Human Chorionic Gonadotropin: Clinical Utility & Diagnostic Considerations

David G. Grenache, PhD, MT(ASCP), DABCC
April 13, 2009
Disclosures

David G. Grenache has no relevant financial interests regarding the material presented today.
Objectives

• Describe the structure, synthesis and function of hCG

• Discuss the clinical utility of hCG testing in the diagnosis and management of pregnancy, malignancy, and Down syndrome

• Discuss the causes of false-positive hCG results and persistently low hCG concentrations and explain investigations that can be used to identify each
Outline

• hCG structure and isoforms

• hCG assays and issues

• Clinical utility

• Persistent, low hCG
Human Chorionic Gonadotropin (hCG)

- Glycoprotein hormone family

- hCG: αβ
- LH: αβ
- FSH: αβ
- TSH: αβ
Human Chorionic Gonadotropin (hCG)

- Glycoprotein hormone family
hCG Structure

• Dimer is ~38,000 daltons
  – 30% of weight due to carbohydrate

• Alpha subunit
  – 92 amino acids
  – 2 N-linked carbohydrate chains
  – 5 disulfide bridges

• Beta subunit
  – 145 amino acids
  – 2 N-linked & 4 O-linked carbohydrate chains
  – 6 disulfide bridges

http://www.chem.gla.ac.uk/protein/glyco/hyper/hcg_act.html
Physiology of hCG

• Extends functional life of corpus luteum

• Maintains high progesterone concentrations in early pregnancy

• Thyrotropic at very high concentrations
hCG Heterogeneity

• Numerous molecular forms of hCG present in pregnancy serum
  – Dissociated or degraded molecules with no biological activity

• Key β-containing isoforms
  – Intact hCG
  – Nicked hCG
  – Free β subunit
  – Nicked free β subunit
  – β-core fragment (urine)

Cole, LA. Clin Chem 1997;43:2233-2243
Hyperglycosylated hCG (HhCG)

• O-linked carbohydrates on β chain larger than normal
  – 74% vs. 16% hexasaccharides

• Synthesized by invasive cytotrophoblasts
  – Implantation blastocysts
  – Choriocarcinoma

• Principal isoform produced in early gestation

Intact hCG

• Synthesized by syncytiotrophoblasts

• Serum concentrations increase progressively in early pregnancy
  – Peak at 7 – 9 weeks of gestation

• Decrease until ~24 weeks then plateau

hCG Assays

• All are FDA approved for assessment of pregnancy status only

• Approximately half of all hCG tests performed for this reason
  – 35% for maternal serum screening for Down’s syndrome
  – 15% for use as a tumor marker

• Quantitative tests should detect intact hCG and free beta subunit
Quantitative Assays

• Immunometric methods designed for use with serum

• Molecular heterogeneity influences assay performance
Assay Antibodies

## Table 1. Commonly identified antibody binding sites (epitopes) on hCG, its free subunits, and degradation products.

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<tr>
<th>Epitope</th>
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<td>Mutual site on hCG, free β, and β-core</td>
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</tr>
<tr>
<td>Anti-common β2</td>
<td>Separate mutual site on hCG and free β (β-core?)</td>
<td>✓ ✓ ✓ ✓ ±⁹</td>
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<tr>
<td>Anti-β C-terminal</td>
<td>Mutual site on hCG and free β only</td>
<td>✓ ✓ ✓ ✓ ✓</td>
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<tr>
<td>Anti-common α</td>
<td>Mutual site on hCG and free α</td>
<td>✓ ✓ ✓ ✓ ✓</td>
</tr>
<tr>
<td>Anti-free β</td>
<td>Free subunit-specific site, hidden on hCG</td>
<td>✓ ✓ ✓ ✓ ✓</td>
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<td>✓</td>
</tr>
<tr>
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<td>Mutual site on free β and β-core fragment</td>
<td>✓ ✓ ✓ ✓ ✓</td>
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<tr>
<td>Anti-β-core fragment</td>
<td>β-core fragment-specific site, hidden on free β</td>
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<tr>
<td>Anti-free α</td>
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</tbody>
</table>

* Some anti-common β antibodies also recognize β-core fragment.

