Laboratory Evaluation of Estrogen Metabolism

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Recent enthusiasm about the potential benefits of hormone replacement therapy (HRT), using conjugated equine estrogens and synthetic progesterins for menopause, has undergone a remarkable reversal. Studies of this form of HRT failed to demonstrate some of the expected advantages of HRT, but did find elevated risk of breast cancer and stroke. Women considering HRT and their healthcare practitioners face a bewildering and often contradictory array of research results and opinions to evaluate. The purpose of this review is to discuss the use of laboratory tests to quantify a patient's estrogen exposure, and efforts that are underway to assess individual patterns of estrogen metabolism in relation to adverse effects.

A number of the most common malignant diseases, including breast and endometrial cancer, seem to be caused by the interaction of multiple genes and environmental factors. A woman's chances of developing one of these cancers can be estimated by determining her individual profile of risk factors, which reflect the various environmental and genetic contributions to the development of the disease. Many of the known risk factors for breast cancer, such as early age at menarche and late menopause, reflect a woman's lifetime exposure to estrogens. Estrogens have the potential to act as both initiators and promoters of malignancies.1

Many studies have been conducted in which women's estrogen concentrations in blood and/or urine were determined, and then the women were followed over many years and monitored for the development of breast cancer. Other studies have measured the hormone concentrations women achieve during HRT in relation to premenopausal values. Recently, a great deal of research has focused on individual patterns of estrogen metabolism. Remarkably, some estrogen metabolites appear to be highly procarcinogenic, while others actually have anti-cancer activity. There is a growing consensus that a woman's estrogen-related cancer risk is intimately related to her individual metabolic handling of estrogens, and the balance of pro- and anti-cancer metabolites produced. New tests are being developed to evaluate a woman's estrogen metabolic profile, which may allow us to identify women at high risk for development of estrogen-related cancers.

Specimens Used to Measure Estrogens

Serum and urine have been the most popular specimens for measuring estrogens. Serum measures circulating hormone levels at the time of the blood draw; 24-hour urine collections give a measure of total daily output. Saliva specimens offer ease of collection, and generally correlate well with serum values for individuals not receiving HRT. However, only serum and urine have been used in large-scale epidemiological studies of cancer risk.

Premenopausal women's estrogen concentrations fluctuate during the menstrual cycle in a predictable pattern, and expected ranges are available for each stage of the cycle.2 Postmenopausal women's estrogen values are more stable. Research has also addressed the issue of how consistent premenopausal women's estrogen concentrations are from cycle to cycle.3,7 In general, the mean value for a group of women, collected at a certain point in the cycle, is quite stable from cycle to cycle. Values for individual women are somewhat more variable from cycle to cycle.

Which Estrogens Are Measured?

Older studies focused on estrone (E1), estradiol (E2), and in some cases estriol (E3). Estradiol is the most important active estrogen physiologically, and it interconverts readily with estrone. Estriol, which is found in very high concentrations during pregnancy, is a terminal estrogen metabolite that is less potent than estradiol (Figure 1).

Recent studies have focused on a number of other estrogen metabolites. Two of these, 4-hydroxyestrone, and 16a-hydroxyestrone, are genotoxic, mutagenic and procarcinogenic.4,5 On the other hand, 2-hydroxyestrone and 2-methoxyestrone have very weak estrogenic properties; 2-methoxyestradiol, which is normally present in the body in trace quantities, has significant anticancer properties, and is currently being evaluated in clinical trials for treatment of several types of cancer.6 (Figure 1)

Estrone, Estradiol, and Estriol and Breast Cancer Risk

Studies have consistently shown that postmenopausal women with higher

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Figure 1

Some Important Estrogen Metabolites

Estradiol (E2)

 Estrone (E1)

CYP1A1

CYP1B1

CYP3A4

2-Hydroxyestrone

4-Hydroxyestrone (Genotoxic)

16a-Hydroxyestrone (Genotoxic)

COMT

2-Methoxyestrone

4-Methoxyestrone

Estriol (E3) (Protective?)

