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Improving Early Detection of Ovarian Cancer

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Bachelor Thesis

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Summary

Worldwide, 239,000 women get affected by ovarian cancer (OC) and it causes 152,000 deaths every year. OC is a disease that is often detected late in 40% of postmenopausal women because symptoms are vague. This results into detection of the cancer in an advanced stage where less than 70% of the women survives 1 year of disease. So, the objective of this thesis is how the early detection of ovarian cancer can be improved in order to increase survival of ovarian cancer patients. The current state of ultrasound screening (TVS), multimodal screening and more extensive (additional) screening programs are being reviewed to give an answer to this question. The best possible screening method was multimodal screening (third-line screening) based on the Risk Of Ovarian Cancer Algorithm (ROCA) with CA125 tests and (additional) TVS as a secondary screen for women with an elevated risk score. Though, more studies are needed to confirm if it can definitely reduce mortality by an earlier detection. Furthermore, we need to learn more about the reproductive, environmental and genetic risk factors associated with OC to determine the specific population requiring primary prevention or screening.

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1. Introduction

OC is an heterogeneous disease that can be classified roughly in hereditary OC and sporadic OC (Kossaï et al., 2018). Almost all malignant ovarian tumours arise from one of three cell types: stromal cells, germ cells and epithelial cells. More than 90% of malignant ovarian tumours are epithelial in developed countries so these are the focus of this thesis (Reid et al., 2017).

25% of all epithelial ovarian cancers (EOCs) have a heritable component. Familial OC is associated with hereditary breast ovarian cancer syndrome (HBOC) and Lynch syndrome (heritable non-polyposis colorectal cancer syndrome, HNPCC) (Bakir & Gabra, 2014).

The HBOC syndrome is 80% of the hereditary OCs and is affiliated with mutations in *BRCA1* and *BRCA2*. These encode proteins that engage in DNA repair, specifically homologous recombination. Almost all tumours have a high-grade serous (HGSC) histology. Furthermore, they respond well to DNA-damaging platinum-based chemotherapy, have a better prognosis than non-*BRCA1*/*BRCA2*-related OCs and have long periods without disease between relapses (Bakir & Gabra, 2014).

Lynch syndrome is 10-15% of the hereditary OCs and is associated with mutations in *MLH1*, *MSH2*, *MSH6* and *PMS2*. These genes encode DNA mismatch repair proteins that correct and recognize short deletions, insertions and single base mismatches. Tumours have a moderately differentiated phenotype and can be of any histology but most common is the non-serous endometroid cancer type. Patients living with the non-serous endometroid cancer for 30 years have a survival rate of 71.5%. This is much better than patients with a *BRCA1*/*BRAC2* mutation (Bakir & Gabra, 2014).

The vast majority of EOCs are sporadic. Sporadic EOCs can be divided into two types of tumours (type 1 and 2). Type 1 tumours are low-grade tumours that are formed through borderline tumours, endometriosis and ovarian surface epithelium. These kind of tumours have somatic mutations in *PTEN*, *BRAF*, *KRAS*, *PIK3CA*, *ARID1A*, *CTNNB1* and *ERBB2*. Generally, they are faineant tumours with low mortality (Bakir & Gabra, 2014; Aggarwal et al., 2016) (fig. 1).

Type 2 tumours are aggressive high-grade with, most of the time, a bad prognosis. The most common mutation is in *TP53* followed by somatic inactivation of *BRCA1*/*BRCA2*. *TP53* encodes p53 and mutations in this gene are found in more than 95% of high-grade serous EOCs (HGSC). *BRCA1*/*BRCA2*

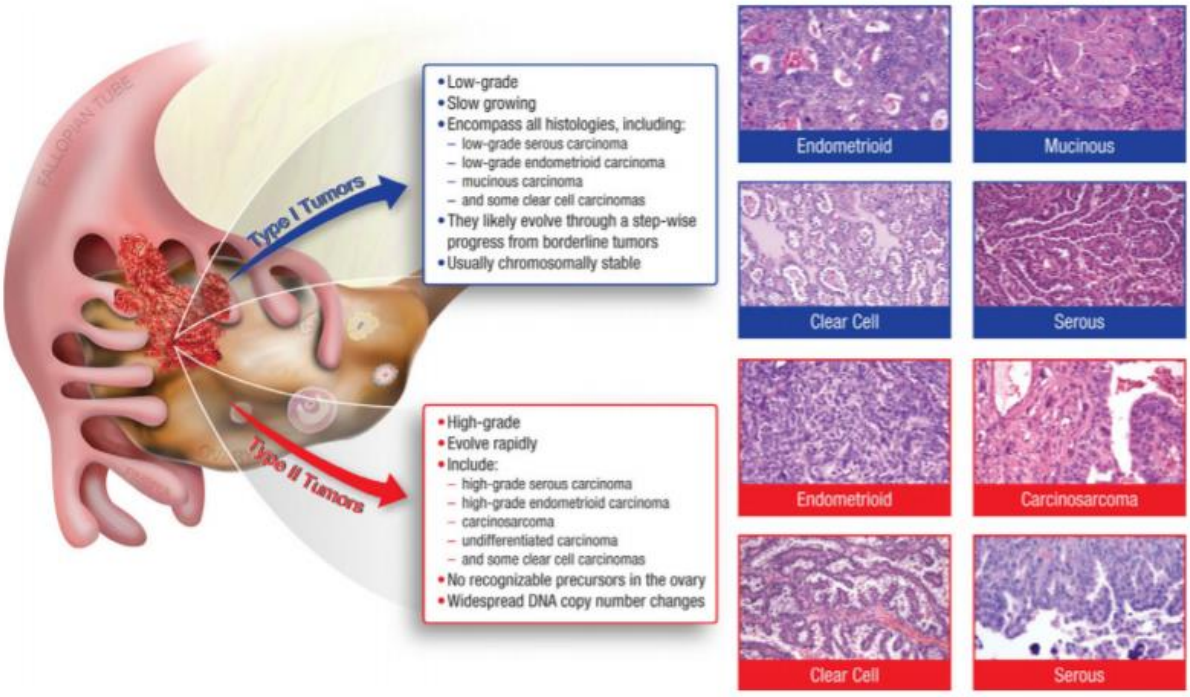


Fig. 1. Type 1 & 2 ovarian tumours (Aggarwal et al., 2016).

is inactivated through hypermethylation or somatic mutations in the gene itself in 40-50% of sporadic HGSCs. So, both *BRCA1/2* and *TP53* are important factors for genetic instability and mutations in these genes lead to tumours that are heterogeneous and chromosomally disordered (Bakir & Gabra, 2014; Aggarwal et al., 2016) (fig. 1).