Cole, LA. *Clin Chem* 1997;43:2233-2243
Assay Antibodies

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**Assay Antibodies**

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<td>Anti-β C-terminal</td>
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* Some anti-common β antibodies also recognize β-core fragment.
CAP Survey (2005)
AT THE HOSPITAL I HAD TO TAKE A PREGNANCY TEST—A WRITTEN PREGNANCY TEST.

1. How many babies are born every minute?
Qualitative Assays

- All can be performed with urine (waived) and some with serum (moderately complex)

- Majority of current tests are immunochromatographic
Qualitative Assays

Anti-β

Anti-α

Anti-Ab

Test zone

Control zone
Qualitative Assays

Anti-β

Anti-α

Anti-Ab

αβαβ

αβαβ

αβαβ
Variation Among Qualitative Assays

• Used for pregnancy detection so are designed to detect dimeric hCG isoforms

• Some detect additional, unexpected isoforms
Case Report

- 24-year-old female with endometrial adenocarcinoma presents to clinic to begin radiation therapy
- Reports being sexually assaulted 2 weeks prior
- Serum hCG of 56 IU/L so no therapy received
- Follow-up hCG tests
  - 4 weeks later: 45 IU/L
  - 6 weeks later: 60 IU/L
- False-positive hCG?
### Qualitative Serum and Urine hCG Results From Different Assays

<table>
<thead>
<tr>
<th>Device Name (Manufacturer)</th>
<th>Serum</th>
<th>Urine</th>
<th>hCG Detection Limits in Serum/Urine (mIU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sure-Vue (Fisher Healthcare, Houston, TX)</td>
<td>Negative</td>
<td>Positive</td>
<td>10/20</td>
</tr>
<tr>
<td>Poly-Stat (Polymedco, Cortlandt Manor, NY)</td>
<td>Negative</td>
<td>Negative</td>
<td>20/20</td>
</tr>
<tr>
<td>QuickVue (Quidel, San Diego, CA)</td>
<td>Negative</td>
<td>Positive</td>
<td>25/25</td>
</tr>
<tr>
<td>Signify (Genzyme Diagnostics, Cambridge, MA)</td>
<td>Negative</td>
<td>Negative</td>
<td>20/20</td>
</tr>
</tbody>
</table>

hCG, human chorionic gonadotropin.

*Performed on week 10 samples (Table 1).

†To calculate Système International units for serum values (IU/L), multiply by 1.0.

### hCG Results From Different Quantitative Assays

<table>
<thead>
<tr>
<th>Sample</th>
<th>DPC Immulite</th>
<th>DPC Free beta</th>
<th>Nichols Advantage ITA</th>
<th>Intact hCG Dimer</th>
<th>Nicked hCG</th>
<th>β Core Fragment</th>
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<tr>
<td>Serum</td>
<td>165</td>
<td>155</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>ND</td>
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<tr>
<td>Urine</td>
<td>683</td>
<td>249</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>&lt;5</td>
<td>1,360†</td>
</tr>
</tbody>
</table>

hCG, human chorionic gonadotropin; ND, not done.

*Performed on week 10 samples (Table 1). All results are in mIU/mL; to calculate Système International units for serum values (IU/L), multiply by 1.0. DPC Immulite and DPC Free beta from Diagnostic Products, Los Angeles, CA; and the Nichols Advantage ITA, Nichols Institute Diagnostics, San Clemente, CA.

†The β core fragment is detected only partially by the DPC Immulite assay.

