FULL PAPER

BJC

British Journal of Cancer (2014) 110, 1759–1766 | doi: 10.1038/bjc.2014.89

Keywords: hCGβ; chemotherapy; cystectomy; prognosis; transitional cell carcinoma; urothelial carcinoma

Serum total hCGβ level is an independent prognostic factor in transitional cell carcinoma of the urothelial tract

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Background: Serum total human chorionic gonadotrophin β subunit (hCGβ) level might have prognostic value in urothelial transitional cell carcinoma (TCC) but has not been investigated for independence from other prognostic variables.

Methods: We utilised a clinical database of patients receiving chemotherapy between 2005 and 2011 for urothelial TCC and an independent cohort of radical cystectomy patients for validation purposes. Prognostic variables were tested by univariate Kaplan–Meier analyses and log-rank tests. Statistically significant variables were then assessed by multivariate Cox regression. Total hCGβ level was dichotomised at $<$ *vs* $\geqslant 2\,\mathrm{IU\,l^{-1}}$.

Results: A total of 235 chemotherapy patients were eligible. For neoadjuvant chemotherapy, established prognostic factors including low ECOG performance status, normal haemoglobin, lower T stage and suitability for cisplatin-based chemotherapy were associated with favourable survival in univariate analyses. In addition, low hCGβ level was favourable when assessed either before (median survival not reached *vs* 1.86 years, $P = 0.001$) or on completion of chemotherapy (4.27 *vs* 0.42 years, $P = 0.000002$). This was confirmed in multivariate analyses and in patients receiving first- and second-line palliative chemotherapy, and in a radical cystectomy validation set.

Conclusions: Serum total hCGβ level is an independent prognostic factor in patients receiving chemotherapy for urothelial TCC in both curative and palliative settings.

Approximately 10 000 new bladder cancers are diagnosed annually in the United Kingdom and over 90% are transitional cell carcinomas (TCC) (Crabb and Wheater, 2010). Perioperative cisplatin-based chemotherapy provides a 5–6% absolute survival advantage for operable muscle invasive bladder TCC and a modest survival gain in metastatic disease (which may include TCC occurring in other parts of the urothelial tract) (Logothetis *et al*, 1990; von der Maase *et al*, 2000; Sternberg *et al*, 2001; Grossman *et al*, 2003; von der Maase *et al*, 2005; Advanced Bladder Cancer (ABC) Meta-analysis Collaboration, 2005a, b; Crabb and Wheater, 2010; International Collaboration of Trialists *et al*, 2011). Improved prognostic characterisation to facilitate stratification for treatment would be valuable.

Various prognostic factors are established for urothelial tract TCC on treatment with chemotherapy. In the neoadjuvant setting (bladder TCC), favourable prognostic factors are pathological complete response in those undergoing cystectomy and lower T stage (Grossman *et al*, 2003). In advanced disease, performance

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Revised 13 January 2014; accepted 23 January 2014; published online 20 February 2014

status and disease extent are key prognostic factors (Mead *et al*, 1998; Bajorin *et al*, 1999). Bajorin *et al* (1999) described a retrospective cohort where Karnofsky performance status $<80\%$ and visceral metastases were independent poor prognostic factors. In a phase III trial comparing cisplatin-based regimens, good prognostic factors included Karnofsky performance status $>70\%$, no M1 disease, low/normal alkaline phosphatase, $\leqslant 3$ disease sites and no visceral metastases (von der Maase *et al*, 2005). Predictive biomarker development for TCC has been unsuccessful to date (Stadler *et al*, 2011). Various prognostic or predictive molecular characterisation models in retrospective cohorts have been proposed warranting prospective validation of their potential for therapy selection (Dyrskjot *et al*, 2003; Takata *et al*, 2005; Sanchez-Carbayo *et al*, 2006; Mengual *et al*, 2009; Mitra *et al*, 2009; Smith *et al*, 2011).

Human chorionic gonadotrophin (hCG) is a heterodimeric glycoprotein secreted by trophoblastic cells during gestation with placental, uterine and fetal regulatory roles. The α subunit is common to hCG, LH, FSH and TSH, whereas the β subunit (hCGβ) is distinct to the variant hCG forms. Serum hCGβ levels are a key tumour marker for trophoblastic and germ cell cancers. In addition, hCGβ is elevated in various solid epithelial malignancies, including TCC, with links in some to poor prognosis (Iles, 2007).

We hypothesised that total hCGβ level would function as an independent prognostic marker in patients undergoing chemotherapy for urothelial TCC and report data to demonstrate this.

