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Is Placental Alkaline Phosphatase (PLAP) a Useful Marker for Seminoma?

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The usefulness of placental alkaline phosphatase (PLAP) as a tumour marker was assessed in 1578 serum samples from 236 patients with seminoma. Smoking habits were known for all but 7 patients (22 samples). Smoking was associated with significantly higher mean levels of PLAP in disease-free patients (28.8 [S.E. 2.1] U/l vs.15.9 [1.3] U/l in non-smokers). Mean PLAP levels were higher in patients with active disease (78.6 [23.5] U/l in non-smokers and 47.2 [18.5] U/l in smokers). The median values showed a similar trend. However, there was considerable overlap between the various groups and differences between mean and median values indicated that PLAP values were distributed asymmetrically. The predictive value of PLAP as a tumour marker was consequently much less than superficial inspection of these values might suggest. In 97 patients on surveillance, only 2 out of 11 patients who relapsed had elevated PLAP at the time of clinically detectable relapse. With the upper limit of normal PLAP quoted by our laboratory (35 U/l), specificity and sensitivity were, respectively, 88% and 45% (all patients) and 96% and 47% (non-smokers). The sensitivity and specificity of PLAP were assessed in more detail for a series of threshold values (normal vs. abnormal) with a graphical method. Only in non-smokers did PLAP seem useful and even in this group the positive predictive value of an "abnormal" test may be low; less than 50% in clinically relevant circumstances. Serum PLAP assay cannot usefully stand alone as a marker for seminoma and its routine estimation contributes little to follow-up.

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INTRODUCTION

PLACENTAL ALKALINE phosphatase (PLAP) is a heat-stable isoenzyme of alkaline phosphatase and is normally expressed by placental syncytiotrophoblasts [1]. Expression of PLAP and PLAP-like activity has also been described in several normal tissues such as testis, cervix, thymus and lung as well as in a number of malignancies, including germ-cell, ovarian and lung tumours [1–3]. Only in seminomas and ovarian tumours is the level of PLAP high enough to be detectable in serum and thus of potential use as a tumour marker [2–7].

The serum level of PLAP may be associated with disease activity in patients with seminoma [4, 6–12], but its usefulness as a tumour marker has not definitively been established. One of the prime requirements for a clinically useful marker is that fluctuations in the level of the marker must correlate in a reasonably precise manner with the presence or absence of disease [13]. Although an association with disease activity in a few selected patients has been described [3, 4, 6, 8, 10, 12, 14], this is insufficient documentation of a role as a marker. The tumour markers alpha fetoprotein (AFP) and beta human chorionic gonadotrophin (βHCG) have proved useful in the management of non-seminomatous germ-cell tumours because in most cases elevated levels accurately reflect the presence of disease [13, 15, 16]. A reliable tumour marker for monitoring seminoma has not been described. Both serum lactate dehydrogenase (LDH) and βHCG may reflect the response of seminoma to treatment [9, 17–19], but their use as markers is hampered by low sensitivity. The lack of comparable markers for seminoma is troublesome, particularly for the surveillance policy now being investigated in many centres for stage I disease.

Serum levels of PLAP depend upon smoking habits [2, 4, 7, 20] which may hamper clinical interpretation. We have investigated the usefulness of PLAP as a tumour marker for seminoma in patients with known smoking habits. A rigorous analytical approach has been used to discover whether any of the difficulties in interpreting PLAP results can be overcome. The emphasis has been on assessing the *clinical* usefulness of PLAP as a marker for seminoma.

