



Original Research Report

Gestational trophoblastic diseases: 3. Human chorionic gonadotropin-free β -subunit, a reliable marker of placental site trophoblastic tumors

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Abstract

Objectives. Placental site trophoblastic tumor (PSTT) commonly presents with low and variable concentration of hCG immunoreactivity in serum which can be difficult to differentiate from early stage choriocarcinoma/gestational trophoblastic neoplasm (GTN) or quiescent gestational trophoblastic disease (quiescent GTD). Nontrophoblastic malignancies such as germ cell tumors or other tumors secreting low hCG must also be considered in the differential diagnosis. Because treatments for these conditions are different, a means of differentiating PSTT from other diagnoses is important. We investigate the usefulness of hCG-free β -subunit to make this discrimination.

Methods. Data collected on cases referred to the USA hCG Reference Service for consultation served as a basis for this retrospective analysis. There were 13 cases with histology proven PSTT and 12 with nontrophoblastic malignancy. hCG-free β -subunit was measured by immunoassay and reported as a proportion of total hCG (hCG-free β -subunit(%)). hCG-free β -subunit(%) results were determined for all histologically proven cases of PSTT and for the nontrophoblastic malignancies. Comparisons of hCG-free β -subunit(%) were made and compared with those of the 82 choriocarcinoma/GTN cases and 69 quiescent GTD cases. The accuracy of hCG-free β -subunit(%) to discriminate these malignancies was analyzed by investigating the areas under receiver-operating characteristics curve \pm standard error.

Results. hCG-free β -subunit(%) was the predominant hCG form in cases of PSTT (mean \pm standard deviation, $60 \pm 19\%$) and nontrophoblastic malignancies ($91 \pm 11\%$), thus discriminating these diagnoses from choriocarcinoma/GTN ($9.3 \pm 9.2\%$) and from quiescent GTD ($5.4 \pm 7.8\%$). The cutoff of $>35\%$ free β -subunit is proposed. At this cutoff, 100% detection at 0% false-positive is achieved. The accuracy of hCG-free β -subunit(%) for this discrimination is $100 \pm 0\%$. At a proposed cutoff of $>80\%$, the free β -subunit test will also distinguish PSTT from nontrophoblastic malignancy, with 77% detection at 23% false-positive or an accuracy of $92 \pm 3.2\%$.

Conclusion. Measurement of the proportion hCG-free β -subunit(%) was found to be useful in the diagnosis of PSTT using proposed cutoff values of $>35\%$ and $>80\%$. While this finding needs to be confirmed by larger studies, it would be reasonable to measure hCG-free β -subunit(%) whenever the diagnosis of PSTT is considered.

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Keywords: hCG-free β -subunit; Placenta site trophoblastic tumor; Nontrophoblastic neoplasm

Introduction

Placental site trophoblastic tumors (PSTT) usually presents with amenorrhea or irregular vaginal bleeding often remotely following a normal pregnancy, spontaneous abortion, or occasional hydatidiform mole [1–3]. The interval between the occurrence of PSTT and the antecedent gestational event is

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unusually long compared with choriocarcinoma and other gestational trophoblastic neoplasms (GTN). The mean is 3.4 years, with a range of <2 to >5 years [1,4]. PSTT is generally associated with significantly lower hCG levels than choriocarcinoma (<200 mIU/ml) that fail to rise sharply over time [1]. Clinically, PSTT can readily be confused with quiescent gestational trophoblastic disease (GTD) or inactive choriocarcinoma [5]. Additionally, the accuracy of the initial pathologic diagnosis may be limited by the small amounts of tissue obtained by endometrial curettage. The definitive diagnosis may be difficult to achieve short of performing a hysterectomy. Although human placental lactogen (hPL) may be useful for diagnosing PSTT, the use is frequently limited immunohistochemistry rather than to serum tumor marker measurements. Because of the difficulty of discriminating malignant PSTT clinically, from quiescent GTD and choriocarcinoma/GTN, a reliable marker for differentiating these conditions is needed. hCG-free β -subunit measurements may fulfill this role.