MATERIALS AND METHODS

Patients and data collection. We undertook retrospective analysis of consecutive patients treated with systemic chemotherapy for invasive urothelial tract cancer at University Hospital Southampton NHS Foundation Trust, UK, between 2005 and 2011. Eligibility criteria were age $\geqslant 18$, confirmed pure or mixed histology TCC, and muscle invasive disease and/or nodal or metastatic spread (staged T_{2-4} and/or N_{1-3} and/or M_1) at first use of chemotherapy. Data collection was through retrospective case note review with data lock on 5 January, 2013. An independent validation set of patients undergoing radical cystectomy for bladder TCC but not perioperative chemotherapy was also created with data lock of 2 August, 2013.

Patients receiving chemotherapy or surgery were managed by oncologists and urologists with specialist interests in urothelial cancer and consistent with regionally approved treatment guidelines. The treating institution undertook specialist multidisciplinary review of all diagnostic and staging investigations for all patients.

Chemotherapy analyses were undertaken in three prospectively defined patient cohorts. The 'neoadjuvant cohort' received chemotherapy before either radical surgery or radiotherapy with curative intent for disease staged T_{2-4} N_0 M_0. The 'first line cohort' either received chemotherapy for newly diagnosed disease staged T_{any} N_{1-3} M_0 or T_{any} N_{any} M_1 or previously received perioperative (adjuvant or neoadjuvant) chemotherapy and were then subsequently treated again at disease relapse. The 'second line cohort' comprised all patients from the first-line cohort treated with subsequent chemotherapy at disease progression.

This research had UK National Research Ethics Service committee approval (10/H0405/99).

Statistical analyses. Overall survival was from the first day of the relevant course of chemotherapy, or the date of cystectomy in the validation set, to death. Progression free survival (first- and second-line cohorts) was from the first day of the relevant course of chemotherapy to disease progression or death from any cause. Relapse free survival (RFS) was from the first day of chemotherapy (neoadjuvant cohort), or cystectomy (validation set), to the first local, regional or distant recurrence or death from any cause. Statistical analysis was performed with SPSS, version 20.0 (IBM, Portsmouth, UK). Univariate analyses of survival outcomes were by the Kaplan–Meier method and log-rank tests. Statistically significant prognostic factors in univariate overall survival analyses were included in multivariate Cox regression analyses to determine hazard ratios as previously described (Crabb *et al*, 2008a, b). P values <0.05 were considered statistically significant.

hCGβ measurement. Total hCGβ levels in serum samples were determined by an accredited UK National Health Service chemical pathology department using a quantitative chemiluminescent immunoassay on a Beckman Coulter DxI immunoassay system (product number 33500, Beckman Coulter, High Wycombe, UK). Blood samples used were those taken as part of routine clinical practice before and immediately following a course of chemotherapy. We prospectively dichotomised hCGβ levels at $<$ *vs* $\geqslant 2\,\mathrm{IU\,l^{-1}}$. hCG$\beta$ levels were the most recent before initiation, or the first following completion, of a course of chemotherapy within a 28-day window. Levels outside these time constraints were excluded.

RESULTS

Chemotherapy cohorts and hCGβ levels. A total of 244 patients received chemotherapy for urothelial TCC between 2005 and 2011, of whom 235 met the inclusion criteria (Figure 1). A total of 92 and 149 received chemotherapy within the neoadjuvant and first-line cohorts, respectively. A total of 16 patients received adjuvant chemotherapy following radical cystectomy. A total of 14 and 8 patients receiving neoadjuvant or adjuvant chemotherapy, respectively, were also treated within the first-line cohort at disease relapse. A total of 63 patients had second-line chemotherapy.

