Urine Biomarkers for the Early Detection of Ovarian Cancer – Are We There Yet?

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Kelly Grayson, Ebony Gregory, Ghazala Khan and Barbara-Ann Guinn

Department of Biomedical Sciences, University of Hull, Hull, UK.

ABSTRACT: Ovarian cancer affects around 7500 women in the United Kingdom every year. Despite this, there is no effective screening strategy or standard treatment for ovarian cancer. If diagnosed during stage I, ovarian cancer has a 90% 5-year survival rate; however, there is usually a masking of symptoms which leads to an often late-stage diagnosis and correspondingly poor survival rate. Current diagnostic methods are invasive and consist of a pelvic examination, transvaginal ultrasonography, and blood tests to detect cancer antigen 125 (CA125). Unfortunately, surgery is often still required to make a positive diagnosis. To address the need for accurate, specific, and non-invasive diagnostic methods, there has been an increased interest in biomarkers identified through non-invasive tests as tools for the earlier diagnosis of ovarian cancer. Although most studies have focused on the identification of biomarkers in blood, the ease of availability of urine and the high patient compliance rates suggest that it could provide a promising resource for the screening of patients for ovarian cancer.

KEYWORDS: Biomarker, ovarian cancer, early detection, diagnosis, urine

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CORRESPONDING AUTHOR: Barbara-Ann Guinn, Department of Biomedical Sciences, University of Hull, Hardy Building, Cottingham Road, Hull HU6 7RX, UK.
Email: B.Guinn@Hull.ac.uk

Introduction

Clinicians and researchers have long sought an effective and non-invasive method for diagnosing ovarian cancer (OC) at the earliest possible stage of disease. Earlier detection is the key to reducing OC mortality as this enables an optimal treatment response and a reduced chance of metastasis, and therefore improved survival rates.[1] Almost 6 in 10 cases of OC are diagnosed in the later stages, stage III and IV, when the 5-year survival rate is below 40%, in comparison with stage I, when the 5-year survival rate is 90%.[2] In addition, 15% of OC cases are not staged often due to the patient being too ill to benefit from staging information and having a 5-year survival rate of 12.5%, suggesting that the impact of a late-stage diagnosis may still be underestimated.[3] Despite breakthroughs in genomics, molecular medicine, and proteomics, a reliable diagnostic method for early-stage OC has yet to be developed, hindering the patient's eligibility for effective treatments and associated with an ever-worsening prognosis.[4] As a result, OC is considered to be the most fatal gynaecological disease. The correlation between survival rates and disease stage at diagnosis supports the need for earlier OC diagnosis and when compared with the 86.6% overall 5-year survival rate of breast cancer, the survival rate for patients with OC is significantly worse.[5,6]

Aspects of OC such as the lack of aetiological understanding, the high cost for treatment which has yet to be standardised, and the lower prevalence of OC in comparison with other cancer types have placed stringent requirements on any screening test.[7] When applying these rigorous standards, none of the biomarkers in clinical use for early-stage OC, including carcinoembryonic antigen (CEA), cancer antigen-125 (CA125), carbohydrate antigen 19-9 (CA19-9), and human epididymis protein 4 (HE4), are effective.[8] This is due to the lack of sensitivity and specificity of the currently available biomarkers for OC, the two key measures of diagnostic accuracy. The sensitivity of a biomarker is measured by its ability to identify a patient with the disease correctly, as it will be present in diseased samples, and the specificity is measured by the ability to not be detected in healthy individuals.[9] A biomarker with only one of these attributes will lead to false positives or false negatives, respectively. Therefore, the ideal biomarker will be both sensitive, positive in samples from patients who do have OC, and specific, negative in samples from healthy individuals, even at the earliest stages of disease (before symptoms appear).

