Biochemical markers and combination testing for the diagnosis of ovarian cancer in women with symptoms or signs suspicious of ovarian cancer

By

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ABSTRACT

Ovarian cancer (OC) has the highest mortality of all gynaecological cancers. A significant contributing factor to the high mortality in OC is delayed diagnosis. Currently, there is no consensus regarding the best test for early diagnosis.

A review of existing systematic reviews about symptoms, biochemical markers and US test used alone or in combination for the diagnosis of OC in symptomatic women demonstrated that existing reviews were variable in quality, applicability and limited by poor reporting. I attempted to address these deficiencies in two reviews on the accuracy of biomarkers alone and symptoms, biomarkers or US in combination for the diagnosis of OC in symptomatic women in generalist settings in pre and postmenopausal women separately.

My thesis finds key methodological issues, e.g., literature is not applicable to generalist settings as studies included women typical of tertiary healthcare settings, some studies excluded borderline tumours which inflates estimates of sensitivity, important differences exist in test performance between pre and postmenopausal women. Main results are 1) reviews not applicable to primary care settings – more research is needed. 2) for biomarkers i) HE4 at the threshold of 60-80pMol/L and 130-150pMol/L is recommended in pre and postmenopausal women for low prevalence settings ii) ROMA or LR2 in premenopausal women to replace RMI in secondary/tertiary setting; continue with RMI for postmenopausal women as it shows comparable accuracy to ROMA and LR2

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ABBREVIATIONS

ADNEX	Assessment of Different NEoplasias in the adneX
AMSTAR	Assessment of Multiple SysTemAtic Review
ASR	Age standardised rate
AFP	Alpha feto protein
ACOG	American College of Obstetricians and Gynaecologists
ASCO	American Society of Clinical Oncology
BOTS	Borderline ovarian tumours
CA125	Cancer Antigen 125
CEA	Carcinoembryonic Antigen
CI	Confidence Interval
DOvE	Diagnosing ovarian cancer early
EFSUMB	European Federation of Societies for Ultrasound in Medicine and Biology
DTA	Diagnostic accuracy test
EBOTs	Endometrioid borderline ovarian tumours
EOCs	Epithelial ovarian cancers
ESGO	European Society of Gynaecological Oncology
FDA	U.S. Food and Drugs administration
FOC	Familial Ovarian cancer
FN	False negative
FP	False positive
GCT	Germ cell tumour
HCG	Human chorionic gonadotrophin
HE4	Human Epididymis protein 4
HGSOC	High grade serous ovarian cancer
HNPCC	Hereditary nonpolyposis colorectal cancer
HSROC	Hierarchical Summary Receiver Operating Characteristic
ICTRP	WHO International Clinical Trials Registry Platform
IGCS	International Gynaecologic Cancer Society
IOTA	International ovarian tumour analysis
LDH	Lactate dehydrogenase
LGSOC	Low grade serous ovarian cancer

LR	Logistic Regression (1 and 2)
MBOTS	Mucinous borderline ovarian tumours
MMT	Mixed mesodermal tumours
NICE	National Institute of Clinical Excellence
OC	Ovarian cancer
PPC	Primary peritoneal cancer
PROBAST	Prediction model risk of bias assessment
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RCOG	Royal College of Obstetricians and Gynaecologists
RMI	Risk of Malignancy Index (1,2,3 and 4)
ROB	Risk of Bias
ROC	Receiver operating characteristic
ROMA	Risk of malignancy algorithm
RRBSO	Risk reducing bilateral Salpingo-oophorectomy
SAS	Statistical analysis system
SBOTs	Serous borderline ovarian tumours
SN	Sensitivity
SP	Specificity
SGO	Society of Gynecologic Oncology
TN	True negative
TP	True positive
SR	Systematic reviews
QUADAS	Quality assessment of diagnostic accuracy studies
US	Ultrasound
UKCRN	UK Clinical Research Network

CHAPTER 1: INTRODUCTION

Ovarian tumours are a group of heterogeneous masses that develop in the ovary. Worldwide about 7% of women have ovarian masses in their lifetime,[2] with up to 10% of women having some form of surgery for an ovarian mass.[3] The epidemiology of ovarian masses is unclear due to inconsistent reporting and spontaneous resolution of many ovarian masses. Causes of ovarian masses range from normal physiological processes to abnormal growth of cells leading to malignancy. They are commonly classified into 3 main subcategories based on their malignant potential: 1) benign; 2) borderline, and 3) malignant tumours. They can occur at any age, but 2/3rd of malignancy occurs in the 40-65 years age group.[4]

Ovarian cysts are more common in premenopausal women due to the physiological function of the ovary. Most are benign functional cysts that resolve spontaneously. Some persistent cysts are caused by benign conditions such as endometriosis, fibromas and cystadenomas which may require surgical intervention; however, the risk of malignancy is low, 1/1000.[3] The risk of malignancy increases in postmenopausal women, 3/1000 at age 50, [3] and is highest in women aged 55-64 years, median age 63 years.[5]

Borderline ovarian cysts are tumours of low malignant potential which are more common in the reproductive age group and typically affect women in their 40s. The incidence of borderline ovarian tumours (BOTs) is low, 1.5 - 2.5 per 100,000 women per year[6] with approximately 30% of these appearing in women younger than 40 years.[7]

A small proportion of ovarian masses can be malignant and are responsible for high case fatality ratio. Ovarian cancers can arise *de novo* or in the background of borderline or benign ovarian tumours.

Asymptomatic smaller benign tumours can be managed conservatively. Surgical management is recommended in symptomatic ovarian tumours, borderline and malignant ovarian tumours; the route, extent and place of surgery are guided by the diagnosis and the need for fertility preservation. Unfortunately, there is no non-invasive method to make a definitive preoperative diagnosis, and optimal management is guided by diagnostic tests providing risk thresholds that allow women classified as ‘low risk’ to be managed in secondary care with fertility preserving and less radical treatment options including laparoscopic surgery or surveillance whilst triaging

women at 'high-risk' for treatment at a cancer specialist centre for appropriate surgery as required.

1.1 Epidemiology of ovarian cancer

1.1.1 Incidence and mortality

Ovarian cancer (OC) is the 8th most common cancer in the women worldwide, and nearly 300,000 new cases were diagnosed in 2018.[8, 9] Incidence rates are lower in less developed countries, Sub-Saharan Africa and Asia, 5 and 5.8 ASR /100,000 respectively and highest in North America and Europe, exceeding 8.4 and 9.5 per 100,000 respectively.[8, 9] It is the commonest cause of death from gynaecological malignancies in the developed world. OC is the eighth most common cause of death in women with 184,799 deaths in 2018.[9]

In the United Kingdom, 7270 new cases in 2015 and 4227 deaths in 2016 from OC were reported.[10] In the United States 22,240 new cases and 14,070 deaths per year per 100,000 women were reported in 2018.[5, 11, 12] The overall survival for OC is poor as nearly 2/3rd of the disease are diagnosed at an advanced stage.

Age-Standardised rates (ASR) for OC for one, five and ten year net survival in women (aged 15 - 99), in England and Wales for 2010 - 2011 are 72.4, 46.2 and 34.5 respectively. Upper control limit for the ASR for one, five and ten year survival for OC are 72.5, 46.4 and 35.3 respectively and lower control limit are 72.4, 45.9 and 33.8 respectively.

1.1.2 Distribution

The incidence of OC increases with age, with a median age of diagnosis at 63 years; more than 75% of OC is diagnosed in women aged 55 years and over.[10, 13] The majority of ovarian cancers are sporadic, but 10-15% of the ovarian cancers are due to inherited mutations in cancer susceptibility genes. OC in women with BRCA mutations tends to develop at a younger age, approximately a decade earlier in comparison to sporadic OC.[14] BRCA mutations are more common in Ashkenazi Jews compared to general population.[15, 16]

1.2 Hypotheses of development of OC

Ovarian cancer development is now categorised into 2 broad categories based on molecular genetic pathways. Type I or low-grade indolent tumours develop from a precursor lesion and show step wise growth from benign, borderline into malignancy; this includes low grade serous OC (LGSOC), endometrioid cancers, clear cell cancer, mucinous cancers and Brenner tumours. Type II or high-grade aggressive tumours arise *de novo* without precursor lesions; high-grade serous OC (HGSOC) being the most common and others including undifferentiated and malignant mixed mesodermal tumours/carcinosarcoma (MMT). The low-grade tumours demonstrate a different genetic profile demonstrating more BRAS, KRAF, β-catenin and PTEN mutations in comparison to HGSOC that demonstrate ubiquitous p53 mutations (Table 1.1). Further morphological, molecular and genetic advancements have shed further light to explain the diversity seen in ovarian tumours. Piek et al. in 2003 described lesions in the fallopian tube that closely resembled HGSOC[17] which was substantiated in subsequent studies.[17-19] It is widely accepted now that serous ovarian tumours arise from the fallopian tube, whereas endometrioid and clear cell tumours arise from endometriosis and mucinous and Brenner tumours arise from intestinal/tubo-mesothelial junction.[20-23]

Table 1. 1: Precursors and molecular genetic alterations of type I and II tumours of the ovary.

Type I tumours	Precursors*	Known molecular genetic alterations
Low grade serous ca	Serous cystadenoma / adenofibroma Atypical proliferative serous tumour Non-invasive low-grade serous ca	67% - BRAF and KRAS mutations
Mucinous ca	Mucinous cystadenoma Atypical proliferative mucinous tumour Intraepithelial ca	>60% - KRAS mutations
Endometrioid ca	Endometriosis Endometrioid adenofibroma Atypical proliferative endometrioid tumour Intraepithelial ca	20% - LOH or PTEN mutations 16-54% β-catenin mutations 4-5 % KRAS mutations 13-50% - microsatellite instability
Clear cell ca	Endometriosis Clear cell adenofibroma Atypical proliferative clear cell tumour Intraepithelial ca	5- 16% - KRAS mutations ≈ 13% - Microsatellite 66% - TGF- β RII instability
Malignant Brenner tumour	Brenner tumour Atypical proliferative Brenner tumour	Unidentified yet
Type II tumours	Precursors	Known molecular genetic alterations
High grade serous ca	Unidentified yet	50-80% - p53 mutations 10-20% - amplification and overexpression ofHER2/neu gene 10-17% - Inactivation of p16 gene
Undifferentiated ca	Unidentified yet	Unidentified yet
Malignant mixed mesodermal tumour	Unidentified yet	>90% - p53 mutations

*atypical proliferative and non-invasive tumours are also called BOTS

1.3 Histological subtypes, molecular, cytogenetics and clinical features of ovarian tumours

1.3.1 Benign ovarian tumours

Ovarian cysts are fluid or solid filled pockets that can be physiological, benign or malignant. They can develop at all ages and stages of life. The increased use of imaging has increased the rate of detection of adnexal masses,[24] many of which are asymptomatic cysts that are largely physiological or benign. Most cysts that appear during the reproductive years are due to hormonal activity and disappear on their own. A nested case control study in USA noted simple

cysts in 23.8% and 13.4% in women <50 and >50 years respectively.[25] Benign ovarian cysts are broadly classified into functional, inflammatory/infectious and benign neoplastic tumours.

Functional ovarian cysts

The pituitary gonadotrophin menstrual feedback pathway regulates the ovarian function. The ovaries form follicles monthly, one of which ruptures to release an egg and form the corpus luteal cyst. These follicular cysts can persist or get large due to hormonal imbalance (polycystic ovaries) and or normal or increased hormonal activity.[26] They are usually asymptomatic and resolve spontaneously unless complicated by rupture, torsion or haemorrhage that can present with symptoms of abdominal pain, distension nausea or vomiting or can be picked up incidentally on ultrasound, commonly as simple cysts.

Follicular cysts can develop in response to intrinsic or extrinsic gonadotrophic stimulation. However, it is unclear if the persistence is either due to failure of an immature follicle to undergo atresia or failure of a dominant follicle to rupture.[27] They are typically 2 - 3 cm and not usually larger than 5 cm. Corpus luteal cysts develop following ovulation and cease to function by undergoing luteolysis at the end of a nonfertilised menstrual cycle.

It can persist as corpus luteum of pregnancy if fertilised. It can grow to a larger size and can rupture either in pregnant / non-pregnant states, causing pain and bleeding. Theca lutein cysts develop due to hypertrophy of theca lutein cells or luteinised granulosa cells or in response to excessive human chorionic gonadotrophin in gestational trophoblastic disease, multiple pregnancies or exogenous hormonal stimulation. Theca lutein cysts are often bilateral and occasionally increase to a very large size called hyperreactio luteinalis.[28] However most of these cysts are unilocular and do not grow over 7 cm.[29] Many of these are seen on ultrasound as simple cysts and unless symptomatic need no treatment.[25]

Inflammatory/infectious ovarian cysts

Inflammatory and infective adnexal masses may also present as complex ovarian masses. They often present acutely with pain and signs of infection and raised inflammatory markers; however, they can have a chronic presentation, e.g. tuberculosis and actinomycosis where the symptoms and signs of acute inflammation and infection are not present. Instead, they could be incidentally detected or have slow, insidious symptoms similar to ovarian cancer such as

abdominal pain, distension, loss of appetite and failure to thrive. They are clinically difficult to distinguish from ovarian malignancies as they have similar symptoms, appear as complex cysts +/- free fluid +/- peritoneal implants on USS and may have associated raised CA125.

Benign neoplastic ovarian tumours

Ovarian neoplastic tumours can grow to an excessive size and have an abnormal growth without showing dysplasia. They are also a group of heterologous tumours due to the pluripotent capacity of ovarian tissue and vary in histology as given in Table 1.2. Nearly 60% of all ovarian tumours are benign.[30] The normal growth control is dysregulated, leading to uncontrolled growth of ovarian tissue but without the capacity to breach stroma or invade other tissues.[31] The most common benign tumours are serous and mucinous cystadenomas.[32] They can also be detected incidentally on ultrasound (US) as they can grow to a large size without causing symptoms or present with symptoms of abdominal pain (due to haemorrhage, rupture, infection, torsion), abdominal distension, vomiting (due to torsion) or urinary or bowel symptoms (due to pressure). They can be seen on US as simple or complex cysts. Occasionally, these cysts have associated solid components or free fluid (which are features seen more with malignancy) due to ovarian accident and inflammation.

Benign neoplastic ovarian tumours can be broadly classified into epithelial, germ cell and stromal cell tumour depending on the tissue of origin. Germ cell tumours are most commonly seen in the adolescent age group, with peak incidence at 18 years. Sex cord tumours are often seen in the 4th and 5th decade and can be associated with feminising or virilising signs due to hormonal production. The various subtypes are detailed in Table 1.2 and Table 1.3.

Epithelial cell tumours

Serous cystadenoma and adenofibroma and cyst adenofibromas: Benign serous tumours account for 53.39% and the commonest of the benign tumours. The peak incidence is in the 3rd, 4th and 5th decades with increasing incidence with advanced age. 12-23% are bilateral, may be unilocular or multilocular, and vary in size from 3-30 cm. The surface may be smooth or may have papillary excrescences called papilloma either in the inner or outer surface. Unlike the malignant counterparts, they do not show surface or peritoneal invasion. Benign serous cystadenomas do not show KRAS and BRAF mutations unlike borderline and low-grade serous tumours.[32]

Mucinous cystadenoma: Mucinous cystadenomas account for 41% of benign ovarian tumours and are also common in the 3rd – 5th decade.[33] They are usually unilateral (only 5% bilateral), loculated, have a smooth surface and often grow to large sizes asymptomatically, sometimes filling the abdomen and pelvis.[34] Complex mucinous tumours may have multiple loculi and daughter cysts, giving appearances of pseudoinvasion. They also may contain florid and complex glandular and papillary arrangement, making invasion very difficult to rule out and posing histological challenges.

Endometrioid tumours: Ovarian cysts can also contain cysts that have walls lined by glandular cells similar to the endometrium and are called endometriomas or chocolate cysts. It is a benign oestrogen-dependent tumour that is caused by endometriosis, which is a condition characterised by growth of endometrial tissue outside the uterine cavity. Endometriomas contain old degenerated blood that oozes dark brown coloured fluid when the cyst is opened or bursts. Endometriosis has a strong correlation with history of infertility and nulliparity. Cyst walls are often thick and fibrotic and densely adherent to other structures. They are multiloculated and anechoic, having solid components on imaging due to fluid blood debris or old clot, making them difficult to distinguish from a malignant tumour. Patients also often have raised CA125 due to the associated inflammation. Despite classification as a benign disease, there is mounting evidence that endometriomas have a potential to become malignant,[35] with associations with clear cell and endometrioid cancers.[36]

Clear cell cystadenomas and adenofibromas: These are rare epithelial cell tumours that may contain several cell types; clear cells and hobnail cells being the most common.[37] Initially, they have been thought to arise from the mesonephric rest cells of the female reproductive system, and later others have suggested a Mullerian origin.[38] The benign tumours tend to be relatively more solid than their malignant counterparts.

Serous cell tends to be the most common epithelial component in cystadenomas and adenofibromas; less commonly they may have endometrioid, mucinous, clear call or mixed cell type[39] and careful histological examination is necessary to enable correct identification.

Brenner tumour: Brenner tumours are a rare, solid ovarian tumours mostly occurring in postmenopausal women. The majority of Brenner tumours are benign and less than 5% of are malignant.[40] They arise from ovarian surface epithelium or the pelvic mesothelium by transitional cell metaplasia.[41] They are well circumscribed tumours with a grey or slightly

yellow surface and hard or fibromatous appearance. They comprise approximately 3% of neoplasms and are asymptomatic and often discovered incidentally.[34]

Germ cell tumours

Germ cell tumours (GCT) are derived from the primordial cells of the ovary and, as the name suggests, contain all 3 embryonic germ cell layers ectoderm, endoderm, and mesoderm. They commonly occur around 10-30 years of age, and comprise 10 - 25% of ovarian tumours, but only 5% are malignant.[42] Malignancy is more common in the childhood and adolescent age group and GCTs contribute 70% of ovarian malignancy in this group.[43] They include dysgerminomas, teratomas, embryonal tumours and endodermal sinus tumour.

All the germ cell tumours are malignant except for teratomas, which have a benign counterpart called dermoid cysts or mature cystic teratomas. Teratomas usually form unilocular cysts lined by squamous epithelium and containing hair follicles and sebaceous material. They may also contain teeth, bone cartilage and thyroid tissues. Mature ectoderm layer (skin and hair) and mesoderm layer (fat and muscle) is the commonest composition.[44] They have a characteristic feature on ultrasound and are often detected incidentally. They can present acutely with abdominal pain, as they are prone to torsion (16%) due to the fat content.[45] Fertility preserving cystectomy is often adequate treatment.

Unlike epithelial tumours, often germ cell tumours are not associated with raised CA125. Different types of biomarkers, such as alpha feto protein (AFP), human chorionic gonadotropin (HCG) and lactate dehydrogenase (LDH) may be raised depending on the sub type of GCT. GCTs are also often symptomatic and identified at an early stage, respond to chemotherapy and have good prognosis when treated appropriately.

Stromal cell tumours

Stromal cell are heterogenous group of tumours in the ovary that arise from the primitive sex cord or stromal cells. They are uncommon and comprise only 7% of all ovarian tumour.[46] The sex cord cells give rise to Granulosa and Sertoli cells tumours, whilst the stromal cells give rise to Thecomas, Fibromas and Leydig cell tumours, and may present on their own or as mixed types. Granulosa cell and Theca cell tumours are oestrogenic and may present as postmenopausal bleeding, whilst Sertoli and Leydig cell tumours are androgenic and may

present with virilising symptoms. They occur in a wide range of women but are more common in younger and older women. They were reclassified in the new WHO classification and are grouped under 3 main categories of pure stromal tumours, pure sex cord stromal tumours and mixed stromal-sex cord tumours.

Sclerosing stromal tumour and Fibromas are hormonally inert. Thecomas and Luteomas often produce oestrogen. Unlike sclerosing ovarian tumours that arise in the first three decades of life, Thecomas are common in the 5th decade and in postmenopausal women. Even though Thecomas are themselves benign they can be associated with oestrogenic sequelae, such as endometrial adenocarcinoma or rarely endometrial stromal cell sarcoma in 20% of thecomas.[47] Less commonly, Luteomas can also produce androgenic hormones. Leydig cell tumours are also benign androgen producing tumours seen more commonly in postmenopausal women. They all appear as solid ovarian tumour, grey-white or red-brown in colour. They can be incidental findings on imaging or present with hormonal symptoms and need to be distinguished from the rare malignancies that can arise in stromal-sex cord tumours.[47] Granulosa tumours are associated with increased levels of Inhibin, which is used as a biomarker to aid diagnosis and surveillance; the levels of inhibin are influenced by the volume of the tumour.[48]

1.3.2 Borderline ovarian tumours

Borderline ovarian tumours (BOT) are tumours of epithelial origin with upregulated cellular proliferation and mild nuclear atypia but without destructive stromal invasion.[49] They were first recognised as an entity in 1929 and incorporated into the WHO classification in 1973.[50] The current recognised WHO classification (2014) for BOTs, and malignant tumours are as given in Table 1.3. According to the 2014 classification the term “tumours of low malignant potential” is no longer recommended and is replaced by atypical proliferation.[51] There are 6 subtypes of BOTs similar to the malignant counterparts serous (SBOTs - 50%) and mucinous (MBOTs - 45%) being most common. The others less commonly occurring types are endometrioid, clear cell, seromucinous and Brenner.[52] Histologically they have mild to moderate atypia, hyperchromasia, epithelial multilayering and detachment of cells into the lumen.

SBOTS and MBOTS generally occur between 33-44 years, unlike the Endometrioid borderline ovarian tumours (EBOT) where the mean age at diagnosis is 57 years. The clinical or ultrasound features of BOTS are no different from those of benign lesions or early malignancy. BOTs may act as a precursor lesion, and continuum of progression to malignancy has been demonstrated. Serous BOTS often give rise to LGSOC rather than HGSOC and share similar genetic profiling with LGSOC, demonstrating BRAS and KRAF mutations. Areas of BOT and LGSOC may coexist.[53, 54] Mucinous tumours are markedly heterogeneous with the coexistence of benign, borderline and invasive components within the same cyst and extensive sampling is required at histology to rule out invasive malignancy. Benign metaplastic endometriosis outside the uterus can undergo stepwise transformation to EBOTs and then develop malignancy (low grade serous or clear cell cancer), although the overall risk of this is low. Clear Cell (CCBOTs) and Brenner borderline ovarian tumours are rare. CCBOTs represent <1% of BOTs and have a high likelihood of association with clear cell carcinoma.

Apart from the potential for malignant transformation, another addition to the recent classification is the use of the term 'micro invasion' to replace non-invasive implants. These are isolated groups of cells no larger than 5 mm in the largest dimension, associated with BOTs. Unlike the malignant epithelial cell tumours, the majority of BOTs are diagnosed in stage 1 (75%) and have a 5 year survival rate of 97%.[55] Although in the minority, serous borderline ovarian tumours are associated with microinvasion, peritoneal implants, lymphadenopathy and progression to invasive cancers. Hannibal et al. showed the micropapillary variant to be associated with more advanced stage at presentation and a worse prognosis.[56] However, overall the clinical significance of all these variants and its impact on survival is debatable with studies varying in its conclusion. The criteria for diagnosis are largely based on the 2003 NIH consensus criteria.[57] They commonly occur in the reproductive age group, and fertility sparing surgery can be advocated. If fertility is not desired the treatment is removal of the reproductive organs (hysterectomy and removal of both tubes and ovaries) with the omentum. However, there remains the preoperative challenge of distinguishing it from the benign and malignant counterparts as blood tests, and imaging can be similarly abnormal, and it is challenging to make a clear distinction in order to tailor the location or extent of surgery. This is of particular relevance when considering issues such as fertility preservation in premenopausal women.

Table 1. 2: WHO histological classification of ovarian tumours (2013).

Epithelial cell tumours	Germ Cell tumours	Sex cord stromal tumours	Metastatic*
Serous carcinoma	Endodermal sinus tumour	Granulosa cell	Breast
Mucinous carcinoma	Embryonal carcinoma	Sertoli-Leydig cell tumour	Colon
Endometrioid carcinoma	Choriocarcinoma	Theca cell tumour Thecoma	Endometrium
Clear cell carcinoma	Dysgerminoma	Fibroma	Stomach
Malignant Brenner	Immature Teratoma	Sex cord tumour with annular tubules	Cervix
Transitional cell carcinoma	Yolk sac tumour	Lipid cell tumour	
Small cell carcinoma	Polyembryoma	Gynandroblastoma	
Mixed mesodermal	Mixed germ cell tumour		
Undifferentiated Carcinoma			

*common sites of origin

1.3.3 Malignant ovarian tumours

Epithelial tumours are the commonest of all ovarian tumours and contribute to 75% of all tumours and 90% of malignancies. Germ cell tumours and stromal cell tumours contribute to 15 - 20% of neoplasms (commoner in adolescents) and 5 - 10% of malignant tumours respectively. Metastatic tumours including Krukenberg tumours, account for 5%.

Table 1. 3: New WHO histological classification of epithelial ovarian tumours.[1]

Previous	NEW (2014)
Serous tumours	
Benign type	
Cystadenoma	
Papillary cystadenoma	Cystadenoma
Surface papilloma	Adenofibroma
Adenofibroma and cystadenofibroma	Surface papilloma
Borderline (SBOT)	
Papillary cystic BOT	Serous BOT/Atypical proliferating tumour
Papillary surface BOT	SBOT micropapillary type/Non-invasive serous, low grade carcinoma
Adenofibrormatous and cystadenofibromatous BOT	
Malignant type	Serous low- grade carcinoma
Adenocarcinoma	Serous high-grade carcinoma
Papillary surface carcinoma	
Adenocarcinofibroma	

Mucinous tumours	
Benign type	
Cystadenoma	
Adenofibroma and cystadenofibroma	
Mucinous type with mural nodules	
Mucinous cystic tumour with pseudomyxoma peritonei	Cystadenoma
Borderline (MBOT)	Adenofibroma
Intestinal type	
Endocervical type	
Malignant type	
Adenocarcinoma	
Adenocarcinofibroma (malignant adenofibroma)	Mucinous BOT/atypical proliferating mucinous tumours
	Mucinous carcinoma
Endometrioid tumours	
Benign type	
Cystadenoma	Endometriosis cyst
Adenofibroma and cystadenofibroma	Endometrioid cystadenoma
Borderline (EBOT)	Endometrioid cystadenofibroma
Cystic tumour	
Adenofibroma and cystadenofibroma	Endometrioid EBOT/Atypical proliferative endometrioid tumour
Malignant type	
Adenocarcinoma NOS	Endometrioid carcinoma
Adenocarcinofibroma (malignant adenofibroma)	
Malignant Mullerian mixed tumour (carcinosarcoma)	
Adenosarcoma	
Endometrioid stromal sarcoma (low- grade)	
Undifferentiated ovarian sarcoma	
Clear cell tumours	
Benign type	
Cystadenoma	
Adenofibroma and cystadenofibroma	
Borderline (CBOT)	Cystadenoma
Cystic tumour	
Adenofibroma and cystadenofibroma	CBOT/atypical proliferating clear cell tumour
Malignant type	
Adenocarcinoma	
Adenocarcinofibroma (malignant adenofibroma)	Clear cell carcinoma
Transitional cell tumours	**Brenner tumours**
Benign type	
Brenner tumour	Brenner tumour
Metaplastic type	
Borderline	Borderline Brenner tumour/Atypical proliferating Brenner tumour
Borderline Brenner tumour	
Proliferative type	
Malignant type	Malignant Brenner tumour
Transitional cell carcinoma	
Malignant Brenner tumour	**Seromucinous tumour**
	Benign type
	Seromucinous cystadenoma
	Seromucinous adenofibroma
	Borderline
	Seromucinous borderline tumour/Atypical proliferating seromucinous tumour
	Malignant type
	Seromucinous carcinoma
Squamous epithelial tumours	
Mixed epithelial tumours	
Undifferentiated and unclassifiable tumours	**Undifferentiated carcinoma**

1.4 Factors influencing risk of ovarian cancer

The cause of OC is not currently known, but several factors have been noted that may increase or reduce the risk of developing OC. Age, inherited gene mutations, reproductive history and family history are some of the factors that have been linked to risk of OC. Understanding and awareness of these are important as the risk profile alludes to the pre test probability of OC.

1.4.1 Age

The risk of developing OC increases with age. According to the American Cancer Society (ACS), more than half of the women who develop OC are older than 63 years, and it is commonest in women aged 75-79 years.[58] The average number of new cases per year and age specific incidence rates per 100.000 females is shown in Figure 1.1. More than 80% of the epithelial ovarian cancer occurs in the perimenopausal and postmenopausal age group (> 40 years of age). Germ cell tumours account for 15-20% of all ovarian tumours, but unlike Epithelial Ovarian Cancers, 2/3rd of them appear in younger women and children, and only 3-5% are malignant.[59, 60] Familial OC occurs at younger ages compared to sporadic OC.[14] With successive generations a birth cohort effect for familial (mainly BRCA related) cancers is seen. i.e. increasing OC risks with successive generations.[61]

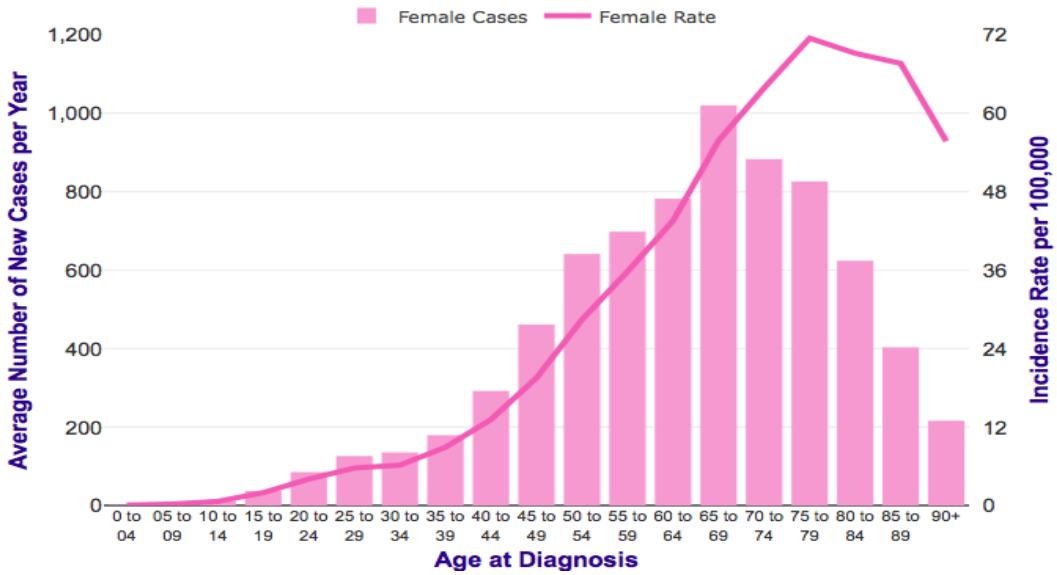


Figure **1. 1: Age-Specific Incidence Rates per 100,000 population, females, UK, 2013-2015.**[10]

1.4.2 Inherited genetic risk

The majority of the ovarian cancers are sporadic and occur due to somatic mutations that are acquired in a person's lifetime. However, a family history of ovarian cancer or breast cancer is considered to be one of the most significant risk factors for development of ovarian cancer. Therefore, family history is one of the referral criteria in the ACOG guidelines. About 15-20% of non-mucinous EOCs are inherited as autosomal dominant mutations in the germ line cells; they are carried in families and passed by either parent. The majority of non-mucinous EOCs occur due to an inherited mutation in breast cancer genes (e.g. BRCA 1 and 2),[14, 62] that increase susceptibility to develop ovarian cancer in comparison to the general population. Serous and Endometrioid are two of the commonest histology types seen in BRCA carriers, whereas BOTs and non-mucinous are less common.[63, 64, 65] Mutations in the Breast cancer genes (BRCA 1 and 2) are the most common heritable mutation and have high penetrance. BRCA 1 and BRCA 2 are tumour suppressor genes and women with first degree relatives (i.e. a mother or sister or daughters) with a history of OC, have an increased risk of developing ovarian cancer especially if the disease has developed at a younger age. The risk reduces with increasing age.[66] The lifetime risk of developing by age 70 years can be up to 63% and 27% in BRCA1 and BRCA2 carriers, respectively.[15, 67] 39% of women with BRCA 1 and 11-17% of women with BRCA2 develop ovarian cancer by 70 years.[68, 69] The risk increases significantly if more than one relative is affected, ranging from 3.0 - 23.5 depending on the number of affected members in the family.[66, 70] Genetic risk for carrying BRCA genes may be identified, and lifetime risk can be calculated using tools such as BOADICEA and Manchester scoring system. Surgical prevention in the form of risk reducing bilateral Salpingo-oophorectomy (RRBSO) in these high-risk women can reduce the risk of ovarian cancer in BRCA carriers by 80-96%, but a 1-6% residual risk of primary peritoneal cancer (PPC) remains.[71]

Another, more commonly known heritable risk factor is families with Lynch syndrome II, also called hereditary nonpolyposis colorectal cancer syndrome (HNPCC). Due to defects in the mismatch repair genes MLH1, MSH2, MSH6 PMS1 and PMS2.[72, 73] The other less known but heritable causes of OC are PTEN tumour hamartoma syndrome (due to mutation of the PTEN gene), Peutz-Jeghers syndrome (mutations in STK11) and MUTYH-associated polyposis. These mutations are associated with increased risk of breast cancer, colon cancer and endometrial cancer, and hence family history of these cancers also indicate high risk of developing OC.

BRIP1 and RAD51c and RAD51D are moderate penetrance mutations in DNA repair genes and are known to be associated with increased inherited risk of OC of 5.8, 5.2 and 12% respectively.[74, 75] A consensus recommendations from the UK genetics group for ovarian cancer panel testing recommend that women with non-mucinous EOC should undergo testing for BRCA1, BRCA2 BRIP1, MLH1, MSH2 MSH6 and RAD51C. The consensus committee does not recommend testing for EPCAM deletions, TP53, or PMS2 based on current evidence, despite the initial majority recommendation in the survey, as the risk was not considered significant [76] in a wider population.

Mainstream testing (i.e. the routine testing of BRCA 1 and 2 genes status in women with HGSOC and CCC) is now standard of care in the UK.

Although epidemiology on reproductive factors, Environmental, dietary and host factors are of interest as risk factors, none have been incorporated into models of diagnostic test accuracy and are not discussed in this thesis**.**

1.5 Diagnosis of ovarian cancer

Survival for OC in England is lower in comparison to Scandinavian and many European countries. This difference is unlikely due to availability of different tests but may be due to different factors such as attitudes or access to health care, better general health or different methods to estimate relative survival. Five thousand or more deaths within the 5 years of diagnosis can be avoided in England in comparison to the European average.[77] This has been attributed to delays in diagnosis, and the delays can be in patient presentation to primary care practitioners, delays in primary care referrals, delays in secondary care referrals or delays in treatment. The first year survival in England is much worse in comparison to their European counterparts with comparable health care systems and seen as reflection of delayed diagnosis.[78] The document "*on route to diagnosis*" suggests that 30% of OC cases in England are diagnosed via the emergency route. These cases show far worse 1 year survival rates (45%) in comparison to the 84% survival at 1 year for women diagnosed by the urgent cancer referral pathway (2 week referral) and the non urgent GP referral route.[79] Of the women who died in their first year after diagnosis, 58% did not receive any form of treatment, reflecting a need to improve symptom awareness, early diagnostic pathways and access to treatment.[80]

The majority of OCs (75%) are diagnosed at an advanced stage and responsible for the highest mortality among gynaecological cancers. The lack of awareness and recognition of symptoms by patients and physicians is considered one of the main factors in delayed diagnosis and poor outcomes. Diagnosis of OC is challenging because of variable presentation, the non-specific nature of symptoms,[81] and low prevalence in primary care (0.09 - 0.18%).[82] The heterogeneity of OC does not lend itself to diagnosis by a single test as they have different clinical presentations. The known tests, such as ultrasound and biomarkers, have varying sensitivity and specificity for different sub groups.

1.5.1 Diagnostic challenges and dilemmas in the diagnosis of ovarian cancer

Historically OC was called a "silent killer". However, we know now that most women with OC have symptoms many months prior to their initial diagnosis.[83, 84] The common presenting symptoms known to be associated with OC are pelvic or abdominal pain, abdominal distension or bloating, loss of appetite or feeling full quickly and urinary frequency and urgency.[85-87]

These symptoms are not only non-specific but mimic symptoms of common benign conditions including gynaecological, non-gynaecological and functional disorders (e.g. menstruation, irritable bowel syndrome, endometriosis, etc.). A symptom index has been designed by Goff et al. and is defined by the presence of any of the following 6 symptoms: pelvic/abdominal pain, abdominal bloating/increased size, reduced appetite or a feeling of early fullness experienced more than 12 times a month and present for less than one year;[84] this is the most validated questionnaire used.[88] The median duration of symptoms from presentation to diagnosis has been noted to be between 3 - 5 months and its value in prompting early detection and effect on improving outcomes have been questioned by some experts.[89-91]

Gilbert et al. performed a pilot study evaluating the role of symptoms which triggered testing for early diagnosis of ovarian cancer in women over 50 years (DOvE).[92] This study found that despite not altering the stage at diagnosis, burden of disease was lower in the cohort of women that were identified by symptom triggered testing. Also, a higher proportion of women in the symptom triggered group achieved optimal cytoreduction. The authors concluded that earlier detection using symptom triggered testing resulted in earlier diagnosis and lower volume disease in women with HGSOC, and further research is awaited. As maximal cytoreduction

and lower residual disease is a the most significant prognostic factor[93-95] in improving ovarian cancer outcomes, the role of symptom triggered testing in the future may play a promising role. Furthermore, nearly 22% of women in the UK diagnosed with OC will not receive any cancer treatment at all; likely due to poor performance status at presentation. Thus, symptom triggered testing may also enable diagnosis at a better functional status, consequently improving survival.

The National Institute of Clinical Excellence (NICE) recommends that the persistent presence of pelvic/abdominal pain, abdominal bloating/increased size, reduced appetite or early fullness, or urinary frequency, especially in women over 50, for more than 2 weeks and less than 12 months duration, should trigger primary care doctors to investigate women with CA125 followed by ultrasound if CA125 is abnormal (>= 35U/ml), and referral to secondary care if both tests are abnormal (see Figure 1.1).

However, CA125 is only elevated in EOCs, not elevated in approximately half of the early stage OCs, and may be normal in mucinous OCs. Hence it may be less useful for early diagnosis.[96] On the other hand, CA125 is an inflammatory marker and not specific to ovarian cancer; therefore CA125 may be elevated in other benign conditions including endometriosis, menstrual disturbances and non gynaecological conditions.[97] A majority of women with these symptoms do not have OC, and a raised CA125 may lead to many unnecessary referrals and overwhelm the services as well as causing patient anxiety. A multicentre audit looking at adherence to NICE recommended pathway in the UK showed that 90% of the GP referrals did not follow the sequential testing and referral guidance prescribed by NICE; however, the symptom triggered referral led to more diagnosis by the urgent referrals dedicated for cancer pathway rather than emergency admissions.[98] A study by Lim et al. identified that 1 in 2 women between the ages of 45 and 70 would present to their GP with an OC symptom once a year. Accurate diagnostic strategies, both in primary care and in secondary care, are vital for the early diagnosis of OC, whilst not overburdening the health service and women with false positive referrals.

Germ cells tumours and sex-cord stromal tumours can also present with severe abdominal pain, distension and fever because of rapid growth of the tumours causing capsular distension, haemorrhage and necrosis which mimics ovarian torsion. The symptoms and signs overlap and hence women may present to primary care, secondary care in gynaecology or other specialities, or acutely to emergency care.

As diagnostic tests, women with symptoms undergo imaging (pelvic or abdominal ultrasound, Computed tomography or Magnetic resonance imaging) or blood test, commonly CA125 (HE4 and OVA1 approved for clinical use in USA). The diagnosis is more difficult in premenopausal women as the prevalence of benign conditions is higher and may have associated raised CA125, confounding the reliability of the test. Non-epithelial ovarian cancers (i.e. germs cell tumours and sex-cord stromal tumours) may not have raised CA125, and instead, biomarkers may be raised (AFP, HCG, LDH, Inhibin) which are not specific to OC. Another problem critically affecting early diagnosis is the absence of raised CA125 in nearly 50% of early stage EOC.[96] Differentiating the more common benign tumours from malignant ovarian tumours on imaging is based on a combination of various morphological parameters, blood flow or pattern recognition, which can be influenced by the operator's experience and skill.

OC is managed by a combination of surgery to remove all visible disease and chemotherapy. Patients with benign tumours can have conservative management, i.e. surveillance or limited surgery. The treatment for OC is radical surgery but benign tumours, if requiring surgery, can have conservative surgery. The outcome of OC is better when operated by a gynaecological oncologist.[99, 100]

An accurate diagnosis is critical to ensure the right women get the right treatment in the right place to have optimal outcome. However, all these influencing factors influence care, making the diagnosis of OC difficult and affect the clinical pathway.

1.5.2 Effect of diagnostic practice on clinical treatment pathway

Current data do not support screening for OC. In the absence of any screening studies the recommendation from UK, Europe and USA is to generally increase awareness of symptoms related to OC in the population and use symptoms as triggers for further testing, with appropriate referral to secondary care (general gynaecologists/ cancer units) or tertiary care (gynaecological oncologists/doctors trained to perform surgery for advanced cancer).

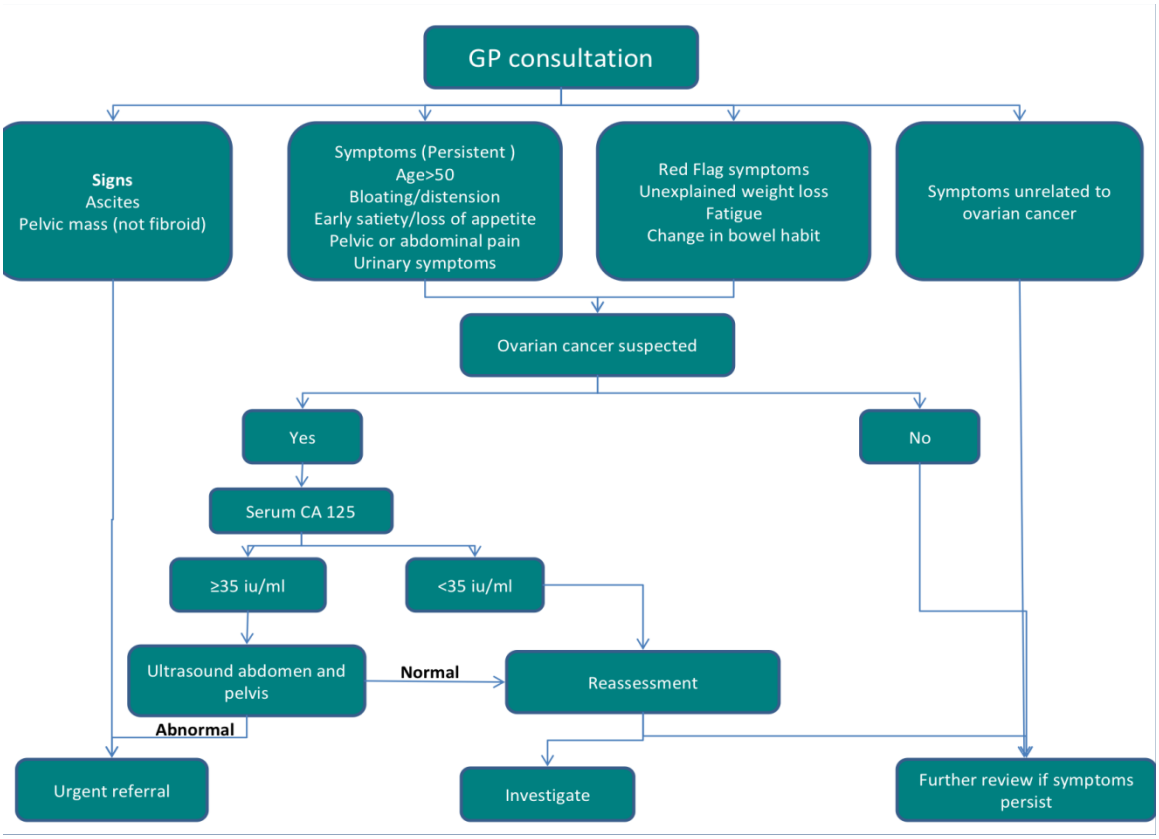


Figure 1. 2: NICE pathway for ovarian cancer referral in primary care.

Unfortunately, as mentioned in previous sections, the symptoms are non specific and there is lack of awareness regarding these symptoms amongst both the population and medical practitioners, leading to unstreamlined diagnostic pathways. This leads to women presenting at different points of health care leading to investigations and referrals, causing diagnostic delays and delays in access to right treatment. In reality, unlike the streamlined pathway prescribed by NICE, the pathways seen in clinical practice are more likely to resemble that shown below (Figure 1.3).

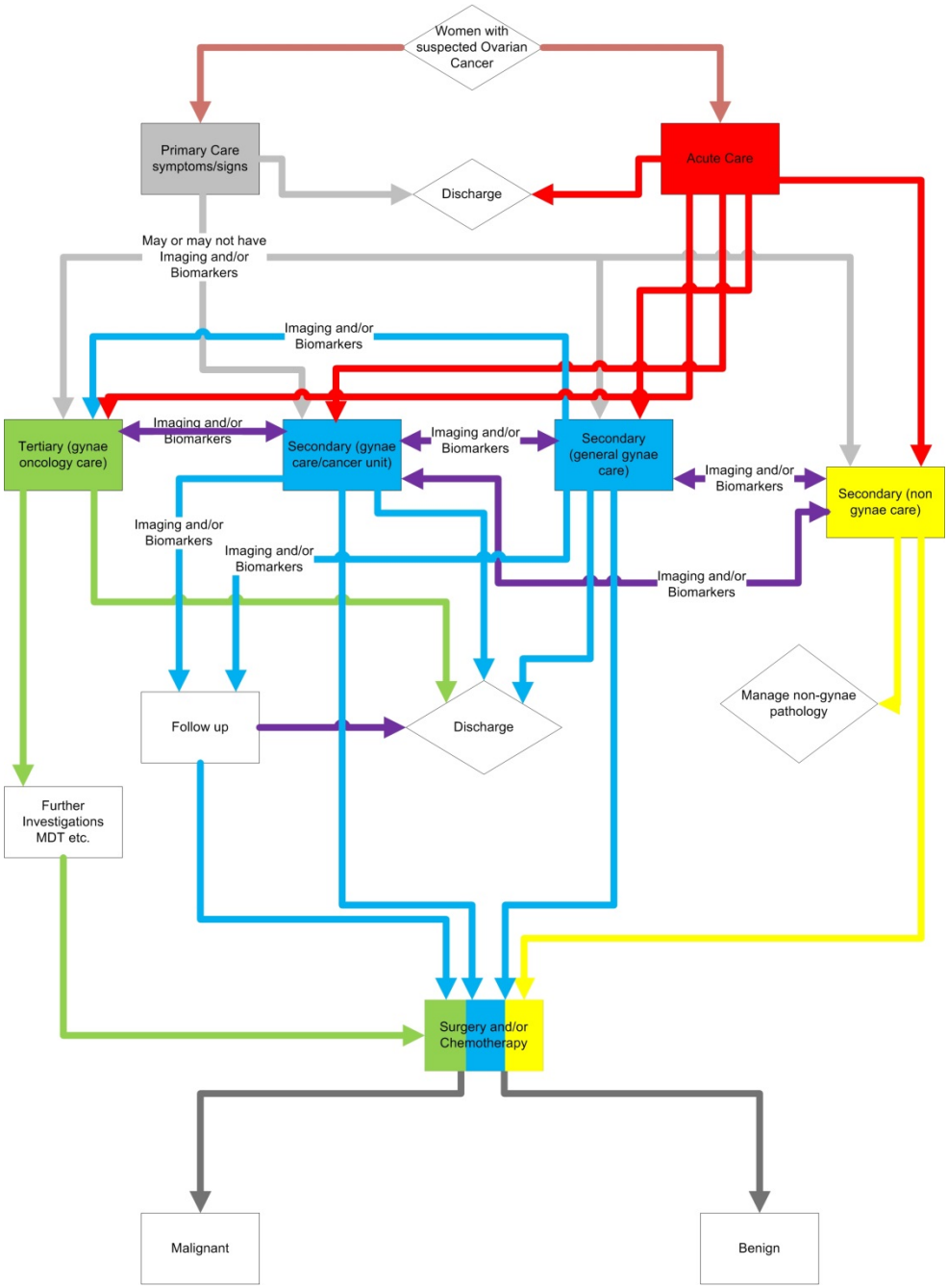


Figure 1. 3: Diagnostic pathway of ovarian cancer in current clinical practice.

As mentioned in previous sections, currently available tests include blood tests, biomarkers (such as CA125, HE4 and OVA1 etc.), imaging (transvaginal ultrasound, Computed tomography, Magnetic resonance imaging etc.) and combination testing using any permutation of symptoms / demographics / family history / blood test / imaging (RMI, ROMA, LR2, ADNEX etc.). However, there is no consensus regarding the test of choice, the point of pathway they are used in or accepted thresholds for test positivity for many of these test(s).

1.6 Aims and objectives

The aims and objectives of my research were to systematically identify the accuracy and applicability of existing tests for OC from existing literature. My research formed part of the broader ROCkeTS project; www.birmingham.ac.uk/rockets which aim to identify best tests for OC in primary care and in secondary care, in pre and postmenopausal women. My research comprised Phase 1 of ROCkeTS. I started my project with a critical evaluation of published systematic reviews and progressed to conduct fresh systematic reviews that addressed the limitations of published reviews. My project also enabled improved design and analysis of ROCkeTS primary study.

1.6.1 Primary objective

i) To critically evaluate published systematic reviews and identify evidence gaps. ii) To establish the accuracy of biomarkers alone or in iii) combination for the diagnosis of OC in pre and postmenopausal women.

Index tests were analysed separately in the following groups:

- Medicines and Healthcare Products Regulatory Agency (MHRA), National Horizon Scanning Centre (NHSC), National Institute for Health and Care Excellence (NICE), European Medicines Agency (EMA), FDA, Royal College of Obstetricians and Gynaecologists (RCOG) and American College of Obstetricians and Gynecologists (ACOG) approved or recommended Biomarkers alone or in combination (CA125, CEA, HE4, OVA1, LDH, HCG, AFP)
- Combinations of tests of more than one modality (biomarkers, imaging) across categories above and or combined with family history / demographics (either as rules or multivariate models
- To compare the accuracy of different tests or test combinations.

1.6.2 Secondary objectives

The following sources of heterogeneity were investigated in my thesis as secondary objectives:

Population:

- Clinical setting (generalist/primary care/community/family practice) versus specialist setting (cancer unit/cancer centre/gynaecological oncology).
- Menopausal status.

Index tests:

- Test positivity threshold.

Target condition:

- Epithelial ovarian cancer versus Non-Epithelial ovarian cancer.
- Early versus late stage.
- Type I versus Type II.

Study quality:

- Case-control versus other study designs.
- Study quality: for study participants not receiving surgery initially following a negative index test result: 12 months follow-up versus less than 12 months follow-up.

CHAPTER 2: METHODS

Diagnostic Test Accuracy (DTA) studies evaluate the accuracy of a test of interest (index test) with the best available method (reference standard) for determining the true presence or absence of the condition of interest (target condition). Systematic reviews of diagnostic test accuracy (DTA) studies play an important role in providing estimates of test accuracy based on all available evidence. The review of studies provides an opportunity to improve the precision of test accuracy and investigate reasons for variation in test results (heterogeneity) by undertaking a meta-analysis. This thesis is an investigation of the comparative accuracy of biomarkers and tests used in combination for the diagnosis of OC.

Development of protocol

In the methods chapter, I describe first the methods planned at the start of the research as per the published generic protocol[101] detailing methods for both the systematic review of biomarker tests and the review of test combinations for the diagnosis of ovarian cancer in symptomatic women. Changes to the protocol during the course of the research and the rationale for these changes are also presented. The methods and results for the systematic review of reviews are presented together in the next chapter.

The methods for this review are based on Cochrane DTA review methods for diagnostic test accuracy systematic reviews. (https://training.cochrane.org/resource/cochrane-handbook-systematic-reviews-diagnostic-test-accuracy) Review methods were tailored to reflect the topic area. In particular, it was anticipated that test accuracy would vary with variation in test positivity threshold (the definition of a positive test result in included studies) and according to histological subtypes of ovarian cancer. For the latter, menopausal status was hypothesised to reflect differences in the prevalence of different histotypes of ovarian cancer (population spectrum). The aim was, therefore, to stratify results based on menopausal status and test positivity threshold. Other factors hypothesised to cause variation in test accuracy were included in planned investigations of heterogeneity or sensitivity analysis and are detailed below.

2.1 Published generic protocol

2.1.1 Question formulation, Inclusion and exclusion criteria

Population (presentation and prior tests)

Adult women aged 18 years or older, irrespective of menopausal status were included. Studies restricted exclusively to populations under 18 were excluded. Studies in pregnant women or women with previous history of ovarian cancer were also excluded.

Prior tests

The reviews are concerned with women in whom a diagnosis of ovarian cancer was suspected based on symptoms, risk factors or signs. As a minimum, women should have self-referred to a healthcare professional on the basis of the presence of symptoms and or risk factors for ovarian cancer. At the point of inclusion, women receive a biomarker or combination test. In practice they may have had prior testing with one or more of history and examination by a healthcare professional, testing with one or more biomarkers or imaging with ultrasound prior to the index test of interest for inclusion. Studies explicitly describing included participants as asymptomatic were excluded, for example where the index test was being applied as a screening test, or where studies included asymptomatic participants and these could not be disaggregated from participants who were symptomatic, had physical signs or were considered at risk of a diagnosis of ovarian cancer. Where the symptom or at risk status of participants was unclear, this was reflected by downgrading a study as part of topic tailored quality assessment of included studies (QUADAS-2) in the patient applicability domain.

Index tests

By definition tests included in the biomarker and combination reviews had to be applicable to both generalist and specialist settings.

Biomarkers

Any of the following recommended or approved biomarkers as detailed in the objectives of the introduction chapter were considered: CA125, CEA, HE4, OVA1, LDH, HCG, AFP. Although

HCG and AFP are not approved for use as tests in ovarian cancer, they are used clinically and recommended by the RCOG and ACOG for women under 40 as additional markers for germ cell ovarian tumours:

- CA125
- CEA
- HE4
- OVA1
- LDH
- HCG
- AFP

Combinations of tests

Any combination of two or more of the following test types 1) symptoms 2) imaging with ultrasound, biochemical markers, 3) risk factors, e.g. family history for ovarian cancer, age. A combination of multiple biomarkers would be a combination of only one test type and therefore considered part of the review of biomarkers; tests were included if used at any threshold and combined in any order. The combination tests included in the review are listed below in Table 2.1.

Reference standards

Histology was used as the reference standard for OC in women who had undergone surgery for an adnexal mass. As it would be unethical to undertake major surgery in women with a low index of suspicion for OC, follow up for a minimum of 6 months of women with a low index of suspicion and not undergoing surgery or women declining surgery was considered acceptable as an alternative reference standard. For studies using clinical follow-up, a year of follow-up was considered of adequate quality. Studies employing follow up for >6 but < 12 months were downgraded as part of quality assessment.

Table 2. 1: Definition of included test combination.

Index Test Combination	Detail	Test positivity thresholds included (reference)
RMI1 ***U* × *M* × CA125**	**Ultrasound (U):** (1 point for each of multilocular cysts, solid areas, metastases, ascites and bilateral lesions) where a total ultrasound point score of 0=0, a point score of 1 =1, and a point score of ≥2 =3 **Menopausal status (M):** premenopausal=1 and postmenopausal =3 **Serum CA125:** CA125U/ml applied directly to the calculation	200, 250 (Jacobs 1993)
RMI2 ***U* × *M* × CA125**	**Ultrasound (U):** (1 point for each of multilocular cysts, solid areas, metastases, ascites and bilateral lesions) where a total ultrasound point score of 0 or 1=1, and a point score of ≥2 =4 **Menopausal status (M):** premenopausal=1 and postmenopausal =3 **Serum CA125:** CA125U/ml applied directly to the calculation	200, 250 (Mol 2001)
RMI3 ***U* × *M* × CA125**	**Ultrasound (U):** (1 point for each of multilocular cysts, solid areas, metastases, ascites and bilateral lesions) where a total ultrasound point score of 0 or 1=1, and a point score of ≥2 =3 **Menopausal status (M):** premenopausal=1 and postmenopausal =3 **Serum CA125:** CA125U/ml applied directly to the calculation	200, 250 (Yamomoto 2009)
RMI4 ***U* × *M* × *S* (size in centimetres) × CA125**	**Ultrasound (U):** (1 point for each of multilocular cysts, solid areas, metastases, ascites and bilateral lesions) where a total ultrasound point score of 0 or 1=1, and a point score of ≥2 =4 **Menopausal status (M):** premenopausal=1 and postmenopausal =4 **Size (S):** tumour size (single greatest diameter) of <7 cm =1, and ≥7 cm =2. **Serum CA125:** CA125U/ml applied directly to the calculation	400, 450 (Yamomoto 2009)
ROMA **Algorithm using CA125, HE4 and menopausal status**	**Premenopausal** PI = −12.0 + 2.38 × LN(HE4) + 0.0626 × LN(CA125) **Postmenopausal** PI = −8.09 + 1.04 × LN(HE4) + 0.732 × LN(CA125) **Predicted probability (ROMA score)** = exp(PI)/[1 + exp(PI)] × 100	Pre 7.4% & Post 25.3% (Bandiera 2011) Pre 12.5% & Post 14.4% (Van Gorp 2011)

Table 2.1 Continued...