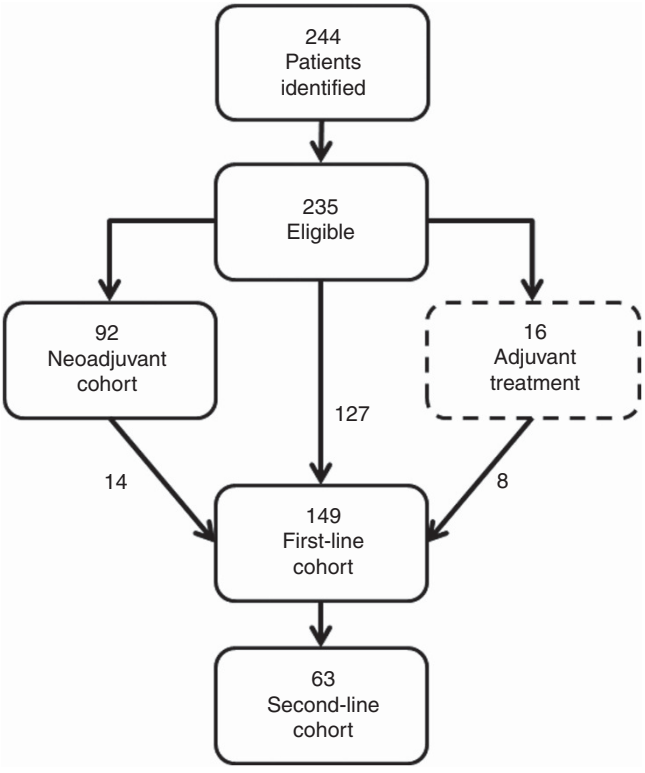

Figure 1. Flow diagram for chemotherapy cohorts used in this study.

Table 1 shows clinico-pathological characteristics and hCGβ levels before, and on completion of, chemotherapy. In the neoadjuvant cohort, 90.2% received gemcitabine/cisplatin (GC) and the rest gemcitabine/carboplatin (GCarbo). For the first-line cohort corresponding figures were 51% and 33.6%, respectively, with the remainder mostly receiving gemcitabine alone due to poor performance status. hCGβ level, where available, was $<2\,IU\,l^{-1}$ before neoadjuvant, first-line and second-line chemotherapy in 68%, 44% and 35%, respectively. The percentage with low ($<2\,IU\,l^{-1}$) hCGβ levels fell with respect to line of treatment with a higher proportion with more advanced disease presenting with intermediate (2 to $<10\,IU\,l^{-1}$) or higher ($\geqslant 10\,IU\,l^{-1}$) levels (Figure 2).

Neoadjuvant chemotherapy. We assessed potential prognostic factors with regard to overall survival following neoadjuvant chemotherapy (Table 2). Favourable factors in univariate analyses for survival were ECOG performance status (0–1 *vs* ⩾2), haemoglobin (⩾ lower limit of normal, LLN), lower T stage (⩽2 *vs* 3 *vs* 4) and suitability for GC (*vs* GCarbo). In addition, hCGβ level $<2\,IU\,l^{-1}$ before neoadjuvant chemotherapy was associated with improved survival (median survival not reached *vs* 1.86 years, $P=0.001$, Figure 3A). Likewise, hCGβ level $<2\,IU\,l^{-1}$ following neoadjuvant chemotherapy was also associated with favourable median survival (4.27 *vs* 0.42 years, $P=0.000002$, Figure 3B).

We undertook multivariate analyses for overall survival incorporating factors reaching statistical significance in univariate analyses including hCGβ level either before, or on completion of, neoadjuvant chemotherapy. hCGβ level in each model remained a statistically significant factor (hazard ratios (HR) 3.41, 95% confidence interval (CI) 1.49–7.83, $P=0.004$ and 15.36, 95% CI 2.13–110.65, $P=0.007$, respectively), along with haemoglobin level in the first model (Table 3).

In addition, low hCGβ level in the neoadjuvant cohort, assessed before chemotherapy was associated with RFS of 7.38 *vs* 1.45 years, but this was not statistically significant ($P=0.07$). However, low hCGβ level on completion of neoadjuvant chemotherapy was associated with RFS of 7.37 *vs* 0.51 years, $P=0.0003$.

First-line chemotherapy. For first-line chemotherapy, favourable ECOG performance status, normal serum alkaline phosphatase (⩽ULN), absence of visceral metastases, receipt of GC (*vs* other regimens), absence of Bajorin risk factors (Bajorin *et al*, 1999) and no prior perioperative chemotherapy were each associated with longer survival in univariate analyses (Table 2).

In addition, hCGβ level $<2\,IU\,l^{-1}$ before, or on completion of, chemotherapy was associated with improved survival (median 1.53 *vs* 0.86 years, $P=0.04$ and 1.68 *vs* 0.84 years, $P=0.00005$, respectively, Table 2, Figure 3C and D).

We undertook multivariate models for overall survival including ECOG performance status and presence of visceral metastases as separate factors (and so omitting the Bajorin index). hCGβ level on completion of first-line chemotherapy remained a statistically significant prognostic factor (HR 3.47, 95% CI 1.97–6.10, $P=0.00002$, Table 4) along with performance status and the presence of visceral metastases. It did not retain statistical significance for hCGβ levels taken before chemotherapy ($P=0.25$, Table 4). We also assessed the impact of hCGβ level on progression free survival and found low levels to be associated with improved outcomes in univariate analysis both before, and on completion of, chemotherapy (0.86 *vs* 0.64 years, $P=0.03$ and 0.86 *vs* 0.50 years, $P=0.0004$, respectively).