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PATIENTS AND METHODS

236 consecutive patients with pure seminomas were studied. The patients were referred for treatment to the Testes Clinic at Princess Margaret Hospital, between January 1983 and December 1989. All patients had been initially treated with radical orchiectomy. The median age of the patients at the time of referral was 34 years (range 20–86). All histological material was reviewed at the Princess Margaret Hospital. 177 patients had classical (seminiferous), 56 had anaplastic and 3 had spermatocytic seminoma. Staging was according to the Royal Marsden system [21] and based on clinical examination, chest X-ray, bipedal lymphography, abdominopelvic computed tomography (CT) scanning and serum tumour markers (AFP, βHCG, LDH). A raised AFP at any point in the clinical course of the disease dictated exclusion from the study. Patients with mixed (seminoma plus non-seminomatous) tumours were not included. Clinical stage was assigned without reference to the serum PLAP levels. 191 patients were stage I, 40 were stage II (A = 21, B = 9, C = 19) and 5 were stage III. Smoking habits during follow-up were known for all but 7 patients.

The treatment policy has been described [22, 23]. As a rule, patients in stage I were either given abdominal radiotherapy (94 patients) or followed up on a surveillance study (97 patients) [23]. Patients in stage II were treated with abdominal radiotherapy except for 3 patients with stage IIC, who were treated with cisplatin-based chemotherapy. All patients with stage III disease were treated with cisplatin-based chemotherapy.

11 patients were followed up for less than 6 months and 16 patients for less than 1 year. 15 patients relapsed: 13 in stage I (2 irradiated, 11 on surveillance) and 2 in stage II. The assessment of relapse was based on clinical examination and imaging. The patients with relapse were treated with either radiotherapy (7) or cisplatin-based chemotherapy (8). Only 2 patients have died; both had advanced disease at presentation.

Serum PLAP analysis

PLAP concentrations in serum were measured at the Department of Biochemistry, Sunnybrook Medical Centre, Toronto, by a kinetic enzyme method depending on the specific characteristics of the enzyme (heat stability at 65°C, pH and specific substrate) as modified by Anstiss *et al.* [24]. The reference threshold level quoted by the laboratory for elevation of serum PLAP was 35 U/l. 1578 serum samples were analysed. The number of samples analysed per patient was 2 or more in 230 patients, 5 or more in 209 patients and 10 or more in 69 patients.

Data analysis

The sensitivity, specificity and positive predictive value (probability of active seminoma when PLAP is elevated) were calculated [25]. Receiver operating characteristic (ROC) curves were constructed by changing the threshold between normal and abnormal test results and then observing the effects these varying thresholds had on the relative proportions of true positive and false positive results [25, 26]. The curve should lie above the 45° diagonal since that line describes a test for which the true-positive rate is always equal to the false-positive rate and which, therefore, has little discriminatory value (Fig. 1). Ideally, a ROC curve should extend as far as possible to the upper left-hand corner of the graph: the proportion of true positives is high while the proportion of false positives is low. The cut-off level is the point on the ROC curve chosen as optimal for the clinical circumstances.

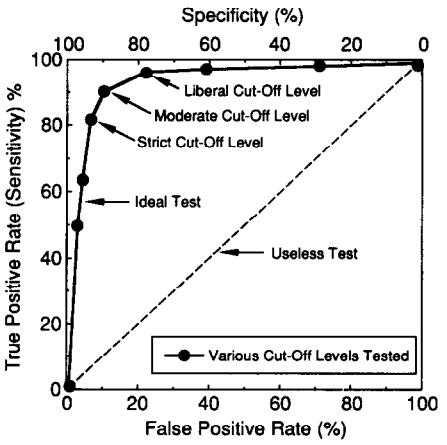

Fig. 1. Hypothetical ROC curve. Liberal cut-off minimises false negative results, moderate cut-off gives acceptable false negative and false positive rates, and strict cut-off minimises false positive results.

RESULTS

Serial PLAP levels from 3 patients with seminoma are shown in Fig. 2: a patient on surveillance relapsing and being successfully treated with chemotherapy; a patient in stage IIC primarily treated with radiotherapy; and a patient in stage III with a short remission after chemotherapy and who subsequently died of the disease. In these 3 patients serum PLAP levels correlate precisely with disease activity. However, all 3 were selected for illustrative purposes—few patients showed such useful correlations.