Choriocarcinoma characteristically comprises hyperglycosylated hCG-producing mononuclear villous-origin cytotrophoblast cells with a variable content of regular hCG-producing multinucleated syncytiotrophoblast cells [1,7–9]. PSTT, in contrast, is a malignancy of nonvillous trophoblasts, a functionally separate, morphologically different tissue with dense eosinophilic cytoplasm [1]. Commonly, PSTT comprises mononucleated tissue with much necrosis [1]. While choriocarcinoma cells predominantly infiltrate through an intravascular mechanism, PSTT characteristically lacks this tendency for early and widespread vascular invasion. This makes PSTT different from the hyperglycosylated hCG-producing invasive cytotrophoblast described in the initial article in this series [9].

The hCG subunits produced in PSTT appear to be produced in insufficient concentrations to produce $\alpha\beta$ dimers, as governed by the law of mass action, thus leading to hCG-free β -subunit production. This is indicated by the high proportions of β -core fragment detected in PSTT patient urine samples [10]. It is well established that hCG-free β -subunit is also produced by some nontrophoblastic malignancies [11–14]. As such, any study considering the clinical use of this test to detect PSTT would also need to consider nontrophoblastic malignancies in the differential diagnosis. With the aim of finding a specific tumor marker to differentiate PSTT from quiescent GTD and choriocarcinoma, we examined the proportions of hCG-free β -subunit, hyperglycosylated hCG, and total hCG in PSTT and in patients with nontrophoblastic malignancies referred to the USA hCG Reference Service.

Methods

Patients

The USA hCG Reference Service evaluates parallel serum and urine samples from women with unusual, idiopathic or questionable hCG results. All cases were those referred to the Reference Service at Yale University ($n = 15$) and the University of New Mexico ($n = 292$), USA, between January 1998 and June 2005, and were retrospectively reviewed. This article concentrates on 13 patients demonstrated by histology to have PSTT.

In 7 cases, the patients had an incidental pregnancy test. This was positive but no intra- or extra-uterine pregnancy was found. Although the patients had no

physical or imaging evidence of disease, they were suspected of having GTN. They were referred to the Reference Service to find out whether the hCG was false-positive or real and to determine the source of hCG. In all seven cases, a predominance of hCG-free β -subunit immunoreactivity was shown, and PSTT or nontrophoblastic malignancy was predicted. PSTT was subsequently confirmed by histology 2–8 weeks after the referral.

In 6 cases with a recent history of PSTT, persistent low levels of hCG immunoreactivity were found. These 4 cases were referred to examine the nature and find the source of the hCG. Real hCG-free β -subunit immunoreactivity was detected, indicating the persistence of PSTT.

This report also describes 12 patients demonstrated by pathology to have a nontrophoblastic malignancy.

Five patients each had an incidental positive pregnancy test and intra- and extra-uterine pregnancy was excluded. They had no physical or imaging evidence of disease and were suspected of having GTN. They were referred to the Reference Service to investigate the nature (false-positive or real hCG) and deduce the source of hCG. Real hCG was shown together with a high proportion of hCG-free β -subunit immunoreactivity, PSTT or nontrophoblastic malignancy was predicted. From feedback information from referring physicians, nontrophoblastic malignancy was demonstrated by histology, 1 week to 2 months after the referral. These 5 cases were one embryonal ovarian malignancy, 3 ovarian dysgerminomas, and 1 parathyroid malignancy.

In 7 cases, nontrophoblastic malignancies had been diagnosed previously. Patients were referred to the Reference Service to determine if hCG results were real or false-positive. In all cases, real hCG immunoreactivity was demonstrated, mainly due to hCG-free β -subunit immunoreactivity.