Second-line chemotherapy. In patients receiving second-line chemotherapy, low hCGβ level on completion of chemotherapy was associated with improved survival (median 1.78 *vs* 0.29 years, $P=0.003$), but not with levels before chemotherapy ($P=0.3$).

Validation cystectomy cohort. Finally we assessed hCGβ level in an independent sample set following radical cystectomy, but without chemotherapy, (Supplementary Table 1) and found that low levels were associated with improved overall survival (median not reached *vs* 2.18 years, $P=0.002$, Figure 4) and RFS (median not reached *vs* 0.87 years, $P=0.00002$).

DISCUSSION

Chemotherapy for muscle invasive TCC improves cure rates in combination with radical treatment options (Grossman *et al*, 2003; Advanced Bladder Cancer (ABC) Meta-analysis Collaboration, 2005a, b; International Collaboration of Trialists *et al*, 2011) and extends survival in metastatic disease (von der Maase *et al*, 2000, 2005). However, outcomes are poor following disease relapse or progression. We sought to extend the prognostic information available on initiation, or completion, of chemotherapy. In bladder cancer/TCC, previous studies indicate elevated hCGβ levels of 30–76% in serum, 35–73% in urine and 35% by immunohistochemistry, and possible associations to grade, stage and survival (Moutzouris *et al*, 1993; Iles, 2007). hCGβ-expressing TCC appears to act in a biologically aggressive manner with poor survival outcomes, increased risk of disease relapse and poor radiotherapy response (Martin *et al*, 1989; Marcillac *et al*, 1993; Moutzouris *et al*, 1993; Dobrowolski *et al*, 1994; Iles, 2007). Cook *et al* (2000) investigated hCGβ level within a tumour marker panel including carcino-embryonic antigen, CA125 and CA19.9, and response to chemotherapy in advanced bladder cancer. Neither clinical response nor survival differed between marker-negative and marker-positive patients, however clinical response was strongly related to marker response. Only 19 patients (24%) were evaluable for hCGβ response and so its relevance in this cohort remains uncertain (Cook *et al*, 2000). Urinary total hCG levels were found to be elevated in a subgroup of patients referred for cystoscopy who were found to have bladder cancer but none of those with benign conditions. It was a poor prognostic factor in those with muscle invasive disease (Iles *et al*, 1996).

Our work establishes raised total hCGβ level as a poor prognostic factor in chemotherapy-treated urothelial TCC and confirms independence from other established prognostic factors. To our knowledge this is the first time this has been demonstrated for a malignancy other than testis/germ cell cancer. We also demonstrated its association with a poor prognosis in patients who undergo cystectomy as a first step towards validation of hCGβ as a prognostic factor. We would now propose prospective validation in a chemotherapy-treated group of patients before clinical utilisation. Such development would be attractive as hCGβ level is routinely available to clinicians as a relatively cheap, commercially available, validated clinical test with known performance characteristics. Thus the path to establishing this biomarker for use in TCC may be less tortuous than other options. It is important to note that our assessment of hCGβ level utilised an automated, routine, clinically available immunoassay to detect total hCGβ. This is, in essence, a surrogate measurement of the free hCGβ presumed to be expressed by the tumour but will have included all forms of hCG present including intact, free, nicked and glycosylated forms. Future work should look to dissect the relevance of these using assays available with the sensitivity and specificity to do so (Cole and Butler, 2012).

Strengths of our study include that it represents a complete set of sequentially treated patients according to common management criteria, with all patients treated where clinically appropriate with cisplatin-based chemotherapy. These are 'real world' data however (which we view as a strength) and so also include those unfit for cisplatin-based regimens who are frequently omitted from research in this disease despite representing 40–50% of the population.