## Qualitative hCG Device

<table>
<thead>
<tr>
<th></th>
<th>Sure-Vue</th>
<th>Clinitest</th>
<th>QuickVue+</th>
<th>Osom</th>
<th>hCG Combo</th>
<th>ICON II</th>
<th>Elecsys (IU/L)</th>
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<tbody>
<tr>
<td><strong>Capture Ab</strong></td>
<td>anti-α</td>
<td>anti-α hCG dimer</td>
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<td>anti-α</td>
<td>anti-α</td>
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<td>Pos</td>
<td>Neg</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>hCGβcf</td>
<td>Neg</td>
<td>Pos (weak)</td>
<td>Pos</td>
<td>Neg</td>
<td>Pos</td>
<td>Neg</td>
<td>Neg</td>
</tr>
</tbody>
</table>
Diagnosing Pregnancy – Home Tests

“More than 99% accurate when used on the day of the expected period”

“Use as early as 4 days before your expected period.”

“Test anytime of day. You do not have to use the first morning urine.”
Diagnosing Pregnancy – Home Tests

- 221 women planning to conceive kept menstrual diaries and froze daily urine for later analysis (151 pregnancies)

- Quantitative hCG determined by high-sensitive assay (0.13 IU/L detection limit)
  - Onset of pregnancy defined as earliest day of sustained hCG elevation

- 90% of pregnancies occurred by the day of the expected period
  - Detecting 99% of pregnancies required +10 days

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Wilcox, et al., JAMA 2001;286:1759-1761
Ectopic Pregnancy

• Extrauterine implantation of blastocyst
  – 98% occur in fallopian tube

• Incidence is estimated at 2% of all pregnancies

• Leading cause of maternal mortality in the 1st trimester
  – 1 death per 2000 ectopic pregnancies

• Diagnostic tools
  – Serial hCG (prolonged doubling time, 87% sensitive)
  – Transvaginal ultrasound (90% sensitive)
Ectopic Pregnancy

- **hCG**
  - <1500 IU/L
    - **Serial hCG**
      - Normal increase
      - >1500 IU/L
        - Ultrasound
          - IUP
            - No IUP
              - Surgical management
        - IUP
          - Abnormal increase
            - Surgical management
      - Ultrasound
        - IUP
          - No IUP
            - Surgical management
  - >1500 IU/L
    - Ultrasound
      - No IUP
        - Surgical management

Maternal Serum Screening for Down’s Syndrome

- **2nd trimester screening (16-18 weeks)**
  - Triple screen: hCG, AFP, uE3
  - Quadruple screen: hCG, AFP, uE3, Inhibin A

- **1st trimester screening (11-13 weeks)**
  - hCG or free beta subunit, PAPP-A, nuchal translucency (ultrasound)

UpToDate, 2007
Gestational Trophoblastic Disease (GTD)

- Heterogeneous group of interrelated lesions derived from an aberrant fertilization event
  - Hydatidiform mole (partial and complete)
  - Persistent/invasive GTD
  - Choriocarcinoma

- All produce hCG and hormone is used in diagnosis and to monitor response to therapy

- Very responsive to chemotherapy and treatment is most often single-agent therapy with methotrexate
hCG in GTD

- Concentrations may exceed 100,000 IU/L
- Assess tumor mass
  - Serum concentration correlates with the number of viable tumor cells
- Monitor therapy
  - Successful treatment leads to progressive decline of hCG, usually within 14 weeks
  - A rise or plateau suggests recurrence or persistence of disease
- Detect recurrence
  - ACOG recommends f/u hCG for 6 months

Schlaerth et al., Obstet Gynecol 1981;58:478
Persistent Low hCG

- Low concentrations of hCG that persist for months to years
  - hCG often <50 IU/L

- Uncommon event attributed to
  - False-positives
  - Pituitary hCG
Persistent Low hCG – The Clinical Problem

• hCG tests performed on women prior to interventions that could harm fetus
  – hCG cutoff of <5.0 IU/L used for pregnancy diagnosis

• Standardized protocols result in the use of hCG testing even in women who are unlikely to be pregnant (e.g. menopausal, hysterectomy)

• Positive results create clinical fusion, may delay needed therapies, or result in unnecessary therapy
Jury awards $15.5 million to woman misdiagnosed with cancer. UW and drug company share blame

