Reviewer's report

Title: Comparison of metabolic ratios of urinary estrogens between benign and malignant thyroid tumors in postmenopausal women

Version: 1 Date: 23 May 2013

Reviewer: Roni Falk

Reviewer's report:

Major compulsory revisions:

1) This study compared levels of urinary estrogen metabolites in women with papillary thyroid carcinoma (PTC) to levels in women with benign thyroid tumors, with the aim of determining whether patterns of estrogen metabolism could successfully discriminate between benign and malignant disease. No biologic rationale for this hypothesis is provided, and except for noting the disproportionately high incidence of thyroid cancer in women vs men, no background supporting a role for estrogen in the promotion or progression of this cancer is discussed. Rather, the authors focus on the lack of sensitivity and specificity of IHC and gene expression profiling for discriminating between benign and malignant thyroid tumors, and postulate that estrogen metabolism may better serve this purpose.

2) This study was not adequately powered to answer this question, as it included only 18 women with PTC (11 stage I, 7 stage III/IV) and 9 with benign tumors. The statistical approach was not appropriate for several reasons. With such a small number of subjects, a non-parametric test is preferable. In my experience hormone data are not normally distributed and data transformations to normalize the distributions are usually required for parametric analyses. Finally, it is preferable to use an analysis of variance approach when comparing multiple case groups to a single control group. I would advise using a non-parametric ANOVA approach (Kruskal-Wallis) for these analyses. No mention is made of the small sample size and the lack of adjustment of p value for multiple comparisons.

3) The statement regarding downregulation of 2-hydroxylase (or other hormone-metabolizing enzymes) in the abstract results and throughout the main paper discussion is misleading. Enzymatic activity is not measured; rather the ratios of metabolites to their precursors is a measure of this activity. I am not convinced of this. Assessing circulating or urinary estrogen metabolites gives a snapshot of the profile of these hormones at a given time, where many hormone-metabolizing enzymes are active simultaneously. Thus, for example, there is dynamic interconversion of estrone and estradiol (by 17#-HSD types 1 and 2), and the ratio of 2-OH-estradiol/estradiol as a marker of 2-hydroxylase activity ignores the likely contribution of levels of estrone as a precursor to 2-OH-estradiol.

4) Figures 2a-2d are redundant. These data are in Table 2.
5) Revised Discussion: This is a pilot study and results need to be confirmed in other independent datasets using appropriate statistical methods. As it stands, the conclusions are not based on appropriate statistical methods. Discussion needs to be revised per reanalysis.

6) Study limitations not addressed.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Not suitable for publication unless extensively edited

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests