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Uterine washings as a novel method for early detection of ovarian cancer: Trials and tribulations

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ABSTRACT

Given the tubal origin of high-grade serous ovarian cancer (HGSC), we sought to investigate intrauterine lavage (IUL) as a novel method of biomarker detection. IUL and serum samples were collected from patients with HGSC or benign pathology. Although CA-125 and HE4 concentrations were significantly higher in IUL samples compared to serum, they were similar between IUL samples from patients with HGSC vs benign conditions. In contrast, CA-125 and HE4 serum concentrations differed between HGSC and benign pathology (P =.002 for both). IUL and tumor samples from patients with HGSC were subjected to targeted panel sequencing and droplet digital PCR (ddPCR). Tumor mutations were found in 75 % of matched IUL samples. Serum CA-125 and HE4 biomarker levels allowed for better differentiation of HGSC and benign pathology compared to IUL samples. We believe using IUL for early detection of HGSC requires optimization, and current strategies should focus on prevention until early detection strategies improve.

There are currently no recommended screening tests for ovarian cancer. Designing effective screening tools is a priority, as most patients are diagnosed with advanced-stage disease and die within 5 years of diagnosis. Various algorithms have been proposed, including combinations of tumor biomarkers, a symptom index, patient age, menopausal status, and pelvic ultrasound (Woodward et al., 2007; Menon et al., 2021); however, none of these methods have demonstrated decreased mortality from ovarian cancer.

High-grade serous ovarian cancer (HGSC) is characterized by clonal pathogenic genetic alterations in *TP53* that are acquired early in tumorigenesis and serve as a molecular marker in cancer development (Bell et al., 2011). The earliest morphologically recognizable form of HGSC is the serous tubal intraepithelial carcinoma (STIC) lesion (Zhang et al., 2019). It arises in the fimbriated end of the fallopian tube, sheds malignant cells, and harbors identical *TP53* mutations to primary tumors (Soong et al., 2018; McDaniel et al., 2015). Theoretically, malignant cells travel down the fallopian tube and into the uterine cavity. Somatic variants have previously been identified in DNA collected via Papanicolaou tests and vaginal samples from patients with endometrial and ovarian cancers (Erickson et al., 2014; Paracchini et al., 2020; Wang et al., 2018). Consequently, intrauterine lavage (IUL) has been proposed as a potential method for early detection of HGSC.

Our group previously investigated IUL as a novel early detection tool for HGSC and found that CA-125 and HE4 biomarkers were elevated in serum and IUL samples from patients with HGSC compared to benign controls (St. Clair et al., 2013). Furthermore, both CA-125 and HE4 were

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detected at substantially higher values in uterine washings compared to serum, suggesting an opportunity for early detection.

Here, we sought to validate our original study using IUL samples from a more modern cohort to detect HGSC. We explored 2 hypotheses – first, we hypothesized that the intrauterine cavity is an accessible location for collecting lavage samples, and that cancer biomarkers would be significantly higher in samples collected from patients with HGSC than unaffected controls. Second, we hypothesized that pathogenic mutations present in the tumor specimen would be detectable in the IUL sample. This study was approved by Memorial Sloan Kettering Cancer Center's institutional review board.

Patients with stage III or IV HGSC planned for primary debulking surgery as well as patients with benign pathology and planned hysterectomy were approached and consented to the protocol between December 1, 2021, and August 1, 2022. Saline IUL samples were obtained before surgical incision. Serum samples for CA-125 and HE4 measurements were also collected from patients intraoperatively prior to incision. After general anesthesia and before vaginal preparation, a sterile metal speculum was inserted, and the cervix was grasped with a tenaculum. The triple-lumen Speiser Catheter (Ovartec GmbH, Illingen, Germany) was introduced transcervically and IUL samples were collected as previously described using 20 cc of normal saline and a 15 mL Falcon tube (Elisabeth et al., 2018; Maritschnegg et al., 2015; Ghezelayagh et al., 2022). Washings were transported on ice from the operating room to the laboratory space and aliquoted to 500 uL cryovials and frozen at −80 °C. Serum samples were collected in a 5 mL serum separator tube and spun at 2000x *g* for 10 min at 4 °C. The supernatant was collected and frozen at −80 °C in 500 uL cryovials. CA-125 and HE4 levels were quantified from the serum and the washings on the Architect i2000 analyzer using a 2-step chemiluminescent microparticle immunoassay (Abbott Laboratories, Abbott Park, IL, USA). CA-125 and HE4 levels from intraindividual serum and IUL samples were compared using the Wilcoxon signed rank test. The Mann-Whitney test was used to compare concentrations of biomarkers between patients with HGSC and those with benign pathology. All cases for which both IUL samples and serum samples were available were included in this analysis.