Figure 3 illustrates the relation between PLAP level and smoking habits in patients with no clinical evidence of seminoma. The mean level of serum PLAP of smokers was higher than that of non-smokers, and the level gradually increased with

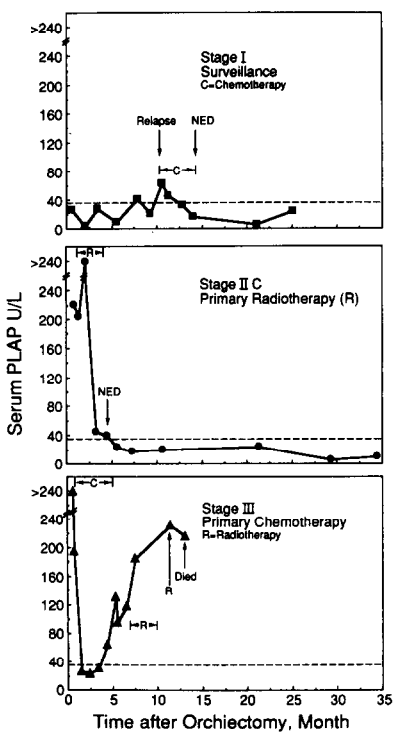

Fig. 2. Serial serum PLAP levels in patients in stage I, IIC and III. NED = no evidence of active seminoma. Broken line = upper limit of normal PLAP quoted by the laboratory (35 U/l).

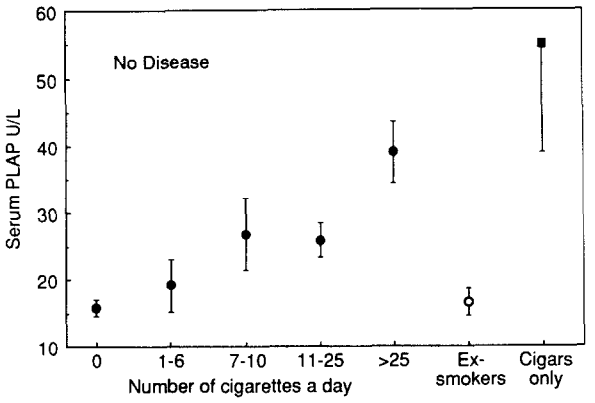

Fig. 3. Effect of smoking habits on mean serum PLAP levels in patients with no active seminoma. • = cigarette smokers, ■ = cigar smokers (only 16 samples) and ○ = exsmokers. Bars = 95% confidence interval.

increasing number of cigarettes smoked per day. The level in cigar smokers seems even higher, but the number of patients was small. PLAP levels in ex-smokers (defined as having stopped smoking at least a year before) did not differ from those of non-smokers. 7 patients changed their smoking habits during the follow-up. 3 ex-smokers resumed cigarette smoking; in 1, serum PLAP increased. 3 patients stopped smoking cigarettes; in 1, an increased PLAP level returned to normal. In the last patient, a cigar smoker who stopped, serum PLAP decreased from 103.3 to 43.5 U/l.

The mean values of serum PLAP in the various groups of patients are summarised in Table 1. In only 7 patients (22 samples) were smoking habits unknown. In patients with no active seminoma the mean level of PLAP was significantly higher in smokers than in non-smokers. It was also higher in patients with active seminoma (primary stage II/III or recurrent disease) than in those with no disease. This difference was more striking in non-smokers than in smokers. The table also shows median levels; although the medians followed the same pattern as the means, the differences between the groups were less striking, especially for smokers compared with non-smokers. The median level of PLAP was highest in non-smokers with active disease. The difference between the mean and median PLAP levels indicate that the PLAP data are skewed and a normal distribution for the values cannot be assumed.

