

**Keywords:** hCG $\beta$ ; chemotherapy; cystectomy; prognosis; transitional cell carcinoma; urothelial carcinoma

# Serum total hCG $\beta$ level is an independent prognostic factor in transitional cell carcinoma of the urothelial tract

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**Background:** Serum total human chorionic gonadotrophin  $\beta$  subunit (hCG $\beta$ ) 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 hCG $\beta$  level was dichotomised at  $<$  vs  $\geq 2\text{IU l}^{-1}$ .

**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 hCG $\beta$  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 hCG $\beta$  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 Wheeler, 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 Wheeler, 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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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,  $\leq 3$  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  $\alpha$  subunit is common to hCG, LH, FSH and TSH, whereas the  $\beta$  subunit (hCG $\beta$ ) is distinct to the variant hCG forms. Serum hCG $\beta$  levels are a key tumour marker for trophoblastic and germ cell cancers. In addition, hCG $\beta$  is elevated in various solid epithelial malignancies, including TCC, with links in some to poor prognosis (Iles, 2007).

We hypothesised that total hCG $\beta$  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  $\geq 18$ , confirmed pure or mixed histology TCC, and muscle invasive disease and/or nodal or metastatic spread (staged T<sub>2-4</sub> and/or N<sub>1-3</sub> and/or M<sub>1</sub>) 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<sub>2-4</sub> N<sub>0</sub> M<sub>0</sub>. The 'first line cohort' either received chemotherapy for newly diagnosed disease staged T<sub>any</sub> N<sub>1-3</sub> M<sub>0</sub> or T<sub>any</sub> N<sub>any</sub> M<sub>1</sub> 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.

**hCG $\beta$  measurement.** Total hCG $\beta$  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 hCG $\beta$  levels at  $<$  vs  $\geq 2$  IU l<sup>-1</sup>. 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 hCG $\beta$  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.

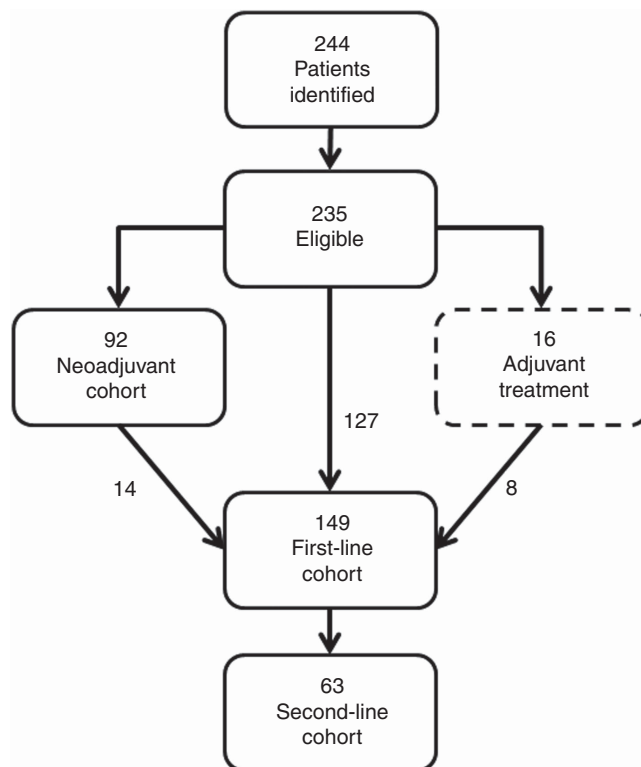


Figure 1. Flow diagram for chemotherapy cohorts used in this study.