In the HGSC cohort (n = 8), the median CA-125 level was 997 U/mL (range, 74–4258) in serum and 5031 U/mL (range, 167–107,032) in IUL samples. CA-125 levels were significantly higher in the IUL samples than serum (P =.023, Fig. 1A). In the benign cohort (n = 5), the median CA-125 values in the serum and IUL samples were 12 U/mL (range, 10–16) and 880 U/mL (range, 436–3454), respectively (P =.063, Fig. 1B).

In the HGSC cohort, the median HE4 concentration was 343 pmol/L (range, 111–703) in serum and 6495 pmol/L (range, 421–25,373) in IUL samples (P =.016, Fig. 1C). For patients with benign pathology, the median HE4 values in the serum and IUL samples were 33 pmol/L (range, 29–53) and 1807 pmol/L (range, 1445–5271), respectively (P =.063, Fig. 1D).

Comparison of IUL samples from patients with HGSC vs benign pathology demonstrated no significant difference in CA-125 (P =.13) and HE4 (P =.09) concentrations (Fig. 1E, 1F). The concentrations of CA-125 and HE4 in the serum of patients with HGSC vs benign conditions differed significantly (P =.002 for both, Fig. 1G, 1H).

In another set of experiments, IUL samples were collected from patients with known or suspected advanced-stage HGSC (n = 4), adnexal masses of uncertain etiology (n = 3), and patients who underwent risk-reducing adnexectomy (n = 6) who were seen preoperatively in clinic and were consented to the research protocol between December 1, 2017, and March 30, 2018. A total of 30 cc of normal saline was injected into the uterus and collected. A standardized sample volume was obtained prior to sample processing. DNA was extracted from tumor tissue and IUL samples and subjected to targeted panel sequencing. In addition, high-sensitivity droplet digital PCR (ddPCR) assays for *TP53* and *ARID1B* were performed for 3 patients and 1 patient, respectively, to detect clonal tumor mutations in IUL samples.

Patient characteristics are summarized in Table 1. The median amount of DNA obtained was significantly higher in patients with HGSC compared to patients with benign pathology (1274 ng vs 148 ng, respectively; P =.03). All 4 HGSCs (100 %) harbored a pathogenic *TP53* mutation, and 3 (75 %) of 4 cases had mutations from their respective matched tumor detected by either sequencing or ddPCR in the IUL sample (Table 2).

In conclusion, although CA-125 and HE4 measurements from patients with HGSC were significantly higher in IUL samples than serum, biomarker values were significantly higher for patients with HGSC compared to benign pathology in serum samples but not in IUL washings. Furthermore, HGSC-specific mutations, specifically *TP53*, were detected in IUL samples via sequencing/ddPCR analysis in 75 % of cases. Limitations of our study include the small sample size due to time and resource constraints; however, strengths include standardized sample collection protocols and pathologic confirmation of both HGSC and benign conditions. Obtaining IUL samples was challenging and time-intensive, and sensitive identification of mutations using this approach must be optimized. The natural variation of CA-125 and HE4 levels over time and throughout the menstrual cycle is poorly understood and may lead to issues with data interpretation, particularly with a small sample size. This is pertinent when comparing levels between older patients with HGSC to younger patients who undergo surgery for benign indications and small sample sizes preclude controlling for age. Consideration could be given to discovering novel biomarkers through perception-based systems such as machine learning (Kim et al., 2022; Yaari et al., 2021). Though there are opportunities for further investigation into IUL as a diagnostic or screening tool, we believe current strategies should focus on prevention and other avenues for early detection.