ACOG version 1 **ACOG v1**	**-Premenopausal**: 1 of CA125 >200; family history positive for either breast ca or OC in first degree relatives; Ascites; abdo or distant mets/ pleural effusion **-Postmenopausal**: 1 of CA125 >35; family history positive for either breast ca or OC in first degree relatives; Ascites; abdo or distant mets/ pleural effusion; nodular or fixed pelvic mass	Presence of any one positive test component (ACOG 2002)
ACOG version 2 **ACOG v2**	**-Premenopausal:** 1 of CA125 >67; family history positive for either breast ca or OC in first degree relatives; Ascites; abdo or distant mets/ pleural effusion **-Postmenopausal:** 1 of CA125 >35; family history positive for either breast ca or OC in first degree relatives; Ascites; abdo or distant mets/ pleural effusion; nodular or fixed pelvic mass	Presence of any one positive test component (Dearking 2007)
ACOG version 3 **ACOG v3 (CA125)**	**-Premenopausal:** 1 of CA125 >67; Ascites; abdo or distant mets/ pleural effusion **-Postmenopausal**: 1 of CA125 >35; Ascites; abdo or distant mets/ pleural effusion; nodular or fixed pelvic mass	Presence of any one positive test component (Dearking 2007)
ACOG version 3 **ACOG v3 (OVA1)**	**-Premenopausal**: 1 of OVA1; family history positive for either breast ca or OC in first degree relatives Ascites; abdo or distant mets/ pleural effusion **-Postmenopausal**: 1 of OVA1; family history positive for either breast ca or OC in first degree relatives Ascites; abdo or distant mets/ pleural effusion; nodular or fixed pelvic mass	Presence of any one positive test component (Miller 2011)
LR2	-age of the woman (in years) -presence of ascites (yes, 1; no, 0) -presence of blood flow within a solid papillary projection (yes, 1; no, 0) -maximum diameter of the solid component of the adnexal mass (expressed in millimetres, but with no increase 950 mm) -irregular internal cyst walls (yes, 1; no, 0) -presence of acoustic shadows (yes, 1; no, 0), The probability of malignancy is	Test positivity threshold to achieve a post test probability of malignancy 10% (Timmerman 2010)
ADNEX	-age (years) -serum CA125 level (log transformed) -type of centre (oncology centres vs other hospitals) -maximum diameter of the lesion (log transformed) -proportion of solid tissue (with quadratic term) -number of papillary projections -more than 10 cyst locules -acoustic shadows	Test positivity threshold to achieve a post test probability of malignancy of 3%, 5%, 10% and 15% (Van Calster 2014)

Target condition

OC, of all stages and histological types, was included. Studies restricted to specific ovarian pathologies were excluded with the exception of studies restricted to only epithelial ovarian cancer (EOC) as this is the most common of the ovarian cancers (> 90% of tumours in postmenopausal women) and is associated with the highest mortality. Studies restricted to metastatic or recurrent ovarian cancer or where data on recurrent tumours could not be disaggregated were excluded. Studies, where it was not possible to disaggregate metastatic tumours from primary ovarian cancer, were downgraded as part of quality assessment. Metastatic tumours are defined as tumours originating from non-ovarian/ adnexal areas, e.g. colon, breast, endometrium that metastasise to the ovary (also see Table 1.2). Metastatic tumours to the ovary are outside the remit of this review as they will represent the primary tumour pathologically and may not secrete the same biomarkers or exhibit the same ultrasound characteristics.

The quality of included studies was downgraded if borderline ovarian tumours (BOTs) or non-epithelial ovarian cancer (non-EOCs) were excluded from or not reported for the purposes of data extraction and analysis in this review. Non-EOCs are germ cell, and stromal cell tumours originating from the ovary and excluding them contribute as a source of bias as they do not secrete the same biomarkers, e.g. CA125, HE4.

Types of studies

Case control (provided the control arm comprised women with benign ovarian pathology and these could be disaggregated from any healthy controls), cross-sectional (retrospective and prospective data collection) and comparative diagnostic test accuracy studies of any design (within person or between person) were included. In view of the low prevalence of OC, it was anticipated that the majority of studies would recruit women who had already undergone the reference standard and index test results would be ascertained retrospectively. We also included studies externally validating multivariable models for the diagnosis of OC. Studies were included if there was sufficient data to extract 2 x 2 tables on diagnostic test performance. Inclusion of studies not providing verification of index test negatives was included where 2 x 2 tables could be constructed by imputation using setting-specific prevalence estimates. However, there were no included studies where index test negatives were not verified.

2.1.2 Study identification and selection

Electronic searches

Sensitive search strategies combining terms for the target condition (ovarian cancer) and each of 3 index tests that might be used alone or in combination: biochemical markers, symptom indices and ultrasound imaging were used. In addition, terms to describe testing models and algorithms were added, including the IOTA (International Ovarian Tumour Analysis) variables. The strategies were designed to run across a range of databases: the Cochrane Central Register of Controlled Trials (CENTRAL), MEDLINE and MEDLINE in Process (Ovid), EMBASE (Ovid), CINAHL / EBSCO, the Cochrane Database of Systematic Reviews (CDSR), Database of Abstracts of Reviews of Effects (DARE), Health Technology Assessment Database (HTA) and SCI Science Citation Index (ISI Web of Knowledge).

A date restriction was applied (1991 onwards) to ensure applicability to current technology. Searches were completed in April 2015. No language restrictions were applied. The search strategy used for MEDLINE (Ovid) biomarkers is shown as an example in Appendix 1.

Searching other resources

To identify ongoing and unpublished studies the following trials registers and conference abstracts and proceedings were searched without date restrictions: ClinicalTrials.gov, UK Clinical Research Network Study Portfolio Database (UKCRN) and WHO International Clinical Trials Registry Platform (ICTRP). Conference proceedings were searched from the European Society of Gynaecological Oncology (ESGO), International Gynaecologic Cancer Society (IGCS), American Society of Clinical Oncology (ASCO) and Society of Gynecologic Oncology (SGO) supplemented by searches of the ZETOC and Conference Proceedings Citation Index (Web of Knowledge). The reference lists of existing systematic reviews and guidelines were identified in the electronic searches as a source of primary studies.

Data collection and analysis

Search results were managed in EndNote. After removal of duplicates study selection at the title and abstract and subsequently full text stage was carried out independently and in duplicate by 2 reviewers (NR, RC, PS) with disagreements resolved by a third reviewer (CD, SS). Data

extraction and quality assessment were performed by one reviewer (NR or PS), and 30% of studies were randomly checked independently by a second reviewer (RC or CD) with disagreements resolved by discussion.

Selection of studies

Unique titles and abstracts were reviewed against predefined criteria to select potentially relevant studies for full-text review. The results of the selection process and reasons for exclusion are documented and summarised using a PRISMA flow diagram.

2.1.3 Study quality assessment and data extraction

Data extraction and management

A pre-defined data collection form (Appendix 2) was used to extract the following data (if available) into an excel database prior to entry into Review Manager version 5.3: study design; country; setting, including single or multi-centre; method of recruitment; reasons for exclusion; number of participants; number of women with a diagnosis of ovarian cancer and borderline ovarian tumours; age; menopausal status (directly or using age over 50 years or history of previous hysterectomy in the absence of information about postmenopausal status); prior tests; index tests and index test threshold(s); index test operator experience (for symptoms and ultrasound imaging); reference standard (including where relevant duration of follow-up); stage and histological subtype of ovarian cancer. Data to derive a 2 x 2 table for each study was extracted in duplicate by either a clinician (NR) or reviewer (PS) and independently by either a methodologist or statistician (CD, JD, SM, KS).

Assessment of methodological quality

Quality assessment was undertaken using the QUADAS-2 checklist tailored according to the topic and detailed in Appendix 3. Key aspects of tailoring for this review included:

Addition of a question concerning specification of the order and rule for combining individual tests in test combinations (index test domain)

Addition of 2 questions in the index test domain relevant to multivariable model / composite index test validation studies drawing on the PROBAST (prediction model risk of bias assessment) tool for diagnostic and prediction models[102]: pre-specification of thresholds and comparable assessment of all model /test components)

Addition of one question in the reference standard domain concerning the ability to disaggregate index test and reference standard results for primary ovarian cancer, metastatic disease and borderline tumours

Addition of a comparative domain for within and between person comparative studies. concerning participant selection (were they likely to be different from non comparative studies) and index test (were index tests interpreted blind to each other and if the interval between index tests < 3 months)

STARD is a reporting tool for primary test accuracy studies. We did not use the tool to assess reporting quality in our reviews as Cochrane methods follow the STARD guidelines implicitly as part of data extraction and quality assessment; lack of clarity of reporting of included studies is reflected by downgrading in quality assessment

2.1.4 Statistical analysis and data synthesis

Index test subgroups

For each index test, where data allowed separate meta-analyses were conducted for:

Patient characteristics: Mixed populations (pre and postmenopausal combined) versus premenopausal versus postmenopausal status.

Index test characteristics: Index test version, (for example data on 4 different versions of RMI were identified for this review). Test threshold where studies reported a common threshold.

Target condition: Appropriate grouping of borderline test results for construction of the 2 x 2 table. For these reviews, based on current clinical practice, it was considered appropriate if included studies classified histologically borderline ovarian tumours as positive (grouped with

histologically malignant ovarian tumours for estimation of test accuracy). It was considered inappropriate, if included studies excluded borderline ovarian tumours from estimation of test accuracy or if the classification of borderline ovarian tumours in included studies was unclear (borderline tumours may not have been identified in study participants, or classification of borderline tumours as positive (malignant) or negative (benign) for the estimation of test accuracy was unclear.

Methods for meta-analysis

Exploratory analyses included forest plots of study estimates of sensitivity and specificity grouped by test threshold, and plotting sensitivity and specificity in ROC space with test thresholds indicated. Analyses were conducted in Stata version 14.[103] Where adequate data were available, and it was considered reasonable to pool results, meta-analyses were performed using hierarchical models to estimate summary effects. These models take into account the correlation between sensitivity and specificity, threshold effects and the fact that factors causing heterogeneity may affect sensitivity differently to specificity. Hierarchical models deal with statistical distributions at two levels. At the lower level, they model the cell counts in the 2×2 tables extracted from each study using binomial distributions and logistic (log-odds) transformations of proportions. This level deals with sampling variability in individual studies and the correlation that exists between sensitivity and specificity. At the higher level, random study effects analysis is used to account for heterogeneity in test accuracy between studies. The weighted means of a test sensitivity (weighted by prevalence) or specificity (weighted by complement of prevalence) provides overall accuracy; this can provide distorted impression of a validity of the test and have potentially negative consequences especially as the difference between sensitivity and specificity increases and making it inferior to the hierarchical models balanced consideration of sensitivity and specificity. Univariate analysis of sensitivity separate to specificity is not recommended because it does not take into account this correlation. Forrest plots are displayed for a visual representation of test accuracy estimates across studies.[104] Random-effects univariate analyses (which ignore any correlation between sensitivity and specificity) were used where pooling was considered an appropriate approach, but bivariate models failed to converge. Summary estimates of sensitivity and specificity were translated into summary estimates of the absolute numbers of true positives, false negatives, false positives and true negatives using a hypothetical population of 1000 women. Three different estimates of disease prevalence (pre test probability) were used reflecting primary care (0.23%)

and specialist settings (secondary care 10% and tertiary care 30%) taken from the published literature and hospital audits.

The meta-analyses are represented by curves that are fitted when the HSROC hierarchical model is used. This model is appropriate where there is not a common threshold used across studies. The curve illustrates the average estimate of test accuracy across the range of thresholds included. Where included studies can be grouped according to the threshold used (as in the biomarker review) the bivariate hierarchical model is used and a pooled point estimate of test accuracy at a particular threshold can be estimated. This point estimate should be indicated on the SROC plot. It is not possible to produce a point estimate of sensitivity and specificity without reference to a single threshold. In the combination review, to overcome this problem, we estimate sensitivity from the curve at a fixed specificity (80% or 90%) based on clinical consensus about an acceptable FP rate.

Narrative synthesis was used where meta-analysis was not considered appropriate due to clinical or methodological heterogeneity or where only a single study was identified.

Estimation of the accuracy of individual index tests

Since the characteristics measured by index tests could be extracted as 2 x 2 tables reported at common index test thresholds, bivariate model including random effects[105, 106] was used. To estimate average sensitivity and specificity at fixed thresholds, the analysis of each index test version was performed by first restricting to studies that reported thresholds recommended in guidelines and or used in clinical practice and secondly to those thresholds most commonly reported across included studies. In addition, for ROMA, studies using thresholds +/- 2 around the most commonly reported thresholds were included. For CA125 the positivity threshold cut off CA125 35U/ml (RCOG recommended) 65U/ml (modified ACOG) and the 200U/ ml as recommended by RCOG for premenopausal women were investigated along with results within a 5U range of the test thresholds (30-40U/ml and 60-70U/ml). For HE4 as there is no consensus on the clinically agreed threshold cut off for test positivity, thresholds cut off as recommended by manufacture and the most commonly reported clinical thresholds of 70pMol/L and 140pMol/L and also two analyses including results within a 10U range of these

test thresholds (60-80pMol/L, 130-150pMol/L). Studies were excluded if no threshold was given for the reported values of sensitivity and specificity.

Comparison of test accuracy

For the review of test combinations an indirect comparison of each version of each index test was undertaken by fitting HSROC models for each of the 3 patient groups: mixed populations (pre and postmenopausal combined), premenopausal only and postmenopausal only. A summary ROC curve for each version of each index test across all thresholds was estimated. Each included study contributed one threshold to the summary ROC curve. Where an individual study reported more than one threshold, the most commonly reported threshold for that index test across all included studies was selected for the meta-analyses. This selection of threshold was only necessary for ROMA studies where the threshold pair 31.1 (+/-2) and 27.2 (+/2) was the most commonly reported across studies. Summary ROC curves which have a common shape were fitted to the data. Differences in accuracy were reported using the ratio of Diagnostic odds ratios with 95% CI using RMI version 1 as the baseline category, RMI I being the combination test currently in routine clinical use in the UK. The difference in specificity fixed sensitivities of 80% and 90% for each index test version compared to RMI version 1 with 95% CI was also reported.

For the review of biomarkers, test accuracy between different tests was compared first by restricting to studies that make head-to-head (direct) comparisons between tests within the same population as this provides the most reliable evidence.[107] Secondly, tests were also compared by including all relevant studies (indirect comparison), particularly when there are few studies comparing tests within the same population and thirdly comparisons were undertaken based only on studies using appropriate grouping of borderline tumours for analysis (grouped with malignant tumours). Test accuracy were compared by adding a covariate for test type and threshold in the bivariate model and calculating differences and 95% confidence intervals using non-linear estimating methods.

Analyses of differences in accuracy between index tests were performed using the NLMIXED procedure in Statistical Analysis System.[108]

2.1.5 Investigations of heterogeneity

Heterogeneity is investigated in test accuracy meta-analysis by fitting covariates to the hierarchical model used. Likelihood ratio tests are used to test for the significance of covariates on estimates of accuracy.

For each index test group, the number of studies with study characteristics specified in the secondary objectives, and their effect by visual inspection of forest plots and summary ROC plots were explored. Investigations of heterogeneity were undertaken, where an adequate number of studies reporting as including or not including these characteristics were present. Potential sources of heterogeneity were added as separate covariates in the bivariate model to assess association with test performance: menopausal status and grouping of borderline results in analysis (with malignant results or excluded/unclear).

Premenopausal versus postmenopausal women

Accuracy for pre and postmenopausal women for commonly reported thresholds of each index test version were compared.

Comparison of test accuracy for appropriate and inappropriate grouping of borderline test results in 2x2 tables

The effect of appropriate and inappropriate grouping of borderline test results on estimates of test accuracy was investigated by comparing summary ROC curves for studies using inappropriate groupings (studies excluding borderline ovarian tumours and studies where the management of borderline ovarian tumours was unclear) with studies using an appropriate grouping (studies combining borderline ovarian tumours with malignant ovarian tumours) as the baseline category.

Estimation of differences in accuracy was performed using the NLMIXED procedure in Statistical Analysis System[108] by including menopausal status and borderline grouping as

covariates in the bivariate model. Differences in accuracy were reported using the ratio of Diagnostic odds ratios with 95% CI and associated p values were computed using Wald tests.

2.1.6 Sensitivity analyses

Sensitivity analyses was considered where sufficient studies to investigate the impact on the summary estimates of (i) including only studies with low concern about applicability in the patient selection domain of QUADAS-2, (ii) leaving out potentially highly influential studies and (iii) classification of borderline tumours as malignant or benign.

2.1.7 Assessment of reporting bias

Formal assessment of reporting bias in were not undertake in this review due to current uncertainty about how to assess reporting bias in diagnostic test accuracy reviews, especially in the presence of heterogeneity.[109]

2.2 Differences between protocol and final review methods

2.2.1 Selection of studies

Language

Searches were not restricted to English Language publications but non-English publications were not considered due to time and resource limitations. The volume of non-English publications not considered by this review is explicit in the results of the search strategy.

2.2.2 Study quality assessment and data extraction

Data extraction and quality assessment

Study characteristic data and quality assessment was extracted by a single reviewer (NR or PS) and 30% of studies were randomly checked independently by a second reviewer (RC). Any differences were resolved by discussion.

Methodological quality data was extracted by a single reviewer (NR or PS) and 30% of studies independently checked by a second reviewer (CD). Any differences were resolved by discussion.

Quality assessment

A separate domain for multivariable models was not considered necessary, particularly as no studies including only reporting development of multivariate models were included. Instead 2 questions were added to the participant domain of QUADAS-2 drawing on the PROBAST (prediction model risk of bias assessment) tool for diagnostic and prediction models 1) Prespecification of thresholds and 2) Comparable assessment of all model /test components.

2.2.3 Methods for meta-analysis

Statistical analysis

Test accuracy in pre and postmenopausal women were compared by adding a covariate in the bivariate model and calculating differences and 95% confidence intervals using non-linear estimating methods, taking advantage of advances in analysis methods compared to simple testing of differences using likelihood ratio tests.

2.2.4 Heterogeneity and sensitivity analyses

The following planned heterogeneity analyses were not carried out due to insufficient studies with differences in the relevant study characteristics or with these study characteristics reported: Generalist (primary care/community/family practice) versus specialist setting (cancer unit/cancer centre/gynaecological oncology), Histological subtype, reference standard QUADAS-2 domain ROB (high/unclear vs low. Case-control study versus other study designs, 12 months follow-up versus > 6 months < 12 months follow-up for study participants not receiving surgery initially following a negative index test result.

Sensitivity analyses were not undertaken for (i) leaving out highly influential studies as this was not considered necessary (ii) including only studies with low concern about applicability in the patient selection domain of QUADAS-2 as there were insufficient studies (iii) classification of borderline tumours as malignant or benign as this proved too simple an

approach given the heterogeneity in approach to management and reporting of borderline tumours in included studies. Instead, where data allowed, a comparison of estimates of the test accuracy of each index test for studies using an inappropriate grouping (studies excluding borderline ovarian tumours and studies where the management of borderline ovarian tumours was unclear) with studies using an appropriate grouping (studies combining borderline ovarian tumours with malignant ovarian tumours) was undertaken using the HSROC model.

CHAPTER 3: REVIEW OF EXISTING REVIEW

Early detection of ovarian cancer and the aim of reducing mortality has been the goal of research efforts aimed at improving diagnostics in ovarian cancer. Improved understanding of the pathophysiology of ovarian cancer combined with advances in technology in imaging and biochemistry (i.e. proteomics, genomics) has also led to the development of new tests and new studies. Whilst there are many existing guidelines for the detection of ovarian cancer both in Europe (NICE, RCOG and ESGO) and North America (ACOG and SGO) consensus is lacking mainly due to the lack of strong evidence favouring any particular test.

The accuracy of a diagnostic test is not fixed and the methodological design and conduct of test accuracy studies can introduce variation in estimation of accuracy. For example, characteristics of the population being tested including their presentation and prior tests received, how the index test was done and the reference standard used to verify the diagnosis can all influence estimated accuracy and the generalisability of the results.[110] Systematic reviews (SR) and meta-analyses of test accuracy studies therefore can be affected by heterogeneity as a result of variation in these factors in included studies. Willis et al. reported that published SRs of DTAS could be uninformative as they are prone to bias and heterogeneous results.[111]

Given recent developments in meta-analytic and quality assessment methods and the potential for heterogeneity in existing SR of test accuracy it was necessary to evaluate existing systematic reviews addressing the accuracy of tests in OC to assess their currency, validity and applicability to my research question. This will not only inform the need, if any, for a new review but also inform the new review objectives in order to address deficiencies of the existing evidence base.

3.1 Aims and objectives

3.1.1 Primary objectives

1) To systematically identify existing Systematic reviews (SR)of test accuracy for the detection of ovarian cancer

2) To assess the currency, applicability and validity of existing SR of test accuracy for the detection of ovarian cancer reviews to the research questions: “symptoms, ultrasound imaging and biochemical markers alone or in combination for the diagnosis of ovarian cancer in women with symptoms suspicious of ovarian cancer”.

3.1.2 Secondary objectives

1) To assess the reporting quality of the existing SRs using the PRISMA-DTA reporting checklist.
2) To evaluate the usability of the PRISMA-DTA tool.

3.2 Materials and methods

3.2.1 Search strategy and data sources

The search strategy and data sources used for this review are detailed in chapter 2. SRs on the index tests of interest were identified as part of the scoping search for the new test accuracy reviews using a systematic reviews filter.

3.2.2 Inclusion criteria

Objective

Diagnostic test accuracy; studies that evaluated screening, prognosis or recurrence was excluded.

Study design

Systematic reviews- Reviews were included (judged systematic) if they had conducted a systematic literature search including at least 1) two electronic databases, 2) had made an attempt at question formulation: Population, (prior tests and presentation), Index test(s), Reference standard and Target condition and had 3) undertaken quality assessment of individual included studies. Studies were excluded if more than one of these criteria was not present.

Index test(s)

Reviews that investigated the accuracy of one or more of symptoms, FDA approved biomarkers, ultrasound or a combination of these were included.

Target condition

Primary ovarian cancer: all stages and histological types

Non-English publications were not translated. Authors were not contacted.

3.2.3 Study selection

Screening of abstracts and titles, followed by selection of studies for inclusion after reviewing full texts was completed by (NR). Any ambiguity was discussed with a 2nd review (CD) and consensus reached after discussion.

3.2.4 Assessment of applicability, quality assessment and assessment of reporting quality

Data extraction

Data extraction for assessment of applicability, quality of review methods and quality of review reporting was undertaken by NR. The published article and all supplementary figures and tables were reviewed to extract information. Any ambiguity was discussed with CD and consensus reached after discussion. Data extraction was undertaken using EXCEL following piloting on 4 reviews by NR and CD independently.

General information about the review regarding year of publication, title, name of first author and country as identified in the corresponding address and the rationale and objectives of the review was extracted.

There is currently no tool for assessment of the quality (validity and applicability) of SRs of test accuracy. Judgements about the applicability and quality of reviews to the new review

question were therefore made based on existing tools for primary test accuracy studies the QUADAS-2 tool[112] and knowledge of the review topic. This is detailed below.

Judgement of applicability

Population (presentation and prior tests)

The aim of this review was to evaluate the accuracy of tests in symptomatic pre and postmenopausal women suspected of ovarian cancer presenting in non-specialist settings to 'triage women for management in either secondary or tertiary care'.

Index test conduct (operator and threshold)

Many of the tests had several possible thresholds for test positivity. As the accuracy of ultrasound is operator dependent, information on the expertise of US operators by accepted grades of classification EFSUMB or study specific definitions was also sought.

Target condition as defined by the reference standard

Tumours of the ovary are heterogeneous with test accuracy affected by sub types and stages of malignancy as well as characteristics of benign and borderline ovarian tumours. Information about histotypes and stage of malignancy included by reviews and the way borderline tumours were classified for analysis (with either benign or malignant tumours) was therefore noted. Histology in index test positives or follow up for a minimum of 6 months for index test negatives or for women declining surgery was judged to be a suitable reference standard for verification of diagnosis.

Assessment of review quality and currency

Review quality was evaluated using the number of databases searched, attempt at question formulation [population, presentation, prior test, Index test, target condition and reference standard (PppITR)] and any form of quality assessment of included studies. If meta-analysis was performed, statistical methods were noted, in particular use of hierarchical models.[113] Currency of reviews was judged according to the date of completion of search.

Reporting quality

The recently published PRISMA-DTA checklist was used to evaluate the reporting quality of the included reviews. The checklist comprises 27 items covering 6 domains: introduction, methods, results, discussion, conclusion and funding. The PRISMA-DTA tool was piloted by 2 reviewers (NR and CD) before a single reviewer (NR) proceeded to evaluate all. During piloting, it emerged that to accurately capture details pertinent to this review question, it was necessary to tailor items in the checklist. Minimally acceptable information in the study that should be reported for certain items to be scored 'yes' were therefore predefined. For example for the item D1-'intended use of test' It was required authors should state where in the pathway it is to be used, e.g. triage for further testing or management/add on to another test to inform decisions about further testing / management. For item 11- 'definitions for data extraction' presentation and prior tests were required as additional information regarding 'clinical setting'. For item 12 -'risk of bias and applicability' authors were required to report components regarding both -'risk of bias and applicability. If some but not all components of an item were not reported fully, then the item was scored 'limited' and if none of the components were reported, the item was scored 'no'. Tailoring of the PRISMA-DTA checklist is based on the data extraction form and the QUADAS tailoring done to address the questions asked in this review as illustrated in appendix 2 and 3, respectively.

3.2.5 Data synthesis

Data extraction for all elements: assessment of applicability, quality assessment and reporting quality was completed for each review in EXCEL. Results are presented narratively and in tables using descriptive statistics.

3.3 Results

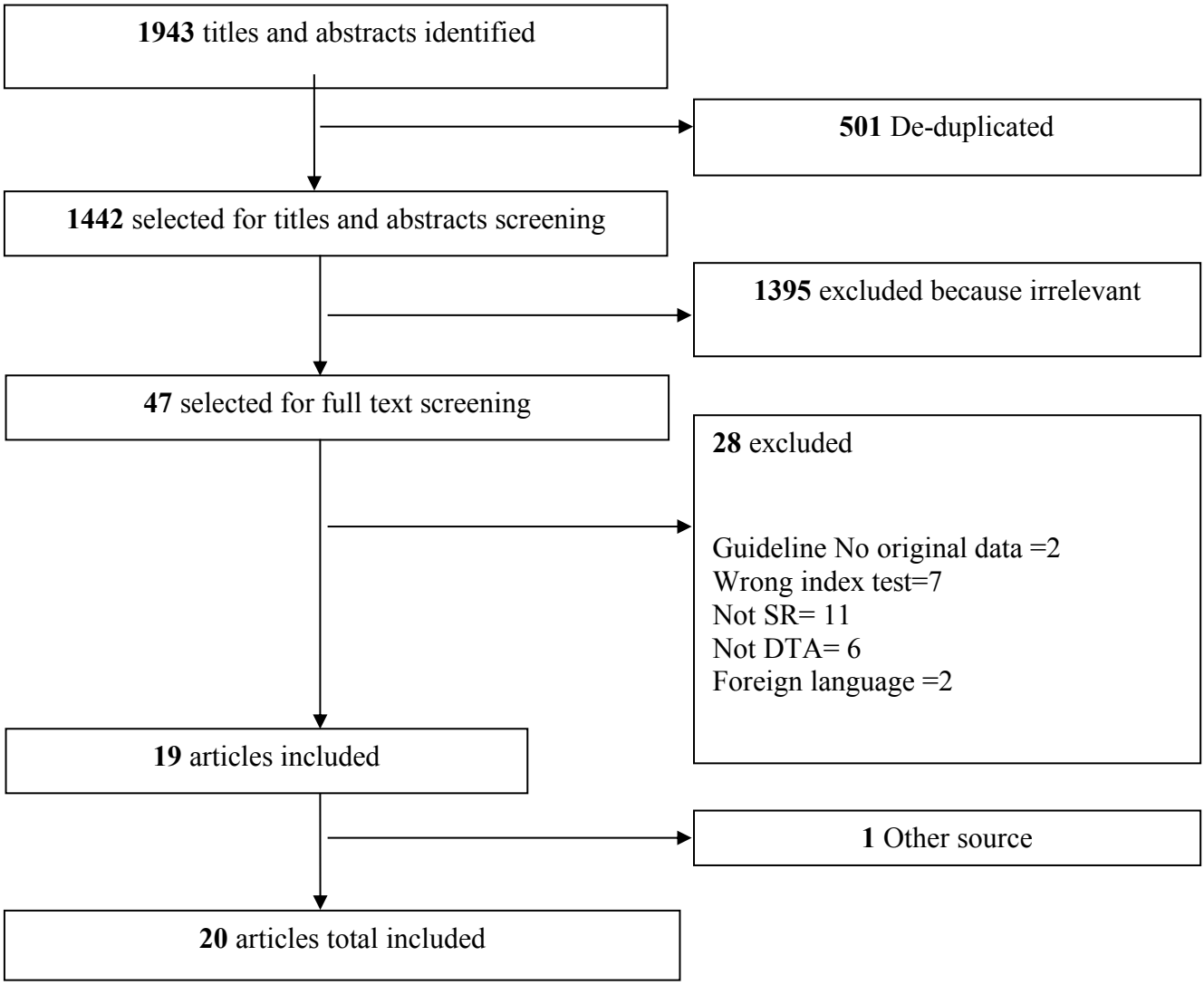


Figure 3. 1: PRISMA of included studies for review of reviews.

