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Urinary Gonadotropin Fragment (UGF) Measurements in the Diagnosis and Management of Ovarian Cancer

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UGF is a small peptide present in the urines and tissues of patients with gynecologic cancers. Published research (which, at present, mainly comes from our laboratory) on the general application of UGF as a tumor marker, and on its use in the diagnosis of ovarian cancer, is reviewed, and new studies on its use, alone and with CA125, in the management of patients with ovarian cancer, are presented. In 234 healthy women, 89 with benign disease, and 79 with ovarian cancer, UGF levels were above 3 fmol/ml (low cut-off) in 12 percent, 7 percent, and 82 percent, respectively, and above 8 fmol/ml (high cut-off) in 1.7 percent, <1.1 percent, and 59 percent, respectively. Similarly, 11 percent, 14 percent, and 70 percent, respectively, had CA125 levels above 35 U/ml (low cut-off), and <1.9 percent, 1.2 percent, and 49 percent had levels above a 200 U/ml (high cut-off). Ideally, the higher UGF and CA125 cut-offs should be used for diagnostic applications, like differentiation of a benign from a malignant pelvic mass (false-positive rate: UGF, <1.1 percent; CA125, 1.2 percent), but raising the cut-offs diminishes sensitivities for malignancy (UGF, 59 percent; CA125, 49 percent). The populations detected by the two markers only partially overlap, however, so that, together, UGF or CA125 can identify 75 percent of malignant pelvic masses. Levels of UGF (cut-off, >3 fmol/ml) and CA125 (35 U/ml) were also monitored in 30 women undergoing therapy for ovarian cancer. Clinical observations were reflected at each clinic visit by UGF alone in 67 percent, by CA125 alone in 57 percent, and by UGF and CA125 together in 87 percent of cases. While separately UGF and CA125 levels predicted 71 percent and 57 percent, together they forecast 86 percent of recurrent cancers prior to clinical manifestations. UGF and CA125 should be used together in the detection and management of ovarian cancers.

BACKGROUND

Human chorionic gonadotropin (HCG) is a glycoprotein hormone composed of a common α-subunit (also found in lutropin, follitropin, and thyrotropin) and a unique β-subunit, which gives the molecule its luteotropic function. HCG is produced by trophoblast tissue and can be detected in the blood and urine of women with pregnancy or trophoblast disease. Free HCG α- and β-subunits are also present in blood and urine of women with pregnancy or trophoblast disease, at levels <1 percent to 40 percent of that of the hormone [1-12].

HCG and HCG free subunit immunoreactivities have also been detected in the serum and tissues of cancer patients [7,13-22]. Recently, Hussa [7] compiled the results of 38 separate studies of HCG detection in cancer patients. As compiled (n = 4,291, with testicular and trophoblastic cancers excluded), 19 percent of subjects with cancer had detectable serum HCG levels. We analyzed 65 serum samples from

Abbreviations: HCG: human chorionic gonadotropin RIA: radioimmunoassay UGF: urinary gonadotropin fragment

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women with active ovarian, cervical, and endometrial cancers. Consistent with the compilation of Hussa [7], only 12 (18 percent) had detectable HCG levels [21]. Furthermore, the levels in the 12 HCG-positive samples (mean = 3.0 mIU/ml) were close to the limit of detection (2 mIU/ml), restricting the range of HCG levels that could be used for monitoring cancer therapy. We concurred with Hussa [7] and concluded that HCG was not a useful marker of non-trophoblastic gynecologic cancers and that other markers with higher cancer sensitivity were needed.

In 1977, Good and colleagues [5], demonstrated, using gel filtration techniques, that a small form of HCG β-subunit was present in pregnancy urines at levels higher than that of HCG. During the following ten years, similar findings were observed by several other researchers [23–29]. Chromatographic studies, and experiments with antibodies directed to different sites on HCG, indicated that this small molecule was composed of segments of β-subunit. As such, the term urinary HCG β-subunit core fragment was coined for this small molecule. In 1987–8, several laboratories generated antibodies against HCG β-subunit core fragment and established specific immunoassays. Akar and collaborators at N.I.H. generated β-subunit core fragment antisera [30]. Krichevsky and colleagues at Columbia University generated a β-subunit core fragment-specific monoclonal antibody [31] (antibody B204), as did Kardana and colleagues in London, England [32]. Using these antibodies in immunoassays, levels of HCG β-subunit core fragment were determined in pregnancy and trophoblast disease patient urines. In pregnancy urines, levels of β-subunit core fragment were shown to exceed HCG levels, by two-(first trimester) to seven-(second trimester)fold. Similarly, β-subunit core fragment levels in patients with trophoblast disease exceeded HCG levels, by as much as fiftyfold [22,30,33,34].

The gel filtration studies by Good and colleagues in 1977 also showed that the small β-subunit fragment was present in placental tissue [5]. Recent studies by Kardana and collaborators have confirmed these observations, by demonstrating that syncytiotrophoblast cells can be specifically stained for HCG β-subunit core fragment with monoclonal antibody 2C2 [34]. Studies in our laboratory have shown that human trophoblast organ cultures (n = 10), from first, second, or third trimester pregnancies secrete HCG β-subunit core fragment at levels similar to or exceeding those of HCG [33]. Studies by Wehmann and Nisula [27] have, however, shown that when HCG β-subunit is administered to humans, β-subunit alone is detected in serum, but both β-subunit and β-subunit core fragment can be detected in urines. This result suggested the presence of an additional pathway for generating HCG β-subunit core fragment, possibly involving renal degradation of molecules.

