EXPRESSION AND PROGNOSTIC VALUE OF HCGβ IN DIGESTIVE TRACT MALIGNANCIES

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Academic Dissertation

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II Louhimo Johanna, Finne Patrik , Alfthan Henrik, Stenman Ulf-Håkan, and Haglund Caj: Combination of hCGβ, CA 19-9 and CEA with logistic regression improves accuracy in gastrointestinal malignancies. Anticancer Res 2002;22:1759-64.

III Louhimo Johanna, Carpelan-Holmström Monika, Alfthan Henrik, Stenman Ulf-Håkan, Järvinen Heikki, Haglund Caj: Serum hCGβ, CA 72-4 and CEA are independent prognostic factors in colorectal cancer. Int J Cancer 2002;101:545-548.

IV Louhimo Johanna, Kokkola Arto, Alfthan Henrik, Stenman Ulf-Håkan, Haglund Caj: Preoperative hCGβ and CA 72-4 are prognostic factors in gastric cancer. Int J Cancer, in press.

V Louhimo Johanna, Alfthan Henrik, Stenman Ulf-Håkan, Haglund Caj: Serum hCGβ and CA 72-4 are stronger prognostic factors than CEA, CA 19-9 and CA 242 in pancreatic cancer. Oncology, in press.

ABBREVIATIONS

AFOS	alkaline phosphatase
AFP	α-foetoprotein
AUC	area under the curve
CEA	carcinoembryonic antigen
95% CI	95% confidence interval
CT	computerized tomography
CV	coefficient of variation
EGTM	European Group on Tumour Markers
5-FU	5-fluorouracil
FSH	follicle stimulating hormone
hCG	human chorionic gonadotropin
hCGα	free alpha subunit of human chorionic gonadotropin
hCGβ	free beta subunit of human chorionic gonadotropin
hCGβcf	β-core fragment of hCGβ
hCGβn	nicked hCGβ
IFMA	immunofluorometric assay
LH	luteinizing hormone
LHβ	free beta subunit of luteinizing hormone
Ln	natural logarithm
LR	logistic regression
MAb	monoclonal antibody
MRI	magnetic resonance imaging
mRNA	messenger RNA
PAb	polyclonal antibody
ROC	receiver operating characteristic
r_s	Spearman rank correlation coefficient
RH	relative hazard
RT-PCR	reverse transcriptase -polymerase chain reaction
TGF-β	transforming growth factor β
TIMP-1	tissue inhibitor of metalloproteinase-1
TPA	tissue polypeptide antigen
TPS	specific tissue polypeptide antigen
TSH	thyroid stimulating hormone
UICC	International Union Against Cancer (Union Internationale Contre le Cancer)
US	ultrasonography

ABSTRACT

Background. Human chorionic gonadotropin (hCG) is a placental glycoprotein hormone consisting of α and β subunits. HCG is in clinical use in the management of pregnancy and in trophoblastic diseases. The free β subunit (hCGβ) has been shown to be expressed in various non-trophoblastic cancers, including digestive tract malignancies, and previous studies have suggested that hCGβ may be a useful tumour marker for these malignancies.

Aims. In the present study, the tissue expression of hCGβ was investigated by immunohistochemistry in various gastrointestinal cancers and benign tissues. In addition, hCGβ in serum was evaluated in gastrointestinal diseases and its diagnostic value was compared with carcinoembryonic antigen (CEA) and CA 19-9. Furthermore, the prognostic value of serum hCGβ was assessed and compared with the prognostic value of tumour markers CEA, CA 19-9, CA 242, and CA 72-4 in colorectal, gastric and pancreatic cancer, three of the most common digestive tract cancer forms in developed countries.

Patients. Tissue samples were obtained from 107 patients with various gastrointestinal malignancies and from 36 patients with benign or normal tissue samples. For the diagnostic evaluation, serum samples were obtained from 142 patients with various malignant and 178 with benign gastrointestinal diseases. For the prognostic evaluation, preoperative serum samples were obtained from 204 patients with colorectal, 146 patients with gastric, and 160 patients with pancreatic cancer.

Methods. A monoclonal antibody (MAb) specific for free hCGβ was applied in the immunohistochemical study to detect the tissue hCGβ. The specificity of the staining was verified and the results were compared with those obtained with a widely used commercial polyclonal antibody (PAb) reacting with both free hCGβ and intact hCG, as well as with β-subunit of luteinizing hormone (LHβ). Serum hCGβ was measured with an in-house immunofluorometric assay (IFMA) based on the same MAb specific for the free hCGβ subunit, with 2 pmol/L as a cut-off value. The quantification of CEA, CA 19-9, CA 242, and CA 72-4 in serum was performed with commercial kits with cut-off values of 5 μg/L for CEA, 35 or 37 kU/L for CA 19-9, 20 kU/L for CA 242, and 6 kU/L for CA 72-4. The diagnostic values of the markers were compared with logistic regression (LR) analysis. Survival analysis was performed with univariate Kaplan-Meier life-tables and log-rank test, and with multivariate Cox regression analysis.

Results. Immunohistochemical expression of hCGβ was observed most frequently in gastric (60%) and pancreatic (56%) carcinomas, and in extrahepatic cholangiocarcinomas (36%). In addition, staining was observed in samples from benign pancreatic and biliary tissues.

In serum, hCGβ level was elevated most frequently in bile duct (56%), pancreatic (45%), and gastric cancer (41%). In benign diseases, the hCGβ concentration in serum was elevated in 10% of the patients. In a LR model, each of the markers CEA, CA 19-9 and hCGβ provided independent diagnostic information, and the calculated probability of cancer index provided better accuracy than the markers alone. In receiver operating characteristic (ROC) curve analysis, the area under the curve (AUC) value of the index was significantly higher than the AUC values of hCGβ, CEA, or CA 19-9 ($p < 0.049$). In univariate survival analysis, hCGβ was observed to be a prognostic factor in colorec-

tal, gastric and pancreatic cancer ($p < 0.001$). In Cox multivariate analysis, hCGβ was found to be the strongest prognostic marker of the studied markers in colorectal, gastric and pancreatic cancer.

Conclusions. HCGβ is expressed in malignant digestive tract tissues including pancreatic adenocarcinoma, as well as in benign pancreatic and biliary tissues. In gastrointestinal malignancies, serum hCGβ provides additive diagnostic information to CA 19-9 and CEA, and the use of an algorithm established by logistic regression analysis improves the diagnostic accuracy compared to the individual markers. Serum hCGβ is an independent prognostic factor in colorectal, gastric and pancreatic cancer. These results substantiate previous findings that hCGβ is a potential serum tumour marker for digestive tract malignancies.

INTRODUCTION

Digestive tract malignancies are important causes of cancer-related morbidity and mortality. In developed countries, the most common digestive tract cancers are colorectal, gastric, and pancreatic cancer. In all gastrointestinal cancers, the role of surgery is essential and radical surgical resection has been the potentially curative treatment with positive impact on survival. Therefore, the anatomic extent of the disease, represented by stage of the tumour, and the possibility of performing surgical resection with intent to cure have been the established prognostic factors. However, with the development of more effective chemo- and radiotherapies, additional prognostic and predictive factors, such as tumour markers, could emerge as useful tools for the clinician in selecting patients for different adjuvant treatment regimens. Apart from the evaluation of prognosis of the disease, tumour markers may be useful in primary diagnosis of cancer and of recurrence of the disease, and in the monitoring of the response to treatment.

Human chorionic gonadotropin (hCG) is a glycoprotein hormone produced at high concentrations by the placenta during pregnancy. It is also a very sensitive marker for trophoblastic tumours. HCG consists of an α and a β subunit, and the free β subunit (hCGβ) has been shown to be expressed in many non-trophoblastic tumours, including gastrointestinal cancers. In addition, it has been suggested that hCGβ may be a prognostic factor in various digestive tract malignancies.

The present study was carried out to verify previous findings and to investigate the tissue and serum expression of hCGβ in gastrointestinal malignancies with a sensitive and specific monoclonal antibody. Furthermore, the aim was to ascertain the prognostic value of serum hCGβ in colorectal, gastric and pancreatic cancer, which are recognized as three of the most common digestive tract cancers in developed countries.

Chemistry and biology of chorionic gonadotropin and its subunits

Human chorionic gonadotropin is a member of a glycoprotein hormone family that includes the three anterior pituitary hormones luteinizing hormone (LH), follicle stimulating hormone (FSH) and thyroid stimulating hormone (TSH). These glycoprotein hormones are heterodimers consisting of two distinct non-covalently bound subunits, α and β. The α-subunits are identical in these glycoprotein hormones, whereas the β-subunits are specific, though homologous, for individual hormones accounting for the biological characteristics and specific activities of the hormones. The 3-dimensional structure of hCG contains a cystine-knot motif that resembles certain protein growth factors, namely nerve growth factor (NGF), transforming growth factor β (TGF-β) and platelet-derived growth factor β (PDGF-β) (Lapthorn et al., 1994).

The hCGα-subunit comprises 92 amino acids and contains two N-linked oligosaccharide side chains attached at amino acid residues 52 and 78. The β-subunit has 145 amino acids and contains two N-linked carbohydrate side chains at residues 13 and 30, and four O-linked side chains at residues 121, 127, 132, and 138 (Morgan et al., 1975). The figure 1 illustrates schematically the linear structure of hCGα and hCGβ and its degradation products nicked hCGβ (hCGβn) and β-core fragment hCGβ (hCGβcf) (Alfthan & Stenman, 1996; Cole, 1997; Berger et al., 2002).

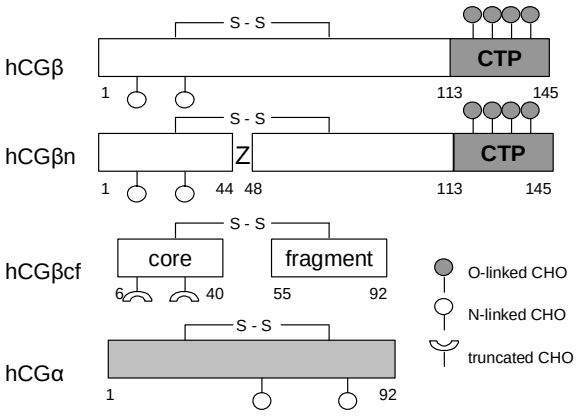

Figure 1. Schematic representation of hCGα and hCGβ and its variants. Numbers indicate the number of an amino acid residue in the polypeptide chain, and S-S illustrates disulfide bonds. hCGβn= nicked hCGβ; hCGβcf= β-core fragment of hCGβ; CTP= carboxy-terminal peptide; CHO= carbohydrate side chain. Adopted and modified from Berger *et al.* (2002) and reproduced with the permission of the publisher S. Karger AG, Basel.

Of the glycoprotein hormones, hCG is the most heavily glycosylated, with approximately 30% carbohydrate content by weight. The total molecular weight of the hCG molecule is 36.7 kDa, of hCGα 14.5 kDa, and of hCGβ-subunit 22.2 kDa (Birken, 1984). In addition, the molecular epitope structure of hCG and its α- and β-subunits has been characterized in detail, along with several monoclonal antibodies (MAbs) directed against hCG and its subunits and their variants (Berger et al., 2002). Some of the epitopes on the hCGβ disappear upon the composition of intact hCG. Consequently, depending on the epitope they identify, the hCGβ MAbs detect either the β-subunit only, or both hCGβ and hCG (Berger et al., 2002).

The α-subunit is encoded by a single gene, which is located in chromosome 6q21.1-23 (Fiddes & Goodman, 1979; Boothby et al., 1981; Jameson

& Hollenberg, 1993). The β-subunit is encoded by a cluster of six non-allelic genes or pseudogenes located in chromosome 19q13.3, which is in the same locus as the β-subunit of LH (LHβ) gene (Boorstein et al., 1982; Policastro et al., 1983; Talmadge et al., 1983; Jameson & Hollenberg, 1993). At least five of the genes encoding the β subunit are transcribed in vivo (Bo & Boime, 1992). With the reverse transcriptase -polymerase chain reaction (RT-PCR) technique it has been shown that hCGβ-genes are expressed in various non-trophoblastic tissues, both in neoplastic and normal tissues (Bellet et al., 1997; Yokotani et al., 1997). In cancerous tissues, only certain hCGβ genes are activated whereas other genes are expressed mainly in normal tissues (Lazar et al., 1995; Bellet et al., 1997). The activation of certain genes is suggested to have prognostic significance in breast cancer (Bièche et al., 1998).

Biological function of hCG and its subunits

HCG is normally secreted by syncytiotrophoblast cells of the placenta and it is an essential hormone in maintaining pregnancy. During the first trimester, it stimulates the progesterone production of the corpus luteum (Yoshimi et al., 1969) and the testosterone production of the foetal testes (Huhtaniemi et al., 1977). In vitro, hCG has been shown to induce proliferation of normal uterine leiomyomal and myometrial smooth muscle cells (Horiuchi et al., 2000). A recent study has also suggested that hCG induces neovascularization and acts as an angiogenic factor in uterine adaptation to early pregnancy and in tumour development (Zygmunt et al., 2002).