Globally 239,000 women get affected by OC and it causes 152,000 deaths every year. It is the leading causal agent of death among gynaecological cancers in almost all cultivated countries (Kossaï et al., 2018). On top of that, symptoms of OC are non-specific and vague and that often leads to late detection in an advanced stage. Only 50-60% of postmenopausal women detect OC in an early-stage (Teh et al., 2018). This leads to a high mortality to incidence ratio so that survival at 1-year decreases from 93.5% at stage II to 71% at stage III (Gupta et al., 2019). HGSC grows over a time of 4 years and can be clinically undetected stage III cancers up to a year prior to diagnosis. They have an average diameter of 3 cm's when they metastasize into stage III or IV. An annual screening must detect these adnexal tumours when they are approximately 1.3 cm's to have a 50% sensitivity for stage I or II (Nash et al., 2020).

Thus, through detection at earlier stages, screening can have a major impact on the survival of women with OC. Unfortunately, there are a lot of challenges in constructing an optimal OC screening test. The incidence of the disease is low and false positive screens can lead to unnecessary surgery. Furthermore, screening must detect OC in a stage where it is more remedial (Gupta et al., 2019). So, what this thesis tries to investigate is how the early detection of ovarian cancer can be improved in order to increase survival of ovarian cancer patients.

To do so, in the next few paragraphs, the current state of first line screening (CA125 serum levels) and second-line screening (ultrasound screening) will be discussed. These screenings often have low sensitivity to detect OC in an early stage. So, to improve the sensitivity of early OC screening, third-line screening (multimodal screening) and more extensive (additional) screening programs are being reviewed in subsequent paragraphs in order to find out the best detection method to detect OC in an early stage.

2. Current state of screening

The first-line screening method of OC is measuring the serum Carbohydrate Antigen 125 (CA125) levels in patients' blood. CA125 is a mucin-type glycoprotein secreted by *MUC16*. It is associated with the cellular membrane. The threshold in pre and postmenopausal women is 35 U/mL. Unfortunately, it has a low sensitivity in early stages of OC. Namely, it's only reported to be risen in 23-50% of the women in stage I of the disease (Dochez et al., 2019). The sensitivity of CA125 in advanced stages of OC is 88.24% (Xi et al., 2017).

The second line screening method is imaging therapy for evaluation of an adnexal tumour called transvaginal sonography (TVS) (Mathieu et al., 2018). When detecting OC, it is often combined with measuring serum CA125 levels. It detects disease directly through characteristics associated with a bigger risk for OC such as increasing ovarian volume or through morphological changes (Nash et al., 2020). TVS visualizes both ovaries in both transverse and longitudinal planes and calculates their volume using the prolate ellipsoid formula (length x width x height x 0.523) (Campbell & Gentry-Maharaj, 2018; van Nagell et al., 2011). Figure 2 indicates the morphology index (value between 0-5) with the corresponding tumour volumes and structures (van Nagell et al., 2011).

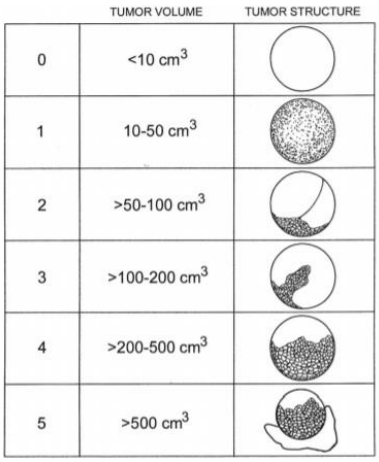


Fig. 2. Morphology index (van Nagell et al, 2011).

2.1 Recent trials & results

There has been done a lot of research in performing first-line screening and second-line screening. One of these trials is The University of Kentucky Ovarian Cancer Screening Trial. This trial had one testing group of patients getting a yearly ultrasound screening. More than 37.000 participants participated in this trial over a period of 24 years with approximately 5.5 scans per participant performed. Women had an average age of 56.6 years. The sensitivity for primary EOC was 86.4%, with 6.8 surgeries carried out per case detected (Campbell & Gentry-Maharaj, 2018). Furthermore, they achieved a positive predictive value (PPV) of 14,5% (Mathieu et al., 2018). When confined to primary invasive EOC the sensitivity is reduced to 79.7% (Campbell & Gentry-Maharaj, 2018).

Three-quarters (75.8%) of the primary OCs were in stage I or II (early stage). In 79% of the patients who had stage III EOC, serum CA125 levels were risen (>35 kU/l) at the time of detection but only in 32% of the patients who had stage I or II EOC. Women in the trial had a substantial longer 5-year survival rate (74,8 ± 6,6%) in comparison with the women who were not screened (53,7 ± 2,3%) (van Nagell et al., 2011). However, it is very likely that the lack of randomization has introduced bias and survival rates instead of mortality were reported which are exposed to lead-time bias (Campbell & Gentry-Maharaj, 2018).

Increased serum CA125 levels caused by for example benign ovarian cysts, endometriosis or leiomyoma result in false positives. Hence, the PPV of serum CA125 is failing to assert its use as an OC screening method (Mathieu et al., 2018). So, TVS is needed to ameliorate specificity and PPV. Furthermore, TVS also has an higher sensitivity for primary than serum CA125 levels (Campbell & Gentry-Maharaj, 2018).

