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# Correlation between basal bilirubin levels and survival in advanced colorectal carcinoma treated with CPT-11-based chemotherapy: A study of the Gruppo Oncologico Italia Meridionale (G.O.I.M.)

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ABSTRACT

*Background:* This study was carried out to evaluate total basal bilirubin levels as a predictive factor for survival and toxicity in patients with advanced colorectal carcinoma treated with CTP-11-based regimens.

*Patients and methods:* The analysis was carried out on a data base including 287 patients affected by advanced colorectal carcinoma all treated with CPT-11 plus bolus and continuous venous infusion intravenous folinic acid and 5-fluorouracil on a biweekly schedule (FOLFIRI regimen). Patients were divided into four groups according to basal bilirubin levels as follows: <0.49 mg/dl; >0.50 and <0.99 mg/dl; >1.00 and <1.49 mg/dl; >1.50 mg/dl. Analysis of overall median survival and time-to-progression were correlated to performance status at entry, volume of liver metastases, and carcinoembrionary antigen.

*Results:* Global statistical analysis showed that bilirubin levels were strongly correlated with time-to-progression and overall survival in a statistically significant fashion ($p < 0.0001$). The size of liver metastases represents the only study parameter which is correlated to basal bilirubin levels in a statistically significant fashion. Neutropaenia and diarrhoea were also correlated to baseline bilirubin levels in a statistically significant manner ($p = 0.001$).

*Conclusions:* Data reported in this study support the observation that basal bilirubin levels are a biomarker able to predict, at least in part, the clinical efficacy and toxicity of CPT-11-based chemotherapy in patients with advanced colorectal carcinoma.



## 1. Introduction

CPT-11 is a semi-synthetic analogue of camptothecin, a vegetal alkaloid derived from *Camptotheca acuminata* which interferes with topoisomerase-I enzymatic system via a complex metabolic pathway.[1] CPT-11 is one of the chemotherapeutic agents most frequently employed in the first- or second-line treatment of advanced colorectal (aCRC),[2,3] gastric[4] and

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pancreatic carcinomas.[5] Regardless of its schedule of administration, myelosuppression and delayed-type diarrhoea are the most common – often unpredictable – side-effects seen in patients treated with this topoisomerase-I inhibitor which may be severe enough to represent a serious clinical concern.[1–5]

The complex metabolism of irinotecan probably contributes to its toxicity variability. CPT-11 is eliminated mainly by hepatic route and in a lesser degree by renal excretion. In the liver CPT-11 is metabolised by a tissue carboxyesterase to an active metabolite – i.e. SN-38 – which has an antineoplastic activity 100–1000 times more effective than the parental compound and into two inactive metabolites – called APC and NPC – by CYP3A4 and CYP3A5 isoenzymes belonging to the P450 cytochrome enzymatic system.[1,6,7] SN-38 is detoxified in the liver to SN-38 glucuronide (SN-38-G) by microsomal glucuronisation enzymes such as uridine-diphosphate glucuronsyltransferase 1A1 isoform (UGT1A1), which is considered the principal effector of the detoxification process[8] UGT1A1 also catalyses the glucuronisation of bilirubin as demonstrated by the altered bilirubin metabolism in patients affected by the Gilbert's syndrome, which is related to UGT1A1 polymorphism. These patients may develop life-threatening toxicity after CPT-11 administration both as grade 4 diarrhoea and neutropaenia.[9] Patients with this genetic modification have a SN-38 glucuronisation activity much lower than that observed in normal individuals.

Delayed-type diarrhoea, defined as diarrhoea occurring more than 24 h after administration of irinotecan, is probably directly mediated by high concentrations of intraluminal SN-38, which is formed partly out of SN-38G by bacterial β-glucuronidases.[10] Severe delayed-type diarrhoea has been reported to occur in up to 30–40% of patients and necessitates hospitalisation for intravenous re-hydration in about 10% of patients and may be a life-threatening or even fatal event.[1–5] Apart from morbidity, this type of diarrhoea results in expanded health-care costs, and even mild diarrhoea may influence continuation of an otherwise active treatment. Therefore there is need for clinical and biological predictive factors able to identify patients at risk for acute and life-threatening side-effects in order to ameliorate treatment efficacy and safety.

