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Prognostic Significance of Serum Proteins in Invasive Bladder Cancer

A Preliminary Report of the E.O.R.T.C. Urological Group*

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Abstract—*This study demonstrates that the survival of patients with UICC category* T3 *and* T4 *bladder cancer is strongly correlated with pre-treatment levels of serum acute phase reactant proteins and albumin. A more accurate assessment of the prognosis is obtained than by considering stage and the type of operation alone.*

INTRODUCTION

A STUDY of the levels of serum acute phase reactant proteins (APRPs) in bladder cancer has demonstrated that they are rarely raised in patients with non-invasive tumours, or tumours with invasion limited to sub-mucosa, and are frequently elevated when invasion involves the muscle or has spread outside the bladder [1]. There is growing evidence that in cancers in which the force of mortality is high, the levels of APRPs at first presentation can carry prognostic information; for example, in stomach cancer [2] and lung cancer [3]. Hollinshead *et al.* [4] have suggested that the ratio of serum prealbumin to α_1-acid glycoprotein carries prognostic potential in bladder cancer. Similarly, in patients with slowly progressing metastatic cancer, a falling serum albumin and rising C-reactive protein are often signs of the onset of the preterminal phase, as illustrated in colon cancer [5] and prostatic cancer [6].

This paper describes an examination of whether plasma protein profiles carry prognostic information in invasive bladder tumours (UICC categories T3 and T4). The study is in part an analysis of data collected originally by Bastable *et al.* [1], and a further study of patients attending the Rotterdam RadioTherapeutisch Instituut (RRTI).

PATIENTS AND METHODS

In the first instance a retrospective study was undertaken on serum samples collected prior to treatment from 82 category T3 and category T4 bladder cancer patients from 3 hospitals in Yorkshire. These sera had been collected in part from patients prior to their initial treatment for bladder cancer and in part from patients who were commencing a treatment having received previous therapy—for example, chemotherapy with an antecedent history of radiotherapy. This set of 82 patients was used to explore any relationship between the levels of acute phase reactant proteins and survival. The lack of uniformity coupled with the dangers of multiple inference when considering many variables made it necessary to be cautious when interpreting statistically significant findings. The approach was a preliminary one, but it motivated a second prospective study to examine the consistency and reproducibility of the findings.

In the second series, serum was collected from 104 patients (63 T3 and 41 T4) prior to treatment at the Dr. Daniel Den Hoed

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Kliniek (RRTI), Rotterdam, The Netherlands. Eighty-six received radiotherapy (47 T3 and 39 T4) and 18 were treated by cystectomy following pre-operative radiotherapy (16 T3 and 2 T4).

Pre-treatment measurements of the following serum proteins were made by radial immunodiffusion (RID) [7]: C-reactive protein (CRP), α_1-antichymotrypsin (ACT), α_1-acid glycoprotein (AGP), albumin (ALB) and haptoglobin (Hp). Haptoglobin concentrations from the RID plates were corrected for phenotype [8]. The phenotype was identified by gradient polyacrylamide gel electrophoresis [9]. Reagents and standards were obtained from the Behring Institute, Marburg/Lahn, F.R.G., and Seward Laboratories, London, U.K. Measurement of the levels of carcinoembryonic antigen (CEA) in pre-treatment sera were made using the Abbott (CEA-RIA) assay on perchloric acid extracted serum. The upper limits of 'normal' were defined as CEA 5 ng/ml, AGP 1.25 g/l, ACT 0.75 g/l, CRP 12 mg/l and Hp 4.5 g/l, in this population of elderly patients, as based on the studies of Bastable *et al.* [1]. A serum albumin of less than 32 g/l was considered to be abnormal.