Table 1. Serum PLAP levels (U/l) according to disease status and smoking habits

Smoking habits	No active disease	Active disease	Primary stage II and III
Non-smokers			
No. of samples	802	60	36
Mean (95% CI)	15.9	78.6	95.6
	(14.6–17.2)	(55.6–101.6)	(68.1–123.1)
Median (range)	14	30	65
	(1–409)	(4–455)	(7–355)
Smokers			
No. of samples	437	89	21
Mean	28.8	47.2	76.3
	(26.7–30.9)	(29.1–65.3)	(11.9–140.7)
Median	22	21	23
	(3–165)	(1–569)	(1–569)
Ex-smokers			
No. of samples	146	13	—
Mean	16.6	76.2	—
	(14.7–18.5)	(26.8–125.6)	—
Median	15	25	
	(1–103)	(1–286)	
Unknown			
No. of samples	18	4	—
Mean	15.9	13.3	—
	(11.7–20.1)	(9.4–17.2)	—
Median	14	14	
	(1–35)	(7–18)	

In non-smokers with no active seminoma almost all patients had PLAP levels below 40 U/l (Fig. 4). In non-smokers with active disease as well as in smokers the PLAP values were more widely distributed, and the threshold level used to define the boundary between normal and abnormal values is important.

To define a possible clinically relevant threshold value ROC analyses were done (Fig. 5). Except for non-smokers the ROC curves obtained were all close to the 45° diagonal, indicating a poor discriminant value of serum PLAP in these groups. The ROC curve for non-smokers was the only graph giving an acceptable true positive level. With the upper limit of normal serum PLAP quoted by our laboratory (35 U/l) the specificity and sensitivity were, respectively, 88% and 45% for all patients and 96% and 47% for non-smokers.

The dependence of the positive predictive value of PLAP (probability of active seminoma when PLAP is elevated) on the prevalence of active seminoma is illustrated in Fig. 6. The lower the prevalence and the lower the threshold level chosen, the lower the positive predictive value of serum PLAP. The highest predictive values were obtained for non-smokers, but even in this group a high positive predictive value could only be obtained by choosing a high cut-off level and thereby accepting a low rate of true positive test results.

The question of whether elevation of serum PLAP offers any means of detecting relapse before it becomes clinically apparent is addressed in Fig. 7. Only in 1 patient (stage IIC) was serum PLAP elevated before relapse was clinically detectable. Only 2 of the 11 patients who relapsed on surveillance had elevated levels of PLAP at the time of recurrence: in both patients the rise in PLAP was synchronous with, and did not precede, clinically obvious recurrence.

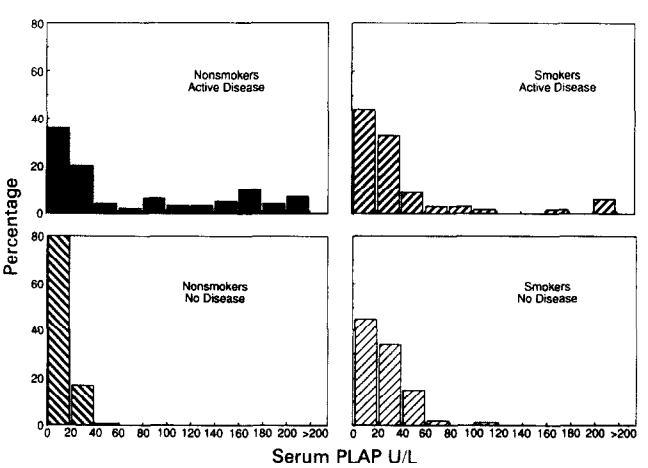

Fig. 4. Distribution of PLAP levels in serum. No. of samples in each group are given in Table 1.

