to their cut-off levels, for PRL <20.0 and >20.0 ng/ml plasma and for CEA <5.0 and >5.0 ng/ml plasma. Patients with PRL >20.0 ng/ml plasma (above upper normal limit) were termed as hyperprolactinemic.

Relationship between disease status and markers
Of the 62 patients, it was possible to follow 39 patients and the rest were lost to follow-up. The details for patients lost to follow-up were noted from the Medical Record Department of the Institute. Therefore, the disease status was known in all cases. On the basis of disease status at the end of 3 years or their death within 3 years, the patients who were
followed serially were divided into two subgroups:

Patients with response (N=19)
Patients who had responded to various treatment modalities at the end of 3 years.

Patients with progressive disease (N=20)
This group included patients who developed recurrence/metastasis within 3 years. Of the 20 patients, 13 (65%) had local recurrence and 7 (35%) had distant metastases. Of the 7 patients, distant metastasis was detected at the sites: lung (N=2), bone (N=2), liver (N=1), oesophagus (N=1) and supraclavicular node (N=1).

The sensitivity, specificity and predictive values (positive and negative) of the markers were calculated according to the method of Tondini et al. (1988) and Caponigro et al. (1990) with the following definitions:

True +ve: Patients in whom marker increased with disease progression.
False -ve: Patients in whom marker decreased with disease progression.
True -ve: Patients in whom marker decreased with response.
False +ve: Patients in whom marker increased with response.

<table>
<thead>
<tr>
<th></th>
<th>True +ve (no. of patients)</th>
<th>True +ve + False -ve (no. of patients)</th>
<th>True -ve (no. of patients)</th>
<th>True -ve + False +ve (no. of patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Specificity (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Predictive value of positive test (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
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<tr>
<td>Predictive value of negative test (%)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

**Immunohistochemical staining of prolactin**

Immunohistochemical localization of prolactin was performed in primary tumors of colon/rectum (N=25). A representative block containing an area of tumor was selected. Five μm thick paraffin embedded sections were cut and dewaxed as per routine histology. Endogenous peroxidase activity was blocked by incubation with 0.6% H₂O₂ in methanol for 5 minutes. The sections were then rinsed with distilled water and placed in 0.05 M Tris-buffered saline (TBS), pH 7.6 for 5 minutes followed by incubation for 20 minutes with normal swine...
serum (DAKO, Denmark) diluted 1:10 in TBS to prevent non-specific binding. The sections were incubated with primary antibody (rabbit anti-human prolactin antibody, DAKO, Denmark) at a dilution of 1:50 in TBS (pH 7.6) at 0°C overnight (16 hours). The second incubation was carried out using biotinylated swine anti-rabbit immunoglobulins (DAKO, Denmark) at a dilution of 1:600 for 40 minutes at room temperature. The third incubation was done using ABC (Streptavidin biotin complex, 1:1:100 dilution with Tris-HCl, pH 7.6, DAKO, Denmark) for 40 minutes at room temperature. The specific immune reaction was revealed using DAB (3,3'-diaminobenzidine tetrahydrochloride) as chromagen and 1% H2O2 as substrate dissolved in 0.2 M Tris-HCl (pH 7.6) for 3 minutes. They were counterstained with haematoxylin (5 minutes), rinsed in tap water, then dehydrated in ascending grades of alcohol and cleared in xylene. The sections were mounted in DPX. Negative control for each tumor section was processed simultaneously with the omission of the primary antibody. Human pituitary was used as positive control. Microphotographs were taken with the help of Zeiss Jena microscope (Germany).
Statistical analysis

Significance was calculated using an exact contingency table test for order data and Fisher's two sided exact test (Mehta and Patel 1983). P values less than 0.05 were considered significant. Correlation between two parameters was calculated using Pearson's correlation coefficient (r) method. Relapse free survival and overall survival time were calculated according to the life table analysis (Kaplan and Meier 1958). Chi square (X^2) statistic was used to assess the prognostic significance of relapse free and overall survival (Pearson and Hartley 1988).
RESULTS

Incidence of PRL

Preoperative PRL levels were significantly higher in patients with colon/rectal cancer as compared to controls (P<0.0001, Table 1). Fifty percent (31/62) patients had hyperprolactinemia (PRL >20.0 ng/ml plasma).

Table 1: Distribution of PRL in controls and in patients with colon/rectal carcinoma (M ± SE).

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>PRL (ng/ml)</th>
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</thead>
<tbody>
<tr>
<td>Controls</td>
<td>30</td>
<td>8.01 ± 0.51</td>
</tr>
<tr>
<td>Colon/rectal cancer patients</td>
<td>62</td>
<td>31.88 ± 4.56</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>1.20 - 195.0</td>
</tr>
</tbody>
</table>

P value: * P<0.0001

Relation of PRL to anatomic site of the lesion

Twenty-six percent (16/62) and 74% (46/62) patients had colon and rectal cancer, respectively. Mean PRL level was higher in patients with colon cancer than in patients with cancer of the rectum. However, the difference was statistically non-significant (Figure 1).
Relation of PRL to Dukes stage

Out of 62 colon/rectal cancer patients, 33 (53%) had Dukes B disease and 29 (47%) had Dukes C disease. Significant difference in PRL was not observed between patients with Dukes B/C disease (Figure 1).