Statistical analysis

Survival curves were estimated using the method of Kaplan and Meier [10]. This takes into account censored data points; that is, the total time on study for a patient who is still alive at the time of analysis. To test whether or not two survival curves were significantly different from each other the log-rank test was used [11]. This had the added advantage of allowing comparisons to be made between different groups, whilst simultaneously adjusting for any imbalances caused by an uneven distribution of a second variable known to have prognostic significance. For instance, ACT had some correlation, though small, with stage and was therefore expected to show some prognostic significance merely because of this relationship. The log-rank test enabled us to investigate any further prognostic significance ACT might have over and above this by making adjustments for stage.

RESULTS

In the Yorkshire study there were 64 category T3 patients and 18 category T4 patients having median survival times of 19 months and 6 months, respectively. The 11 T3 patients who were treated by pre-operative radiotherapy and cystectomy had, as expected, a better survival (median time greater than 42 months) than the other treatment groups which included radiotherapy only, chemotherapy and no treatment (median survival time 18 months). The patients, regardless of their T category or treatment, were divided into two groups solely on the basis of whether their CRP value was considered to lie within normal limits (less than 12 mg/l) or to be raised (greater than 12 mg/l). The raised group had an estimated median survival time of 10 months whereas the normal group had an estimated median survival time of 20 months and this difference was statistically significant ($P<0.5$) (Fig. 1). Similar results were obtained with ACT and AGP where the normal limits were defined as 0.75 and 1.25 g/l, respectively (Fig. 2). However, because of the established correlation between different acute phase reactant proteins, this was not surprising.

In the Rotterdam study the pre-treatment serum levels of AGP, ACT, CRP, ALB, Hp and CEA were measured. The distribution of CEA showed a low incidence of raised values in both T3 (6/33) and T4 (2/20) and was not considered further in the analysis. The patients (T3 and T4 together) were then divided into 2 groups according to whether the particular protein level was considered normal or abnormal on the basis of the levels given earlier. The survival of each group was then followed prospectively. The group with an elevated level of CRP had a median survival time of about 8 months whereas the group with a level within normal limits had a median survival time greater than 28 months. This difference was statistically significant ($P<0.001$). The log-rank test enabled us to verify that this difference was not associated only with stage, and in fact the survival of these two groups differed significantly when considered separately in T3 and T4 patients (Figs. 3 and 4). Once again, as may have been expected, similar results held for ACT and AGP. Serum haptoglobin levels were less effective in separating the groups. However, albumin, when used in this way, identified subgroups with significantly differing survival ($P<0.01$). The group with a low pre-treatment level of albumin (less than 32 g/l) had a median survival time of about 10 months whereas the group with levels lying within normal limits had a median survival time greater than 28 months. Adjusting for the effects of stage the survival of the two subgroups still differed significantly ($P<0.05$).

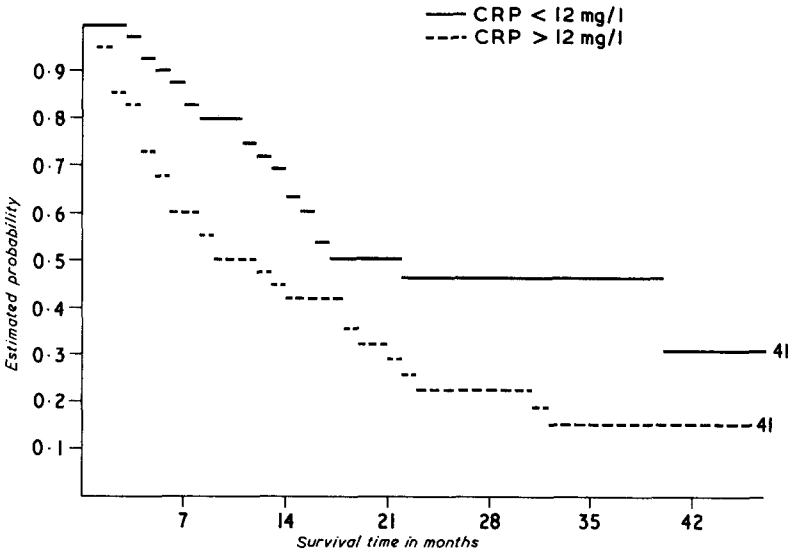

Fig. 1. Kaplan Meier plots of the survival of T3 *and* T4 *bladder cancer (Yorkshire series) according to the level of CRP at presentation.*