Table 1. Patient characteristics in the chemotherapy cohorts

	Neoadjuvant chemotherapy cohort, $n=92$	First-line chemotherapy cohort, $n=149$
Age		
Median	69	69
Range	48–84	34–92
⩽70	55 (59.8%)	77 (51.7%)
>70	37 (40.2%)	72 (48.3%)
Sex		
Male	65 (70.7%)	109 (73.2%)
Female	27 (29.3%)	40 (26.8%)
ECOG PS		
0 or 1	84 (91.3%)	106 (71.1%)
⩾2	5 (5.4%)	28 (18.8%)
Unknown	3 (3.3%)	15 (10.1%)
Hb		
Median ($g\,l^{-1}$)	135	125
Range	94–177	80–170
⩾LLN	61 (66.3%)	69 (46.3%
<LLN	29 (31.5%)	74 (49.7%)
Unknown	2 (2.2%)	6 (4.0%)
ALP		
Median ($U\,l^{-1}$)	88	104
Range	36–342	38–1181
⩽ULN	79 (85.9%)	96 (64.4%)
>ULN	9 (9.8%)	43 (28.9%)
Unknown	4 (4.3%)	10 (6.7%)
LDH		
Median ($IU\,l^{-1}$)	429	439
Range	321–703	272–2115
⩽ULN	21 (22.8%)	42 (28.2%)
>ULN	8 (8.7%)	23 (15.4%)
Unknown	63 (68.5%)	84 (56.4%)
Grade		
2	10 (10.9%)	14 (9.4%)
3	81 (88.0%)	122 (81.9%)
Unknown	1 (1.1%)	13 (8.7%)
Primary tumour site		
Bladder	92 (100%)	111 (74.5%)
Renal pelvis	—	22 (14.8%)
Ureteric	—	13 (8.7%)
Urethral	—	3 (2.0%)
T stage		
⩽2	44 (47.8%)	40 (26.8%
3	35 (38.0%)	48 (32.2%)
4	13 (14.1%)	16 (10.7%)
X	0	43 (28.9%)
N stage		
0	92 (100%)	70 (47.0%)
1	—	24 (16.1%)
2	—	54 (36.2%)
3	—	1 (0.7%)
M stage		
0	92 (100%)	62 (41.6%)
1	—	87 (58.4%)
Visceral metastases		
No	—	95 (63.8%)
Yes	—	54 (36.2%)
Bajorin risk factors (Bajorin *et al*, 1999)		
0	—	70 (47.0%)
1	—	47 (31.5%)
2	—	17 (11.4%)
Unknown	—	15 (10%)
Chemotherapy regimen		
GC	83 (90.2%)	76 (51.0%)
GCarbo	9 (9.8%)	50 (33.6%)
Other	0	23 (15.4%)
Prior chemotherapy		
No	—	127 (85.2%)
Yes	—	22 (14.8%)
Radical local therapy		
Surgery	48 (52.2%)	10 (3.4%)
Radiotherapy	28 (30.4%)	5 (6.7)
None	14 (15.2%)	134 (89.9%)
Unknown	2 (2.2%)	0
hCGβ level before chemotherapy		
$<2\,IU\,l^{-1}$	58 (63.0%)	61 (40.9%)
$\geqslant 2\,IU\,l^{-1}$	27 (29.3%)	77 (51.7%)
Unknown	7 (7.6%)	11 (7.4%)
hCGβ level on completion of chemotherapy		
$<2\,IU\,l^{-1}$	28 (30.4%)	56 (37.6%)
$\geqslant 2\,IU\,l^{-1}$	5 (5.4%)	40 (26.8%)
Unknown	59 (64.1%)	53 (35.6%)

Abbreviations: ALP = alkaline phosphatase; ECOG PS = Eastern Cooperative Oncology Group performance status; GC = gemcitabine/cisplatin; GCarbo = gemcitabine/carboplatin; Hb = haemoglobin; LDH = lactate dehydrogenase; LLN = lower limit of normal for the treating institution's reference range; ULN = upper limit of normal for the treating institution's reference range.

Thus our results are relevant to current standard-of-care management for this disease rather than the rarefied world of a clinical trial.

A number of questions arise from our data. We demonstrated a prognostic impact for hCGβ levels at completion of, as well as before, chemotherapy. This is despite higher numbers where hCGβ level was not recorded (64.1% neoadjuvant, 35.6% metastatic) which we acknowledge holds potential for bias. Thus there might be a role for hCGβ level as a predictive biomarker for chemotherapy benefit, either as an absolute level before, or following, chemotherapy, or in a dynamic sense as hCGβ 'normalisation' during treatment. One could also consider what hCGβ dynamics might imply for required duration or type of chemotherapy or if subsequent hCGβ rise might be utilised to detect disease recurrence/relapse. Anecdotally we have experience

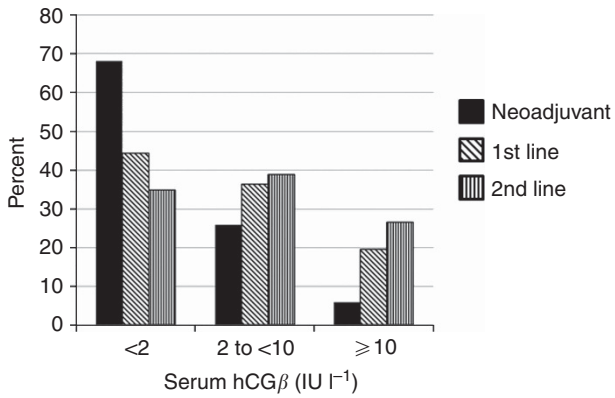

Figure 2. Total hCGβ levels before chemotherapy for patients undergoing neoadjuvant, first-line or second-line chemotherapy.