Figure 1: Histogram showing distribution of PRL according to clinicopathologic parameters in patients with colon/rectal cancer. C-colon; R-rectum; A-adenoacarcinoma; O-others; W-well differentiated; M-moderately differentiated; P-poorly differentiated.
Relation of PRL to histologic type of the tumor

Ninety-four percent (58/62) patients had adenocarcinoma whereas the remaining 6% (4/62) included patients with squamous cell carcinoma (N=3) and transitional cell carcinoma (N=1). Due to the small number of patients in the latter group the data was not analysed statistically (Figure 1).

Relation of PRL to histologic grade of the tumor

Ten percent (6/62), 61% (38/62) and 29% (18/62) patients had histologic grade I (well differentiated), II (moderately differentiated) and III (poorly differentiated) tumors, respectively. The mean level of PRL was higher in patients with grade I tumors than in patients with grade II or III tumors (Figure 1). Ninety percent patients had histologic grade II or III tumors. Ninety-one percent (30/33) and 90% (26/29) patients with Dukes B and C disease had grade II + III tumours, respectively.

Prognostic value of PRL

Patients with Dukes B and C disease

The patients when subgrouped according to the cut-off level of preoperative PRL, it was observed that
hyperprolactinemic patients had significantly poorer overall survival compared to those with PRL levels <20.0 ng/ml plasma \( (X^2 = 7.80, \text{df}=1, P<0.01); \text{Figure 2}). \)

\[ \text{Figure 2: Hyperprolactinemic patients had unfavourable prognosis than patients with PRL <20.0 ng/ml plasma.} \]
Prognostic significance of PRL was also evaluated according to Dukes stage.

Patients with Dukes B disease

Sixty-one percent (11/18) patients with PRL > 20.0 ng/ml plasma developed recurrence whereas only 7% (1/15) patients with PRL < 20.0 ng/ml plasma developed recurrence within 3 years. Relapse free survival was significantly shorter in patients with hyperprolactinemia than those with PRL < 20.0 ng/ml plasma ($X^2 = 3.23$, df = 1, $P < 0.0014$; Figure 3).

Dukes B patients with hyperprolactinemia also had significantly poorer overall survival than their counterpart ($X^2 = 2.69$, df = 1, $P < 0.008$; Figure 3).
Figure 3: Hyperprolactinemic patients with Dukes B disease had shorter relapse free and overall survival time than those with PRL <20.0 ng/ml plasma.
Patients with Dukes C disease

Patients when subgrouped according to the cut-off level of PRL, the difference in overall survival time between the two subgroups was statistically significant \( (X^2 = 2.15, df=1, P<0.031; \text{Figure 4}). \)

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure4.png}
\caption{Hyperprolactinemic patients with Dukes C disease had significantly poorer overall survival period than those with PRL < 20.0 ng/ml plasma.}
\end{figure}
Correlation of PRL with disease status

Patients with response (N=19)

Patients who responded to various therapeutic modalities, showed decreased PRL levels at the end of 3 years as compared to their preoperative levels (P<0.01; Figure 5).

Figure 5: Histogram showing PRL levels in patients with response (P-preoperative; R-at response) and in patients with progressive disease (P-preoperative; PR-preceding; PD-at progression).
Patients with progressive disease (N=20)

On sequential estimation, the preoperative PRL levels reduced at response whereas with the appearance of local/distant metastasis, the PRL levels increased significantly compared to the preceding levels (P<0.02; Figure 5). It was observed that the rise in PRL levels preceded disease progression by approximately 2-3 months. Moreover, PRL levels also remained elevated throughout the disease course in patients who did not respond to adjuvant therapy (Figures 6-7).
Figure 6: Patient was operated (S) and histopathological report was moderately differentiated adenocarcinoma of the rectum. He had Dukes B disease. He was treated with postoperative chemotherapy (CT). Nine months after surgery, he developed recurrence (R). The tumor was excised and he was treated with chemotherapy (CT). Inspite of chemotherapy, after 12 months he developed mass in hypogastrium (R) and again local recurrence (R). PRL levels correlated well with the disease course.
Figure 7: Patient having Dukes C disease was operated (S) and his histopathological report was moderately differentiated adenocarcinoma of the rectum. Postoperative chemotherapy (CT) was given for two months. At the end of first year, he developed lung metastasis (LUNG +). Second line chemotherapy (CT) was instituted but he did not respond to it and finally expired (EXP) after 14 months. Excellent correlation was observed between PRL and the course of the disease. PRL even showed a lead time of 2 months.
Relationship between site of failure and PRL

Patients who later on developed progressive disease within 3 years were further subgrouped according to the site of failure [local (N=13) and distant (N=7)]. The preoperative PRL levels were elevated in 54% (7/13) and 43% (3/7) patients with local and distant metastases, respectively. However, the mean PRL levels were similar in both the subgroups (Table 2).