Developing diagnostic tests for OC with the capacity to sensitively and specifically predict cancer in its earliest stages increases the likelihood of effective responses to therapy that could protect fertility and maximise survival rates.[10] Surgery to remove only the affected ovary, fallopian tube, and surrounding tissue (unilateral salpingo-oophorectomy) can be given to stage IA patients, but subsequent stages typically involve the removal of both ovaries and fallopian tubes (bilateral salpingo-oophorectomy) or a hysterectomy.[11] It is not recommended even for the earliest stage patients to keep both ovaries due the potential for microscopic metastasis. Rapidly proliferating cancer cells are also only temporarily chemo-sensitive, rendering many OC patients ineligible for chemotherapy, radiotherapy, and other treatments, contributing further to a poor patient prognosis. Life-changing surgery for many women could be circumvented by earlier diagnosis, facilitated by specific and sensitive biomarkers.

Biomarkers are biological characteristics that can be objectively measured to indicate a healthy or pathological state, the

stage of a disease and/or predict therapy response.[12] These definitions categorise biomarkers as diagnostic, prognostic, and predictive, respectively. However, the evolving definition of a biomarker extends to include any substance, structure, or process that can be measured and influence or predict the occurrence or outcome of disease.[13] Advancements in proteomics and molecular biology have allowed for the number of viable biomarkers to grow at an exponential rate, with blood plasma and serum (plasma excluding clotting factors) long being the main focus for biomarker discovery. However, attention is now being paid to urine, an easily accessible waste material, due to its lower complexity proteome and lack of homeostatic mechanisms in comparison with blood.[14] Urine offers a fortuitous resource for analysis and novel biomarker discovery that can contribute towards the development of personalised medicine. In particular, urine biomarkers would be useful for diagnosing OC due to its non-invasive collection, as well as providing an indicator for responses to therapy and a baseline for each patient from which disease progression can be tracked.

This review aims to explore how the use of biomarkers has influenced OC detection, treatment, and mortality and to investigate the demand for novel biomarkers for early stage I and II OC. In particular, urine biomarkers will be discussed as a source for early-stage OC biomarker discovery. We will compare biomarkers based on their usage, utility, and clinical significance. Diagnostic and prognostic procedures routinely used for OC will also be considered, which may add weight to the need for effective urine biomarkers to facilitate screening of women for early OC detection. Mortality rates and treatment response statistics will be used to stress the paramount need for a prompt, early-stage diagnosis for OC, and how, if at all, the use of current biomarkers had influenced these statistics within the last decade.

Current OC Diagnostics

Diagnostic technologies are being refined to detect OC at the earliest point in disease progression such that treatment is initiated at the earliest possible stage, increasing the associated cure rate.[10] A high specificity is also important to decrease the need for surgical confirmatory procedures which can be costly and lead to complications such as infection.[15] Moreover, a highly sensitive and specific screening procedure is associated with a high positive predictive value (PPV) and a high negative predictive value (NPV), thus minimising the occurrence of false positive/negative readings which result in false referral or failure of cancer detection, respectively.[16] Procedures such as pelvic examination, transvaginal ultrasonography (TVUS), and laparoscopy have been developed in an attempt to screen high-risk women for the earlier detection of OC.[17]

A physical examination is the first step in evaluating a patient with a known or suspected OC diagnosis.[18] A pelvic examination consists of a manual examination of the abdomen and pelvic area to feel for any abnormal nodules, bumps, swelling, enlarged ovaries, or fluid accumulation. It is often the case that women are misdiagnosed when the pelvic examination does not indicate any swelling, lumps, or tenderness and subsequently patients are not then referred for further tests. A pelvic examination, internal and external, can give a potential diagnosis for OC as well as other gynaecological diseases, but it cannot reliably exclude the presence of cancer.

TVUS are accepted as an integral component of the OC screening process, often used as an initial screening test or as a secondary examination in high-risk females.[19] Performed using a 5 to 7.5 MHz vaginal probe, TVUS produces ovarian images by applying ultrasound waves across the vaginal wall to visualise the pelvic cavity and any masses. This is an easily performed technique that can be used to identify early/abnormal morphological changes to the ovaries that are non-identifiable upon physical examination.[20] However, neither an examination nor ultrasound can determine whether the mass is a tumour, or whether a tumour is malignant or benign.