Another study called The Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial performed a non-sequential, multimodal strategy with annual TVS scans and annual screening for single risen values of serum CA125. The PLCO had 78,216 women with an average age of 64 that underwent annual TVS and serum CA125 level screening (n = 39.105) or received conventional care (n

= 39.111). The trial achieved a PPV of 2,6% using single serum CA125 levels at or above a fixed threshold of 35 U/mL in the first four annual screening rounds. But a much higher PPV of 20% was achieved combining TVS and positive CA125 (Mathieu et al., 2018). Unfortunately, there was no significant effect on mortality; the control arms and screening arms included 100 and 118 deaths respectively. The mortality rate ratio was 1,18. So this showed that combining TVS and positive CA125 didn't reduce mortality from OC (Campbell & Gentry-Maharaj, 2018).

Also, the Japanese Shizuoka Cohort Study of Ovarian Cancer Screening, combined TVS and positive serum CA125 using a cut-off of 35 kU/l screening women with an average age of 58 years. The trial was carried out on 82.487 low-risk postmenopausal women for a period of 13 years. Women were screened on average about 5,4 times. The trial had a sensitivity for OC of 77,1% and a specificity of 99,9%. In the screened group, the proportion of stage I OCs was higher (63%) than in the control group (38%), but the difference was not statistically significant. Furthermore, it has not yet been reported what the effect of this study is on mortality (Campbell & Gentry-Maharaj, 2018).

2.2 Limitations

Also mentioned above, these trials have a few limitations. For example, the low PPV of 2,6% in the first four sessions of the PLCO trial, shows a relatively high false-positive rate of TVS for the assessment of adnexal masses (Partridge et al., 2009). Next to this, in both The University of Kentucky Ovarian Cancer Screening Project and PLCO, primary borderline epithelial neoplasms of the ovary were triaged as false positives as they are associated with lower mortality rates and have little potential to become malignant (Jacobs et al., 2016).

So, screening with TVS is more effective in detecting the more faineant, type I tumours in early stage. In addition, a substantial fraction of HGSC cancers are believed to arise in the fimbriae of the fallopian tubes as minor tumours before the cancer proceeds to an advanced stage, so far there is little to no registered knowledge in imaging this anatomy (Mathieu et al., 2018).

3. Multimodal screening

In patients with negative biomarker screens before a positive ultrasound scan, additional third-line modality (multimodal screening) could give a clearer diagnosis (Mathieu et al., 2019). Multimodal screening using the Risk of Ovarian Cancer Algorithm (ROCA) is based on initial CA125 level and estimates age-specific OC incidence (Rosenthal et al., 2017). The trial using this algorithm is further described below.

3.1 Recent trial & results

A trial that performed multimodal screening to determine the effect of screening on disease mortality is The United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS). In this trial, more than 2 million postmenopausal women in the age of 50-74 years were randomized to either no screening (control), ultrasound screening strategy or multimodal screening in a 2:1:1 manner for 7-11 years (Jacobs et al., 2016). In the multimodal group, CA125 was taken applying the ROCA to classify the women into low-, intermediate- and elevated-risk groups. Women at intermediate risk had a repeat CA125 in 12 weeks, whereas women with elevated risk were sent for a TVS scan and another CA125 in 6 weeks. The ROC algorithm classified 91% as low risk and were referred to annual screening, in the prevalence screen. Only 9% of women needed a repeat CA125 test and a TVS scan and 0,2% had surgery. For detection of primary OC, the multimodal method had excellent sensitivity (89,4%), specificity (99,8%) and PPV (23%) compared to just screening with TVS (sensitivity 84,9%, specificity 98,2%, PPV 5%) (Campbell & Gentry-Maharaj, 2018).

Major advantages of this research in comparison with the PLCO Cancer Screening Trial are that there was a main supervision of all facets of the trial by the trial management team, mortality was developed within 3 years of the end of the study and the ROCA was applied to define the CA125 rise (Jacobs et al., 2016).

3.2 Limitations

There are some weaknesses in this trial. For example, there was a late effect in mortality with an average reduction in years 7-14 of 21% in the ultrasound group and 23% in the multimodal group (Jacobs et al., 2016). The late effect of screening in the statistical design is not anticipated in this trial and is likely due to the relatively good short-term survival of women with primary invasive epithelial neoplasm of the ovary (8 years in the non-screened group) (Gupta et al., 2019; Jacobs et al., 2016). Otherwise, the weighted log-rank test could have been in the same timeframe as the PLCO Cancer Screening Trial (Jacobs et al., 2016; Buys et al., 2011).

Furthermore, high-risk women would be persuaded they are safe from cancer due to screening and do not have to undergo far more effective contraceptive surgery. The current screening methods showed that HGSCs developed in high genetic risk individuals are infrequently detected. So, because of this, the efficacy of screening in high-risk women is maybe not very suitable. Contraceptive surgery is highly effective in high-risk individuals so carrying out this screening trial in this setting would be difficult from an ethical point of view (Gupta et al., 2019).

Overall, multimodal screening may improve the early detection of OC but more research is needed to appraise the circumference of the death rate reduction before standing-one conclusions can be drawn on the cost-effectiveness and long-term competence (Jacobs et al., 2016). Women who prefer not to have surgery, multimodal screening using TVS and ROCA every 3 months (at an interval computed by ROCA), seems to be a greater alternative than symptom consciousness alone. Such screening should not be looked at as a substitution of surgery, but it seems to offer a better chance of evading a

diagnosis of advanced incompletely resectable fallopian tube cancer/OC in between (Rosenthal et al., 2017).