2-Methoxyestradiol (Anticancer)
levels of estradiol and estrone in their serum and/or urine have a moderately elevated risk of developing breast cancer. Postmenopausal women in the top quartile for urinary estrone excretion have about 2.5 times the risk of developing breast cancer, compared to women in the bottom quartile. Women in populations with low breast cancer risk have lower estrogen concentrations, both before and after menopause, than women in higher risk groups. For example, the higher breast cancer risk for US Caucasian and African-American women, compared to Chinese women living in Singapore, may be explained by the difference in urinary estrogen excretion rates: US women excrete 162% more estrone, 152% more estradiol, and 92% more estradiol than Singapore Chinese women.

Of the three primary estrogens, estrone seems to have the strongest relationship with elevated breast cancer risk, and estradiol has the weakest. In 1969, Cole and MacMahon suggested that estradiol may act to protect cells from the proliferative action of estradiol. It has been proposed that higher values for the estrogen quotient (E3/E1+E2) are associated with reduced breast cancer risk (see Wright and Morgenthaler for a review of the literature). Not all studies support the "estradiol hypothesis." But the fact remains that the estrogen quotient is higher in some lower risk populations, such as the Chinese women living in Singapore discussed above. Laboratory studies of the interaction of estradiol and the estrogen receptor also lend some support to the estradiol hypothesis.

Finally, a recent abstract by Siterri et al. describes a study in which the estradiol concentration during pregnancy was determined in 15,000 women who were subsequently followed for a period of 40 years. Remarkably, women in the highest quartile for urinary estradiol excretion during pregnancy had a 58% lower incidence of breast cancer during the follow-up period, when compared to women in the lowest quartile. Because alternative HRT regimens often include estradiol alone or in combination with other natural estrogens, further research on the estrogen quotient is urgently needed.

### Measurement of Estrogens in Women Receiving HRT

In 1994, Tepper et al. published a paper entitled, "Estrogen replacement in postmenopausal women: Are we currently overdosing our patients?" They showed that a majority of women receiving the recommended 2 mg of estradiol orally reached peak serum concentrations higher than the peak ovulatory levels normally measured in premenopausal women. As pointed out by Cohen in Overdose: The Case Against the Drug Companies, recommended doses for many pharmaceuticals are too high, because of problems with clinical trial designs. According to Cohen, an oral dose of 0.5 mg estradiol is sufficient for many women. At this lower estradiol dose, serum and urinary estrogen concentrations are more closely matched to premenopausal values. A recent study in JAMA reported improved bone mineral density at estradiol doses of 0.25 mg/day. Women with renal disease may require even lower oral doses. Lower estradiol doses are also possible with transdermal administration.

Cohen observes a similar pattern with Premarin. From 1974-1987, the recommended dose was 1.25 mg/day. In 1988, the recommended dose was lowered to 0.625 mg/day, and in 2003, the FDA approved a 0.3 mg formulation. Current guidance emphasizes use of the lowest possible dose of estrogens for HRT, based on the unproven but reasonable assumption that adverse effects will be less common at lower doses.

The route of administration has a major impact on the urinary hormone profiles of women receiving estrogens for HRT. Urinary estrogens are typically higher in women who receive them orally, compared to women receiving the same dose via creams prepared by compounding pharmacies. In our experience, the urinary profiles of women receiving the cream formulations are more closely matched to those of premenopausal women (Table 1).

### Estrogen Metabolism

Further research is needed to clarify the effect of route of administration on estrogen bioavailability. Based on the urinary profiles we see at Meridian Valley Laboratory, it appears that some women taking typical doses of TriEst (80% estradiol, 10% estrone, 10% estradiol) or EiEst (80% estradiol and 20% estradiol) orally may reach excessive levels of estrone.