Used independently, neither pelvic examination nor TVUS is sufficient in giving an accurate and reliable early diagnosis of OC.[20] Pelvic examinations are useful for prompt referrals and ruling out physical abnormalities; however, there is a risk that cancerous abnormalities may be overlooked due to the small size of the ovaries and their location within the pelvis.[21,22] In addition, stage I and stage II OC can be asymptomatic and therefore a pelvic examination would give a false negative result, increasing the burden on diagnostic techniques and the need for a way to easily and reliably screen for OC in the general population.[23]

Although TVUS meets certain criteria such as ease, time efficiency, sensitivity, and low risk, it is an expensive procedure, unreliable in differentiating benign from malignant ovarian tumours and has a low PPV.[19] Developments in the use of TVUS have led to morphology indexing and contrast-enhanced TVUS using microbubble contrast-agent particles.[24] Such developments give light to morphological alterations and tumorigenesis which predict metastasis and cancer staging.

Current Biomarkers for OC

CEA was used as the first biomarker for OC in 1976, but was quickly replaced by CA125 as, in comparison, CEA showed lower sensitivity and specificity for detecting OC using patient serum.[25] With the largest wealth of research, CA125 is currently the only serological biomarker in routine use for the management of patients with OC.[26] CA125 levels are found to be elevated ($\geqslant$35 units/mL) in 83% of OC patients.[27] Preoperatively, CA125 serum readings are useful to predict malignant potential and higher levels correlate with an increased risk of mortality.[28] Despite CA125 being one of the highest in terms of specificity and sensitivity among existing OC biomarkers, many weaknesses render CA125 ineffective during early-stage screening.[29] CA125 is a particularly poor biomarker, in terms of specificity and sensitivity, most notably

Table 1. Stage I ovarian cancer tumour biomarker sensitivity in patient serum.

BIOMARKER(S)	SENSITIVITY AT 95% SPECIFICITY (%)	SENSITIVITY AT 98% SPECIFICITY (%)
HE4	45.9	30.8
CA125 + HE4	39.5	38.4
CA125 + osteopontin	15.3	15.3
CA125	15.1	7.7
Osteopontin	14.7	7.6

Adapted from Moore et al[43].

in early-stage OC development.[30] In addition, the histology type of OC also affects the concentration of CA125, with most epithelial OC (EOC) showing the highest levels and mucinous tumours showing low expression levels of CA125.[31] A number of other conditions are also characterised by elevated CA125 levels, including endometriosis, pelvic trauma, and ovarian cysts, and in the majority of studies, only 50% to 60% of stage I OC patients have increased CA125 levels.[30,32] Furthermore, with serum CA125 requiring the use of phlebotomy, collection is invasive and uncomfortable, introducing a very small risk of infection and increased costs predominantly in terms of staff time.[26]

CA19-9 is most commonly used as a biomarker for pancreatic and gastrointestinal cancers,[33] but has shown the potential to support a diagnosis of OC in a number of small trials. OC patients with elevated CA19-9 levels typically have mucinous tumours, whereas CA125 is frequently less elevated in these patients, and therefore, CA19-9 levels could be a useful biomarker for this histotype.[34] However, there have not been enough studies, or studies with enough patients, at present, to determine whether CA19-9 is a reliable biomarker for OC, and these studies still require the invasive collection of patient serum.[35]

HE4 is a proteinase inhibitor that was found to have increased expression in OC in 1999 and has been argued as a superior biomarker when compared with CA125 for several years.[36,37] A systematic analysis was conducted to evaluate the diagnostic value for HE4 in detecting OC.[38] The quality of studies included in the analysis was assessed using the Quality Assessment of Diagnostic Accuracy Studies (QUADAS-2) tool. Seven publications were used in the analysis that encompassed 413 OC patients and 573 controls. The authors found the use of urinary HE4 to be sensitive and highly specific for the diagnosis of OC and suggested that diagnosis for OC using this non-invasive method could be highly efficient.[38,39] It was also demonstrated that HE4 can be detected in the urine of OC patients at a specificity level of 94.4%, including those with stage I/II disease. This data indicated that measuring HE4 in the urine may aid early-stage diagnosis and help to monitor the response of therapy leading to the effective generation of treatment plans for patients. However, the study concluded that a prospective study would be necessary to validate and expand the findings.[40] This investigation was one of the first to report that HE4 may be a promising urine biomarker for OC; however, of the 413 OC patients used in this analysis, less than 16% had stage I OC. Therefore, further studies using a much larger sample size that focuses on early disease stage would be essential to investigate the ability of HE4 to accurately and sensitively diagnose early-stage OC. At present, CA125, CA19-9, and HE4 are unsatisfactory as biomarkers for effective screening methods that would enable an early OC diagnosis, but in combination, these biomarkers merit further investigation.[41]