Table 2: Relationship between site of failure and preoperative PRL levels (M ± SE).

<table>
<thead>
<tr>
<th>Site of failure</th>
<th>N</th>
<th>Preoperative PRL (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local</td>
<td>13</td>
<td>27.71 ± 6.90</td>
</tr>
<tr>
<td>Distant</td>
<td>07</td>
<td>27.11 ± 8.59</td>
</tr>
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</table>
Sensitivity, specificity and predictive values of PRL

The sensitivity, specificity, and positive and negative predictive values of PRL in monitoring the disease course were 100% (Table 3).

Table 3: Sensitivity, specificity and predictive values of PRL.

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<table>
<thead>
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<tbody>
<tr>
<td>Sensitivity</td>
<td>100%</td>
</tr>
<tr>
<td>Specificity</td>
<td>100%</td>
</tr>
<tr>
<td>Positive Predictive value</td>
<td>100%</td>
</tr>
<tr>
<td>Negative Predictive value</td>
<td>100%</td>
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</tbody>
</table>
Pre- and post-operative PRL levels in patients with response and in patients with progressive disease

In patients with response, the postoperative PRL levels decreased significantly as compared to their preoperative levels (P<0.05). On the other hand, in patients who later on developed progressive disease, postoperative PRL levels decreased as compared to their preoperative levels but the difference was statistically non-significant (Table 4).

Table 4: Comparison between pre- and post-operative PRL levels in patients with response and in patients who developed progressive disease (N ± SE).

<table>
<thead>
<tr>
<th>Patients</th>
<th>N</th>
<th>PRL (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with response</td>
<td>19</td>
<td></td>
</tr>
<tr>
<td>Preoperative</td>
<td></td>
<td>23.66 ± 5.55 *</td>
</tr>
<tr>
<td>Postoperative</td>
<td></td>
<td>11.79 ± 1.58</td>
</tr>
<tr>
<td>Patients with progressive disease</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Preoperative</td>
<td></td>
<td>26.44 ± 6.24</td>
</tr>
<tr>
<td>Postoperative</td>
<td></td>
<td>15.23 ± 1.81</td>
</tr>
</tbody>
</table>

P value: * P<0.05
Overall survival in relation to postoperative PRL

To examine the prognostic value of postoperative PRL, patients were subgrouped according to the cut-off level of PRL (20.0 ng/ml plasma). The overall survival did not differ significantly between the two subgroups ($X^2 = 1.70$, $df=1$, $P<0.089$; Table 5).

Table 5: Overall survival in relation to postoperative PRL.

<table>
<thead>
<tr>
<th>Cut-off level of PRL</th>
<th>N</th>
<th>Percent patients died</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;20.0 ng/ml</td>
<td>33</td>
<td>30</td>
</tr>
<tr>
<td>&gt;20.0 ng/ml</td>
<td>06</td>
<td>50</td>
</tr>
</tbody>
</table>

P value: * $P < 0.089$

Comparison of PRL in tumor draining venous blood with PRL in peripheral blood (N=17)

The concentration of PRL in the tumor draining venous blood ($M \pm SE$, 119.67 ± 27.71 ng/ml serum) was 10 fold higher when compared with PRL in the peripheral blood ($M \pm SE$, 12.30 ± 1.63 ng/ml plasma; $P<0.001$). A weak inverse correlation was observed between PRL levels in
tumor draining venous blood and in the peripheral blood ($r=-0.14$; Figure 8).

Figure 8: Scatterogram showing the distribution of PRL in peripheral blood (PB) and tumor draining venous blood (TDVB) in patients with colon/rectal cancer. A weak inverse correlation was observed between them.
Immunohistochemical localization of PRL in colon/rectal tumors

Twenty-five primary tumor sections, (colon, \(N=7\); rectum, \(N=18\)) were stained immunohistochemically. Brown staining indicated the presence of PRL in the tumor cells.

**Figure 9:**


[B] Negative control: A section of colon carcinoma tissue with the omission of primary antibody, magnification: 128X.
Figure 9: [C] A section of well differentiated colon carcinoma immunostained for PRL showing cytoplasmic staining, magnification: 128X.
Incidence of CEA

The preoperative CEA levels were significantly higher in patients with colon/rectal cancer as compared to controls (P<0.0001, Table 6). Sixty-one percent (38/62) patients had CEA levels above the upper normal limit (5.0 ng/ml plasma).