4. First-line screening improvements

4.1 Alternative biomarkers

First-line screening measuring only serum CA125 levels is often too short-sighted. So, the sensitivity of using CA125 test for detecting early-stage OC could be enhanced by taking additional blood-based biomarkers like human epididymal protein 4 (HE4), since CA125 is expressed in only 80% of OCs. HE4 is a glycoprotein and is overexpressed in malignant ovarian tissues at a substantial higher rate than that of benign tumours and normal ovarian tissue (Huang et al, 2018). The *N*-glycosylated protein is a product of the *HE4* gene, which is secreted into the extracellular environment and can be detected in the bloodstream of OC patients (Scaletta et al., 2017).

This is a non-invasive method because the only thing that is required is the patient's blood (Simmons et al., 2016). Furthermore, measurements of HE4 serum levels will also help in monitoring the therapeutic efficacy of OC treatment (Teh et al., 2018).

A study performed by Chang, *et al*, used DNA methylation biomarkers in cervical scrapings to detect endometrial and ovarian cancer. For 14 genes in DNA pools of endometrial and OC tissues, the methylation status was tested using quantitative methylation-specific PCR. Tissues of endometrial cancer/normal endometrium and OC/normal ovary were validated in training set using cervical scrapings of 10 OC patients, 10 endometrial cancer patients and 10 control patients. They were further validated in the testing set using independent cervical scrapings in 30 OC patients, 30 endometrial cancer patients and 30 control patients. The study developed cut-off values of methylation index (M-index) from cervical scrapings to differentiate between cancer patients and control patients. The genes *PTGDR*, *HS3ST2*, *POU4F3* and *MAGI2* displayed hypermethylation in endometrial cancer tissue and OC tissue and they were validated in training set. The average M-index of *POU4F3* is 78.28 in endometrial cancer and 20.36 in OC which are greater than that in the controls and so is the value of MAGI2 (246.0) in endometrial cancer and 12.2 in OC which are also significantly greater than in controls (fig. 3). The specificity and sensitivity of *POU4F3*/*MAGI2* were 61% and 62%-69% for detection of OC and 83%-90% and 69%-75% for detection of endometrial cancer. So, the results of this study are promising for the detection of endometrial and ovary cancer but a limitation is the small sample size and so the specificity must be determined in a bigger cohort (Chang et al., 2018).

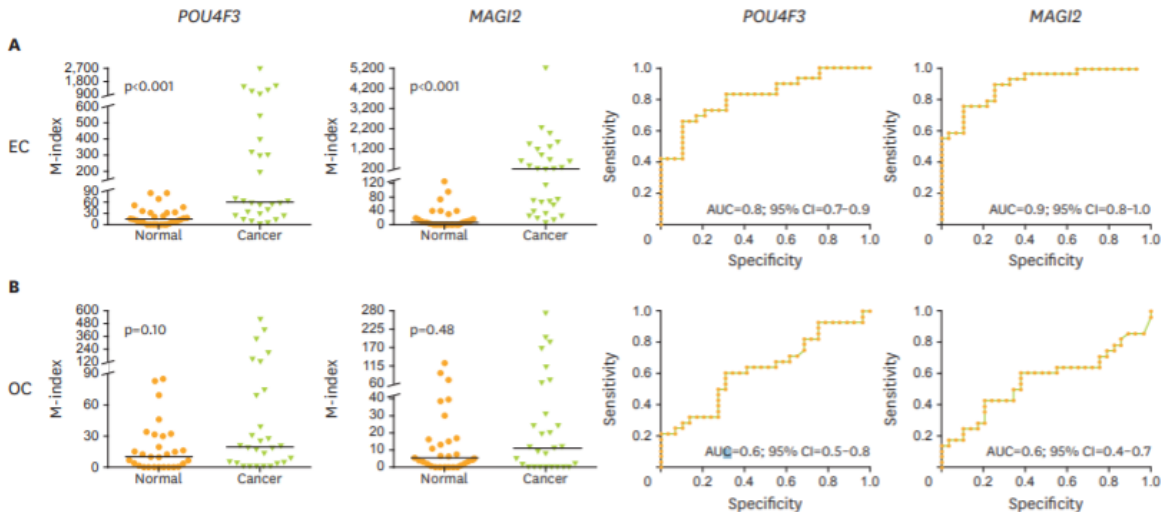


Fig. 3. The M-index of POU4F3 and MAGI2 from endometrial cancer (EC) patients and OC patients and normal control patients in testing set in cervical scrapings. (A) Endometrium and (B) ovary (Chang et al., 2018).

4.2 Liquid biopsies

Another possibility of improving first-line screening is taking liquid biopsies from patients, which detect circulating tumour DNA (ctDNA) mutations, elevations in the overall level of cell-free DNA (cfDNA), DNA methylation or other epigenetic biomarkers and circulating tumour cells (CTCs) in the extracted body fluids. Comparing ctDNA with CTCs liquid biopsies, ctDNA offers greater sensitivity for early detection because it has higher profit and ctDNA is more frequent in liquid biopsy samples (Mathieu et al., 2019).

ctDNA is released from tumour cells mainly through apoptosis. The ability to detect specific mutations, DNA hypermethylation, loss of heterozygosity (LOH), copy number variation and the presence of single nucleotide variants is because of the competence to execute deep sequencing and droplet digital PCR (ddPCR) on scrupulous amounts of ctDNA. Traditional PCR can also identify *TP53* mutations in cfDNA. Tumour-specific *TP53* sequences were detected in 21 (30%) plasma or serum samples of the 69 cases with somatic *TP53* mutations. Yet, mutant *TP53* was only in one case of stage I cancer detected (Elias et al., 2019).