Several investigators have addressed the potential relationships between patients clinical characteristics to efficacy and toxicity of CPT-11 in aCRC.[11,12] The aim of this study was to explore the possible relationship between patients' clinical characteristics, basal bilirubin levels and survival.

## 2. Patients and methods

### 2.1. Study design and patients selection

This secondary analysis was carried out in a series of 287 patients treated during three consecutive controlled trials of the GOIM which have been published recently.[13–16] All patients were chemonaive and required to have biopsy-proven histologically confirmed aCRC. The other eligibility criteria included the following: bi-dimensionally measurable disease, age between 18 and 75 years, life expectancy of at least 3 months, performance status (PS) 0–2 according to the ECOG scale, adequate bone marrow (platelets count $\geqslant$100.000/mm$^3$, WBC count >4000/mm$^3$, granulocyte count of $\geqslant$1500/mm$^3$, a haemoglobin level of $\geqslant$10.0 g/mm$^3$), renal (serum creatinine concentration $\leqslant$2.0 mg/dl), and hepatic functions (serum bilirubin level $\leqslant$2.0 mg/dl and AST three or less times the normal level in the absence of liver involvement with cancer or up to five times the institutional normal level when cancer was present in the liver). No concurrent uncontrolled medical illness was allowed. Patients had to be previously untreated for advanced disease, but prior adjuvant chemotherapy was allowed if $\geqslant$6 months had relapsed since discontinuation of treatment. Patients were excluded if these criteria were not met and also in the presence of active infections, cerebral metastases, evidence of congestive heart failure, serious cardiac arrhythmias, symptoms of coronary artery disease, history of thromboembolic disease or other malignancy (apart from adequately treated non-melanotic skin cancer and carcinoma in situ of the uterine cervix), or any psychological condition that precluded treatment or adequate follow-up. Radiotherapy was allowed only in sites other than those measurable for response evaluation. Patients had to agree to, and sign, a statement of informed consent prior to entry in this study. Informed consent was previously approved by the Scientific Committee of the GOIM and ethics committees of each individual institution.

### 2.2. Chemotherapy schedule

All patients included into the analysis had been treated with the FOLFIRI regimen: CPT-11 180 mg/m$^2$ on day 1 with LV5FU2 regimen: leucovorin (LV) at 100 mg/m$^2$ administered as a 2-h infusion before 5FU at 400 mg/m$^2$ as an i.v. bolus, and 5FU at 600 mg/m$^2$ as a 22-h infusion immediately after 5FU bolus injection on day 1 and 2. The treatment was repeated every 2 weeks. Globally considered these series of patients achieved an overall response rate of 34.5% (95% confidence interval [CI]: 29–40%) with a median time-to-progression, median duration of response, and survival of 7 months, 10.5 months, and 14 months, respectively.

### 2.3. Statistics

Baseline serum levels were determined by the participating institution's laboratory. As previously described,[11] patients were divided into four groups according to basal bilirubin levels recorded just before the first cycle of chemotherapy: group 1 < 0.49 mg/dl, group 2 > 0, 50 e < 0.99 mg/dl, group 3 > 1.00 e < 1.49 mg/dl, and group 4 > 1.50 mg/dl. No enrolled patient had bilirubin >2 mg/dl. Analysis of time-to-progression (TTP) and global overall survival (OS) was carried out according to the Kaplan–Meier method and compared by a two-tailored log-rank test employing a statistical software S-Plus 6.0 Professional (Insightful Corporation ©).[17] Multivariate analysis was carried out according to Cox regression model.[18] Variables that were considered for the models included age, performance status, prior adjuvant treatment, site of metastatic disease, and baseline carcinoembryonic antigen (CEA). All statistical tests were two-sided.