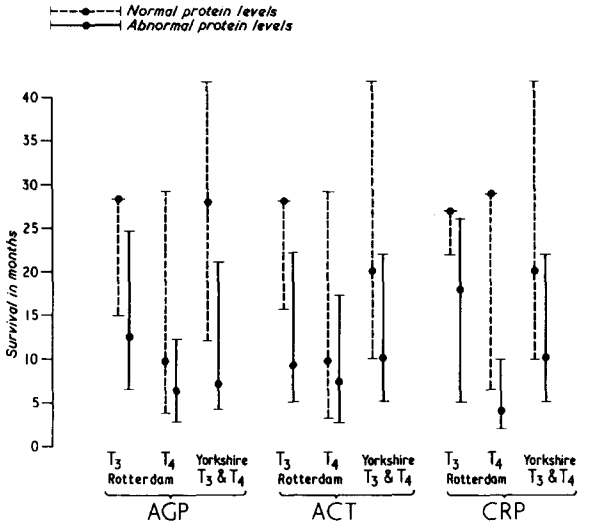

Fig. 2. Median survival (●) and interquartile range (|——| |····|) of 63 T3 *and* 41 T4 *bladder cancers from Rotterdam and* 82 T3 *and* T4 *from Yorkshire. Groups sub-divided according to whether AGP, ACT and CRP were normal or raised. Where the median survival and upper interquartile range coincide, more than* 50% *of patients were surviving at the time of analysis.*

The prognostic significance of albumin is of particular interest because it is not highly correlated with the acute phase reactant proteins. This suggested that a combination of albumin and one of the acute phase reactant proteins might separate the patients more powerfully into groups with different prognoses. Indeed, the survival of that subgroup of patients having either one of these proteins abnormal was very similar to that subgroup where both proteins were abnormal. On the other hand, the subgroup having both proteins normal had significantly better prognoses. In T3 patients the separation into good and poor prognosis achieved by measuring the AGP : prealbumin ratio was comparable to that given by acute phase reactant proteins alone.

DISCUSSION

The acute phase protein response to acute and chronic injury is a well-known phenomenon; its general features have been described by Koj [12], and the behaviour of these proteins in cancer have been discussed by several authors (see [13] for review). Much remains unknown about the precise control of the hepatic synthesis of these proteins and to what extent their rise in response to certain types of tissue damage in cancer is beneficial or detrimental to the host. Nevertheless, this study and those made in stomach cancer [2], lung [3] and prostate cancer [6] indicate that elevation of acute phase reactants, in the absence of infection, is a sign of aggression that is indicative of a poorer prognosis than when these protein levels are normal. It is clearly not the presence of the tumour *per se*, or even its spread, that is the stimulus for the rise of APRPs [14]. Longitudinal studies in large bowel and prostate cancer indicate that the rise of APRPs is but one of a series of catenated reactions that occur as the overall biochemical status of the host declines. The precise moment in the evolution of an invasive tumour when an APRP response is