Recently, our laboratory collaborated with that of Steven Birken at Columbia University and determined the fine peptide and carbohydrate structure of HCG β-subunit core fragment. As was shown [26], β-subunit core fragment is composed of HCG β-subunit residues 6-40 attached by four disulfide linkages to HCG β-subunit residues 55-92, with two attached N-linked sugar units. The N-linked sugar units lacked the sialic residues that are found on HCG oligosaccharides. The molecular weight of HCG β-subunit core fragment was just 10,300, or 28 percent of that of HCG (36,700). Recently, Wehmann and colleagues [35] showed that HCG β-subunit core fragment is cleared from the circulation in humans 120 times faster than HCG (if equal amounts were produced, circulating β-subunit core fragment levels would be 0.8 percent of those of HCG). The rapid clearance rate is not surprising, when the absence of sialic acid (which maintains the circulatory half-life of the glycoprotein hormones

[20]) on HCG β-subunit core fragment and the small size, relative to HCG, are considered. The rapid clearance rate may explain why β-subunit core fragment is more readily detected in urine than in serum samples (serum concentration is 70 times lower than that in urine [36]).

Kardana and colleagues [34] used antibodies 2C2, INN13 (hCG-specific), and 1E5 (free β-subunit-specific), with histochemical methods, to stain fixed slices of non-trophoblastic cancer tissues. While none of 83 tissue slices was stained with the HCG or the free β-subunit antibodies, 77 (93 percent) were stained with the fragment-specific antibody, 2C2, at a distinctly higher level than surrounding control tissue. Consistent with these findings, our laboratory has used immunoassays with antibodies B204, 1E5, and B109 (HCG-specific) and gel filtration methods to indicate the absence of HCG and free β-subunit, and presence of HCG β-subunit core fragment in five of five tissue pieces from ovarian cancer [20,36]. Other researchers have used chromatographic methods [24–26,37] or, in more recent studies, immunoassays with monoclonal antibodies 2C2 or B204 [21,22,34,38–41], to show that HCG β-subunit core fragment-like molecules are present in the urines of as many as 92 percent of cancer patients. The finding that HCG β-subunit core fragment is produced by cancer cells, in the absence of detectable HCG or β-subunit, indicated a detachment in their production. As such, the terms HCG and HCG β-subunit core fragment were detached, and the latter called urinary gonadotropin fragment (UGF) [21,34,38–41].

In the past two years, we have published several articles on UGF assays, and on applications of UGF and of UGF and CA125 measurements in the diagnosis of gynecologic cancers [21,38–41]. This article reviews our published data and supplements it with new observations on the use of UGF alone, and of UGF with CA125, in the management of ovarian cancer and on the early detection of recurrent disease.

DEVELOPMENT OF UGF ASSAY

A specific immunoradiometric assay was developed for UGF. Urine, 200 μl, was added to tubes coated with 900 ng monoclonal antibody B204. UGF was bound by the coated tube, and urine removed. Radiolabeled monoclonal antibody HCO514 (directed to a common β-subunit epitope) was then added, which labeled the bound UGF. After removing excess labeled antibody, radioactivity was determined. A linear relationship was found between UGF in urine and radioactivity. The cross-reactivity of HCG in this assay was <0.2 percent; with HCG free β-subunit, was 7.5 ± 0.54 percent; and with lutropin or lutropin free β-subunit, was <0.1 percent. Over a six-month period the intra-assay and inter-assay coefficients of variation averaged 7.8 ± 1.9 percent and 12.7 ± 1.7 percent, respectively, and the sensitivity (concentration different from 0 fmol/ml standard in Student's *t*-test) averaged 2.1 ± 0.31 fmol/ml. Details of these methods, and of the effects of varying antibody concentrations and incubation conditions, are published elsewhere [40].

ESTABLISHMENT OF CANCER SENSITIVITY AND SPECIFICITY, AND CUT-OFF LEVELS

UGF levels were measured in 323 control samples, from 234 healthy women, and from 89 with benign disease (Table 1). Thirty-three of the 323 women providing control urines had UGF levels which exceed 3 fmol/ml; only four, however, exceed 8 fmol/ml, or 4 standard deviations among the mean. A cut-off of 8 fmol/ml was indicated for screening-type applications, where a low false-positive rate (1.2 percent)

TABLE 1
Incidence of False-Positive UGF Levels in Healthy Individuals, and Women with Benign Disease

	Number of Patients	Number of False-Positives	
		>3 fmol/ml	>8 fmol/ml
Healthy women			
Pre-menopause	94	3 (3.2%)	0
Post-menopause	140	24 (17%)	4 (2.9%)
Subtotal	234	27 (12%)	4 (1.7%)
Women with benign disease			
Endometriosis	16	3 (18%)	0
Ovarian neoplasm	14	0	0
Leiomyomata uteri	13	1 (8%)	0
Condyloma	15	0	0
Other	31	2 (6%)	0
Subtotal	89	6 (7%)	0
Total	323	33 (10%)	4 (1.2%)

is critical. The higher false-positive rate, using the 3 fmol/ml cut-off (10 percent), is lower than that reported for CA125 (14 percent and 35 U/ml cut-off [41,42]) and is appropriate for application where sensitivity is less critical, such as monitoring the progress of patients with established cancer.