Seattle Post-Intelligencer
Saturday, June 30, 2001
False-positive hCG

• Patient consulted doctor about irregular bleeding between menstrual periods
  – Positive serum hCG but no apparent intra-uterine pregnancy

• 11 month ordeal to identify cause

• Treatments
  – Laparoscopy for presumed ectopic pregnancy (none)
  – D&C (normal)
  – Chemotherapy, single and multi-agent (no hCG change)
  – Hysterectomy (no hCG change)
  – Surgery for removal of lung nodules found by CT (no disease)

• hCG eventually found to be false-positive due to interfering antibody in patient’s serum

Interfering Antibodies

**Heterophile antibodies**
- Antibodies with broad but weak reactivity for many different antigens
- Naturally occurring and originate from early stages of B-cell immunoglobulin synthesis
- Distinct from antibodies produced against specific animal immunoglobulins

**Human anti-animal antibodies (HAAA)**
- Produced after exposure to a defined antigen
- Exposure to therapeutic animal immunoglobulin or pharmaceutical agents derived from animal sources
- Immunogen is often unknown and its source remains unclear
- Human anti-mouse antibody (HAMA) is the most common HAAA
Mechanism of Interference - Positive

Real hCG present

Interfering antibody cross-links reagent antibodies

Falsely increased result
Mechanism of Interference - Negative

Real hCG present

Interfering antibody binds only 1 reagent antibody and prevents binding of hCG

Falsely decreased result
Detection

• Suspicion of interfering antibodies should be high when immunoassay results are inconsistent with the clinical scenario.

• Lab personnel are frequently unaware of clinical condition of patient
  – Difficult for lab to identify independently.

• What to do when asked “could this result be falsely increased?”
Urine hCG

- Real hCG should be detectable in the urine
- Antibodies too large to be filtered by kidney
- Negative urine hCG suggests interfering antibody present
Serial Dilution

- Interfering antibodies are reactive against the assay reagents and not the measured analyte.

- Serial dilution of specimen may not produce the expected linear response.
Different Assay

- Repeat test using an immunoassay that utilizes antibodies produced from a different animal species

- Not fool-proof as some interfering antibodies are not species-specific
  - May cross react with multiple animal species and interfere with multiple assays
Blocking Agents

• Remove interfering antibody through use of material that binds to it
  – Substantial change in concentration after use suggests interfering antibody present

• Non-immune globulin from the same species of animal used to produce the assay antibodies

• Effectiveness depends on interfering antibody class, specificity, and concentration

• Immunoassay reagents often contain blocking agents
Pituitary hCG

- First reported 30 years ago
- Gonadotrope cells of pituitary gland produce small amounts of hCG
Pituitary hCG

- First reported 30 years ago
- Gonadotrope cells of pituitary gland produce small amounts of hCG
Pituitary hCG –
The Clinical Problem

• JP is a 49-year-old female with ESRD secondary to polycystic kidney disease. She has been on peritoneal dialysis for 2 years and is admitted for a deceased-donor renal transplant.

• Pre-transplant screening reveals an hCG of 12 IU/L.

• The renal team calls to ask what this means.
1. Determine upper hCG limit in peri- and post-menopausal women

2. Evaluate the utility of serum FSH to assist in the interpretation of a positive hCG result

hCG in Aging Women

• 4 cohorts
  – Pregnant ($\geq$18 y)
  – Non-pregnant, pre-menopausal (18 – 40 y)
  – Non-pregnant, peri-menopausal (41 – 55 y)
  – Non-pregnant, post-menopausal (>55 y)

• No history of trophoblastic disease or ectopic pregnancy

• Menopause status defined by age alone

hCG is Correlated with Age

hCG Reference Intervals by Age

hCG >5.0 IU/L
Peri-menopausal, N=3
Post-menopausal, N=16

Table 1. hCG concentration ranges and the 97.5 percentile values for the nonpregnant cohorts in the study.

