Fallacious hCGβ in patients with choriocarcinoma

Zweifelhafte hCGβ-Sekretion bei Patientinnen mit Chorionkarzinom

Jeanne S. E. Dericks-Tan, H.-D. Taubert

Abteilung für Gynäkologische Endokrinologie, Z.F.G., Klinikum der J. W. Goethe Universität, Frankfurt am Main

Summary:

The serum level of hCG and total hCGβ (hCG plus hCGβ) was measured by a manual (hCG_1 and β_1) and an automated (hCG_2 and β_2) immunometric assay in two therapy-resistant patients with choriocarcinoma (ChCA). In contrast to hCG_1 and hCG_2, the results of β_1 and β_2 differed clearly. Total hCGβ was always higher than hCG. The ratio of total hCGβ/hCG in patient A with initially high serum hCG ranged between 1.1 and 5.3, while that of patient B with low serum hCG increased with time ranging between 1.1 and 550. The qualitative analysis of the hCG- and hCGβ-activities was performed by gel chromatography of serum samples on Sephadex-G100 and Superdex-75 during various stages of the disease, and compared to the pattern of gel chromatography of nonradioactive hCG and hCGβ or serum from a pregnant woman. After gel chromatography of serum samples from the two patients with ChCA only one fraction showed hCG-activity. There was also a variety of total hCGβ-activity representing little and big-hCG or hCGβ. The total hCGβ activity measured by β_1 and β_2 in the fractions obtained by gel chromatography of serum samples showed different patterns. The hCG-activity in the gel chromatogramm of the serum samples of patient B decreased with progress of the disease, whilst the total hCGβ-activities increased in the eluates.

It was concluded that the discordant measurements of total hCGβ in ChCA were probably not due to hCGβ but to heterogeneity in the secretory pattern of hCG- and hCGβ-like substances, which also varied with time. Moreover, the total hCGβ-activities measured with different methods were shown to be considerably at variance.

Keywords:

hCGβ – total hCGβ – choriocarcinoma – gel chromatography – serum

Zusammenfassung:

Die Serumkonzentrationen von hCG und Gesamt-hCGβ (hCG+hCGβ) wurde mit manuellen (hCG_1 und β_1) und automatisierten (hCG_2 und β_2) immunometrischen Verfahren an Blutproben von zwei Frauen mit einem Therapie-resistenten Chorionkarzinom bestimmt. Die Ergebnisse des hCG_1 und hCG_2 unterscheiden sich kaum voneinander, während β_1 und β_2 große Unterschiede aufwiesen. Die Konzentration des Gesamt-hCGβ lag jedoch stets höher als die des hCG. Das Verhältnis von Gesamt-hCGβ/hCG schwankte bei der Patientin A (mit anfänglich hohem hCG) zwischen 1,1 und 5,3, bei Patientin B (mit konstant niedrigem hCG) zwischen 1,1 und 550. Bei der letzteren nahm die Serumkonzentration von Gesamt-hCGβ, nachdem es zur Metastasierung gekommen war, bei Verwendung von β_1, vor allem aber von β_2, stark zu, während hCG_1 und hCG_2 gleichbleibend niedrig blieb (<26 IE/l).

Eine qualitative Analyse des Gesamt-hCGβ und hCG im Serum wurde mit Hilfe der Gel-Chromatographie über Sephadex-G100 und Superdex 75 während verschiedener Stadien der Erkrankung durchgeführt, und mit dem Elutionsprofil nicht-radioaktiven hCG und hCGβ bzw. dem Serum einer Schwangeren verglichen. Nach der Gel-Chromatographie der Seren dieser Patientinnen wies nur eine Fraktion hCG-Aktivität auf, deren Elutionsprofil dem des authentischen hCG oder Serum einer Schwangeren glich. Dazu kam noch ein weites Spektrum von Gesamt-hCGβ-Aktivität, die jedoch nicht mit hCGβ identisch war. Bei der Bestimmung der Gesamt-hCGβ-Aktivität mit der β_1- und β_2-Methode in den Eluaten ergaben sich beträchtliche Unterschiede. Der Anteil des hCG im Gel-Chromatogramm von Patientin B nahm mit der Dauer der vierjährigen Erkrankung ab, während die mit der β_1- und β_2-Methode darstellbaren Aktivitäten zunahmen.

