Repurposing the vaginal tampon for endometrial cancer detection

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A device that was first described in the earliest medical literature in the 15th century BC is being made ‘new again’ with contemporary technology. Leveraging its acceptance as a hygiene product and proximity in placement to the endometrium with rapidly evolving sensitivity of molecular detection techniques, the intravaginal tampon may be the ideal biospecimen collection device for endometrial cancer (EC) screening and early detection.

The lower female reproductive tract has been at the center of one of medicine’s greatest screening triumphs – the Papanicolaou cervical smear to detect cervical cancer and dysplasia was initially introduced in 1928. This test evolved over decades to a liquid-based, automated cytologic test, then a co-test with high-risk human papillomavirus DNA detection. Most recently, evidence suggests high-risk human papillomavirus DNA testing alone may offer appropriate cervical cancer and dysplasia screening [1]. Unfortunately, while an abnormal Pap test may indicate the presence of a noncervical gynecologic cancer, cervical cytology is not highly sensitive for upper female reproductive tract cancers [2] and is not a screening test for EC.

However, from an anatomic perspective, biospecimens from the cervix and vagina hold promise as targets for upper female reproductive tract cancer detection. There is direct physical continuity from the uterine cavity to the cervix and vagina. These organs are the conduits through which the most common initial symptom of EC – bleeding – presents. Asymptomatic states of endometrial

pathology may also shed signs of disease into the lower reproductive tract. Technological advances have introduced sensitive molecular detection methods and tumor DNA and proteins can be identified even in biospecimens with limited intact tumor cells [3–7]. Thus, cytologic evidence may not be needed to identify cancer. Molecular changes that occur in the process of carcinogenesis, such as DNA mutations, epigenetic changes, copy number variants and the introduction of microorganism genomes, may all be potential signals that are detectable in the downstream endometrial effluent. Additionally, as technology has evolved, multiplexing has improved testing efficiency, data may be processed in a high throughput fashion, assay costs have fallen, and novel methods to enhance nucleotide and peptide capture from various body fluids are also in development [8].

DNA alterations in primary EC tumors have been shown to be identifiable in both cervical and vaginal samples [3,4]. Kinde *et al.* performed a proof-of-concept study showing that DNA mutations from primary EC tumors could be detected in corresponding cervical Pap tests. At least one mutation from the primary endometrial tumor was identified in corresponding Pap tests in 100% of cases. Interestingly, this methodology was also expanded to a small cohort of women with a new diagnosis of ovarian cancer. For a cancer with high mortality and no screening test, they found that 41% had primary ovarian cancer mutations detected in the Pap test solvent [4]. Diagnostic endometrial sampling prior to Pap test may have biased the muta-

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tion detection in EC; however, the findings in ovarian cancer suggest that molecular tumor markers from the upper reproductive tract spontaneously shed and can be detected in the lower tract.

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Which brings us to vaginal self sampling for upper female genital tract cancer detection. To date, only two proof-of-principle studies on the intravaginal tampon as a collection device for EC detection have been published [3,6]. In 2004, Feigl *et al.* explored methylation of 35 genes in vaginal fluid samples collected via tampon from women with EC and women with benign endometrium. Refining the panel to five genes, they found that with a threshold of three methylated genes there was 100% sensitivity and >97% specificity for EC [6]. Over a decade later, we validated the findings of Feigl in a larger cohort of EC [3]. In addition, we identified significantly higher methylation of several novel EC genes [9] and other genes implicated in EC carcinogenesis in vaginal fluid collected via tampon from women with EC compared with women with benign endometrium. Methylation findings were similar in the vaginal fluid and the cellular brushings from the primary tumor and benign endometrium [3]. Pairing the tampon with DNA analyses for upper female reproductive tract cancer detection has also been reported in a single study of women undergoing surgery for a pelvic mass suspicious for ovarian cancer. Primary tumor-specific *TP53* mutations were detected in 60% (three of five cases) of women with ovarian cancer and intact fallopian tubes. However, among those with ovarian cancer who had undergone remote tubal ligation (three cases), no *TP53* mutations were identified in tampon samples [7]. This finding supports the importance of tubal patency in assessing the uppermost aspect of the gynecologic tract through downstream biospecimens. In ovarian cancer, it makes clinical sense that anatomic alterations, such as a prior tubal ligation or hysterectomy, will physically block DNA from the upper reproductive tract from reaching the vagina. However, in EC, prior gynecologic procedures are less likely to interfere with testing; women with a prior endometrial ablation appear to have no differences in presenting symptoms and time to EC diagnosis compared with those without prior endometrial ablation [10].