<table>
<thead>
<tr>
<th>Nonpregnant cohort</th>
<th>n</th>
<th>hCG range, IU/L</th>
<th>97.5 percentile, IU/L</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premenopausal, 18–40 years</td>
<td>240</td>
<td>&lt;2.0 to 4.6</td>
<td>5</td>
<td>2.5</td>
</tr>
<tr>
<td>Perimenopausal, 41–55 years</td>
<td>240</td>
<td>&lt;2.0 to 7.7</td>
<td>8</td>
<td>4.8</td>
</tr>
<tr>
<td>Postmenopausal, &gt;55 years</td>
<td>240</td>
<td>&lt;2.0 to 13.1</td>
<td>14</td>
<td>7.7</td>
</tr>
</tbody>
</table>

a Compared with the nonpregnant premenopausal cohort.
b Compared with the nonpregnant premenopausal and nonpregnant perimenopausal cohorts.

FSH vs. Age

FSH by Cohort & hCG Status

- FSH cutoff of 20 IU/L differentiates hCG of pregnancy from pituitary hCG

Interpreting Low hCG Results

hCG 5.0 – 14.0 IU/L

Age 18-40 y

Age 41-55 y

Age >55 y

Measure serum FSH

FSH ≤20 IU/L

Possible pregnancy

FSH >20 IU/L

Pregnancy unlikely
Study Limitations

• Analytical variation among hCG assays
• Interfering antibodies
• Age as criteria for menopause status
• Estrogen therapy
• 3 peri-menopausal patients with low pos hCG
  – Need to validate FSH of 20 cutoff in peri-menopausal group
Follow-up Investigation

• Validate the 20 IU/L FSH cutoff for excluding pregnancy in 41-55 yo with hCG 5-14 IU/L

• 100 patients desired
  – Need ~25,000 hCG results
  – 7 medical center laboratories
  – 80% of hCG and FSH assays represented

Inclusion/Exclusion Algorithm

MD-ordered hCG
N=39,742

Female sex
N=4,415 (11%)

Excluded

Male sex
OR
Female ≤40 or ≥55 y
N=35,327 (89%)

Female sex
41 – 55 y
N=4,415 (11%)

Excluded

hCG ≤5 or ≥14 IU/L
N=4,256 (96%)

Chart available
For review
N=100

hCG 5 – 14 IU/L
N=159 (3.6%)

Excluded

Chart not available
OR
QNS for FSH
N=59

Results

- 77 hCG not of placental origin
- 23 hCG of placental origin
  - 17 resolving abortion/miscarriage
  - 4 GTD
  - 2 early pregnancies

<table>
<thead>
<tr>
<th>Cutoff</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 IU/L</td>
<td>83%</td>
<td>84%</td>
</tr>
<tr>
<td>45 IU/L</td>
<td>100%</td>
<td>76%</td>
</tr>
</tbody>
</table>

Fig. 2. Scatter plot of hCG vs FSH concentrations. Open circles represent nonplacental hCG; n = 77. Closed circles represent placental hCG; n = 23. The dashed line represents a FSH cutoff of 45 IU/L.

Summary

• hCG is a heterogeneous molecule that exists as numerous isoforms.

• hCG assays show considerable variability in the isoforms of hCG that they detect.

• There is a biological limit regarding the early detection of urinary hCG in pregnancy.

• Serum hCG is useful in conjunction with ultrasound for the diagnosis of ectopic pregnancy.

• hCG is an essential biomarker in screening for Down’s syndrome and as a monitor of GTD.
Summary

• False-positive hCG tests are infrequently encountered but can have significant consequences if not recognized.

• Serum hCG increases with age in non-pregnant women.

• A cutoff of higher than the often used 5 IU/L should be utilized when interpreting hCG results in women >55 years of age.

• Pregnancy is unlikely in peri-menopausal women 41-55 years of age with an hCG between 5.0 and 14.0 IU/L if serum FSH is >45 IU/L.
Case Follow-up

49 yo renal transplant patient with hCG of 12 IU/L

• FSH = 215 IU/L
  – More consistent with menopausal status than pregnancy

• Renal transplant was performed
## Acknowledgements

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