Combining biomarkers for use in a panel has been shown to have a higher diagnostic sensitivity than using single markers. A study of serum from patients with benign ($n = 262$) or malignant ($n = 196$) ovarian tumours was analysed alongside healthy donors ($n = 386$) for CEA, CA125, CA19-9, and HE4 levels, alone and in combination with each other. This study noted the highest sensitivity achieved when CA125 and HE4 were used in combination.[42] These results mimic those of a previous study, which also showed that the same biomarkers in combination gave the best result and no further improvement was achieved by the addition of other biomarkers including osteopontin (Table 1).[43]

Studies that combine biomarkers in diagnostic screens now include the use of multivariate index assays (MIA), such as OVA1, that detect biomarkers CA125, prealbumin, apolipoprotein A-1, β_2-microglobulin, and transferrin and generates an index score, from 0 to 10, to determine whether there is a high or low probability of malignancy. The sensitivity of OVA1 has been shown to be between 92.2% and 94% in studies, and 98.1% in combination with TVUS and pelvic examination, which is significantly higher than the standard test for CA125 at 76%.[44,45] Although OVA1 showed a very high sensitivity rate, the specificity for detecting OC was far lower than compared with testing for CA125 in patient serum at 35%.[46] The second-generation MIA (MIA2G), named Overa, was released in 2016 and combines CA125, HE4, apolipoprotein A-1, follicle stimulating hormone, and transferrin in an effort to maintain the assay sensitivity and increase specificity for OC.[46] While MIA2G has increased the specificity of detection to 64%, it has not reached the criteria to stand alone as a diagnostic tool for OC.[45]

Despite the wealth of biomarkers and MIA that are already available for OC, most are considered to be insufficient in their ability to detect early-stage disease. A proposal was made that for an effective screening technique to achieve an early-stage diagnosis, it must obtain a specificity greater than 99.6%, to achieve a PPV larger than 10%, and a sensitivity greater than 75%.[47] In addition, the assay would be easily quantifiable, clinically

Table 2. Summary table for the most commonly used serum biomarkers for OC detection.

BIOMARKERS	NO. OF OC CASES ANALYSED	SENSITIVITY (%)	SPECIFICITY (%)	PPV (%)	REFERENCES
Leptin, prolactin, osteopontin, and insulin-like growth factor-II	100	95	95	95	Mor et al[48]
Serum proteomic profile	50	100	95	94	Petricoin et al[49]
CA125 and prostasin	37	92	94		Mok et al[50]
CA125 and apolipoprotein A1, transthyretin, and inter-α-trypsin inhibitor heavy chain H4	41	74	97		Zhang et al[51]
CA125	75 000	65	97-99	4.6	Zhang et al[51]
CA125 and soluble IL-2Rα	39	88.5	27.1		Sedlaczek et al[52]

Abbreviations: OC, ovarian cancer; PPV, positive predictive value; TVUS, transvaginal ultrasonography.
Each biomarker is evaluated in terms of specificity and sensitivity to judge their ability to detect the presence of OC effectively and accurately. Comparisons can be made between CA125 used independently and in conjunction with TVUS. Sensitivity refers to the probability of correctly identifying that OC is present. Specificity refers to the probability of correctly determining when OC is not present.

Table 3. Summary table for urine biomarkers for ovarian cancer.

BIOMARKER(S)	SENSITIVITY (%)	SPECIFICITY (%)	REFERENCE
N,*N*-diacetylspermine	86.5	65.2	Niemi et al[57]
HE4	76	92	Jia et al[38]
Eosin-derived neurotoxin + COOH-terminal osteopontin fragments	72	93	Ye et al[58]
Mesothelin	42	95	Badgwell et al[59]
CA125	3.3	98	Moore et al[43]

validated, and non-invasive, with many current biomarkers failing to meet these standards (Table 2).[53] To fill this gap, different sources of biomarkers, such as urine, must be investigated further to increase the opportunity for novel biomarker discovery.

