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Evaluation of Ki-67 Expression as a Prognostic Feature in Hepatocellular Carcinoma in Cirrhosis

M. Colleoni, M.R. Biasin, L. Boni, P. Nelli, P. Manente, F. Gaion, A. Perasole and D. Infantolino

HEPATOCELLULAR CARCINOMA (HCC) accounts for less than 2% of all malignancies in the U.S.A. and a similar incidence has been recorded in Europe [1]. Although a number of factors in HCC correlating with survival have been identified (including performance status, impaired liver function, age, sex and stage), additional variables are needed in order to better profile these patients [2–5].

Important indications have been recently provided by indices of cell proliferation in solid tumours [6, 7], but limited data are available in HCC. An approach is represented by Ki-67 antibody immunostaining, which has been demonstrated to react with a nuclear antigen associated with cell proliferation [6]. Recent studies, comparing the results obtained with paraffin-embedded sections with those obtained on frozen sections, reported similar findings [8]. We, therefore, retrospectively analysed the prognostic role of Ki-67 expression, along with 18 other variables in paraffin-embedded sections from 45 patients with HCC and associated cirrhosis.

There were 33 men (73%) and 12 women (27%), with a median age of 66 years (range 44–79). 21 cases had hepatitis C virus (HCV) positivity (47%), 14 previous alcohol abuse (31%), and 14 previous or concomitant hepatitis B virus (HBV) infection (31%). In 13 patients, chemo-emolisation with epirubicin had been performed and one patient had undergone surgery. Each biopsy specimen (>10 × 1.5 mm) was fixed in 3.5% formalin buffer and embedded in paraffin wax by conventional procedures. Polyclonal antibody (AO47 Dako, Milan, Italy) and peroxidase anti-peroxidase immunohistochemical techniques were used to detect Ki-67 expression. Ki-67-positive nuclei were evaluated at a magnification of ×250 on a Zeiss microscope. A minimum of 800 cells, evaluated through a minimum of 200 cells per field in four different fields, were considered in each case. The Ki-67 rate was obtained by determining the proportion of Ki-67-positive cells. The other variables analysed in the study (sex, age, TNM (tumour, node, metastasis) stage, Child's status, Okuda stage, ECOG performance status (PS), vascular invasion, HbsAg and HCV positivity, alcohol abuse, previous chemoembolisation, bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase, α-feto protein, platelets) were chosen because an influence on prognosis was found in a previous study [9] or reported by other authors [2–4].

The median follow-up at the time of the study was 17.5 months, with a median survival time of 9 months (range 1–60). The percentage of Ki-67-positive cells of all the HCC tumours ranged between 0.33 and 35.4%. Three Ki-67 categories were established according to the percentage of positivity of stained cells (low positivity, 0–2%; medium positivity, 2–6%; high positivity >6%). The three groups of patients were numerically well balanced (low, 14 cases; medium, 12 cases; high, 19 cases). The difference in survival for the three groups (low, 14.6 months; medium, 9 months; high, 7 months) did not reach statistical significance at univariate analysis ($P = 0.40$). At multivariate analysis with the Cox regression model, PS according to ECOG scale ($P < 0.001$), TNM stage ($P = 0.001$), and age ($P = 0.01$) were found to correlate significantly with survival (Table 1).

The value of cell proliferation indices in solid tumours has been well documented in many tumour types [6]. In spite of this, the results of this study with Ki-67 antigen failed to show any significant correlation with survival, (for this proliferative expression), although patients with a low positivity tended to survive longer than patients with high proliferative expression (14.6 versus 7 months). However, the number of patients analysed may have been too small to detect minimal differences in the survival curve. The absence of significance may also be related to the fact that HCC patients often die as a result of complications of liver failure with or without massive bleeding, or to other factors not related to the proliferative index of the tumour such as cachexia, malnutrition, infection and sepsis.

Interestingly, in this study, clinical parameters were found to significantly correlate with survival. Performance status (PS), according to the ECOG scale, has been reported to be an independent prognostic factor in HCC [3]. Such data are confirmed in this study, with a median survival of 25.6 months for patients with a PS of 0–1 and 3.4 months for patients with a PS of 2–3. The significance of TNM staging in HCC has already been demonstrated [10]. The present study confirmed longer survival for patients with early stage of disease (TNM I–II), with a significant difference in survival versus the other stages (22 versus 5 months). Vascular invasion has been found to predict survival in a group of 295 patients submitted to hepatectomy [5]. The authors suggested that the presence of vascular invasion is a susceptible condition for extrahepatic spread. In the group of patients reported here, only 4 cases had vascular invasion and only 1 died of distant metastases, so no correlation could be made with these limited data.

Table 1. Variables of prognostic significance in HCC patients (results of Cox's regression model)

Variable	Regression coefficient	*P* value	Relative risk (95% CI)
Stage III–IVB (TNM)	1.9	0.001	7 (2–24)
ECOG PS 2–3	2.6	<0.001	14 (5–40)
Age ≥ 60 years	1.2	0.01	3 (1–9)

PS, performance status; TNM, tumour, node, metastasis.