Appropriate glycosylation is a prerequisite for the full expression of the biological activity of hCG in vivo. The hormonally active form of hCG is the intact heterodimer of α (hCGα) and β (hCGβ) subunits, whereas the free subunits lack gonadotrophic effects (Rayford et al., 1972; Catt et al., 1973). HCGβ and LHβ are highly homologous; furthermore, hCG and LH elicit their hormonal actions interacting with a common LH/hCG receptor. The receptor is a G-protein coupled transmembrane receptor with a large extracellular domain, which constitutes the binding site of hCG and LH hormones (McFarland et al., 1989). Subsequent to receptor binding, the hormones bring forth their the biological effects by a ligand-induced change in the conformation of the receptor followed by activation of intracellular adenylate cyclase. This results in an increase in adenosine 3',5'-monophosphate (cAMP) content which finally leads to an increase in steroid synthesis and production (Segaloff & Ascoli, 1993; Puett et al., 1996).

The true biological function of the free subunits of hCG in neoplastic disease is unknown. Nevertheless, it has been shown that in vitro hCGβ stimulates the phospholipid methylation in Leydig cells (Ronco et al., 1993), stimulates the growth of tumour cells (Gillott et al., 1996), and inhibits apoptosis (Butler et al., 2000). Furthermore, expression of hCGβ has been proposed as a phenotypic characteristic of neoplastic cells (Acevedo et al., 1995; Regelson, 1995). Endogenous hCGβ has been suggested to have an auto- or paracrine effect on tumour cell growth (Gillott et al., 1996), but the mechanism for this possible action is unclear because hitherto no receptors have been identified for the free hCGβ. In addition, ββ-homodimers have been shown to exist in vitro, but the possible biological activity and functions of this homodimer remains to be determined (Butler et al., 1999).

Clinical use of hCG determinations

Normal pregnancy

The measurement of hCG is in widespread use in the diagnosis and follow-up of pregnancy and pregnancy related disorders. Pregnancy can be diagnosed by detecting an elevated concentration of hCG in the serum or urine after the first missed menstrual period. Following conception, the concentration of hCG in serum starts to increase 7 to 11 days after ovulation. During the first six weeks after the last menstrual period, the increase is nearly exponential with a doubling time of 1.5 days. The peak in hCG concentration is evident at 7-10 weeks of pregnancy. Thereafter the concentrations decrease until the beginning of the second trimester, wherein a moderate and gradual increase occurs towards term (Marshall et al., 1968; Braunstein et al., 1976; Lenton et al., 1982; Pittaway et al., 1985). The concentrations of hCGβ in serum during pregnancy have a similar profile to concentrations of intact hCG (Alfthan et al., 1988). However, following delivery the concentration of intact hCG returns to baseline in about 21 days, while the elimination of hCGβ is markedly slower (Korhonen et al., 1997).

Pregnancy related disorders

The incidence of an early spontaneous abortion is 20-25% of all conceptions (Wilcox et al., 1988). In relation to early foetal loss, the hCG concentration may initially increase normally before its obvious decline after abortion (Braunstein et al., 1978). However, it is noteworthy that following a spontaneous abortion, the return of hCG levels into the reference range may take up to 35 days (Steier et al., 1984). Nonetheless, hCG continues to be the most relevant marker of pregnancy and therefore also of spontaneous pregnancy loss (O'Connor et al., 1994).

Ectopic pregnancy is usually diagnosed by ultrasonography (US) with no visible intrauterine gestational sac in patients with elevated hCG values (Cacciatore et al., 1990). In patients with ectopic pregnancy the serum hCG concentration may be lower than during a normal pregnancy, but it may also be normal for a long time (Rayford et al., 1972; Braunstein et al., 1978).

In foetal trisomy 21 (Down's syndrome), maternal serum concentration of hCG is commonly elevated, whereas the concentrations of α-foetoprotein (AFP) and oestriol are simultaneously decreased. The combined measurement of these markers has been applied in the screening of trisomy 21 (Wald et al., 1988). However, sensitivity of the free hCGβ has been shown to be superior to intact hCG in detecting Down's syndrome pregnancies (Spencer, 1991). Furthermore, the combination of maternal age, and AFP and hCGβ serum measurements (Crossley et al., 1991; Spencer et al., 1992; Nørgaard-Pedersen et al., 1994), as well as the combined measurement of hCGβ and inhibin A have improved the diagnostic accuracy (Wald et al., 1996).

Gestational trophoblastic disease and germ cell tumours

Neoplastic cells of trophoblastic origin produce hCG, which makes it a useful tumour marker for choriocarcinoma and other embryonal tumours such as teratomas (Bagshawe, 1992; O'Connor et al., 1994; Alfthan & Stenman, 1996). Compared to normal pregnancies, the molecular diversity of hCG is more extensive in trophoblastic pregnancies (Cole et al., 1994). The proportion of free hCGβ and intact hCG can be applied in differentiating benign

molar disease (ratio less than 5%) from choriocarcinoma (ratio more than 6%). The ratio of hCGβ to hCG in trophoblastic diseases, from the highest to lowest, are choriocarcinoma, benign complete hydatidiform mole, partial hydatidiform mole, and normal pregnancy (Fan et al., 1987; Ozturk et al., 1988; Rinker & Tietz, 1989).

Approximately 20% of ovarian and 90% of testicular tumours are of germ cell origin (Talerman, 1985). In ovarian germ cell tumours, AFP and hCG are applied routinely in the diagnosis and management of the disease (Zalel et al., 1996). Testicular germ cell tumours are further classified into seminomas and non-seminomatous germ cell tumours and both types produce hCG and/ or hCGβ, which are recommended for monitoring the disease (Braunstein et al., 1973; Saller et al., 1990; Marcillac et al., 1992; Madersbacher et al., 1994). Recently hCGβ was suggested to be superior to hCG in sensitivity in seminomas, whereas hCG was more sensitive than hCGβ in non-seminomatous testicular tumours (Hoshi et al., 2000).

Expression of free β-subunit of human chorionic gonadotropin

HCGβ in healthy subjects and in benign conditions

The serum levels of hCGβ in healthy subjects are normally low (< 2 pmol/L) (Alfthan et al., 1992a). HCGβ is expressed at low levels in the pituitary (Hoermann et al., 1990) and positive immunostaining of hCGβ has also been reported in normal and inflammatory gastric tissues (Manabe et al., 1985; Yakeishi et al., 1990), and in normal pancreatic ductal epithelium (Graeme-Cook et al., 1990). In addition, normal tissues of breast, prostate, skeletal muscle, bladder, adrenal glands, thyroid, colon, and uterus, have been shown to express hCGβ genes (Bellet et al., 1997). The same report also suggested that some immunoassays for hCGβ fail to be sensitive enough to detect the secretion of hCGβ by normal cells, which have low transcriptional activity of hCGβ genes.

Table 1. Upper reference limits (pmol/L) for hCG and hCGβ in serum and in urine in healthy subjects (Alfthan *et al.*, 1992a).

Age (years)	*< 50*	*≥50*	*<50*	*≥50*
Serum	Women		Men	
hCG	8.6	15.5	2.1	6.1
hCGβ	1.6	2.0	1.9	2.1
Urine				
hCG	8.8	11.5	2.9	8.4
hCGβ	1.7	4.3	1.3	3.6

Moderately elevated serum levels of intact hCG have been demonstrated in patients with enteritis, ulcerative colitis, gastric and duodenal ulcers, and in liver cirrhosis (Vaitukaitis et al., 1976; Hoermann et al., 1992). The concentration of free hCGβ subunit in serum has been reported to be elevated in some benign conditions, such as pancreatitis and benign biliary obstruction (Alfthan et al., 1992b; Syrigos et al., 1998).

HCGβ in malignant diseases

Various non-trophoblastic malignancies have been shown to express hCG or its free β subunit (Braunstein et al., 1973; McManus et al., 1976; Hattori et al., 1978; Blackman et al., 1980; Marcillac et al., 1992). HCGβ has been proposed to be a useful tumour marker in gastrointestinal, bladder, renal, ovarian, and lung cancers, with high serum levels associating with adverse prognosis (Iles & Chard, 1991; Marcillac et al., 1992; Webb et al., 1995; Carpelan-Holmström et al., 1996a; Webb et al., 1996; Syrigos et al., 1998;

Lundin et al., 2001; Vartiainen et al., 2001; Hotakainen et al., 2002). Tumours producing hCGβ have also been suggested to be more aggressive in nature and more resistant to therapy than hCGβ negative tumours (Campo et al., 1987; Moutzouris et al., 1993). In addition, the role of hCG has been evaluated in differentiating malignant from benign effusions in patients with hematological, gastrointestinal, gynecological, pulmonary, urological, and other miscellaneous malignant and benign diseases. With an assay detecting both free hCGβ and intact HCG, measurement of hCGβ was shown to be useful in ascitic and pleural effusions improving diagnostic accuracy particularly in cytology-negative effusions (Lamerz et al., 1999). In another report on several non-trophoblastic malignant tumours, an assay for the free hCGβ was shown to be useful in distinguishing malignant from benign ascites (Hoermann et al., 1992).

HCGβ in digestive tract malignancies

A positive hCGβ immunoreactivity in tissues has been observed in several digestive tract malignancies, including in 21 to 33% of oesophageal squamous cell carcinomas (Burg-Kurland et al., 1986; Trias et al., 1991), in 14 to 53% of gastric carcinomas (Manabe et al., 1985; Wittekind et al., 1986; Fukayama et al., 1987b; Yakeishi et al., 1990; Webb et al., 1996), in 17 to 52% of colorectal carcinomas (Shousha et al., 1986; Campo et al., 1987; Fukayama et al., 1987a; Yamaguchi et al., 1989; Connelly et al., 1993; Webb et al., 1995; Kido et al., 1996), in 15 to 23% of cholangiocarcinomas (Nakanuma et al., 1986; Nonomura et al., 1989), and in 2% of hepatocellular carcinoma (Nakanuma et al., 1986). However, to the best of the author's knowledge, the incidence of tissue expression of hCGβ has not previously been reported in pancreatic cancer.

Elevated levels of hCGβ in serum in digestive tract malignancies have been demonstrated in 21% to 52% of patients with gastric (Birkenfeld et al., 1989; Webb et al., 1996), 16% to 36% with colorectal (Mercer & Talamo, 1985; Birkenfeld et al., 1989; Webb et al., 1995), and in 42% with pancreatic cancer (Syrigos et al., 1998). In most studies, however, the applied hCGβ antibodies have been polyclonal which are known to cross-react with intact hCG. Studies on antibodies specific for free hCGβ have shown elevated levels in 9% of gastric (Marcillac et al., 1992), 17% of colorectal (Carpelan-Holmström et al., 1996a), 11% of hepatocellular (Marcillac et al., 1992), 86% of biliary (Alfthan et al., 1992b), and in 30% to 72% of pancreatic cancer patients (Alfthan et al., 1992b; Marcillac et al., 1992).

Molecular detection of hCGβ in neoplasms

With the improvement in molecular cloning techniques, more sensitive molecular detection of hCGβ has become possible. Expression of hCGβ messenger RNA (mRNA) has been demonstrated with RT-PCR on circulatory metastatic cells in patients with breast and pancreatic cancer (Hoon et al., 1996; Bilchik et al., 2000; Hu & Chow, 2000; Taback et al., 2001), and with trophoblastic and germ cell tumours (Hautkappe et al., 2000; Taniguchi et al., 2000; Hara et al., 2002; Suzuka et al., 2002). In colorectal cancer sentinel nodes have been evaluated with molecular profiling, and expression of hCGβ mRNA was evident in the nodes along with other molecular markers (Bilchik et al., 2001; Bilchik et al., 2002). In future, these techniques may be clinically applicable in assessing the prognosis and identifying patients with high risk of recur-

rence, as well as in the earlier detection of disease progression and monitoring response to treatment.

Colorectal cancer

Epidemiology of colorectal cancer

Worldwide, colorectal cancer is the most common gastrointestinal cancer and it is an important cause of cancer related death having accounted for more than 490,000 deaths in year 2000 (International Agency for Research on Cancer, 2001). In developed countries its age-adjusted incidence was 37.3/ 100 000 in males and 25.4/ 100 000 in females (International Agency for Research on Cancer, 2001). In Finland in the last few decades, only moderate changes have occurred in the incidences of colon or rectal cancer (Finnish Cancer Registry, 2003). However, over a longer time span of about 50 years, the incidences have been gradually increasing. In the year 2000 colon cancer was the fourth most common cancer form in males after prostate, lung and urinary tract cancers, with an age-adjusted incidence rate of 13.4 per 100 000 person-years. Rectal and anal cancer showed an incidence of 10.9/ 100 000 in males and presented as the sixth most common cancer form. Similarly in females for the same period, colon cancer was the third most common cancer after breast and uterus cancers with an age-adjusted incidence rate of 12.0 per 100 000 person-years. Rectal and anal cancer in females ranked ninth with an incidence of 6.8/ 100 000 (Finnish Cancer Registry, 2003).