Smoking appears to have a major impact on estrogen metabolism. Smoking stimulates the metabolism of estrogens, resulting in reduced effects. Compensating for accelerated metabolism by increasing the estrogen dose may be dangerous, because some of the estrogen metabolites produced may be especially toxic and pro-carcinogenic.

### The 2/16 Estrogen Metabolite Ratio Test

As shown in Figure 1, in addition to the three classical estrogens, a number of other estrogen metabolites are important physiologically. The 4-hydroxy and 16-a hydroxy estrogen metabolites are genotoxic, and may be directly involved in carcinogenesis. On the other hand, 2-hydroxy estrone and its downstream metabolites are only weakly estrogenic. (As noted above, 2-methoxy estradiol, normally present in trace quantities, actually has anticancer properties.) Based on the different effects of these estrogen metabolites, Bradlow and colleagues developed the urinary estrogen metabolite ratio test, to evaluate a patient's balance of "good" (2-hydroxy) and "bad" (16-a hydroxy) estrogens. Studies in premenopausal Caucasian women showed that a higher 2/16 metabolite ratio was associated with a decreased risk of estrogen-related cancers. Further studies showed that the 2/16 ratio can be increased by a diet rich in cruciferous vegetables and supplements containing compounds which induce formation of additional CYP1A1, an enzyme responsible for producing, among other important metabolites, 2-hydroxy estrogens.

Studies of the 2/16 estrogen metabolite ratio in post-menopausal and non-Caucasian women have painted a more complex picture. In one major study conducted in Italy, higher 2/16 estrogen metabolite ratios were associated with a reduction in breast cancer in premenopausal women, but higher ratios in postmenopausal women were...

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**Table 1 - Influence of Route of Administration on Urinary Estrogen Profiles**

<table>
<thead>
<tr>
<th>Route of Administration</th>
<th>Estrone</th>
<th>Estradiol</th>
<th>Estradiol</th>
<th>Quotient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal range (Luteal phase)</td>
<td>3-62</td>
<td>1-27</td>
<td>9-60</td>
<td>&gt;1.0 desirable</td>
</tr>
<tr>
<td>Patient 1 Transdermal</td>
<td>33</td>
<td>17</td>
<td>165</td>
<td>3.3</td>
</tr>
<tr>
<td>Patient 2 Oral</td>
<td>30</td>
<td>23</td>
<td>366</td>
<td>7</td>
</tr>
<tr>
<td>Patient 3 Transdermal</td>
<td>88</td>
<td>37</td>
<td>1264</td>
<td>10.2</td>
</tr>
<tr>
<td>Patient 4 Oral</td>
<td>81</td>
<td>24</td>
<td>935</td>
<td>8.9</td>
</tr>
</tbody>
</table>
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associated with an increased risk of breast cancer. Post-menopausal women with high-density mammographic patterns, indicative of markedly elevated risk of breast cancer, actually had higher 2/16 ratios than women without them. However, another study found that four postmenopausal women with recently diagnosed breast cancer had lower mean 2/16 estrogen metabolite ratios than a group of post-menopausal women without breast cancer. These studies are difficult to reconcile, because the 2/16-metabolite ratios in the women without breast cancer in some studies are comparable with the ratios for the women with breast cancer in others, even though the assay methodology seems to be the same.

Adlercreutz and coworkers found that premenopausal Finnish women presumed to be at relatively high risk for breast cancer had 2/16-metabolite ratios 4.5 times higher than Asian women presumed to be at low breast cancer risk. They speculated that the increased breast cancer risk in Finnish women was due to their higher total estrogen levels, rather than alterations in metabolite ratios. The higher estrogen levels found in Finnish women were attributed to a higher fat diet, higher estrogen production, and lower fecal estrogen excretion. Korean women recently diagnosed with thyroid cancer had very high 2/16 ratios, which returned to normal values after surgery. Studies of the variation of the 2/16 ratio between different ethnic groups are continuing. Another factor that needs to be considered is that the 2/16 ratio may vary with assay methodology. Values determined by enzyme immunoassay are correlated with values determined by gas chromatography-mass spectrometry (GC-MS), but results from the two methods are not identical. Ratios tend to run lower when determined by GC-MS than by enzyme immunoassay, especially for postmenopausal women.