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>CEA (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>30</td>
<td>1.44 ± 0.22</td>
</tr>
<tr>
<td>Colon/rectal cancer patients</td>
<td>62</td>
<td>13.83 ± 3.44</td>
</tr>
<tr>
<td>Range</td>
<td></td>
<td>0.60 - 185.0</td>
</tr>
</tbody>
</table>

Table 6: Distribution of CEA in controls and in patients with colon/rectal cancer (N ± SE).

P value: * P<0.0001

Relation of CEA to anatomic site of the lesion

The mean CEA level was significantly higher in patients with cancer of the rectum than in patients with colon cancer (P<0.05, Figure 10). Moreover, 31/46 (67%) patients with cancer of the rectum and 7/16 (44%) patients with colon cancer had CEA >5.0 ng/ml plasma.
Relation of CEA to Dukes stage

CEA was higher in patients with Dukes C disease as compared to patients with Dukes B disease. However, the difference was statistically non-significant (Figure 10).

Figure 10: Histogram showing the distribution of CEA in patients with colon/rectal cancer according to clinicopathologic parameters. C—colon; R—rectum; A—adenocarcinoma; O—others; W—well differentiated; M—moderately differentiated; P—poorly differentiated.
Relation of CEA to histologic type of the tumor

Patients with adenocarcinoma had high CEA levels when compared to others (squamous cell carcinoma or transitional cell carcinoma). Due to the small number of patients in the latter group the data was not analysed statistically (Figure 10).

Relation of CEA to histologic grade of the tumor

Intergroup variation of CEA was not observed according to histologic grade of the tumor. This may be due to the fact that 90% patients had either histologic grade II or III tumors (Figure 10).
Prognostic value of CEA

Patients with Dukes B and C disease

The patients when subgrouped according to the cut-off level of preoperative CEA, it was observed that patients with CEA $>5.0$ ng/ml plasma had shorter overall survival than patients with CEA $<5.0$ ng/ml plasma. However, the difference was statistically non-significant ($X^2 = 1.08$, df=1, P < 0.25; Figure 11). Forty-two percent (10/24) and 55% (21/38) patients with CEA $<5.0$ ng/ml and CEA $>5.0$ ng/ml plasma, respectively, died within 3 years.

Figure 11: Lack of influence of preoperative CEA ($<5.0$ and $>5.0$ ng/ml plasma) on overall survival in patients with colon/rectal carcinoma.
Patients with Dukes B disease

Twenty-five percent (3/12) and 43% (9/21) patients with CEA <5.0 ng/ml and CEA >5.0 ng/ml plasma, respectively, developed recurrence within 3 years. It was noted that patients with CEA <5.0 ng/ml plasma had a better recurrence free survival than their counterpart but the difference was statistically non-significant ($X^2 = 1.05$, df=1, $P<0.29$; Figure 12).

The overall survival did not differ significantly between the two subgroups of CEA ($X^2 = 1.28$, df=1, $P<0.20$; Figure 12).
Figure 12: In Dukes B patients, significant difference in relapse free and overall survival was not observed when subgrouped according to the cut-off level of CEA.

Patients with Dukes C disease

Patients when subgrouped according to the cut-off level of CEA, the difference in overall survival time between the two subgroups was statistically non-significant \( (X^2 = 0.56, \text{df}=1, P<0.57; \text{Figure 13}). \) However, there was a
trend towards better overall survival for patients with CEA < 5.0 ng/ml plasma than those with CEA > 5.0 ng/ml plasma.

Figure 13: Overall survival curves of patients with Dukes C disease when subgrouped according to the cut-off level of CEA. Significant difference was not observed between the two subgroups.
Correlation of CEA with disease status

Patients with response (N=19)
Patients who responded to various therapeutic modalities at the end of 3 years, showed a trend towards a decrease in CEA levels when compared to their preoperative levels (Figure 14). However, in 5/19 (26%) patients with response, non-progressive elevation of CEA was observed at the end of 3 years (Figure 16).

Patients with progressive disease (N=20)
At the time of disease progression, 60% (12/20) patients had elevated CEA levels when compared to their preceding levels whereas in 40% (8/20) patients the CEA levels decreased. The difference was statistically non-significant (Figures 14-16).
Figure 14: Histogram showing CEA levels in patients with response (P-preoperative; R-at response) and in patients with progressive disease (P-preoperative; PR-preceding; PD-at progression).
Figure 15: Patient had Dukes B disease, was operated (S) and the histopathology report of the tumor was moderately differentiated adenocarcinoma of the rectum. Due to the rising titre of postoperative CEA, chemotherapy (CT) was instituted. Despite of chemotherapy, he developed recurrence (R). The tumor was excised and he was treated with chemotherapy (CT). After 1 year, he developed mass in hypogastrium (R) and again local recurrence (R). CEA correlated well with the course of the disease.
Patient had Dukes C disease, was operated (S) and the histopathology report of the tumor was well differentiated adenocarcinoma of the rectum. He was treated with postoperative chemotherapy (CT). Four months after chemotherapy, he developed local recurrence (R). The tumor was excised and he was treated with chemotherapy followed by radiotherapy (RT). He was without any complaints for 12 months. Thereafter, he developed local recurrence (R) and was treated with second line chemotherapy (CT). The persistent high level of CEA at response indicated the lack of specificity of the test.
In a patient who developed liver metastasis (N=1), CEA level increased significantly at the time of metastasis and correlated excellently with the disease course. It also showed a lead time of 2-3 months (Figure 17).