All PSTT and nontrophoblastic malignancy cases referred to the USA hCG Reference Service are included, with the exception of 10 additional cases for whom no follow-up information or firm diagnosis became available. In these cases, Health Insurance Portability and Accountability (HIPAA) rules precluded us from obtaining outcome information.

For comparison in this study, we have included the 82 cases with choriocarcinoma or GTN, and the 69 with quiescent gestational trophoblastic disease described in the second article in this series [5]. Evaluation of the databases of the Reference Service data and examination of patient records were all approved by the University of New Mexico Human Research Review Committee (protocols 99-349 and 02-548).

The USA hCG Reference Service evaluates parallel serum and urine samples from women with unusual, idiopathic, or questionable hCG results. In all cases, records are carefully evaluated, and hCG and hCG-related molecule tests are performed [5,15–18]. These have always included our basic tests for total hCG (all forms of hCG-related molecule), a repeated total hCG test at multiple serum dilutions (to confirm results), a total hCG test after treatment with heterophilic antibody blocking agent (HBR, to consider false-positive results). An hCG-free β -subunit only test was performed at multiple dilutions to confirm results [15–18]. Concentrations of hCG-free β -subunit were converted into molar units (pmol/l) and the molar percentage of immunoreactivity due to hCG-free β -subunit (pmol/l \div pmol/l) was calculated. This percentage is called hCG-free β -subunit(%). In addition, a hyperglycosylated hCG (hCG-H) test was performed. This was also repeated at multiple dilutions, where possible, to confirm results. The percentage of hCG-H in relation to total hCG (hCG-H(%)) was calculated as described in the preceding article [5]. Additional tests were performed as needed. These were an intact hCG only test, a nicked hCG only test, a nicked free β -subunit only test, and a β -subunit core fragment only test [17,18]. This article is restricted to our basic tests, total hCG, hCG-free β -subunit, and hCG-H.

Laboratory tests

All laboratory testing was performed in the Reference Service laboratories. This is certified by the Department of Health and Human Services for performing clinical tests for patient records (CLIA certification 32D0972561). The consistency of the tests is monitored by the College of American Pathologists (CAP certification 7176750-01).

In all cases, parallel serum and urine samples were received. The samples were shipped frozen and thawed and tested immediately upon arrival. All basic testing involved automated assays using preformulated reagent packs. Serum total hCG was measured using the robotic chemiluminescence DPC Immulite hCG tests (DPC Inc., Los Angeles, CA). This assay detects hCG, hCG-H, and

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t1.1 Table 1

t1.2 Summary of cases with PSTT and nontrophoblastic neoplasms

t1.3		hCG, mIU/ml	hCG-free β (%)	hCG-H (%)	Reason for referral
t1.4	1. Cases having histology proven nontrophoblastic malignancy, $n = 12$				
t1.5	1	119	81	4	Myeloma shown, confirm real hCG
t1.6	2	474	68	2	Pancreatic cancer shown, confirm real hCG
t1.7	3	165	88	0	Endometrial cancer shown, confirm real hCG
t1.8	4	41	100	0	Karposi's sarcoma shown, confirm real hCG
t1.9	5	8.7	78	0	Dysgerminoma shown, confirm real hCG
t1.10	6	26	100	0	Ovarian embryonal shown, confirm real hCG
t1.11	7	40	100	0	Ovarian serous shown, confirm real hCG
t1.12	8	14	87	0	NED, quiescent or false-positive hCG or GTD? ^a
t1.13	9	160	100	0	NED, quiescent or false-positive hCG or GTD? ^a
t1.14	10	8.0	100	0	NED, quiescent or false-positive hCG or GTD? ^a
t1.15	11	2.9	100	0	NED, quiescent or false-positive hCG or GTD? ^a
t1.16	12	4.2	88	0	NED, quiescent or false-positive hCG or GTD? ^a
t1.17	Mean \pm SD	95 \pm 140 ^{b,c}	91 \pm 11 ^{b,c}	0.55 \pm 1.3 ^{b,c}	
t1.18	Range	2.9–474	68–100	0–4	
t1.19	2. Cases having histology proven PSTT, $n = 13$				
t1.21	13	28	69	16	NED, quiescent or false-positive hCG or GTD? ^d
t1.22	14	8.5	47	34	NED, quiescent or false-positive hCG or GTD? ^d
t1.23	15	231	38	37	NED, quiescent or false-positive hCG or GTD? ^d
t1.24	16	35	50	0	NED, quiescent or false-positive hCG or GTD? ^d
t1.25	17	12.8	68	0	NED, quiescent or false-positive hCG or GTD? ^d
t1.26	18	94	48	0	NED, quiescent or false-positive hCG or GTD? ^d
t1.27	19	13	62	0	NED, persistent mole suspected ^d
t1.28	20	0.77	46	0	PSTT history, active disease?
t1.29	21	3.3	39	0	PSTT history, active disease?
t1.30	22	236	82	0	PSTT previously shown, confirm real hCG
t1.31	23	25	90	0	PSTT previously shown, confirm real hCG
t1.32	24	138	97	5	PSTT previously shown, confirm real hCG
t1.33	25	31	52	0	PSTT previously shown, confirm real hCG
t1.34	Mean \pm SD	65 \pm 84 ^{e,f}	60 \pm 19 ^{e,f,g}	7.1 \pm 13 ^{e,f}	
t1.35	Range	3.3–236	38–97	0–37	
t1.36	3. Cases with choriocarcinoma/GTN, $n = 82$ [4]				
t1.38	Mean \pm SD	13380 \pm 30747	9.3 \pm 9.2	54 \pm 41	
t1.39	Range	5.9–144627	0–35	2.0–100	
t1.40	4. Cases with quiescent GTD, $n = 69$ [4]				
t1.42	Mean \pm SD	54 \pm 103	5.4 \pm 7.8	0.47 \pm 2.1	
t1.43	Range	0.5–144	0–30	0–12	

t1.44 All cases are those referred to the USA hCG Reference Service, no case was excluded. All cases are those having previously diagnosed disease or those predicted to have disease by the Reference Service and confirmed by histology shortly after referral. Approximately half of the cases were referred to the Reference Service to confirm the nature of the hCG, real versus false-positive, among other investigative alternative. For comparison, we summarize data from cases with quiescent gestational trophoblastic disease (quiescent GTD). These are the 69 cases described in Table 2 of the companion paper [4]; details not repeated. These are also 82 cases with pathology proven choriocarcinoma or GTN described in Table 1 of the companion paper [4]; details not repeated. hCG results, hCG-free β (%), and hyperglycosylated hCG (%) are presented, together with the reason for the referral.

^a Nontrophoblastic malignancy or PSTT predicted by presence of significant hCG-free β -subunit (%) by USA hCG Reference Service. Feedback confirmed nontrophoblastic malignancy by histology, 1 week to 2 months after the referral. In cases 8–12, a new parathyroid malignancy, new embryonal ovarian malignancy, and 3 new dysgerminoma cases identified, respectively.

^b In a t test, nontrophoblastic malignancy compared with choriocarcinoma/GTN, no significance observed with hCG ($P > 0.05$), significant difference observed with hCG-free β subunit(%) and hyperglycosylated hCG ($P < 0.00000001$, $P < 0.00000001$).

^c In a t test, nontrophoblastic malignancy compared with quiescent GTD, no significance observed with hCG or hyperglycosylated hCG ($P > 0.05$ and $P > 0.05$), significant difference observed with hCG-free β subunit(%) ($P < 0.00000001$).

^d Nontrophoblastic malignancy or PSTT predicted by presence of significant hCG-free β (%) by USA hCG Reference Service. Feedback confirmed PSTT by histology following hysterectomy or hysteroscopy within 2 weeks to 2 months after the referral (cases 13–19).