But there is currently almost no prospective appraisal of the usefulness of ctDNA in a screening setting despite the evidence of measurable concentrations of ctDNA in patients with diagnosed disease, implying that liquid biopsies may be effective for detecting early-stage OC. Moreover, there is a big variance in the concentration of ctDNA in liquid biopsy samples in patients even at the same disease stage, contrary to the belief that concentration is comprehend to tumour load (Mathieu et al., 2019). Thirdly, ctDNA can only be investigated at the genomic level with molecular DNA assays but CTC's can be investigated at genomic, transcriptomic and proteasomal level (Tan et al., 2016). So, more research is needed to define the variance in the concentration of ctDNA, the association between disease circumference and ctDNA concentration to define if early detection is feasible (Mathieu et al., 2019).

Another liquid biopsy approach is CancerSEEK. This is a blood-based assay, performed by Cohen, *et al*, that looks at a combination of tumour DNA and protein biomarkers to identify the presence of relatively early cancers and to localize the organ from which these cancers started to grow. They tried to evaluate a panel of gene markers and a panel of protein to detect many solid tumours at a stage prior to the development of distant metastases. CancerSEEK was applied to 1005 patients with nonmetastatic, clinically detected cancers of the liver, pancreas, colorectum, stomach, oesophagus, lung, breast and also the ovaries. None of the patients received neo-adjuvant chemotherapy before blood sample collection and none had conspicuous distant metastasis at the start of the trial. The healthy control group consisted of 812 individuals with an average age of 55 with no history of illness. CancerSEEK had a median sensitivity of 73% for stage II cancers and is similar for stage III cancers (78%) and 43% for stage I cancers (Cohen et al., 2018).

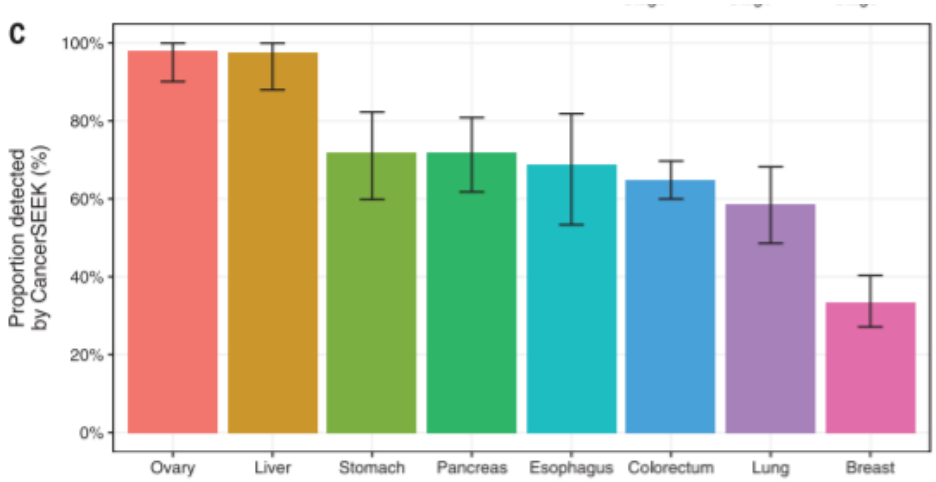


Fig. 4. Performance of CancerSEEK. Sensitivity per tumour type (Cohen et al., 2018).

What's interesting in this trial is that the sensitivity for stage I and II ovary cancer is nearly 100% (fig. 4) (Cohen et al., 2018).

However, CancerSEEK had a patient group with individuals with known cancers. Most individuals in a normal screening setting would have less advanced disease and the sensitivity of detection is usually a little lower than claimed here. Furthermore, this trial used healthy controls with no history of illness whereas in a normal cancer screening setting, some individuals might have diseases. This could result in more false-positive results than observed in the trial. All in all, more research needs to be done for all incident cancer types in a large population to achieve clinical application and to demonstrate that it can reduce worldwide mortality (Cohen et al., 2018).

4.3 Autoantibodies

Autoantibodies are unique early detection markers for OC. These are interesting diagnostic biomarkers because they circulate at higher concentrations than their antigen (Fortner et al., 2017). Moreover, small amounts of cancer in the fallopian tube or ovary can stimulate autoantibodies to mutated proteins, facilitating greater sensitivity and earlier detection than CA125. Autologous antibodies can be manufactured against mutant TP53 protein. The most prevalent genetic mutation in OCs, seen in 95% of HGSCs, is modification in *TP53* (Cancer Genome Atlas Research Network, 2011). Autoantibodies could be detected in 21-30% of serum samples from patients with HGSC at a specificity of 97% (Yang et al., 2017).

When comparing autoantibodies with repulsed protein antigens, autoantibodies have greater sensitivity. An immune response that is detectable before diagnosis could already be induced with small amounts of tumour associated antigen. Autoantibodies to *TP53* have been risen 8 months before an elevation in CA125 and 22 months before clinical diagnosis in patients who did not have an elevation in CA125 (Simmons et al., 2019).

Kaaks, *et al*, executed an analysis on a chosen panel of four antibodies against *CTAG1A*, *CTAG2*, *TP53* and *NUDT11*. They used serum samples of epithelial invasive ovarian, peritoneal cancers or fallopian tube cancers from 194 patients collected up to 36 months prior to diagnosis and 705 matched control participants (Kaaks et al., 2018). Sensitivity for early detection ranged from 19-23% for the four antibodies at 98% specificity with lead times less than/equal to 6 months. However, the study lasted longer than 1 year and this leads to a sensitivity that varies only 1-11%. Furthermore, adding the four autoantibodies to CA125 did not enhance sensitivity for detection at 98% specificity.

The study of Fortner, *et al*, that investigated 6 individual autoantibodies against *EpCAM*, *PLAT*, *c-Myc*, *IL-8*, *MDM2* and *HOXA7* showed 39-67% sensitivity at 98-100% specificity for detecting OC at all stages and from all histologies (Fortner et al., 2017). These autoantibodies need to be further examined in order to be able to use them for the early detection of OC (Elias et al., 2019).