Figure 17: Patient had Dukes B disease, was operated (S) and the histopathology report of the tumor was moderately differentiated adenocarcinoma of the rectum. Postoperative chemotherapy (CT) was instituted. At the end of 6th month his abdominal sonography report showed presence of liver metastasis (LI+). He was treated with combination chemotherapy (CT). After completion of seven courses of chemotherapy, his sonography report showed increased liver metastasis (LI++). CEA levels correlated excellently with the disease course. It also showed a lead time of 2 months.
Relationship between site of failure and CEA

Patients who later on developed progressive disease within 3 years were further subgrouped according to the site of failure [local (N=13) and distant (N=7)]. Patients who developed distant metastases showed a trend towards higher preoperative CEA levels than patients who developed with local recurrence (Table 7).

Table 7: Relationship between site of failure and preoperative CEA levels (M ± SE).

<table>
<thead>
<tr>
<th>Site of failure</th>
<th>N</th>
<th>Preoperative CEA (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local</td>
<td>13</td>
<td>5.12 ± 1.20</td>
</tr>
<tr>
<td>Distant</td>
<td>07</td>
<td>12.49 ± 7.10</td>
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</tbody>
</table>
Sensitivity, specificity and predictive values of CEA

The sensitivity, specificity, and positive and negative predictive values of CEA in monitoring the disease course were 60%, 74%, 70% and 64% respectively which were lower when compared with those of PRL (Table 8).

Table 8: Sensitivity, specificity and predictive values of CEA

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<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Sensitivity</td>
<td>60%</td>
</tr>
<tr>
<td>Specificity</td>
<td>74%</td>
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<tr>
<td>Positive Predictive value</td>
<td>70%</td>
</tr>
<tr>
<td>Negative Predictive value</td>
<td>64%</td>
</tr>
</tbody>
</table>
Pre- and post-operative CEA levels in patients with response and in patients with progressive disease

In patients with response, the postoperative CEA levels decreased significantly as compared to their preoperative levels (P<0.01). On the other hand, in patients who later on developed progressive disease, postoperative CEA levels decreased but the difference was statistically non-significant when compared to their preoperative levels (Table 9).

Table 9: Comparison between pre- and post-operative CEA levels in patients with response and in patients with progressive disease (M ± SE).

<table>
<thead>
<tr>
<th>Patients with response</th>
<th>N</th>
<th>CEA (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative</td>
<td>19</td>
<td>8.11 ± 1.41</td>
</tr>
<tr>
<td>Postoperative</td>
<td></td>
<td>3.57 ± 0.36</td>
</tr>
<tr>
<td>Patients with progressive disease</td>
<td>20</td>
<td>8.62 ± 3.35</td>
</tr>
<tr>
<td>Preoperative</td>
<td></td>
<td>3.26 ± 0.48</td>
</tr>
</tbody>
</table>

P value: * P<0.01
Overall survival in relation to postoperative CEA

To examine the prognostic value of postoperative CEA, patients were subgrouped according to the cut-off level of CEA (5.0 ng/ml plasma). The overall survival did not differ significantly between the two subgroups ($X^2 = 1.60, \text{df}=1, \text{P}<0.109$; Table 10).

Table 10: Overall survival in relation to postoperative CEA.

<table>
<thead>
<tr>
<th>Cut-off level of CEA</th>
<th>N</th>
<th>Percent patients died</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;5.0 ng/ml</td>
<td>30</td>
<td>27</td>
</tr>
<tr>
<td>&gt;5.0 ng/ml</td>
<td>09</td>
<td>55</td>
</tr>
</tbody>
</table>

P value: * \( \text{P}<0.109 \)

Comparison of CEA in tumor draining venous blood with CEA in peripheral blood (N=17)

The concentration of CEA in tumor draining venous blood was 3 fold higher than in peripheral blood. Significant difference in the mean concentration of CEA in tumor draining venous blood ($M \pm SE, 22.29 \pm 9.88 \text{ ng/ml serum}$) and in peripheral blood ($M \pm SE, 6.95 \pm 2.23 \text{ ng/ml plasma}$) was not observed. However, a significant linear
correlation was observed between CEA levels in tumor draining venous blood and in the peripheral blood (r=+0.84, P<0.001; Figure 18).