Current Urine Biomarkers for OC

The earlier diagnosis of OC using urine biomarkers would circumvent invasive screening methods that are required for the detection of serum and plasma biomarkers.[38] Urine is the more favourable body fluid for biomarker detection by ease of access, volume available, and a protein profile that is less complex than that of plasma or serum. In addition, the proteins or peptides excreted with urine tend to maintain a greater stability.[27,54] Potential urine biomarkers, such as HE4 and Bcl-2 (B-cell lymphoma 2), for OC may also be able to distinguish between benign and malignant tumours.[55] However, insufficient sensitivity and specificity readings have so far left the ability to differentiate between malignant and benign debatable, with HE4 having only a specificity of 87% and sensitivity of 74%.[56] HE4 is still under consideration for use as an OC urine biomarker pending larger trials, and due to being novel, under development and not yet fully established. The previously described meta-data analysis of urine HE4 in comparison with serum showed that there was high heterogeneity in the patient studies in the seven trials found to include urinary HE4, with the largest number of participant in one trial being 92 OC patients against 187 healthy donors.[39] Therefore, currently, there is very little evidence to suggest that HE4 has the potential to improve mortality rates and treatment response statistics (Table 3).[38]

Urinary polyamines

Research has uncovered that potential polyamine biomarkers for OC that may be able to distinguish between benign and malignant urine may be used as a biomarker for OC diagnosis.[57] In this prospective study, 71 postmenopausal women and 22 control women provided morning preoperative urine samples. CA125 serum levels were also determined. Of all polyamines analysed, only *N*,*N*-diacetylspermine was found to be statistically significant.[57] *N*,*N*-diacetylspermine was elevated in malignant tumours when compared with benign tumours ($P < 0.001$) and even in early stage. Despite showing potential for early-stage OC detection, applications for the use of this polyamine are arguable due to having unsatisfactory sensitivity and specificity values.[60] To avoid the occurrence of false positives and false negative results, it is paramount that any diagnostic method used leaves little room for error. It was shown that *N*,*N*-diacetylspermine has a higher sensitivity (86.5%) than CA125

but a lower specificity (65.2%) for distinguishing between benign and malignant OC tumours.[57] These values are lower than what is considered acceptable for a diagnostic technique to be accepted for routine use.[47] Consequently, further research should be undertaken to reassess how urinary polyamines can be used as an effective and reliable diagnostic tool.[57]

Protein/peptide profiling

Protein and peptide profiling is a two-step proteomic approach to identify candidate proteins or peptides within the urine of OC patients for their potential use as a screening tool. Mass spectrometry, two-dimensional gel electrophoresis, and liquid chromatography were used to detect differences in the urine of OC patients ($n = 128$) in comparison with those with benign conditions ($n = 52$), other cancers ($n = 44$), and healthy donors ($n = 188$).[58] Two potential urinary biomarkers were identified: glycosylated forms of eosin-derived neurotoxin (EDN) and COOH-terminal osteopontin fragments.[58] Immunoprecipitation showed that a hyperglycosylated form of EDN was detected at levels two times greater in OC urine samples when compared with urine samples from patients with benign conditions. Two different osteopontin fragments (~20 kDa) were present only in OC urine samples, which supports previously published data showing that OC exhibits different osteopontin expression to healthy donors and between different OC histotypes.[61]

Due to both osteopontin and EDN not being cancer-specific markers, they may be detected in conditions including systemic inflammation and eosinophilia due to other cancers.[62] When used in combination, urinary osteopontin and EDN had a sensitivity of 72% and specificity of 95% for OC against controls.[58] Specificities were higher for the biomarkers when used together in comparison with independent use (47% for osteopontin and 63% for EDN). However, these figures would be considered inadequate for use as biomarkers in diagnostic tests for OC. Accuracy and reliability are vital elements of a screening tool for early OC diagnosis as this disease type is highly asymptomatic and consequently, more evidence would need to be provided regarding the robustness of osteopontin and EDN before confidence can be invested into their use in combination for an earlier OC diagnosis.[63] Nevertheless, the use of proteomics to identify urinary biomarkers should not be disregarded.

