Published in final edited form as:

Sci Transl Med. 2013 January 9; 5(167): . doi:10.1126/scitranslmed.3005411.

Repurposing the Pap Smear: One Step Closer to Gynecologic Cancer Screening

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Cervical cancer was the leading cause of cancer-related death among women in the early 20th century. The introduction of routine screening for cervical cancer with cytology, and more recently Human Papilloma Virus (HPV) DNA, has made a tremendous impact in the United States by reducing cervical cancer incidence and mortality by 75% and 70%, respectively(1).

Unfortunately, the success of screening programs for endometrial and ovarian cancer has been modest. Endometrial and ovarian cancers are now the most common gynecologic malignancies in the United States and caused more than 22,000 deaths in 2012(2). Screening for endometrial cancer has focused on the use of transvaginal ultrasonography (TVU) to measure endometrial thickness. A recent assessment of TVU to detect endometrial hyperplasia or malignancy in asymptomatic patients achieved a maximum sensitivity and specificity of 77.1% and 85.8%, respectively. Given the burden of further diagnostic procedures incurred by false positive results, the authors advocated the consideration of screening for only high-risk women(3). Options for routine ovarian cancer screening generally have included TVU and serum CA125 levels. An annual intervention including simultaneous use of both methodologies was evaluated in a large prospective cohort study of low risk women. Unfortunately, this trial failed to demonstrate a mortality benefit to screening, and in fact, there were more complications in the screening arm due to surgical evaluation of false positive patients(4). Results from a large trial (UKCTOCS) of the assessment of the change in CA125 followed by TVU in patients with rising CA125 are anticipated. Outside of patients with hereditary cancer syndromes such as BRCA mutation or Lynch Syndrome, most professional guidelines recommend against routine screening for endometrial or ovarian cancer due to lack of sensitivity and in particular due to failure to demonstrate survival benefit in any current studies.

Given the lack of an effective screening option for endometrial and ovarian cancer, there is great interest to establish new approaches. In the accompanying manuscript, Kinde and colleagues propose to leverage the existing cervical screening strategy with assessment of molecular aberrations commonly found in ovarian and endometrial cancers to provide a

*Corresponding Author: Shannon N. Westin, MD, MPH, Department of Gynecologic Oncology and Reproductive Medicine, University of Texas M.D. Anderson Cancer Center, 1155 Herman Pressler Drive, Houston, TX 77030; swestin@mdanderson.org. The use of existing cervical screening DNA technology has the potential to revolutionize screening for other gynecologic malignancies.

cost-effective screening approach for endometrial and ovarian cancer (need Kinde et al reference).

Established Cytologic Methods for Cervical Cancer Screening

Papanicolaou (Pap) smear

Since the introduction of the Pap smear in the 1950s, the technology has rapidly evolved. Two methods are available in the United States for cytology screening, the conventional Pap smear and liquid-based cytology. In regards to detection of cytologic abnormalities, studies have not found a significant difference between the two methodologies(5). Liquid-based cytology has the added advantage of the option to perform concurrent HPV testing to further guide treatment planning.

To date, the utility of Pap smear cytology to diagnose endometrial and ovarian cancer has been limited. For endometrial cancer, conventional cervical cytology yields sensitivities of approximately 30–40%(6) and liquid-based screening may perform only slightly better in this regard(7). The presence of psammoma bodies in a Pap smear may be associated with ovarian cancer(8), however, this cancer is even more likely to escape detection by cervical cytology with only 15% sensitivity reported(9). However, the ability to even occasionally detect these cancers during cervical cytology suggested that DNA present from ovarian or endometrial cancer cells could be obtained and tested in cytology preparations.

HPV Testing

The use of polymerase chain reaction (PCR) to detect high-risk subtypes of HPV has further expanded the detection of early cervical abnormalities and can reduce the risk of cervical dysplasia and cancer at subsequent evaluation by 40%(10). This testing is performed as an adjunct to liquid-based cytologic screening and provides additional information regarding risk and need for additional testing such as colposcopy. This relatively simple acquisition of DNA for testing has been repurposed by Kinde and colleagues to assess for genomic abnormalities present in endometrial and ovarian cancer (Figure).