Similar false-positive results were observed for two groups with similar age distribution, women pre-menopause with no history of disease (0 of 94 exceed 8 fmol/ml) and women with benign gynecologic lesions (0 of 89 exceed 8 fmol/ml). The similarity of the results between these two groups indicates that benign pelvic lesions such as endometriosis, benign ovarian neoplasm, and leiomyomata uteri do not falsely elevate UGF levels. False-positive results were higher among women who were post-menopause than pre-menopause (false-positive rates 2.9 percent and <1.1 percent, respectively, at 8 fmol/ml cut-off). Recent studies have shown that some healthy post-menopausal women have low levels of HCG (0–10 mIU/ml) and HCG free α- and free β-subunits in their circulation, of pituitary origin [43,44]. We postulate that the 2.9 percent of post-menopausal women who have false-positive UGF levels in urine are those whose pituitary produces cross-reacting HCG free β-subunit.

UGF levels were examined in 156 women with proven gynecologic cancers (Table 2). While UGF levels in 102 patient samples (65 percent), exceed 3 fmol/ml, only 70 (45 percent) exceed the 8 fmol/ml cut-off. The mean UGF level in cancer patients was 44 fmol/ml, the range of levels was 3–714 fmol/ml (240-fold different), which is

TABLE 2
Incidence of True Positive UGF Levels in Individuals with Gynecologic Cancer

	Number of Patients	Number of True Positives	
		>3 fmol/ml	>8 fmol/ml
Ovary	71	58 (82%)	42 (59%)
Endometrium	33	20 (61%)	12 (36%)
Cervix	52	24 (46%)	16 (31%)
Total	156	102 (65%)	70 (45%)

TABLE 3
Detection of UGF and CA125 Levels in Parallel Urine and Plasma Samples from Healthy Individuals, and from Those with Benign and Malignant Pelvic Masses

		UGF		CA125		UGF or CA125	
	Number of Patients	>3	>8 fmol/ml	35	200 U/ml	>3 35	>8 fmol/ml 200 U/ml
Healthy women	234[a]	12%	1.7%	11%	0 (<1.9%)		
Women with benign pelvic mass (myoma, ovarian cyst, or endometrioma)	443[b]	9.3%	0 (<2.3%)	14%	1.2%		
Patients with malignant pelvic mass, pre-surgery or at initial clinic visit	71	82%	59%	70%	49%	96%	75%
Serous carcinoma	43	81%	60%	79%	56%	95%	79%
Endometrioid, mucinous, or other ovarian carcinoma	28	82%	57%	57%	39%	96%	68%

[a]Only 53 plasma samples collected for CA125 measurements
[b]Only 43 urine samples collected for UGF measurements

similar to that of CA125 (35–9,000 U/ml, 260-fold different [45]). The organ specificity of UGF, as a cancer marker, was also similar to that of CA125 (Table 2). While 82 percent, 61 percent, and 46 percent of ovarian, endometrial, and cervical cancers, respectively, were detected by UGF (cut-off >3 fmol/ml), 76 percent, 59 percent, and 18 percent were reported to be detected by CA125 (cut-off 35 U/ml [45]); however, the tissue specificities of UGF and CA125 for ovarian cancers were markedly different. While UGF is impartial and detected a similar proportion of serous (81 percent) and endometrioid/mucinous/other (82 percent) ovarian cancers (Table 3), CA125 had a distinct preference for detecting serous malignancies (sensitivity 79 percent, vs. 57 percent for other ovarian malignancies). We conclude that UGF is a marker of gynecologic cancers. Like CA125, UGF levels preferentially mark ovarian malignancies. The sensitivity and specificity of UGF (81 percent and 10 percent, cut-off >3 fmol/ml) and CA125 (79 percent and 14 percent, cut-off 35 U/ml) are similar for serous ovarian cancers. While CA125 has reduced sensitivity for other types of ovarian cancer, however, the sensitivity of UGF remains the same (82 percent).

APPLICATION 1: IN DIFFERENTIATING BENIGN AND MALIGNANT PELVIC MASSES

The distinction of a benign from a malignant pelvic mass is a difficult problem for the gynecologist. Physical examination may be of little use, and ultrasound, while distinguishing an adnexal mass from a uterine mass, has limited use in differentiating a benign pelvic mass from a malignancy [46–49]. Tumor markers, such as CA125, are also insufficiently accurate to help in the differential diagnosis of benign and malignant disease. In studies at Yale–New Haven Hospital (Table 3), CA125 detected 79 percent of serous malignancies, but also detected 14 percent of patients with benign pelvic masses. Studies at other centers show that CA125 detects 76 percent to 91 percent of serous malignancies, but also 6 percent to 40 percent of benign pelvic lesions [42,50–52].