At this point, the balance of evidence suggests that a higher 2/16 estrogen metabolite ratio may be associated with reduced risk of estrogen-related cancers in premenopausal Caucasian women. The 2/16 ratio is not useful as a measure of breast cancer risk in post-menopausal women. The 2/16 ratio varies between ethnic groups, and Asian women with relatively low breast cancer risk may have lower mean 2/16 ratios.

4-Hydroxy Estrogens and CYP1B1

The 2/16 estrogen metabolite ratio test has a compelling rationale and promising preliminary data, but at this point it appears to be useful for only certain subsets of patients. A great deal of attention in the current scientific literature is focused on another group of genotoxic estrogens, 4-hydroxyestrone and 4-hydroxyestradiol. Research by Cavalleri and coworkers has shown that these compounds can be further metabolized to quinones that attack DNA, leading to mutations. Accumulated mutations are thought to lead to the development of malignancies. It is possible that the problem with the 2/16-metabolite ratio is that it does not focus on the most dangerous 4-hydroxy estrogens.

The 4-hydroxyestrogens are primarily produced by CYP1B1, an enzyme localized in tissues, including breast. CYP1B1 is also involved in the metabolic activation of some environmental carcinogens. CYP1B1 activity varies according to several genetic polymorphisms. Women with high-activity CYP1B1 polymorphisms are at elevated risk for breast cancer (Chinese women), ovarian cancer, and endometrial cancer; men with high-activity CYP1B1 are at elevated risk for prostate cancer. In Caucasian women, CYP1B1 polymorphisms were not associated with breast cancer risk, but were associated with estrogen receptor status in women with breast cancer. More evidence implicating elevated 4-hydroxyestrogens in human cancer comes from tissue studies. Human breast cancer tissue produces much higher levels of 4-hydroxyestrogens than 2-hydroxyestrogens, while normal breast tissue produces approximately equal amounts of the two metabolites. CYP1B1 was also found at high activity in human colorectal cancer biopsy tissue. A recent study from the Karolinska Institute in Sweden found that women using HRT who have the CYP1B1*3/*3 genotype had twice the risk of developing breast cancer compared to other HRT users.

It may also be important that equilin, a non-human estrogen that is a major component of conjugated equine estrogens, undergoes 4-hydroxylation and further metabolism to potent cytotoxic quinones, which are capable of attacking DNA, and causing mutations.

Recent research also suggests that the DNA damage caused by 4-hydroxyestrogens can be mitigated to some extent by taking antioxidant supplements.

Routine lab tests for 4-hydroxyestrogens and CYP1B1 polymorphisms are not currently available. The value of urine or blood tests for 4-hydroxyestrogens is uncertain, because CYP1B1 is located in the tissues, rather than in the liver, where other key estrogen-metabolizing enzymes are located. Because of this, it is not known how closely urine or blood levels would reflect tissue levels. Studies are underway to determine urinary 4-hydroxyestrogen levels in population samples, which will be followed prospectively for the subsequent development of estrogen-related cancers. Since the high activity polymorphism of CYP 1B1 is associated with so many different malignancies, routine tests may be of value in identifying high-risk patients.

Deterministic vs. Probabilistic Tests

It is important to recognize that all the tests described in this review are probabilistic, rather than deterministic. In other words, none of these tests can tell a woman whether or not she will develop breast or other estrogen-related cancers. Rather, they may help determine if a woman is at low, moderate or high risk. The risk factor approach follows from the current view that breast cancer is caused by multiple genetic and environmental factors. Since, in most cases, there is no single cause, it follows that no single test is capable of giving a "yes or no" answer. Critics of the risk factor approach point out that there may be a main cause, which is simply undiscovered at this time.