^e In a t test, PSTT compared to choriocarcinoma/GTN, no significant difference observed with hCG ($P > 0.05$), significant difference observed with hCG-free β -subunit(%) and hCG-H (%) ($P < 0.00000001$, $P < 0.00000001$).

^f In a t test, PSTT compared with quiescent GTD, no significance observed with hCG or hyperglycosylated hCG ($P > 0.05$ and $P > 0.05$), significant difference observed with hCG-free β subunit(%) ($P < 0.00000001$).

^g In a t test, PSTT compared with nontrophoblastic malignancy, no significance observed with hCG or hyperglycosylated hCG ($P > 0.05$ and $P > 0.05$), significant difference observed with hCG-free β subunit (%) ($P = 0.000008$).

164 hCG-free β -subunit on an equal molar basis. When the concentrations of pure
165 hCG, hCG-H, and hCG-free β -subunit were determined in molar units (nmol/l)
166 by absorbance at 278 nm, near-identical results were observed (in mIU/ml) in
167 the DPC Immulite hCG test (H-hCG result 99% and free β -subunit result 100%
168 of hCG standard concentration) [2,4,7].

169 Serum samples were tested for H-hCG using the Nichols Institute
170 Diagnostics robotic chemiluminescence hCG-H assay (Nichols Institute
171 Diagnostics, San Clemente, CA). This assay has <0.1% cross-reactivity with
172 hCG [7]. This assay is calibrated in ng/ml using a choriocarcinoma hCG-H
173 standard. As published, hCG-H mass values can be converted to hCG equivalents
174 (in mIU/ml) by multiplying by 11 [2,4,7]. hCG-H(%) was calculated as the
175 proportion of total hCG immunoreactivity due to hCG-H, or hCG-H / total hCG.

176 Serum hCG-free β -subunit was measured using the robotic chemilumines-
177 cence DPC Immulite hCG tests (DPC Inc., Los Angeles, CA). This assay is
178 calibrated in ng/ml.

179 It should be noted, that while the total hCG, hCG-H, and hCG-free β -subunit
180 assay are all commercially available and are all FDA-approved tests, they are
181 only approved for pregnancy applications. Gestational trophoblastic diseases
182 can be considered as pregnancy- or gestation-related applications, but these
183 cancer-related applications should be considered as “off-label” applications. We
184 have carefully evaluated all 3 tests and demonstrated their particular suitability
185 and accuracy, compared with other commercial hCG tests, for gestational
186 trophoblastic disease applications [15–18].

187 Data analysis

188 In June 2005, all accrued test results from 1998 to 2005, including dates,
189 ages, diagnoses, antecedent gestation data, and pertinent treatment histories,
190 were digitized by entry into Microsoft Excel 2003 spreadsheet (Microsoft Inc.,
191 Redmond, WA). Basic mean, range, and standard deviation statistics and *t*
192 statistics were determined in the Excel 2003 spreadsheet. Data groups were
193 ranked and nonparametric centiles were determined, and detection rates were
194 calculated at corresponding false-positive rates. Receiver-operating character-
195 istics (ROC) curves were plotted and areas under ROC curves determined as an
196 indicator of test accuracy, and their asymptotic standard errors calculated using
197 AccuROC software, version 2.4 (Accumetric Corp., Montreal, QC).

198 Results

199 In the serum from 13 cases with histologic proven PSTT, 4
200 new cases and 9 at different stages of primary therapy or
201 recurrent disease, the major portion of the total hCG
202 immunoreactivity was due to hCG-free β -subunit: mean
203 $60 \pm 19\%$, range 38–97% (Table 1). The levels of total hCG
204 (hCG + hCG-H + hCG-free β -subunit) were 3.3–263 mIU/ml
205 (Table 1). Minimal or no hCG-H was detected (9 cases with no
206 detectable hCG-H and 4 cases with 5–37% hCG-H,
207 mean \pm standard deviation $7.1 \pm 13\%$). Thus, PSTT cases are
208 marked by a middling proportion of hCG immunoreactivity due
209 to hCG-free β -subunit.