## 3. Results

### 3.1. Patients population

Overall 287 caucasian patients with advanced CRC were assessed. The main clinical characteristics of evaluated patients according to basal bilirubin level are shown in Table 1. Briefly, there were 208 evaluable patients (109 males and 99 females) with a median age of 61 years (range 32–75), all treated with FOLFIRI regimen every two weeks. One hundred twenty-eight patients had a PS of 0, 73 patients had a PS of 1, and four patients had PS of 2. Three patients had not received PS evaluation before the first chemotherapy cycle. Median basal bilirubin level of the whole series was 0.79 mg/dl (range 0.1–6.2 mg/dl). Fifty-one patients (24.5%) had a basal bilirubin level ⩽49 mg/dl, 108 patients (51.9%) had a bilirubin level between ⩾0.5 and ⩽0.99 mg/dl, 38 (18.2%) ⩾ 1 and ⩽1.49 mg/dl, and 11 patients (5.3%) had bilirubin ⩾1.5 mg/dl. Forty-nine patients had no disease in the liver, while 157 patients had hepatic metastases. Among the latter group 118 patients had liver lesions >10 cm. (H+ > 10), 39 patients had smaller liver disease ⩽10 cm (H+ ⩽ 10). Median CEA level was 368 ng/dl (range 0–6204 ng/dl). Basal bilirubin levels were not significantly correlated to age, baseline PS, primary site of tumor, baseline CEA, and haemoglobin levels. In addition, there were no appreciable differences by prior pelvic irradiation, prior adjuvant 5FU-based chemotherapy, number of organs involved, and baseline WBC count (data not shown).

### 3.2. Survival analysis

Data concerning TTP are depicted in Fig. 1. Patients ($n = 11$) with bilirubin higher >1.5 mg/dl had a median time-to-progression of 2.91 months as compared to 7.0, 7.19, and 8.11 months of patients with bilirubin >1.0–<1.5 mg/dl ($n = 38$), ⩾0.5–<0.99 mg/dl ($n = 107$) and <0.5 mg/dl ($n = 51$).

Fig. 2 shows the Kaplan–Meier distributions for OS by bilirubin category for the entire series.

OS correlated to basal bilirubin levels in a statistically significant manner ($p < 0.0001$). The median survival of patients ($n = 11$) with basal bilirubin levels >1.5 mg/dl was 4.93 months, that of patients ($n = 38$) with bilirubin levels 1.0–1.49 mg/dl was 12.43 months, while that of patients with bilirubin within the normal limits was 13.87 months and 14.96 months, respectively, for the group of patients ($n = 107$) with bilirubin in the 0.49–1.0 mg/dl range and for the group ($n = 51$) with bilirubin <0.49 mg/dl (Table 2). In this series of patients median survival is strongly correlated to basal bilirubin levels.

As shown in Table 2 multivariate Cox analysis (15) of sex, age, PS, basal CEA levels, and presence of hepatic metastases stratified according to basal bilirubin levels showed a statistically significant correlation only for the presence of hepatic lesions ($p = 0.019$). Overall survival analysis of patients according to liver metastasis burden is shown in Fig. 3 which shows that survival is correlated to the presence and the size of hepatic metastases (log-rank 0.0356).

### 3.3. Toxicity analysis

Toxicity grades of diarrhoea and neutropaenia recorded in the series were plotted against baseline bilirubin levels. We evaluated rates and grade of toxicities, employing the worst degree of toxicity of each patient. As shown in Fig. 4, both neutropaenia and diarrhoea were correlated to baseline bilirubin levels in a statistically significant manner ($p = 0.001$). By the first four cycles of chemotherapy, 75% of patients bilirubin >1.5 mg/dl required a toxicity-related dose-reduction as compared to that with a lower initial bilirubin.