However, in developed countries the age-adjusted mortality from colorectal cancer in males and females was 17.4/ 100 000 and 12.3/ 100 000 respectively (International Agency for Research on Cancer, 2001). In Finland in 1999, colon cancer was the fifth most common cause of cancer related deaths, while rectal and anal cancer the seventh most common in males with mortalities of 6.5 and 5.6 per 100 000 respectively (Finnish Cancer Registry, 2003). In females, colon cancer ranked fourth, while rectal and anal cancer were eighth with mortalities of 4.7 and 3.0/ 100 000, respectively (Finnish Cancer Registry, 2003). The predicted five-year survival in the last five years has been 57% for colon cancer and 53% for rectal and anal cancer (Finnish Cancer Registry, 2003).

Established risk factors for colorectal cancer are inflammatory bowel disease, adenomas and polyps, inherited disorders such as familial adenomatous polyposis and hereditary non-polyposis colorectal cancer, as well as dietary factors (Potter et al., 1993; Wilmink, 1997).

Staging of colorectal cancer

Tumour stage is generally considered to be the strongest prognostic factor in colorectal cancer (Gospodarowicz et al., 2001). Stage of the tumour in colorectal cancer is commonly determined either according to the modified Dukes' classification (ACPS system) (Davis et al., 1984) or the UICC (International Union Against Cancer) TNM-classification (Sobin & Wittekind, 2002), which are compared in table 2. The Dukes' classification is generally the staging system for colorectal cancer in Finland.

Table 2. Modified Dukes' classification of colorectal cancer compared to TNM-stage (Sobin & Wittekind, 2002).

	Dukes' classification (Davis et al., 1984)	*TNM stage (UICC 2002) (Sobin & Wittekind, 2002)*
A vs. I	Tumour in bowel wall not extending beyond muscularis propria	Tumour invades submucosa (T1) or muscularis propria (T2) T1-2, N0, M0
B vs. II	Tumour extends beyond muscularis propria directly into adjacent tissues or organs	Tumour invades into subserosa or non-peritonealized pericolic or perirectal tissues (T3), or invades directly other organs or structures, or perforates visceral peritoneum (T4) T3-4, N0, M0
C vs. III	Lymph node metastasis	Regional lymph node metastasis (≤ 3 N1, ≥ 4 N2) T1-4, N1-2, M0
D vs. IV	Any cancer remaining locally or at a distance after treatment	Distant metastasis (M1) T1-4, N0-2, M1

Diagnosis and treatment of colorectal cancer

The most important diagnostic method for colorectal cancer is colonoscopy, because apart from visualization of the tumour, it provides a definitive histological diagnosis from biopsies obtained during the procedure. Diagnostic methods for colorectal cancer also include barium enema; other radiological imaging techniques may serve as tools for evaluating the extent of disease. Distant metastasis may be detected with chest x-ray and abdominal US, or alternatively with computerized tomography (CT), and local invasion of rectal tumours may be evaluated with magnetic resonance imaging (MRI).

The only fully curative treatment of colorectal cancer is radical surgical resection, which can be achieved particularly during the early stages of disease. This is reflected in the rather encouraging five-year survival rates for patients with Dukes' A (about 90%) and B (about 65 to 70%) colorectal cancer. The five-year survival rate for Dukes' C cancer is approximately 40 to 45%. Therefore, adjuvant treatment has been studied extensively particularly in Dukes' B and C cancers where the rate of recurrence is clinically relevant. The consensus statement of the National Cancer Institute from 1990 stated that adjuvant chemotherapy should be the standard treatment for patients with node-positive (Dukes' C, stage III) resected colon cancer (NIH consensus conference, 1990). A chemotherapy regimen consisting of 5-fluorouracil (5-FU) and leucovorin has been suggested as the treatment of choice (Wolmark et al., 1993; Wolmark et al., 1999). However, the benefit of adjuvant chemotherapy is not yet established for patients with Dukes' B (or stage II) colorectal cancer (IMPACT B2, 1999; Mamounas et al., 1999)

Serum tumour markers in colorectal cancer

Carcinoembryonic antigen (CEA) has been the most extensively studied and best documented tumour marker in the management of colorectal cancer. CEA is a cell membrane associated antigen originally identified in foetal colon and colon adenocarcinoma in 1965 by Gold and Freedman (Gold & Freedman, 1965). CEA is a

glycoprotein belonging to the immunoglobulin superfamily (Paxton et al., 1987). It has a molecular weight of about 200 kDa containing approximately 50% carbohydrate in complex oligosaccharide chains (Coligan et al., 1972; Westwood et al., 1974; Hammarström et al., 1975).
CEA has been postulated to function as a cell adhesion molecule (Benchimol et al., 1989; Oikawa et al., 1992; Zhou et al., 1993). Although the sensitivity of CEA is insufficient for purposes of screening or primary diagnosis, several studies have shown CEA to have prognostic significance in colorectal cancer (Wanebo et al., 1978; Blake et al., 1982; Chu et al., 1991; Webb et al., 1995; Harrison et al., 1997; Carriquiry & Piñeyro, 1999; Duffy, 2001). Based on these findings, recently published guidelines of the European Group on Tumour Markers (EGTM) proposed that CEA has clinical utility in prognosis and monitoring of colorectal cancer (Duffy et al., 2003). This suggestion is in agreement with the consensus statements of the College of American Pathologists from 1999 and of the American Joint Committee on Cancer (Compton et al., 2000a; Compton et al., 2000b).

Other potential serum markers widely studied in colorectal cancer include carbohydrate antigen CA 19-9, which was initially characterized by a monoclonal mouse antibody obtained by immunizing mice with a human colorectal carcinoma cell line SW 1116 (Koprowski et al., 1979). The antigen, recognized by the antibody of the same name, is an oligosaccharide corresponding to Lewis^{a} blood group antigen, sialylated lacto-N-fucopentaose II (Magnani et al., 1982; Falk et al., 1983). The CA 19-9 antigen is expressed in tissues predominantly as a monosialoganglioside and in serum it is associated with circulating mucins, high molecular weight glycoproteins rich in carbohydrate (Magnani et al., 1982; Magnani et al., 1983). However, 5% of the population lacks the Lewis gene encoding the fucosyltransferase enzyme that is required for CA 19-9 production (Koprowski et al., 1982; Ritts et al., 1984).
Although CA 19-9 is less sensitive than CEA in diagnosing colorectal cancer (Duffy, 1998), several studies have suggested that elevated levels correlate with adverse survival (Lindmark et al., 1995; Nakayama et al., 1997; Reiter et al., 1997b; Reiter et al., 2000). Moreover, a study with multivariate survival analysis indicated that CA 19-9 provides independent prognostic information in colorectal cancer (Filella et al., 1992). The recent EGTM guidelines concluded that CA 19-9 may provide independent prognostic information, but conveys only little additive information to that of CEA in the follow-up of colorectal cancer (Duffy et al., 2003). The American consensus statements declared that CA 19-9 is a potential prognostic factor in colorectal cancer but at present has not been studied thoroughly enough (Compton et al., 2000a; Compton et al., 2000b).

CA 242 is a sialylated antigen closely associated with CA 19-9 as it is found in serum on the same mucin complex (Baeckström et al., 1991; Johansson et al., 1991a; Johansson et al., 1991b; Nilsson et al., 1992). CA 242 is defined by a mouse monoclonal antibody (C242) that was raised against a human colorectal carcinoma cell line COLO 205 (Lindholm et al., 1983). Several studies have indicated that CA 242 provides independent prognostic information complementing CEA in the management of colorectal cancer (Nilsson et al., 1992; Hall et al., 1994; Carpelan-Holmström et al., 1996a; Carpelan-Holmström et al., 1996b; Carpelan-Holmström et al., 1996c; Hasholzner et al., 1999).

Tissue polypeptide antigen (TPA) and specific tissue polypeptide antigen) (TPS) are proteins associated with cytokeratins 8, 18 and 19, and are produced by rapidly growing tissues such as placenta and malignancies (Björklund & Björklund, 1957; Björklund & Björklund, 1983; Bonfrer et al., 1994; Sundström & Stigbrand, 1994). TPA and TPS have been shown to correlate with aggressive disease and to convey prognostic information (Lindmark et al., 1995; Carpelan-Holmström et al., 1996a; Lindmark et al., 1996).
Tissue inhibitor of metalloproteinase-1 (TIMP-1) is a glycoprotein involved in inhibiting metalloproteinase activity, stimulating cell growth and inhibiting apoptosis (Chambers & Matrisian, 1997). Furthermore, TIMP-1 has also been suggested to predict poor outcome in colorectal cancer (Holten-Andersen et al., 2000).
More recently the EGTM guidelines on the serum tumour markers CA 242, TPA, TPS, and TIMP-1 concluded that they seem to convey independent prognostic information, but sufficient data is currently lacking to recommend their routine use for prognostic evaluation and monitoring of colorectal cancer (Duffy et al., 2003).

CA 72-4 antigen (also called tumour associated glycoprotein 72, TAG-72) is a mucin-like glycoprotein defined by a monoclonal antibody B72.3 obtained by immunization with a hepatic metastasis cell line of breast carcinoma (Colcher et al., 1981; Johnson et al., 1986). In colorectal cancer, elevated CA 72-4 in serum has been suggested to correlate with adverse survival and to supplement CEA in detecting recurrence of the disease (Guadagni et al., 1993; Lindmark et al., 1996). The American consensus statements have proposed that CA 72-4 is a potential prognostic factor yet lacking sufficient evidence in colorectal cancer (Compton et al., 2000a; Compton et al., 2000b).

Other prognostic factors in colorectal cancer

Prognostic factors could serve as tools in clinical practice to reliably identify those patients with a low or high risk of recurrence, thus helping in the prospective selection of therapy for an individual patient: patients with only a low risk could be spared the hardship of adjuvant treatment, whereas those patients with a significant risk of recurrence could be treated efficiently. However, it is unclear whether distinct prognostic factors also have predictive value in correlating with the response to treatment.

The consensus statement of the College of American Pathologists from 1999 and the American Joint Committee on Cancer defined four categories of prognostic factors: (I) factors with definitely proven prognostic importance; (II) extensively studied factors with repeatedly shown prognostic value but lacking the statistical data to be included in the first category; (III) putative factors which have not yet been sufficiently studied; (IV) well studied factors with no prognostic value (Compton et al., 2000a; Compton et al., 2000b).

Prognostic factors for each category according to the consensus statements are outlined below.
Category I factors included the pathologically assessed local extent of the tumour (pT), regional lymph node metastasis (pN), blood or lymphatic vessel invasion, residual tumour after surgery (R classification), and preoperative CEA. The recent American consensus statements concluded that an elevated preoperative serum level of CEA has been shown to have prognostic significance independent of tumour stage, and furthermore should be included in the staging system reported as either CX

(CEA level not assessed), C0 (CEA < 5 μg/L), or C1 (CEA ≥ 5 μg/L).

Category II factors comprised tumour grade, radial margin status, TNM-classification of the tumour after neoadjuvant treatment, histological type and features associated with microsatellite instability, high degree of microsatellite instability, certain chromosomal alterations, and tumour border configuration.

Category III factors included DNA content, perineural invasion, microvessel density, peritumoural fibrosis and inflammatory response, focal neuroendocrine differentiation, nucleolar organizing regions, proliferation indices, and tumour cell associated proteins and carbohydrates such as tumour markers CA 19-9 and CA 72-4. In addition, tumour tissue molecular markers, such as K-ras, p27, p21, Bcl-2, p53, TGF-β1, VEGF, and CD44, were included in this category. Molecular markers have provided promising preliminary results on diagnostic, prognostic or predictive aspects in management of colorectal cancer, but the data to date remains insufficient for making recommendations for their use in clinical practice.

Category IV comprised those factors with no prognostic value in colorectal cancer such as tumour size and gross tumour configuration.

Gastric cancer

Epidemiology of gastric cancer

Gastric cancer is the second most common digestive tract malignancy after colorectal cancer. In 2000 its age-adjusted incidence was 24.6/ 100 000 in males and 11.0/ 100 000 in females from developed countries (International Agency for Research on Cancer, 2001). In Finland, the incidence has diminished dramatically in the last five decades. In 2000, gastric cancer was the eighth most common cancer in males and the eleventh most common in females. The age-adjusted incidence rate was 9.9/ 100 000 in males and 5.7/ 100 000 in females, whereas during the 1950's the incidence was about six times higher for both sexes (Finnish Cancer Registry, 2003).

Although the incidence has been decreasing, gastric cancer continues to be a clinically important cause of morbidity and mortality with rather poor outcome. In 2000 gastric cancer was the leading cause of cancer-related death of digestive tract malignancies worldwide accounting for more than 640,000 deaths. In developed countries, its age-adjusted mortality in males and females was 16.2/ 100 000 and 7.7/ 100 000 (International Agency for Research on Cancer, 2001). In Finland in 1999, the age-adjusted mortality of gastric cancer was 8.1/ 100 000 in males, being the third most common cause of cancer death after lung and prostate cancers. In females the mortality was 3.6/ 100 000, being the sixth most common cause of cancer death after cancers of breast, lung, pancreas, colon, and ovaries. The predicted 5-year relative survival rate in the last five years has been 26% (Finnish Cancer Registry, 2003).