Table 1 shows clinico-pathological characteristics and hCG $\beta$  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. hCG $\beta$  level, where available, was  $<2\text{ IU l}^{-1}$  before neoadjuvant, first-line and second-line chemotherapy in 68%, 44% and 35%, respectively. The percentage with low ( $<2\text{ IU l}^{-1}$ ) hCG $\beta$  levels fell with respect to line of treatment with a higher proportion with more advanced disease presenting with intermediate (2 to  $<10\text{ IU l}^{-1}$ ) or higher ( $\geq 10\text{ IU l}^{-1}$ ) 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  $\geq 2$ ), haemoglobin ( $\geq$  lower limit of normal, LLN), lower T stage ( $\leq 2$  vs 3 vs 4) and suitability for GC (vs GCarbo). In addition, hCG $\beta$  level  $<2\text{ IU l}^{-1}$  before neoadjuvant chemotherapy was associated with improved survival (median survival not reached vs 1.86 years,  $P=0.001$ , Figure 3A). Likewise, hCG $\beta$  level  $<2\text{ IU l}^{-1}$  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 hCG $\beta$  level either before, or on completion of, neoadjuvant chemotherapy. hCG $\beta$  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 hCG $\beta$  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 hCG $\beta$  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 ( $\leq$ 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, hCG $\beta$  level  $<2\text{ IU l}^{-1}$  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). hCG $\beta$  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 hCG $\beta$  levels taken before chemotherapy ( $P=0.25$ , Table 4). We also assessed the impact of hCG $\beta$  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 hCG $\beta$  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 hCG $\beta$  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 hCG $\beta$  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). hCG $\beta$ -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 hCG $\beta$  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 hCG $\beta$  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 hCG $\beta$  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 hCG $\beta$  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 hCG $\beta$  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 hCG $\beta$  level utilised an automated, routine, clinically available immunoassay to detect total hCG $\beta$ . This is, in essence, a surrogate measurement of the free hCG $\beta$  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
<b>Age</b>		
Median	69	69
Range	48–84	34–92
≤70	55 (59.8%)	77 (51.7%)
>70	37 (40.2%)	72 (48.3%)
<b>Sex</b>		
Male	65 (70.7%)	109 (73.2%)
Female	27 (29.3%)	40 (26.8%)
<b>ECOG PS</b>		
0 or 1	84 (91.3%)	106 (71.1%)
≥2	5 (5.4%)	28 (18.8%)
Unknown	3 (3.3%)	15 (10.1%)
<b>Hb</b>		
Median (g l <sup>-1</sup> )	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%)
<b>ALP</b>		
Median (UI l <sup>-1</sup> )	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%)
<b>LDH</b>		
Median (IU l <sup>-1</sup> )	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%)
<b>Grade</b>		
2	10 (10.9%)	14 (9.4%)
3	81 (88.0%)	122 (81.9%)
Unknown	1 (1.1%)	13 (8.7%)
<b>Primary tumour site</b>		
Bladder	92 (100%)	111 (74.5%)
Renal pelvis	—	22 (14.8%)
Ureteric	—	13 (8.7%)
Urethral	—	3 (2.0%)
<b>T stage</b>		
≤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%)
<b>N stage</b>		
0	92 (100%)	70 (47.0%)
1	—	24 (16.1%)
2	—	54 (36.2%)
3	—	1 (0.7%)

Table 1. (Continued)

	Neoadjuvant chemotherapy cohort, n = 92	First-line chemotherapy cohort, n = 149
<b>M stage</b>		
0	92 (100%)	62 (41.6%)
1	—	87 (58.4%)
<b>Visceral metastases</b>		
No	—	95 (63.8%)
Yes	—	54 (36.2%)
<b>Bajorin risk factors (Bajorin et al, 1999)</b>		
0	—	70 (47.0%)
1	—	47 (31.5%)
2	—	17 (11.4%)
Unknown	—	15 (10%)
<b>Chemotherapy regimen</b>		
GC	83 (90.2%)	76 (51.0%)
GCarbo	9 (9.8%)	50 (33.6%)
Other	0	23 (15.4%)
<b>Prior chemotherapy</b>		
No	—	127 (85.2%)
Yes	—	22 (14.8%)
<b>Radical local therapy</b>		
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
<b>hCG<math>\beta</math> level before chemotherapy</b>		
<2 IU l <sup>-1</sup>	58 (63.0%)	61 (40.9%)
≥2 IU l <sup>-1</sup>	27 (29.3%)	77 (51.7%)
Unknown	7 (7.6%)	11 (7.4%)
<b>hCG<math>\beta</math> level on completion of chemotherapy</b>		
<2 IU l <sup>-1</sup>	28 (30.4%)	56 (37.6%)
≥2 IU l <sup>-1</sup>	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 hCG $\beta$  levels at completion of, as well as before, chemotherapy. This is despite higher numbers where hCG $\beta$  level was not recorded (64.1% neoadjuvant, 35.6% metastatic) which we acknowledge holds potential for bias. Thus there might be a role for hCG $\beta$  level as a predictive biomarker for chemotherapy benefit, either as an absolute level before, or following, chemotherapy, or in a dynamic sense as hCG $\beta$  'normalisation' during treatment. One could also consider what hCG $\beta$  dynamics might imply for required duration or type of chemotherapy or if subsequent hCG $\beta$  rise might be utilised to detect disease recurrence/relapse. Anecdotally we have experience