## 4. Discussion

We studied the influence of baseline serum bilirubin levels on the activity and toxicity of chemotherapy in a cohort of 287 patients with aCRC, all treated in multicenter randomised trials with the FOLFIRI regimen on a biweekly schedule. Data reported in the present study confirm that basal bilirubin levels of patients at entry are correlated to time-to-progression and median overall survival (Figs. 1 and 2; $p < 0.0001$). Mild increase in total baseline bilirubin levels (up to 1.5 mg/dl) did not influence significantly the activity of CPT-11 in terms of survival parameters, but further increase in bilirubin was strongly correlated with reduced time-to-progression and

**Table 1 – Patients clinical and demographical characteristics**

| | Bilirubin <0.49 | Bilirubin >0.5–<0.99 | Bilirubin >1–<1.49 | Bilirubin >1.5 |
|---|---|---|---|---|
| No of patients | 51 (24.5%) | 108 (51.9%) | 38 (18.2%) | 11 (5.3%) |
| Median age | 61.05 (32–74) | 59.33 (35–74) | 61.94 (32–75) | 58.00 (43–74) |
| Sex | 27♂–24♀ | 55 ♂–53 ♀ | 25 ♂–13♀ | 2 ♂–9 ♀ |
| PS | | | | |
| 0 | 31 | 77 | 19 | 1 |
| 1 | 18 | 28 | 19 | 8 |
| 2 | 1 | 1 | 0 | 2 |
| NA | 1 | 2 | 0 | 0 |
| $H^{+/-}$ | | | | |
| 0 | 26 | 62 | 22 | 8 |
| 1 | 11 | 17 | 9 | 2 |
| 2 | 9 | 17 | 4 | 0 |
| 3 | 4 | 11 | 3 | 1 |
| NA | 1 | 1 | 0 | 0 |
| CEA | 256.58 (0.21–4508) | 344.96 (0.00–4950.00) | 314.23 (0.00–2831.00) | 1230 (0.00–6204.00) |