Established risk factors for gastric cancer are helicobacter pylori infection, atrophic gastritis and pernicious anaemia, intestinal metaplasia, previous gastric surgery, gastric ulcers, ionizing radiation, certain dietary as well as hereditary factors (Kelley & Duggan, 2003).

Staging of gastric cancer

One of the most important prognostic factors influencing the survival of patients with gastric cancer has been the anatomic extent of the disease represented by tumour stage (Gospodarowicz et al., 2001). The stage

of gastric tumours is commonly determined according to the UICC TNM-classification, which is described in table 3 according to the two latest editions of UICC TNM-classification (Sobin & Wittekind, 1997; Sobin & Wittekind, 2002).

Table 3. TNM stage of gastric cancer according to UICC 1997 and 2002 (Sobin & Wittekind, 1997; Sobin & Wittekind, 2002).

Stage I	T1, N0-1, M0 T2, N0, M0	T1 Tumour invades lamina propria or submucosa T2 Tumour invades muscularis propria or subserosa N1 Metastasis in 1 to 6 regional lymph nodes
Stage II	T1, N2, M0 T2, N1, M0 T3, N0, M0	T3 Tumour penetrates serosa N2 Metastasis in 7 to 15 regional lymph nodes
Stage III	T2-3, N2, M0 T3, N1, M0 T4, N0, M0	T4 Tumour invades adjacent structures
Stage IV	T4, N1-3, M0 T1-3, N3, M0 T1-4, N0-3, M1	N3 Metastasis in more than 15 regional lymph nodes M1 Distant metastasis

Histological types of gastric cancer

The histological type of the tumour is regarded as an essential prognostic factor in gastric cancer (Gospodarowicz et al., 2001). Several systems exist for classification of the histological type. One of the most commonly applied is the Laurén classification, which distinguishes intestinal adenocarcinoma from diffuse mucocellular type (Laurén, 1965). The intestinal carcinoma usually presents glandular structures with little or no mucus production, whereas diffuse type tumour cells are scattered as solitary cells or as cell clusters with mucus secretion in nearly all of the tumour cells. The intestinal type tumours typically spread to a distinctly defined area, while the diffuse type forms less defined tumours.

The WHO classification differentiates the histological type of gastric cancers into adenocarcinomas, adenosquamous carcinomas and undifferentiated carcinomas (Oota & Sobin, 1977). Adenocarcinomas are tumours of glandular epithelium, adenosquamous carcinomas show both adenocarcinomatous and squamous elements in an individual tumour, whereas undifferentiated tumours are epithelial tumours with no definite features of any specific line of differentiation. In addition, the WHO classification further divides the histology of adenocarcinomas into subgroups of papillary, tubular, mucinous, or signet-ring cell types, or alternatively by grade into well, moderately or poorly differentiated carcinomas.

According to the Ming classification gastric carcinomas are categorized either as expanding or infiltrative type (Ming, 1977). The expanding tumour seems to grow by forming discrete tumour nodules with glandular structures being common. The growth pattern of the infiltrative tumour is more diffuse with individually penetrating tumour cells often accompanied by mucus production. The classification criteria of expanding and infiltrative tumours correspond closely to the Laurén classification of intestinal and diffuse types (Ming, 1977).

Diagnosis and treatment of gastric cancer

The most important diagnostic procedure is gastroscopy, which provides the possibility of obtaining biopsies for definitive histological diagnosis. Radiological imaging techniques (US, CT, MRI) are mainly applied for assessing the spread of the disease.

The only curative treatment for gastric cancer is radical surgical resection. Most current adjuvant treatments have provided only some improvement in survival. Adjuvant chemotherapy has been evaluated in meta-analyses: Hermans et al. observed no benefit in survival (Hermans et al., 1993), whereas Earle et al. and Mari et al. detected some improvement in survival in favour of adjuvant chemotherapy compared to surgery alone (Earle & Maroun, 1999; Mari et al., 2000). Adjuvant radiotherapy alone has been shown to have no positive effect on outcome of patients with gastric cancer (Hallissey et al., 1994). Adjuvant chemoradiotherapy with 5-FU and leucovorin, however, has been suggested to convey significant improvement on outcome of patients with resectable gastric cancer (Macdonald et al., 2001). According to a recent Canadian practice guideline, the adjuvant treatment of choice is chemoradiotherapy based on 5-FU and leucovorin (Earle et al., 2002).

Serum tumour markers in gastric cancer

The most widely studied tumour markers in gastric cancer have been CEA, CA 19-9 and CA 72-4. Of these three, CA 72-4 has been proposed to be the most sensitive and specific (Guadagni et al., 1992). However, multivariate survival analysis shows conflicting data for their prognostic value and hitherto no tumour markers have emerged into clinical practice for the diagnosis and management of gastric cancer. Several studies have demonstrated CEA or CA 19-9 to have independent prognostic value (Maehara et al., 1994; Nakane et al., 1994; Kodera et al., 1996; Reiter et al., 1997a; Tachibana et al., 1998; Tocchi et al., 1998; Ishigami et al., 2001), but in other studies no significant correlation with survival was observed (Kodera et al., 1996; Webb et al., 1996; Gaspar et al., 2001; Ishigami et al., 2001; Lai et al., 2002; Nakagoe et al., 2002). Furthermore, CA 72-4 has been suggested to be a stronger prognostic factor than CEA and CA 19-9 in gastric cancer (Gaspar et al., 2001), whereas in other studies CA 72-4 has lacked independent prognostic significance (Tocchi et al., 1998; Lai et al., 2002). In addition the sialyl Tn antigen (α2,6-N-acetylgalactosamine, STN), a less established gastrointestinal marker, has been proposed as a prognostic marker in gastric cancer (Takahashi et al., 1994; Nakagoe et al., 2001). The carbohydrate associated sialyl Tn antigen is found on abnormal mucin-like glycoproteins (Hakomori, 1989).

Other prognostic factors in gastric cancer

The established prognostic factors influencing survival of patients with gastric cancer are histological type of the tumour, anatomic extent of the disease reflected by the TNM-classification and stage, as well as the possibility of curative surgical resection (Gospodarowicz et al., 2001). In addition, a recent report suggested that the stage and eosinophilic infiltration of tumour stroma are independent prognostic factors in potentially curable gastric cancer (Cuschieri et al., 2002).

Similar to colorectal cancer, various tissue molecular markers have also been studied in gastric cancer. Some adhesion molecules and matrix met-

alloproteinases may be considered as new and promising prognostic factors (Gospodarowicz et al., 2001), although the data has not been conclusive enough to merit a citation in international guidelines for clinical practice (EGTM, 1999; Fleisher et al., 2002).

Pancreatic cancer

Epidemiology of pancreatic cancer

Despite recent advances in the diagnosis and treatment of cancer, pancreatic cancer retains a rather dismal prognosis. In the last 20 years, its age-adjusted incidence in Finland has been 5-10/ 100 000 with nearly 100% mortality (Finnish Cancer Registry, 2003). In 1999 pancreatic cancer was the fourth leading cause of cancer-related deaths in males, and the third in females. The predicted 5-year relative survival rate of pancreatic cancer, including both exocrine and endocrine malignancies, is only 2% (Finnish Cancer Registry, 2003), and of exocrine malignancies less than 0.5% (Gudjonsson, 1987; Alanen & Joensuu, 1993).

Worldwide in the year 2000, pancreatic cancer accounted for more than 210,000 deaths, and in developed countries its age-adjusted incidence and mortality were 7.8/ 100 000 and 7.7/ 100 000 in males, and 5.1/ 100 000 and 5.1/ 100 000 in females, respectively (International Agency for Research on Cancer, 2001). In developed countries, pancreatic cancer was the third most common digestive tract malignancy after colorectal and gastric cancers and it was also the third leading cause of cancer death from digestive tract malignancies (International Agency for Research on Cancer, 2001).

The aetiology of pancreatic cancer is unknown, but generally accepted risk factors are chronic and hereditary pancreatitis, diabetes, some hereditary syndromes, cigarette smoking and some dietary factors, as well as exposure to certain carcinogens (DiMagno et al., 1999).

Staging of pancreatic cancer

The staging of pancreatic tumours is commonly assessed according to the UICC TNM-classification. The staging criteria of the UICC TNM-system has varied markedly for pancreatic cancer particularly in the last three editions (Hermanek & Sobin, 1987; Sobin & Wittekind, 1997; Sobin & Wittekind, 2002); table 4 outlines the two latest editions. This variation reflects the degree of interest and research as well as the growing knowledge of factors influencing prognosis of patients with pancreatic cancer. Accordingly, tumour-related factors, such as stage of the disease at the time of diagnosis, and thereby the possibility of curative resection, have been the most important prognostic factors in pancreatic cancer (Gospodarowicz et al., 2001).

Table 4. Comparison of TNM stage according to UICC 1997 and 2002 in pancreatic cancer (Sobin & Wittekind, 1997; Sobin & Wittekind, 2002).

	TNM stage UICC 1997 (Sobin & Wittekind, 1997)	*TNM stage UICC 2002 (Sobin & Wittekind, 2002)*
Stage I	T1-2, N0, M0 T1 Tumour limited to pancreas, ≤ 2 cm T2 Tumour limited to pancreas, > 2 cm	T1-2, N0, M0 T1 Tumour limited to pancreas, ≤ 2 cm T2 Tumour limited to pancreas, > 2 cm

Stage II	T3, N0, M0 T3 Tumour extends directly into duodenum, bile duct or peripancreatic tissues	T3, N0, M0 T1-3, N1, M0 T3 Tumour extends beyond pancreas without involvement of coeliac axis or superior mesenteric artery N1 Regional lymph node metastasis
Stage III	T1-3, N1, M0 N1 Regional lymph node metastasis	T4, N0-1, M0 T4 Tumour involves coeliac axis or superior mesenteric artery
Stage IV	T4, N0-1, M0 T1-4, N0-1, M1 T4 Tumour extends directly into stomach, spleen, colon, adjacent large vessels M1 Distant metastasis	T1-4, N0-1, M1 M1 Distant metastasis

Diagnosis and treatment of pancreatic cancer

The diagnosis of pancreatic cancer at early stages of the disease is problematic due to the lack of specific symptoms. This is compounded by a tendency for early metastasis to regional lymph nodes and liver, which is an unfortunate characteristic feature of pancreatic cancer. Consequently, a majority of cases are diagnosed at advanced stages of the disease, which in turn yields the over-all dismal prognosis. Various radiological imaging techniques (US, CT, MRI, endoscopic US) serve as diagnostic methods for pancreatic cancer. Endoscopic retrograde cholangiopancreaticography (ERCP) may also provide useful diagnostic information on tumours of the pancreas.

The only treatment with impact on survival in pancreatic cancer is radical surgical resection. However, the patient outcome remains poor even after a successful apparently curative resection. Therefore, adjuvant treatments have been studied in the hope of improving survival. Adjuvant chemotherapy regimens have been based on 5-FU or gemcitabine; the latter has been proposed to be the more effective of the two (Rothenberg et al., 1996; Burris et al., 1997). Nevertheless, both regimens have shown only minimal improvement in long-term survival in advanced disease (Cullinan et al., 1990; Rothenberg et al., 1996; Burris et al., 1997). In addition, the radiosensitization properties of chemotherapeutic agents have also been under investigation. A small study with 5-FU based chemoradiation adjuvant treatment demonstrated significant improvement in survival (Kalser & Ellenberg, 1985). However, another somewhat larger trial concluded that no demonstrable benefit arose from 5-FU based chemoradiation (Klinkenbijl et al., 1999). Furthermore, apart from adjuvant chemotherapy and chemoradiation treatment, also chemoimmunotherapy has been evaluated. The addition of interleukin-2 to the chemotherapy regimen was suggested to convey a significant positive impact on outcome (Lygidakis et al., 2002). The adjuvant treatment modalities continue to be of considerable scientific interest with several ongoing randomised trials.

Serum tumour markers in pancreatic cancer

In pancreatic cancer, the role of tumour markers is not established in clinical practice. However, CA 19-9 has been the most extensively studied tumour marker and is suggested to be the "gold standard marker" in diagnosis and follow-up of pancreatic cancer (EGTM, 1999). The prognostic value of tumour markers CA 19-9 and CEA is well documented, and both of them have been associated with adverse outcome (Kalser et al., 1978; Glenn et al., 1988; Taylor et al., 1992; Tian et al., 1992; Lundin et al., 1994; Yasue et al., 1994; Gattani et al., 1996; Nakao et al., 1998; Safi et al., 1998; Takeuchi et al., 1998). Other possibly useful, but less established markers include CA 195 and CA 242, which have been shown to predict poor prognosis in pancreatic cancer (Taylor et al., 1992; Lundin et al., 1995). CA 195 is a carbohydrate antigen recognized by a monoclonal mouse antibody raised against a human hepatic metastasis cell line of colon cancer; the antibody reacts with sialylated Lewis^a and the Lewis^b antigens (Fukuta et al., 1987).