New methods are needed to distinguish masses and to identify malignancies pre-operatively. With these methods, appropriate additional studies could be arranged which may influence the type of surgery scheduled. If a malignancy is expected, then a bowel prep and the necessary surgical expertise can be scheduled pre-operatively. In this way, accurate staging procedures and complete tumor removal can be done, both of which are very important for selection of the appropriate post-operative management program.

We investigated the levels of UGF in patients with pelvic masses and its ability to complement CA125 results. As shown in Table 3, UGF at a cut-off of >3 fmol/ml, like CA125 at 35 U/ml, falsely detected a high proportion (9.3 percent and 14 percent, respectively) of benign pelvic masses. When the UGF and CA125 cut-off levels were raised to >8 fmol/ml and to 200 U/ml, respectively, the number of benign pelvic masses detected was significantly reduced (to <2.3 percent and 1.2 percent, respectively). The cancer sensitivities of UGF and CA125 were reduced also (59 percent and 49 percent, respectively). The populations detected by the two markers only partially overlap, however, so that, together, UGF or CA125 can identify 79 percent of serous and 68 percent of other malignant pelvic masses.

The latter finding indicated new procedures for the evaluation of patients presenting with a pelvic mass. A urine specimen should be collected for UGF measurements and a parallel plasma sample collected for CA125. Using two assay results, with UGF at a cut-off of >8 fmol/ml and CA125 at a cut-off of 200 U/ml, 75 percent of malignancies should be detected. The false-positive rate is 1.2 percent for CA125 alone, <2.3 percent for UGF alone, and <1.2 percent when, as in the majority of cases, UGF and CA125 are detected. These false-positive rates may be low enough for use in the evaluation of pelvic masses. If elevated levels are detected (>8 fmol/ml or ≥200 U/ml), cancer should be assumed and appropriate measures taken.

APPLICATION 2: IN MONITORING THE THERAPY OF OVARIAN CANCER

The clinical evaluation of tumor burden in ovarian cancer patients undergoing therapy and follow-up is problematic. Currently, second-look surgical procedures are commonly used to determine whether therapy should be halted or modified, or new procedures instituted. The discovery of sensitive tumor markers which accurately reflect the tumor burden may help to monitor clinical status better, alleviate the necessity of second-look surgery, and possibly permit the detection of recurrent disease prior to the appearance of clinical signs, when therapy may be too late to be of value.

CA125 is found in the plasma of a high proportion of patients (reports vary from 76 percent to 91 percent) with serous ovarian cancers [42,50–52]. Several centers have examined the use of plasma CA125 measurements in monitoring therapy of ovarian cancer. Early reports showed that CA125 levels diminished and became undetectable as ovarian cancer patients responded to treatment [53–55]. This finding suggested that CA125 levels reflected tumor burden, and applications for CA125 in monitoring the efficacy of therapy and in the early detection of recurrent disease [53–55]. More recent studies have found limitations in the use of CA125 in ovarian cancer management. For instance, reports indicate that >40 percent of patients who are clinically free of disease, with CA125 levels (<35 U/ml) in the normal range, have persistent cancer when evaluated by second-look surgery procedures [45,56,57]. Furthermore, it has been suggested that diminishing CA125 levels in ovarian cancer patients do not

TABLE 4
Detection of UGF and CA125 Levels in Parallel Urine and Plasma Samples from Patients with Malignant Pelvic Masses: Breakdown by Stage (F.I.G.O.)

	Number of Patients	UGF		CA125	
		>3	>8 fmol/ml	35	200 U/ml
Stages I and II	5	80%	40%	40%	0
Stage III	40	75%	50%	60%	43%
Stage IV	10	100%	70%	90%	60%
Stage X	4	100%	100%	100%	100%
Recurrence	12	83%	75%	92%	67%
Total	71	82%	59%	70%	49%

necessarily indicate diminishing disease [58]. Additional markers are needed to back up CA125 and to detect non-serous ovarian malignancies.

As described earlier, UGF is also produced by a high proportion of serous ovarian carcinomas. In our experience (Table 3), CA125 (cut-off, 35 U/ml) detects in plasma 79 percent, and UGF (cut-off, >3 fmol/ml) in urine a similar proportion (81 percent) of serous cystadenocarcinomas. Like CA125, UGF sensitivity, and levels [41], increase with advancing stage (Table 4). This finding suggests that UGF levels, like those of CA125, may reflect tumor burden. UGF, however, unlike CA125, similarly detects serous, mucinous, and endometrioid malignancies and, as described earlier, complements CA125 detection of serous cystadenocarcinoma (Table 3: UGF, 81 percent; CA125, 79 percent, and together they detect 95 percent of serous cancers). The use of UGF alone, and of UGF and CA125, was investigated, in following the therapy of patients with ovarian cancer.