Perhaps the greatest value of the intravaginal tampon for EC detection is in its nearly ubiquitous use as

a hygiene product in developed nations. As evidenced by the approximately \$15 billion annually spent on tampons in the USA, it is a highly accepted personal hygiene product. As a collection device, the tampon is essentially noninvasive, does not require a provider for placement or collection, lends itself to frequent, serial sampling and may lead to higher compliance with the appeal of self sampling. In fact, self sampling of vaginal fluid for cancer detection is highly acceptable to women [11,12]. While self sampling with intravaginal swabs have been described for human papillomavirus detection [13], DNA yields for these devices have not to date been described in the setting of abnormal uterine bleeding or EC screening/detection test development. Tampons are highly absorbent, can be retained in the vagina longer than a swab, and have been shown to collect ample amounts of DNA for both sequencing and methylation assays [3,6,7,11]. Pairing self sampling with the stability of DNA suggests such a screening test could be an at-home collection with samples mailed to a testing center.

An exciting example of the successful development of a unique noninvasive screening test for cancer is the story of Cologuard (Exact Sciences, WI, USA), a multitarget stool DNA test for colorectal cancer screening that was approved for clinical use by the US FDA in August 2014. The molecular targets are methylated *BMP3* and *NDRG4*, β -actin, mutant *KRAS* and hemoglobin. In combination, this panel identified >92% of colorectal cancers and >42% of precancerous lesions from self-collected stool samples [14]. The design of this test addressed all elements for effective cancer detection, perhaps most importantly the comfort and ease of self collection. As noted, the panel is highly sensitive for its target lesions. In addition, tumor site does not affect the sensitivity of the test and the molecular assay is automated. However, the importance of access and compliance was paramount. From an access standpoint, stool is collected in a simple device designed for mailing and can be collected in the patient's own home. In contrast to colonoscopy, no dehydrating cathartic is needed in preparation, the test requires no diet or medication restrictions, and there is no anesthetic or visit to a clinician skilled in colonoscopy. Simplifying the biospecimen collection process has great potential to enhance compliance with colorectal cancer screening.

Of course the tampon is not yet ready for prime time as an EC screening/early detection device. Thus far, all studies have been pilot, proof-of-principle studies with limited numbers. Studies of both the tampon and cervical Pap tests have included women with EC who have biopsy-proven cancer. As such, prior diagnostic endometrial sampling may have influenced the yield of pri-

mary tumor cells and DNA from the vagina secondary to physically dislodging portions of the primary tumor. Cytology evidence supports the fact that at minimum low levels of spontaneous cellular shedding occurs in EC [15] and an ongoing Mayo Clinic clinical study is aiming to identify and quantify spontaneous shedding of tumor DNA into the vagina in women with EC and precancerous endometrial lesions (ClinicalTrials.gov identifier: NCT01793545). Additionally, data are needed regarding whether asymptomatic EC or hyperplasia can be detected via molecular markers in the vaginal fluid of women with prior endometrial ablation as well as in the setting of cervical stenosis or prior pelvic radiation.

In summary, the tampon, a well-accepted hygiene product is a highly promising biospecimen collection device that may allow for the development of the first

screening and/or early detection test for EC. Testing samples self-collected with an intravaginal tampon has the potential to enhance patient's comfort, compliance and access to care. While a screening test is still early in development for EC, promising results in colorectal screening development may be a foreshadowing of the future of EC detection and prevention.

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