Other prognostic factors in pancreatic cancer

The established prognostic factors in pancreatic cancer are tumour-related factors, such as the anatomic extent of the disease represented by TNM classification and stage, as well as the possibility of curative surgical resection (Gospodarowicz et al., 2001).

Tissue molecular markers such as tumour suppressor genes (p53, p16, DPC4, Bcl-2), proto-oncogenes (Ki-67), oncogenes (K-ras) and growth factors (VEGF, EGF/ EGFR, TGFβ, FDG-PET) have been proposed as new and promising prognostic factors (Gospodarowicz et al., 2001). However, further studies are needed to establish their true value and role in clinical practice.

AIMS OF THE STUDY

The aims of the present study were threefold:

1. To investigate the tissue expression of hCGβ in various digestive tract malignancies and benign pancreatic and biliary tissues by immunohistochemistry. The purpose was also to verify the specificity of the staining and to compare the results with those obtained from a widely used commercial polyclonal antibody against hCG (I).

2. To assess the expression of hCGβ in serum from patients with various digestive tract malignancies and benign conditions and to compare the diagnostic value of serum hCGβ with two commonly applied markers for gastrointestinal malignancies, CEA and CA 19-9. In addition, the aim was to evaluate the use of an algorithm derived from logistic regression analysis as a diagnostic test (II).

3. To elucidate the preoperative hCGβ and its relationship with prognosis and to compare it with more established serum tumour markers CEA, CA 19-9, CA 242, and CA 72-4 in patients with colorectal, gastric, and pancreatic cancer, three of the most common digestive tract cancer forms in developed countries (III-V).

MATERIALS AND METHODS

Patients and samples

Tissue samples (I)

Formalin-fixed and paraffin-embedded tissue sections from diagnostic biopsies or surgically removed tissue samples were obtained from 143 patients treated at the Department of Surgery, Helsinki University Central Hospital; 107 samples were from patients with various digestive tract malignancies and 36 samples from patients with pancreatitis, normal pancreas, and benign biliary ducts.

Serum samples (II-V)

Preoperative serum samples were obtained from patients treated at the Department of Surgery, Helsinki University Central Hospital. The samples were taken within 30 days prior to surgery, or if the patient was not operated on, within 30 days prior to histo- or cytological confirmation of the diagnosis. Serum samples were stored at -20°C or at -80°C prior to analysis.

Samples included serum from 142 patients with different gastrointestinal malignancies and 178 with various benign digestive tract diseases (II); from 204 patients with primary colorectal cancer (III); from 146 patients with primary gastric cancer (IV); and from 160 patients with primary ductal adenocarcinoma of the pancreas (V). The diagnosis of malignancy was verified either histologically from biopsies or surgical specimens, or cytologically from fine needle biopsies. Tumour stage was determined according to the modified Dukes' classification for colorectal (Davis et al., 1984) (II, III) or according to the 1997 UICC TNM-classification for the other cancers (Sobin & Wittekind, 1997) (II, IV, V) based on patient data on radiological findings, clinical findings at operation, and histology. Table 5 shows clinicopathological characteristics of patients with colorectal, gastric, or pancreatic cancer (III-V).

Patients with meta- or synchronous malignancies, and patients who had received neoadjuvant treatment were excluded from the studies. The clinical data and survival and cause of death data were obtained from patient records, the Population Register Centre of Finland, and the Statistics Finland.

The hospital ethics committee has approved the study.

Table 5. Clinicopathological characteristics of 204 patients with colorectal (III), 146 with gastric (IV), and 160 with pancreatic cancer (V) (NA= Not available).

Variables	*Colorectal cancer n=204*	*Gastric cancer n=146*	*Pancreatic cancer n=160*
Male	108 (53%)	73 (50%)	79 (49%)
Female	96 (47%)	73 (50%)	81 (51%)
Age	22.7-92.5 years, median 66.6 years	31.6- 88.4 years, median 63.8 years	40.7-87.5 years, median 63.8 years
Stage	31 (15%) Dukes A	29 (20%) Stage I	10 (6%) Stage I
	70 (34%) Dukes B	11 (8%) Stage II	25 (16%) Stage II
	49 (24%) Dukes C	42 (29%) Stage III	24 (15%) Stage III
	54 (26%) Dukes D	64 (44%) Stage IV	101 (63%) Stage IV

Resected for cure	150 (74%)	78 (53%)	29 (18%)
Tumour location	102 (50%) Colon	16 (11%) Cardia or fundus	130 (81%) Caput
	102 (50%) Rectum	68 (47%) Corpus or angulus	26 (16%) Corpus or cauda
		41 (28%) Antrum	4 (3%) Whole pancreas
		18 (12%) Whole stomach	
		3 (2%) Amputated stomach	
Tumour histology	190 (93%) Adenocarcinoma	57 (39%) Intestinal type	160 (100%) Ductal adenocarcinoma
	14 (7%) Mucinous carcinoma	88 (60%) Diffuse type	
		1 (1%) NA	
Tumour size	NA	45 (31%) <5cm	50 (31%) ≤3cm
		38 (26%) ≥5cm, <10cm	69 (43%) >3cm, ≤6cm
		54 (37%) ≥10cm	32 (20%) >6cm
		9 (6%) NA	9 (6%) NA

Methods

Immunohistochemical staining procedure (I)

For the immunohistochemical staining of hCGβ, two antibodies were compared, a MAb (clone 9C11) (Alfthan et al., 1988), and a commercial polyclonal antibody (PAb) (rabbit anti-hCG, code no. A 231, Dakopatts, Glostrup, Denmark). Benign tissue sections were stained with three additional in-house anti-hCGβ MAbs (clones 6G5, 6E11, and 7E3, characterized and produced in the laboratory of Ulf-Håkan Stenman and Henrik Alfthan). MAbs 9C11, 6E11, and 7E3 react specifically with the free β-subunit, whereas MAb 6G5 and the polyclonal antibody also react with intact hCG (data not shown). In addition, the polyclonal antibody has been reported to cross-react with LHβ (Morrish et al., 1987). Paraffin sections mounted on 3-aminopropyl-triethoxy-silane coated slides (APES) (Sigma, St. Louis, MO, USA) were deparaffinized with xylene and rehydrated in graded concentrations of ethanol to distilled water. The sections were pre-treated with 0.5% trypsin (Difco Laboratories, Detroit, MI, USA), followed by incubation with 0.5% hydrogen peroxide in methanol to block endogenous peroxidase. To reduce non-specific staining, the slides were pre-incubated with normal horse serum (for staining with MAbs) or with normal goat serum (for PAb) before staining (dilutions 1:67, Elite ABC Kit, Vectastain, Vector Laboratories, Burlingame, CA, USA). The sections were incubated with the primary antibodies overnight at room temperature with the following dilutions: 1:1000 for MAbs 9C11 and 6G5, and 1:500 for MAbs 6E11, 7E3 and the polyclonal antibody.

Bound antibody was visualized with the ABC immunoperoxidase technique (avidin-biotin complex) (Elite ABC Kit). The sections were first incubated with biotinylated second layer antibody and then with peroxidase-labelled avidin-biotin complex. Visualization of peroxidase activity was performed with 3-amino-9-ethyl-carbazole (Sigma, St. Louis,

MO, USA) in acetate buffer containing 0.03% hydrogen peroxide. The sections were counterstained in Mayer's haematoxylin and mounted in aqueous mounting media (Aquamount, BDH, Poole, UK).
Sections of normal human placenta or hCG positive ovarian carcinoma were included as positive controls. The negative controls included substitution of the primary antibody with PBS (phosphate-buffered saline, pH 7.2) or with a MAb against muscle actin recognizing actin filaments of skeletal, cardiac, and smooth muscle (dilution 1:50, mouse IgG1 anti-muscle actin, clone HHF35, Enzo Diagnostics, Farmingdale, NY, USA).
To establish the specificity of the immunostaining, the antibody was absorbed by blocking its binding sites with exogenous hCG. MAb 6G5 was incubated with a 10-fold molar excess of hCG purified from a partially purified hCG preparation (Pregnyl®, Organon, Oss, The Netherlands). The mixture was separated by gel filtration and fractions of high molecular weight (>100 kDa), corresponding to the antibody-hCG complex, were collected and used in immunohistochemistry to replace the primary antibody at a final dilution of 1:1000.
Red cytoplasmic staining with blue counterstaining of the nuclei was considered positive. The staining result was graded on a scale of 0 to 3 based on the percentage of positive cells: absent (0) <1%, weak (1) 1-10%, moderate (2) 11-30%, and strong (3) > 30%. The intensity of the stain had no influence on the score.

Serum Assays (II-V)

In the present study, the measurement of hCGβ in serum was performed by a highly sensitive non-commercial time-resolved immunofluorometric assay (IFMA) utilizing monoclonal antibodies specific for the free β-subunit of hCG (Alfthan et al., 1988). The majority of patients with non-trophoblastic cancer have a very low concentration of hCGβ in serum. However, the commercial hCGβ assays are mainly designed for screening and management of pregnancy related disorders when the concentration of hCGβ is usually relatively high. The detection limit of the IFMA is 0.27 pmol/L with an inter-assay coefficient of variation (CV) of 10.7% at the concentration of 97 pmol/L (Alfthan et al., 1992a). The applied cut-off value for hCGβ (based on the 97.5 percentile of healthy blood donors) was 2 pmol/L (Alfthan et al., 1992a).

The serum levels of CEA, CA 19-9, CA 242, and CA 72-4 were measured by commercial assays. The serum levels of CEA and CA 19-9 (II) were measured on the Bayer Immuno 1 system (Bayer, Tarrytown, New York, USA). The applied cut-off values were 5 μg/L for CEA and 35 kU/L for CA 19-9 (cut-off values recommended by the manufacturer). CEA (III-V) was measured by a time-resolved IFMA (AutoDELFIA®, Wallac, Turku, Finland). The detection limit of the assay is 0.2 μg/L and the inter-assay CV is <3% in the concentration range 3-90 μg/L (total CV <4%). CA 19-9 and CA 72-4 (III-V) were quantified by immunoenzymometric assays (Immuno1®, Bayer, Tarrytown, NY). Detection limits of the assays are 0.8 kU/L and 0.3 kU/L, respectively. The total CV for CA 19-9 is <3% in the concentration range 15-230 kU/L and for CA 72-4 <6% in the range 6-51 kU/L. CA 242 (III-V) was measured by a manual immunoenzymometric assay (CanAg Diagnostics, Gothenburg, Sweden). The detection limit is 1 kU/L and inter-assay CV is <7% in the concentration range 13-134 kU/L. The applied cut-off values for the tumour markers (III-V) were 5 μg/L for CEA, 37 kU/L for CA 19-9, 6 kU/L for CA 72-4, and 20 kU/L for CA 242.

Statistical analysis (II-V)

The association between elevated tumour marker serum levels (serum value above the specified cut-off value) and tumour stage, size and location was assessed with the χ^2-test. The Mann-Whitney U-test was applied to determine the significance of the difference in tumour marker concentrations between the sexes, between malignant and benign disease groups (II), the colonic and rectal tumours (III), the intestinal and diffuse histological type (Laurén classification) (IV), and between jaundiced and non-jaundiced patients (V). The correlations between serum levels of individual markers, as well as between marker levels and age, and between markers and other routine laboratory tests, were assessed by the Spearman Rank correlation test.

The diagnostic accuracies of hCGβ, CEA and CA 19-9 were compared with McNemar's test (II). Logistic regression (LR) analysis was applied to evaluate and compare the diagnostic information provided by the tumour markers and their combinations (Hosmer & Lemeshow, 2000) (II). The LR model was established with hCGβ, CEA and CA 19-9 as predictor variables, while cancer status (malignant or benign disease) was the binary outcome variable. Several transformations of the predictor variables were tested, and the best model was achieved with marker values as continuous variables with logarithmic transformation of the serum concentrations. The best fit of the model with different marker combinations was assessed with Likelihood Ratio -test. The probability of cancer for each patient was calculated with the LR model, and it served as a diagnostic test. Receiver operating characteristic (ROC) curves were constructed and the differences in the area under the curve (AUC) values were determined between the tumour markers and the calculated probability of cancer (Hanley & McNeil, 1983).

Life-tables were calculated (III-V) with the Kaplan-Meier method and the log-rank test was applied to test the significance of differences in survival between subgroups of patients. Patients alive at the end of the follow-up period, patients lost to follow-up due to emigration, and patients who died from unrelated causes were treated as censored cases. In addition, patients who died postoperatively (within 30 days from operation) were censored (III, IV).

Multivariate survival analysis (III-V) was performed with the Cox proportional hazards regression model. Clinical variables included as covariates in the model were age, gender, stage, and tumour location. In addition, histological type of the tumour (III, IV), tumour size and curative resection (IV, V), and jaundice (V) were also included as covariates. The serum tumour marker levels of hCGβ and CEA, CA 19-9, CA 242 and CA 72-4 were entered as continuous variables, and a logarithmic transformation was applied on account of the non-Gaussian distribution of the marker values. For selection of variables, the multivariate Cox regression analysis was performed with both backward and forward stepwise procedure applying the likelihood ratio test with a limit of $p = 0.05$ for exclusion or inclusion of a variable.