Figure 18: Scatterogram showing the distribution of CEA in peripheral blood (PB) and tumor draining venous blood (TDVB) in patients with colon/rectal cancer. A significant linear correlation was observed between them.
DISCUSSION

The goal of preoperative assessment of PRL and CEA levels in patients with colon/rectal cancer is to define operative risk factors and to identify low-risk and high-risk groups of patients, so that high-risk patients can be followed more intensely and treated accordingly. In the present study, it was observed that fifty percent of the patients with colon/rectal cancer had hyperprolactinemia. Molitch et al. (1981), in contrast, did not notice hyperprolactinemia in a series of 215 patients with various malignancies including those of the large intestine.

CEA levels were elevated in 61% patients. The mean level of CEA was significantly low in patients with colon cancer than those with cancer of the rectum. Contradictory to this finding, Gold and Freedman (1965), and Go and Zamcheck (1982) have observed elevated levels of CEA in patients with colon cancer. However, various investigators have found elevated CEA levels in 20-90% patients with colorectal cancer (Khojasteh et al. 1990, Guadagni et al. 1991a).

Dukes stage has long been acknowledged as an important
prognostic instrument for patients with colon/rectal cancer. Intergroup variation in PRL was not observed when the patients were grouped according to Dukes stage whereas a trend of high concentration of CEA was noted in patients with Dukes C disease. On the other hand, Wanebo et al. (1978), Arnaud et al. (1980) and Moertel et al. (1986) have shown correlation of CEA with disease stage.

In the present study significant intergroup variation in PRL and CEA was not observed when the patients were grouped according to histologic grade of the tumor. This may be due to the fact that 90% patients had histologic grade II or III tumors. On the contrary, Goslin et al. (1981) reported that CEA in serum of poorly differentiated carcinoma may not be elevated because of reduced ability of tissues to produce CEA. Bordes et al. (1973) and Rognum et al. (1982) have found tumoral CEA content to be more abundant in moderately or poorly differentiated tumors.

The next important aspect of this study was to determine whether preoperative PRL and CEA levels could provide valuable prognostic information in addition to
the conventional prognostic features and to identify low-risk and high-risk groups of patients. The patients were divided into two subgroups according to the cut-off level of PRL and the results indicated that preoperative PRL levels indeed had a significant prognostic value. Colon/rectal cancer patients with hyperprolactinemia had poorer overall survival time than did their counterpart. The data indicated that hyperprolactinemia is a particularly ominous sign. As 55% and 45% patients with Dukes B and C disease, respectively were hyperprolactinemic, the next question to be addressed was: Can PRL add to the prognostic significance of Dukes stage? Suprisingly, it was observed that hyperprolactinemic patients with Dukes B disease had a shorter relapse free and poorer overall survival time than did patients with PRL <20.0 ng/ml plasma. Even though patients with Dukes B disease are considered to be in an early stage of the disease, if they are hyperprolactinemic at diagnosis, they should be considered high-risk patients for developing metastatic disease and hence, should be treated accordingly. Similarly, hyperprolactinemic patients with Dukes C disease had poorer overall survival time. This
suggests that elevated preoperative PRL levels were predictive of aggressive colon/rectal cancer.

On the other hand, preoperative CEA levels failed to discriminate patients with favourable and unfavourable prognosis. Even in patients with Dukes B disease CEA levels did not appear to identify patients with better or worse relapse free survival time. The lack of prognostic significance of CEA levels in patients with colon/rectal carcinoma is in agreement with the findings of Albè et al. (1990). He observed that preoperative CEA was not longer related to prognosis in Dukes stage A, B or C tumor groups. Contradictory to the above finding, Wolmark et al. (1984), Norton and Fraker (1989) and Sener et al. (1989) suggested CEA as a marker of prognosis which is partially independent of clinical staging. Recently, Barone et al. (1990) and Chu et al. (1991) reported that high CEA levels can identify a poor prognostic group of patients likely to benefit from adjuvant treatment. Furthermore, preoperative CEA levels have been shown to be of prognostic value when used in conjunction with Dukes staging (Zamcheck et al. 1975). Wanebo et al. (1978) found a significant difference in rate and time of
recurrence between two CEA groups (<5.0 ng/ml and >5.0 ng/ml) in patients with Dukes B/C disease.

In colorectal carcinoma, locoregional control remains a major problem among patients with Dukes B or C disease (Bentzen et al. 1992). Hence, the clinical utility of PRL and CEA in monitoring the disease course was studied. Whether PRL and CEA are associated with recurrence of disease and can they be used as therapeutic monitors? Which is a better marker? To answer these questions PRL was estimated sequentially in patients with colon/rectal cancer, and it was observed that serial determinations of PRL accurately predicted disease remission/progression. PRL levels decreased significantly in patients who responded to various therapeutic modalities as compared to their preoperative levels. On the other hand, PRL levels increased with disease progression. The view of PRL being an indicator of progressive disease is supported by the fact that in case of non-responders, a rise in PRL preceded clinical symptoms by 2-3 months. This is an important finding as it may provide a way out from a despairing situation, that recurrent disease which is symptomatic or which can be detected on routine examination is likely to
be advanced and not usually curable. Thus, serial estimations of PRL are useful in early diagnosis of progressive disease.