Correspondence to M. Colleoni at the Service of Medical Oncology, City Hospital, Via dell'Ospedale 1, 31033 Castelfranco Veneto, Italy.
M. Colleoni, P. Manente and F. Gaion are at the Service of Medical Oncology, M.R. Biasin, A. Perasole and D. Infantolino are at the City Hospital, Castelfranco Veneto; and L. Boni is at the Division of Clinical Epidemiology, National Cancer Institute, Genova, Italy.
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The influence of age on HCC has been reported in two studies [3, 4]. The authors suggested a possible influence of the fibrolamellar variant (more frequent in young subjects) as partially responsible for the data. In our experience, no fibrolamellar carcinoma was detected, suggesting that other factors related to young age are responsible for a better survival.

In conclusion, the present study, although it failed to show a correlation between Ki-67 expression and prognosis, is helpful for better definition of the prognostic variables influencing results in clinical trials. Proper selection of patients for the various treatment modalities should avoid inclusion of poor-risk patients in clinical trials, but should facilitate comparison of therapeutic options and may improve clinical results.

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Patterns of Gastric Cancer Care by Age. A Registry-based Study in Romagna, Italy

L. Bucchi, M. Serafini, G. Rosti, F. Zumaglini and O. Nanni

THE ROLE of cancer registries in informing the medical community about major aspects of cancer in the elderly has recently been emphasised [1]. In Europe, despite a steady incidence decrease observed over the last decades, gastric cancer (GC) (ICD-9 151) still comprises 8–9% of all new cases of cancer [2]. In particular, GC has a major impact on geriatric oncology. In Europe, such an important health problem has never been the subject of population-based studies of patterns of care. We have reviewed the medical records of GC cases notified to the Romagna Cancer Registry [3, 4] for residents of Ravenna (northern Italy) during 1987–1989. This is one of the areas at highest risk in Europe [3].

The 317 eligible patients were treated in 11 public hospitals and three private clinics. There were 185 males (median age 72 years, range 34–93) and 132 females (median age 77 years, range 35–95). The original case records were obtained for 293 (92%) patients. To minimise the probability of misclassification, data collection was focused on a few variables each having essential modalities. Cases were staged according to the 1992 TNM criteria of the UICC [5]. T categories were based on postresection (pT) information. N classification was based on pathological and surgical data. For the M classification, any available information was used to classify a case as M_1 with the M_0 class being based on surgical data. Age was categorised as shown in Table 1. The extended Mantel–Haenszel χ^2 test for trend [6] was used to assess the relationship between age and (a) the proportion of patients given an essential set of diagnostic and therapeutic procedures, and (b) the TNM distribution of cases.

We observed an inverse relationship between age and the proportion of patients undergoing endoscopy [coding age groups as in Table 1: A 50/53 (94%), B 67/72 (93%), C 91/101 (90%), D 48/67 (72%), $\chi^2 = 14.2$, $P = 0.0002$] and ultrasound [A 47/53 (89%), B 62/72 (86%), C 86/101 (85%), D 46/67 (69%), $\chi^2 = 7.7$, $P = 0.0054$] with no significant age trend in the proportion of patients examined by barium X-ray and by computed tomography.

The proportion of patients surgically cured decreased with age, paralleled by an increase in the frequency of cases not undergoing any surgical procedure (Table 1). The downward trend in the frequency of palliative/explorative approaches was of borderline significance. Restricting analysis to the two categories of patients not cured, the progressive shift from palliative/explorative surgery to "no surgery" was highly significant ($\chi^2 = 27.0$, $P = 0.0000$). Age was also a determinant of the probability of receiving palliative chemotherapy [A 4/16 (25%), B 4/24 (17%), C 2/44 (5%), D 0/49, $\chi^2 = 13.8$, $P = 0.0002$) and adjuvant chemotherapy [A 9/37 (24%), B 5/48 (10%), C 0/56, D 0/17, $\chi^2 = 16.1$, $P = 0.0001$].

TNM distribution by age was closely related to age trend in patterns of clinical and surgical assessment (Table 1). Because of the increasing frequency of T_X, N_X and M_X cases with increasing age, the significant reductions in the T_{1-2}, N_0 and M_0 categories were not coupled with reverse trend in the proportions of more advanced lesions, i.e. T_{3-4}, N_{1-2} and M_1. For the N_{1-2} cases the opposite was observed, with a significant age-dependent reduction.

Admittedly, our findings regarding diagnosis modalities might be biased by missing information on tests performed (if any) on an outpatient basis and not reported in hospital records. In fact,

Correspondence to L. Bucchi.
L. Bucchi and M. Serafini are at the Romagna Cancer Registry (Ravenna Unit), Cancer Prevention Centre; G. Rosti and F. Zumaglini are at the Medical Oncology Department, St Maria delle Croci Hospital, Via Missiroli 10, I-48100 Ravenna; and O. Nanni is at the Medical Oncology Department, Morgagni-Pierantoni Hospital, Forli, Italy.
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