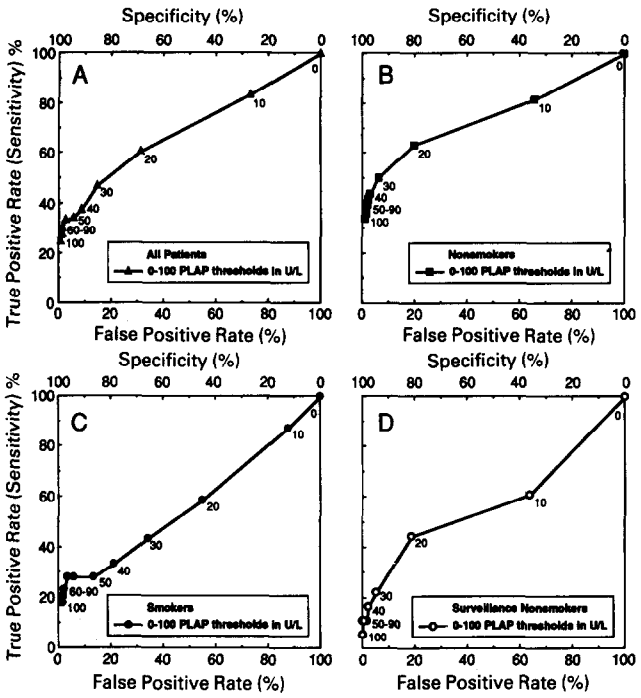

Fig. 5. ROC curves plotted for all patients studied (A), non-smokers (B), smokers (C) and non-smoking patients on surveillance (D). Numbers along the curves correspond to threshold values of PLAP in U/l.

DISCUSSION

We confirmed the association between smoking and elevated PLAP levels in healthy males with no evidence of active testicular cancer [2, 4, 7, 20]. There was a suggestion of a dose-response relation (Fig. 3). The high PLAP levels associated with cigar smoking are interesting since most cigar smokers do not inhale the smoke deeply and previous hypotheses to explain elevated PLAP levels in smokers have implicated direct damage to the alveolar lining cells by inhaled smoke [2, 4].

Various methods have been used to estimate PLAP activity in serum [24, 27–29] with different sensitivities and specificities [27]. Claims that use of monoclonal antibodies offers improved performance [27] have not been substantiated. Without evidence

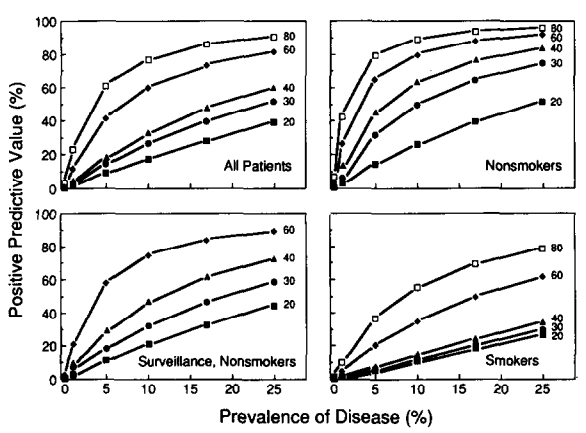

Fig. 6. Positive predictive value as function of prevalence of active seminoma for all patients, non-smokers, non-smokers on surveillance, and smokers. Each curve represents data calculated at threshold value of PLAP (U/l) given by number on the curve. Prevalence values tested were chosen arbitrarily. Estimates of clinically relevant point prevalence values are given in Table 2.

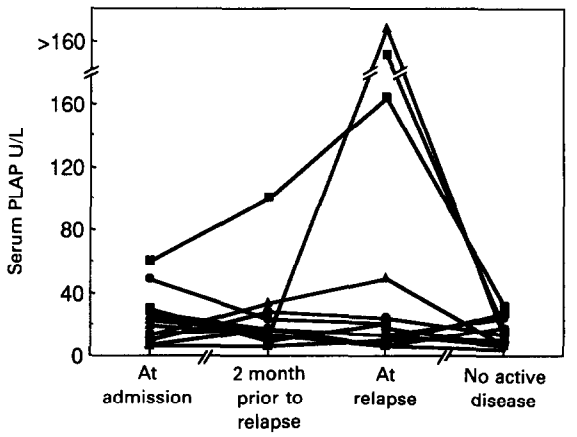

Fig. 7. Serum PLAP levels in patients who relapsed with seminoma. Levels are shown at time of first admission, 2 months before detection of relapse, at relapse (detectable clinically or by imaging), and at first visit with no detectable disease following treatment of relapse.

to the contrary the results and conclusions from the methods we used can be considered generally applicable.