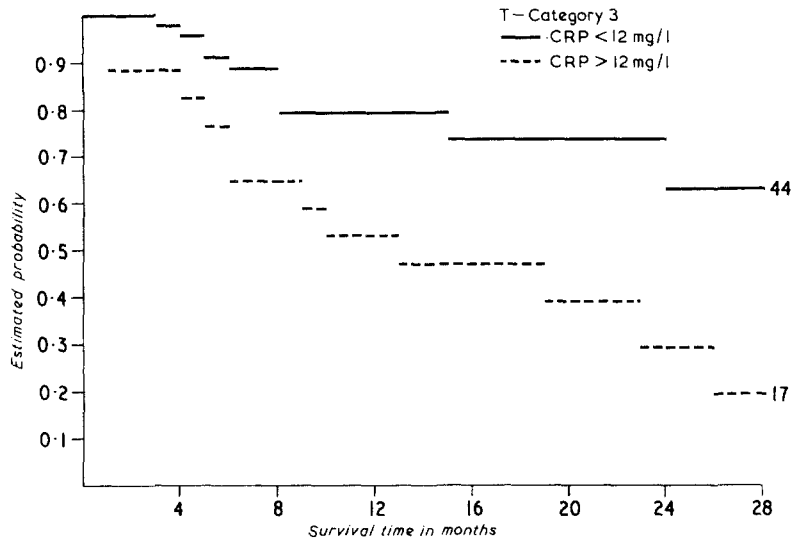

Fig. 3. Kaplan and Meier plot of survival of T3 (Rotterdam) according to the level of CRP at presentation.

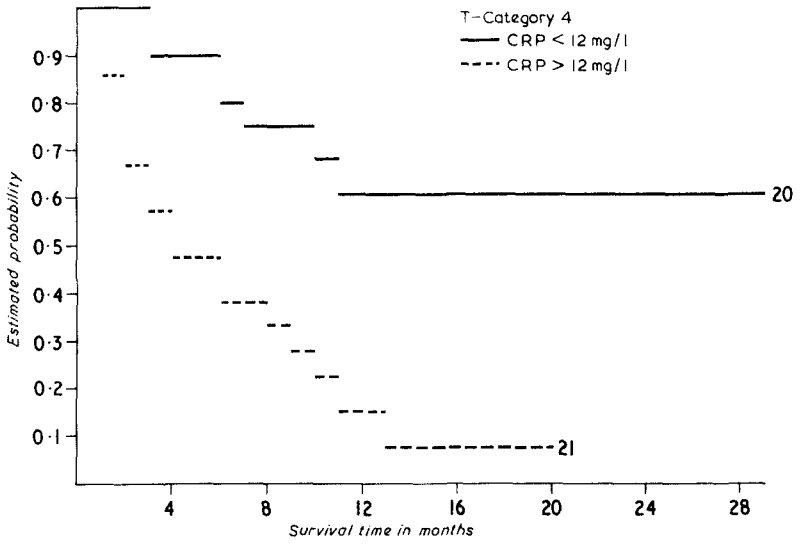

Fig. 4. Kaplan and Meier plot of survival of T4 (Rotterdam) according to the level of CRP at presentation.

initiated is variable; it can occur as part of a loco-regional response to invasion, as seen in bladder and cervix cancer [1, 14, 15], or may be delayed until late in the progression of metastatic disease, as in large bowel and breast cancers [14].

The prognostic information contained in the levels of APRPs was such that a relatively crude split of the patients (T3 and T4) into those with a value above a certain level and those with a value below a certain level was enough to produce two groups with different survival rates. The log-rank analysis was able to indicate the statistical significance of any apparent differences whilst, if necessary, making adjustments for other explanatory variables such as stage. From a practical viewpoint, the analysis has indicated that the measurement of CRP, ACT or AGP have very similar discriminatory effects but haptoglobin is less effective and is more expensive as phenotypic identification is necessary in order to correct the values obtained by radial immunodiffusion. With the increasing use of nephelometric systems for the determination of plasma proteins, commercial assays are now readily available for CRP and AGP determination. The contribution of albumin is as yet uncertain and will probably need a larger series to see whether this helps to identify any particular subsets of patients who have invasive bladder cancer. However, it may be

worth considering other factors when identifying markers. For instance, although our study indicated the greater prognostic value of CRP compared to the other serum proteins, it should be noted that there is more error associated with the measurement in the region of normal values than is usual with the other proteins.

In the light of this experience a prospective trial has been designed and is in progress, with 80 patients entered so far. This trial will take into account more of the well-established clinical and pathological prognostic factors. Hence, a multi-variate analysis, either employing a parametric model [16] if indicated by the data, or a non-parametric approach such as Cox's regression model [17], will be used. This latter approach has been used recently in identifying prognostic factors in renal cell carcinoma [18].

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