Es wird gefolgert, daß die im Vergleich zu hCG höhere Konzentration von Gesamt-hCGβ im Serum von Frauen mit metastasierenden Chorionkarzinomen nicht durch hCGβ verursacht wird, sondern durch ein Spektrum qualitativ und quantitativ verschiedener Substanzen, die auf Grund ihrer Heterogenität von verschiedenen Testsätzen in unterschiedlicher Weise erfaßt werden.

Schlüsselwörter:

hCGβ – Gesamt hCGβ – Chorionkarzinom – Gel-Chromatographie – Serum

Introduction

The determination of hCG in serum for diagnosing a choriocarcinoma (ChCA) and monitoring therapy can yield confounding results, particularly when unusually low values are being found [1–3]. Discordant measurements of hCG had i.e. been obtained in a case of ChCA when two different immunometric assays were used. This was thought to be due to variations in the secretion of hCGβ [4]. A falsely-low hCGβ value was also obtained because of a high dose hook effect in a patient with ovarian choriocarcinoma using an immunometric assay [5]. This is, however, a technical problem which can readily be solved by validating the assay. As the secretion of free β subunit of hCG (naturally occurring hCGβ) and its ratio to hCG in patients with ChCA was found to be higher than during pregnancy [6–13], it was postulated that the determination of total hCGβ (hCG plus hCGβ) in an additive manner would be more advantageous than that of hCG or hCGβ by a competitive immunoassay.

The secretion of hCG and total hCGβ was followed in 2 therapy-resistant patients with ChCA from the time of diagnosis to their demise after 7 months and 4 years, respectively. The quantitative determination of hCG and total hCGβ in serum and the qualitative analysis by gel chromatography using Sephadex-G100 and Superdex-75 was carried out by means of a manual and automated immunometric assay during various stages of the disease.

Material and Methods

Patients

Patient A: the 28 years old patient was admitted as a case of emergency because of occlusion of the aorta by a chorionepithelioma, one year after a molar pregnancy had been terminated by suction evacuation. Even though intensive cytostatic therapy was carried out subsequent to initially successful surgical intervention using methotrexate and actinomycin D, the patient expired 7 months later from the sequelae of cerebral metastasis.

Patient B: 39 years old women, Gravida 3, Para 1. The last pregnancy terminated as a spontaneous abortion 4 years ago. She was admitted to the hospital on 12 June, 1987 (Day 0) because of suspected ectopic pregnancy as the hCG-Test was positive and sonography failed to show an intrauterine chorionic vesicle. The histologic examination of the endometrial tissue obtained by curettage revealed a choriocarcinoma. After hysterectomy, hCG declined to unmeasurable levels (<1 IU/l). Two years later, an exploratory laparotomy revealed the presence of metastases in paraaortical lymph nodes. Soon there were brain metastases, and on 7 June 1991, the patient succumbed to the disease.

Gel chromatography

The composition of hCG and hCGβ in serum of these patients was characterized by gel chromatography on Sephadex-G100 and Superdex-75 (Pharmacia, Freiburg) and measurement of hCG and total hCGβ in the eluates. The characteristics of the elution patterns and immunoactivities were compared to the patterns of gel chromatograms of nonradioactive authentic substances (hCG and hCGβ), and of a serum sample obtained from a pregnant woman.

A column (1.6×90 cm) was packed with Sephadex-G100 ultrafine (uf) and eluted with PBS-buffer after addition of 0.1% NaN_3 and equilibrated at 4°C. The flow rate was 0.09 ml/min, and the total bed volume (Vt) was 168 ml. The Superdex-75 was a prepacked 1.6×60 column (HiLoad Superdex-75: Pharmacia, Freiburg). Elution was accelerated by means of a peristaltic pump with a flow rate of 0.85 ml/min. One ml of serum or 5 µg hCG or hCGβ were applied to the column and fractions of 1 ml collected for the determination of hCG and total hCGβ (β_1 and β_2).