Most recent studies [3, 4, 6, 8, 10, 12, 14] have reported precise correlation of serial PLAP levels with clinical course in selected patients with seminoma. However, many of these studies have relied upon the statistical demonstration of differences in mean PLAP values between patients with and without disease to demonstrate the potential usefulness of PLAP as a tumour marker. Unfortunately the use of such comparisons is only valid if PLAP values are normally distributed [26]. Our findings on medians and frequency distributions do not support such an assumption: non-parametric methods should be used. This general use of parametric statistics may explain why some of the initial promise of PLAP as a marker for seminoma has not been fulfilled. The evolution of PLAP as a marker for seminoma in some respects epitomises the natural history of the assessment of a potential tumour marker [13]. Initial enthusiasm, based on careful selection of "ideal" demonstrations (Fig. 2) and an uncritical acceptance of the relevance of mean values is later tempered by clinical disenchantment and more critical review.

ROC curves facilitate assessment of a potential tumour marker [25, 26] by permitting rapid visual estimation of the effects of changing diagnostic thresholds: too high a level and the number of false negative results vitiates the test; too low a level and false positives swamp the truly positive results. In our study only the ROC curve calculated for non-smokers indicated a clinically acceptable discriminating value of serum PLAP, but even in this group of patients the number of true positives (sensitivity) was low. The difference in the discriminatory performance of PLAP in the various groups of patients is self-evident. There is therefore no indication for more sophisticated analysis of the ROC curves.

The performance of even a highly sensitive, highly specific diagnostic test depends critically upon prevalence [25]. When prevalence is low the positive predictive value of a test is much lower than when the disease is common. This relation is particularly steep at low prevalence rates (Fig. 6). Clinically relevant point-prevalence rates for patients followed up for seminoma can be fairly easily calculated from the overall relapse rate and the shape of the cumulative time-to-relapse curve. With current data [21–23, 30–33] the prevalence of disease for patients on surveillance and for patients followed up after retroperitoneal

Table 2. Point prevalence rates for seminoma stage I estimated for each clinic visit during the specified time interval*

Time interval (yr)†	Point prevalence rate‡ (%)	
	Surveillance	Post-irradiation
Start–1	1.50	0.30
1–2	0.40	0.13
2–2½	0.30	0.10
2½–3	0.24	0.08

* From refs. 21–23 and 30–33.
† Assumed clinic visits every 2 months per year in 0–2 years and every 3 months per year thereafter.
‡Assumed overall relapse rates = 15% (surveillance) and 5% (post-irradiation).

irradiation for stage I seminoma was calculated (Table 2). Comparison of these figures with the graphs in Fig. 6 demonstrates that the positive predictive value of PLAP in clinically relevant circumstances is low. Not even the use of a high threshold value of PLAP, accepting a low true positive rate, will increase the predictive value of an abnormal test to more than 50%. This shortcoming would be of less concern if elevated PLAP levels significantly preceded clinically evident relapse, but this was not the case (Fig. 7). The poor predictive value of an elevated PLAP level cannot be excused on the basis that it permits an earlier diagnosis of relapse. This objection is particularly relevant to the use of PLAP in surveillance studies.

An abnormal elevation of PLAP may have a useful corroborative role in a patient suspected, on other grounds, of having active disease. For example, if a patient on surveillance for stage I disease were found to have a single enlarged retroperitoneal node on routine abdominal CT, most clinicians would be unwilling to assume immediately that this represented metastatic disease: other evidence would be required. If the patient were a non-smoker and PLAP was above 40 U/l, then this could be regarded as adequate corroboration. In the absence of information on PLAP level, invasive methods, such as fine needle aspiration or biopsy, might be necessary to confirm the clinical suspicion of relapse.