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CRediT authorship contribution statement

Tiffany Y. Sia: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Zvi Yaari:** Writing – review & editing, Methodology, Data curation, Conceptualization. **Ron Feiner:** Writing – review & editing, Data curation. **Evan Smith:** Writing – review & editing, Methodology, Formal analysis, Data curation, Conceptualization. **Arnaud Da Cruz Paula:** Formal analysis. **Pier Selenica:** Formal analysis. **Sital Doddi:** Data curation. **Dennis S. Chi:** Writing – review & editing, Methodology. **Nadeem R. Abu-Rustum:** Writing – review & editing, Methodology. **Douglas A. Levine:** Writing – review & editing, Methodology, Conceptualization. **Britta Weigelt:** Writing – review & editing, Methodology, Formal analysis. **Martin Fleisher:** Writing – review & editing, Methodology. **Lakshmi V. Ramanathan:** Writing – review & editing, Methodology, Conceptualization. **Daniel A. Heller:** Writing – review & editing, Methodology, Conceptualization. **Kara Long Roche:** Writing – review & editing, Methodology, Formal analysis, Data curation, Conceptualization.

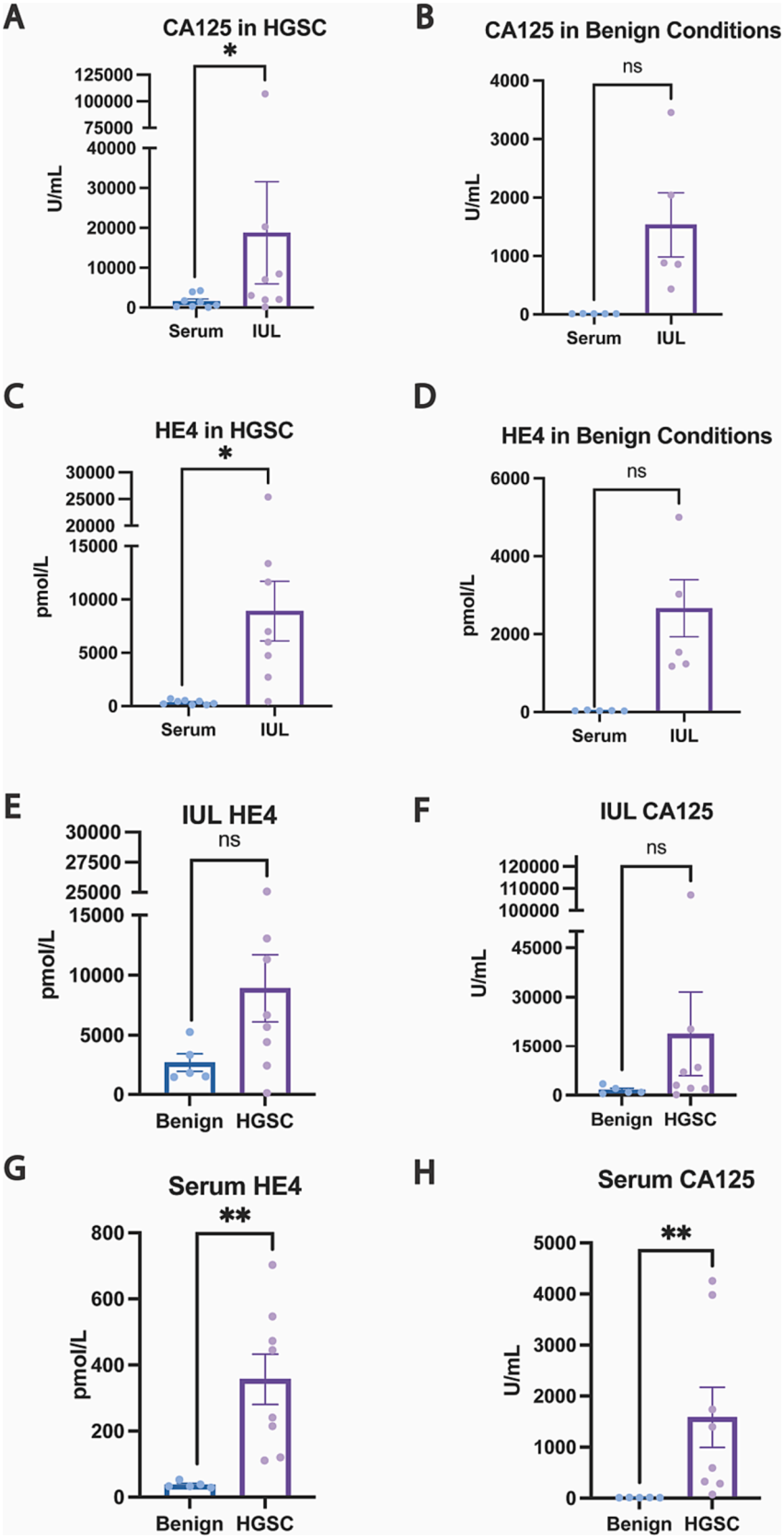

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Fig. 1. Comparison of CA-125 and HE4 biomarker levels in serum vs intrauterine lavage (IUL) samples from patients with high-grade serous ovarian carcinoma (HGSC) or benign conditions. **A**: CA-125 levels in serum vs IUL samples from patients with HGSC; **B**: CA-125 levels in serum vs IUL samples from patients with benign conditions; **C**: HE4 levels in serum vs IUL samples from patients with HGSC; **D**: HE4 levels in serum vs IUL samples from patients with benign conditions; **E**: HE4 levels in IUL samples from patients with benign conditions compared to HGSC; **F**: CA-125 levels in IUL samples from patients with benign conditions compared to HGSC; **G**: HE4 levels in serum from patients with benign conditions compared to HGSC; **H**: CA-125 levels in serum from patients with benign conditions compared to HGSC; * $P < .05$; ** $P < .01$; ns: not significant.

Table 1
Cases with intrauterine lavage (IUL) sample collection, grouped by preoperative diagnosis.

Case ID	Age (y)	Preoperative diagnosis	Final pathology	FIGO stage	Total DNA from IUL (ng) *
KLR1	77	Suspected HGSC	HGSC	IIIC	744