Chemicals

Nonradioactive hCG (immunological activity: 9000 IE/mg 1.IRP 75/537) and hCGβ (chemically dissociated from hCG: containing hCGα <0.1%) were purchased from Boehringer Mannheim (Germany). Phosphate buffer saline (PBS) pH 7.4 and the proteins for calibration of the molecular weights were supplied by Serva (Heidelberg, Germany).

Immunoassay

The manual system for determination of hCG (hCG1) was a time-resolved fluoroimmunometric assay (Delfia®-hCG: Pharmacia-LKB, Freiburg). The capture antibodies directed against a specific antigenic site on the β-subunit of hCG, and the europium-labelled antibodies directed against a specific antigenic site on the α-subunit were used as signal antibodies. The cross reaction to hCGβ was 2.4% (probably due to contamination with hCG). The automated system for determination of hCG (hCG2) was an enzyme fluoroimmunometric assay: Stratus®-hCG (Baxter, Munich) (Table 1).

The total hCGβ was determined in a manual system (β_1) by means of a radioimmunometric assay with oligoclonal

Table 1: Immunoassay systems used for quantitative and qualitative measurements.

analyte designation	hCG hCG_1	hCG hCG_1	total hCGβ β_1	total hCGβ β_2
cross reaction:				
hCG	100%	100%	100%	100%
hCGβ	2.4%	1.4%	100%	100%
LH,FSH	undetectable	undetectable	undetectable	undetectable
assay	time-resolved fluoro-immunometric	enzyme-fluoro-immunometric	radioimmunometric	microparticle enzyme-fluoro-immunometric
ligand	europium chelate	alkaline phosphatase + 4-methylumbelliferyl-phoshate	J^{125}	alkaline phosphatase + 4-methylumbelliferyl-phosphate
standard range*)	0–10,000 IU/l	0–500 IU/l	0–1,000 IU/l	0–1,000 IU/l
sensitivity	<1 IU/l	2 IU/l	1.5 IU/l	2 IU/l
interassay CV**)	7.8%	6%	6%	6.5%
system	manual	automated	manual	automated
kit	Delfia®-hCG	Stratus®-hCG	hCG + β-IRMA	Total β-hCG IMx
supplier	Pharmacia	Baxter	Medgenix	Abbott

**) high-dose hook effect in this range not observed.
**) CV = coefficient of variation.

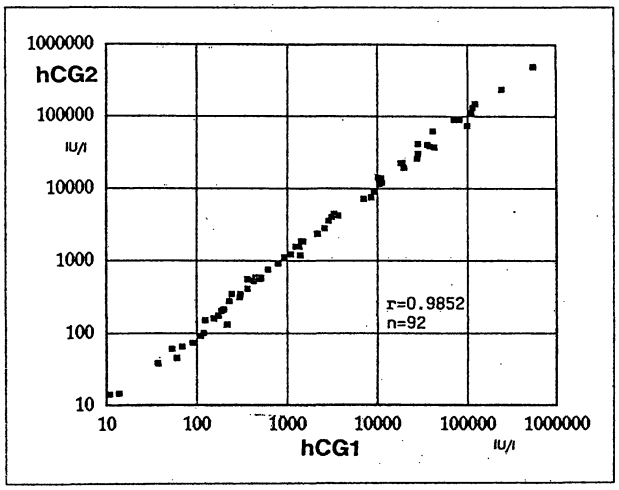

Figure 1: Concentration of hCG in serum samples from pregnant women and patients with chorioncarcinoma measured by a manual system (Delfia®-hCG = hCG_1) and an automated system (Stratus®-hCG = hCG_2).