of the latter representing early indication of disease activity. These are hypotheses however and should be tested prospectively. It would also be of interest to investigate the relevance of hCGβ level in other settings, for example in non-muscle invasive disease to test risk for relapse and progression. In this, and the neoadjuvant/adjuvant chemotherapy settings, the question of whether a raised hCGβ level reflects those with micro-metastases arises and warrants further prospective work, possibly with comparison with experimental imaging methodologies. A further question is the relevance of hCGβ expression in the primary tumour which we are investigating in our radical cystectomy cohort.

Our work raises the question of the biological role of hCGβ in TCC. hCGβ may have a functional role in cancer progression as a transforming growth factor, an immunosuppressive agent, an inducer of metastasis or as an angiogenic factor (Iles, 2007; Cole, 2010). hCGβ, but not intact hCG, hCGα or hCGβ core fragment, stimulated TCC cell line growth which could be inhibited by hCGβ

Table 2. Univariate analyses to assess individual potential prognostic factors with respect to overall survival following chemotherapy

		Neoadjuvant chemotherapy		First-line chemotherapy	
Factor	**Division**	**Median OS, years (95% CI)**	***P*-value**	**Median OS, years (95% CI)**	***P*-value**
Age	⩽70 >70	NR 3.36 (0.91–5.81)	0.08	1.13 (0.66–1.61) 0.98 (0.68–1.30)	0.21
Sex	Male Female	5.26 (4.37–6.15) 4.05 (0.88–7.23)	0.49	1.16 (0.79–1.53) 1.02 (0.71–1.33)	0.84
ECOG PS	0 or 1 ⩾2	5.26 (4.52–6.01) 0.42 (0.33–0.50)	0.000008	1.49 (1.08–1.90) 0.63 (0.47–0.79)	0.0000005
Hb	⩾LLN <LLN	NR 1.22 (0.94–1.51)	0.00005	1.39 (0.97–1.82) 0.96 (0.69–1.24)	0.15
ALP	⩽ULN >ULN	NR 1.14 (0.87–1.41)	0.08	1.35 (1.05–1.65) 0.66 (0.52–0.80)	0.003
LDH	⩽ULN >ULN	4.27 (0.10–8.44) NR	0.34	1.23 (0.80–1.66) 1.04 (0.53–1.55)	0.58
Grade	2 3	4.05 (2.71–5.40) 5.26 (4.49–6.03)	0.90	1.02 (0.44–1.90) 1.14 (0.87–1.41)	0.87
Primary tumour site	Bladder Other	— —		1.07 (0.78–1.35) 1.07 (0.66–1.49)	0.57
T stage	⩽2 3 4	NR 5.27 (4.15–6.39) 1.09 (0.61–1.57)	0.006	— — —	
Visceral metastases	No Yes	— —		1.49 (1.03–1.95) 0.78 (0.67–0.88)	0.004
Chemotherapy regimen	GC GCarbo Other	NR 0.89 (0.48–1.31) —	0.001	1.49 (1.05–1.93) 0.80 (0.41–1.20) 0.57 (0.40–0.74)	0.00005
Bajorin risk factors (Bajorin *et al*, 1999)	0 1 2	— — —		1.74 (1.41–2.08) 0.98 (0.78–1.17) 0.47 (0.31–0.65)	0.0000001
Prior perioperative chemotherapy	No Yes	— —		1.15 (0.90–1.41) 0.60 (0.50–0.70)	0.001
hCGβ level before chemotherapy	<2 IU l^{-1} ⩾2 IU l^{-1}	NR 1.86 (0.51–3.21)	0.001	1.53 (1.17–1.89) 0.86 (0.67–1.05)	0.04
hCGβ level on completion of chemotherapy	<2 IU l^{-1} ⩾2 IU l^{-1}	4.27 (1.65–6.89) 0.42 (0.14–0.70)	0.000002	1.68 (1.25–2.11) 0.84 (0.68–1.00)	0.00005

Abbreviations: ALP = alkaline phosphatase; CI = confidence interval; ECOG PS = Eastern Cooperative Oncology Group performance status; GC = gemcitabine/cisplatin; GCarbo = gemcitabine/carboplatin; Hb = haemoglobin; LDH = lactate dehydrogenase; LLN = lower limit of normal for the treating institution's reference range; NR = not reached; ULN = upper limit of normal for the treating institution's reference range.