Deterministic tests are possible when a single gene, pathogen, or other environmental factor is the primary cause of a disease. On the other hand, when a disease appears to be caused by multiple genetic and environmental factors, a test for any single factor (i.e. serum cholesterol in relation to
cardiovascular disease), can only give us an estimate of the probability of getting the disease.

One approach to improving estimates of disease risk in conditions involving multiple risk factors is to attempt to combine the multiple risk factors into a global estimate of risk. Thus for cardiovascular disease, combining the measures of HDL and LDL cholesterol, triglycerides, C-reactive protein, fibrinogen, homocysteine and other tests is thought to allow a more reliable prediction of the risk of heart attack. At this time, such a systematic approach to laboratory measures of estrogen-related cancer risk has not been attempted, but this should be a fruitful area for future research. For example, would women with a high-activity CYP1B1 polymorphism, which predisposes to formation of toxic 4-hydroxyestrogens, who also have high plasma and urinary estrone concentrations, and low estriol concentrations, be at especially high risk for estrogen-related cancers?

**Summary**

A number of laboratory tests can help in assessing a patient’s risk of estrogen-related cancers. Most research has focused on tests of various estrogen metabolites in urine or serum. Use of saliva for estrogen testing is also popular, but there is a smaller research database on salivary estrogens to draw from. It is hoped that more research on salivary estrogens will become available in the future.

Higher levels of estradiol (E2), and especially estrone (E1), in serum and urine have been consistently associated with a moderately elevated risk of breast cancer. There is some evidence that higher levels of estriol (E3) are associated with a reduced risk of breast cancer. Higher values for the estrogen quotient (E3/E1+E2) have been associated with a reduced risk of breast cancer in some, but not all studies. Many women are seeking alternatives to HRT comprised of conjugated equine estrogens. Popular alternatives include estriol alone or in combination with other natural estrogens. Thus further research on the roles of estriol and the estrogen quotient in assessing breast cancer risk are urgently needed.

Laboratory monitoring of HRT has provided a number of useful insights, which may help healthcare practitioners to individualize dosing to achieve the minimum estrogen exposure consistent with symptom relief. Recommended estrogen doses for HRT have been reduced dramatically during the last decade.

Studies of estrogen metabolites show that some are highly toxic, mutagenic, and procarcinogenic; others strongly stimulate cell proliferation, still others are only weakly estrogenic, and some even have anticancer activity.

The 2/16 estrogen metabolite ratio test has a compelling rationale and very promising preliminary data. This test gives a ratio of "good" to "bad" estrogens in urine. Higher values are associated with a reduced risk of breast cancer in premenopausal Caucasian women. However, data from studies of post-menopausal women do not show the same pattern. The 2/16 estrogen metabolite ratio varies from one ethnic group to another, and some lower-risk groups actually have lower, rather than the expected higher ratios, when compared to high-risk groups.

The area of greatest current research interest concerns toxic and mutagenic 4-hydroxyestrogen metabolites, which...
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are produced by the enzyme CYP1B1. Women with the high activity polymorphism for CYP1B1, who produce excessive 4-hydroxyxestrogens, are at increased risk for several estrogen-related cancers. Studies of malignant tissues also demonstrate excess 4-hydroxyxestrogens. Laboratory studies suggest that the toxicity of 4-hydroxyxestrogens can be mitigated to some extent by antioxidant supplementation. Tests for CYP1B1 polymorphisms are not currently routinely available, but may provide a valuable screen for persons at high risk for estrogen-related cancers.

Estrogen-related cancers are thought to result from multiple genetic and environmental factors. Thus all of the tests discussed are probabilistic in nature. They provide information on relative risks, but cannot give "yes or no" answers. In the future, use of several of the tests discussed in this review in combination may improve our ability to estimate cancer risk.

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References