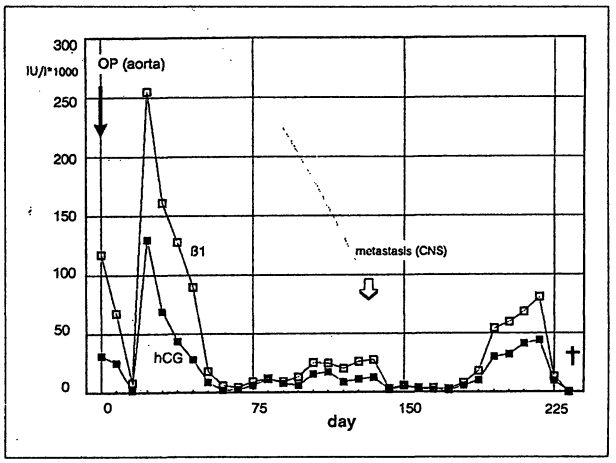

Figure 2: Quantitative measurements of hCG and total hCGβ (β_1-assay) in serum of patient A with initially high serum hCG during a period of 7 months.

antibodies (hCG+β-IRMA: Medgenix, Düsseldorf). The capture antibodies used for coating the tubes had a low affinity. Addition of J^{125}-labelled antibody (signal antibody) triggered the immunological reaction. The automated total hCGβ (β_2) was a microparticle enzyme immunometric-assay (Total hCGβ: Abbott-IMx, Wiesbaden) (Table 1).

The results of the measurements were expressed in IU/l hCG, calibrated against the 3rd International Standard WHO (IS no. 75/537), previously known as the 1st International Reference Preparation WHO (IRP no. 75/537). The characteristics of the kits used are listed in Table 1.

Results

The measurement of hCG with the manual (hCG_1) and automated (hCG_2) system in 92 serum samples of patients with ChCA and pregnant women with a concentration ranging between 10–550000 IU/l showed a high degree of correlation (coefficient of correlation r = 0.9853) and did not differ significantly (paired t-test: t = 0.342, p > 0.05) (Fig. 1).

Follow-up of quantitative measurements in serum

Patient A: As expected the serum level of total hCGβ (β_2) was higher than that of hCG. After the chorionepithelioma had been removed from the aorta, three courses of treat-

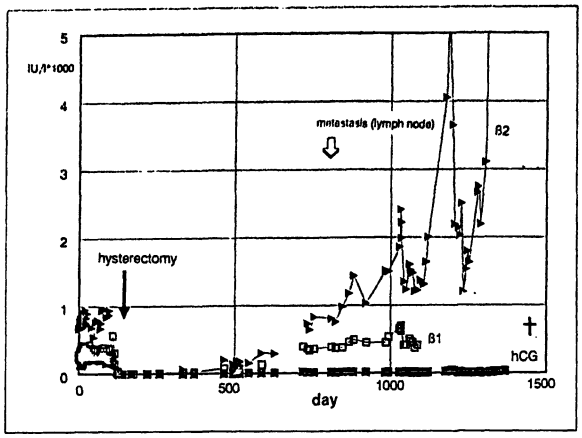

Figure 3: Quantitative measurements of hCG, total hCGβ (β_1-, β_2-assay) in serum of patient B with permanently low serum hCG during a period of 4 years.

ment with methotrexate were carried out. This brought about just a temporary reduction of hCG and total hCGβ (Fig. 2). The concentration of hCG in serum correlated well with that of total hCGβ ($r = 0.96455$, $n = 32$, $p < 0.001$). It is shown in Fig. 2 that the serum level of hCG varied between 1,500 and 250,000 IU/l during the following 7 months of observation. The total hCGβ and hCG ratio varied between 1.1 and 5.3.