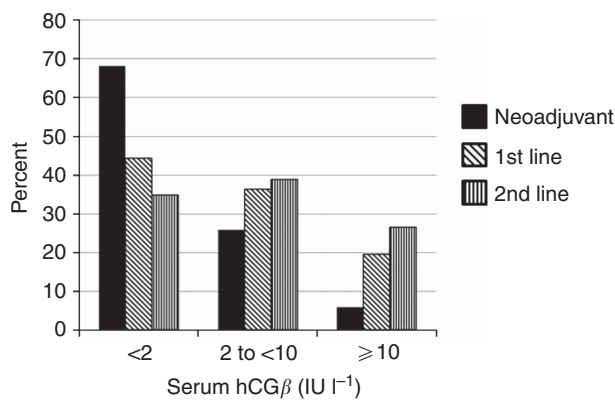


Figure 2. Total hCGβ levels before chemotherapy for patients undergoing neoadjuvant, first-line or second-line chemotherapy.

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/adjvant 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

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

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.

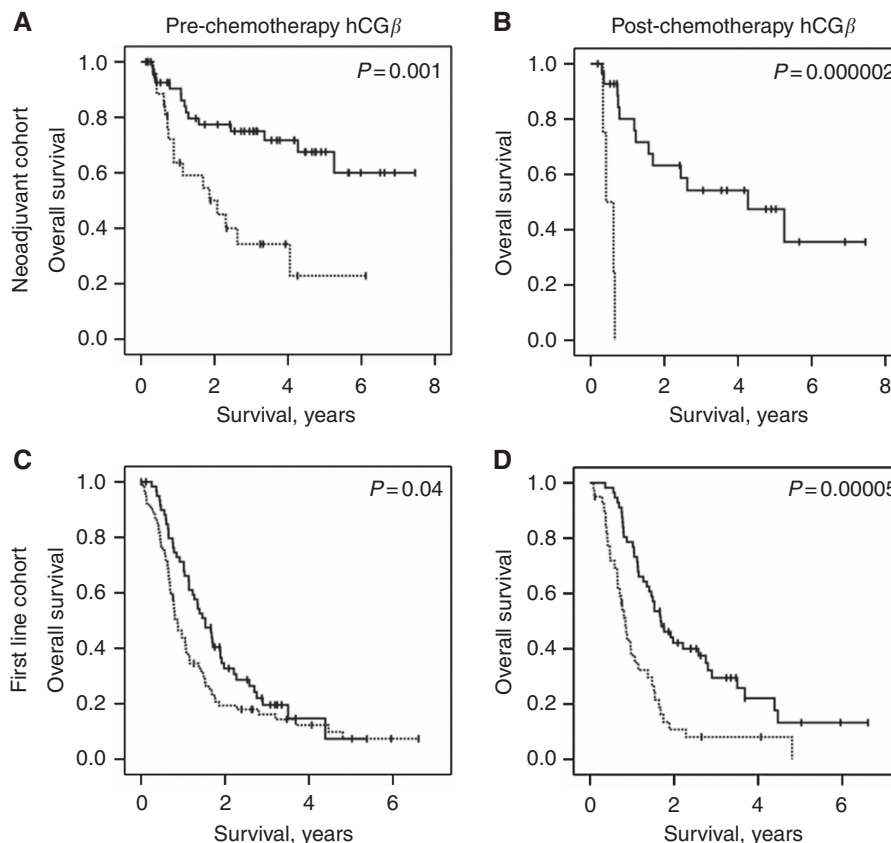


Figure 3. Kaplan–Meier plots to show overall survival according to total hCGβ level in the neoadjuvant (A, B) or first-line (C, D) chemotherapy cohorts either before (A, C), or on completion of (B, D) chemotherapy. Broken line – hCGβ level ≥ 2; continuous line – hCGβ level < 2.