3.3.1 Included reviews

After deleting duplicates, 1943 articles underwent title and abstract screening resulting in 47 articles for full text screening (see PRISMA Figure 3.1). 20 publications reporting 23 separate systematic reviews were included. Two reviews investigated the accuracy of symptoms (both individual and in combination), 27 studies included in these reviews investigated the accuracy of a range of biomarkers (CA125, HE4, OVA1) and 35 studies included in these reviews investigated the accuracy of test combinations (RMI1-4, ROMA, ACOG, LR1 and LR2). Where reviews included more than one test assessments of applicability, quality and quality of reporting were undertaken for each test. Date of publication ranged between 2006 [82] and 2016[114]. The reviews were spearheaded from USA, Europe and China. A total of 64 independent syntheses of tests were undertaken across included reviews; These covered single and multiple symptoms (s) (2 syntheses); biomarkers: a total of 27 individual test cohorts that include CA125, HE4 and OVA1; and test combinations: 35 syntheses of individual test combinations including RMI1, RMI2, RMI3, RMI4, ROMA, LR1, LR2 and ACOG. The synthesis of these reviews and assessment of applicability, review quality and currency of the existing reviews are detailed below (also see Table 3.1 and Table 3.2).

3.3.2 Applicability

A total of 64 independent syntheses of tests were undertaken across included reviews; These covered single and multiple symptoms (s) (2 syntheses); biomarkers: CA125 (13 syntheses), HE4 (12 syntheses) and OVA1 (2 syntheses) and test combinations: RMI1 (7 syntheses), RMI2 (7 syntheses), RMI3 (5 syntheses), RMI4 (1 synthesis), ROMA (5 syntheses), LR1 (3 syntheses), LR2(5 syntheses) ACOG (2 syntheses). (see Table 3.1)

Table 3. 1: Review characteristics – applicability to review question(s).

Author and publication date	Population				Index test(s) included		Results can be /have been stratified by:			
	Presentation (A)Symptomatic	Prior tests	Health care setting (Primary/Secondary/ Tertiary)	Biomarkers	Test combination	Symptoms	Menopausal status / Age	BOT are or can be grouped with malignant	Threshold	Histological subtypes
E. R. Myers / Feb 2006	Reported as unable to assess due to lack of information in primary studies	Reported as unable to assess due to lack of information in primary studies	Reported as unable to assess due to lack of information in primary studies	CA125			Yes	Yes	Yes	NR
					RMI1,2 and 3, LR1 and 2		Yes	NR	NR	NR
P. Geomini / Feb 2009	NR	NR	NR		RMI1 and 2		NR	NR	for RMI1	NR
L. R. Medeiros / Feb 2009	NR	NR	NR	CA125			NR	Yes	NA Single threshold included	NR
NICE April /2011 (a) sym	Symptomatic	NR Assume 'no' as presenting in primary care	Primary care			Single and combination symptoms	NR	NR	NR	NR
NICE April /2011 (b) Bio	Noted in summary that results do not directly apply to symptomatic women	'Women with a confirmed adnexal mass or ascites.'	Secondary care	HE4, CA125			NR	NR	NR	NR

Table 3.1 Continued...

NICE April /2011 (c) combi	NR	NR	NR	Yes	RMI1 & 2 and physical examination+ CA125+USS studies from different reviews assuming conditional independence		NR	NR	NR	NR
S. Yu/ Feb 2012	NR	NR	NR	CA125, HE4	No	No	NR	N	Yes for HE4 only	NR
J. E. Dodge/ Apr 2012	NR	NR	NR	CA125	RMI1 and 2 RMI3, LR1, LR2, ACOG	No	NR	NR	NR	NR
F. Li / June 2012	NR	NR	NR		ROMA		Yes*	Yes	NR	EOC Vs OC
				HE4, CA125			Not pooled analysis but represented in Forest plot (as lee than 3 studies)- same done for early vs late	No	NR	
L. Wu/ Sept 2012	NR	NR	NR	HE4	No	No	NR	NR	NR threshold effect found on investigation	NR

Table 3.1 Continued...

J. Lin / Dec 2012	NR	NR	NR	CA125, HE4	ROMA	No	NR	NR	Yes (ideal Vs suggested) for HE4	NR
S.Ferraro/ Feb 2013	NR	NR	Yes (44% in gyn onc setting)	CA125, HE4	No	No	NR (4 studies but not enough to perform pooled analysis)	NR	NR	NR
Z. Yang/ Jul 2013	NR	NR	NR	HE4	No	No	NR	NR	NR	NR
J. Lin / Dec 2013	NR	NR	NR	CA125, HE4	ROMA	No	NR	NR	Yes (ideal Vs suggested) for HE4	NR
J. Kaijser/ Dec 2013	NR	NR	NR	OVA1	Many models including ROMA, RMI (1-4), LR 2	No	Where reported in primary studies	NR	Yes	No (but studies excluded if restricted to specific histological
J.Wang/ Mar 2014	NR	NR	NR	CA125, HE4	ROMA	No	Yes	Yes (EOC VS EOC+BOT)	No (best test threshold reported by study was chosen)	NR§§
S. Zhen /Apr 2014	NR	NR	Yes (60% of the studies- Gyn onc setting)	CA125, HE4	No	No	NR	NR	NR	NR
X. Y. Liao / Nov 2014	NR	NR	NR	CA125	No	No	NA Only postmenopausal women included	NR	NR	NR
A.C.L. Macedo / Sept 2014	NR	NR	NR	HE4	No	No	Yes	Yes	Yes	NR§
M. Stukan / Feb 2015 (no meta analysis	NR	NR	NR	CA125, HE4, OVA1	ROMA, RMI1,2 and 3, LR1, LR2, ACOG	No	Yes	Yes (only studies including borderline with malignant included)	Yes	Yes
M.Ebell / Mar 2016	Symptomatic	NR	NR	No	No	Individual symptoms, combi symptoms	Yes	NR	No/NA	NR

Table 3.1 Continued...

E.M.J. Meys/ May 2016	NR	NR	Yes (LR2 studies 3 studies with 1 tertiary and 2 mixed, RMI -18 studies 9 in secondary or mixed setting); metaregression analysis for heterogeneity with less or more than 25% prevalence	No	LR2, RMI1,2 and 3	No	Yes	Borderline grouped with benign excluded	NR	Studies not reporting all histological subgroups excluded

Note: In the absence of menopausal status age >< 50 used to stratify results

*Additional analyses by stage, methods (low vs high quality);

§early vs late

§§ (also sub group for early vs late and diff testing platforms

**AAN- Artificial Neural network.

Population (presentation and prior tests)

With the exception of the 2 reviews investigating the accuracy of symptoms themselves only 2 reviews concerned with combination and biomarker tests [82, 115] commented on the absence of information on symptom status in included studies.

Only one review discussed the relevance of information on prior tests (conducted before the index test).[82] A further 3 reviews Ferraro[116] (44% study participants in tertiary care setting), Zhen[117] (60% participants in tertiary care setting) and Meys[114] (3 LR2 studies-1 tertiary care and 2 mixed; 18 RMI studies 9/18 in secondary or mixed setting and unknown in 9/18)[114] included information on the clinical setting of included primary studies and provided stratified results according to ovarian cancer prevalence (< or > 25%).

Menopausal status, index test positivity threshold and target condition

Nine reviews stratified test accuracy estimates by menopausal status.[82, 88, 114, 118-122] Seven reviews provided stratified results by test positivity threshold.[82, 119, 121-124] Two reviews presented stratified results according to histological subtype (2)[65, 122] and 6 reviews explicitly reported how borderline ovarian tumours had been categorised for analysis.[82, 118, 120-122, 125, 126]

Important potential sources of heterogeneity for the accuracy of tests for ovarian cancer include population characteristics (presentation and tests received prior to the index test under evaluation), menopausal status, the test positivity threshold used for biomarkers and inclusion of all histological subtypes of ovarian cancer including borderline tumours. A total of 9 reviews did not report study characteristics or undertake investigations of heterogeneity for any of these variables.

3.3.3 Review quality and currency

Currency

The search dates in the included reviews covered the period from database inception[115] to 2016.[114] The search dates of the included reviews had a wide range depending on the data source; the search end dates for different reviews ranged from September 2004[82] to July 2015.[114] (see Table 3.2)

Database coverage

With the exception of 3 reviews,[88, 127, 128] 2 or more bibliographic databases were searched; Medline (15 reviews) and Embase (12 reviews) were the most commonly searched databases followed by the Cochrane library (11). Two reviews searched Pubmed only[88, 128] and 1 review[127] covered Medline alone. Thirteen of 23 reviews did not specify if unpublished literature was searched.

Table 3. 2: Review characteristics – quality.

Author and publication date	Index test(s) included			Search dates	Electronic databases	Study selection (PppIRT)	Quality assessment included studies (tool)	Synthesis employed hierar chical models unless not appropriate to review question or evidence base*
	Biomarkers	**Test combinations**	**Symptoms**					
E.R. Myers / Feb 2006	Yes	Yes	No	1969 - Sept 2004	Cochrane and MEDLINE	No prior tests	Yes (review specific tool)	No
P. Geomini / Feb 2009	No	Yes	No	Inception to Mar 2008	MEDLINE and EMBASE	PppR not reported	Yes (review specific tool)	Yes
L. R. Medeiros / Feb 2009	Yes	No	No	JAN 1985 - DEC 2007	MEDLINE, CANCERLIT, LILACS, EMBASE, Cochrane	Ppp not reported	Oxford evidence level Studies with level 4&5 excluded	No
NICE April /2011 (a) sym	Individual and combination			1806 – 2010	MEDLINE, PreMEDLINE, EMBASE, Cochrane, CINAHL, BNI, PsycINFO, Web of Science, BioMed Central	PppR Not reported	Yes QUADAS	No meta analyses. Forest plots of sensitivity and specificity
NICE April /2011 (b)Bio	CA125, HE4			1806 – 2010	MEDLINE, PreMEDLINE, EMBASE, Cochrane, CINAHL, BNI, PsycINFO, Web of Science, BioMed Central	Ppp not mentioned in methods but results summary notes that results do not directly apply to symptomatic women and timing of tests not reported (page 64)	Yes QUADAS	No
NICE April /2011 combi	Yes	Yes	Yes	Same	Same	PpR(presentation and prior test) After search SR for PE+CA125+ USS combined and Geomini for RMI and 1 additional updated study for RMI and combined tests results from different studies assuming conditional independence	Yes QUADAS	Not Done
S. Yu/ Feb 2012	Yes	No	No	1974-2011	PubMed, MEDLINE, EMBASE, EBSCO, Science Direct, Cochrane, CNKI	Ppp not reported	Yes QUADAS	No
J. E. Dodge/ Apr 2012	Yes	Yes	No	SRs and guidelines 1999-2009 and Jan 2004 - March 2009 ALL studies Myer's SR from 2004 updated	MEDLINE	Details not reported except Core methodology same as Meyer's	Yes AMSTAR for SR Review specific tool for primary studies	Yes

Table 3.2 Continued...

F. Li / June 2012	Yes	Yes	No	NO START DATE - UP TO 22/12/2011	MEDLINE, EMBASE, Web of Science, Google Scholar, Cochrane, Clinical Trials.gov	presentation and p prior test not reported	Yes QUADAS	Limited (univariate analysis was performed to determine if multivariate analysis appropriate based on heterogeneity (I2))
L. Wu/ Sept 2012	Yes	No	No	JAN 1990 - AUG 2011	PubMed	Ppp not reported	Yes QUADAS	No
J. Lin/ Nov 2012	Yes	No	No	SEPT 1995 - NOV 2011	MEDLINE, EMBASE, Cochrane	PppR not reported	Yes (Selected from QUADAS)	No
J.Lin/ Dec 2012	Yes	Yes	No	2009 - June 2012 (Studies before 2009 excluded due to progressive development in assay according to author)	EMBASE, MEDLINE, Cochrane	PPPRT not reported	Yes (Selected from QUADAS)	
S.Ferraro/ Feb 2013	Yes	No	No	Medline (since 1966) and Embase (since 1993) upto Jan 2012	MEDLINE, EMBASE,	Ppp not reported (Chinese women)	Yes QUADAS	2 studies for CA125 and 3 for HE4 were excluded as they were outliers
Z. Yang/ Jul 2013	Yes	No	No	JAN 2000 - MAY 2013	PubMed and EMBASE	PppR not reported	Yes QUADAS	Yes
J.Lin/ Dec 2013	Yes	Yes	No	Until June 2012	EMBASE, MEDLINE, Cochrane	PppR not reported	Yes (Selected from QUADAS)	No
J. Kaijser / Dec 2013	Yes	Yes	No	2008-2013 + added Models from Geomini	MEDLINE and EMBASE	Ppp reported	Yes QUADAS	Yes
J.Wang/ Mar 2014	Yes	Yes	No	Period NR; Only last date of search reported-15-Nov-13	MEDLINE, Science Direct, Cochrane	presentation and prior test not reported	Yes QUADAS	Yes
S. Zhen /Apr 2014	Yes	No	NO	2008-2012		PppT not reported	Yes QUADAS	No
X. Y. Liao / Nov 2014	Yes	No	No	NR	PubMed, WANFANG, CNKI	PppR not reported	NR	
A.C.L. Macedo / Sept 2014	Yes	No	No	1990-APRIL 2013	MEDLINE, EMBASE, Cochrane, Central Register of Controlled Trials, IBEX, BIOSIS, Web of Science, SCOPUS, Congress, Abstracts, Grey Literature	presentation and prior test not reported	Yes QUADAS	No

Table 3.2 Continued...

M. Stukan / Feb 2015	Yes	Yes	No	1984-April 2004	MEDLINE, EMBASE, CINAHL	Ppp not reported	Yes QUADAS	NA
M.Ebell / Mar 2016	No	No	Yes	2001-2014 (only provided in abstract not methods)	PubMed	Ppp not reported	Yes QUADAS	Yes
E.M.J. Meys/ May 2016	No	Yes	No	1990 - 31 JULY 2015	MEDLINE, EMBASE, Cochrane, Central Register of Controlled Trials	Prior test and presentation not reported	Yes QUADAS	Yes

Notes: PppIRT Population, prior tests, presentation, Index test & threshold, Reference Standard and Target condition defined (specified) in objectives or methods

*Meta-analyses in diagnostic test accuracy reviews require multivariate models (bivariate and hierarchical summary receiver operating characteristic), which allow for the trade off between sensitivity and specificity with changes in test positivity threshold and also for variability across studies by incorporating random effects. Univariate methods may be used if the review question is concerned with estimates of sensitivity at a fixed specificity or vice versa. Meta-analysis may be precluded by low volume or heterogeneity of the evidence base

Question formulation

Inclusion criteria were not clear or comprehensive in any of the (23/23) of reviews. Details regarding one of more of population presentation, prior testing, menopausal status, index test threshold and conduct, details of the target condition (histological subtypes including borderline tumours) and details on the methods used to verify of diagnosis (reference standard) were missing from all reviews. Only nine of the reviews [114, 121, 120, 82, 115, 118, 127] explicitly specified inclusion of both pre and postmenopausal women. One review restricted inclusion to only postmenopausal women.[129] No reviews provided information on presentation or prior testing leading up to inclusion in the study cohort.

Index test information provided in reviews was limited to the type of index test and with the exception of one study did not specify thresholds for inclusion.[126] However, 17/23 reviews reported test positivity information in their results.

Seven of 23 reviews did not define the reference standard in the inclusion criteria or provide details of the reference standard used in included studies (histology or duration of clinical follow up). Histological conformation of diagnosis for both index test positive and index test negative was the prerequisite inclusion criteria in the remaining 16 reviews. Histological subtypes or stages of ovarian cancer to be included were not defined in any review although 13/23 of the reviews reported some histological information in their results.

Quality assessment of studies included in reviews

Quality assessment of included studies was undertaken in 22 reviews with 15 of them using the QUADAS tool in its entirety and 2 using only a selection of items rather than the full tool.[124, 130] 6 reviews used a quality assessment tool not specific for test accuracy studies; 3 reviews used a review specific quality assessment tool[82, 125] and one AMSTAR[127] Liao et al was the only review[129] that did not undertake quality assessment of included studies.

Statistical synthesis

Meta-analyses in diagnostic test accuracy reviews require multivariate models (bivariate and hierarchical summary receiver operating characteristic), which not only allows for the trade off between sensitivity and specificity with changes in test positivity threshold but can also take

into consideration variability across studies by incorporating random effects. Univariate methods may be used if the review question is concerned with estimates of sensitivity at a fixed specificity or vice versa. Meta-analysis may be precluded by a low volume of evidence or heterogeneity. Twelve of the reviews did not use hierarchical models for meta-analysis without explanation. One review used hierarchical models in a limited way.[118] Three reviews in 2 publications[115, 122] provided reasons for not undertaking meta-analysis either because included reviews were confined to SRs or because of a low volume of included primary studies.

3.3.4 Quality of review reporting

Quality of reporting using the PRISMA DTA checklist is detailed below. The tables summarising the quality of reporting on the methods used and results reported for the included reviews are presented in appendix 4 and 5 respectively.

Review background

Eight reviews were published by European groups (1 from UK) and 8 from China (all biomarkers) and 4 from America (1 from USA, 1 from Canada and 2 from Brazil).

Twelve of 20 reviews identified themselves as a SR whilst 5 identified themselves as a meta-analysis. Two of the reviews not identifying themselves as a SR were guidelines.[82, 115]

The clinical background was poorly described by the majority of reviews including the role of the test, the potential effect of test results on management and the minimal acceptability level of test accuracy. The majority of reviews in their introduction and rationale for review are limited to only stating "a review of diagnostic tests to diagnose ovarian malignancy in women with adnexal mass". In summary 14 reviews were scored as 'no' and 4 as 'limited' for this section of the reporting tool. With the exception of one study[114] none of the protocols for any of the reviews were registered.

With the exception of one review[115] reporting of the review objectives was scored as "limited" or "no" due to the paucity of information about the population limited to "women".

Methods

The study eligibility criteria that should be reported by a test accuracy review include study characteristics: population, index test, target condition, reference standard and study design. Information on index test (23 scoring 'yes') and target condition (23 scoring 'yes') were consistently reported whilst information on population (14 scoring 'No'), reference standard (8 scoring 'No') and setting (20 scoring 'No') were poorly reported. Study design and language were not reported in 9 reviews. Only one review (NICE "biomarker reviews") reported all eligibility criteria whilst 2 reviews omitted to report one of the above items (NICE "symptoms review" (reference standard) and Myers review (setting). All other reviews had more than 1 eligibility item-missing ranging from 2 items (7 reviews) to 5 items (3 reviews).

Information sources and search

Sub items included for reporting checklist for this item included details of databases included, search dates including date of last search, search strategy, details regarding contact with authors and other sources and any search limits (such as date or study design) applied.

Some information regarding data sources, search period and key words were reported by all reviews. However this reporting item was scored as 'limited' in 19 reviews because the last search date (8 reviews), contact with authors (16 reviews) or both (4 reviews [116, 125, 124, 129] were missing. Search limits were not reported in 5 reviews. Only 8 reviews reported their search strategy whilst 1 other review made it available on request;[121] All sub items were reported in 2 reviews.[82, 115] One review[120] did not report any information and was scored 'no' for this item.

Study selection

All sub items of study selection were reported in 19/23 reviews. Three reviews were scored 'limited' as eligibility for meta-analysis was not reported and 1 review[122] did not report any of the sub items and was scored 'no'.

Data extraction

20/23 reviews were scored as 'Limited' on this item based on the omission of one of the sub items "piloting of data extraction form". Three reviews did not report any sub items and were

scored 'no'. [115, 122, 127] The NICE "combination review" was screened by a single reviewer and included only the SR by Geomini et al along with an other RMI study by Raza et al was regarding definition of terms for data extraction only 2 reviews scored 'yes'.[119, 114] The remaining reviews were scored as "limited" as one or more of the variables index test, target condition, reference standard and study design were undefined for data extraction; target condition was defined by the least number of reviews (17/23) and the reference standard by the most reviews (16/23).

Quality assessment

The quality assessment tool used was reported in 22/23 reviews.

Planning statistical analysis

Regarding definition of the unit of assessment for meta-analysis this was not explicitly stated in any of the reviews. Only one review[119] reported methods on how authors intend to group index tests for comparison. Only 2 reviews did not report methods for meta-analysis and accuracy measures to be derived [122] and the NICE "symptoms review".[115]

Fourteen reviews reported a-priori plans for additional analyses; 2/14 of the reviews reported plans for subgroup and sensitivity analyses and 12 reviews reported additional analyses for investigating heterogeneity, threshold effects and publication bias.

Results

All sub items of study selection were reported in 12 reviews and scored 'yes'. Stukan and Dodge et al[122, 127] did not report any sub items and scored "No". 11 of the reviews did not report a PRISMA diagram. In 3 reviews[114, 122, 117] the reason for excluding studies from the meta analysis was not provided.

Study characteristics

With respect to reporting study characteristics of included studies one review[120] scored "No" as none of the individual study characteristics (patient symptom status, prior test, study design, index test and threshold, target condition, reference standard, sample size and funding) were

reported. The remaining 22 reviews were scored as 'limited' mostly (18 reviews) due to the nonreporting of the sub items regarding patient symptomatology (18), prior testing (21) and funding (22). 13 reviews had 4 or more sub items unreported.

Quality of the primary studies included in the reviews

Six of the reviews reported both results of risk of bias and applicability assessment of individual studies and included a summary table or figure and were scored "Yes"; Three reviews reported a narrative summary of the quality of included studies without detail for individual studies in either narrative or figure form and were scored "No". 2 reviews reported the intention to do Quality assessment in their methods but did not report the quality assessment results. 9 reviews were scored "Yes" and 8 as 'Limited' either due to risk of bias or applicability judgements not being reported.

Numerical results

The PRISMA DTA numerical results items for quantitative analysis include reporting results of individual studies (TN, TP, FN, FP, diagnostic measure e.g. Sensitivity and specificity with confidence intervals, threshold for test positivity, ROC and/ or Forest plot), synthesis across studies (narrative summary or pooled estimates of meta analysis and confidence intervals) and any additional analyses performed or not performed with reasons.

17 reviews lacked 1 or more sub items relating to reporting of results for included studies. Sensitivity and Specificity, threshold for test positivity and ROC curve/Forest plot were not reported in 10, 12 and 3 reviews respectively.

With the exception of one study[122] the results of statistical synthesis were well reported (summary estimates of sensitivity and specificity with confidence intervals).

Additional analyses in reviews are undertaken for investigation of heterogeneity (sub group analyses or meta-regression) or to test the robustness of results (sensitivity analyses). It is often not possible to undertake planned additional analyses due to limitations of quality and / or volume of data in a review. In the case that reviews indicate a plan to undertake additional analyses in their methods the results of these should be reported or reasons provided for why they were not performed. Across included reviews reporting of the results of additional

analyses was scored as “Limited" (15 reviews) and “No” (8 reviews). Additional analyses that were reported included subgroup analyses (17 reviews), sensitivity analyses (6 reviews) and meta-regression (4 reviews). 9 reviews planned and reported additional analyses and 1 review[119] reported planned subgroup analysis by menopausal status but inability to conduct this due to paucity of studies and provided individual data of 2 studies, which were available. 1 review provided subgroup results of menopausal status for biomarkers without an a priori plan in the methods[82] and 3 reviews reported a plan to perform heterogeneity results but did not provide the results or the reasons for not performing it.[125, 127, 88]

None of the reviews reported the funding of the included studies and therefore lacking transparency in industry funded studies or conflict of interest. The funding source was declared in 11 of the reviews.

3.4 Discussions

Systematic reviews of diagnostic test accuracy undertaken outside of the Cochrane collaboration are increasing in number as a resource for evidence based diagnosis and are an important resource of guideline development. Poor quality reporting of DTA reviews limits assessment of their quality and applicability, which in turn can mislead the guidelines and their recommendations drawn from these reviews and hinder policy development and assessing the need for further research. McInnes et al. published the Preferred Reporting Items for a Systematic Review and Meta-analysis of Diagnostic Test Accuracy Studies-The PRISMA-DTA statement to address this deficiency in 2018 [131]. To date, there is no tool for the quality assessment of systematic reviews of test accuracy.

3.4.1 What is the applicability of existing SRs for OC diagnosis?

Judgment of the applicability of existing reviews for OC diagnosis was difficult to assess in a large number of included reviews. For the majority of reviews where pertinent information was not available (symptomology, prior tests received and healthcare setting), no reason for its omission was provided. Only a minority (2 reviews) cited difficulty as a result of lack of information in included studies. Based on information reported the studies included in the reviews largely included patient populations from specialist settings presenting with adnexal

masses, with no details provided on presentation (presence of absence of symptoms) and the point in the clinical pathway.

Ovarian Cancer often presents with symptoms or may be detected incidentally during investigation for other conditions. The presenting symptoms are variable, non-specific, often mimic bowel symptoms and range in intensity, duration and frequency. The nature of symptoms will influence the healthcare setting in which a woman first presents (primary care, emergency setting, and the specialist team they are referred to), tests that will have been done prior to using an index test and the prevalence and severity of disease at the time of index testing (the further along a referral pathway – the higher prevalence and greater proportion of more advanced disease is encountered). The performance of the same test is, therefore expected to vary according to the healthcare setting in which it is used. Absence of reporting of this information in systematic reviews precludes judgements about applicability to a particular healthcare setting and contributes to unknown sources of heterogeneity when undertaking meta-analyses. The importance of information about patient characteristics can be illustrated using included reviews that did report relevant information. Meys[114] presented stratified for prevalence less than and greater than 25% based on knowledge of the range of prevalence in included populations: Of 24 studies included in the analyses the lowest prevalence was 9.5%, 3 studies reported a prevalence less than 15% and 6 studies reported a prevalence over 40%. The prevalence of OC varies on the setting and menopausal status and there is no published literature alluding to it. In generalist settings the prevalence of ovarian cancer is in the region of 0.03%. The implications for making judgements about the applicability of the existing systematic review evidence presented here is clearly crucial.

The ratio of the prevalence of ovarian cancer to benign conditions in women presenting with symptoms suspicious for an adnexal mass increases with age and after attaining menopause. Ovarian cancer is a heterogeneous disease and tests are known to perform differently according to histological subtypes. Similarly, borderline ovarian tumours (of low malignant potential) can display features of benign or malignant tumours with either US testing or testing for tumour markers. Tests would therefore be expected to perform differently in populations where the distribution of tumour types is different, for example, pre and post menopause. A lack of information on different histotypes identified in included along with the absence of information on participant age and/ or menopausal status also impacts on the applicability judgements of an evidence base.

3.4.2 Quality: How reliable are the results of existing SRs for OC diagnosis

In the absence of DTA specific quality tool consensus criteria for systematic review conduct was taken from the critical appraisal tool for systematic reviews, AMSTAR, combined with best practice guidance from the Cochrane Diagnostic Test Accuracy Review Handbook.