Patient B: During the first four months of observation prior to hysterectomy, the serum level of hCG and total hCGβ fluctuated within a relatively narrow range:

hCG	130–260 IU/l
total hCGβ (β_1)	250–420 IU/l
total hCGβ (β_2)	670–950 IU/l

The serum concentration of total hCGβ both in the β_1- and β_2-assay was higher than that of hCG, the β_2 exceeding the level of either one. After hysterectomy, a rapid decline in the concentration of hCG and total hCGβ could be shown by all three methods (Fig. 3). Three months later, hCG_1 and hCG_2 rose minimally to levels ranging between 5 and 26 IU/l and remained at that until the end of the 4 years period of follow-up. In contrast, the level of total hCGβ in the β_1 and β_2-assay increased markedly for 2 years, the latter reaching a level of more than 10,000 IU/l during the final stage of the disease. The total hCGβ (β_1 and β_2)/hCG ratio fluctuated before hysterectomy between 1.1 and 5.9. It increased rapidly to a value of 550:1 after lymphatic metastasis had occurred. There was a highly significant correlation between the level of β_1 and β_2 ($r = 0.9329$, $n = 58$), but neither one did correlate with hCG.

Qualitative analysis to characterize the composition of hCG and hCGβ

Authentic substances and serum from a pregnant woman

Gel chromatography of nonradioactive hCG over Sephadex-G100 showed one main peak which was eluted in

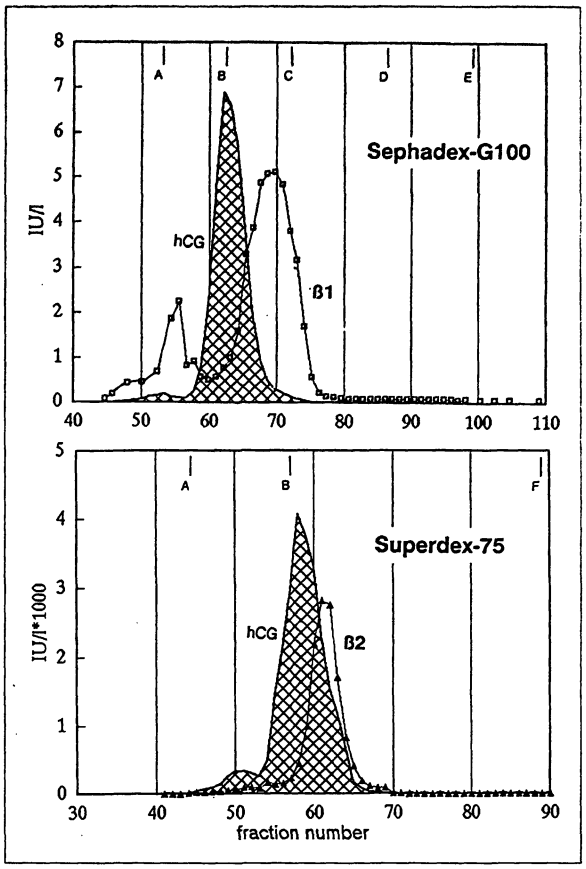

Figure 4: Elution profile of nonradioactive hCG and standard hCGβ after gel chromatography on Sephadex-G100 ultrafine: column = 1.6×86 cm (flow rate 0.09 ml/min) (upper panel) and Hiload Superdex-75 column = 1.6×60 cm (flow rate 0.85 ml/min) (lower panel). PBS buffer pH 7.4 was used for elution. One milliliter of fractions were collected and the immunoactivity of hCG and total hCGβ (β_1- and β_2-assay) were measured in the eluates. The lines above the versals indicate the peak of the fraction the calibrators for molecular weight (MW) were eluted: A = Ferritin (480,000), B = bovine serum albumin (67,000), C = Ovalbumin (43,000), D = Chymotrypsin (25,000), E = Ribonuclease A (13,700) and F = Cytochrom C (12,300).

fraction no. 62 and over Superdex-75 in fraction no. 57 when hCG was measured by means of the hCG-assay (Fig. 4). A small fraction with hCG-immunoactivity corresponding to a larger molecular weight (big-hCG) was also present both after gel chromatography over Sephadex-G100 or Superdex-75.