Although, factors or mechanisms which lead to high PRL levels are unknown, it is a fact that in patients with breast cancer, high PRL is associated with progression of the disease and the levels of PRL increases with tumor progression (Holtkamp et al. 1984, Bhatavdekar et al. 1990). Moreover, it is known that the intact "little prolactin" (Garnier et al. 1978) which forms approximately 85% of circulating PRL can be cleaved by tissue enzymes resulting into a "16K" fragment which is thought to contain "mitogenic potential" contributing to the pathogenesis of breast cancer (Mitra 1980, Clapp 1987). This suggests that elevated PRL has to do with metabolic processes of the metastatic tumor. A similar mechanism might be operating in patients with colon/rectal cancer. PRL probably modulates the effect of other hormones on tumor growth in colon/rectal carcinoma. An early rise in PRL in colon/rectal carcinoma is an important finding and may offer a sensitive means to predict the presence of recurrent disease which is often difficult
to evaluate. Colon/rectal cancer often metastasizes to bone and other sites that are difficult to evaluate for response. Thus, serial PRL measurements may be a more sensitive indicator for assessing response.

Although, CEA assay is well established as a single method for evaluating disease recurrence, there is some controversy about the adequacy of CEA as a monitor of disease activity in colon/rectal cancer. Wood et al. (1980), Go and Zamcheck (1982), Stabb et al. (1985) and Behbehani et al. (1990) have found CEA to be a useful marker for monitoring patients with colorectal cancer whereas Moertel et al. (1978), Denstman et al. (1986), Ovaska et al. (1990) and Angel et al. (1992) have found that it lacks predictive power, and is less sensitive and therefore, unsatisfactory for clinical use in patients with colorectal cancer. The present study, however, suggested that CEA may be of little practical value in patients with local/distant metastasis, except for patients with liver metastasis. In 30% patients who developed recurrence(s), CEA remained <5.0 ng/ml plasma throughout the disease course. Moreover, non-progressive elevation of CEA was seen in 26% patients at response which is an extreme
example of this phenomenon. Similar results were observed by Rittgers et al. (1978). Despite the lack of specificity for colon/rectal cancer, CEA demonstrated an excellent correlation in patients who developed liver metastasis (Steele et al. 1982, De Brauw et al. 1987, Chang et al. 1989, Lorenz et al. 1989, Bhatavdekar et al. 1992, Hohenberger et al. 1994). In an attempt to improve the sensitivity of markers, the use of combination of markers with CEA have been proposed. Tsavaris et al. (1993) observed that the combination of CEA and CA 19-9 had some utility in follow-up, without significantly improving CEA results. Guadagni et al. (1991a, b) recommended the use of TAG-72 and CEA serum markers in diagnosis of recurrent disease.

In the present study, the sensitivity, specificity, and positive and negative predictive values for CEA were 60%, 74%, 70% and 64%, respectively which were lower than those found for PRL. The observed low predictive value of CEA in the present study may be related to the greater incidence of moderately or poorly differentiated tumors. Thus, PRL remains a superior marker in monitoring disease response to treatment or disease progression for patients with colon/rectal cancer.
As observed, PRL levels decreased postoperatively and showed an elevation at the time of recurrence, it was hypothesized that colon/rectal tumors might be producing autoregulatory PRL or PRL-like molecule(s). It was observed that the concentration of PRL in the tumor draining venous blood was 10 fold higher than that in the peripheral blood. Such a high gradient was not observed for CEA. Tabuchi et al. (1991) reported that colorectal cancer patients with a high-risk of hematogenous metastasis and/or recurrence are thought to be more effectively checked by the determination of tumor draining venous blood and draining-plasma CEA gradient than that of plasma CEA. The high concentration of tumor draining venous blood PRL was further substantiated by immunohistochemical localization of PRL using rabbit anti-human PRL antibody. This confirmed that PRL or a molecule closely resembling it, was produced ectopically by the tumor itself. Therefore, increased PRL level in patients with colon/rectal cancer was probably due to the ectopic production of PRL by tumor cells. Hsu et al. (1992) in a study on uterine cervical cancer and Bhatavdekar et al. (1994a, b) in patients with breast and tongue cancers showed
evidence of ectopic PRL production by immunohistochemical localization. This strengthens and confirms the hypothesis that colon/rectal tumors produce PRL or PRL-like molecule(s) and "this ectopically produced PRL might be acting as one of the major local peptide growth promoter via autocrine/paracrine mechanism(s)". To confirm the endocrine nature of colon/rectal tumor, the neurosecretory granules were studied with electron microscopy. However, neurosecretory granules were not observed in colon/rectal tumors (Bhatavdekar et al. unpublished data).