In the present study only the usefulness of serum PLAP was analysed, and an analysis of the predictive value of PLAP in conjunction with LDH and βHCG will be reported separately. It has not escaped our attention that no similarly rigorous analysis has been done on traditionally accepted tumour markers (e.g. AFP and βHCG in non-seminomatous tumours and acid phosphatase and PSA for prostate cancer).

Seminomas are too rare for tumour markers to be of much use in screening asymptomatic populations for occult disease [13]. The rarity of the disease, coupled with the low probability of relapse, also makes the question of prognostic significance of serum PLAP impossible to answer from the present data. A potential criticism of the present data is the lack of preoperative serum PLAP values. The results cannot, therefore, specifically answer the question whether serum PLAP is a useful marker in the primary diagnosis of seminoma.

Our intuition, based on experience in the Testes Clinic, has been that we cannot use PLAP values for clinical decision making. Analysis of our data now provides a firm foundation for this subjective assessment. The interpretation of serum PLAP values in patients with seminoma is so fraught with difficulty that routine measurement of levels of PLAP in serum can play no useful role in management.

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Folinic Acid plus High-dose 5-fluorouracil with Allopurinol Protection in the Treatment of Advanced Colorectal Carcinoma

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Protection by prolonged administration of allopurinol against high-dose 5-fluorouracil (5-FU) administered with folinic acid in 74 patients with colorectal cancer was investigated. The dose of 5-FU was 700 mg/m^2 per day for 5 days. Of 41 patients without previous chemotherapy, 1 had a complete response and 4 had partial responses (total 12%), 15 remained stable and 21 progressed. Mean duration of response was 7.4 (1.8–12.6) months. The most frequent toxicities were decreased granulocytes (13%), diarrhoea (37%), and stomatitis (35%), which were similar to the frequencies of other studies with lower doses of 5-FU without allopurinol. Prolonged administration of allopurinol thus gives some protection to patients with colorectal cancer who receive folinic acid plus high-dose 5-FU but responses were not better than those with conventional doses.
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INTRODUCTION

FOLINIC ACID pretreatment enhances 5-fluorouracil 5-FU efficacy and toxicity by an interesting biochemical mechanism. The folinic acid metabolite, 5,10-methylenetetrahydrofolate, tightly binds the active 5-FU metabolite, fluorodeoxuridine monophosphate (FdUMP), to thymidylate synthetase, inactivating the enzyme completely and shutting down *de novo* thymidilate synthesis [1].

Early trials of folinic acid plus 5-FU in patients with metastatic colon cancer were reported in 1982, with responses in patients with cancers refractory to 5-FU and improved responsiveness over that expected with 5-FU alone in previously untreated patients [1, 2]. Toxicity was substantial (neutropenia, diarrhoea, stomatitis and neurological symptoms). Many institutions have tested folinic acid and 5-FU combinations with similar results [3, 4].

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We have tried to obtain a sufficient response with low and acceptable toxicity by using an ordinary dose of folinic acid with high-dose 5-FU. In addition, for more protection, we added allopurinol because metabolites of allopurinol were expected to inhibit 5-FU catabolism by orotidine phosphorybosyl transferase in normal tissues but not in tumour tissues [5, 6]. There are, however, doubts about the efficacy of this combination [7, 8], although our previous work [9] showed some protection by allopurinol. There are no comparative or randomised studies [10].

PATIENTS AND METHODS

Between June 1987 and September 1989, 74 patients with advanced colon cancer, mean age 61, entered the study (Table 1). Eligibility criteria included: (1) biopsy-proven adenocarcinoma of the colon or rectum; (2) measurable metastatic disease; (3) Karnofsky status of 60 or better, with life expectancy of at least 2 months; and (4) no brain metastases.

The treatment schedule was as follows. Allopurinol 300 mg, three times per day orally was given for 17 days starting 2 days