5. Second-line screening improvements

5.1 TVS refinements

Improving TVS is important for diminishing repeat scanning for OC due to unsatisfactory tests. One way to improve this is through the implementation and development of quality procedures, which led to better visualization rates of the ovaries among enrolled centres throughout the UKCTOCS. This inclination was observed even among experienced sonographers who had performed more than 1000 UKCTOCS screens (Mathieu et al., 2019).

In addition to this, guidelines and standards like the guidelines from the Society of Radiologists in Ultrasound, also should be followed. They should be used in the evaluation of asymptomatic cysts (Levine et al., 2010). Implementing such guidelines would aid physicians to acknowledge that many postmenopausal women have ovarian cysts, often in the size of 3-4 cm, which demand only annual or biannual controls and not actual treatment. Moreover, strict standards should be implemented for malignancy of complex masses, where mural nodules with elevated blood flow is the most crucial component. Besides, haemorrhagic material should also not be confused with septations (Mathieu et al., 2019).

The International Ovarian Tumor Analysis (IOTA) group has established specific standards based on TVS characteristics to help distinguish unique sonographic features among disease sub-types (Timmerman et al., 2008). These standards unfortunately do not help with earlier detection of OC, such as the identification of typical characteristics in later stage tumours as presence of a complex (solid and cystic) mass. But it may help to reduce false positives (Mathieu et al., 2019).

Another possibility of improvement could be performing check-ups on premenopausal women at high risk for OC. Moreover, clinically triaged postmenopausal women could have cyclically-changing ovarian cysts. So then perimenopausal is a more accurate label for these women. In both premenopausal and perimenopausal women, false positives are usually because of inadequate diagnosis of physiologic cysts (e.g., haemorrhagic or corpus luteum) as possible invasive cancers. In these non-malignant cysts, proliferation of blood vessels at the margins as well as mural irregularity and haemorrhage are often confused for the complex neovascularity and nodularity of OC cancer in early stages. In these circumstances, the suspicion of potential malignancy leads to further diagnostics and maybe surgery in patients without OC. Therefore, TVS of high-risk premenopausal women should be carried out in the first ten days of a new menstrual cycle to prevent development of the corpus luteum (Mathieu et al., 2019).

When a dubious lesion has been seen, another TVS should be carried out 6-8 weeks after the first scan. This can correctly diagnose the lesion and thus avoid false-positive diagnoses by operators who are comparatively inexperienced in performing a TVS. To further avoid false-positive and false-negative diagnosis, experts should personally scan the patient to make sure they are going to use the most optimal technique. This will result in less inter-observer variation, more accurate interpretation of the performed tests and improve the overall performance of TVS (Mathieu et al., 2019).

5.2 TVS with photoacoustic imaging

Photoacoustic spectral characteristics such as high-resolution detection of angiogenesis and active neovascularisation have shown to be promising tools in early-stage OC diagnosis (Mathieu et al., 2019; Amidi et al., 2019). Moreover, non-invasive photoacoustic imaging can be implemented together with TVS. This combination technique was performed by Amidi, *et al*, on 39 ovaries of 24 women in the age of 54. The ovarian lesions were categorised in benign/normal ovaries (n=27), invasive epithelial cancers (n=9) and other types of neoplasms (n=3). Photoacoustic and ultrasound (PAT/US) imaging of a benign fibrothecoma and an ovary with HGSC is shown in fig. 5. Greater local deviations in pixel values are seen in the benign ovary compared to the malignant ovary which results in a bigger contrast and lower correlation value for the benign ovary. These results show that using functional characteristics enhances discriminating normal/benign ovarian lesions from all types of tumours (Amidi et al., 2019).

Limitations such as a deterioration in spatial resolution with rising depth and limited tissue penetration depth can be overcome when it is integrated with TVS. Although, further research is needed to improve the photoacoustic signal and visualization of tumour margins in OC models so it could be beneficial for future detection of OC in an early stage (Mathieu et al., 2019).

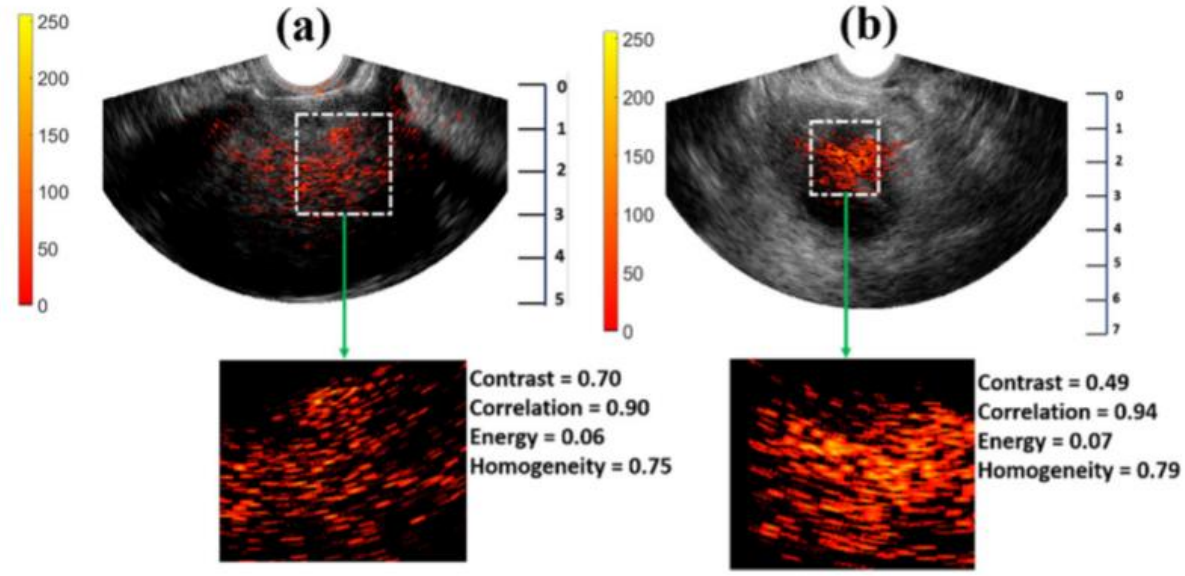


Fig. 5. PAT and US images. (a) ovary with benign fibrothecoma and (b) ovary with epithelial cancer (HGSC) (Amidi et al., 2019).

