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Letter to the Editor

Letter to Editor in response to "Detection of endometrial cancer via molecular analysis of DNA collected with vaginal tampons" by Bakkum-Gamez *et al.* (Gynecol Oncol. 2015)

We read with great interest the article by Bakkum-Gamez et al. "Detection of endometrial cancer via molecular analysis of DNA collected with vaginal tampons" in the recent edition of Gynecologic Oncology (Bakkum-Gamez et al., 2015).

According to Authors, we believe that endometrial cancer (EC) will become an even more important public health issue in next years. In developed countries, the incidence of EC has raised only slightly over the past two decades although the number of annual estimated deaths from this disease has more than doubled since the 1980s. Recent findings suggest that the increase in mortality over the last 14 years may be related to an increased rate of advanced-stage cancers, higher tumor grade and non-endometrioid histology (Ueda et al., 2008). For these reasons scientists, doctors, and public health professionals are more concerned than ever before with identifying effective preventive measures for this condition, which has a readily available treatment with a potential for cure that increases with early detection. Along with primary prevention through modification of lifestyle and risk factors, we believe that next generation strategies should include periodical screening in high-risk subjects.

Interestingly Bakkum-Gamez et al. demonstrate the feasibility of detecting EC by combining minimally-invasive specimen collection techniques with sensitive molecular testing (Bakkum-Gamez et al., 2015). Intravaginal tampons and Tao brush were used respectively to collect vaginal pool and liquid-based endometrial cytology (LBEC) prior to hysterectomy for EC or benign conditions. Tampon and brush DNA underwent separately pyrosequencing assays showing a higher gene-level methylation of both well-studied and recently identified markers for EC. Although the higher methylation among the EC group compared to BE group, a subset of EC methylation overlaps with the distribution of BE methylation. Additional discovery efforts to identify other novel candidate biomarkers to improve test sensitivity and predictive value are warranted.

HOXA9, previously reported as methylated in normal endometrium of women presenting with ovarian cancer (Widschwendter et al., 2009), was included in the panel of genes demonstrating a methylation pattern comparable with RASSF1, a gene methylated in nearly 90% of ECs.

Homeobox (HOX) genes encode transcriptional factors involved in embryonic development. During development of the female reproductive system HOXA9, HOXA10, HOXA11, and HOXA13 are expressed uniformly along the Müllerian duct axis, although in adults their expression becomes spatially restricted to particular organs. HOXA9 becomes expressed in the fallopian tubes; HOXA10 is expressed in the developing uterus, HOXA11 in the lower uterine segment and cervix and HOXA13 in the upper vagina (Taylor et al., 1997).

Several studies have demonstrated that HOXA10 is cyclically expressed in endometrial glands and stroma throughout the menstrual cycle and that aberrant expression of HOXA 10 by the epigenetic mechanism of methylation is associated with infertility and endometriosis (Taylor et al., 1998; Andersson et al., 2014; Fambrini et al., 2013a).

Recently, our group has reported the methylation profile of HOXA10 promoter gene in EC and normal endometrial tissues obtained in different phases of menstrual cycle (Fambrini et al., 2013b). There were statistically significant differences in mean methylation between EC and normal endometrium, suggesting a possible role of epigenetic changes in HOXA10 gene regulation in EC. This data are in agreement with other studies (Yoshida et al., 2006) indicating that HOXA 10 is aberrantly expressed in EC, and that its deregulation significantly contributes to tumor progression. Therefore, we believe that HOXA10 should be included in the panel of genes selected by the Authors to improve test performance.

Concerning DNA collection method, vaginal tampon can potentially enable self-collection and delivery to a testing laboratory by mail, extending access to EC early detection methods into setting with limited resources. However the impact of previous biopsy on tumor cell and cell-free DNA shedding in unknown and the interpretation of study is limited by collection of samples following prior endometrial instrumentation. Specimens of the study included also endometrial brushing collected via Tao brush and suspended in liquid-based solution. The median amount of DNA collected from endometrial brushing was higher respect to vaginal pool via tampon, even if both collection techniques yielded sufficient DNA quantities for methylation analysis and methylation levels between EC and BE were similar. However, an utmost relevant point is that LBEC provides the opportunity to obtain a morphological diagnosis according to accurate and standardized diagnostic criteria. Since 2003, the use of LBEC has been described by several authors in different study populations. Overall, cumulative literature on nearly four thousand patients revealed sensitivity of 78–100%, a specificity of 95–1000%, a positive predictive value (PPV) of 78–100%, and a negative predictive value (NPV) of 96–100% (Buccoliero et al., 2007, 2008; Fambrini et al., 2008, 2012, 2014; Kipp et al., 2008).

We reported a very high diagnostic accuracy of LBEC in early detection of EC, even in troublesome subgroups of women such as tamoxifen users (Buccoliero et al., 2008), post-menopausal subjects (Buccoliero et al., 2007) or patients with low-risk endometrial polyps (Fambrini et al., 2008). In speculating on the role of LBEC as first line investigation, we combined LBEC and transvaginal ultrasounds in women with post-menopausal bleeding observing a 100% sensitivity with 100% NPV in EC identification (Fambrini et al., 2012).

LBEC meets most of the ideal screening test requirements such as the low rate of inadequate specimens, the reasonable cost (comparable to a liquid-based pap test), the good acceptability and the ease of execution in an office setting.

We thank Bakkum-Gamez et al. for giving the attention to early detection of EC, supporting a screening policy in high-risk population. Further studies evaluating cost-effectiveness are needed to assess the best screening modalities in developed countries. However, we believe that LBEC combined with molecular techniques, such as DNA methylation

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analysis, could be used as an outpatient testing for early detection of EC in groups of women with a higher overall incidence of disease.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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