Levels of UGF and CA125 were monitored in 30 women undergoing therapy for ovarian cancer. Twenty-one of the group (70 percent) had true positive UGF (>3 fmol/ml) and 19 of the group (63 percent) had true positive CA125 levels (≥35 U/ml) at entry into the study or when cancer became clinically evident. Levels of UGF accurately (at each clinic visit) reflected clinical observations during therapy in 20 of the 21 (95 percent) true positives, or 67 percent of the 30 patients (Table 5). CA125 levels reflected clinical observations in 17 of the 19 (89 percent) true positives, or 57 percent of the 30 patients. Interestingly, UGF levels reflected the clinical course in a similar proportion of the 19 CA125 true positives (64 percent) and 11 CA125 true negatives (68 percent), suggesting the independence of the two markers. While CA125 levels were most useful in monitoring patients with serous cancers (levels were concordant with clinical observations in 67 percent of women with serous and 33 percent of those with other ovarian malignancies), UGF levels were best in the management of those with endometrioid and other ovarian malignancies (levels reflected clinical observations in 62 percent of those with serous and 77 percent of patients with other cancers). These results further indicate that the two markers complement each other, and the appropriateness of using them together, to back up each other's false-negatives. Using UGF and CA125 together, therapy was appropriately monitored at each clinic visit in 26 of the 30 (87 percent) patients.

We investigated the use of UGF alone, CA125 alone, and the two markers together in the early detection of recurrent disease. Rising UGF and rising CA125 levels each separately predicted four of seven recurrences in patients clinically free of disease.

TABLE 5
Correlation of UGF and CA125 Levels with Clinical Observations

Case	Histology	Stage/ Grade	Therapy and Clinical Observations[a] Visit Number: 1 2 3 4 5 6 7 8 9	UGF Levels (fmol/ml) at Visits 1 through 9	CA125 Levels (U/ml) at Visits 1 through 9	Clinical Correlation		
						UGF	CA125	Either
Patients with True Positive UGF Levels								
1	Endometrioid	II/2	S→C↓C•C•C•	30, 2, 0, 0, 0	5, 6, 6, 5, 5	+	–	+
2	Endometrioid	III/3	C→C→N•N•N↑	5, 3, 4, 6, 8	33, 8, 5, 6, 22	+	–	+
3	Endometrioid	III/3	S→C↓C→C•C•C↑	15, 0, 0, 0, 4, 13	27, 8, 5, 8, 4, 6	+	–	+
4	Imm. teratoma	III/3	C→C→C→C↓C•C•C•	9, 10, 10, 0, 0, 0, 2	1, 7, 1, 10, 5, 5, 5	+	–	+
5	MMT	III/3	S↓C↑C↑	19, 0, 3	33, 5, 1	–	–	–
6	Mucinous	X	C↓C↓C↑	7, 4, 6	1,580, 1,430, 1,360	+	–	+
7	Mucinous	X	S↓C↓C↓	80, 9, 8	600, 210, 21	+	+	+
8	Serous	III/2	S↓C•C•C•C•	11, 3, 4, 4, 0	1, 7, 8, 10, 6	+	–	+
9	Serous	III/2	S•C•C↑C↑	2, 2, 6, 21	6, 84, 56, 84	+	–	+
10	Serous	II/1	↑C↓C•N•N↑S↓	5, 0, 0, 0, 4, 4	1, 5, 5, 25, 5, 5	+	–	+
11	Serous	IV/3	S↓C↓C↓C•	13, 10, 8, 0	6, 7, 7, 6	+	–	+
12	Serous	R/3	C→C↑C↑	5, 10, 15	40, 350, 360	+	+	+
13	Serous	X	C→C•C•C↑C↑	12, 6, 8, 7, 56	200, 180, 190, 270, 1,220	+	+	+
14	Serous	III/3	S→C•C•C↑	2, 9, 8, 14	66, 57, 250, 860	+	+	+
15	Serous	III/3	↑S↓C•C•C•	6, 0, 0, 0, 0	1,000, 40, 7, 7, 7	+	+	+
16	Serous	III/3	C→C→C↓N•N•N•	6, 4, 2, 0, 2, 3	490, 24, 15, 11, 12, 13	+	+	+
17	Serous	R/2	↑S↓C•C•	9, 11, 0, 0	40, 56, 32, 30	+	+	+
18	Serous	III/2	↑S↓C→C↑C→	29, 19, 7, 22, 12	4,880, 100, 100, 850, 180	+	+	+
19	Serous	R/3	↑C↓C•N•N↑	20, 13, 0, 0, 10	2,000, 5, 6, 6, 60	+	+	+
20	Serous	III/2	C•C•C•C•C↑S↓C•C•C↑	0, 0, 0, 0, 3, 0, 5, 3, 9	5, 5, 7, 5, 21, 0, 34, 55, 98	+	+	+
21	Theca cell	II/2	S↓C•C•C•	6, 3, 0, 0	40, 8, 8, 7	+	+	+
					UGF True Positives	95%	52%	95%

Patients with False-Negative UGF Levels								
22	Endometrioid	R/3	C↑R↑C→	0, 0, 2	300, 1,300, 870	−	+	+
23	Serous	III/1	C→C↑C→	0, 0, 0	125, 150, 83	−	+	+
24	Serous	III/3	C→C↑C↑	0, 0, 0	230, 580, 1,750	−	+	+
25	Serous	R/3	C↑C→C↑	0, 12, 5	2,050, 3,430, 5,260	−	+	+
26	Serous	III/3	S→C→C↑C↑	0, 0, 0, 0	410, 400, 460, 1,860	−	+	+
27	Serous	IV/3	S→C↑C↓C→C↑	0, 5, 0, 0, 3	220, 700, 1,300, 990, 3,760	−	+	+
28	Serous	III/3	S→C→C→	0, 0, 0	23, 11, 3	−	−	−
29	Serous	III/3	C→C↑C↑C↑C↑	0, 0, 0, 0, 41	7, 31, 35, 27, 1,200	−	−	−
30	Serous	III/3	S→C→C→C→S→	3, 3, 0, 0, 6	5, 2, 6, 9, 25	−	−	−
					UGF False-Negatives	0	67%	67%
					Overall	67%	57%	87%

[a]A brief patient history is presented for the study period. The symbols ↑, →, ↓, and • mark the clinical observations at each visit/sample collection and represent progressive, stable, and decreasing tumor mass, and no evidence of disease, respectively. Therapy between clinic visits/sample collections is indicated by the letters S, C, R, and N, which refer to surgery, chemotherapy, radiotherapy, and no therapy, respectively. The time between clinic visits/sample collections averaged 32 days (range, 5–210 days). The symbols in the clinical correlation columns indicate whether changing levels of UGF, CA125, or both are (+) or are not (−) concordant with clinical observations (at all clinic visits).

Using either marker, however, seven of seven recurrences were detected when first clinically evident, and six of seven at a clinic visit prior to that.

We conclude that UGF is a good back-up marker for CA125 and that it should be used in monitoring all patients with ovarian cancer. For mucinous and other non-serous ovarian cancers, UGF should be the marker of choice, and, for serous malignancies, the use of both UGF and CA125 is recommended. In our experience while following the progress of 21 patients with serous cancer, changes in CA125 and UGF levels do not conflict with each other, so that, in patients clinically free of disease, an elevation of either CA125 or UGF indicates a recurrence, and the need to start appropriate therapy.

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REFERENCES

1. Cole LA, Kroll TG, Ruddon RW, Hussa RO: Differential occurrence of free α and free β subunits of human chorionic gonadotropin in pregnancy sera. J Clin Endocrinol Metab 58:1200–1202, 1984
2. Cole LA, Hartle RJ, Laferla JJ, Ruddon RW: Detection of the free α-subunit of human chorionic gonadotropin in cultures of normal and malignant trophoblast cells, pregnancy sera, and sera of patients with choriocarcinoma. Endocrinology 113:1176–1178, 1983
3. Cole LA, Restrepo-Candelo H, Lavy G, DeCherney A: hCG free beta-subunit an early marker of outcome of in vitro fertilization clinical pregnancies. J Clin Endocrinol Metab 64:1328–1330, 1987
4. Elegbe RA, Pattillo RA, Hussa RO, Hoffmann RG, Damole IO, Finlayson WE: Alpha subunit and human chorionic gonadotropin in normal pregnancy and gestational trophoblastic disease. Obstet Gynecol 63:335–337, 1984
5. Good A, Ramos-Uribe M, Ryan R, Kempers RD: Molecular forms of human chorionic gonadotropin in serum, urine and placental extracts. Fertil Steril 28:846–850, 1977
6. Hay DL: Discordant and variable production of human chorionic gonadotropin and its free alpha and beta subunits in early pregnancy. J Clin Endocrinol Metab 61:1195–1200, 1985
7. Hussa RO: Human chorionic gonadotropin, a clinical marker: Review of its biosynthesis. Ligand Rev 3:1–43, 1981
8. Rozmus VM, Skalba P: Die konzentrationsbestimmung des choriogonadotropin (HCG) und seiner freien untereinheiten im plazentagewebe in verschiedenen perioden der normalen schwangerschaft. Zbl Gynakol 106:834–844, 1984
9. Vaitukaitis JL: Changing placental concentrations of human chorionic gonadotropin and its subunits during gestation. J Clin Endocrinol Metab 38:755–760, 1974
10. Ashitaka Y, Nishimura R, Takamori M, Tojo S: Production and secretion of HCG and HCG subunits by trophoblastic tissue. In Chorionic Gonadotropin. Edited by SJ Segal. New York, Plenum Press, 1980, pp 147–176
11. Ozturk M, Bellet D, Manil L, Hennen G, Frydman R, Wands J: Physiologic studies of human chorionic gonadotropin (hCG), alpha hCG, and beta hCG as measured by specific monoclonal immunoradiometric assays. Endocrinology 120:549–558, 1987
12. Khazaeli MB, Hedayat MM, Hatch KD, To ACW, Soong SJ, Shingleton HM, Boots LR, LoBuglio AF: Radioimmunoassay of free beta subunit of human chorionic gonadotropin as a prognostic test for persistent trophoblastic disease in molar pregnancy. Am J Obstet Gynecol 155:320–327, 1986
13. Hussa RO, Fein HG, Pattillo RA, Nagelberg SB, Rosen SW, Weintraub BD, Perini F, Ruddon R, Cole