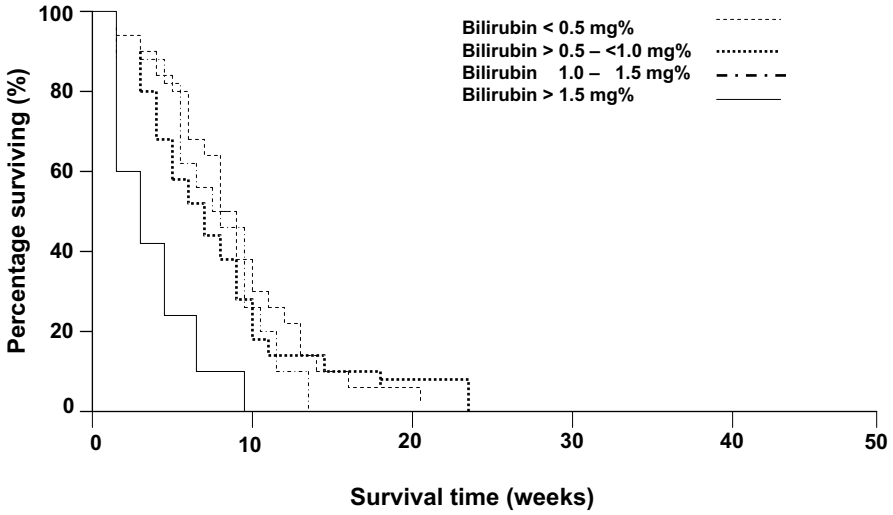


**Fig. 1 – Time-to-progression according to basal bilirubin levels.**

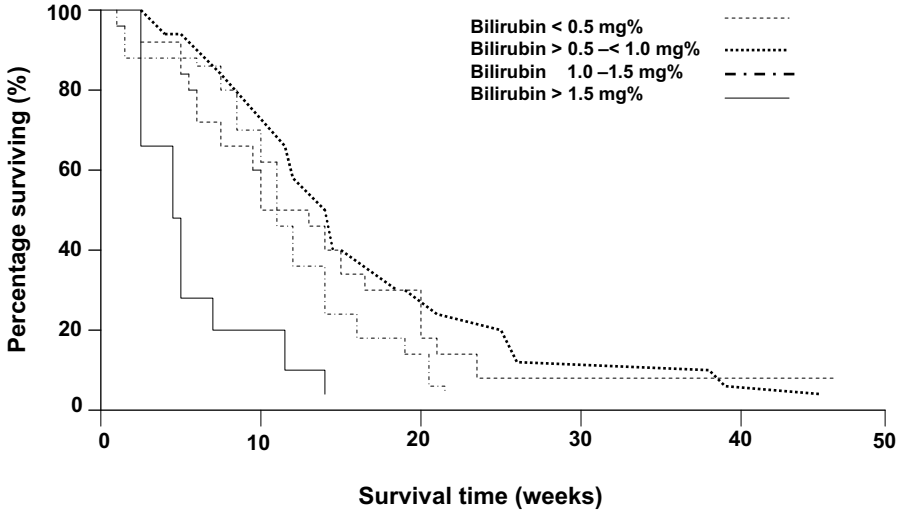


**Fig. 2 – Patients survival according to basal bilirubin levels.**

**Table 2 – Multivariate Cox analysis of sex, age, performance status, basal CEA levels and presence of hepatic metastases stratified according to basal bilirubin levels**

| | Coef | Exp (coef) | SE (coef) | z | *p* | Exp (coef) | Exp (–coef) | Lower.95 | Upper.95 |
|---|---|---|---|---|---|---|---|---|---|
| Sex | –0.0036575 | 0.996 | 0.2710 | –0.0135 | 0.990 | 0.996 | 1.00 | 0.586 | 1.695 |
| Age | –0.0053868 | 0.995 | 0.0140 | –0.3839 | 0.700 | 0.995 | 1.01 | 0.968 | 1.022 |
| PS | –0.0087000 | 0.991 | 0.2773 | –0.0314 | 0.970 | 0.991 | 1.01 | 0.576 | 1.707 |
| Mts | –0.3464447 | 0.707 | 0.1473 | –2.3522 | 0.019 | 0.707 | 1.41 | 0.530 | 0.944 |
| CEA | –0.0000282 | 1.000 | 0.0002 | –0.1407 | 0.890 | 1.000 | 1.00 | 1.000 | 1.000 |

overall survival. Moreover, our data show that overall survival is also correlated to the presence and the size of hepatic metastases (Fig. 3; log-rank 0.0356).

Analysis of the relationship between baseline bilirubin levels and CPT-11-induced diarrhoea and irinotecan-induced neutropaenia showed a significant correlation for both side-effects (Fig. 4, $p = 0.001$). The importance of basal bilirubin levels in predicting CPT-11-related toxicity is further strengthened by the observation that dimension of liver metastases is the only clinical parameter correlated with bilirubin levels (Table 2). These observations are also meaningful if we consider the increasing importance of CPT-11 in the management of aCRC, and its use in combination with bevacizumab in the first-line treatment and with cetuximab in the second-line setting.

