Improved Methods for Detecting β-Core in Normal and Cancer Patient Urines, Akira Tanaka and Laurence A. Cole1 (Dept. of Obstet. and Gynecol., Yale University, Cedar St., New Haven, CT 06520; 1 author for correspondence: fax 203-785-6367)

β-Core fragment (also referred to as β-core, Urinary Gonadotropin Peptides, UGF, and UGP) is the postsecretion degradation product of the β-subunit of human chorionic gonadotropin (hCG). In 1988, our laboratory used a specific immunooassay (from O'Connor, Krichevsky, and Canfield) to demonstrate β-core in 50 urines from women with gynecological cancers and to determine that its concentrations rise and fall concordant with tumor mass (1, 2). This observation has now been confirmed by other centers (1–4). Commercial kits have been developed for detecting this tumor marker. The Ciba-Corning Diagnostics (Alameda, CA) UGP kit is sold in Europe and the US for research purposes. This kit, and the β-core kit from Wako Chemicals (Osaka, Japan), are approved by the government in Japan and are used for detecting and monitoring gynecological cancers. A third kit, UGP from Toagosei Co. (Tokyo, Japan), is waiting approval.

All β-core kits have a significantly higher false-positive rate for postmenopause than premenopause urine samples (1–5). For example, Nam et al. (2) indicated 3.2% false-positive results for healthy premenopause and 17% for postmenopause urines; deMedeiros et al. (4) indicated 5.6% false positives for healthy premenopause and 19% for postmenopause urines; and Maruo et al. (3) indicated 2.4% false positives for women <50 years and 15% for those older. hCG β-core closely resembles the luteinizing hormone (LH) β-core produced by the healthy pituitary gland. Sequence analysis indicates <12% difference in the amino acid sequence of the two molecules (5). Thus, antibodies in β-core kits may also detect LH β-core. It has been suggested that LH β-core causes the greater false-positive rates in postmenopause urines (6).

Ciba Corning Diagnostics recently refined their UGP kit (current kit). They incorporated an LH β-subunit scavenger antibody into the assay to reduce detection of LH β-core and decrease the false-positive rate for detecting hCG β-core. With concern about the β-core postmenopause false-positive problem, we acquired this new kit, compared it with the first-generation UGP kit, and determined to what extent the added scavenger antibody solves the high rate of false-positives issue.

β-Core tests are used with other marker methods to test women suspected of gynecological cancer, to differentiate benign disease and cancer, and to monitor the progress of therapy in those with proven malignancy. Test groups were selected to allow evaluation of these three applications for β-core measurements. Urines were collected from 132 individuals suspected of cancer who were referred to the gynecology oncology clinic at Yale University and found to be free of disease ("healthy" test group). Samples were also collected from 180 individuals suspected of cancer who were referred to the clinic and found to have benign gynecologic disorders ("benign disease" test group). Further urine samples were collected at the clinic from women treated for proven gynecological cancers and found, at the time of collection, to clinically have no evidence of disease (N.E.D. test group). Urine samples were also collected at the gynecology oncology clinic at Yale University, and at the Asan Medical Center and the Korea Cancer Center in Korea, from 240 individuals with active gynecological cancers (cancer group). Samples were collected in accordance with the ethical standards of Yale University Human Investigations Committee.

Samples were tested with the first-generation and the current UGP kits from Ciba Corning Diagnostics. The assay procedures were those described in the manuals, and the customary cutoff value of 3 pmol/L was adopted (2).

Table 1 shows the false-positive rates for premenopause and postmenopause urine samples at the customary cutoff value. The false-positive rates for the postmenopause women in the healthy, benign disease, and N.E.D. groups tested with the first-generation UGP kit were 13%, 17%, and 18%, respectively, and 16% overall. For the same groups tested with the refined UGP kit, the rates were lower: 5.7%, 13%, and 12%, respectively, and 9.9% overall. The improvement in false-positive rate was less apparent for premenopause women, being 4.3% overall with the first-generation kit and 3.9% overall with the refined UGP kit. Examining all control groups (healthy, benign, and N.E.D.) pre- and postmenopause, we determined that the false-positive rate was 10% with the first-generation kit (n = 293) and 6.9%, or almost one-third lower, with the refined UGP assay incorporating the scavenger antibody.

We then compared the sensitivities of the two UGP kits for cancer samples. The true-positive rates for 240 cervical, ovarian, and endometrial cancers (mostly early-stage) were 27% with the first-generation UGP kit and 33% with the refined kit. The small difference in true-positive rates was considered attributable to population error. We inferred that the refinements made to the UGP kit to lower the false-positive rate do not adversely affect the sensitivity of the test for detecting cancer.