Molecular aberrations in endometrial and ovarian cancer provide opportunity

The comprehensive molecular analysis of cancer offers insight into tumor-specific alterations required for tumorigenesis and has potential to direct new research avenues for screening prevention and therapy. Due to its high mortality rate, ovarian cancer was an early target of The Cancer Genome Atlas (TCGA) effort. Reports from the TCGA as well as others(11) describe almost universal p53 mutations in high grade serous ovarian cancers, the most lethal form of ovarian cancer. Less common histologies have high rates of PI3K, K-RAS and B-RAF mutations.

The TCGA project on endometrial cancer is underway and results are anticipated soon. Several smaller, yet comprehensive studies have been completed and are consistent with data presented in the Kinde et al. In contrast to ovarian cancer, endometrial cancers, especially those of endometrioid histology, are rich with mutations with some of the highest mutational rates of all cancers(12). These studies are notable for high mutation rates in multiple members of the PI3K pathway; RAS and B-Catenin mutations are also common(13). Interestingly, papillary serous cancer, a rare and aggressive histology of endometrial cancers, is similar to ovarian cancer in type and spectrum of mutations and copy number changes(14).

Based on a combination of published and newly generated data, Kinde and colleagues generated a list of 12 genes that represent common mutations in endometrial and ovarian cancers. This multiplexed molecular assay termed “Papgene” included 12 genes: APC, AKT1, BRAF, CTNNB1, EGFR, FBXW7, KRAS, NRAS, PIK3CA, PPP2R1A, PTEN and TP53. Briefly, specific PCR probes with attached unique identifiers were designed to regions of these genes and used to amplify DNA collected from pap liquid. These amplicons, representing normal and mutated targets, were sequenced in mass with next generation sequencing technology. Pap samples from patients with known endometrial and ovarian cancers were evaluated and mutations were identified at rates as low as 0.01% mutant copies. Mutations were not detected in pap samples from patients without cancer. Might these exploratory studies be the foundation of a new genomic based screening test for endometrial and ovarian cancers?

What constitutes a good screening test??

The proof of concept studies by Kinde and colleagues should be considered in the context of guidelines from the World Health Organization and NCI Early Detection Research Network (EDRN) for a good screening test. The test should be easy to perform, feasible to complete in large-scale situations, reliable, and cost-efficient. The test should be tolerable for the patient and have a high positive predictive value. Sensitivity and specificity should be maximized to capture the largest proportion of those affected with disease while minimizing false positive results(15)

The strengths of the “Papgene” test include that mechanism for routinely obtaining cervical cytology is well established, feasible, and tolerable for women. The additional DNA analysis should be relatively easy to implement in the context of an annual gynecologic examination. The test demonstrates a strong sensitivity for endometrial cancers, however, this cancer is typically diagnosed quite early in the disease process secondary to the presence of early symptoms including vaginal bleeding. Unfortunately, the test only captured 40% of patients with known ovarian cancer. Arguably, this is the disease where a screening test could have the most impact, given the insidious nature of ovarian cancer and that it is often diagnosed at a later stage when it is no longer curable. It is possible that the sensitivity for detection of ovarian cancer could be improved with the addition of mutations to increase the molecular coverage of aberrations found in ovarian cancer. However, it may be that low detection rate of ovarian cancer is not due to the failure of the test to detect ovarian cancer DNA, but a reflection of ovarian cancer biology. Perhaps only a proportion of ovarian cancers would have cells or DNA that are shed in sufficient quantities for detection at the cervical os. Serous type endometrial cancers, which are often asymptomatic and metastasize early in their development, provide a similar opportunity for impact of a successful screening program. These tumors were not tested in sufficient numbers to determine sensitivity.