6. Third-line screening additional options

Additional possibilities to improve third-line screening even further are the application of techniques like hyperpolarized MRI or magnetic relaxometry.

6.1 Hyperpolarized MRI

Hyperpolarized MRI is a technique that can display enhanced sensitivity in comparison with TVS. In this technique, hyperpolarized [1-^{13}C]pyruvate can be utilized as an imaging tracer to define cancer metabolism and can cause a signal in the pre-tumours of mouse models before the development of a primary tumour. Hyperpolarized MRI has 50,000-fold better sensitivity for *in vivo* metabolic imaging than TVS (Hu et al., 2011).

Ravoori, *et al.,* performed an *in vivo* assessment in a mouse model to look if early or late components of glucose metabolism, illustrated by inter alia hyperpolarized MRI, can be indicators of response in OC to multityrosine kinase inhibitor pazopanib. Two days after beginning therapy with pazopanib, they found a substantial rise in hyperpolarized ^{13}C-lactate signal compared to the ^{13}C-pyruvate substrate signal in vivo in a SKOV3 human ovarian tumour model (fig. 6). This is in relation with tumour growth inhibition, which indicates that in the OC model, hyperpolarized MRI can be an early response indicator to pazopanib therapy. This is a marker for treatment response, but if we could implement this method in screening for OC, we can make a lot of progress in detecting OC in an early stage based on the results already achieved (Ravoori et al., 2017). It already has a great effect within tumours of prostate cancer patients, but there hasn't been done a lot of research in OC patients (Mathieu et al., 2019). So, more research needs to be done if this can significantly improve the early detection of OC.

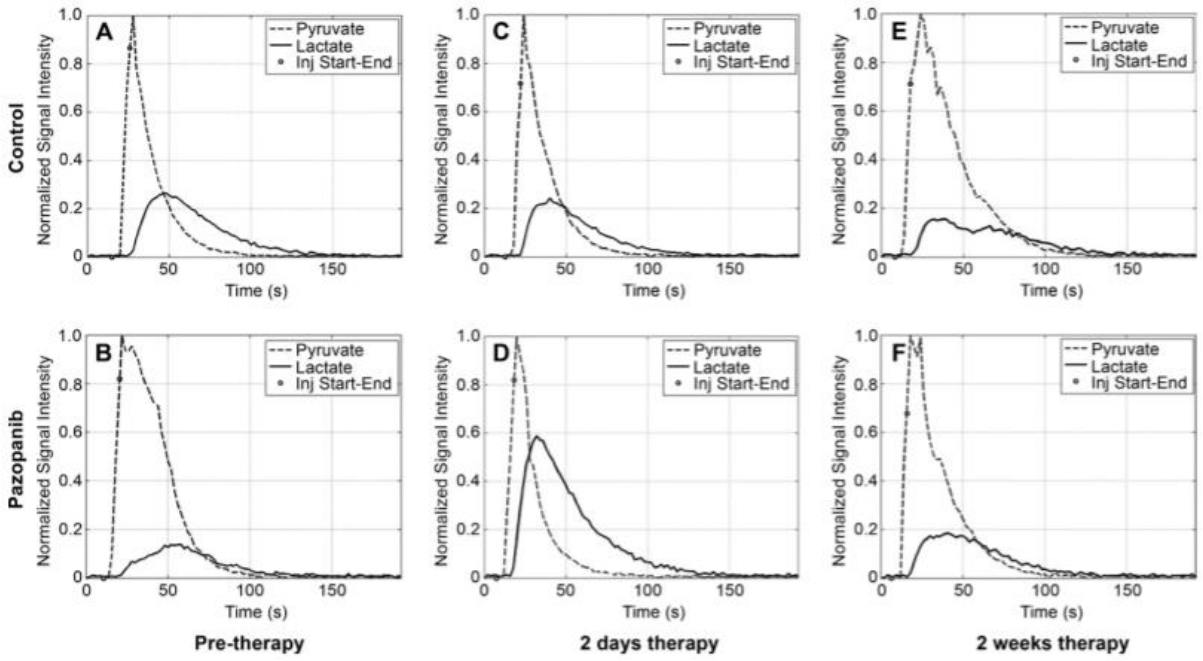


Fig. 6. MR spectroscopic imaging of hyperpolarized ^{13}C-pyruvate and ^{13}C-lactate prior (A & B) or 2 days (C & D) and 2 weeks (E, F0 after control (A, C & E) or pazopanib (B, D & E) treatment. To peak signal for each injection (inj), the average signal for lactate and pyruvate was normalized. By summing signal from hyperpolarized ^{13}C-pyruvate and hyperpolarized ^{13}C-lactate, the total hyperpolarized ^{13}C signal was approximated (Ravoori et al., 2017).