210 In the serum from 12 cases with histologic proven
211 nontrophoblastic malignancy, 5 new cases and 7 at different
212 stages of therapy, the total hCG immunoreactivity was due to
213 hCG-free β -subunit: mean $91 \pm 11\%$, range 68–100% (Table 1).
214 Variable amounts of total hCG were also detected, 2.9–
215 474 mIU/ml. Therefore, the majority of nontrophoblastic
216 neoplasm are associated with a high proportion of hCG
217 immunoreactivity due to hCG-free β -subunit.

218 The range of total hCG concentrations and proportions of
219 hCG-H in quiescent GTD cases overlapped with those observed
220 with PSTT and nontrophoblastic hCG malignancy cases (*t* test:
221 $P > 0.05$ and $P > 0.05$, respectively).

Table 2
Usefulness for an hCG-free β -subunit(%) test

Test comparison	AU-ROC	Cutoff >35%	Cutoff >80%
PSTT vs. quiescent GTD	100 \pm 0%	100% at 0%	
PSTT vs. choriocarcinoma/GTN	99 \pm 1.7%	100% at 0%	
Nontrophoblastic malignancy vs. quiescent GTD	100 \pm 0%	100% at 0%	
Nontrophoblastic malignancy vs. choriocarcinoma/GTN	100 \pm 0%	100% at 0%	
PSTT vs. nontrophoblastic malignancy	92 \pm 3.2%		77% at 23%

We examined the test at 2 arbitrary cutoff values, >35% and >80%. With each a
detection rate is given at a corresponding false-positive rate. We also
investigated the utility of the test independent of an arbitrary cutoff by
receiver-operating characteristic (ROC) analysis. This analysis plots infinite
cutoff points and their detection rates against the corresponding false-positive
rate. The area under the ROC curve (AU-ROC) is a direct measure of test
accuracy. AU-ROC curve \pm standard error (SE) results are presented.

We investigated the usefulness of hCG-free β -subunit(%) to
differentiate PSTT, nontrophoblastic malignancies, choriocar-
cinoma/GTN, and quiescent GTD (Table 2). This test was
100 \pm 0% accurate in differentiating PSTT from quiescent GTD,
99 \pm 1.7% for PSTT from choriocarcinoma/GTN, 100 \pm 0% for
differentiating nontrophoblastic malignancy from quiescent
GTD and choriocarcinoma/GTN, and 92 \pm 3.2% accurate for
differentiating PSTT and nontrophoblastic malignancy. The
results indicate the use of a >35% hCG-free β -subunit cutoff for
differentiating PSTT and nontrophoblastic malignancy from
quiescent GTD and from choriocarcinoma/GTN and >80%
hCG-free β -subunit cutoff for separating PSTT and nontropho-
blastic malignancy.

The usefulness of the free β -subunit(%) test was demon-
strated by the Reference Service experience with 12 patients
(Table 1), with an uncertain diagnosis. In all 12 cases,
nontrophoblastic neoplasm or PSTT was predicted correctly
by the finding of predominantly hCG-free β -subunit. In all 12
cases, nontrophoblastic neoplasm (5 patients) and PSTT (7
patients) were later confirmed by histology.

Discussion

The finding presented here indicate that hCG-free β -subunit
(%) determination can be used to definitively differentiate a
nontrophoblastic malignancy from a choriocarcinoma/GTN
diagnosis, in a patient with a history of pregnancy, hydatidi-
form mole or choriocarcinoma/GTN. hCG-free β -subunit(%)
can also point to a nontrophoblastic malignancy in patients
presenting with persistent low levels of hCG. hCG-free β -
subunit(%) can accurately discriminate a PSTT compared with
a choriocarcinoma/GTN and PSTT compared with quiescent
GTD in a patient presenting with persistent low levels of hCG
or with symptoms that suspect GTD. With less absolute
certainty, hCG-free β -subunit(%) can also differentiate a PSTT
from a nontrophoblastic malignancy. This retrospective
analysis was based on stratifying the laboratory values based
on the “gold standard” histologic confirmation of PSTT and
nontrophoblastic malignancies within the 7-year experience of