Search strategy

Regarding the search strategy of a review it is important to know the dates and range of information searches interrogated and the use of search limits such as date, language or study design. The search period was provided in all the reviews allowing assessment of currency. With the exception of 3 reviews a minimum of 2 electronic databases and citation checking was provided by the other included reviews. The concept of publication bias is not well understood for DTA studies but current guidance advocates searching for unpublished literature in DTA reviews; however more than 50% of included reviews did not report searching for unpublished literature in their methods.

Question formulation (review this section)

Question formulation is a critical step for the review that follows as it provides clarity on the objectivity of the review by predefining what forms the basis for the inclusion criteria. It provides the framework and objectivity for including and excluding studies and provides transparency and quality control of included studies in the review. The question formulation in a DTA review typically includes PppITR so that it reflects and defines the inclusion criteria that allow one to determine applicability of the review and contextualises the results and conclusion drawn.

The relevance regarding applicability of the reviews to the relevant population in clinical practice could not be drawn due to the lack of information and clarity missing for details for inclusion criteria regarding population, presentation and prior testing. The review by Meyers et al. (AHRQ)[82] also commented on this. However, the review that followed do not provide further clarity or address this weakness.

Even though the type of index test was reported in all reviews, 39% of review did not prespecify test positivity thresholds to be included. As test accuracy varies with test positivity, if index test thresholds are not predefined there is a risk that thresholds will be chosen that optimise test performance in a particular population, therefore potentially introducing bias into the review. This also potentially introduces heterogeneity to results of the review when the meta analyses that follow do not take this into account and prespecify the plan to deal with multiple different thresholds from included studies. The reviews also in their results/ conclusion did not make any recommendation of the clinically useful threshold for testing; the results of accuracy are meaningless when the threshold at which it can be applied, and this precludes decision on what is an acceptable false positive or false negative for the patient, clinician or stakeholder.

Similarly, information on target condition was not defined and limited to broadly just stating ovarian cancer in majority of the reviews thus showing an inability to understand the heterologous nature of ovarian tumours and its attendant effect on test accuracy. The lack of predefined criteria on target condition and the following lack of predefined plan on how to deal with borderline tumours, different histotypes or stages also makes the results less useful as again the patients, clinicians or stakeholders cannot infer its applicability. With the exception of one review[82], the lack of clarity on the spectrum of ovarian tumours included (benign, borderline and malignant and different histotypes) considered (inclusion and investigation of heterogeneity) was unclear. Without information about the range of histological subtypes included in a review and how these have been dealt with for meta-analyses (for example have borderline tumours been excluded) or has review authors selectively included studies with histological subtypes in which the index test is known to perform better, for example, HE4 is suggested to perform better for EOC,[132] applicability cannot be determined and recommendations made.

The quality of the reference standard used in test accuracy studies is key to the validity of estimates of test accuracy generated. Poor quality reference standards may underestimate or overestimate test accuracy. Reference standards that share characteristics of the index test may be chosen to inflate the observed test accuracy of an index test. In addition, inclusion of more than one type of reference standard in reviews is a potential source of heterogeneity for estimates of test accuracy.

Quality assessment in reviews

Assessment of the quality of included studies in a review is necessary so that appropriate inferences can be made about the validity of review results and the impact of study quality on heterogeneity can be investigated as appropriate. With the exception of one review[129] review, authors undertook quality assessment of included studies. The original DTA-specific quality assessment tool, QUADAS (QUality Assessment of Diagnostic Accuracy Studies) tool was published in 2003[112] and was superseded by QUADAS-2 in 2011;[133] All reviews published after 2011 included QUADAS as the quality assessment tool. Six of the reviews undertaking quality assessment did not provide details and presented these findings only with a figure.

Statistical analysis

Because of the correlation between sensitivity and specificity induced by threshold effects, meta-analysis of test accuracy outcomes requires hierarchical models. Hierarchical models deal with statistical distributions at two levels. At the lower level, they model the cell counts in the 2×2 tables extracted from each study using binomial distributions. This level deals with sampling variability in individual studies and the correlation that exists between sensitivity and specificity. At the higher level, random study effects analysis is used to account for heterogeneity in test accuracy between studies. The Bivariate and HSROC hierarchical models were developed between 2001 and 2005 and have been shown to be equivalent under many circumstances.[134] Hierarchical models are now advocated over separate (univariate) analysis of sensitivity and specificity.[135] Univariate analysis fails to take into account the correlation between sensitivity and specificity and is known to underestimate both metrics. Despite all included reviews being published after 2006 (earliest Myers Feb 2006) Only 8 of the (insert number of reviews undertaking meta-analysis as denominator here) reviews undertaking meta-analysis used hierarchical models or justified why they were considered inappropriate meaning that summary estimates of test accuracy may not be valid.

3.4.3 Reporting quality

There was consistent poor reporting regarding the methodology employed for most of the sub items such as rationale for review, protocol registration, search strategy, pilot data forms, defining index test thresholds, minimally acceptable test accuracy, definition of DTA elements,

plan for results synthesis on handling differences in primary studies during meta-analysis and funding of individual studies. Information on database searched, period included, eligibility criteria and personnel involved in data extraction were reported with key details missing thus lacking clarity on population (menopausal status), Index test (assay method, threshold for test positivity) spectrum of target condition. Lack of apriori plan or poor reporting of a apriori plan on how to how handle differences in different thresholds, different definitions of the target condition, indeterminate results or other potential variation across studies contribute to potential source of bias in review conduct and impacts on applicability and accuracy of the results.

The poor quality of reporting was as also reflected in the reporting of results with lack of information on data extraction, data on presentation, prior testing, patient characteristics, target condition and reference standard. The results of the meta-analyses for SN, SP, CI and ROC were well reported but subgroup / sensitivity analyses and stratification of results by menopausal status, threshold cut off or borderline or staging are poorly reported.

Methodologically, this lack of information not only introduces potential sources of heterogeneity but the lack of transparency prevents comparing or reproducing these results, which basically infers to poor quality scientific evidence.

Stukan et al. reported study specific results of the accuracy of index tests that included combination tests and biomarkers in epithelial ovarian cancer compared to other histotypes when available in included studies. Fake Li et al[118] is the only review that undertook meta-analyses and reported a stratified result for epithelial ovarian cancer compared to other histotypes for the accuracy of HE4 (biomarker). Poor reporting on accuracy of results without reporting stratification and adequate assessment of quality and or heterogeneity affects accuracy, transparency and applicability.

Information is lacking regarding presentation and clinical setting and limited to women in surgical setting; this affects the prevalence and spectrum of disease, information crucial to assessment of the applicability of review results to clinical practice apart from affecting accuracy of the index tests

These generalist results also cannot be applied to the population in a meaningful way, thus contributing to the continuing debate and lack of consensus on the clinical utility of these results.

While piloting the PRISMA–DTA tool, it was also noted that methodological and topic specific clinical expertise is required for meaningful interpretation of questions which may limit its usability to stakeholders. Secondly, the word count in most journals could be a stumbling block and inclusion of this information in the appendix apart from providing transparency is also likely to help potential reviewers, stakeholders and future researchers to identify relevant information to avoid reduplication of work and concentrate resources where the gaps are identified.

A requirement by journals to adhere to the reporting items as minimal standard as is already the case for many journals for RCTs (CONSORT) and systematic reviews of interventions (PRISMA). Adherence to PRISMA-DTA as well as a greater use of web-based material accompanying publications would improve the reporting of DTA reviews and therefore their usability.

3.5 Conclusion

The many identified SR were variable in quality. The poor quality of reporting precludes, making judgement on generalisability and applicability and therefore underlining the need for a review, which is systematic and compare tests across the board.

The poor reporting and where this could be assessed, deficiencies in quality and lack of applicability to the planned reviews (biomarkers and combination) led to the conclusion that updating an existing review was not an option. The lack of information in the reviews could have been either due to lack of the concerned review or the primary studies, and hence an attempt was made to not only look for all relevant information in the primary studies but also provide transparency to the review process.

The gaps in current knowledge discovered during the review of reviews were used to refine the review question and define the PppITR inclusion criteria. The information distilled from review of reviews was used to inform the methodology and tailor the quality assessment questions of the planned review to identify gaps in review and report test accuracy results giving

consideration to menopausal status, test positivity thresholds, status of borderline tumours and improve the reporting applicability of the review to make it more clinically relevant.

The QUADAS 2 tool was tailored to evaluate the primary review to evaluate heterogeneity, transparency and applicability of the studies to enable assessment and also contextualise results to aid patient and stakeholders alike.

The newly published DTA reporting PRISMA guide will be used to help complete reporting.

CHAPTER 4: RESULTS OF COMBINATION REVIEW

4.1 Screening and selection of studies

The original primary searches and subsequent updates retrieved a total of 36,005 records after de-duplication. After reviewing titles and abstracts, 34,968 records were subsequently excluded. Full-text copies of 1,037 potentially relevant reports were obtained and screened for inclusion, of which 72 were deemed eligible for inclusion for combination test. Figure 4.1 shows the PRISMA diagram of the study selection process. Studies were excluded if they failed to meet one or more of the specified inclusion criteria with regard to study design, participants, target conditions or ability to derive a 2x2 table. In this systematic review index tests or test combinations that did not report test positivity threshold or, had been superseded (for example LR1) or that had not been used at some point in clinical practise were excluded.

The test combinations included in the review were RMI (versions 1-4), ROMA, LR2, ACOG and modifications of ACOG and ADNEX (Table 2.1). A total number of 72 studies were identified for inclusion in this review (see section quantity of evidence). 48 studies including 15542 women were identified investigating the accuracy of RMI; 31 studies including 12335 women for RMI I, 19 studies including 4989 women for RMI II, 19 studies including 5945 women for RMI III and 6 studies including 1971 women for RMI IV. 22 studies, including 6497 women, were identified investigating the accuracy of ROMA, 4 studies including 4888 women, were identified investigating the accuracy of LR2. Four studies, including 2787 women, were identified investigating the accuracy of 4 different versions of ACOG. Three studies, including 1301 women for the ACOG version 1, 3 studies including 837 women for ACOG version 2, 3 studies including 2123 women for ACOG version 3 incorporating CA125 and 1 study including 516 women for ACOG version 3 incorporating OVA1. One study, including 2124 women, was identified investigating the accuracy of ADNEX. Reason for full text study exclusions are detailed in Appendix 6.

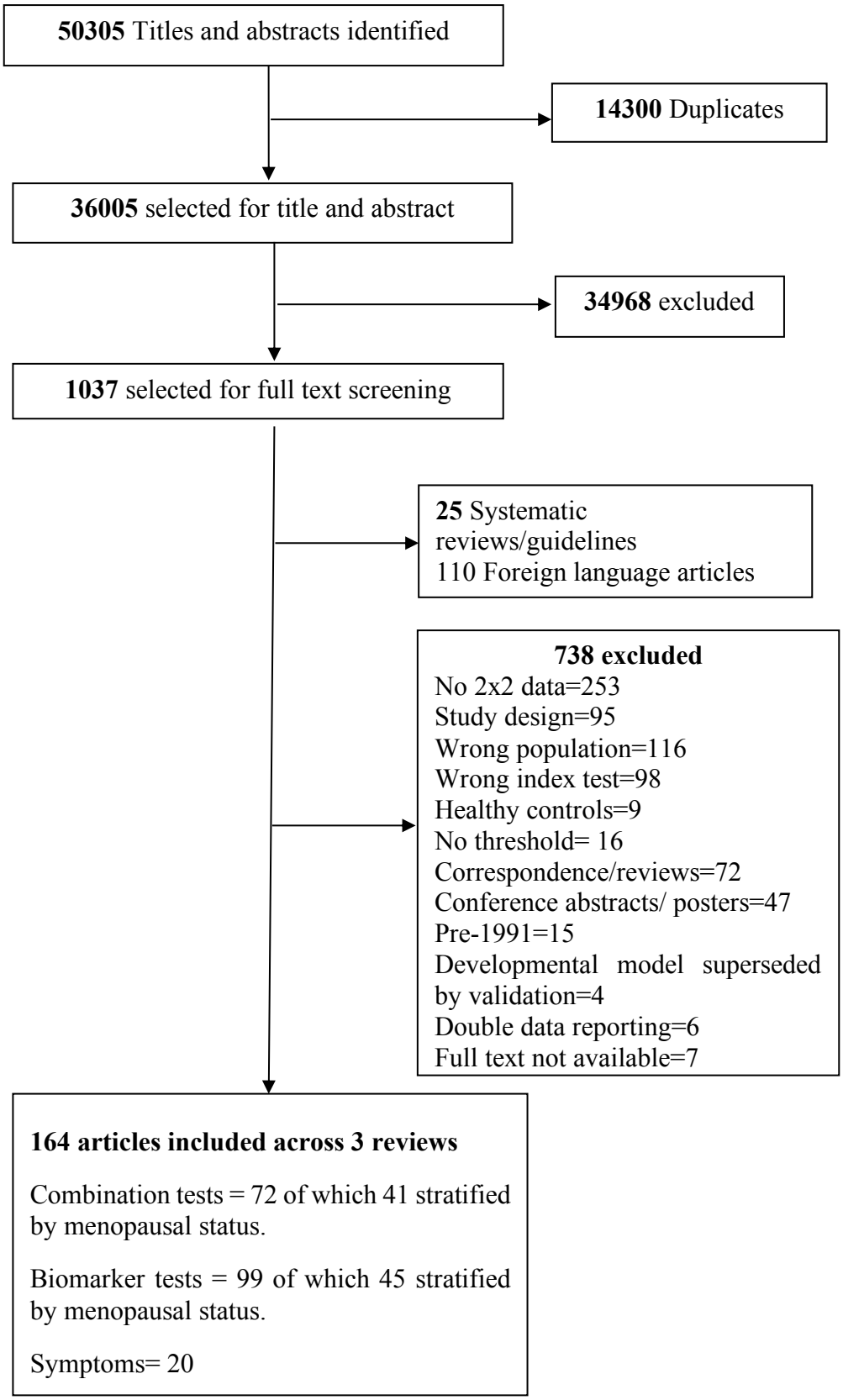


Figure 4. 1: PRISMA of included studies for the combination and biomarker review. See Appendix 6 for reasons of exclusion.

Forty-one of 72 included studies allowed separation of results by menopausal status and are included in the analysis. Details about included test combinations are detailed in Table 2.1, 4.1, 4.2, 4.3, 4.4 and 4.5. Where multiple publications were available for the same cohort of patients, publications reporting accuracy by menopausal status were chosen over non-differentiated results to reflect well-documented differences in spectrum in pre and postmenopausal women as detailed in the introduction chapter. Similarly, publications reporting accuracy on the basis of clinical assessment in primary care were preferred over those reporting accuracy on the basis of clinical assessment by gynaecologists in secondary care or gynaecological oncologists in tertiary care and specialist ultrasonographers. This was to reflect the setting in which index tests were to be used.

Seven of the included studies compared two index tests. Five patient cohorts reported in 6 studies compared ROMA with RMI [136, 137, 138, 139-141]. Two studies compared RMI with LR2 for both pre and postmenopausal women.[142, 143]

4.2 Study characteristics and quality of included studies

4.2.1 Study characteristics of included studies

The clinical pathways of patients from presentation to the decision/referral for surgical intervention were not detailed in any included studies. Testing prior to surgical intervention in this patient group will have included clinical history and examination, biomarker measurement and imaging. These tests can be carried out in primary care, by Gynaecologists/other specialities, by Gynaecological oncologists or a mixture of both. In clinical practice in the UK, clinical assessment and biomarker testing are most commonly initially performed in primary care. Ultrasound assessment may be initiated by a primary care practitioner but conducted in a secondary care setting. If the primary care practitioner decides to refer a woman for further management because of a suspicion of ovarian cancer, clinical assessment and ultrasound assessment or CT/MRI may be used as adjuncts to aid in decision-making and management plan in secondary or tertiary care.

The characteristics of all included studies, baseline characteristics of participants and the nature of the index test combination are described below under the headings RMI, ROMA, LR2, ACOG and ADNEX.

RMI and RMI variation studies

(Menopausal status, US findings and serum CA125). See also Table 2.1

The study characteristics of 48 studies, including 15542 women evaluating RMI for the diagnosis of ovarian cancer in pre and or postmenopausal women, are described in Table 4.1. Of 48 studies, 7 studies were multicentre, conducted within Europe and participating women were recruited mostly from mixed clinical settings (secondary or tertiary) [144, 142, 143, 145, 146, 147, 148] or from secondary care.[149] The remaining 41 studies were single centre, mostly conducted in secondary care (24 studies), 14 in tertiary car and in 3 studies the setting was not clear.[150, 151, 152] The majority of single centre studies (23/41) were conducted in Europe [153, 154, 150, 155, 156, 157, 158, 159, 160, 161, 162, 138, 163, 151, 164, 165, 166, 167, 137, 168, 169, 170, 140] 15 were conducted in Asia[171, 172, 173, 174, 175, 176, 177 177, 152,178, 179, 180, 181, 141, 182, 183] and one each were conducted in Canada[184,] Australia[185] and Brazil.[136]

Studies used various versions of RMI (1-4) and at different test positivity thresholds. RMI version 1 at threshold of 200 was the most commonly reported threshold (31/48 studies) followed by RMI version 2 at a threshold of 200 (19/48 studies) and RMI version 3 at a threshold of 200 (19/48 studies). RMI versions 1, 2 or 3 at thresholds of 250 were reported in 17 studies. RMI version 4 at a threshold of 400 or 450 was reported in 6 studies.[153, 178, 147, 182, 183, 149]

Table 4. 1: Study characteristics: RMI I.

Author Year Country	Setting	Participants characteristics	Index test threshold
Akturk 2011 **Turkey**	**Study criteria:** Women with pelvic mass undergoing laparotomy or laparoscopy **Clinical setting:** Secondary care **Prior tests:** Unclear **Exclusions:** Borderline tumours **Centre:** Single centre	**N:** 100 **Postmen n (%):** 16 (16) **Ovarian cancer n (%):** 18 (18) **Age:** Not reported **Separated by menopausal status:** No	**Threshold:** 200, 250 **Prespecified:** No
Anton 2012 **Brazil**	**Study criteria:** Women referred with pelvic mass diagnosed by US, CT or MRI with signs of carcinomatosis undergoing surgery or image-guided biopsy. **Clinical setting:** Secondary care **Prior tests:** Unclear **Exclusions:** None reported **Centre:** Single	N: 120 **Postmen n (%):** 73 (60) **Ovarian cancer n (%):** 30 (25) **Borderline n (%):** 17 (14) **Age mean:** -Malignant: 54.7 -Borderline: 56.4 -Benign: 50.7 **Separated by menopausal status:** Yes	**Threshold:** 200 **Prespecified:** Yes
Arun-Muthuvel 2014 **India**	**Study criteria:** women with pelvic tumours scheduled for surgery **Clinical setting:** Tertiary care **Prior test:** Unclear **Exclusions:** Nil **Centre:** Single centre	**N:** 467 **Postmen n (%):** 105 (22.4) **Ovarian cancer n (%):** 82 (18) **Borderline n (%):** 16 (3) **Age mean:** 39 **Separated by menopausal status:** No	**Thresholds:** 200, 250 **Prespecified:** No

Table 4.1 Continued...

Asif 2004 **India**	**Study criteria:** Women who underwent elective surgical exploration for ovarian mass **Clinical setting:** Secondary care **Prior test:** Unclear **Exclusions:** Unclear **Centre:** Single centre	**N:** 100 **Postmen n (%):** 30 (30) **Ovarian cancer n (%):** 55 (55) **Borderline n (%):** Not reported **Age mean:** - Malignant: 45 - Benign: 37 **Separated by menopausal status:** No	**Thresholds:** 200, 250 **Prespecified:** No
Aslam 2000 a **UK**	**Study criteria:** Women undergoing surgery for adnexal mass was examined preoperatively by US **Clinical setting:** Tertiary care **Prior test:** Unclear **Exclusions:** Nil **Centre:** Single centre	**N:** 61 **Postmen n (%):** 25 (41) **Ovarian cancer n (%):** 19 (31) **Borderline n (%):** 4 (7) **Age mean:** - Malignant: 53 - Benign: 43 **Separated by menopausal status:** No	**Thresholds:** 200 **Prespecified:** Yes
Bouzari 2011 **Iran**	**Study criteria:** Women with pelvic masses admitted for laparotomy **Clinical setting:** Secondary **Prior test:** Unclear **Exclusions:** Not reported **Centre:** Single centre	**N:** 182 **Postmen n (%):** 21 (12) **Ovarian cancer n (%):** 23 (13) **Borderline n (%):** 8 (4) **Age mean:** 39.9 **Separated by menopausal status:** No	**Thresholds:** 200 **Prespecified:** No

Table 4.1 Continued...

Clarke 2009 **Canada**	**Study criteria:** Women with adnexal masses who underwent surgery **Clinical setting:** Tertiary **Prior test:** Unclear **Exclusions:** Nil **Centre:** Single centre	**N**: 163 **Postmen n (%):** 102 (63) **Ovarian cancer n (%):** 58 (36) **Borderline n (%):** 1 (1) **Age mean:** - Malignant: 57 - Benign: 54 **Separated by menopausal status:** No	**Thresholds:** 200 **Prespecified:** No
Davies 1993 **UK**	**Study criteria:** Women undergoing surgery for an adnexal mass **Clinical setting:** Secondary **Prior test:** Unclear **Exclusions:** unclear **Centre:** Single centre	**N:** 124 **Postmen n (%):** 58 (47) **Ovarian cancer n (%):** 28 (23) **Borderline n (%):** 7 (6) **Age mean:** Not reported **Separated by menopausal status:** No	**Thresholds:** 200, 250 **Prespecified:** No
Di Legge 2012 **European countries**	**Study criteria:** Women who underwent surgery within 120 days after ultrasound examination for adnexal mass **Clinical setting:** Mixed **Prior test:** Unclear **Exclusions:** Nil **Centre:** Multicentre	**N:** 2445 **Postmen n (%):** 945 (39) **Ovarian cancer n (%):** 398 (16) **Borderline n (%):** 131 (5) **Age mean:** Not reported **Separated by menopausal status:** No	**Thresholds:** 200 **Prespecified:** Yes

Table 4.1 Continued...

Dotlic 2011 **Serbia**	**Study criteria:** adnexal masses hospitalised for surgery **Clinical setting:** Secondary **Prior test:** Unclear **Exclusions:** Nil **Centre:** Single centre	**N:** 81 **Postmen n (%):** 30 (37) **Ovarian cancer n (%):** 30 (37) **Borderline n (%):** Not reported **Age mean:** 44.13 **Separated by menopausal status:** No	**Thresholds:** 200 **Prespecified:** Yes
Enakpene 2009 **Germany**	**Study criteria**: Women with suspicious adnexal mass undergoing surgery **Clinical setting:** Tertiary **Prior test:** Unclear **Exclusions:** Women with obvious clinical symptoms of malignant disease and ultrasound features of metastases were excluded Centre: Single centre	**N:** 302 **Postmen n (%):** 137 (45) **Ovarian cancer n (%):** 127 (42) **Borderline n (%):** 31 (10) **Age mean:** Not reported **Separated by menopausal status:** No	**Thresholds:** 250 **Prespecified:** Yes
Huchon 2012 **France**	**Study criteria:** Women who presented with an adnexal mass requiring surgery **Clinical setting:** Tertiary **Prior test:** Unclear **Exclusions:** Nil **Centre:** Single centre	**N:** 99 **Postmen n (%):** Not reported **Ovarian cancer n (%):** 6 (6) **Borderline n (%):** 5 (5) **Age mean:** 45.8 - Benign: 45.1 - Borderline: 52.3 - Malignant: 49.9 **Separated by menopausal status:** No	**Thresholds:** 200 **Prespecified:** No
Irshad 2013 **Pakistan**	**Study criteria:** Unclear (ovarian masses) **Clinical setting:** Secondary **Prior test:** Unclear **Exclusions:** Unclear **Centre:** Single centre	**N:** 36 **Postmen n (%):** 36 (100) **Ovarian cancer n (%):** 24 (37) **Borderline n (%):** Not reported **Age mean:** 58 **Separated by menopausal status:** Yes	**Thresholds:** 250 **Prespecified:** Yes

Table 4.1 Continued...

Jabeen 2015 **Pakistan**	**Study criteria:** Women with adnexal mass undergoing surgery **Clinical setting:** Secondary **Prior test:** Unclear **Exclusions:** only women over 30 were included **Centre:** Single centre	**N:** 60 **Postmen n (%):** Not reported **Ovarian cancer n (%):** 17 (28) **Borderline n (%):** 1 (2) **Age mean:** 50.8 **Separated by menopausal status:** No	**Thresholds:** 200 **Prespecified:** Yes
Jacobs 1993 **UK**	**Study criteria:** Women undergoing laparotomy for suspected adnexal mass Clinical setting: Secondary **Prior test:** Unclear **Exclusions:** Nil **Centre:** Single centre	**N:** 143 **Postmen n (%):** Not reported **Ovarian cancer n (%):** 37 (26) **Borderline n (%):** 4 (3) **Age mean:** Not reported **Separated by menopausal status:** No	**Thresholds:** 200 **Prespecified:** No
Kader Ali Mohan 2010 **Australia**	**Study criteria:** Women undergoing surgery for abdominopelvic mass **Clinical setting:** Tertiary **Prior test:** Unclear **Exclusions:** Only women over 30 included **Centre:** Single centre	**N:** 224 **Postmen n (%):** 124(55.3) **Ovarian cancer n (%):** 40 (18) **Borderline n (%):** 22 (10) **Age mean:** Not reported **Separated by menopausal status:** No	**Thresholds:** 200 **Prespecified:** Yes
Manjunath 2001 **India**	**Study criteria:** Women with a pelvic mass admitted for Laparotomy/ Laparoscopy **Clinical setting:** Secondary **Prior test:** Unclear **Exclusions:** BOT excluded from analysis **Centre:** Single centre	**N:** 152 **Postmen n (%):** 43(65) **Ovarian cancer n (%):** 91 (60) **Borderline n (%):** (excluded from analysis) 4 (3) **Age :** Not reported **Separated by menopausal status:** No	**Thresholds:** 200, 250 **Prespecified thresholds:** No

Table 4.1 Continued...

Mol 2001 **Netherlands**	**Study criteria:** women who had surgery for adnexal mass **Clinical setting:** Unclear **Prior test:** Unclear **Exclusions:** Nil **Centre:** Single centre	**N:** 170 **Postmen n (%):** 61 (36) **Ovarian cancer n (%):** 30 (18) **Borderline n (%):** Not reported **Age mean:** Not reported **Separated by menopausal status:** No	**Thresholds:** 200 **Prespecified:** No
Moolthiya 2009 **Thailand**	**Study criteria:** women with pelvic mass admitted for laparotomy **Clinical setting:** Secondary **Prior test:** Unclear **Exclusions:** only women >30 years included **Centre:** Single centre	**N:** 209 **Postmen n (%):** 88 (42) **Ovarian cancer n (%):** 60 (29) **Borderline n (%):** 11 (5) **Age mean:** 50 **Separated by menopausal status:** No	**Thresholds:** 200 **Prespecified:** Yes
Morgante 1999 **Italy**	**Study criteria:** Women over 30 years undergoing surgery for ovarian mass **Clinical setting:** Secondary **Prior test:** Unclear **Exclusions:** Only women over 30 years included **Centre:** Single centre	**N:** 124 **Postmen n (%):** 55 (44) **Ovarian cancer n (%):** 27 (22) **Borderline n (%):** 2 (2) **Age mean:** Not reported **Separated by menopausal status:** No	**Thresholds:** 200 **Prespecified:** No
Obeidat 2004 **Jordan**	**Study criteria:** Women with a pelvic mass admitted for laparotomy **Clinical setting:** Tertiary **Prior test:** Unclear **Exclusions:** Unclear **Centre:** Single centre	**N:** 100 **Postmen n (%):** 73 (73) **Ovarian cancer n (%):** 56 (56) **Borderline n (%):** 16 (16) **Age mean:** Not reported **Separated by menopausal status:** No	**Thresholds:** 200, 250 **Prespecified:** No

Table 4.1 Continued...

