Early Detection of Ovarian Cancer, is it Possible?

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Ovarian cancer is the 5th most common cancer in females, with the highest mortality of any gynaecological malignancy today [1,2]. Despite modern advances in treatment over the last 40 years, up to 70% of diagnosed females present with late stage disease in whom the UK 5-year survival remains unchanged [3,4]. Unlike other female cancers including endometrial, breast and cervical cancer, which can be diagnosed early through appropriate clinical examination and biopsy, ovarian cancers can only be diagnosed by invasive investigations such as omental biopsy, laparoscopy and laparotomy [2,4].

Ovarian cancers are overwhelmingly epithelial derived (~90%) and can be broadly classified into two types. Type 1 ovarian cancers are low grade slow growing cancers with often mucinous and dear cell histology and can be associated with underlying endometriosis. In contrast, type 2 ovarian cancers are of high grade serous histology and present at a later stage and confer increased mortality [5,6]. Current evidence suggests that ovarian cancers and peritoneal cancers may originate from the fallopian tubes as shown by the high proportion of tubular intraepithelial carcinoma (TIC) present in women undergoing risk reducing ovarian surgery. Therefore, opportunistic salpingectomy without ovariectomy is now considered in all abdominal hysterectomies to reduce future ovarian cancer risk.

The focus of recent research has been the identification of at risk individuals to try to alleviate the burden of this disease. Of those risk factors documented a first-degree family history of either ovarian/breast cancer (Relative Risk: 3.6/1.4 respectively) is the most significant. This is present in 10-15% of women with ovarian cancer, and is even greater in hereditary cancer syndromes such as Familial BRCA1/2 mutation and Lynch syndrome [1]. In addition, up to ~10% of ovarian cancers occur in women positive for BRCA1 and BRCA2 mutations (carrier frequency 1 in 500-800) and incurs a lifetime risk of ovarian cancer in the order of 40% and 18% respectively [7]. Other established risk factors, related to hormonal exposure, include early menarche, late menopause and obesity while protective factors include pregnancy, hysterectomy+salpingectomy, hormone replacement therapy and combined oral contraceptive use. Indeed, it is the routine use of combined oral contraception that accounts for the overall decreasing incidence of ovarian cancer, as every 5 years of use results in a risk reduction of 50% which is maintained decades after their cessation [7].

A key issue that limits early diagnosis of ovarian cancer is its wide-ranging presentations. In a recent systematic review exploring this concept it was found that ovarian cancer present similarly and with significant overlap to irritable bowel syndrome, dyspepsia and menopause. More importantly, it was found that there are no symptoms that can effectively rule out ovarian cancer [6]. Therefore, the aim is to design a screening test which does not rely on physical symptoms but instead allows for early identification of ovarian malignancy through biomarker and imaging measures and thus shift the stage of diagnosed disease to stage 1 where 5-year survival is >90%. Early work in this area has provided some debate regarding the screening of high-risk individuals in whom results have been conflicting and has not yet demonstrated a significant improvement in survival and mortality [7]. However, the utility of screening in average risk individuals who make up the majority of patients is yet to be determined.

A key concept in cancer screening is that the test offered should be minimally invasive and that there must be a suitable time interval between detectable early-stage disease and the presence of metastasis. Currently, many clinical biomarkers exist in the field of cancer screening, although they are not specific to this setting. The challenge for research is to identify a defined cut-off for these biomarkers which discriminates between benign and malignant disease. In the setting of ovarian cancer, it has been demonstrated that a screening test must achieve a minimum specificity of 99.6% and sensitivity of ≥ 75% if a positive predictive value (PPV) of 10% is to be achieved. This latter statistic is important so as to limit the number of unnecessary operations required to detect each case of ovarian cancer. To achieve such an effective screening test may require the use of multiple tests as part of a screening programme [8]. The use of multiple tests does have a significant impact on a screening programme’s performance, for instance, when tests are combined in series the specificity is improved at the cost of the sensitivity; while when tests are performed in parallel then the reverse is true [8].

One biomarker that has attracted much attention for screening purposes is CA-125. This mucinous glycoprotein expressed by adult tissues derived from coelomic and mullerian epithelium can be elevated up to 10-60 months before ovarian cancer diagnosis and is used clinically to detect recurrence following treatment.
The issues arise from the fact that CA-125 levels fluctuate greatly in healthy women and can be elevated in many benign and physiological conditions including endometriosis, uterine fibroids and menstruation [7]. In addition CA-125 is differentially expressed according to the underlying stage of ovarian disease and disease subtype with only 50% of patients with stage 1 disease demonstrating elevated levels versus 80% of patients with advanced disease [8]. Currently a cut-off value of 35U/mL is used to differentiate between benign and malignant disease, although it has failed to demonstrate a reduction in ovarian cancer mortality even in high-risk groups. This is due to the inadequate sensitivity and the high false-positive rate that CA-125 displays in asymptomatic individuals [5]. It is because of this that in the recent Prostate Lung Colorectal and Ovarian (PLCO) trial [9], which utilised concurrent transvaginal ultrasound (TVUS) and CA-125, that after a 13-year follow-up period no decrease in mortality was observed. This study suggested that up to one third of ovarian cancer cases could have been detected earlier by evaluating CA-125 velocity versus annual single measurements [10]. Indeed, when CA125 velocity was explored alongside several covariates including first-degree family history, age at baseline and the time interval between screening tests, it was found that this approach demonstrated statistically significant predictions of ovarian cancer in asymptomatic individuals with greatly improved specificity and PPV when used as part of a multiple logistic regression model [10].

