

Methods: A case-control study was performed including all cases of recurrent stage I, grade 1 endometrioid endometrial cancer at a single institution in a ten-year period. Cases were matched to controls in a 1:2 ratio. Controls were matched by age, BMI, weight and stage. Controls had clinical surveillance lasting at least 6 months beyond the longest time to recurrence of the cases. Molecular testing was performed on archival tumor specimens: microsatellite instability testing with Promega MSI Analysis, POLE mutational status with Sanger sequencing, and mutational status of 67 genes with next-generation sequencing using the ArcherDx VariantPlex Solid Tumor kit. Patient and tumor characteristics and molecular results were compared using chi-square and Fisher's exact tests.

Results: 311 stage I, grade 1 endometrioid endometrial cancers were identified; 15 cases had recurrent disease and available tumor specimens. Cases and controls were similar in median age (57 vs. 59, $p=0.53$), BMI (33.1 vs. 34.2, $p=1.00$), weight (83.5 vs. 87.1kg, $p=1.00$) and stage (IA 86.7% vs. 93.1%, IB 13.3% vs. 6.9%, $p=0.60$). Recurrence location was vaginal in 60% of cases, pelvic in 20% and abdominal in 20%. Mutations identified at high frequency among cases included PTEN (80%), PIK3CA (67%), CTNNB1 (60%). Both CTNNB1 and MSI-H were present at significantly higher rates in cases than in controls (CTNNB1 60% vs. 27.6%, $p=0.04$, MSI-H 46.7% vs. 13.8%, $p=0.03$), while PTEN, PIK3CA, KRAS and TP53 all had equivalent distribution (p values 0.68, 0.34, 0.74 and 0.65 respectively). POLE mutations were found in 0.0% of cases vs. 6.9% of controls ($p=0.54$). Among specimens demonstrating microsatellite stability (MSS), 87.5% of cases vs. 24.0% of controls had CTNNB1 mutations ($p=0.003$). CTNNB1 wild type tumors were MSI-H in 83.3% of cases vs. 9.5% of controls ($p<0.001$).

Conclusions: Compared to controls, CTNNB1 mutation is present at significantly higher rates in recurrent stage I, grade 1 endometrioid endometrial cancers and is found most commonly in MSS tumors. This marker may be useful for prognostic risk stratification and adjuvant therapy decision-making in this otherwise low risk population.

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Abstract #25

Combining methylation markers, genomic instability, and next generation sequencing as a panel for endometrial cancer detection via intravaginal tampon collection

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Objectives: We aimed to develop and evaluate a molecular panel for the early detection of endometrial cancer (EC) from intravaginal tampons by methylation markers, genomic instability, and next generation sequencing (NGS).

Methods: Tampons were collected from women undergoing hysterectomy for EC and women undergoing hysterectomy for benign indications. Extracted tampon DNA underwent the following: 1) low-coverage whole genome sequencing (LC-WGS) using the Illumina HiSeq 2500 according to 51 base-pair single-end sequencing protocol and evaluated for copy number gains and losses (genomic instability) using an in-house bioinformatics pipeline (<http://bioinformaticstools.mayo.edu/research/wandy/>); 2) pyrosequencing to measure promotor methylation of HOXA9, RASSF1, and CDH13; and 3) NGS using PCR amplification primers followed by Ion Torrent and orthogonal Illumina covering 19 genes commonly found in EC. Sensitivity and specificity for each test and combinations of tests were calculated.

Results: Data from all 3 molecular approaches is presented in Table 1. Sensitivity and specificity for EC via methylation was as follows:

25 benign and 36 EC tampon samples had HOXA9 methylation (42% sensitivity; 100% specificity); 22 benign and 35 EC tampon samples had RASSF1 methylation (40% sensitivity; 100% specificity); 20 benign and 36 EC tampon samples had CDH13 methylation (39% sensitivity; 100% specificity). Genomic instability was identified in tampons from 9 (64%) of 14 ECs and 1 (11%) of 9 benign endometrial controls. 18 benign samples and 21 EC samples underwent NGS with mutations detected in 11 (52%) EC samples and 3 (17%) benign samples. When combining the three tests, 11/12 (92%) ECs and 1/7 (14%) controls were positive for either genomic instability, methylation or NGS, resulting in a sensitivity of 92% and specificity of 86%. Interestingly, the benign endometrial control that was positive for genomic instability had an underlying leiomyosarcoma uncovered at hysterectomy.

Conclusions: When combined, genomic instability, methylation, and NGS have a high sensitivity and specificity in detecting EC; however, the combination of genomic instability and methylation provides comparably high sensitivity. Validation of the molecular biomarker combinations and further methylation marker discovery, especially among the rarer EC histologies, are needed to further develop a tampon-based EC detection test.

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Abstract #26

MLH-1 hypermethylation is associated with lower recurrence free survival in patients with endometrial cancer

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Objectives: Approximately 25% of endometrial cancers (EC) are characterized by microsatellite instability due to MLH1 promotor hypermethylation. This study evaluates the prognostic importance of MLH1 hypermethylation among EC patients undergoing universal screening for Lynch Syndrome (LS).

Methods: From August 2012- August 2016, all patients with EC underwent screening for LS using immunohistochemistry (IHC) for mismatch repair (MMR) proteins. Tumors with lack of expression of PMS2 or MLH1 were assessed for MLH1 promotor methylation. Tumors were classified as MMR-I (intact expression of MMR proteins) and MMR-DM (MMR deficient due to MLH1 methylation) Clinical and pathologic factors associated with MMR-DM evaluated using univariate and multivariate analysis. Overall survival (OS) and recurrence free survival (RFS) assessed using cox proportional hazards for patients with at least 2 years of follow up.

Results: Among 720 EC samples evaluated for MMR, 516 (71.6%) were MMR-I, 172 (23.8%) MMR-DM and 32 (4.4%) MMR-DU. Patients with MMR-DM tumors were older ($p<0.001$) and had a lower BMI ($p=0.03$) vs. MMR-I patients. MMR-DM tumors were higher grade (grade 2/3, $p<0.001$), had positive LVSI ($p<0.01$), and myometrial invasion $>50%$ ($p=0.002$). There was no significant difference in stage, adnexal involvement, or cervical stromal invasion between groups. Lymphadenectomy was performed in 316 patients (43.9%), specifically 44.2% of MMR-DM and 41.8% of MMR-I groups. On multivariable analysis, older age (OR 1.03, CI (1.01-1.05), $p=0.001$), endometrioid histology (OR 5.60, CI (1.71-17.88), $p=0.004$), and tumor size < 2 cm (OR 0.50 CI (0.31-0.80), $p=0.004$) were independently associated MMR-DM. Recurrences were observed in 11.2% (19/169) MMR-DM and 5.9% (30/509) of MMR-I patients ($p=0.02$). Evaluating by FIGO stage, recurrences at early stage (I/II) were seen in 6.6% of MMR-DM (9/137) vs. 2.9% of MMR-I (12/421)