6.2 Magnetic relaxometry

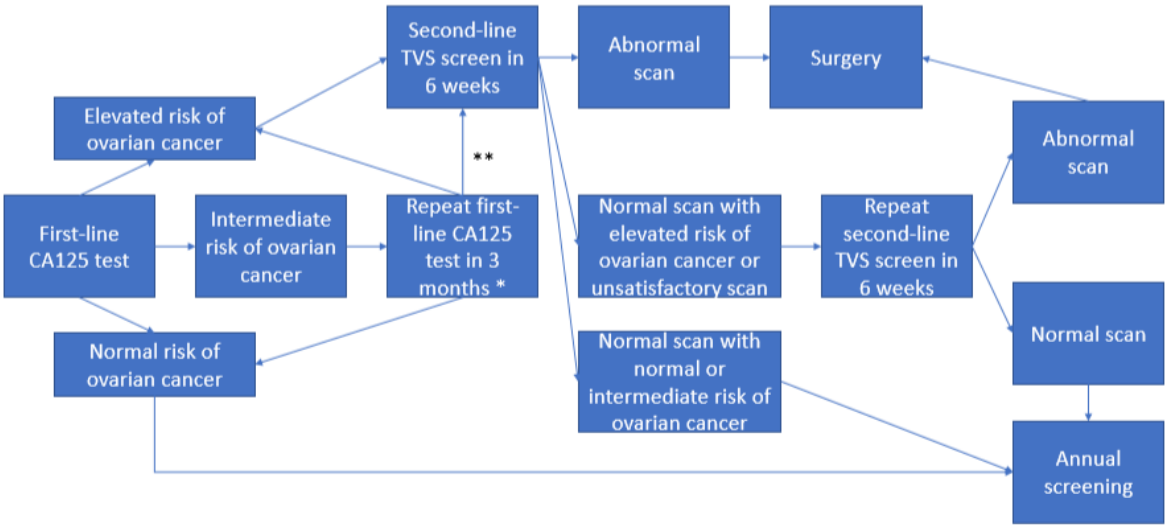
Magnetic relaxometry (MRX) is another method that can improve sensitivity of third-line screening by two orders of magnitude (Elias et al., 2019). This *in vivo* technique relies on the detection of the binding between targeted iron oxide nanoparticles and tumour vessels or cancer cells. It has been used already for detecting minimal residual disease in leukaemia patient bone marrow biopsies, breast cancer detection in mice and measuring nanoparticle amassing in biological samples (Flynn & Bryant, 2005). A few researches on magnetic relaxometry in mouse models point out that one million OC cells bound to superparamagnetic iron oxide nanoparticles conjugated with anti-ovarian cancer associated antigens can be detected. These are a lot of cancer cells that can be detected. Magnetic relaxometry can be very useful for the detection, not imaging, small cancers of the ovary or fallopian tube, it has a higher sensitivity than TVS and therefore must be further investigated to translate this research into the clinic (Mathieu et al., 2019).

7. Discussion, conclusion and future implications

What this thesis tried to investigate is how we can improve the early detection of ovarian cancer in order to increase survival of ovarian cancer patients. Yet, third-line multimodal screening based on longitudinal CA125 profile and second-line TVS improves, in the best manner, the early detection of OC (fig. 7).

A major outcome of the UKCTOCS trial that performed screening in a multimodal manner, was that it had better sensitivity, specificity and positive predictive value than TVS. Nevertheless, the cost-effectiveness of the screening method is very important because this method can potentially improve the performance of the early detection of OC in the future. A recent analysis of Moss, *et al*., extrapolated the survival curves of the UKCTOCS trial and their outcome was that this screening method might be cost-effective in the United States (Moss et al., 2017). But this is only certain when the size of any mortality benefit is known along with the costs of the CA125 algorithm (Nash & Menon, 2020). Similarly, an analysis of individual trial data from the UKCTOCS implied that a national programme of multimodal screening would come near the National Institute for Health and Care Excellence (NICE) threshold of cost effectiveness for England (Nash & Menon, 2020). Although, third-line screening modalities are more costly than TVS, but the group of patients getting this screening is very modest (for example, less than 1% of patients during the prevalence screen of UKCTOCS). So this does not have a big effect on the cost effectiveness (Mathieu et al., 2019).

However, this screening trial has not been shown to reduce OC mortality. This is due to that the trial did not anticipated the statistical design and is likely due to the relatively good short-term survival of women with primary invasive epithelial neoplasm of the ovary (8 years in the non-screened group). So, further follow-up is needed in the UKCTOCS trial to definitely confirm if it can develop mortality reduction and can be implemented in the early detection of OC.



* If women are again triaged as intermediate risk of ovarian cancer after this test, then after 3 months another repeat first-line CA125 test will be performed.
** If they are still triaged as intermediate risk of ovarian cancer, then women will undergo second-line TVS screening in 6 weeks.

Fig. 7. Overview third-line (multimodal) screening.

In general, the main frailty of TVS is the high false-positive rate which, in most cases, results in the detection of a benign adnexal tumour. An adnexal tumour can be cancerous and may not have the classic features of an advanced tumour so they need comprehensive follow-up until they are proven to be harmless.

Clinical trials like the University of Kentucky Ovarian Cancer Screening Trial or PLCO using TVS and CA125 have shown almost no benefit. This is because the low PPV of 2,6% in the first four sessions of the PLCO trial which results in a relatively high false-positive rate of TVS for the assessment of adnexal masses. Moreover, in both PLCO and The University of Kentucky Ovarian Cancer Screening Project, primary borderline epithelial neoplasms of the ovary were triaged as false positives as they are associated with lower mortality rates and have little potential to become malignant.

Research into the use of alternative biomarkers like HE4 helps monitoring the therapeutic efficacy of OC treatment but have yet not improved OC screening. Although, promising is traditional PCR performed on liquid biopsies that can identify *TP53* mutations in cfDNA. Tumour-specific *TP53* sequences were detected in 21 (30%) plasma or serum samples of the 69 cases with somatic *TP53* mutations. But yet, mutant *TP53* was only in one case of stage I cancer detected. More research focusing on detecting *TP53* in more advanced stages of OC could be useful to eventually implement in the early detection of OC. Furthermore, autoantibodies and ctDNA must be further evaluated to enhance the sensitivity of first-line screening.

To conclude, third-line screening based on ROCA with CA125 tests and (additional) TVS as a secondary screen for women with an elevated risk score is the best way to improve the early detection of OC. The main factor that recent trials could not improve OC screening, is the lack of ability to detect a big portion of early disease that can be treated effectively with few false positives. More study is needed to reduce complications associated with false positives and we need to develop that ability to detect early disease in large numbers. All in all, to determine the specific population of women requiring primary prevention or screening, we need to learn more about the reproductive, environmental and genetic risk factors associated with OC.

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