LA: A distinctive form of human chorionic gonadotropin beta subunit-like material produced by cervical carcinoma cells. Cancer Res 6:1948–1954, 1986
14. Kahn CR, Rosen SW, Weintraub BD, Fajans SS, Gorden P: Ectopic production of chorionic gonadotropin and its subunits by islet-cell tumors. N Engl J Med 297:565–569, 1977
15. Weintraub BD, Rosen SW: Ectopic production of the isolated beta subunit of human chorionic gonadotropin. J Clin Invest 52:3135–3142, 1973
16. Nagelberg SB, Cole LA, Rosen SW: A novel form of ectopic human chorionic gonadotropin beta subunit in the serum of a woman with epidermoid cancer. J Endocrinology 107:403–408, 1985
17. Papapetrou PD, Nicopoulou SC: The origin of a human chorionic gonadotropin beta-fragment in the urine of patients with cancer. Acta Endocrinologica 112:415–422, 1986
18. Cole LA, Hussa RO, Rao CV: Discordant synthesis and secretion of human chorionic gonadotropin and subunits by cervical carcinoma cells. Cancer Res 41:1615–1619, 1981
19. Vaitukaitis JL: Immunologic and physical characterization of human chorionic gonadotropin (hCG) secreted by tumors. J Clin Endocrinol Metab 37:505–514, 1973
20. Cole LA: Occurrence and properties of glycoprotein hormone free subunits. In Microheterogeneity of Glycoprotein Hormones. Edited by HE Grotjan, BA Keel. New York, CRC, 1988, pp 53–73
21. Cole LA, Wang Y, Elliott M, Latif M, Chambers JT, Chambers SK, Schwartz PE: Urinary human chorionic gonadotropin free β-subunit and β-core fragment: A new marker of gynecologic cancers. Cancer Res 48:1356–1360, 1988
22. O'Connor J, Schlatterer JP, Birken S, Krichevsky A, Armstrong EG, McMahon D, Canfield R: Development of highly sensitive immunoassays to measure human chorionic gonadotropin, its β-subunit, and β core fragment in the urine: Application of malignancies. Cancer Res 48:1361–1366, 1988
23. Schroeder HR, Halter CM: Specificity of human β-choriogonadotropin assays for the hormone and for an immunoreactive fragment present in urine during normal pregnancy. Clin Chem 29:667–671, 1983
24. Masure HR, Jaffee WL, Sickel MA, Birken S, Canfield RE, Vaitukaitis JL: Characterization of the small molecular weight urinary immunoreactive human chorionic gonadotropin (hCG)-like substance produced by normal placenta and by hCG-secreting neoplasm. J Clin Endocrinol Metab 53:1014–1020, 1981
25. Vaitukaitis JL: Immunologic and physical characterization of human chorionic gonadotropin (hCG) secreted by tumors. J Clin Endocrinol Metab 37:505–514, 1973
26. Birken S, Armstrong EG, Kolks MAG, Cole LA, Agosto GM, Krichevsky A, Canfield RE: The structure of the human chorionic gonadotropin beta core fragment. Endocrinology 123:572–583, 1988
27. Wehmann RE, Nisula BC: Characterization of a discrete degradation product of human chorionic gonadotropin beta subunit in humans. J Clin Endocrinol Metab 51:101–105, 1980
28. Blithe DL, Akar AH, Wehmann RE, Nisula BC: Purification of β-core fragment from pregnancy urine and demonstration that its carbohydrate moieties differ from those of native human chorionic gonadotropin-β. Endocrinology 122:173–180, 1988
29. Kato Y, Braunstein GD: β-core fragment is a major form of immunoreactive urinary human chorionic gonadotropin in human pregnancy. J Clin Endocrinol Metab 66:1197–1201, 1988
30. Akar AH, Wehmann RE, Blithe DL, Blacker C, Nisula B: A radioimmunoassay for the core fragment of the human chorionic gonadotropin beta-subunit. J Clin Endocrinol Metab 66:538–545, 1988
31. Krichevsky A, Armstrong EG, Schlatterer J, Birken S, O'Connor J, Bikel K, Silverberg S, Lustbader J, Canfield R: Preparation and characterization of antibodies to the urinary fragment of the human chorionic gonadotropin β-subunit. Endocrinology 123:584–593, 1988
32. Kardana A, Taylor ME, Rowan AJ, Read DA, Bagshawe KD: Characterization of antibodies to urinary gonadotropin peptide. J Immunol Methods 118:53–58, 1989
33. Cole LA, Birken S: Origin and occurrence of human chorionic gonadotropin β-subunit core fragment. Mol Endocrinol 2:825–830, 1988
34. Kardana A, Taylor ME, Southall PJ, Boxer GM, Rowan AJ, Bagshawe KD: Urinary gonadotropin peptide—isolation and purification, and its immunohistochemical distribution in normal and neoplastic tissues. Br J Cancer 58:281–286, 1988
35. Wehmann RE, Blithe DL, Flack MR, Akar AH, Nisula BC: Metabolic clearance rate and urinary clearance of β-core in humans (Abstract I-12). In International Symposium on Glycoprotein Hormones. Newport Beach, March 1988
36. Cole LA, Kardana A, Birken S: The isomers, subunits and fragments of HCG (Abstract). In Symposium on Structure-Function Relationship of Gonadotropins. Paris, May 1988
37. Papapetrou PD, Nicopoulou SC: The origin of a human chorionic gonadotropin β-subunit core fragment in the urine of patients with cancer. Acta Endocrinologica 112:415–422, 1986