KLR7	60	HGSC	HGSC	IIIC	1437
KLR13	73	HGSC	HGSC	IIIC	8557
KLR20	61	Suspected HGSC	HGSC	IIIC	1110
KLR12	47	Adnexal mass	Benign leiomyoma	N/A	3303
KLR17	45	Adnexal mass	Benign ovary cyst	N/A	173
KLR21	41	Adnexal mass	Benign ovary cyst	N/A	123
KLR2	37	HBOC	Benign	N/A	148
KLR3	36	HBOC	Benign	N/A	110
KLR4	34	HBOC	Benign	N/A	844
KLR5	40	HBOC	Benign	N/A	134
KLR8	38	Lynch syndrome	Benign	N/A	34
KLR15	40	HBOC	Benign	N/A	184

* a standardized volume of IUL sample was used for processing. IUL: intrauterine lavage; HGSC: high-grade serous ovarian cancer; FIGO: International Federation of Gynecology and Obstetrics; HBOC: hereditary breast and ovarian cancer; N/A: not applicable.

Table 2
Results from panel sequencing and droplet digital PCR (ddPCR) for patients with high-grade serous ovarian cancer (HGSC).

Sample ID	Clonal somatic tumor mutation of interest	Tumor mutations in IUL sample by panel sequencing	Tumor mutations in IUL sample by ddPCR
KLR1	*TP53 (p.D281E)*	Not identified	Not detected
KLR7*	*ARID1B (p.G326)*	**Identified**	**Detected**
KLR13	*TP53 (p.X261_splice)*	Not identified	**Detected**
KLR20	*TP53 (p.R175H)*	**Identified**	Not detected

* Case also harbored a subclonal *TP53* (p.P191Cfs*46) mutation. IUL: intrauterine lavage; HGSC: high-grade serous ovarian cancer; ddPCR: droplet digital PCR.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: D.A.H. is a co-founder and officer with equity interest of Lime Therapeutics Inc., co-founder with equity interest of Selectin Therapeutics Inc. and Resident Diagnostics, Inc., and a member of the scientific advisory boards of Concarlo Therapeutics Inc., Nanorobotics Inc., and Mediphage Bioceuticals Inc. B.W. reports research funding by Repare Therapeutics, outside of the submitted work. E.S. reports honoraria from Dilon Technologies, Inc. D.A.L. is a full-time employee of Merck & Co., Inc. (Rahway, NJ, USA) and a co-founder with equity interest of Resident Diagnostics, Inc. N.A.R. reports grant funding from GRAIL paid to the institution. D.S.C. reports personal fees from Apyx Medical, Verthermia Inc., Biom 'Up, and AstraZeneca, as well as recent or current stock/options ownership of Apyx Medical, Verthemia, Intuitive Surgical, Inc., TransEnterix, Inc., Doximity, Moderna, and BioNTech SE. K.L.R. reports travel support from Intuitive Surgical. T.Y.S and M.F. have no conflicts of interest to report.