MicroRNAs

MicroRNAs (miRNAs) are an emerging source of biomarkers for many cancer types due to their key involvement in post-transcriptional regulation of gene expression.[64] The close relationship between miRNA and tumour development, as oncogenes, also known as oncomirs, or oncosuppressors, known as anti-oncomirs, and the nature of the highly conserved molecules, would make them ideal biomarkers.[65] While there are many questions surrounding miRNAs as biomarkers for OC, they have been found in the urine of OC patients and have shown promising results in facilitating early-stage disease detection.[66] A study investigating miRNAs using microarrays analysed urine samples from 39 ovarian serous adenocarcinoma patients, 26 patients with benign gynaecological disease, and 30 healthy controls. It was found that the miRNA, miR-30a-5p, was increased in serous adenocarcinoma patients when compared with healthy samples, which correlated with early-stage disease. For further validation, miR-30a-5p was found to be reduced in 20 gastric cancer and 20 colon carcinoma patients suggesting at least some OC specificity.[67] In addition, miR-92a and miR-200b were found to be upregulated and miR-106b and miR-100 were found to be downregulated in OC.[68] Previous research on patients with EOC may show that miR-200 is the most promising candidate. Using miR-200a, miR-200b, and miR-200c in combination yielded 100% specificity and 83% sensitivity for EOC ($n = 20$) against healthy donors ($n = 32$).[69] With this, the development of a high-throughput diagnostic kit for miRNA in OC will also have to be developed and validated.[70]

Matrix metalloproteinase

Matrix metalloproteinases (MMPs) have been linked to tumour progression, migration, and poor patient prognosis in a variety of cancer types. MMPs are extracellular matrix (ECM) remodelling enzymes which act on many components including gelatins, fibronectins, collagens, and elastin, as well as having roles in angiogenesis, apoptosis, cell proliferation, migration, and many other cell regulatory functions.[71–73] MMP-1, 2, 3, 7, 8, 9, 13, 14, and 15 have all been linked to the progression, migration, and metastasis of OC,[74] increasing in expression with disease stage and poor prognosis. For an in-depth review of MMPs, please see the works of Zhang and Chen[74] or Al-Alem and Curry.[75] In 2011, a study of 178 women showed that patients with OC ($n = 97$) had higher levels of MMP-2 and MMP-9 than healthy donors ($n = 81$).[76] When used in combination with patient age, these urinary biomarkers had an 82% sensitivity and a specificity of 75%. However, this study focussed on patients with late-stage OC leaving their potential as early-stage biomarkers to be explored.

Urine as a Source for Novel Biomarkers

Identifying novel urine biomarkers would be a breakthrough for this area of gynaecological cancer research due to the improvements in survival and treatment response rates that could be seen if OC was detected sooner, with urinary metabolites potentially proving useful in this regard[77] by virtue of its ease of collection. However, the question remains as to whether urine biomarkers could be superior to those found in patient blood and, if so, why looking for novel biomarkers in urine has been less prevalent.

Blood has traditionally been the main subject for biomarker discovery due to the wealth of knowledge of the dynamic blood proteome which is more complex than the urine proteome, relative uniformity of volume, and concentration between patients and a large amount of blood samples are collected as part of routine tests.[14] In addition, the collection of blood relies on a phlebotomist rather than the patients themselves and has a very limited chance for bacterial infection, unlike urine which can be easily contaminated. Patient gender, habits, diet, age, hormones, and genetics, alongside environmental factors, can influence the urine proteome of each person, making identifying individual patient's baselines difficult.[78] The lack of high-throughput techniques for urine analysis has also affected biomarker discovery, as the labour intensive techniques including two-dimensional gel electrophoresis and enzyme-linked immunosorbent assays (ELISA) have been overlooked in favour of high-throughput assays and transcriptomics, both of which are readily available for plasma and serum proteomes.[79]