Ong 2013 **Singapore**	**Study criteria:** Women admitted to the hospital for surgery due to ovarian mass **Clinical setting:** Secondary **Prior test:** Unclear **Exclusions:** Nil **Centre:** Single centre	**N:** 228 **Postmen n (%):** 66(2.9) **Ovarian cancer n (%):** 6 (3) **Borderline n (%):** 11 (5) **Age mean:** Not reported **Separated by menopausal status:** No	**Thresholds:** 200 **Prespecified:** Yes
Radosa 2011 **Germany**	**Study criteria:** women with adnexal mass who subsequently underwent surgery were selected **Clinical setting:** Tertiary **Prior test:** Unclear **Exclusions:** Nil **Centre:** Single centre	**N:** 442 **Postmen n (%):** 141 (32) **Ovarian cancer n (%):** 79 **Borderline n (%):** 19 **Age mean:** 43.3 **Separated by menopausal status:** Yes	**Thresholds:** 200 **Prespecified:** Yes
Sayasneh 2013a **UK**	**Study criteria:** Women presenting with adnexal mass and undergoing surgery within 120 days after examination **Clinical setting:** Mixed **Prior test:** Unclear **Exclusions:** Nil **Centre:** Multi centre	**N:** 255 **Postmen n (%):** 117 (46) **Ovarian cancer n (%):** 48 (19) **Borderline n (%):** 18 (7) **Age mean:** 46 **Separated by menopausal status:** Yes	**Thresholds:** 200 **Prespecified:** Yes
Sayasneh 2013 (secondary publication)		**N:** 301 **Postmen n (%):** 116 (39) **Ovarian cancer n (%):** 65 (22) **Borderline n (%):** 18 (6) **Age mean:** 47 **Separated by menopausal status:** No	

Table 4.1 Continued...

Simsek 2014 **Turkey**	**Study criteria:** Women referred with adnexal mass and managed surgically Clinical setting: Tertiary **Prior test**: Unclear **Exclusions:** Nil **Centre:** Single centre	**N:** 569 **Postmen n (%):** 164 (29) **Ovarian cancer n (%):** 234 (41) **Borderline n (%):** 8 (1) **Age mean:** - Benign: 35.23 - Malignant: 50.78 **Separated by menopausal status:** No	**Thresholds:** 200 **Prespecified:** Yes
Terzic 2013 **Serbia**	**Study criteria:** Women treated for adnexal tumours **Clinical setting:** Secondary **Prior test:** Unclear **Exclusions:** Nil **Centre:** Single centre	**N:** 689 **Postmen n (%):** 138 (20) **Ovarian cancer n (%):** 112 (16) **Borderline n (%):** 33 (5) **Age mean:** - Benign: 42.8 - Borderline: 53.6 - Malignant: 57.25 **Separated by menopausal status:** Yes	**Thresholds:** 250 **Prespecified:** Yes
Testa 2014 **European countries**	**Study criteria:** Women presenting with adnexal mass and undergoing TVS by one of the principal investigators and surgery within 120 days after examination **Clinical setting:** Mixed **Prior test:** Unclear **Exclusions:** Nil **Centre:** Single centre	**N:** 2403 **Postmen n (%):** 1049 (44) **Ovarian cancer n (%):** 701 (29) **Borderline n (%):** 153 (6) **Age mean:** Not reported **Separated by menopausal status:** Yes	**Thresholds:** 200 **Prespecified:** Yes

Table 4.1 Continued...

Tingulstad 1996 **Norway**	**Study criteria:** Women with pelvic mass undergoing laparotomy **Clinical setting:** Secondary **Prior test:** Unclear **Exclusions:** women under 30 years were excluded **Centre:** Single centre	**N:** 173 **Postmen n (%):** 39 (27) **Ovarian cancer n (%):** 45 (26) **Borderline n (%):** 6 (3) **Age mean:** Not reported **Separated by menopausal status:** No	**Thresholds:** 200, 250 **Prespecified:** No
Ulusoy 2007 **Turkey**	**Study criteria:** Women undergoing surgery for an adnexal mass Clinical setting: Tertiary **Prior test:** Unclear **Exclusions:** Not reported **Centre**: Single centre	**N:** 296 **Postmen n (%):** 39.2 (49) Ovarian cancer n (%): 84 (28) **Borderline n (%):** 15 (5) **Age mean:** 42 **Separated by menopausal status:** No	**Thresholds:** 200, 250 **Prespecified:** Yes
Van Calster 2012 **European countries**	**Study criteria:** Women presenting with adnexal mass and undergoing surgery within 120 days **Clinical setting:** Mixed **Prior test:** Unclear **Exclusions:** Nil **Centre:** Multicentre	**N:** 1938 **Postmen n (%):** 742 (38) **Ovarian cancer n (%):** 472 (24) **Borderline n (%):** 111 (6) **Age mean:** Not reported **Separated by menopausal status:** No	**Thresholds:** 250 **Prespecified:** Yes

Table 4.1 Continued...

Van Gorp (IOTA) 2012 **Belgium**	**Study criteria:** Women with a pelvic mass, scheduled for surgery **Clinical setting:** Secondary **Prior test:** Unclear **Exclusions:** Nil **Centre:** Single centre	**N:** 374 **Postmen n (%):**196 (52) **Ovarian cancer n (%):** 94 (25) **Borderline n (%):** 31 (8) **Age mean:** - Benign: 46.2 - Malignant: 57.7 **Separated by menopausal status:** Yes	**Thresholds:** 200 **Prespecified:** Yes
Van Holsbeke 2007 **Belgium, Sweden, Italy, UK**	**Study criteria:** Women with persistent adnexal mass undergoing surgery within 120 days **Clinical setting:** Mixed **Prior test:** Unclear **Exclusions:** Unclear **Centre:** Multicentre	**N:** 1066 **Postmen n (%):** 431 (40) **Ovarian cancer n (%):** 266 (25) **Borderline n (%):** Not reported **Age mean:** Not reported **Separated by menopausal status:** No	**Thresholds:** 200 **Prespecified:** Yes
Yamamoto 2009 **Japan**	**Study criteria:** Women with pelvic mass scheduled for laparotomy or laparoscopy **Clinical setting:** Secondary **Prior test:** Unclear **Exclusions:** Nil **Centre:** Single centre	**N:** 253 **Postmen n (%):** 39 (15) **Ovarian cancer n (%):** 32 (13) **Borderline n (%):** 8 (3) **Age mean:** - Benign: 39.8 - Malignant: 54 **Separated by menopausal status:** No	**Thresholds:** 200, 250 **Prespecified:** No

Table 4.1 Continued...

Yamamoto 2015 **Japan**	**Study criteria:** Women with pelvic mass scheduled for laparotomy or laparoscopy **Clinical setting:** Secondary **Prior test:** Unclear **Exclusions:** Nil **Centre:** Single centre	**N:** 296 **Postmen n (%):** 103 (35) **Ovarian cancer n (%):** 47 (16) **Borderline n (%):** 19 (6) **Age mean:** - Benign: 43.6 - Malignant: 56.3 **Separated by menopausal status:** No	**Thresholds:** 200 **Prespecified:** No
Yavuzcan 2013 **Turkey**	**Study criteria:** women with adnexal mass who underwent surgery Clinical setting: Unclear Prior test: Unclear **Exclusions:** Unclear **Centre:** Multicentre	**N:** 153 **Postmen n (%):** 54 (35) **Ovarian cancer n (%):** 24 (16) **Borderline n (%):** 8 (5) Age mean: 46 **Separated by menopausal status:** Yes	**Thresholds:** 200, 250 **Prespecified:** No

Thresholds extracted for RMI I: 200 and 250

Spectrum: presentation

With the exception of one study, all included studies recruited patients undergoing surgical investigation of an adnexal mass. In one study[138] women underwent surgery because of a 'high index of suspicion' for ovarian cancer, which may or may not have included the presence of an adnexal mass. This was the only RMI study where authors explicitly acknowledged that included women might have been symptomatic. However, the proportion of symptomatic women was not reported. Participants were recruited from a secondary care setting (general gynaecology unit), a tertiary care setting (specialist gynaecology oncology unit) or a mixed setting that included both general gynaecology and specialist gynaecology oncology units. None of the included studies recruited participants from a primary care setting. These highly selected populations are not representative of unselected, symptomatic women presenting in primary care where testing is initiated because of a suspicion of ovarian cancer.

Spectrum: target conditions included

The types of ovarian malignancy reported in included studies varied. Two studies reported including only epithelial ovarian cancer (EOC) and other benign ovarian tumours.[137, 141] Excluding certain tumour types changes the spectrum of disease in the study population, and this is known to affect estimates of test accuracy. For example, CA125 and HE4 are known to have a higher sensitivity in EOC compared to other types of ovarian tumour such as stromal and germ cell tumours. Similarly, 9 of the included studies either explicitly excluded those with borderline tumours [153, 174, 137] or did not identify borderline tumours in the study cohort.[150, 172, 158, 151, 166, 148]

Spectrum: age and menopausal status

1. Information on participant age was reported in 36/48 RMI studies. Twenty eight studies reported mean age which varied between 26.0 years[149] and 55.0 years.[184, 160, 166] Age range was reported in 16 studies and ranged from 10 years[178] to 94 years.[147, 144] Four of 48 RMI studies included women less than 16 years old among their participants.[171, 144, 178, 147] Nine studies restricted inclusion to women over 30 years of age.[154, 177, 185, 152, 180, 164, 169, 143, 146] Twelve studies did not report any information about the age of included participants.

Menopausal status is a risk factor for ovarian cancer. In addition, the spectrum of disease (the severity of ovarian cancer and the range of differential diagnoses) observed in postmenopausal women are different to those of premenopausal women. For example, in premenopausal women the normal menstrual cycle and benign pathology such as endometriosis can result in false positive test results. The accuracy of tests is expected to vary between pre and post menopause and therefore I consider that stratifying test performance by menopausal status an important feature of studies. Only eight of 48 RMI studies stratified results by menopausal status.[136, 160, 165, 142, 137, 168, 143, 140]

ROMA studies

(Menopausal status, serum HE4 and CA125). See also Table 2.1

The study characteristics of 22 studies including 6489 women using ROMA for the diagnosis of ovarian cancer in pre and postmenopausal women are described in Table 4.2

Table 4. 2: Study characteristics: ROMA.

Author Year	Setting	Participant characteristics	Index test threshold*
Anton 2012 **Brazil**	**Study criteria:** Women presenting with signs of carcinomatosis with a pelvic mass diagnosed by US, CT or MRI undergoing surgery or image-guided biopsy. **Clinical setting:** Secondary care **Prior tests:** Not reported **Exclusions**: None reported **Centre:** Single centre	**N:** 120 **Postmen n (%):** 73 (60.8%) **Ovarian cancer n (%):** 30 (25%) **Borderline n (%):** 17 (14%) **Age mean:** -Malignant: 54.7 -Borderline: 56.4 -Benign: 50.7 **Separated by menopausal status:** Yes	**Thresholds:** -Premen 13.1; -Postmen 27.7 **Pre-specified:** Yes
Bandiera 2011 **USA**	**Study criteria:** Not reported **Clinical setting:** Tertiary care **Prior tests:** Not reported **Exclusions:** Non Epithelial Ovarian Cancer excluded **Centre:** Single	**N:** 278 **Postmen n (%):** 183 (65.8) **Ovarian cancer n (%):** 113 (41) **Borderline n (%):** Not reported **Mean age:** Premenopausal: -Malignant: 44.7 -Benign: 41.5 Postmenopausal: -Malignant: 66.3 -Benign: 64.0 **Separated by menopausal status:** Yes	**Thresholds:** -Premen 7.4; -Postmen 25.3 **Pre-specified:** Yes

Table 4.2 Continued...

Chan 2013 **Countries in Asia-Pacific region**	**Study criteria:** Women over 18 years diagnosed with adnexal mass diagnosed by any Imaging method (US, CT or MRI) **Clinical setting:** Unclear **Prior test:** Unclear **Exclusions:** Nil **Centre:** Multicentre	**N:** 414 **Postmen n (%):** 26 (108) **Ovarian cancer n (%):** 74 (18) **Borderline n (%):** 16 (4) **Age mean:** Not reported **Separated by menopausal status:** Yes	**Thresholds:** -Premen 7.4; -Postmen 25.3 **Pre-specified:** Yes
Chen 2015 **China**	**Study criteria:** Women with pelvic masses scheduled for surgery **Clinical setting:** Unclear **Prior test:** Unclear **Exclusions:** Nil **Centre:** Single	**N:** 232 **Postmen n (%):** Not reported **Ovarian cancer n (%):** 60 (26) **Borderline n (%):** Not reported **Age mean:** - Benign: 33 - Malignant: 53 **Separated by menopausal status:** Yes	**Thresholds:** - Premen 11.4 **Pre-specified:** Yes
Chen 2014 **China**	**Study criteria:** Women with EOC and Benign lesions **Clinical setting:** Tertiary **Prior test:** Unclear **Exclusions:** Women with non EOC excluded **Centre:** Single	**N:** 192 **Postmen n (%):** 84 (44) **Ovarian cancer n (%):** 123 (64) **Borderline n (%):** Not reported **Age mean:** not reported **Separated by menopausal status:** Yes	**Thresholds:** - Premen 12.2; - Postmen 25.8 **Pre-specified:** Yes
Farzaneh 2014 **Iran**	**Study criteria:** Women with adnexal mass undergoing surgery and having attained menarche 12 months before presenting with adnexal mass **Clinical setting:** Secondary **Prior test:** Unclear **Exclusions:** Excluded non EOC **Centre:** Single	**N:** 99 **Postmen n (%):** 31 (31) **Ovarian cancer n (%):** 43 (43) **Borderline n (%):** Not reported **Age mean:** - Benign: 39 -Malignant (EOC): 51 **Separated by menopausal status:** Yes	**Thresholds:** - Premen 11.5; - Postmen 25.5 **Pre-specified:** Yes

Table 4.2 Continued...

Fujiwara 2015 **Japan**	**Study criteria:** Women with adnexal mass diagnosed on Ultrasound/CT/MRI/PET and scheduled for surgery **Clinical setting:** Unclear **Prior test:** Unclear **Exclusions:** Women with previous history of any gynaecological disease and NON EOC excluded **Centre:** Multicentre	**N:** 221 **Postmen n (%):** 90 (41) **Ovarian cancer n (%):** 71 (32) **Borderline n (%):** 19 (9) **Age mean:** - Benign: 43 - Borderline: 47 - Malignant: 54 **Separated by menopausal status:** Yes	**Thresholds:** 8.8 (premen and postmen) **Pre-specified:** Yes
Grenache 2015 **USA**	**Study criteria:** Women with abnormal adnexal mass detected on physical examination and Imaging Included Ultrasound, CT or MRI) followed by surgery **Clinical setting:** Unclear **Prior test:** Unclear **Exclusions:** Unclear **Centre:** Multicentre	**N:** 146 **Postmen n (%):** 76 (52) **Ovarian cancer n (%):** 19 (13) **Borderline n (%):** 7 (5) **Age mean:** 52 **Separated by menopausal status:** Yes	**Thresholds:** - Premen 8.6, 13.1; - Postmen 27.7 **Pre-specified:** Yes
Karlsen 2012 **Denmark**	**Study criteria:** Women admitted to surgery for pelvic mass or pelvic pain potentially caused by malignant disease or endometriosis **Clinical setting:** Secondary **Prior test:** Unclear **Exclusions:** Nil **Centre:** Single	**N:** 1218 **Postmen n (%):** 621 (51) **Ovarian cancer n (%):** 261 (21) **Borderline n (%):** 79 (6) **Age mean:** Not reported **Separated by menopausal status:** Yes	**Thresholds:** - Premen 7.4; - Postmen 25.3 **Pre-specified:** Yes
Kadija 2012 **Serbia**	**Study criteria:** Women diagnosed with adnexal mass scheduled to undergo surgery **Clinical setting:** Secondary **Prior test:** Unclear **Exclusions:** Nil **Centre:** Single	**N:** 108 **Postmen n (%):** 41 (38) **Ovarian cancer n (%):** 24 (22) **Borderline n (%):** 5 (5) **Age mean:** Not reported **Separated by menopausal status:** Yes	**Thresholds:** - Premen 12.5; - Postmen 14.4 **Pre-specified:** No

Table 4.2 Continued...

Kim 2011 **S. Korea**	**Study criteria:** Women diagnosed with adnexal mass on the first visit to the gyn oncology clinic and underwent surgery **Clinical setting:** Tertiary **Prior test:** Unclear **Exclusions:** Only EOC included **Centre:** Single	**N:** 159 **Postmen n (%):** 108 (68) **Ovarian cancer n (%):** 68 (43) **Borderline n (%):** 10 (6) **Age mean:** - Benign: 35.7 - Malignant: 51.7 **Separated by menopausal status:** **Yes	**Threshold:** - Premen: 7.6 **Pre-specified:** Yes
Molina 2011 **Spain**	**Study criteria:** Not reported **Clinical setting:** Unclear **Prior test:** Unclear **Exclusions:** Nil **Centre:** Single	**N:** 396 **Postmen n (%):** 143 (36) **Ovarian cancer n (%):** 111 (28) **Borderline n (%):** Not reported **Age mean:** Not reported **Separated by menopausal status:** Yes	**Thresholds:** - Premen 13.1; - Postmen 27.7 **Pre-specified:** Yes
Montagnana 2011 **Italy**	**Study criteria:** women with pelvic mass scheduled to have radical surgery **Clinical setting:** Secondary **Prior test:** Unclear **Exclusions:** only EOC included **Centre:** Single	**N:** 104 **Postmen n (%):** 53 (51) **Ovarian cancer n (%):** 55 (53) **Borderline n (%):** Excluded **Age mean:** **-** Malignant: 56.9 - Benign: 42 **Separated by menopausal status:** Yes	**Thresholds:** - Premen 12.5; - Postmen 14.4 **Pre-specified:** Yes
Moore 2009 **USA**	**Study criteria:** Women with ovarian cyst scheduled to undergo surgery **Clinical setting:** Unclear **Prior test:** Unclear **Exclusions:** Nil **Centre:** Multicentre	**N:** 513 **Postmen n (%):** 150 (29) **Ovarian cancer n (%):** 143 (28) **Borderline n (%):** 22 (4) **Age mean:** 54 **Separated by menopausal status:** Yes	**Thresholds:** - Premen 13.1; - Postmen 27.7 **Pre-specified:** Yes

Table 4.2 Continued...

Moore 2011 **USA**	**Study criteria:** Women with ovarian cyst scheduled to undergo surgery **Clinical setting:** Mixed **Prior test:** Unclear **Exclusions:** Nil **Centre:** Multicentre	**N:** 472 **Postmen n (%):** 217 (46) **Ovarian cancer n (%):** 68 (14) **Borderline n (%):** 19 (4) **Age mean:** 50.3 **Separated by menopausal status:** Yes	**Thresholds:** - Premen 13.1; - Postmen 27.7 **Pre-specified:** Yes
Novotny 2012 **Czech Republic**	**Study criteria:** Women with pelvic abnormalities **Clinical setting:** Secondary **Prior test:** Unclear **Exclusions:** premenopausal women excluded **Centre:** Single	**N:** 256 **Postmen n (%):** 256 (100) **Ovarian cancer n (%):** 21 (8) **Borderline n (%):** Not reported **Age mean:** - Benign: 65.28 - Malignant: 64.37 **Separated by menopausal status:** Yes	**Thresholds:** - Postmen 26.3 **Pre-specified:** No
Ortiz-Munoz 2014 **Spain**	**Study criteria:** Women with gynaecological symptoms, diagnosed with primary ovarian cancer **Clinical setting:** Tertiary **Prior test:** Symptoms **Exclusions:** Nil **Centre:** Single	**N:** 148 **Postmen n (%):** 104 (70) **Ovarian cancer n (%):** 29 (20) **Borderline n (%):** Not reported **Age mean:** not reported **Separated by menopausal status:** **Yes	**Thresholds:** - Premen 11.4; - Postmen 29.9 **Pre-specified:** Yes
Partheen 2011a **Sweden**	**Study criteria:** Women with complex cystic mass and suspicious of malignancy undergoing surgery **Clinical setting:** Tertiary **Prior test:** Unclear **Exclusions:** Solid and unilocular mass were excluded **Centre:** Single	**N:** 374 **Postmen n (%):** 276 (74) **Ovarian cancer n (%):** 108 (29) **Borderline n (%):** 45 (12) **Age mean:** Not reported **Separated by menopausal status:** **Yes	**Thresholds:** - Premen 17.3 - Postmen 26.0 **Pre-specified:** Yes

Table 4.2 Continued...

Sandri 2013 **Italy**	**Study criteria:** Women aged 18 years or older presenting to a gynaecologist undergoing surgery at a gyn oncology centre **Clinical setting:** Unclear **Prior test:** Unclear **Exclusions:** Nil **Centre:** Multicentre	**N:** 349 **Postmen n (%):** 191 (55) **Ovarian cancer n (%):** 160 (46) **Borderline n (%):** 25 (7) **Age mean:** 51.6 **Separated by menopausal status:** No	**Thresholds:** - Premen 7.4; - Postmen 25.3 **Pre-specified:** Yes
Van Gorp 2011 **(Van Gorp 2012- secondary publication- smaller cohort)** **Belgium**	**Study criteria:** All patients diagnosed with pelvic mass undergoing surgery **Clinical setting:** Unclear **Prior test:** Unclear **Exclusions:** Ni **Centre:** Single	**N**: 389 **Postmen n (%):** 161 (41) **Ovarian cancer n (%):** 161 (41) **Borderline n (%):** Not reported **Age mean:** - Benign: 46.3 - Malignant: 57.8 **Separated by menopausal status:** Yes	**Thresholds:** - Premen 12.5; - Postmen 14.4 **Pre-specified:** Yes
Winarto 2014 **Indonesia**	**Study criteria:** Women diagnosed with ovarian tumour by physical examination and TVS **Clinical setting:** Secondary care **Prior test:** Unclear **Exclusions:** Women with non EOC and unresectable tumours excluded **Centre:** Single	**N:** 128 **Postmen n (%):** 42 (33) **Ovarian cancer n (%):** 50 (39) **Borderline n (%):** 17 (13) **Age mean:** - Benign: 41 - Malignant: 44 **Separated by menopausal status:** No	**Thresholds:** - Premen 7.4; - Postmen 25.3 **Pre-specified:** No

Notes to table: *ROMA thresholds most commonly reported and included:-**Premenopausal** 7.4(+- 2); 12.5; 13.1(+- 2); **Postmenopausal** 25.3 (+-2); 14.4; 27.7 (+-2)

**Threshold for premenopausal women OR postmenopausal women reported in the study not included in analysis. EOC: Epithelial ovarian cancer

Six of the 22 included ROMA studies were multicentre and conducted across Asia-pacific [186, 187], the USA [188, 189, 190,] and Italy.[191] The remaining 16 studies were single centre; the majority conducted in Europe[138, 137, 192 139, 193-197], 5 in Asia[198,141, 199, 200] and one each in the USA[201] and Brazil.[136]

In 7/22 studies women were recruited from secondary care.[136, 138, 195, 197, 200 141, 196] In 5 studies, women were recruited from tertiary care[194, 198, 201, 192, 137] and in one study, women were recruited from mixed hospital settings (secondary or tertiary).[190] In nine studies the hospital setting for recruitment (secondary or tertiary) was unclear.[186, 202, 199, 187, 188, 193, 189, 191, 139]

Of the 22 ROMA included studies, 14 provided results for premenopausal women and for postmenopausal women separately using different thresholds. Four studies only included accuracy data for premenopausal women [202, 187, 198, 194] and 2 studies only provided accuracy data for postmenopausal women.[196, 192] Two studies did not stratify accuracy by menopausal status.[191, 141]

In premenopausal women a test positivity threshold of 13.1 (+/-2) was most commonly used (13/18 studies)[136, 202, 199, 200, 188, 197, 193, 195, 189, 190, 194, 137, 139] followed by a threshold of 7.4 (+/-2) (5/18 studies).[201, 186, 187, 138, 198] In postmenopausal women, a test positivity threshold of 27.7 was used in 5/16 studies,[136, 188, 193, 189,] thresholds between 25.5 to 27.8 in 5/16 studies,[199, 200, 196, 192, 137] a threshold of 25.3 in 3/16 studies[201, 186, 138] and a threshold of 14.4 in 3/16 studies.[197, 195, 139]

Spectrum: presentations

All the ROMA studies included women with an adnexal mass scheduled for surgery for suspected ovarian cancer. Only three studies specified the presence of symptoms including 'gynaecological symptom's[194], pelvic pain[138] and vaginal bleeding, pain, distension and weight loss.[200] However, it is unclear whether the presence of symptoms prompted women's health seeking behaviour or whether the presence of symptoms was recorded following identification of an adnexal mass. The majority of studies (18/22) did not detail the imaging modality leading to the diagnosis of pelvic mass. Four studies reported that an adnexal mass was identified following investigation with one of either ultrasound, MRI or CT.[186, 136, 188, 187]

Spectrum: target conditions included

The types of ovarian malignancy reported in included studies varied. Eight studies included only EOC.[201, 199, 200, 187, 198, 195, 137, 141] In two other studies it was not clear if germ cell tumours were purposively excluded or not identified in the study cohort.[194, 188] Eight studies either excluded borderline tumours[195, 137] or did not identify borderline tumours in the study cohort.[201, 199, 202, 200, 193, 194, 196, 139]

Spectrum: age and menopausal status

Information on participant age was reported in 18/22 ROMA studies. Mean age varied from 42.5[141] to 65 years.[196] The age range of participants was reported in 12/18 studies and ranged across studies from 14 years[198] to 93 years.[196] Four studies did not report any information about the age of included participants.

A total of 18 studies provided test accuracy estimates for ROMA in premenopausal women only and 16 studies for postmenopausal women only. Only 2 studies did not stratify results by menopausal status.[141, 191]

LR2 studies

(Age, US findings). See also Table 2.1

The study characteristics of four studies including 4888 women[142, 143, 203, 204] in pre and or postmenopausal women are described in Table 4.3. Three of the studies were multicentre studies conducted across Europe[143, 204] and in the UK[142] where women were recruited from mixed clinical settings (secondary or tertiary care). One study was single centre and conducted in the UK in a secondary care setting.[203] All studies used LR2 at a test positivity threshold corresponding to a 10% probability of malignancy. For studies with overlapping data, the study with the most complete data was chosen after checking the studies for duplicate data.

Table 4. 3: Study characteristics: LR2.

Author Year	Study criteria and setting*	Participants characteristics	Index test threshold
Nunes 2013 **UK**	**Study criteria:** Women referred with US evidence of adnexal mass and undergoing surgery within 120 days **Clinical setting:** Secondary care **Prior tests:** Not reported **Exclusions:** None reported **Centre:** Single	**N:** 292 **Postmen n (%):** 137 (46.9) **Malignant n (%):** 90 (30.8) **Borderline n (%):** 17 (5.8) **Mean age (+/-SD):** 51 (not reported) **Median age (range):** Not reported (16-91) **Separated by menopausal status:** No	**Threshold:** 10% post test probability of malignancy **Prespecified:** Yes
Sayasneh 2013 (a) **Secondary study:** **Sayasneh 2013** **UK**	**Study criteria:** Women presenting with adnexal mass and undergoing surgery within 120 days after examination **Clinical setting:** Mixed secondary and tertiary care **Prior tests:** Not reported **Exclusions:** None reported **Centre:** Multicentre	**N:** 255 **Postmen n (%):** 117 (45.9) **Malignant n (%):** 48 (18.8) **Borderline n (%):** 18 (7.1) **Mean age (+/-SD):** 46 (not reported) **Separated by menopausal status:** Yes	**Threshold:** 10% post test probability of malignancy **Prespecified threshold:** Yes
Testa 2014 **Europe**	**Study criteria:** Women presenting with adnexal mass on TVS and undergoing surgery within 120 days. **Clinical setting:** Mixed secondary and tertiary care **Prior tests:** Not reported **Exclusions:** None reported **Centre:** Multicentre	**N:** 2403 **Postmen n (%):** 1049 (43.7) **Malignant n (%):** 701(18.8) **Borderline n (%):** 153 (6.4) **Median age (range):** -Malignant:57(33-66) -Benign: 44 (not reported) **Separated by menopausal status:** Yes	**Threshold:** 10% post test probability of malignancy Prespecified threshold: Yes
Timmerman 2010 **Secondary study:** **Di Legge 2012** **Eurpoe**	**Study criteria:** Women with persistent adnexal mass undergoing surgery within 120 days **Clinical setting:** Mixed secondary and tertiary **Prior tests:** Not reported **Exclusions:** None reported **Centre:** Multicentre	**N:** 1938 **Postmen n (%):** 742 (38.0) **Malignant n (%):** 373 (19.2) **Borderline n (%):** 111 (5.7) **Mean age (+/-SD):** 46 (not reported) **Median age (range):** Not reported Separated by menopausal status: Yes	**Threshold**: 10% post test probability of malignancy Prespecified threshold: Yes

Spectrum: presentation

All LR2 studies included women with an adnexal mass scheduled for surgery.

Spectrum: target conditions included

All 4 studies reported a range of ovarian cancer tumour types following histology including borderline tumours. No studies explicitly excluded any type of ovarian cancer or benign ovarian pathology.