We used the customary cutoff of 3 pmol/L in these studies; changing this value would, of course, modify the true- and false-positive results. To compare the first-generation and refined assays independent of an arbitrary cutoff, we used receiver-operating characteristic (ROC) curve analysis, which measures the effectiveness of an assay to discriminate two populations. A value of 0.5 for the area under an ROC curve indicates no discrimination, whereas 1.0 shows complete discrimination (7). The area under the ROC curve value, postmenopause controls vs cancer, was 0.71 for the first-generation kit and 0.80 for the refined kit: This suggested a slight improvement in the discrimination capabilities of the refined kit. The value for postmenopause controls vs cancer was 0.52 for the first-generation kit and 0.73 for the refined kit, which suggests a major improvement from almost no discrimination to significant discrimination of postmenopause and cancer samples. The ROC curve data showed that the refined kit better discriminated healthy and cancer populations (0.74 vs 0.64 ROC area), and benign disease and cancer (0.78 vs 0.61 ROC area), than did the first-generation kit. Significant improvement in the assay was suggested (t-test; ROC statistics of old and
Table 1. False-positive rates of first-generation and refined UGP kits for healthy and benign disease urine samples.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>First-generation UGP kit</th>
<th>Refined UGP kit (current assay)</th>
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<tbody>
<tr>
<td></td>
<td>No. of samples</td>
<td>No. (%) with beta-core ≥3 pmol/L</td>
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<tr>
<td>Control groups</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy</td>
<td></td>
<td></td>
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<tr>
<td>Premenopause</td>
<td>50</td>
<td>3 (6.0)</td>
</tr>
<tr>
<td>Postmenopause</td>
<td>47</td>
<td>6 (13)</td>
</tr>
</tbody>
</table>
| Total                      | 97           | 9 (9.3)                           | 0.64      | 132          | 6 (4.6)                           | 0.74*
| Benign disease             |              |                                   |           |              |                                   |           |
| Premenopause               | 91           | 3 (3.3)                           |           | 103          | 4 (3.9%)                          |           |
| Postmenopause              | 71           | 12 (17)                           |           | 77           | 10 (13)                           |           |
| Total                      | 162          | 15 (9.3)                          | 0.61      | 180          | 14 (7.8)                          | 0.78*
| N.E.D. after cancer, postmenopauseb | 34 | 6 (18)                            |           | 34           | 4 (12)                            |           |
| Total premenopause         | 141          | 6 (4.3)                           | 0.71      | 165          | 6 (3.6)                           | 0.80*
| Total postmenopause        | 152          | 24 (16)                           | 0.52      | 181          | 18 (9.9)                          | 0.73*
| All control groups, pre- and postmenopause | 293 | 30 (10)                           | 0.60      | 346          | 24 (6.9)                          | 0.80*
| Gynecological cancer, totalc | 240          | 64 (27)                           |           | 206          | 68 (33)                           |           |

* Area under ROC curve: results for pre- and postmenopause controls compared with results for gynecological cancer.

b All are postmenopausal after cancer surgery.

c Gynecological cancer group was 24% ovarian, 27% endometrial, and 49% cervical cancers, and most were early-stage disease (42% stage I, 26% stage II, 24% stage III, and 8% stage IV).

Refined assays, \( P < 0.001 \). Examining all control groups pre- and postmenopause, the area under ROC curve value was 0.60 for the first-generation kit and 0.80 for the refined kit, which suggests an overall improvement in the discrimination capabilities of the kit (\( t \)-test, ROC statistics of old and refined assays, \( P < 0.001 \)).

Most new cases of gynecological cancers occur after menopause. The high false-positive rates for postmenopause urines, found with all beta-core immunoaassays, has limited the use of beta-core determinations in cancer detection and in the differential diagnosis of benign and malignant disease. In this comparative study, we show that the refined UGP kit with an LH beta-subunit scavenger antibody to reduce the false-positive rate better discriminates cancer and control postmenopause urine samples. We believe that this advance in the detection of beta-core will partly surmount the false-positive problem that has limited its use as a tumor marker.

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References


Cystic fibrosis (CF) is one of the most common autosomal recessive disorders in Brittany, with 1 in 1600 to 1 in 2500 affected newborns and a carrier frequency close to 1 in 20 (1).

In the Celtic population, 80% of the CF chromosomes carry a 3-bp deletion that results in the loss of a phenylalanine residue at position 508 (\( \Delta F508 \)) of the CF transmembrane conductance regulator protein (CFTR). Among the non-\( \Delta F508 \) mutations identified in the Celtic population, one of the most frequently seen (5.7%) is the loss of a single thymine nucleotide at position 1078 in exon 7 of the CFTR cDNA (1078 delT) (2, 3). This mutation may be detected rapidly in a whole sample from a single population by polymerase chain reaction (PCR)-mediated site-directed mutagenesis (PSM). When the molecular defect neither creates nor destroys a restriction enzyme cutting-site, PSM transforms any alteration in DNA sequence into an allele.