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References

Bell, D., Berchuck, A., Birrer, M., Chien, J., Cramer, D.W., Dao, F., et al., 2011. Integrated genomic analyses of ovarian carcinoma. Nature 474 (7353), 609–615.
Elisabeth, M., Florian, H., Nina, P., Jirí, B., Fabian, T., Christoph, G., et al., 2018. Uterine and tubal lavage for earlier cancer detection using an innovative catheter: A feasibility and safety study. Int. J. Gyneco. Cancer. 28 (9), 1692.
Erickson, B.K., Kinde, I., Dobbin, Z.C., Wang, Y., Martin, J.Y., Alvarez, R.D., et al., 2014. Detection of somatic TP53 mutations in tampons of patients with high-grade serous ovarian cancer. Obstet. Gynecol. 124 (5), 881–885.
Ghezelayagh, T.S., Kohrn, B.F., Fredrickson, J., Manhardt, E., Radke, M.R., Katz, R., et al., 2022. Uterine lavage identifies cancer mutations and increased TP53 somatic mutation burden in individuals with ovarian cancer. Cancer Res. Commun. 2 (10), 1282–1292.
Kim, M., Chen, C., Wang, P., Mulvey, J.J., Yang, Y., Wun, C., et al., 2022. Detection of ovarian cancer via the spectral fingerprinting of quantum-defect-modified carbon nanotubes in serum by machine learning. Nat. Biomed. Eng. 6 (3), 267–275.
Maritschnegg, E., Wang, Y., Pecha, N., Horvat, R., Van Nieuwenhuysen, E., Vergote, I., et al., 2015. Lavage of the uterine cavity for molecular detection of Müllerian duct carcinomas: A proof-of-concept study. J. Clin. Oncol. 33 (36), 4293–4300.
McDaniel, A.S., Stall, J.N., Hovelson, D.H., Cani, A.K., Liu, C.J., Tomlins, S.A., et al., 2015. Next-generation sequencing of tubal intraepithelial carcinomas. JAMA Oncol. 1 (8), 1128–1132.
Menon, U., Gentry-Maharaj, A., Burnell, M., Singh, N., Ryan, A., Karpinskyj, C., et al., 2021. Ovarian cancer population screening and mortality after long-term follow-up in the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS): a randomised controlled trial. Lancet 397 (10290), 2182–2193.
Paracchini, L., Pesenti, C., Delle Marchette, M., Beltrame, L., Bianchi, T., Grassi, T., et al., 2020. Detection of TP53 clonal variants in Papanicolaou test samples collected up to 6 years prior to high-grade serous epithelial ovarian cancer diagnosis. JAMA Netw Open. 3 (7), e207566.
Soong, T.R., Howitt, B.E., Miron, A., Horowitz, N.S., Campbell, F., Feltmate, C.M., et al., 2018. Evidence for lineage continuity between early serous proliferations (ESPs) in the fallopian tube and disseminated high-grade serous carcinomas. J. Pathol. 246 (3), 344–351.
St. Clair, C., Ducie, J., Zhou, Q., Dao, F., Kauff, N., Spriggs, D., et al., 2013. Uterine washing biomarkers as a novel screening tool for high-grade serous carcinoma. Gynecol. Oncol., 130(1):e71.
Wang, Y., Li, L., Douville, C., Cohen, J.D., Yen, T.-T., Kinde, I., et al., 2018. Evaluation of liquid from the Papanicolaou test and other liquid biopsies for the detection of endometrial and ovarian cancers. Sci. Transl. Med. 10 (433).
Woodward, E.R., Sleightholme, H.V., Considine, A.M., Williamson, S., McHugo, J.M., Cruger, D.G., 2007. Annual surveillance by CA125 and transvaginal ultrasound for ovarian cancer in both high-risk and population risk women is ineffective. BJOG 114 (12), 1500–1509.
Yaari, Z., Yang, Y., Apfelbaum, E., Cupo, C., Settle, A.H., Cullen, Q., et al., 2021. A perception-based nanosensor platform to detect cancer biomarkers. Sci. Adv. 7 (47).
Zhang, S., Dolgalev, I., Zhang, T., Ran, H., Levine, D.A., Neel, B.G., 2019. Both fallopian tube and ovarian surface epithelium are cells-of-origin for high-grade serous ovarian carcinoma. Nat. Commun. 10 (1), 5367.