Spectrum: age and menopausal status

Information on participant age was reported for all 4 studies. The age range of participants ranged from 11 to 94 years.[143, 203, 204] One study only included women who were older than 30 years.[143] Where reported the mean age was similar across studies varying between 46 years and 51 years.[142, 203, 204]

Three of the 4 included LR2 studies stratified results by menopausal status[142, 143, 204] whilst one study[203] only reported test accuracy for all participating women regardless of menopausal status.

ACOG and ACOG variation studies

(Serum CA125 or OVA1, US findings and for ACOG v1 family history of breast or ovarian cancer). See also Table 2.1

The study characteristics of four studies (2787 women) using ACOG and variations of ACOG[205-208] for the diagnosis of ovarian cancer in pre and or postmenopausal women are described in Table 4.4.

All four ACOG studies were conducted in the USA. Three studies were multicentre and women were recruited from mixed clinical settings (secondary or tertiary care).[205, 207, 208] The test positivity threshold for ACOG is defined as the presence of any one of the variables being measured (positive family history of breast or ovarian cancer, raised biomarker level, any one ultrasound finding) The 4 included ACOG papers evaluated the accuracy of four different versions of ACOG. The first modification entailed changing the threshold of serum CA125

used in premenopausal women, the second modification entailed removing family history of ovarian or breast cancer and the third modification entailed replacing serum CA125 with OVA1 (see Table 4.4). Three of 4 ACOG studies evaluated more than one version of ACOG. The original version of ACOG was reported in three studies;[206-208] modified ACOG version 1 in three studies;[206, 208, 209] modified ACOG version 2 in three studies;[206-208] and modified ACOG version 3 in one study.[208]

Table 4. 4: Study characteristics: ACOG V3 (CA125) and ACOG V3 (OVA1).

Author Year	Study criteria and setting*	Participants characteristics	Index test version and threshold **
Bristow 2013 Secondary publications Bristow 2013a, Goodrich 2014, Longoria 2014 Ueland 2011 USA	**Study criteria:** Women undergoing surgery within 3 months of a documented pelvic mass on imaging (CT, US or MRI) **Clinical setting**: Mixed secondary and tertiary care **Prior tests:** Not reported **Exclusions:** None reported **(Longoria 2014**: Women with advanced disease, ascites and metastatic disease excluded**)** **Centre:** Multicentre	**N:** 770 **Postmen n (%):** 217 (43.9) **Malignant n (%):** 115 (15) **Borderline n (%):** 29 (3.8) **Mean age (+/-SD):** 49 (13.97) **Median age (range):** 48 (18-90) **Separated by menopausal status:** Yes	ACOG v3 (CA125)
Dearking 2007 USA	**Study criteria:** Women who fulfilled ACOG/SGO guidelines criteria for referral and undergoing surgery **Clinical setting:** Tertiary care **Prior tests:** Not reported **Exclusions:** Women aged < 35 **Centre:** Single	**N:** 837 **Postmen n (%):** 597 (71) **Malignant n (%):** 277 (33) **Borderline n (%):** Not reported **Mean age (+/-SD):** -Malignant: 64.9 (10.5) -Benign: 44.7 (4.9) **Age range:** (35-91) **Separated by menopausal status:** Yes	ACOG v3 (CA125)
Ware Miller 2011 USA	**Study criteria:** Women undergoing surgery within 3 months of a documented pelvic mass on imaging (CT, US or MRI) **Clinical setting:** Mixed secondary and tertiary care **Prior tests:** Not reported **Exclusions:** None reported **Centre:** Multicentre	**N:** 516 **Postmen n (%):** 281 (54) **Malignant n (%):** 115 (22) **Borderline n (%):** 28 (5) **Age range:** (18-92) **Separated by menopausal status:** Yes	ACOG v3 (CA125) ACOG v3 (OVA1)

* Setting: Secondary care: dedicated gynaecologist in a general hospital; Tertiary care – gynaecological oncology centre.

American College of Obstetrics and Gynaecologists (ACOG) v3 (CA125): **Premenopausal: 1 of CA125 >67; Ascites; abdo or distant mets/ pleural effusion. **Postmenopausal**: 1 of CA125 >35; Ascites; abdo or distant mets/ pleural effusion; nodular or fixed pelvic mass.

American College of Obstetrics and Gynaecologists (ACOG) v3 (OVA1): **Premenopausal**: 1 of: OVA1 positive; US: presence of ascites, abdominal or distant mets, pleural effusion. **Postmenopausal**: 1 of OVA1 positive; US: presence of ascites, abdominal or distant metastases, pleural effusion, nodular or fixed pelvic mass.

Threshold: any one positive test result.

Spectrum: presentation

All 4 ACOG studies included women with an adnexal mass scheduled for surgery.

Spectrum: target conditions included

All 4 studies reported a range of ovarian cancer tumour types following histology. One study either excluded borderline tumours or did not identify borderline tumours in the study cohort.[207]

Spectrum: age and menopausal status

Information on participant age was reported in all 4 studies and ranged from 18 years to 92 years. One study[206] restricted inclusion to women above 35 years old only. Mean age varied across studies in these studies between 49 and 52 years old.

All four included studies stratified results by menopausal status.

ADNEX studies

(Age, serum CA125, referral centre (tertiary or secondary), US findings). See also Table 2.1

One study evaluating ADNEX for the diagnosis of ovarian cancer in pre and or postmenopausal women[210] is described in Table 4.5. The ADNEX model was developed on 3506 patients recruited between 1999 and 2007, temporally validated on 2403 patients recruited between 2009 and 2012, and then updated on all 5909 patients. For the purposes of this review results were considered from validated data on 2403 participants. This was a multicentre study conducted across 10 countries in Europe. The study evaluated 4 test positivity thresholds: 3%, 5%, 10% and 15% probabilities of malignancy.

Table 4. 5: Study characteristics: ADNEX.

Author Year	Study criteria and setting*	Participants characteristics	Index test threshold
Van Calster 2014 **Europe**	**Study criteria:** Women presenting with adnexal mass on US and selected for surgery **Clinical setting:** Mixed secondary and tertiary care **Prior tests:** Not reported **Exclusions:** None reported **Centre**: Multicentre	**N:** 2403 **Postmen n (%):** 1049 (43.7)** **Malignant n (%):** 827 (34.4) **Borderline n (%):** 153 (6.4) **Age:** Not reported **Separated by menopausal status:** Yes**	**Threshold:** 3, 5, 10 and 15% post test probability of malignancy **Prespecified threshold:** Yes

* Setting: Secondary care: dedicated gynaecologist in a general hospital; Tertiary care – gynaecological oncology centre.

** Contact with authors

Spectrum: presentation

Women presenting with an adnexal mass scheduled for surgery were recruited from mixed settings (secondary and tertiary care).

Spectrum: target conditions included

The study reported a range of ovarian cancer tumour types following histology including borderline tumours. No type of ovarian cancer or benign ovarian pathology was explicitly excluded.

Spectrum: age and menopausal status

The study did not provide information on participant age in the validation data set (n=2403). Results were not stratified by menopausal status.

4.2.2 Methodological quality of included studies

The methodological quality of all studies evaluating one or more of RMI, ROMA, LR2 and ACOG and ADNEX studies is summarised in Figure 4.2. Separate Figures summarise study quality grouped by index tests RMI, ROMA, LR2 and ACOG (Appendix 7, Appendix 8,

Appendix 9, and Appendix 10. Methodological quality of the 41 studies reporting test accuracy data separately for premenopausal and postmenopausal women was not notably different to the 31 studies where accuracy was not reported separately according to menopausal status.

The data was extracted using a modified QUADAS-2 criteria proforma (Appendix 3) focusing on four domains of methodological quality: patient selection; index test; reference standard; and flow and timing. A comparative domain was completed for studies comparing different index tests. Tailoring of QUADAS-2 to the clinical topic required consideration of the following.

The target population was unselected women in whom a diagnosis of ovarian cancer was suspected. Thus, studies were considered to be at high risk of bias if they excluded certain types of malignant or benign pathology that might affect the accuracy of index tests specifically for detecting primary ovarian cancer. Examples include endometriosis (which for example causes raised CA125 levels) and borderline ovarian tumours (which although managed as malignant tumours in current clinical practice may not result in a positive test result). Additionally, restricting populations by age was considered to place studies at high risk of bias as age alters disease spectrum. For example, epithelial malignant tumours are more prevalent in older women and germ cell tumours in younger women and it is known that index test performance differs in different types of malignancy.[211, 212] The target population was unselected, symptomatic women in whom testing was initiated and conducted in a primary care setting and resulted in a suspicion of ovarian cancer. Thus, studies were considered to be at high risk of applicability in the presence of one or more of the following characteristics. If index tests were not performed on the basis of presentation and prior testing in primary care and / or if women were not symptomatic at presentation.

Menopausal status is a risk factor for ovarian cancer. In addition, the spectrum of disease (the severity of ovarian cancer and the range of differential diagnoses) observed in postmenopausal women are different to those of premenopausal women. For example, in premenopausal women the normal menstrual cycle and benign pathology such as endometriosis can result in false positive test results. I therefore considered distinguishing test performance in pre and postmenopausal women an important feature of studies. For this reason, the quality of studies that stratified test results by menopausal status is presented separately.

The review included a variety of index tests, themselves all comprised of several tests covering demographic information (such as age and family history of ovarian cancer), biochemical testing and ultrasound examination. Studies were considered at high risk of bias if ultrasound assessment (as a relatively subjective test) was not conducted blind to the results of other index test components (such as biochemical marker results). Similarly, where index tests included ultrasound as a component studies were considered at high risk of bias if the index test was not conducted and interpreted blind to the disease status/reference standard result. Studies that did not prespecify the test positivity threshold were also considered at high risk of bias because this usually results in over optimistic test accuracy estimates that are not replicable outside of the study sample. For composite index tests based on multivariable models (ACOG and ADNEX) additional factors resulting in a judgement of high risk of bias were whether all model components and thresholds were not prespecified or if individual test components were not assessed in a similar way (for example in similar healthcare settings / by individuals with similar levels of expertise). Assessment of applicability of index tests comprised consideration of the expertise of clinicians undertaking more subjective tests such as history taking, examination and ultrasonography.

Histological diagnosis or clinical follow up for a minimum of 6 months (in the absence of histological diagnosis) are both likely to correctly classify the target condition (therefore a low risk of bias) and therefore this was considered to be a valid reference standard for the purpose of this review. Most included studies were conducted in women with an adnexal mass scheduled for surgery and so histological diagnosis was available for all participants. In a minority of studies women with a negative index test result (considered to have a benign condition) were followed up. In these studies, risk of bias was considered high if follow up was less than 6 months and unclear if the duration of follow up was not reported. Concerning the applicability of the target condition /reference standard, assessments were based on how authors had dealt with borderline tumours in their analysis and the implications this had for meta-analysis. Within the constraints of a 2x2 table and reflecting current clinical practice borderline tumours classified as malignant was considered as appropriate for the purposes of estimation of a test's accuracy in this review. Thus, studies either grouping borderline tumours with malignant tumours or reporting results in a way which allowed this grouping for the purpose of meta-analysis were considered to be of low applicability concern. Studies excluding borderline tumours from their analysis were considered as a high applicability concern as this supposes that borderline tumours would not be encountered in a typical patient population

being tested for ovarian cancer and is likely to lead to an overoptimistic estimation of a test's accuracy.

Concerning the flow and timing domain studies, risk of bias was increased if more than one reference standard (histology and follow up) was used in an individual study, if the interval between index test and reference standard application was > 3 months or if some patients were not included in the analysis.

Methodological quality of studies including RMI and variations of RMI

48 studies included an evaluation of the accuracy of RMI. The majority (39 of 48) RMI studies did not stratify women according to menopausal status. Appendix 7 summarise quality assessment for all 11 studies stratifying by menopausal status.

Risk of bias

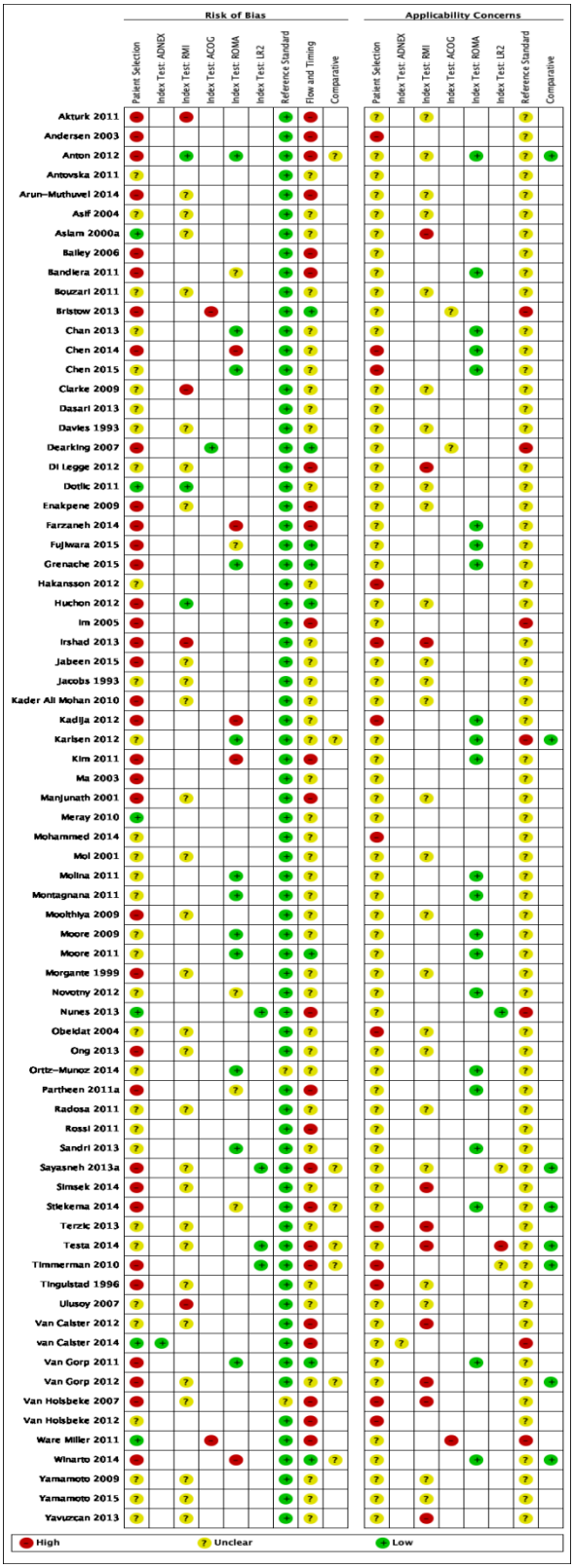
Patient recruitment was prospective in 18 studies,[150, 153, 155, 158, 160, 161, 171, 173, 138, 142, 151, 163, 166, 177, 140, 147, 168, 182] retrospective in 19 studies[144, 152, 157, 159, 175, 184, 159, 174, 178, 180, 146, 179, 181, 137, 167, 141, 143, 148, 183, 149] and was unclear in 11 studies.[156, 172, 176, 213, 162, 164, 165, 169, 170, 185]

For the participant selection domain 22/48 studies were judged at high risk of bias and 23 at unclear risk of bias. Only 3 studies were judged at low risk of bias[155, 158, 163] on the basis that authors explicitly reported consecutive sampling in a representative population (representative on the basis of reporting of patient characteristics (age range, menopausal status) and comprehensive listing of tumour types / pathology identified at histology. For studies rated as at unclear risk of bias this was most often because of a lack of an explicit mention of consecutive sampling in combination with a limited range of tumour types and benign pathology presented. For the index test and flow and timing domains the majority of studies were judged as at unclear risk of bias (40/48 unclear). The most common reason for an unclear risk of bias judgement in the index test domain was lack of clarity about blinding of the reference standard result to the index result. This is important because ultrasound forms a component of RMI, thus there is the potential for knowledge about true disease status to affect index test interpretation. Four of 48 studies were judged at high risk of bias either because of a lack of blinding of the index test to the reference standard or because of lack of prespecification of the index test positivity threshold. For the reference standard domain, the majority of studies

(47/48) were judged as low risk of bias. One study was judged at unclear risk of bias[154] because the length of follow up for 10% of participants was not reported. For the flow and timing domains the majority of studies were judged as at unclear risk of bias (31/48). Fifteen of 48 studies were judged at high risk of bias. The most common reason for an unclear risk of bias judgement in the flow and timing domain was lack of clarity of the interval between the conduct of the index test and the reference standard whereas those studies judged at high risk of bias in this domain did not include all patients in their analysis, for example excluding borderline tumours.

Risk of applicability

No studies were judged as having a low applicability concern for any of the participant selection, index test and reference standard domains. For participant selection this was due to the fact that study participants did not obviously represent symptomatic women presenting in primary care. For the index test domain this was due to fact that ultrasound was conducted by specialist sonographers or their level of specialism was unclear. The accuracy of US estimated from RMI studies could therefore not be assumed to be achievable in non-specialist, primary care settings. For the reference standard domain 47 of 48 RMI studies were judged as having an unclear applicability concern as it was unclear how borderline tumours had been handled for the paper's analysis. One study excluded borderline tumours from analysis and was judged to be of high applicability concern.[138]



Empty cells indicate that an index test was not evaluated by a study.

Figure 4. 2: Risk of bias and applicability concerns for 72 individual included studies for index tests: ADNEX: Assessment of Different NEoplasias in the adneXa model; RMI 1: Risk of Malignancy Index 1; ACOG: American College of Obstetrics and Gynaecology

Guidelines ; ROMA: The Risk of Ovarian Malignancy Algorithm; LR2: Logistic Regression 2 model.

Methodological quality of studies including ROMA

Twenty two studies included an evaluation of the accuracy of ROMA. Fourteen of 22 ROMA studies did not stratified women according to menopausal status. Appendix 8 summarise quality assessment for the 14 studies stratifying by menopausal status.

Risk of bias

Patient recruitment was prospective in 10 studies,[138, 186, 187, 197, 200, 139, 189-192] retrospective in 6 studies[188, 193, 194, 199, 137, 141] and unclear in 6 studies.[136, 195, 196, 198, 201, 202]

For the participant selection domain 12/22 were judged at high risk of bias and 10 studies at unclear risk of bias. The reason for unclear or high risk of bias in the patient selection domain was non-consecutive sampling and evidence of inappropriate exclusions or lack of clarity about these aspects of study conduct. For the index test domain 5 studies were judged as at high risk of bias because of lack of prespecification of the index test positivity threshold and three studies were judged at unclear risk because this was unclear. The majority of studies (21/22) were judged as low risk of bias for the reference standard domain and one study at unclear risk[194] because the duration of follow up was not reported for 15% of participants. For the flow and timing domains the majority of studies were judged as at high (6/22) or unclear (11/22) risk of bias because of exclusion of participants from analysis and a lack of clarity of the interval between the conduct of the index test and the reference standard respectively.

Risk of applicability

All studies were judged as being of unclear applicability concern for the patient selection domain due to the fact that study participants did not obviously represent symptomatic women presenting in primary care. For the index test domain all studies were judged as being of low applicability concern because all components of ROMA are biomarker tests and not likely to be subject to the influence of operator experience and skill. All studies were judged as being of unclear applicability in the reference standard domain because it was how borderline tumours had been handled for the paper's analysis.

Methodological quality of studies including LR2

Four studies included an evaluation of the accuracy of LR2. Only 1 of the 4 LR2 studies[203] did not stratify women according to menopausal status. Appendix 9 summarises quality assessment for all 4 studies.

Risk of bias

Patient recruitment was prospective in 3 studies[203, 142, 204] and retrospective in 1 study.[143]

For the participant selection domain 2 studies were judged at high risk of bias[142, 204] and 1 study was judged as at unclear risk of bias[143] as a result of non consecutive sampling and evidence of inappropriate exclusions or lack of clarity with respect to this aspect of study conduct. One study was judged to be at low risk of bias in the participant selection domain.[203] All studies were judged as low risk of bias for the index test and reference standard domains. All 4 studies were judged at high risk of bias for the flow and timing domain on the basis that not all study participants were included in the analysis and the interval between conduct of the index test and reference standard was not within 3 months.

Risk of applicability

For the patient selection domain 1 study was judged as being of high concer[204] and the remaining studies of unclear concern[142, 143, 203] due to the fact that study participants did not obviously represent symptomatic women presenting in primary care. For the index test domain 1 study was judged as being of high concern because US was performed by specialists,[143] 2 of unclear concern because it was unclear whether ultrasonographers were specialists[142, 204] and therefore the performance of US could not be assumed to be applicable to that achievable in primary care. One study was of low concern because ultrasonographers were not specialists.[203] For the reference standard domain 1 study was judged as being of high concern because borderline tumours were excluded from the study[203] and in the remaining 3 studies it was unclear whether borderline tumours had been excluded and if not excluded how they had been classified in the paper's analysis.[142, 143, 204]

Methodological quality of studies including ACOG and variations of ACOG

Four studies included an evaluation of the accuracy of ACOG. All 4 of the ACOG studies stratified women according to menopausal status. Appendix 10 summarises quality assessment for all 4 studies.

Risk of bias

Patient recruitment was prospective in 1 study,[208] retrospective in 2 studies[207, 209] and unclear in 1 study.[206]

Three of 4 studies were judged at high risk of bias for the participant selection domain[206, 207, 209] with the remaining study judged to be of unclear risk[208] as a result of non-consecutive sampling and evidence of inappropriate exclusions. For the index test domain 2 studies were judged at high risk of bias[208, 209] because individual components of the multivariable model were not prespecified. One study was judged at unclear risk of bias[207] because it was not clear if index test results were interpreted without knowledge of disease status. One study was judged at low risk of bias in the index test domain.[206] All studies were considered at low risk of bias for the reference standard domain. For the flow and timing domain 2 studies were judged at high risk of bias[207, 208] because not all participants were included in the analysis and 2 studies were judged to be at low risk of bias.[206, 209]

Risk of applicability

For the patient selection domain all studies were judged as being of unclear concern due to the fact that study participants did not obviously represent symptomatic women presenting in primary care. For the index test domain 1 study was judged as being of high concern[208] and the remaining 3 studies of unclear concern[206, 207, 209] because US was not performed by non specialised sonographers or this was not clear and therefore the performance of US could not be assumed to be applicable to that achievable in primary care. For the reference standard domain all studies were judged as being of high concern because borderline tumours were excluded from analysis.

Methodological quality of the single ADNEX study

Only one study was identified which used the ADNEX model.[210]

Risk of bias

This prospective study did not stratify women according to menopausal status. Figure 4.2 includes quality assessment for this single study.

The study was judged at unclear risk of bias for patient selection because the method of patient recruitment was not clearly reported. For the index test, reference standard and index test domains the study was judged at low risk of bias. For the index test domain this judgement was importantly based on the fact that all components and thresholds of the nine individual test components of ADNEX were prespecified and assessed in a similar way (for example similar healthcare setting) for all participants.

Risk of applicability

The study was of unclear concern in the patient domain on the basis that study participants did not obviously represent symptomatic women presenting in primary care. The index test domain was judged as of unclear concern on the basis that the skill of the sonographer undertaking US examinations was not reported and therefore the performance of US could not be assumed to be applicable to that achievable in primary care. The study was judged as being at low concern in the reference standard domain because borderline tumours could be grouped with malignant tumours for the purpose of analysis.

4.3 Results of meta-analyses

4.3.1 Test positivity thresholds used in included studies

For RMI I accuracy was estimated at 2 different thresholds (200 and 250), for RMI II at two thresholds (200 and 250), for RMI III at two thresholds (200 and 250) and for RMI IV at two thresholds (400 and 450). For ROMA accuracy was estimated at three different test positivity pairs (7.4 +/- 2 for premenopausal women and 25.3 +/- 2 for postmenopausal women; 12.5 for premenopausal women and 14.4 for postmenopausal women; 13.1 +/- 2 for premenopausal

women and 27.7 +/- 2 for postmenopausal women). For LR2 accuracy was estimated at a threshold to achieve a post test probability of malignancy of 10%. For ACOG, referral of women with an adnexal mass to specialist care is recommended if either CA125 is raised (threshold menopausal status dependent) or any one of a list of US features suggestive of malignancy are present. For ADNEX estimation of accuracy was reported at 4 different thresholds to achieve post test probabilities of malignancy of 3%, 5%, 10% and 15%.

4.3.2 Accuracy in premenopausal versus postmenopausal women

Where data allowed, for individual index test versions at defined test positivity thresholds, accuracy was compared between premenopausal women and postmenopausal women. This comparison was possible for RMI I (thresholds of 200 and 250), RMI III (threshold of 200), LR2, ROMA (all 3 threshold pairs), ACOG version 1, ACOG version 3 (incorporating CA125) and ACOG version 3 (incorporating OVA1) (Appendix 11).

In the populations of women included in this review a consistent difference was observed in sensitivity (higher in postmenopausal women) and specificity (lower in postmenopausal women) across all versions of all index tests at all thresholds analysed. For RMI I at a threshold of 200 (5 studies) and 250 (3 studies) sensitivity was significantly higher and specificity significantly lower for postmenopausal women compared to premenopausal women. RMI I at a threshold of 200: sensitivity post compared to premenopausal ROR 2.71 (95% CI 1.92 to 3.83) p =0.0001 and specificity post compared to premenopausal ROR 0.42 (95% CI 0.19 to 0.94) p=0.03; RMI I at a threshold of 250: sensitivity post compared to premenopausal [ROR 3.88 (95% CI 1.61 to 9.34)] p=0.003 and specificity post compared to premenopausal [ROR 0.52 (95% CI 0.30 to 0.90)] p= 0.02. For LR2 (3 studies) sensitivity was significantly higher and specificity significantly lower for postmenopausal women compared to premenopausal women; sensitivity post compared to premenopausal [ROR 3.02(95% CI 2.04 to 4.49) p=0.0001] and specificity post compared to premenopausal [ROR 0.21(95% CI 0.11 to 0.43) p=0.0001].

Subsequently, where study reporting allowed, sensitivity and specificity for all index test versions and all test positivity thresholds were estimated separately for premenopausal women only and postmenopausal women only. Estimates of test accuracy for pre and postmenopausal

women combined for the 41/ 72 studies where 2x2 tables could not be extracted separately according to menopausal status are also presented in Appendix 12.

It is of note that this pattern of test performance suggests a population selected on the basis of prior testing (i.e. representative of specialist settings). At earlier points in the testing pathway for ovarian cancer, it would be reasonable to expect tests to perform with lower specificity in premenopausal women compared to postmenopausal women, reflecting the greater possibility of false test positives from physiological alterations in premenopausal women that cause raised CA125 and ovarian cysts (e.g. the menstrual cycle) and conditions such as endometriosis that are commoner in premenopausal women.

Test accuracy is described based on estimates for premenopausal separate to postmenopausal women for those index test versions considered relevant to clinical practice and at test positivity thresholds relevant to clinical practice, or, in the absence of clinical consensus those thresholds most commonly reported in included studies are presented in Table 4.6 and Figure 4.6, Figure 4.7, Figure 4.8, Figure 4.9, Figure 4.10. Test versions and thresholds described include RMI I at thresholds of 200 and 250; ROMA at each of 3 thresholds pairs reported in included studies: 7.4 +/- 2 for premenopausal women and 25.3 +/- 2 for postmenopausal women; 12.5 for premenopausal women and 14.4 for postmenopausal women; 13.1 +/- 2 for premenopausal women and 27.7 +/- 2 for postmenopausal women); LR2 at a threshold to achieve a post test probability of malignancy of 10%, ACOG v3 (incorporating CA125), ACOG v3 (incorporating OVA1) and ADNEX at thresholds to achieve a post test probability of malignancy of 5% and 10%.

Table 4. 6: Sensitivity, False Negatives, Specificity and False positives of RMI I, ROMA, LR2, ACOGv3 (CA125), ACOGv3 (OVA1) and ADNEX.

Test	Threshold / Version	Women, Cases (%) (Studies)		Sensitivity (95% CI)		False Negative rate N (%) of cancers missed for every 100 women with ovarian cancer		Specificity (95% CI)		False Positives rate N (%) of women tested/treated unnecessarily for every 100 women without ovarian cancer	
		Premenopause	Postmenopaue	Premenopause	Postmenopause	Premenopause	Post menopause	Pre menopause	Post menopause	Pre menopause	Postmenopause
RMI 1	**200**	2634, 502 (19%) (5)	1879, 862 (46%) (5)	52.2 (45.9, 58.5)	75.0 (69.5, 79.8)	48 (41 to 54)	25 (20 to 30)	95.4 (92.5, 97.3)	90.1 (83.1, 94.4)	5 (3 to 7)	10 (5 to 17)
	250	356, 31 (9%) (1)	220, 97 (44%) (2)	54.8 (36.0, 72.7)	82.5 (73.6, 88.8)	45 (27 to 64)	17 (11 to 26)	88.3 (84.3, 91.6)	79.7 (71.6, 85.9)	12 (8 to 16)	20 (14 to 28)
ROMA	**7.4, 25.3**	1269, 173 (14%) (6)	1396, 544 (40%) (7)	82.6 (71.6, 89.9)	92.2 (87.4, 95.2)	17 (10 to 18)	8 (5 to 13)	80.0 (68.4, 88.1)	81.1 (71.8, 88.8)	20 (12 to 32)	19 (11 to 28)
	12.5, 14.4	302, 68 (23%) (3)	299, 177 (59%) (3)	63.5 (51.0, 74.4)	88.0 (80.6, 92.8)	36 (26 to 49)	12 (7 to 19)	89.3 (80.8, 94.3)	68.3 (57.4, 77.5)	11 (6 to 11)	32 (22 to 43)

Table 4.6 Continued...