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
<b>ECOG PS</b>						
≥ 2 vs 0 or 1	2.10	0.41–10.89	0.38	2.14	0.31–14.95	0.44
<b>Hb</b>						
< LLN vs ≥ LLN	3.56	1.69–7.46	0.001	1.17	0.25–5.45	0.84
<b>Chemotherapy regimen</b>						
GCarbo vs GC	1.48	0.40–5.42	0.56	1.79	0.42–7.57	0.43
<b>T stage</b>						
T3 vs T2	1.29	0.55–3.00	0.56	0.98	0.23–4.15	0.98
T4 vs T2	1.76	0.47–6.61	0.40	0.66	0.07–6.04	0.71
<b>hCGβ level before chemotherapy</b>						
≥ 2 vs < 2	3.41	1.49–7.83	0.004	—	—	—
<b>hCGβ level on completion of chemotherapy</b>						
≥ 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.

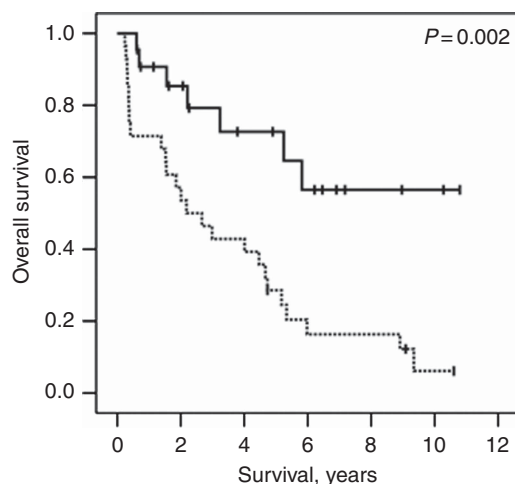
antibodies (Gillott *et al*, 1996). There is some evidence to suggest that, in part, hCGβ might act in TCC, and potentially other malignancies, by inhibition of TGFβ-induced apoptosis by virtue of their structural homology (reviewed by Iles (Iles, 2007)).

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 hCG $\beta$  level either before, or on completion of, chemotherapy

Factor	HR	95% CI	P-value	HR	95% CI	P-value
<b>ECOG PS</b>						
$\geq 2$ vs 0 or 1	2.39	1.38–4.13	0.002	2.09	1.01–4.33	0.047
<b>ALP</b>						
<LLN vs $\geq$ LLN	0.98	0.59–1.60	0.92	1.44	0.78–2.68	0.24
<b>Visceral metastases</b>						
Yes vs no	2.01	1.29–3.13	0.002	2.65	1.49–4.70	0.001
<b>Chemotherapy regimen</b>						
GCarbo vs GC	0.83	0.83–0.66	0.11	1.86	0.52–6.66	0.34
<b>Prior perioperative chemotherapy</b>						
Yes vs no	2.16	0.81–5.79	0.82	1.07	0.60–6.66	0.34
<b>hCG<math>\beta</math> level before chemotherapy</b>						
$\geq 2$ vs <2 IU l <sup>-1</sup>	1.28	0.84–1.96	0.25	—	—	—
<b>hCG<math>\beta</math> level on completion of chemotherapy</b>						
$\geq 2$ vs <2 IU l <sup>-1</sup>	—	—	—	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 hCG $\beta$  level in a radical cystectomy cohort. Broken line – hCG $\beta$  level  $\geq 2$ ; continuous line – hCG $\beta$  level < 2.

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

We utilised a prospectively defined hCG $\beta$  cut point of < vs  $\geq 2$  IU l<sup>-1</sup>. 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 hCG $\beta$  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 hCG $\beta$  levels exists from this sample set.

In conclusion, serum hCG $\beta$  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.

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