After gel chromatography of nonradioactive hCGβ over Sephadex-G100 or Superdex-75, the peak of the main fraction was detected in fraction no. 70 and 61, respectively, when assayed by the β_1 or β_2. The elution profile of hCGβ over Sephadex-G100 showed another fraction with a peak in fraction no. 55 when assayed with the β_1-kit. This fraction is probably similar to the large molecular form found in placental extracts, which showed cross reaction to antibodies to hCG and its subunits, but could not be

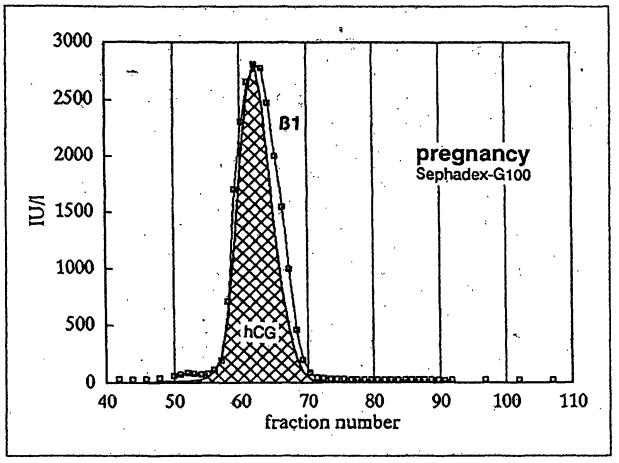

Figure 5: Elution profile of serum from a normal pregnant woman (11th week of pregnancy) after gel chromatography on Sephadex-G100, and the activity of hCG and total hCGβ (β_1-assay) in the fractions (see legend Fig. 4).

dissociated to subunits [14]. The elution profile after gel chromatography of serum from a pregnant woman on Sephadex-G100 showed only one main peak in fraction no. 62, when the samples were examined by both the hCG- and the β_1-kit (Fig. 5).

Patient A: The fraction with hCG activity in the eluates after gel chromatography of serum from day 89 and day 198 on Sephadex-G100 or Superdex-75 showed a peak in fraction no. 62 and 58 respectively (Fig. 6). In contrast to this, the total β_1 and β_2-activities in the eluates showed a broad spectrum, which was due to the presence of a cohort of smaller and larger sized hCG-like moieties (ca. 30,000 and 100,000 daltons). The qualitative analysis of the eluates with the β_1- and β_2-assay showed a rather different pattern in detection of the hCG-like moieties in the gel chromatography of serum from day 198.

Patient B: The fraction with hCG activity in the eluates after gel chromatography of serum day 33 on Sephadex-G100 showed a peak in fraction no. 62. There was minimal hCG-activity in serum obtained on day 370 but none in serum from day 1329 (Fig. 7). In contrast to this, the pattern of hCG-moieties detected with the β_1- and β_2-kit in the eluates after gel chromatography of serum from day 33, day 370 or day 1329 showed a different and broader spectrum. The cohort of substances detected by β_1- and β_2-kit represented smaller and larger sized hCG or hCGβ-moieties (20,000–100,000 daltons). The total hCGβ-moieties after gel chromatography detected by the β_1- and β_2-kit showed a qualitative different pattern.

Discussion

It could be shown, that the concentration of total hCGβ measured by 2 different methods (β_1 and β_2) was higher than that of hCG in serum of the patients with ChCA. The higher ratio of total hCGβ/hCG was, however, not to be