Specificity is a key component of a screening test for disease such as ovarian and endometrial cancer where the prevalence is relatively low to avoid misdiagnosis and overtreatment. While Kinde et al performed studies to begin to evaluate this aspect of “Papgene”, there is much more work remaining. The “Papgene” test did not detect mutations in 14 normal controls, suggesting that mutations are not common in a normal population. Further testing of much larger numbers of patients will be needed to determine assay specificity. More importantly, cancers often develop in the context of other non-cancerous disease states: endometrial cancers occur within fields of endometrial hyperplasia or atrophy; clear cell ovarian can develop from regions of endometriosis. Though newer more comprehensive and more sensitive mutation detection technologies have been utilized for tumor analysis, most have used normal samples as controls. It has been the presumption that mutations are specific to cancer states, but there is evidence to suggest that this is not always

the case. In a study completed with older, less sensitive molecular technologies, PTEN mutations were detected in a measurable number of endometrial hyperplasias that did not progress to malignancy(16). Though it may well be that the detection of common tumor mutations will identify the population at most risk for cancer development, this needs to be tested.

The cost of a genomic-based screening test should also be considered. Next generation sequencing is still expensive, although the overall cost has fallen markedly, allowing for more broad application. Developing a cost effective CLIA-compliant assay based on this platform could prove challenging. Further, the success of a screening test is directly related to the prevalence of a disease in the population. The implementation of this test would need careful consideration in regards to the populations chosen for screening and the impact on test utility. The younger population currently undergoing Pap screening for cervical cancer is at relatively low risk for ovarian and endometrial cancers, which generally occur in older post-menopausal women. Further, recent recommendations endorse expanding the screening interval to every 5 years and stopping after three negative tests. Thus, women at risk for endometrial and ovarian cancer may not be screened as frequently. If the approach was to be used for screening for ovarian and endometrial cancer, new guidelines for collection of cytology would need to be developed and implemented bypassing one of the arguments for cost effectiveness: piggybacking on a current test.

Another opportunity for DNA detection for cancer screening

The concept of screening for tumor DNA to detect cancer is rapidly expanding and assessment of DNA in the blood may have potential utility for detection of endometrial and ovarian cancer. Cell free DNA, the presence of DNA in the plasma or serum of patients has garnered interest for cancer diagnosis. It is hypothesized that the tumor releases the free DNA through apoptosis versus lysis of intact tumor cells present in the circulation. Once extracted from the serum/plasma, cell free DNA can be analyzed for genomic aberrations including mutations, microsatellite instability, and epigenetic changes including DNA hypermethylation(17). Cell free DNA is currently being explored as method to screen for cancer, identify the primary tumor site, and monitor for relapse. In colorectal and breast cancers, genomic aberrations found in cell free DNA have high concordance with those found in the primary tumor(18, 19). Current issues with the development of cell free DNA screening tests include the variation of cell free DNA concentration based on the protocols for processing blood, the interval of time between specimen attainment and analysis and determining the site of mutation origin.

Prevention and early detection remain essential to decreasing cancer mortality. Inherent to the success of our prevention programs and cancer screening tests for cervical cancer, is a fundamental understanding of its tumor development and progression, namely HPV and cellular atypia. The etiologies and early development of ovarian and endometrial cancers remain less understood. Though explored as a diagnostic test, a modified “Papgene” might be further utilized for probing the essential in situ mutational events that dictate ovarian and endometrial tumor development. In turn, these discoveries could offer further opportunity for prevention, directed screening and other life saving interventions.

Acknowledgments

Funding: NIH 2P50 CA098258-06 SPORE in Uterine Cancer

SU2C-AACR-DT0209 - Stand Up to Cancer Dream Team Translational Research Grant, a Program of the Entertainment Industry Foundation

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Figure.
DNA evaluation of cervical cytology is possible through genomic analysis of the liquid fixative of the liquid-based test.