Our results suggest that PRL plays a significant role in the pathogenesis of colon/rectal cancer. Hyperprolactinemia provides additional prognostic information and may be useful as an independent predictor of short-term survival. CEA determination would seem to add very little to the prognostic information obtained from surgical pathologic staging. The changes in PRL levels gave significantly higher predictive power than did CEA levels for detection of disease recurrence as well as response to treatment in patients with Dukes B or C colon/rectal cancer. PRL also
showed a lead time of 2-3 months. CEA was found to be a better marker for monitoring patients who developed liver metastasis. Hence, it is suggested that the use of plasma PRL can help to identify low-risk and high-risk subgroups of patients, so that high-risk patients may be followed up more intensively and treated accordingly. Moreover, it was observed that PRL is produced ectopically by colon/rectal tumors. The ectopically produced PRL does raise the possibility of anti-prolactin treatment as an adjunct, and which may improve the prognosis of patients with colon/rectal cancer.

A B S T R A C T

In this chapter, the aim was to investigate the usefulness of the markers i.e. prolactin (PRL) and carcinoembryonic antigen (CEA) in patients with colon/rectal cancer: (i) as prognosticators, (ii) to find out a better marker to differentiate low-risk and high-risk subgroups of patients, (iii) serial estimations for detection of recurrence/metastasis, (iv) comparison of markers in the tumor draining venous blood with peripheral blood levels and (v) immunohistochemical
localization of PRL.

Part A:
Preoperative levels of plasma PRL were significantly higher in patients with colon/rectal cancer as compared to controls (P<0.0001). Fifty percent patients (31/62) had hyperprolactinemia (PRL >20.0 ng/ml plasma, upper normal limit). Intergroup variations in PRL were not observed when considering the clinicopathologic parameters (anatomic site, Dukes stage, and histopathologic type and grade).

Patients with preoperative hyperprolactinemia had significantly poorer overall survival than those with PRL <20.0 ng/ml plasma (X^2 =7.80, df=1, P<0.01). A similar trend was maintained when the patients were further subgrouped according to Dukes B (X^2 =2.69, df=1, P<0.008) or C (X^2 =2.15, df=1, P<0.031) disease. In addition, the relapse free survival was significantly shorter for Dukes B patients with hyperprolactinemia as compared to Dukes B patients with PRL <20.0 ng/ml plasma (X^2 =3.23, df=1, P<0.0014).

For the serial estimations of PRL and CEA, it was possible to follow only 39/62 (63%) patients, and the
rest were lost to follow-up. However, the Medical Record Department had the survival details of patients lost to follow-up. Based on their disease status at the end of three years, the colon/rectal cancer patients were classified into: (1) patients with response (N=19) and (2) patients with progressive disease (N=20).

On sequential estimation, in patients with response, PRL levels decreased significantly at the end of 3 years as compared to their preoperative levels (P<0.01). On the other hand, in patients with progressive disease, the PRL levels decreased at response but with appearance of local/distant metastasis, the PRL levels increased significantly compared to the preceding levels (P<0.02). The positive and negative predictive values of PRL were 100%.

Preoperative PRL levels were higher in patients who developed progressive disease than in patients who responded to treatment. Furthermore, circulating PRL level was compared with tumor draining venous blood. A 10 fold increase was observed in the tumor draining venous blood than peripheral blood. Presence of PRL in the colon/rectal tumors was further confirmed by immunohistochemically.
Part B:
The preoperative CEA levels were significantly higher in patients with colon/rectal cancer as compared to controls (P<0.0001) and 61% (38/62) patients had elevated CEA levels (>5.0 ng/ml plasma). The mean concentration of CEA was significantly higher in patients with cancer of the rectum than patients with cancer of the colon (P<0.05). CEA was higher in patients with Dukes C disease as compared to Dukes B disease. Significant intergroup variations in the mean levels of CEA were not observed considering the histopathologic type and grade of the tumor.

Patients with CEA >5.0 ng/ml had a shorter overall survival than patients with CEA <5.0 ng/ml. A similar trend was maintained when the patients were further grouped according to the disease stage. In addition, the relapse free survival was better for Dukes B patients with CEA <5.0 ng/ml than Dukes B patients with CEA >5.0 ng/ml.

On sequential estimation, CEA levels decreased in patients with response at the end of 3 years compared to their preoperative levels. However, the difference
was statistically non-significant. Furthermore, CEA levels correlated with disease progression but the difference was statistically nonsignificant. The positive and negative predictive values of CEA for monitoring disease course were 70% and 64% respectively. The preoperative CEA level did not differ in patients with response and patients who developed progressive disease. The levels of CEA in the tumor draining venous blood were 3 fold higher when compared with peripheral blood.

PRL is a better overall marker for monitoring disease course than CEA in patients with Dukes B or C colorectal cancer. Hyperprolactinemia provides additional prognostic information and may be useful as an independent predictor of short-term survival in patients with Dukes B or C disease.
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