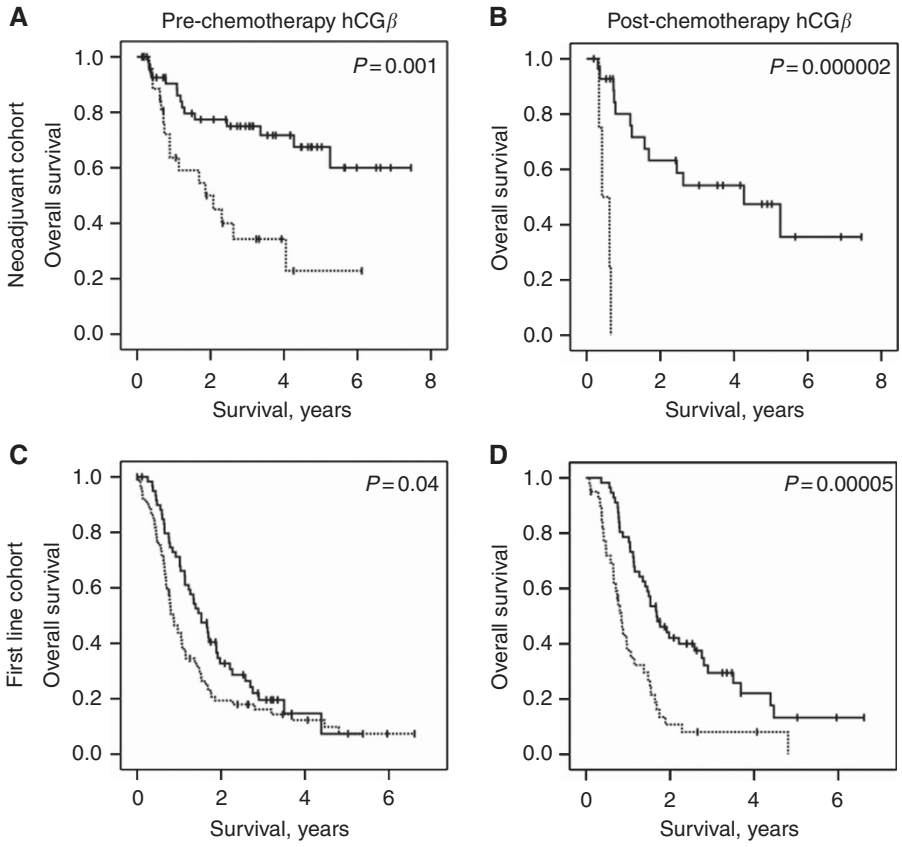

Figure 3. Kaplan–Meier plots to show overall survival according to total hCGβ level in the neoadjuvant (**A**, **B**) or first-line (**C**, **D**) chemotherapy cohorts either before (**A**, **C**), or on completion of (**B**, **D**) chemotherapy. Broken line – hCGβ level ⩾2; continuous line – hCGβ level <2.

Table 3. Multivariate analyses of potential prognostic factors for overall survival in patients undergoing neoadjuvant chemotherapy incorporating hCGβ level either before, or on completion of, chemotherapy

Factor	HR	95% CI	*P*-value	HR	95% CI	*P*-value
ECOG PS						
⩾2 *vs* 0 or 1	2.10	0.41–10.89	0.38	2.14	0.31–14.95	0.44
Hb						
<LLN *vs* ⩾LLN	3.56	1.69–7.46	0.001	1.17	0.25–5.45	0.84
Chemotherapy regimen						
GCarbo *vs* GC	1.48	0.40–5.42	0.56	1.79	0.42–7.57	0.43
T stage						
T3 *vs* T2 T4 *vs* T2	1.29 1.76	0.55–3.00 0.47–6.61	0.56 0.40	0.98 0.66	0.23–4.15 0.07–6.04	0.98 0.71
hCGβ level before chemotherapy						
⩾2 *vs* <2	3.41	1.49–7.83	0.004	—	—	—
hCGβ level on completion of chemotherapy						
⩾2 *vs* <2	—	—	—	15.36	2.13–110.7	0.007

Abbreviations: CI = confidence interval; ECOG PS = Eastern Cooperative Oncology Group performance status; GC = gemcitabine/cisplatin; GCarbo = gemcitabine/carboplatin; Hb = haemoglobin; HR = hazard ratio; LLN = lower limit of normal for the treating institution's reference range.