Additional algorithm based approaches to analyse single CA-125 values include the recent UK based ROCa study (Risk of Ovarian Cancer Algorithm) [10], allowing for patient assignment to low, medium and high-risk groups. This algorithm works by interpreting a person’s longitudinal CA-125 levels in comparison to values obtained from confirmed ovarian cancer cases. This study utilised >200,000 premenopausal women assigned to multimodal screening (annual CA-125 screening with analysis by ROCa algorithm), TVUS or a control non-intervention group (n=101,359). It was found that by using the ROCa algorithm twice the number of cancers was detected (n=154) versus a single CA-125 threshold. More encouragingly the sensitivity and specificity of this method was 85.8% and 99.8% respectively which satisfies the statistical criteria put forth earlier. Moreover, of those tumours that were successfully identified, 41.4% were stage I/II demonstrating the ability of this method to diagnose early disease where survival is likely [10].

To further enhance the sensitivity and specificity of CA-125 efforts have been made to discover new serum biomarkers with which it can be combined. One method of achieving this is Proteomics-the study of gene products i.e. proteins, which has successfully led to the discovery of many promising serum biomarkers (Table 1). These include Human Epidydimal Protein E4 (HE4) and those of a seven-biomarker panel (transthyretin; apolipoprotein A1; Transferrin; Beta-2-Microglobulin; Hepcidin; interalpha trypsin inhibitor heavy chain 4 and connective tissue activating protein 3) [3,11].

HE4 is a protein which is elevated in both early and late stage ovarian cancer but not expressed in benign gynaecological conditions. While HE4 has very high sensitivity and specificity (72.9% and 95% respectively) as a single marker in comparison to other studied biomarkers when combined with CA-125a synergistic effect is observed in both the accuracy and sensitivity obtained. Indeed, when ELISA was used to identify HE4, CA125 and both in 37 serum samples from known ovarian cancer patients, the results of successful identification of ovarian cancer were 30/37,29/37 and 33/37 respectively. Unfortunately while promising, HE4 has shown little ability to discriminate between early stage ovarian cancer and benign disease on its own (sensitivity-45.9%; specificity-95%). As such, while encouraging, it is still far from the minimum sensitivity, specificity and PPV required [7]. In comparison, individual biomarkers derived from the seven-biomarker panel in combination with CA-125 have also been unable to satisfy these requirements although, they increase the specificity to 97% with the sensitivity of CA-125 being unchanged [11].

The limitation of many studies that attempt to identify such biomarkers is that they are performed in patients with known ovarian cancer and so offer little information regarding their screening potential. In one study that tried to address this problem using patients prior to diagnosis, it was found that the seven-biomarker panel did not improve the sensitivity that CA-125 provided. In addition, versus post-diagnostic sampling of these markers, it was found that these substances are often down regulated in the setting of ovarian cancer due to their acute-phase reactant properties [11]. The use of currently available multiple biomarkers has failed to satisfy the criteria required for an effective screening test for ovarian cancer and therefore, new approaches are needed to identify better biomarkers. These include investigating epigenetic changes regulated by microRNAs of which 56 have been found to be differentially expressed between control and ovarian cancer patients [3]. Further research focused on nucleic acid biomarkers including free DNA, mRNA, microRNA and circulating tumour DNA (ctDNA) may result in early diagnosis and personalized cancer treatment while limiting the need for invasive methods to obtain tissue biopsy. This could include the screening of ctDNA for mutations of key oncogenes such as mutated TP53 which is known to be the most common genetic change in ovarian cancers and at particularly high frequency in type 2 ovarian cancers. Such an approach would be useful as small molecules that target mutant TP53 are already in development (e.g. Aprea AB) and are known to be able to inhibit the growth of tp53 mutant ovarian cancer cells [12].