Statistical analyses were performed with StatView 5.0 (II) software (SAS Institute Inc., Cary, NC, USA) or with SPSS 10.07 (III) or 11.0.1(IV, V) software (SPSS Inc., Chicago, Illinois, USA).

RESULTS

Tissue expression of hCGβ in benign and malignant digestive tract diseases (I)

Differences in the tissue staining of hCGβ were observed both in benign and malignant samples between the MAb 9C11 and the polyclonal antibody (Dakopatts). Table 6 shows the distribution of hCGβ expression in malignant and benign gastrointestinal tissues. Gastric and pancreatic carcinomas, and extrahepatic cholangiocarcinomas were most frequently stained with MAb 9C11. With the PAb, positive staining was most frequent in small intestinal carcinoids, small intestinal adenocarcinomas, oesophageal adenocarcinomas, and extrahepatic cholangiocarcinomas. Hepatocellular carcinomas, intrahepatic cholangiocarcinomas, and intestinal lymphomas were negative for hCGβ with both antibodies.

Table 6. Frequency of immunohistochemical expression of hCGβ in malignant and benign gastrointestinal tissues with two different antibodies (I).

Malignancy	*n*	*MAb 9C11*	*PAb Dakopatts*
Oral squamous cell carcinoma	7	14%	0%
Oesophageal adenocarcinoma	9	11%	56%
Oesophageal squamous cell carcinoma	10	0%	30%
Gastric carcinoma	15	60%	40%
Small intestinal adenocarcinoma	7	29%	57%
Small intestinal carcinoid	10	20%	70%
Colorectal carcinoma	13	15%	0%
Pancreatic adenocarcinoma	9	56%	33%
Hepatocellular carcinoma	9	0%	0%
Intrahepatic cholangiocarcinoma	3	0%	0%
Extrahepatic cholangiocarcinoma	11	36%	55%
Intestinal lymphoma	4	0%	0%
	107	24%	32%

Benign disease	*n*	*MAb 9C11*	*PAb Dakopatts*
Acute pancreatitis	8	63%	0%
Chronic pancreatitis	10	40%	0%
Normal pancreas	10	40%	0%
Benign biliary tract	8	50%	0%
	36	47%	0%

The staining result of the benign pancreatic and biliary samples was verified by additional stainings with two MAbs (clones 6E11 and 7E3) specific for the free hCGβ and a MAb (clone 6G5) reacting with both the intact hCG and the free β subunit. The findings were analogous with the staining results of MAb 9C11. In addition, the immunostaining in benign pancreatic sections was successfully blocked by preincubating MAb 6G5 with purified hCG. In placental and carcinoma sections the staining was also strongly reduced further confirming the specificity of the immunoreactivity.

Non-specific staining of the islets of Langerhans was observed with all antibodies in some benign pancreatic samples, which were otherwise immunohistochemically negative for hCGβ. The frequency of positive islets increased with higher antibody concentrations, but the staining was unaffected by absorption of the antibody with hCG.

In both benign and malignant cells the staining pattern of hCGβ was intracytoplasmatic. Samples with positive immunohistochemical staining of hCGβ with both MAb 9C11 and the PAb, showed uniform and homogeneous staining, whereas samples positive only with MAb 9C11, had granular cytoplasmic staining in appearance. Negative controls lacked appropriate staining; the muscle actin antibody failed to stain exocrine or endocrine pancreatic cells in benign pancreas tissue sections.

Expression of hCGβ in serum and its diagnostic value in digestive tract diseases (II)

HCGβ in patients with benign and malignant digestive tract diseases

Table 7 shows the frequency of elevated serum concentrations of hCGβ, CEA, and CA 19-9 in patients with digestive tract diseases. The concentration of hCGβ was elevated in 35% of the patients with gastrointestinal malignancies, and it was most frequently elevated in patients with bile duct (56%), pancreatic (45%), and gastric cancer (41%). The median serum concentration of hCGβ was 1.3 pmol/L (range <0.2 – 3018 pmol/L). In benign digestive tract diseases, the hCGβ level in serum was elevated in 10% of the patients, and most frequently in patients with benign pancreatic (16%), colorectal (13%), and gastric (13%) diseases. The median serum concentration was 0.6 pmol/L (range <0.2 – 18.6 pmol/L).

Table 7. The number of patients with elevated serum hCGβ, CEA, and CA 19-9 in 142 patients with gastrointestinal malignancies and in 179 patients with benign digestive tract diseases (II).

Malignancy	*n*	*hCGβ >2 pmol/L*	*CEA >5 μg/L*	*CA 19-9 >35 kU/L*
Oesophageal	5	1	0	0
Gastric	17	7	4	8
Small bowel	3	0	1	1
Colorectal	34	4	16	12
Liver (hepatocellular)	20	7	2	8
Liver (bile duct)	7	5	3	7
Biliary (extrahepatic)	20	10	3	17
Ampulla of Vater	3	1	0	2
Pancreatic	33	15	10	26
	142	50	39	81

Benign disease	*n*	*hCGβ >2 pmol/L*	*CEA >5 μg/L*	*CA 19-9 >35 kU/L*
Oesophageal	4	0	0	0
Gastric	8	1	1	1
Small bowel	22	1	2	0
Colorectal	15	2	0	0
Liver	21	2	2	9
Biliary	59	6	0	14
Ampulla of Vater	1	0	0	0
Pancreatic	38	6	2	8
Abdominal pain	10	0	0	1
	178	18	7	33

In the benign disease group, elevated serum levels of hCGβ had no association with any specific diagnosis. Elevated values were observed in acute and chronic pancreatitis, gallstones, acute and chronic cholecystitis, primary biliary and liver cirrhosis, diverticulosis and diverticulitis, Crohn's disease, and in one patient with several bleeding gastric ulcers and jaundice caused by gallstones. Some of the patients had additional chronic diseases, such as asthma, chronic obstructive bronchitis, rheumatoid arthritis, gout, glomerulonephritis, IgA nephropathy, focal glomerulosclerosis, polycystic kidneys, renal insufficiency, and benign polyps. Of the 18 patients with benign disease and elevated hCGβ level, six had died by December 2000, all from various benign diseases with no indication of malignancy.

The concentration of hCGβ in serum was significantly higher in patients with malignancies than with benign diseases ($p < 0.001$), but between males and females the difference in serum hCGβ level was insignificant ($p = 0.507$). Modest correlation was evident between serum hCGβ and CEA, hCGβ and CA 19-9, and CEA and CA 19-9 ($r_s = 0.288$ - 0.460, $p < 0.001$). The correlation was poor between the concentrations of hCGβ and aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), γ-glutamyl transferase (γ-GT), alkaline phosphatase (AFOS), bilirubin, amylase, creatinine, albumin, and C-reactive protein (CRP) ($r_s = -0.323 – 0.261$ ($p < 0.001$)).

Comparison of diagnostic value of hCGβ, CEA and CA 19-9

The accuracy (the proportion of true positive and true negative results) was 66% for hCGβ, 66% for CEA, and 71% for CA 19-9 with the recommended cut-off values; the difference in accuracies was insignificant ($p > 0.102$). Furthermore, the accuracies of hCGβ, CEA, and CA 19-9 were compared with ROC curve analysis yielding insignificant differences in AUC values ($p = 0.458 – 0.885$).

The markers hCGβ, CEA and CA 19-9 all provided significant diagnostic information when included simultaneously in a logistic regression model, thus indicating that their combination improves accuracy (Table 8). The best model was achieved incorporating the three markers as continuous variables with a logarithmic transformation of the marker values (Likelihood Ratio test $p < 0.01$). With the logistic regression model, the probability of having cancer (the probability of cancer index, P) was calculated for each patient ($P = e^x/(e^x + 1)$; $x = –1.198 + 0.474*\ln(hCG\beta) + 0.512*\ln(CEA) +$

0.226*ln(CA 19-9)). The index served as a diagnostic test, and its diagnostic validity was compared with hCGβ, CEA, and CA 19-9 with ROC curve analysis. The AUC value of the probability of cancer index was significantly higher than the AUC values for hCGβ, CEA, or CA 19-9 (p= 0.009 – 0.049).

Table 8. Logistic regression equation based on 142 patients with gastrointestinal malignancies and 178 patients with benign digestive tract diseases (II).

Variable	*Coefficient*	*SE*	*p-value*	*Deviance increase*[b]	*OR*[c]	*95% CI*
Ln hCGβ[a]	0.474	0.141	<0.001	6.23	1.61	1.22-2.12
Ln CEA[a]	0.512	0.142	<0.001	7.75	1.67	1.26-2.20
Ln CA 19-9[a]	0.226	0.064	<0.001	6.77	1.25	1.11-1.42
Constant	-1.198	0.229				

[a]= natural logarithm of marker concentration, [b]= deviance increase of the model if the variable is excluded, [c]= OR represents a 1-unit increase on the logarithmic scale
SE= standard error, OR= odds ratio, CI= confidence interval

Prognostic value of hCGβ in colorectal, gastric and pancreatic cancer (III-V)

Serum hCGβ in colorectal, gastric and pancreatic cancer (III-V)

Table 9 shows the frequencies of elevated serum tumour marker levels of hCGβ, CEA, CA 19-9, CA 242, and CA 72-4 and their association with stage in patients with colorectal (III), gastric (IV) and pancreatic (V) cancer. CEA was found to be the most sensitive marker in colorectal cancer, and hCGβ in gastric and CA 19-9 in pancreatic cancer. The correlation between elevated marker concentrations and advanced disease was significant, except for CA 19-9 in gastric and pancreatic cancer (borderline significant), and for hCGβ and CEA in pancreatic cancer.

Table 9. The frequency of tumour marker concentrations above the specified cut-off value, and the association of elevated marker values and stage with χ^2*-test* in 204 patients with colorectal (III), 146 patients with gastric (IV), and 160 patients with pancreatic cancer (V).

	hCGβ >2 pmol/L	*CEA >5 μg/L*	*CA 19-9 >37 kU/L*	*CA 242 >20 kU/L*	*CA 72-4 >6 kU/L*
Colorectal cancer	16%	44%	26%	36%	27%
Correlation with stage	*p*= 0.001	*p*< 0.001	*p*< 0.001	*p*< 0.001	*p*< 0.001
Gastric cancer	36%	18%	31%	34%	34%
Correlation with stage	*p*= 0.027	*p*= 0.014	*p*= 0.056	*p*= 0.015	*p*= 0.001
Pancreatic cancer	63%	46%	87%	68%	39%
Correlation with stage	*p*= 0.174	*p*= 0.329	*p*= 0.074	*p*= 0.009	*p*= 0.002

In colorectal cancer, median serum hCGβ level was 0.7 pmol/L (range <0.3 – 2600 pmol/L), and hCGβ was significantly higher in females than in males (p= 0.002). In gastric and pancreatic cancers, the median serum concentrations of hCGβ were 1.2 pmol/L (range <0.3– 237.4 pmol/L) and 2.8 pmol/L (range <0.5 – 1109 pmol/L), respectively, with no correlation between gender and hCGβ level (p> 0.243). The correlation between age and hCGβ level was strongest in colorectal cancer (r_s= 0.575, p< 0.001), but modest or poor in gastric and pancreatic cancer

(r_s= 0.349, p< 0.001 and r_s= 0.094, p= 0.235). The association between tumour location and elevated hCGβ was insignificant in colorectal and pancreatic cancers (p> 0.601), but significant in gastric cancer (p= 0.047), the least frequently elevated marker values in patients with tumours located in the corpus. Tumour size lacked correlation with elevated hCGβ in gastric and pancreatic cancer (p>0.184). In gastric cancer, hCGβ was significantly higher in patients with intestinal type tumours (p= 0.009). In pancreatic cancer, hCGβ had no correlation with clinical jaundice (p=0.550), nor with serum AFOS (r_s= 0.002, p= 0.982) or bilirubin (r_s= 0.047, p= 0.573). In all these cancer forms, the correlation between hCGβ and markers CEA, CA 19-9, CA 242, and CA 72-4 was modest (r_s= 0.102– 0.334, p< 0.221).

Univariate survival analysis of the prognostic value of hCGβ (III-V)

In colorectal cancer (III), the overall disease specific 5-year survival was 55%, and in Dukes' A, B, C, and D tumours it was 89%, 77%, 52%, and 3%, respectively. With Kaplan-Meier life-tables and log-rank-test, stage, resectability for cure, and tumour markers hCGβ, CEA, CA 19-9, CA 242, and CA 72-4 were found to be prognostic factors (p< 0.001, table 10).

In gastric cancer (IV), the disease specific cumulative 2-year survival was 41%. In univariate analysis, prognostic factors were tumour stage, size, location and histology according to the Laurén classification, resectability for cure, as well as markers hCGβ, CA 19-9, CA 242, and CA 72-4 (p< 0.035, table 10). CEA showed borderline significance (p= 0.069).

In pancreatic cancer (V), the overall disease specific 2-year survival was 10%. Stage, tumour location and size, resectability for cure, and tumour markers hCGβ, CEA and CA 72-4 were prognostic factors in univariate analysis (p< 0.026, table 10).