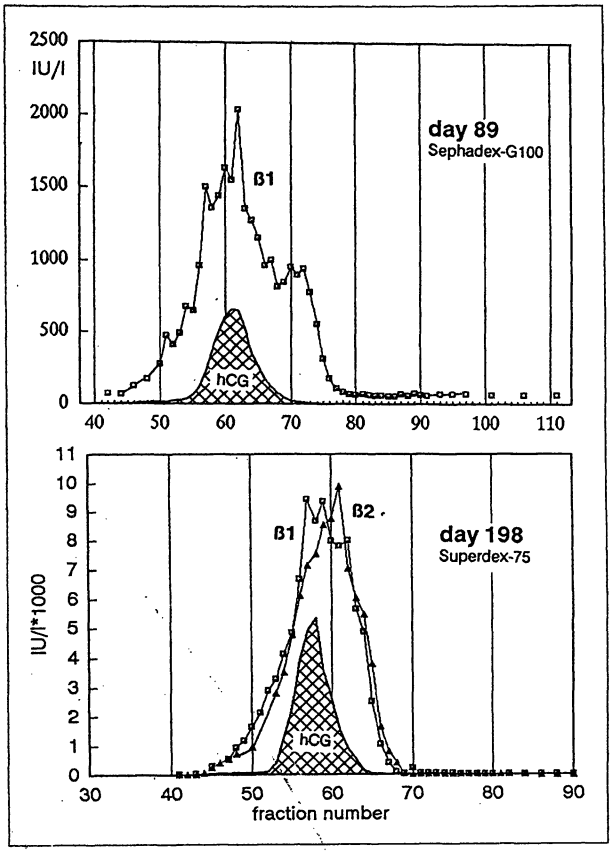

Figure 6: Elution profile of serum from patient A on day 89 (Sephadex-G100, upper panel) and on day 198 (Superdex-75, lower panel), and the activity of hCG and total hCGβ (β_1- and β_2-assay) in the fractions (see legend Fig. 4).

caused by a higher production rate of hCGβ, but by the presence of a certain number of other hCG-moieties. There was a quantitative and qualitative difference discernible in the concentrations of the hCG and hCGβ species depending on the type (β_1 and β_2) of assay being used. Contrary to that, no difference between hCG1 and hCG2 could be shown.

It has become customary in clinical practice to use the measurement of hCG in the diagnosis of early pregnancy, and that of the free β-subunit (hCGβ) as a tumor-marker. A competitive radioimmunoassay (RIA) using anti-hCGβ and J^{125}-labelled hCG or hCGβ, can detect either free hCGβ or intact hCG. This method is, however, not suited for the additive measurement of hCG and hCGβ. The immunometric assay with mixed monoclonal antibodies is a specific and sensitive method for determination of hCG [15] or hCGβ [16]. Falsely-positive or falsely-negative results have, however, been observed in an immunometric assay for hCGβ in the presence of high levels of hCG [17]. On the other hand, the sum of hCG plus hCGβ can be measured quantitatively using a sophisticated mixture of monoclonal antibodies as it is available in the β_1 and β_2-assay.