Transvaginal ultrasound scan (TVUS) is an effective imaging technique used commonly for the investigation of gynaecological problems. This non-invasive method, when used to image the ovary, allows for accurate and detailed assessment of ovarian morphology, echogenicity and, when used alongside Doppler, provides information on ovarian blood supply. TVUS has 90% sensitivity in stage 1 ovarian disease (PPV 7.4-9.9%) but is limited by being a very technician-dependent investigation and one hampered by patient habitus. It is important to note that the presence of an ovarian mass on TVUS is in itself not uncommon one hampered by patient habitus. It is important to note that the presence of an ovarian mass on TVUS is in itself not uncommon.
Table 1: Biomarkers identified and tested in the setting of ovarian cancer screening *PPV=Positive Predictive Value [3,5,10].

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV*(%)</th>
<th>Raised in other conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA-125</td>
<td>45-65</td>
<td>97-98</td>
<td>&lt;10</td>
<td>YES</td>
</tr>
<tr>
<td>MUC1</td>
<td>70</td>
<td>98</td>
<td>93</td>
<td>YES</td>
</tr>
<tr>
<td>Lysophosphatidic Acid</td>
<td>95</td>
<td>89</td>
<td>93</td>
<td>YES</td>
</tr>
<tr>
<td>Osteopontin</td>
<td>66</td>
<td>88</td>
<td>43</td>
<td>YES</td>
</tr>
<tr>
<td>Kallikrein (KLKS)</td>
<td>52</td>
<td>95</td>
<td>93</td>
<td>YES</td>
</tr>
<tr>
<td>Claudin 4</td>
<td>51</td>
<td>98</td>
<td>N/A</td>
<td>YES</td>
</tr>
<tr>
<td>Transthyretin+ApoA1</td>
<td>52</td>
<td>97</td>
<td>35</td>
<td>YES</td>
</tr>
</tbody>
</table>

Future directions regarding diagnostic imaging of ovarian cancer may soon include optical approaches such as autofluorescence imaging [13]. This relatively new method of studying epithelia in high resolution may allow for early identification of precancerous and cancerous lesions due to the fact that as epithelial cells undergo their transition to cancer (epithelial-mesenchymal transition) they will lose their autofluorescence. This technology is already being applied in the dental setting where it has been found to be a reliable method for identifying severe dysplasia and invasive carcinoma of the buccal cavity and after their resection, dramatically reduce the risk of their recurrence. With regards to early malignant change of the fallopian tube, autofluorescence imaging has demonstrated a sensitivity 73-100%, specificity 83-92% and PPV of 50-78%. While these figures are not to the required level for this method to be an established screening test, it does show the power of this technique to diagnose early disease in the gynaecological setting. In addition, through greater understanding of normal tubular epithelial structure, interpretation of AF imaging and isolation of the epithelia during surgical biopsy it is thought these statistical measures may be greatly improved upon [13].

It may also seem that existing technologies used to detect other gynaecological malignancies may have an application in ovarian cancer detection. The Papanicolau (Pap) test is a well-established gynaecological test that allows for early identification and treatment of cervical cancer in screened women [14]. It has been demonstrated that pap smears can yield enough ovarian cancer cells for a diagnosis to be made; although this is not always the case with only 41% of ovarian cancers considered to be identifiable by this method. Moreover, of those cells isolated by the Pap test microscopic confirmation of ovarian cancer remains challenging as it can be relatively indistinguishable from cervical cancer and more common benign conditions. Recent advances in cervical cancer screening have led to the routine testing of samples for Human Papilloma Virus (HPV). As part of this process DNA purification techniques are employed which may instead be used to identify commonly mutated genes that are implicated in cancer pathogenesis through sequencing methods. As such this due to the high number of pap smear tests performed globally this method could help prevent many cases of advanced ovarian disease [15]. Other methods employing existing technologies include hysteroscopic and laparoscopic based approaches for brush cytologic sampling of the fallopian tube epithelium followed by immunohistochemistry for p53 ad KI-67 to allow the diagnosis of ovarian cancer to be made [16].

In summary, ovarian cancer screening is an important research area that could help improve the prognosis of this common and devastating disease. Like other screening programmes the identification of a precursor lesion or early-stage malignancy is key for it to be effective. Due to current understanding regarding ovarian cancer aetiology it may be that identifying Tubular Intraepithelial Carcinoma (TIC) effectively and noninvasively may limit late-stage ovarian disease. Traditional markers such as CA-125 have shown limited ability in this regard, however, using velocity measures in combination with other biomarkers and TVUS, improvements in specificity, sensitivity and PPV can be achieved. Currently, no one biomarker has been identified which alone can achieve the aforementioned screening test criteria. Through the use of emerging technologies, including proteomics and cDNA analysis, additional markers may be identified in pursuit of this aim. In my opinion I believe that perhaps the best strategy is to use in high risk individuals identified by both family history and TVUS and CA125 findings, new optical imaging techniques such as autofluorescence imaging. This could allow for easy, reliable and safe identification of early disease. However, while ovarian cancer screening continues to be investigated it remains important that physicians remind themselves the varied presentations of under diagnosed disease.

REFERENCES