Table 10. Kaplan-Meier life-table and log-rank test analyses of the correlations between preoperative characteristics and survival of 204 patients with colorectal cancer (III), of 146 patients with gastric cancer (IV), and of 160 patients with pancreatic cancer (V) (NA= not available).

	Colorectal cancer		*Gastric cancer*		*Pancreatic cancer*	
Clinicopathological variable	χ^2 *Statistic*	*p-value*	χ^2 *Statistic*	*p-value*	χ^2 *Statistic*	*p-value*
Gender	0.20	0.654	0.16	0.693	1.40	0.237
Stage	112.54	<0.001	79.90	<0.001	21.8	<0.001
Tumour location	1.66	0.197	4.43	0.035	4.93	0.026
Tumour size	NA	NA	55.78	<0.001	6.37	0.012
Tumour histology	0.19	0.666	4.49	0.034	NA	NA
Resectability for cure	150.93	<0.001	70.57	<0.001	32.5	<0.001
Clinical jaundice	NA	NA	NA	NA	1.99	0.159
hCGβ >2 pmol/L	29.53	<0.001	16.34	<0.001	42.9	<0.001
CEA >5 μg/L	42.98	<0.001	3.30	0.069	5.44	0.020
CA 19-9 >37 kU/L	48.60	<0.001	7.76	0.005	1.31	0.252
CA 242 >20 kU/L	37.33	<0.001	6.54	0.011	2.72	0.099
CA 72-4 >6 kU/L	36.58	<0.001	19.97	<0.001	11.8	0.0006

Multivariate survival analysis of the prognostic value of hCGβ (III-V)

In colorectal cancer (III), with Cox stepwise multivariate analysis, the strongest prognostic factor was found to be Dukes' stage. Other independent prognostic factors were rectal tumour location and preoperative serum markers hCGβ, CEA, and CA 72-4 (Table 11). When including only patients treated with curative resection (Dukes A, B, and C tumours), the same independent prognostic factors emerged, the strongest being CEA (Wald statistic 31.33, p< 0.001, relative hazard (RH) 2.08), followed by rectal tumour location (Wald statistic 11.40, p= 0.001, RH 3.21), hCGβ (Wald statistic 7.59, p= 0.006, RH 1.53), stage (Wald statistic 8.06, p= 0.018), and CA 72-4 (Wald statistic 4.23, p= 0.040, RH 1.24).

Table 11. The independent prognostic factors in Cox stepwise regression analysis in 204 patients with colorectal cancer. CA 19-9, CA 242, age, gender, and histological type had insignificant prognostic value in the model (III).

Covariate	*Wald statistic*	*p-value*[b]	*RH*[c]	*95% CI*[d]
Dukes' stage	58.12	< 0.001		
Dukes A				
Dukes B	1.70	NS	2.28	0.66-7.88
Dukes C	8.08	0.004	5.73	1.72-19.10
Dukes D	24.21	< 0.001	21.83	6.39-74.58
Rectal tumour location	9.40	0.002	1.98	1.28-3.05
Ln hCGβ[a]	9.43	0.002	1.28	1.09-1.50
Ln CEA[a]	7.80	0.005	1.20	1.06-1.36
Ln CA 72-4[a]	8.68	0.003	1.21	1.07-1.38

[a] Ln= natural logarithm of the marker concentration, [b] Significance level, [c] RH= Relative hazard, [d] CI= Confidence interval at 95% level

In gastric cancer (IV), with multivariate analysis, the strongest prognostic factor was found to be stage, followed by serum hCGβ, histological type according to Laurén classification and CA 72-4 (table 12). Performing the Cox multivariate regression analysis either in a backward or forward stepwise manner resulted in an identical outcome. Other clinical variables and tumour markers were found to convey insignificant prognostic information by comparison. Exclusion of disseminated disease (stage IV) from the analysis yielded similar results; independent prognostic factors were stage (Wald statistic 21.10, p< 0.001), hCGβ (Wald statistic 14.05, p< 0.001, RH 2.36), and histological type of the tumour (Wald statistic 11.31, p= 0.001, RH 4.31). Performing the analysis with advanced disease only (stages III and IV), hCGβ (Wald statistic 15.53, p< 0.001, RH 1.51), tumour histology (Wald statistic 13.14, p< 0.001, RH 2.48), stage (Wald statistic 12.66, p< 0.001, RH 2.37), and CA 72-4 (Wald statistic 8.21, p= 0.004, RH 1.20) emerged as independent prognostic factors. Evaluating the tumour markers individually while adjusting the multivariate model for stage, hCGβ and CA 72-4 provided significant prognostic information (p< 0.007), contrary to CEA, CA 19-9 and CA 242 (p> 0.212).

Table 12. Independent prognostic factors in Cox stepwise regression analysis in 146 patients with gastric cancer. CEA, CA 19-9, CA 242, age, gender, tumour location and size, and resectability for cure provided insignificant prognostic information (IV).

Covariate	*Wald statistic*	*p-value*[b]	*RH*[c]	*95% CI*[d]
Stage	47.37	< 0.001		
Stage I				
Stage II	3.41	0.065	3.72	0.92- 14.96
Stage III	19.41	< 0.001	11.05	3.79- 32.15
Stage IV	36.35	< 0.001	28.13	9.51- 83.23
Tumour histology	13.82	< 0.001	2.47	1.53- 3.98
Ln hCGβ[a]	16.87	< 0.001	1.52	1.25- 1.86
Ln CA 72-4[a]	6.68	0.010	1.17	1.04- 1.33

[a] Ln= natural logarithm of the marker concentration, [b] Significance level, [c] RH= Relative hazard, [d] CI= Confidence interval at 95% level

In pancreatic cancer (V), hCGβ, CA 72-4 and stage emerged as the factors providing independent prognostic information (table 13). Other clinical characteristics and tumour markers contributed only insignificant prognostic information. The Cox multivariate regression analysis was performed both in a backward and forward stepwise manner with consistent results. Adjustment of the model for resectability for cure provided the following independent prognostic factors: curative resection (Wald statistic 17.47, p< 0.0001, RH 0.35), hCGβ (Wald statistic 41.60, p< 0.0001, RH 1.45), and CA 72-4 (Wald statistic 15.77, p< 0.0001, RH 1.28). In addition, excluding stage IV from the analysis, hCGβ (Wald statistic 18.29, p< 0.001, RH 1.83) and curative resection (Wald statistic 10.82, p= 0.001, RH 0.34) emerged as independent prognostic factors. Evaluating the markers hCGβ, CEA, CA 19-9, CA 242, and CA 72-4 individually but adjusting for stage, all markers were found to convey significant prognostic information in the multivariate model (p< 0.011). Further adjustment of the model for the presence of jaundice did not alter the results.

Table 13. Independent prognostic factors in Cox stepwise regression analysis in 160 patients with pancreatic cancer. CEA, CA 19-9, CA 242, gender, age, tumour location and size, and presence of jaundice provided insignificant prognostic information (V).

Covariate	Wald statistic	p-value[b]	RH[c]	95% CI[d]
Stage	8.74	0.033		
Stage I				
Stage II	3.20	0.074	2.03	0.93-4.42
Stage III	2.63	0.105	1.91	0.87-4.17
Stage IV	7.60	0.006	2.73	1.34-5.58
Ln hCGβ[a]	47.96	< 0.001	1.46	1.31-1.63
Ln CA 72-4[a]	14.83	< 0.001	1.26	1.12-1.42

[a] Ln= natural logarithm of the marker concentration, [b] Significance level, [c] RH= Relative hazard, [d] CI= Confidence interval at 95% level

DISCUSSION

Tissue and serum expression of hCGβ in digestive tract diseases

The present study confirmed previous findings that various gastrointestinal malignancies express hCG or its free β subunit (Braunstein et al., 1973; McManus et al., 1976; Hattori et al., 1978; Blackman et al., 1980; Marcillac et al., 1992). Current results demonstrated positive hCGβ tissue immunostaining that was most frequent in gastric and pancreatic carcinomas and extrahepatic cholangiocarcinoma. Accordingly in serum, the hCGβ level was elevated most frequently in patients with bile duct, pancreatic, and gastric cancer.

The sensitivities observed in the present study were also in concordance with previous findings on the expression of hCGβ. In gastric cancer, the hCGβ tissue expression has been reported up to about half of the cases both in tissues and serum (Manabe et al., 1985; Wittekind et al., 1986; Fukayama et al., 1987b; Birkenfeld et al., 1989; Yakeishi et al., 1990; Marcillac et al., 1992; Webb et al., 1996). Although elevated serum concentrations of hCGβ have been shown to be common in pancreatic cancer (Alfthan et al., 1992b; Marcillac et al., 1992; Syrigos et al., 1998), the incidence of immunohistochemical expression of hCGβ has not been reported in this disease. In biliary cancer, the incidence of hCG-immunoreactivity has been found in up to about one fourth of the cases (Nakanuma et al., 1986; Nonomura et al., 1989), and the frequency of elevated serum levels has been shown to be as high as 86% (Alfthan et al., 1992b). In colorectal cancer, the tissue expression of hCGβ has been reported in up to about half of the cases (Shousha et al., 1986; Campo et al., 1987; Fukayama et al., 1987a; Yamaguchi et al., 1989; Connelly et al., 1993; Webb et al., 1995; Kido et al., 1996), and the serum expression in up to about one third of the cases (Mercer & Talamo, 1985; Birkenfeld et al., 1989; Webb et al., 1995; Carpelan-Holmström et al., 1996a).

Positive immunostaining has previously been reported in normal and inflammatory gastric tissues (Manabe et al., 1985; Yakeishi et al., 1990), and in normal pancreatic ductal epithelium with the Dako anti-hCG PAb (Graeme-Cook et al., 1990). In addition, moderately elevated serum levels of hCG or hCGβ have been shown in patients with gastric and duodenal ulcers, enteritis, ulcerative colitis, liver cirrhosis, pancreatitis, and benign biliary obstruction (Vaitukaitis et al., 1976; Alfthan et al., 1992b; Hoermann et al., 1992; Syrigos et al., 1998).

The current results on benign digestive tract diseases were consistent with the previous findings. Tissue expression of hCGβ was observed in benign pancreatic acinar cells and biliary tract epithelium. The findings were also confirmed with three additional monoclonal antibodies, and the specificity of the immunoreactivity was verified in benign tissues by blocking the staining through absorption of the antibody with hCG. In addition, moderately elevated serum levels of hCGβ were evident in patients with gastric ulcers, Crohn's disease, diverticulosis, liver cirrhosis, cholecystitis and gallstones, and pancreatitis. The patient presenting with elevated serum hCGβ in the benign gastric disease group was hospitalized due to several bleeding ulcers; however, she had biliary obstruction as well, which may have in

part accounted for the elevated level of hCGβ.

In the present study, the same monoclonal antibody was applied both in immunohistochemistry and serum assays (MAb 9C11). The MAb identifies specifically the free β subunit of hCG, and it has been shown to be sensitive for the free hCGβ in serum assays (Alfthan et al., 1988; Alfthan et al., 1992b). Previously Bellet et al. (1997) suggested that some hCGβ immunoassays fail to be sensitive enough to detect the hCGβ secretion of normal cells, which have a low transcriptional activity of hCGβ genes.
The present study confirmed that the MAb is also suited for immunohistochemistry and apparently is able to detect the low hCGβ expression in benign tissues. Current results also provided further support for the suggestion that free hCGβ is secreted not only by the pituitary gland (Hoermann et al., 1990), but also by normal and benign non-trophoblastic cells of varying histological origin (Bellet et al., 1997), which in turn may reflect the low levels of hCGβ observed in sera of healthy non-pregnant individuals.

The tissue staining of MAb 9C11 was compared in the present study with a commercial PAb. The differences observed in both benign and malignant tissues between the two antibodies may be explained by the different sensitivity and specificity of the antibodies. Most of the previous studies on immunohistochemical expression of hCGβ in gastrointestinal cancers have been performed with the PAb from Dakopatts. However, this PAb has also been reported to cross-react with intact hCG, hCGα, LH, TSH, FSH, LHβ, TSHβ, and FSHβ (Morrish et al., 1987), which may explain the more frequent and diffuse staining observed in some cancers. In addition, neuroendocrine tumours, such as carcinoids and islet cell carcinomas, have been shown to express the α subunit of hCG (Blackman et al., 1980), which may account for the findings in small intestinal carcinoids in the present study. The expression of other glycoprotein hormones and their subunits has not been reported in gastrointestinal tissues; consequently it remains to be determined whether the crossreactivity of the PAb with other hormones can explain the findings.