Although blood has been the first choice for biomarker discovery, it has several disadvantages in comparison with urine. We have already mentioned the homeostasis mechanisms involved in maintaining correct body temperature, pH, composition, and many other factors.[80] Homeostasis is an essential and continuous process that is constantly reacting to a variety of signals to maintain and regulate variables in the human body and blood.[81] It could therefore be surmised that some biomarkers are removed from the blood as part of these natural processes. This is a particular issue for early-stage disease biomarkers as they may only be present and detectable in the blood for a limited amount of time, and most likely in low concentrations, at any one time point in the disease progression.[14] Many blood biomarkers for cancer have been antibody responses to cancer rather than cancer-specific proteins per se. In contrast, the urine is a product of the homeostasis mechanism, and it would therefore be more probable to find changes in chemical composition from most body sites in urine, making it a viable source for biomarkers. Protein modification and degradation is known to occur in the blood and at the time of its collection, while degradation is not thought to be induced by the urine collection process itself.[82,83] These factors, in addition to the ability to collect urine non-invasively, make urine promising resource for new biomarker discovery.

Discussion

Currently available screening procedures for OC, including CA125, TVUS, and other biomarkers lack the necessary sensitivity and specificity to deliver cost-efficient, accurate, and reliable screening for the general female population, or even high-risk individuals.[27] Despite the variety of conventional screening methods in routine use, OC remains the most common form of gynaecological malignancy with the highest mortality rate. The identification and validation of early detection urine biomarkers such as polyamines, peptides, and HE4 are required to inaugurate non-invasive screening methods for the earlier diagnosis of OC at a more treatable and curable stage of disease. Although there are promising candidates for OC urine biomarkers, their utility regarding specificity and sensitivity is questionable especially at the earliest stages of disease. The ability to use readily accessible urine samples to detect early-stage OC would be beneficial for both clinicians and patients, compared with the more complex and invasive clinical procedures currently used.[84] Evaluation of promising urinary biomarkers for OC opens up a new scope of research that could contribute to existing biomarker candidates, thus improving diagnostic techniques for OC in its earliest stages of disease.[85]

CA125, when used independently, has been disappointing in terms of sensitivity and specificity.[86,87] Consequently, a movement towards multi-biomarker panels aimed at augmenting the sensitivity and specificity of biomarkers including CA125, urine HE4, and mesothelin has been developed to compensate for the limitations of current OC biomarkers.[53,88] For example, CA125 levels are non-specific for OC detection as they are seen to be elevated in other cancer sub-types and benign conditions, in disease states such as endometriosis and during ovulation.[63] When used alongside biomarkers, such as HE4, the diagnostic ability improves.[89] Combining the multi-biomarker panel with screening for germline BRCA1 and BRCA2 mutations has shown to provide more accurate diagnosis for high-risk populations who are genetically predisposed to OC development.[63] However, with this novel diagnostic approach for OC under development, it has not yet been optimised for the earliest stages of disease. In the future, it is likely that a combination of tests, a panel of urine biomarkers, non-urine biomarkers, as well as TVUS and physical examination(s), will be required to ensure sufficient sensitivity and specificity, as using single biomarker tests in isolation has so far been limited in the information it has provided.

Conclusions

The tests for OC are currently inadequate in their ability to provide an early-stage diagnosis. The current diagnostic methods using CA125 and TVUS together only detect 30% to 45% of women with early-stage OC, and therefore, the majority of women are still being diagnosed at a later stage[1,90] when the symptoms of OC are apparent. Current urine biomarkers are insufficient for the effective detection of OC at stage I and II, but may be superior in their diagnostic ability when used alongside other non-urinary biomarkers and TVUS. Urine biomarkers therefore require further validation for their independent use as an OC diagnostic tool to be considered adequate, reliable, specific, and sensitive. Future work to elucidate urine biomarkers specific for early-stage OC could enable the development of routine screening tests, enable early diagnosis, and significantly reduce the poor survival rates associated with the later detection and treatment of OC.

Author Contributions

KG visualised and wrote the manuscript, and prepared tables 1-3. EG was involved in initial draft preparation. GK revised the manuscript pre-publication. BG conceptualised and edited the manuscript.

ORCID iD

Barbara-Ann Guinn https://orcid.org/0000-0003-0639-4541

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