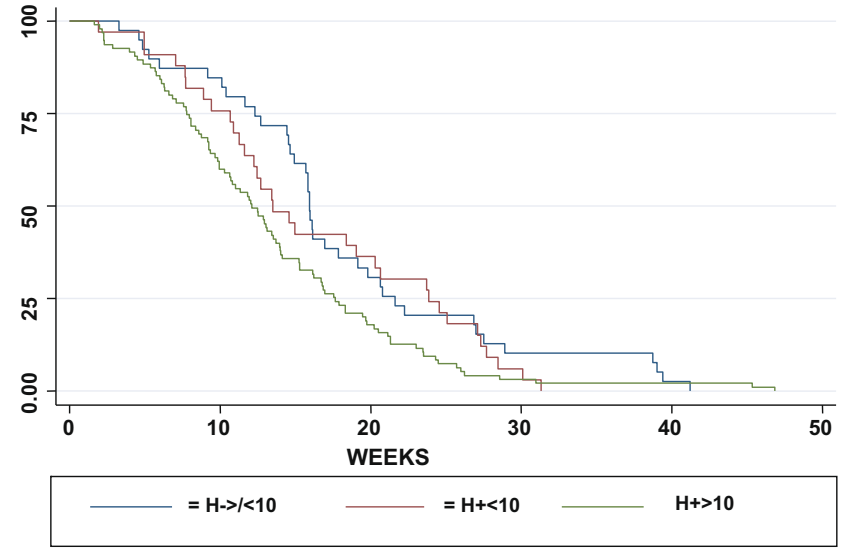


**Fig. 3 – Overall survival according to degree of liver involvement.**

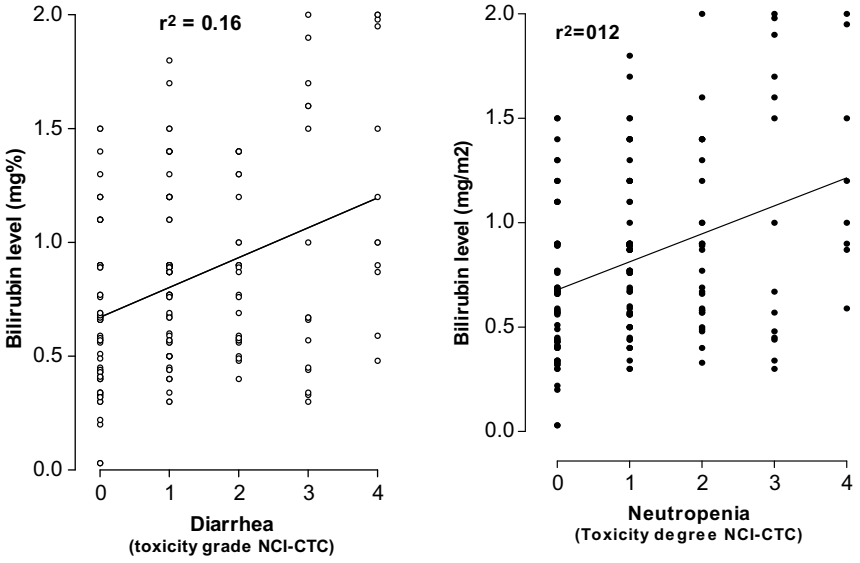


**Fig. 4 – Correlation between baseline bilirubin levels and degree of CPT-11-related toxicity.**

These data are in accord with most of the data published in medical literature. Recent studies[19–21] have shown that the toxicity experienced by patients with aCRC treated with CPT-11-based regimens is correlated with basal bilirubin levels and therefore with its metabolism and its genotype. Moreover, the presence of an altered hepatic metabolism may reduce the antineoplastic activity of CTP-11. Innocenti et al.[22] have shown that UGT1A1 genotypes and total bilirubin basal levels are strongly associated with an excess of severe neutropaenia, suggesting that both parameters may be employed as predictive factors to identify patients potentially exposed to severe toxicity if treated with CTP-11.

The data presented in our study are in accord with those reported by Raymond et al.[22] that showed that bilirubin levels 3 times higher than normal limits cause an exponential reduction of CPT-11 clearance and an increase in neutropaenia rate which require a dose-reduction of chemotherapy drugs.[23] Unfortunately, this study did not report correlation between bilirubin and survival parameters. Since CPT-11 metabolism is tightly correlated to the UGT1A1 enzymatic polymorphism[24–26], responsible for metabolic variability, analysis of genetic profile could be important in planning the treatment of patients with CRC and to predict the toxicology profile. Patients with omozygosis for the 28 allele (UGTA1A1*28) display a higher risk of severe toxicity to CPT-11.

In conclusion, our data strongly support previous reports suggesting that in routine clinical practice patients with baseline bilirubin levels above the normal limits should not be treated with CPT-11-based regimen since the expected severe toxicity could negatively by influence survival. This consideration is further strengthened by the relatively shorter survival of patients with abnormal bilirubin levels as compared to those with normal values.

## Conflict of interest statement

None declared.

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