Diagnostic value of hCGβ and logistic regression algorithm in digestive tract diseases

Traditionally the value of a diagnostic test has been assessed by sensitivity, specificity and accuracy of the variable at certain predetermined cut-off levels. Moreover, ROC curves have been constructed and accuracies of two diagnostic tests have been compared by calculating and comparing the AUC values of the ROC curves (Hanley & McNeil, 1982; Hanley & McNeil, 1983). However, with these models it is difficult to combine and compare the value of two or more diagnostic variables and tests, such as tumour markers.
Consequently, multivariate statistical methods have been developed to address this problem. Logistic regression is a multivariate model assessing the probability of binary outcomes, such as the patient having malignant or benign disease (Hosmer & Lemeshow, 2000). The LR model quantifies the contribution of individual variables without predetermined cut-off values and is suitable for comparing the validity of several diagnostic variables or tests, not only tumour markers, but also other factors such as clinicopathological patient characteristics. The LR model combines the variables optimally in constructing diagnostic algorithms, which can be used to estimate the probability of a disease yielding a

diagnostic risk index on a scale from 0 to 1. This risk index can be in turn applied as a diagnostic test determining its accuracy for example with ROC-curve analysis. Previously, risk calculation based on logistic regression or neural networks has been shown to be useful in the diagnosis of prostate cancer (Finne et al., 2000).
In the present study, only insignificant differences in diagnostic accuracy were observed between the individual markers hCGβ, CEA and CA 19-9. Yet each marker provided significant diagnostic information in logistic regression analysis. With an algorithm based on the LR model, the combination of hCGβ, CA 19-9 and CEA moderately improved the accuracy in this heterogeneous group of gastrointestinal malignancies. However, these results are preliminary and the possible diagnostic value of hCGβ and tumour marker combinations in each disease group must be ascertained in a larger group of patients. It is also noteworthy that although the LR model may be a promising mathematical method and diagnostic tool to improve accuracy, and in future it may help the clinician in deciding the patient examination protocol, the definitive diagnosis of digestive tract cancers will always rely on cyto- or histological findings from fine needle biopsies or biopsies obtained by endoscopic procedures.

Prognostic value of hCGβ in colorectal, gastric and pancreatic cancer

The anatomic extent of the spread of the disease reflected by tumour stage is generally considered to be the strongest prognostic factor in digestive tract malignancies such as colorectal, gastric and pancreatic cancers (Gospodarowicz et al., 2001). To find additional tools and potential prognostic factors to help select patients for possible adjuvant therapies, the role and prognostic value of tumour markers has also been evaluated, with CEA and CA 19-9 as the most studied markers in gastrointestinal cancers.

CEA has been shown to have independent prognostic value in colorectal cancer (Webb et al., 1995; Carriquiry & Piñeyro, 1999) and in gastric cancer (Maehara et al., 1994; Nakane et al., 1994; Reiter et al., 1997a; Tachibana et al., 1998; Tocchi et al., 1998; Ishigami et al., 2001). In pancreatic cancer, CEA has been suggested to provide significant prognostic information (Kalser et al., 1978; Taylor et al., 1992; Yasue et al., 1994; Takeuchi et al., 1998). CA 19-9 has been shown to be an independent prognostic factor in colorectal cancer (Filella et al., 1992), in gastric cancer (Kodera et al., 1996; Reiter et al., 1997a; Tocchi et al., 1998) and in pancreatic cancer (Gattani et al., 1996).
Of the other tumour markers evaluated in the present study, CA 242 has been previously shown to have independent prognostic value in colorectal cancer (Carpelan-Holmström et al., 1996a), and in pancreatic cancer, elevated levels of CA 242 have been suggested to indicate poor prognosis (Lundin et al., 1995). CA 72-4 has been suggested to correlate with adverse outcome in colorectal cancer (Lindmark et al., 1996), and in gastric cancer, CA 72-4 has been reported to be an independent prognostic factor (Gaspar et al., 2001).
Serum hCGβ has been previously shown to provide independent prognostic information in colorectal cancer (Webb et al., 1995; Carpelan-Holmström et al., 1996a; Lundin et al., 2001), in advanced gastric cancer (Webb et al., 1996), and in pancreatic cancer (Syrigos et al., 1998). However, to the best of the author's knowledge, no studies exist comparing the prognostic value of the five

tumour markers, CEA, CA 19-9, CA 242, CA 72-4, and hCGβ, in gastrointestinal cancers.

The current results suggest that hCGβ has prognostic value in digestive tract malignancies. Serum hCGβ was found to be a prognostic factor in univariate survival analysis in colorectal, gastric and pancreatic cancer. Furthermore, in these cancers hCGβ was found to be the strongest prognostic tumour marker in multivariate analysis compared with markers CEA, CA 19-9, CA 242, and CA 72-4. It is also noteworthy that hCGβ provided independent prognostic information in colorectal, gastric and pancreatic cancer even if disseminated disease (Dukes D' or stage IV tumours) was excluded from the analysis.

In colorectal cancer, the variables providing independent prognostic information in the multivariate analysis were (in order of importance) Dukes' stage, serum hCGβ, rectal tumour location, and serum CA 72-4 and CEA. The results were similar also in gastric cancer: the strongest prognostic factor was stage, followed by hCGβ, tumour histology and CA 72-4. When including only patients with stage III and IV gastric tumours in the analysis, hCGβ, CA 72-4 and resectability for cure emerged as independent prognostic factors concurring with the previous report on prognostic value of hCGβ in advanced gastric cancer (Webb et al., 1996).

In pancreatic cancer, the strongest independent prognostic factor in the multivariate survival analysis was preoperative hCGβ followed by CA 72-4 and stage. The observation that markers were stronger prognostic factors than stage most probably reflects the stage distribution of the patient material of the present study. The majority of patients had stage IV disease, with a rather small number of stage I to III tumours. Unfortunately this is a reflection of clinical practice whereby most pancreatic cancer patients present with advanced disease already at the time of diagnosis. However, it is likely that stage would emerge as the strongest prognostic factor in a study comprising more patients with local disease. Most likely as another reflection of the stage distribution of the present study, the possibility of curative resection was observed to be a stronger prognostic factor than stage in pancreatic cancer. However, inclusion of resectability for cure in the model did not alter the results on prognostic value of the markers, hCGβ and CA 72-4 still remained as independent prognostic factors together with curative resection.

Value of hCGβ measurement in digestive tract diseases

Previously hCGβ was considered to be a marker for trophoblastic disease only. Therefore, commercial assays for quantification of free β subunit of hCG are mainly designed for screening and management of pregnancy related disorders when the concentration of hCGβ is usually relatively high. However, hCGβ can also serve as a marker for non-trophoblastic disorders when the majority of patients have very low concentrations of hCGβ in serum. Therefore, the accuracy and clinical value of hCGβ measurement is highly dependent on the detection limit and the specificity of the assay in use. The hCGβ immunoassay (IFMA) applied in the present study is based on a monoclonal antibody and is highly sensitive and specific for the free hCGβ subunit (Alfthan et al., 1988; Alfthan et al., 1992a; Alfthan et al., 1992b). It has no significant crossreactivity with hCG, which may occur at concentrations up to 20 pmol/L in non-pregnant patients with benign condi-

tions (Alfthan et al., 1992a). Since the hCG immunoreactivity expressed by non-trophoblastic tumours is almost exclusively hCGβ, it is likely that this IFMA can provide optimal diagnostic accuracy.

Current results indicate that hCGβ is expressed in various gastrointestinal diseases, both in tissues and in serum. HCGβ was also observed to be the strongest prognostic tumour marker compared with CEA, CA 19-9, CA 242, and CA 72-4, in the three most common digestive tract malignancies of developed countries, namely colorectal, gastric and pancreatic cancers. It remains to be investigated whether elevated serum hCGβ levels in digestive tract malignancies signify increased production of hCGβ by tumour cells or alternatively enhanced shedding or release into circulation. Increased hCGβ production might denote aggressive behaviour of tumour cells associating with mechanisms of tumour invasion. However, enhanced shedding might indicate that the tumour has spread through the natural barriers of distinct histological structures.

Although it has been suggested that hCGβ has auto- or paracrine effect on tumour cell growth (Gillott et al., 1996), the true biological function of hCGβ and the mechanisms of its action continue to be intriguing questions and warrant further analysis. More studies are also required to corroborate the current findings and to validate the prognostic value of hCGβ particularly in local disease with a possibility of curative treatment. Furthermore, it remains to be shown whether hCGβ has also predictive value and could be applied in the choice and monitoring of treatment in patients with digestive tract cancers.

CONCLUSIONS

1. Monoclonal antibodies specific for the free β subunit of hCG are sensitive and specific for detection of hCGβ in tissues. In the present study, the tissue expression of hCGβ was evident most frequently in gastric and pancreatic carcinomas and extrahepatic cholangiocarcinoma. However, positive immunostaining was also observed in benign pancreatic acinar cells and biliary tract epithelium. The results confirm previous findings on hCGβ expression in gastrointestinal diseases and also provide further support for hCGβ as a potential tumour marker for digestive tract malignancies (I).

2. The serum hCGβ level was elevated most frequently in bile duct, pancreatic and gastric cancer, thus confirming the findings with immunohistochemistry. The diagnostic value of hCGβ serum test was additive to that of CEA and CA 19-9 in digestive tract cancers. The probability of cancer index based on logistic regression analysis provided better accuracy as a diagnostic test than the markers alone. Although the LR algorithm may be a promising method to obtain additional diagnostic information, the definitive diagnosis of digestive tract cancers continues to rely on cyto- or histological findings from biopsies (II).

3. In colorectal cancer, Dukes' stage was the strongest independent prognostic factor with preoperative serum hCGβ as the strongest prognostic tumour marker followed by CA 72-4, and CEA (III).

4. In gastric cancer, stage and serum hCGβ had independent prognostic value in addition to tumour histology and serum CA 72-4. HCGβ was observed to be the strongest prognostic tumour marker (IV).

5. In pancreatic cancer, hCGβ, CA 72-4 and stage provided independent prognostic information. Serum hCGβ was found as the strongest prognostic tumour marker (V).

SUMMARY

Tumour markers may be useful in the primary diagnosis of cancer, in the follow-up of disease and monitoring the response to treatment, as well as in the prognostic evaluation of cancer patients. HCG is a glycoprotein hormone normally secreted not only by placenta, but also by neoplastic trophoblastic cells. HCG has been in clinical use in pregnancy and pregnancy related disorders, as well as in choriocarcinoma and testicular germ cell tumours. HCG is composed of two distinct subunits, α and β. The free β subunit of hCG has been shown to be expressed in various non-trophoblastic malignancies, such as bladder and lung cancers, and also in digestive tract malignancies. In the present study, the tissue and serum expression of hCGβ was investigated in various gastrointestinal cancers, wherein the same MAb specific for the free hCGβ was applied both in immunohistochemical method and in serum assays. The prognostic value of serum hCGβ was assessed in colorectal, gastric and pancreatic cancers, the three most common digestive tract cancer forms in developed countries.

In the present study, tissue expression of hCGβ was observed in various gastrointestinal cancers, as well as in benign pancreatic and biliary tissues. The positive immunostaining was most frequent in gastric and pancreatic carcinomas and in extrahepatic cholangiocarcinoma; the incidence of tissue expression of hCGβ had not been reported previously in pancreatic adenocarcinoma. Monoclonal antibodies specific for the free hCGβ, previously applied in serum assays, were also demonstrated to be sensitive and specific for detection of hCGβ in tissues. The results confirmed previous findings on hCGβ expression and provided further support for the role of hCGβ as a tumour marker in digestive tract malignancies.

The expression of hCGβ was observed also in serum of patients with digestive tract malignancies with the hCGβ serum test having additive diagnostic value to that of CEA and CA 19-9. As a diagnostic test, the probability of cancer index based on a logistic regression model and combining the three markers, improved the accuracy compared with the markers alone. In the present study, the hCGβ concentration in serum was elevated most frequently in bile duct, pancreatic, and gastric cancers. However, further studies are warranted to establish the diagnostic value of serum hCGβ compared to other markers in the individual cancer forms. Furthermore, at present the clinical relevance of the current findings is moderate, because although the LR model may be a promising diagnostic tool to improve accuracy, the definitive diagnosis of digestive tract cancers must be based on cyto- or histological findings from biopsies.

HCGβ was observed to be an independent prognostic factor in colorectal, gastric and pancreatic cancers, furthermore, it was found to be the strongest prognostic factor compared with CEA, CA 19-9, CA 242, and CA 72-4. Other factors providing independent prognostic information in colorectal cancer were Dukes' stage, rectal tumour location, and tumour markers CEA and CA 72-4. In gastric cancer, in addition to hCGβ, stage, histology of the tumour according to the Laurén classification, and CA 72-4 had independent prognostic value. In pancreatic cancer, in addition to hCGβ, the strongest independent prognostic factors were observed to

be CA 72-4 and stage of the tumour. In colorectal, gastric and pancreatic cancer, hCGβ remained as an independent prognostic factor also when disseminated disease (Dukes' stage D or stage IV) was excluded from the analysis.

Further studies are required to establish the prognostic value of hCGβ in local disease with a possibility of curative treatment, as well as its predictive value and possible usefulness in selection of patients for adjuvant therapies in digestive tract malignancies.

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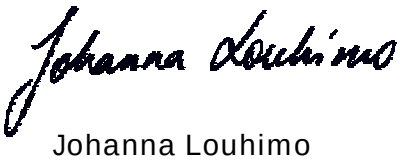

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