antibodies (Gillott *et al*, 1996). There is some evidence to suggest that, in part, hCGβ might act in TCC, and potentially other malignancies, by inhibition of TGFβ-induced apoptosis by virtue of their structural homology (reviewed by Iles (Iles, 2007)). Whether these putative biological mechanisms are relevant, or if hCGβ simply acts as a surrogate for poorly differentiated, biologically aggressive disease remains uncertain and further investigation of the biological role in TCC is warranted. hCGβ

Table 4. Multivariate analyses of potential prognostic factors for overall survival in patients undergoing first-line chemotherapy incorporating hCGβ level either before, or on completion of, chemotherapy

Factor	HR	95% CI	*P*-value	HR	95% CI	*P*-value
ECOG PS						
⩾2 *vs* 0 or 1	2.39	1.38–4.13	0.002	2.09	1.01–4.33	0.047
ALP						
<LLN *vs* ⩾LLN	0.98	0.59–1.60	0.92	1.44	0.78–2.68	0.24
Visceral metastases						
Yes *vs* no	2.01	1.29–3.13	0.002	2.65	1.49–4.70	0.001
Chemotherapy regimen						
GCarbo *vs* GC	0.83	0.83–0.66	0.11	1.86	0.52–6.66	0.34
Prior perioperative chemotherapy						
Yes *vs* no	2.16	0.81–5.79	0.82	1.07	0.60–6.66	0.34
hCGβ level before chemotherapy						
⩾2 *vs* <2 IU l^{-1}	1.28	0.84–1.96	0.25	—	—	—
hCGβ level on completion of chemotherapy						
⩾2 *vs* <2 IU l^{-1}	—	—	—	3.47	1.97–6.10	0.00002

Abbreviations: ALP = alkaline phosphatase; CI = confidence interval; ECOG PS = Eastern Cooperative Oncology Group performance status; GC = gemcitabine/cisplatin; GCarbo = gemcitabine/carboplatin; HR = hazard ratio; LLN = lower limit of normal for the treating institution's reference range.

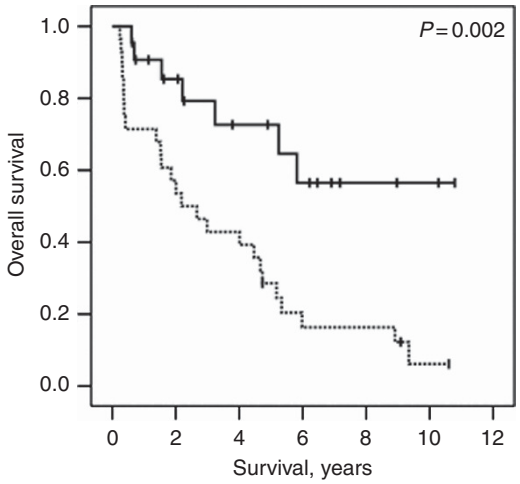

Figure 4. Kaplan–Meier plot to show overall survival according to total hCGβ level in a radical cystectomy cohort. Broken line – hCGβ level ⩾2; continuous line – hCGβ level <2.

might also represent a therapeutic target with vaccination strategies currently under development (Delves *et al*, 2007).

We utilised a prospectively defined hCGβ cut point of < *vs* ⩾2 IU l^{-1}. This was in part pragmatic as, during the period in question, this was the lower level of quantification at our institution. It would be of interest to undertake future analysis either of other cut points to optimise a dichotomous variable or to analyse as a continuous variable.

Certain limitations of our data exist. First, these were retrospective analyses. Second, patients were included on the basis of receipt of chemotherapy for urothelial TCC. The study therefore holds bias for those suitable to commence chemotherapy and our patient cohorts are somewhat heterogeneous, which future prospective validation should seek to address and control for. Our validation cohort was a cystectomy-treated group. We chose this primarily for pragmatic reasons as, to our knowledge and after some effort to find an alternative, there is no current sample set available of chemotherapy-treated patients with hCGβ data available. Prospective validation in both treatment settings is therefore now required and warranted. Finally our cohort was not randomised to treatment and so we cannot establish whether a predictive factor role for hCGβ levels exists from this sample set.

In conclusion, serum hCGβ level is an independent prognostic factor for outcome in patients undergoing chemotherapy for TCC of the urothelial tract. Prospective validation is warranted to determine its value for patient stratification in this disease.

ACKNOWLEDGEMENTS

CC was part funded by the Southampton National Institute of Health Research (NIHR) Wellcome Trust Clinical Research Facility.

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Supplementary Information accompanies this paper on British Journal of Cancer website (http://www.nature.com/bjc)