38. Cole LA, Schwartz PE, Wang Y: Urinary gonadotropin fragments (UGF) in cancers of the female reproductive system. I. Sensitivity and specificity, comparison with other markers. Gynecol Oncol 31:82–90, 1988
39. Wang Y, Schwartz PE, Chambers JT, Cole LA: Urinary gonadotropin fragments (UGF) in cancers of the female reproductive system. II. Initial serial studies. Gynecol Oncol 31:91–100, 1988
40. Nam JH, Cole LA, Chambers JT, Schwartz PE: Urinary gonadotropin fragment, a new tumor marker: I. Assay development and cancer specificity. Gynecol Oncol, in press
41. Cole LA, Nam JH, Chambers JT, Schwartz PE: Urinary gonadotropin fragment, a new tumor marker: II. For differentiating a benign from a malignant pelvic mass. Gynecol Oncol, in press
42. Di-Xia C, Schwartz PE, Xinguo L, Zhan Y: Evaluation of CA125 levels in differentiating malignant from benign tumors in patients with pelvic masses. Obstet Gynecol 72:23–27, 1988
43. Odell WD, Griffin J: Pulsatile secretion of human chorionic gonadotropin in normal adult. N Engl J Med 317:1688–1695, 1987
44. Stenman U-H, Alfthan H, Ranta T, Vartiainen E, Jalkanen J, Seppala M: Serum levels of human chorionic gonadotropin in nonpregnant women and men are modulated by gonadotropin-releasing hormone and sex steroid. J Clin Endocrinol Metab 64:730–736, 1987
45. Schwartz PE, Chambers SK, Chambers JT, Gutmann J, Katopolis N, Foemmel R: Circulating tumor markers in monitoring of gynecologic malignancies. Cancer 60:353–361, 1987
46. Schwartz PE: Gynecological cancer. In Clinical Medicine. Edited by J. Spitell. Philadelphia, Harper and Row, 1985, pp 1–43
47. Finkler NJ, Benacerraf B, Lavin PT, Wojciechowski C, Knapp RC: Comparison of serum CA125, clinical impression, and ultrasound in the pre-operative evaluation of ovarian masses. Obstet Gynecol 72:659–666, 1988
48. Meire HB, Farrant P, Guha T: Distinction of benign from malignant ovarian cysts by ultrasound. Br J Obstet Gynecol 85:893–899, 1978
49. Requard CK, Mettler FA, Wicks JD: Preoperative sonography of malignant ovarian neoplasms. Radiology 137:79–84, 1981
50. Vasilev SA, Schlaerth JB, Campeau J, Morrow CP: Serum CA125 levels in preoperative evaluation of pelvic masses. Obstet Gynecol 71:751–756, 1988
51. Fukazawa I, Inaba N, Ota Y, Sato N, Shirotake S, Iwasawa H, Sato T, Takamizawa H, Wiklund B: Serum levels of six tumor markers in patients with benign and malignant gynecological disease. Arch Gynecol Obstet 243:61–67, 1988
52. Malkasian GD, Knapp RC, Lavin PT, Zurawski VR, Podratz KC, Stanhope CR, Mortel R, Berek JS, Bast RC, Ritts RE: Preoperative evaluation of serum CA 125 levels in premenopausal and postmenopausal patients with pelvic masses: Discrimination of benign from malignant disease. Am J Obstet Gynecol 159:341–346, 1988
53. Bast RC, Klug TL, St. John E, Jenison E, Niloff JM, Lazarus H, Berkowitz R, Leavitt T, Griffiths T, Parker L, Zurawski VR, Knapp RC: A radioimmunoassay using a monoclonal antibody to monitor the course of epithelial ovarian cancer. N Engl J Med 309:883–887, 1983
54. Klug TL, Bast RC, Niloff JM, Knapp RC, Zurawski VR: A monoclonal antibody immunoradiometric assay for an antigenic determinant (CA 125) associated with human epithelial ovarian carcinomas. Cancer Res 44:1048–1053, 1984
55. Canney PA, Moore M, Wilkinson PM, James RD: Ovarian cancer antigen CA125: A prospective clinical assessment of its role as a tumor marker. Br J Cancer 50:765–769, 1984
56. Atack DB, Nisker JA, Allen HH, Tustanoff ER, Levin L: CA125 surveillance and second-look laparotomy in ovarian carcinoma. Am J Obstet Gynecol 154:287–289, 1986
57. Krebs HB, Goplerud DR, Kilpatrick SJ, Myers MB, Hunt A: Role of CA125 as tumor marker in ovarian carcinoma. Obstet Gynecol 67:473–477, 1986
58. Alvarez RD, To A, Boots LR, Shingleton HM, Hatch KD, Hubbard J, Soong SJ, Potter ME: CA125 as a serum marker for poor prognosis in ovarian malignancies. Gynecol Oncol 26:284–289, 1987