259 the Reference Service. It is of note that in 12 of 12 cases, the
 260 predicted histologies by the Reference Service from hCG-free
 261 β -subunit data were subsequently confirmed to be correct.
 262 Most of the data indicated an absolute $100 \pm 0\%$ discrimina-
 263 tion. We use the term indicate, however, because of the
 264 relatively small number of cases ($n = 25$) analyzed in this
 265 study. We invite larger confirmatory studies to confirm these
 266 findings.

267 The optimal use of hCG-free β -subunit is as hCG-free β -
 268 subunit(%), or that expressed as a molar percentage of total
 269 hCG [15–18]. Using a cutoff of $>35\%$, an absolute (based on
 270 $n = 25$) discrimination was made between PSTT plus
 271 nontrophoblastic malignancies and other GTD-related diagno-
 272 ses (choriocarcinoma/GTN and quiescent GTD). Using an
 273 alternative cutoff of $>80\%$, the discrimination of a nontropho-
 274 blastic malignancy from a PSTT can be achieved but with less
 275 certainty.

276 The primary purpose of this study was to identify a reliable
 277 means of diagnosing a PSTT other than definitive histology,
 278 compared with a choriocarcinoma/GTN, or a quiescent GTD in
 279 a patient presenting with persistent low levels of hCG, or
 280 symptoms of GTD. Treatment (hysterectomy, chemotherapy or
 281 expectant management) decisions are dependent on an accurate
 282 diagnosis [1,4]. When endometrial curettage suggests the
 283 diagnosis of PSTT in a patient desirous of future fertility, the
 284 patient and clinician are confronted by difficult and poignant
 285 choices. Hitherto, hysterectomy with histology has been
 286 recommended as the only means of making a definitive
 287 diagnosis. There is pressing need to be able to establish
 288 appropriate diagnosis without sterilizing surgery. Based on the
 289 present data, hCG-free β -subunit(%) may reliably fill this role.
 290 Some nontrophoblastic malignancies, however, also produce
 291 hCG-free β -subunit [11–14]. Not-surprisingly, production of
 292 hCG-free β -subunit is also present in a proportion of gonadal
 293 and germ cell malignancies [12,13], which may present with
 294 nodules in the uterus like GTN. As such, it is important to
 295 exclude nontrophoblastic neoplasms in patients with a history of
 296 pregnancy or hydatidiform mole, or among those presenting
 297 with persistent low levels of hCG. In our prospective experience
 298 with 12 cases suspected of GTN, one with history of
 299 hydatidiform mole, PSTT was confirmed by pathology in 7
 300 cases. Ovarian germ cell malignancies were identified in 4 cases
 301 (3 dysgerminoma and 1 embryonal malignancy), and 1 had a
 302 parathyroid malignancy. Based upon this experience, it may be
 303 as likely to find a germ cell tumor as the origin of the elevated
 304 free β -subunit as to find a PSTT.

305 We conclude that hCG-free β -subunit(%) discriminates
 306 PSTT and nontrophoblastic malignancies from other GTN
 307 possibilities, with extreme sensitivity. The statistical analysis
 308 also supports the use of hCG-free β -subunit(%) to differentiate
 309 PSTT from nontrophoblastic malignancy. Overall, hCG-free β -
 310 subunit(%) accurately differentiates PSTT from all other GTN
 311 and from nontrophoblastic malignancy option. These studies,
 312 however, are limited to 25 cases. While larger studies are
 313 needed to confirm these results, it is reasonable for clinicians to

start evaluating free β -subunit(%) measurements whenever 314
 PSTT is considered. 315

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