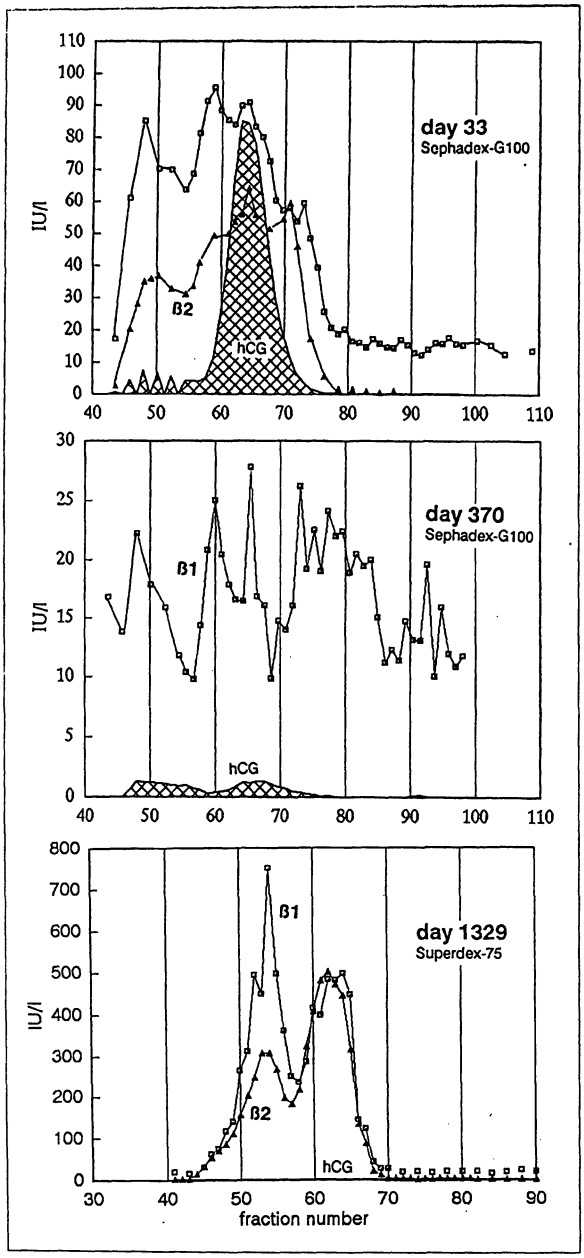

Figure 7: Elution profile of serum from patient B after gel chromatography on day 33 and day 370 (Sephadex-G100) and on day 1329 (Superdex-75). The immunoactivity of hCG, total hCGβ (β_1- and β_2-assay) were measured in the fractions (see legend Fig. 4).

The minute amounts of authentic hCG found in the blood and urine of healthy and nonpregnant women detected by immunometric assays, represent a physiologic phenomenon of unknown relevance. It appears that the genome for hCG is capable of expressing itself outside the trophoblast in various organs such as the pituitary [18], liver, kidney etc. [19–21] and also in some malignant tumours during all phases of life. In postmenopausal women who produce physiologically large amounts of FSH and LH, the secretion of hCG into urine is also somewhat higher than in menstruating subjects or men [22, 23]. For diagnostic purposes, a cut-off level of 5 IU/l is commonly used to minimize the risk of "false-positive" results. Another inherent technical problem of immunometric assays is the occurence of false low levels of hCG caused by the high dose hook effect [5]. It can easily be overcome in manual systems by diluting the sample and in automated systems by a built-in computerized break.

There is growing evidence that the hCG-species produced by the placenta differ to a great deal from those secreted by a choriocarcinoma. Discordant results were e.g. obtained in a case of ChCA, when an immunometric assay for hCG and total hCGβ was used [4]. In contrast, no significant difference was found when serum hCG was measured during pregnancy with an immunometric assay for hCG or total hCGβ [24]. The discrepancy was probably due to the higher production rate of the naturally occuring hCGβ in ChCA than during pregnancy, resulting in a higher ratio of hCGβ/hCG [6–13]. Nevertheless, immunochemical staining of the tumor for hCGβ in the patient with positive total hCGβ in serum was found to be negative [4]. The biological activity of hCG in serum of patients with ChCA was relatively higher than the immunological activity as compared to that during pregnancy [13]. This indicates that hCGβ produced by the tumor was not identical with standard hCGβ (chemically dissociated). Moreover, there was a striking difference in the prevalence of larger molecular species of hCG, and a higher acidic charge in serum than in urine from a patient with ChCA [25]. The molecular heterogeneity of hCG and its subunits was also apparent when plasma and other biological fluids such as ascites, urine, and tumor extracts from oncologic patients were analyzed [26]. Apart from differences in molecular size [25, 27], median charge [28], and amino-acid sequence [29], there were particular quantitative and qualitative differences in the composition of the sugar chains not only in the N-linked but also in the O-linked oligosaccharide chain as in hCG obtained from ChCA and pregnant individuals [30, 31, 32]. Similarly, the naturally occurring hCGα from normal pregnant women was found to be different than the chemically dissociated hCGα [33–35].

The results of the present study corroborate the observations of others [13, 26, 27, 31, 32, 34, 36] that the difference between total hCGβ and hCG from ChCA is not identical with chemically dissociated hCGβ, but represents a whole cohort of hCG and hCGβ moieties without a constant pattern. This emphasizes the fact that there are considerable qualitative and quantitative difference between the kits provided for the measurement of total hCGβ. Finally, as postulated by Hay [37], the ratio of "hCGβ"/hCG seems to increase with the advent of the disease, although the concentration of hCG remain quite low (Patient B).

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Anschrift der Verfasser:
PD Dr. Jeanne S. E. Dericks-Tan
Prof. Dr. H.-D. Taubert
Klinikum der J. W. Goethe Universität
Abt. für Gyn. Endokrinologie, Z.F.G.
Theodor-Stern-Kai 7
60590 Frankfurt am Main