	13.1, 27.7	1487, 307 (21%) (13)	1497, 610 (41%) (9)	78.3 (71.2, 84.0)	90.7 (86.4, 93.7)	22 (16 to 29)	9 (6 to 14)	83.3 (78.2, 87.5)	79.1 (74.6, 83.1)	17 (12 to 22)	21 (17 to 25)
LR2	**PTProb OC 10%**	2715, 588 (22%) (3)	1861, 1008 (54%) (3)	83.6 (78.6, 87.6)	93.9 (92.3, 95.3)	16 (12 to 21)	6 (5 to 8)	90.4 (81.3, 95.3)	63.9 (60.6, 67.0)	10 (5 to 19)	36 (33 to 39)
ACOG	**V3 CA125**	899, 150 (17%) (3)	1224, 486 (40%) (3)	78.0 (70.4, 84.1)	84.6 (76.0, 90.5)	22 (16 to 30)	15 (9 to 24)	83.9 (65.1, 92.6)	78.1 (71.0, 83.9)	16 (7 to 35)	22 (16 to 29)
	V3 OVA1	235, 45 (19%) (1)	281, 116 (41%) (1)	91.1 (78.8, 97.5)	94.8 (89.1, 98.1)	9 (2 to 21)	5 (2 to 11)	43.2 (36.0, 50.5)	25.5 (19.0, 32.8)	57 (49 to 64)	74 (67 to 81)
ADNEX	**PTProb OC 5%**	1354, 378 (28%) (1)	1049, 602 (57%) (1)	97.6 (95.5-98.7)	98.8 (97.6-99.4)	2 (1 to 4)	1 (1 to 2)	69.5 (66.5-72.3)	37.4 (33.0-41.9)	30 (28 to 33)	63 (48 to 67)
	PTProb OC 10%	1354, 378 (28%) (1)	1049, 602 (57%) (1)	94.7 (92.0-96.6)	97.7 (96.1-98.6)	5 (3 to 8)	2 (1 to 4)	78.6 (75.9-81.1)	55.5 (50.9-60.0)	21 (19 to 24)	44 (40 to 49)
	PTProb OC 15%	1354, 378 (28%) (1)	1049, 602 (57%) (1)	90.5 (87.1-93.0)	96.5 (94.7-97.7)	11 (7 to 13)	3 (2 to 5)	83.4 (80.9-85.6)	63.5 (59.0-67.9)	16 (14 to 18)	36 (32 to 41)

Threshold – ROMA was reported at threshold pairs of 7.4 (+/-2) pre and 25.3 (+/-2) postmenopausal; 12.5 pre and 14.4 postmenopausal; 13.1 (+/-2) pre and 27.7 (+/-2) postmenopausal. PTProb OC: Test threshold used in studies included in the review to achieve the described post test probability of ovarian cancer.

Accuracy of RMI I

RMI I premenopausal women (threshold 200 and 250). (See Figure 4.3)

RMI1 threshold 200 in premenopausal women (5 studies, 2634 women of whom 502 had a diagnosis of ovarian cancer).

The sensitivity of RMI1 in premenopausal women at a threshold of 200 was 52.2% (95%CI 45.9 to 58.5). For every 100 women with an actual diagnosis of ovarian cancer an estimated 52 (46 to 59) would have their cancer identified whilst an estimated 48 (41 to 54) would have their cancer missed (test negative in error).

The specificity of RMI1 in premenopausal women at a threshold of 200 was 95.4% (95% CI 92.5 to 97.3). For every 100 women without an actual diagnosis of ovarian cancer, 95 (93 to 98) would correctly be identified as negative by the test whilst 5 (2 to 7) would receive a positive diagnosis of ovarian cancer in error (a false positive result).

RMI1 threshold 250 premenopausal women (1 study, 356 women of whom 31 had a diagnosis of ovarian cancer):

Based on a single study the sensitivity of RMI1 in premenopausal women at a threshold of 250 was 54.8% (95% CI 36.0 to 72.7). For every 100 women with an actual diagnosis of ovarian cancer an estimated 55 (36 to 73) would have their cancer identified whilst an estimated 45 (27 to 64) would have their cancer missed (test negative in error).

The specificity of RMI I in premenopausal women at a threshold of 250 was 88.3% (95% CI 84.3 to 91.6). For every 100 women without an actual diagnosis of ovarian cancer 88 (84 to 92) would correctly be identified as negative by the test whilst 12 (8 to 16) would receive a positive diagnosis of ovarian cancer in error (a false positive result).

RMI I postmenopausal women (threshold 200 and 250)

RMI1 threshold 200 in postmenopausal women (5 studies, 1879 women of whom 862 had a diagnosis of ovarian cancer).

The sensitivity of RMI1 in postmenopausal women at a threshold of 200 was 75.0% (95% CI 69.5 to 79.8). For every 100 women with an actual diagnosis of ovarian cancer an estimated 75 (70 to 80) would have their cancer identified whilst an estimated 25 (20 to 30) would have their cancer missed (test negative in error).

The specificity of RMI1 in postmenopausal women at a threshold of 200 was 90.1% (95% CI 83.1 to 94.4). For every 100 women without an actual diagnosis of ovarian cancer, 90 (83 to 95) would correctly be identified as negative by the test whilst 10 (52 to 17) would receive a positive diagnosis of ovarian cancer in error (a false positive result).

RMI1 threshold 250 in postmenopausal women (2 studies, 220 women of whom 97 had a diagnosis of ovarian cancer).

The sensitivity of RMI1 in postmenopausal women at a threshold of 250 was 82.5% (95% CI 73.6 to 88.8). For every 100 women with an actual diagnosis of ovarian cancer an estimated 83 (74 to 89) would have their cancer identified whilst an estimated 17 (11 to 26) would have their cancer missed (test negative in error).

The specificity of RMI1 in postmenopausal women at a threshold of 250 was 79.7% (95% CI 71.6 to 85.9). For every 100 women without an actual diagnosis of ovarian cancer 80 (72 to 86) would correctly be identified as negative by the test whilst 20 (14 to 28) would receive a positive diagnosis of ovarian cancer in error (a false positive result).

The expected trade off between sensitivity and specificity with changes in threshold was observed; with an increase in test positivity threshold, sensitivity increased and specificity decreased. There was no evidence of a significant difference in sensitivity between the 2 test positivity thresholds reported in included RMI studies. Estimated specificity was lower (88.3% (95% CI 84.3 to 91.6) at a threshold of 250 compared to 95.4% (95% CI 92.5 to 97.3) at a threshold of 200 in premenopausal women which appeared to be of borderline significance. However, this finding should be interpreted with caution as it is based on a small number of studies.

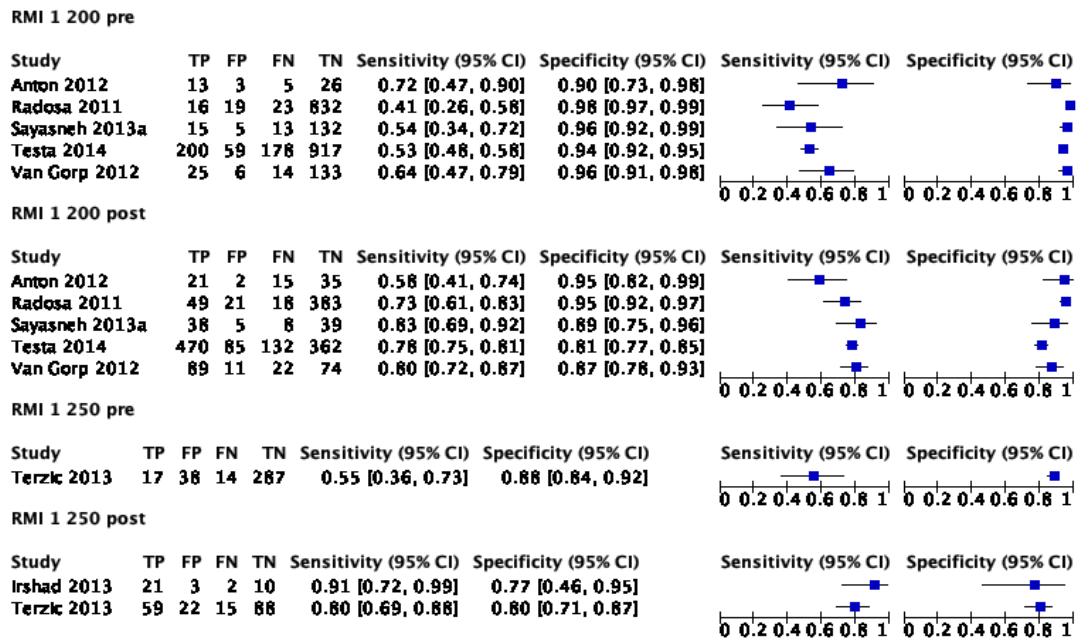


Figure 4. 3: Forest plot of tests: RMI1 at thresholds of 200 and 250, separately for premenopausal and postmenopausal women.

Accuracy of ROMA (See Figure 4.4)

The test accuracy of ROMA was investigated at three test positivity threshold pairs: 12.5 for premenopausal women and 14.4 for postmenopausal women; 13.1 +/- 2 for premenopausal women and 27.7 +/- 2 for postmenopausal women; 7.4 +/- 2 for premenopausal women and 25.3 +/- 2 for postmenopausal women.

ROMA premenopausal women (thresholds 7.4 and 13.1)

Sensitivity in premenopausal women varied from 82.6% (95 % CI 71.6 to 89.9) at a threshold of 7.4 (+/-2) to 78.3% (95% CI 71.2 to 84.0) at a threshold of 13.1 (+/-2). For every 100 women with an actual diagnosis of ovarian cancer an estimated 78 (71 to 84) to 83 (72 to 90) would have their cancer identified whilst an estimated 17 (10 to 28) to 22 (16 to 29 would have their cancer missed (test negative in error).

Specificity varied from 80.0% (95% CI 68.4 to 88.1) at a threshold of 7.4 (+/-2) to 83.3% (95% CI 78.2 to 87.5) at a threshold of 13.1 (+/-2). For every 100 women without an actual diagnosis of ovarian cancer, 83 (78 to 88) to 80 (68 to 88) would correctly be identified as negative by

the test whilst 17 (22 to 12) to 20 (32 to 12) would receive a positive diagnosis of ovarian cancer in error (a false positive result).

ROMA postmenopausal women (thresholds 14.4 and 27.7)

Sensitivity in postmenopausal women varied from 88.0% (95% CI 80.6 to 92.8) at a threshold of 14.4 to 90.7% (95% CI 86.4 to 93.7) at a threshold of 27.7 (+/-2). For every 100 women with an actual diagnosis of ovarian cancer an estimated 88 (81 to 93) to 91 (86 to 94) would have their cancer identified whilst an estimated 11 (6 to 14) to 12 (7 to 19) would have their cancer missed (test negative in error).

Specificity in postmenopausal women varied from 68.3% (95% CI 57.4 to 77.5) at a threshold of 14.4 to 79.1% (95% CI 74.6 to 83.1) at a threshold of 27.7 (+/-2). For every 100 women without an actual diagnosis of ovarian cancer, 68 (57 to 78) to 79 (75 to 83) would correctly be identified as negative by the test whilst 21 (17 to 25) to 32 (28 to 43) would receive a positive diagnosis of ovarian cancer in error (a false positive result).

The expected trade off between sensitivity and specificity with changes in threshold was observed; with an increase in test positivity threshold, sensitivity increased and specificity decreased. There was no evidence of a significant difference in accuracy between thresholds reported in included ROMA studies. It is not possible to recommend a specific ROMA threshold for use in clinical practice from the evidence reviewed.

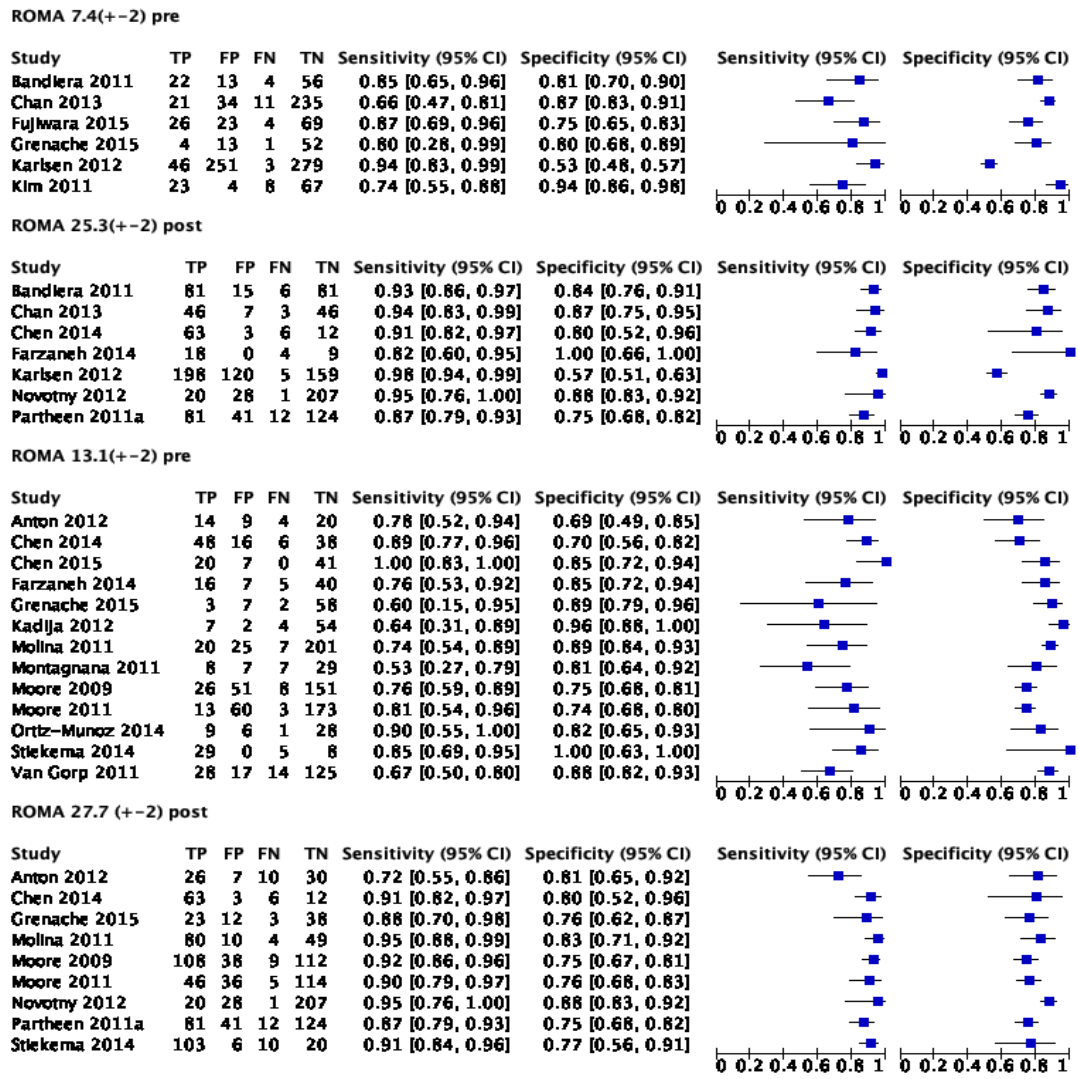


Figure 4. 4: Forest plot of tests: 1 ROMA in at (Thresholds of 7.4(+-2) and 13.1(+-2) in premenopausal women, and at thresholds of 25.3(+-2) and 27.7 (+-2) in postmenopausal women.

Accuracy in LR2 (See Figure 4.5)

All LR2 studies included a threshold to achieve a post test probability of malignancy of 10%. Only one study also included a threshold to achieve a post test probability of malignancy of 6.5% (Nunes 2013).

LR2 post test probability of malignancy 10% premenopausal women

LR2 post test probability of malignancy 10% in premenopausal women (3 studies, 2715 participants of whom 588 had ovarian cancer).

The sensitivity of LR2 in premenopausal women for a post test probability of malignancy of 10% was 83.6% (95% CI 78.6 to 87.6). For every 100 premenopausal women with an actual diagnosis of ovarian cancer an estimated 84 (79 to 88) would have their cancer identified whilst an estimated 16 (12 to 21) would have their cancer missed (test negative in error).

The specificity of LR2 in premenopausal women for a post test probability of malignancy was 90.4% (95% CI 81.3 to 95.3). For every 100 premenopausal women without an actual diagnosis of ovarian cancer, 90 (81 to 95) would correctly be identified as negative by the test whilst 10 (5 to 19) would receive a positive diagnosis of ovarian cancer in error (a false positive result).

LR2 post test probability of malignancy 10% postmenopausal women

LR2 post test probability of malignancy 10% in postmenopausal women (3 studies, 1861 participants of whom 1008 had ovarian cancer).

The sensitivity of LR2 in postmenopausal women for a post test probability of malignancy of 10% was 93.9% (95% CI 92.3 to 95.3). For every 100 postmenopausal women with an actual diagnosis of ovarian cancer an estimated 94 (92 to 95) would have their cancer identified whilst an estimated 6 (5 to 8) would have their cancer missed (test negative in error).

The specificity of LR2 in postmenopausal women for a post test probability of malignancy of 10% was 63.9% (95% CI 60.6 to 67.0). For every 100 postmenopausal women without an actual diagnosis of ovarian cancer, 64 (61 to 67) would correctly be identified as negative by the test whilst 36 (33 to 39) would receive a positive diagnosis of ovarian cancer in error (a false positive result).

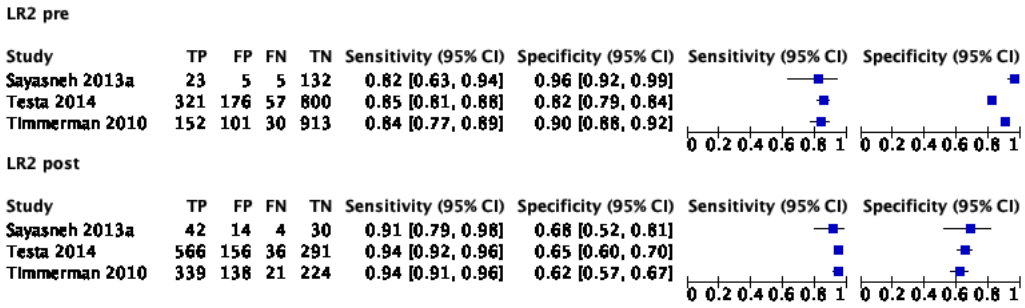


Figure 4. 5: Forest plot of tests: LR2 separately for premenopausal and postmenopausal women.

Accuracy in ACOG v3 (incorporating CA125). See Figure 4.6

ACOG vs (CA125) premenopausal women

ACOG vs (CA125) in premenopausal women (3 studies, 899 participants of whom 150 had ovarian cancer).

The sensitivity of ACOG v3 (CA125) in premenopausal women was 78.0% (95 CI 70.4 to 84.1). For every 100 premenopausal women with an actual diagnosis of ovarian cancer an estimated 78 (70 to 84) would have their cancer identified whilst an estimated 22 (16 to 30) would have their cancer missed (test negative in error).

The specificity of ACOG v3 (CA125) in premenopausal women was 83.9% (95% CI 65.1 to 92.6). For every 100 premenopausal women without an actual diagnosis of ovarian cancer, 84 (65 to 93) would correctly be identified as negative by the test whilst 16 (7 to 35) would receive a positive diagnosis of ovarian cancer in error (a false positive result).

ACOG vs 3 (CA125) in postmenopausal women

ACOG vs 3 (CA125) in postmenopausal women (3 studies, 1224 participants of whom 486 had ovarian cancer).

The sensitivity of ACOG v3 (CA125) in postmenopausal women was 84.6% (95% CI 76.0 to 90.5). For every 100 postmenopausal women with an actual diagnosis of ovarian cancer an

estimated 85 (76 to 91) would have their cancer identified whilst an estimated 15 (9 to 24) would have their cancer missed (test negative in error).

The specificity of ACOG v3 (CA125) in postmenopausal women was 78.1% (95% CI 71.0 to 83.9). For every 100 postmenopausal women without an actual diagnosis of ovarian cancer, 78 (71 to 84) would correctly be identified as negative by the test whilst 22 (16 to 29) would receive a positive diagnosis of ovarian cancer in error (a false positive result).

ACOG v3 (incorporating OVA1). See Figure 4.6

ACOG v3 (incorporating OVA1) premenopausal women

ACOG v3 (incorporating OVA1) in premenopausal women (one study, 235 participants of whom 45 had ovarian cancer).

The sensitivity of ACOG v3 (OVA1) in premenopausal women was 91.1% (95% CI 78.8 to 97.5). For every 100 premenopausal women with an actual diagnosis of ovarian cancer an estimated 91 (79 to 98) would have their cancer identified whilst an estimated 9 (2 to 21) would have their cancer missed (test negative in error).

The specificity of ACOG vs (OVA1) in premenopausal women was 43.2% (95% CI 36.0 to 50.5). For every 100 premenopausal women without an actual diagnosis of ovarian cancer, 43 (36 to 51) would correctly be identified as negative by the test whilst 57(49 to 64) would receive a positive diagnosis of ovarian cancer in error (a false positive result).

ACOG v3 (incorporating OVA1) postmenopausal women

ACOG v3 (incorporating OVA1) in postmenopausal women (one study, 281 participants of whom 116 had ovarian cancer.

The sensitivity of ACOG v3 (OVA1) in postmenopausal women was 94.8% (95% CI 89.1 to 98.1). For every 100 postmenopausal women with an actual diagnosis of ovarian cancer an estimated 95 (89 to 98) would have their cancer identified whilst an estimated 5 (2 to 11) would have their cancer missed (test negative in error).

The specificity of ACOG v3 (OVA1) in postmenopausal women was 25.5% (95 CI 19.0 to 32.8). For every 100 postmenopausal women without an actual diagnosis of ovarian cancer, 25 (19 to 33) would correctly be identified as negative by the test whilst 75 (67 to 81) would receive a positive diagnosis of ovarian cancer in error s(a false positive result).

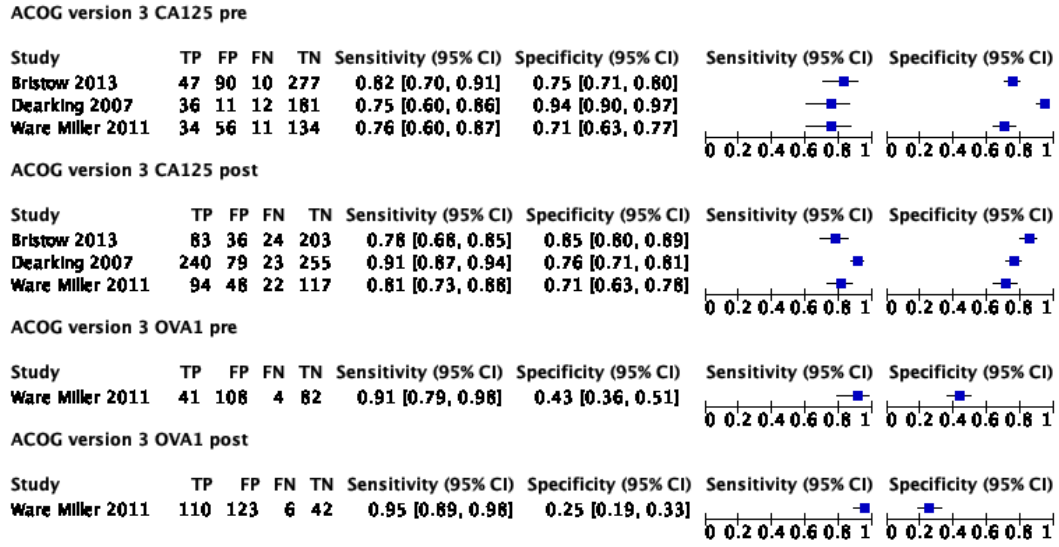


Figure 4. 6 Forest plot of tests: ACOG version 3 CA125 and ACOG version 3 OVA1 separately for premenopausal and postmenopausal women.

Accuracy in ADNEX See Figure 4.7

The test accuracy of ADNEX was investigated at four thresholds to achieve post test probabilities of malignancy of 3%, 5%, 10%, 15% (1 study). Results for post test probabilities of 5% and 10% are presented below.

ADNEX post test probability of malignancy 5% premenopausal women

ADNEX post test probability of malignancy 5% in premenopausal women (one study,1354 participants of whom 378 had ovarian cancer).

The sensitivity of ADNEX in premenopausal women at a threshold to achieve a post test probability of malignancy of 5% was 97.6% (95% CI 95.5 to 98.7). For every 100

premenopausal women with an actual diagnosis of ovarian cancer an estimated 98 (96 to 99) would have their cancer identified whilst an estimated 2 (1 to 4) would have their cancer missed (test negative in error).

The specificity of ADNEX in premenopausal women at a threshold to achieve a post test probability of malignancy of 5% was 69.5% (95% CI 66.5 to 72.3). For every 100 premenopausal women without an actual diagnosis of ovarian cancer, 70 (67 to 72) would correctly be identified as negative by the test whilst 30 (28 to 33) would receive a positive diagnosis of ovarian cancer in error (a false positive result).

ADNEX post test probability of malignancy 5% postmenopausal women

ADNEX post test probability of malignancy 5% in postmenopausal women (one study, 1049 participants of whom 602 had ovarian cancer).

The sensitivity of ADNEX in postmenopausal women at a threshold to achieve a post test probability of malignancy of 5% was 98.8% (95 CI 97.6 to 99.4). For every 100 postmenopausal women with an actual diagnosis of ovarian cancer an estimated 99 (98 to 99) would have their cancer identified whilst an estimated 1 (1 to 2) would have their cancer missed (test negative in error)

The specificity of ADNEX in postmenopausal women at a threshold to achieve a post test probability of malignancy of 5% was of 37.4% (95% CI 33.0 to 41.9). For every 100 postmenopausal women without an actual diagnosis of ovarian cancer, an estimated 38 (33 to 42) would correctly be identified as negative by the test whilst 62 (48 to 67) would receive a positive diagnosis of ovarian cancer in error (a false positive result).

ADNEX post test probability of malignancy 10% premenopausal women

ADNEX post test probability of malignancy 10% in premenopausal women (one study, 1354 participants of whom 378 had ovarian cancer).

The sensitivity of ADNEX in premenopausal women at a threshold to achieve a post test probability of malignancy of 10% was 94.7% (95% CI 92.0 to 96.6). For every 100 premenopausal women with an actual diagnosis of ovarian cancer an estimated 95 (92 to 97)

would have their cancer identified whilst an estimated 5 (3 to 8) would have their cancer missed (test negative in error).

The specificity of ADNEX in premenopausal women at a threshold to achieve a post test probability of malignancy of 10% was 78.6% (95% CI 75.9 to 81.1). For every 100 premenopausal women without an actual diagnosis of ovarian cancer, an estimated 79 (76 to 81) would correctly be identified as negative by the test whilst 21 (19 to 24) would receive a positive diagnosis of ovarian cancer in error (a false positive result).

ADNEX post test probability of malignancy 10% postmenopausal women

ADNEX post test probability of malignancy 10% in postmenopausal women (one study,1049 participants of whom 602 had ovarian cancer).

The sensitivity of ADNEX in postmenopausal women to achieve a post test probability of malignancy of 10% was 97.7% (95% CI 96.1 to 98.6). For every 100 postmenopausal women with an actual diagnosis of ovarian cancer an estimated 98 (96 to 99) would have their cancer identified whilst 2 (1 to 4) would have their cancer missed (test negative in error).

The specificity of ADNEX in postmenopausal women to achieve a post test probability of malignancy of 10% was 55.5% (95% CI 50.9 to 60.0). For every 100 postmenopausal women without an actual diagnosis of ovarian cancer, an estimated 56 (51 to 60) would correctly be identified as negative by the test whilst 46 (40 to 49) would receive a positive diagnosis of ovarian cancer in error (a false positive result).

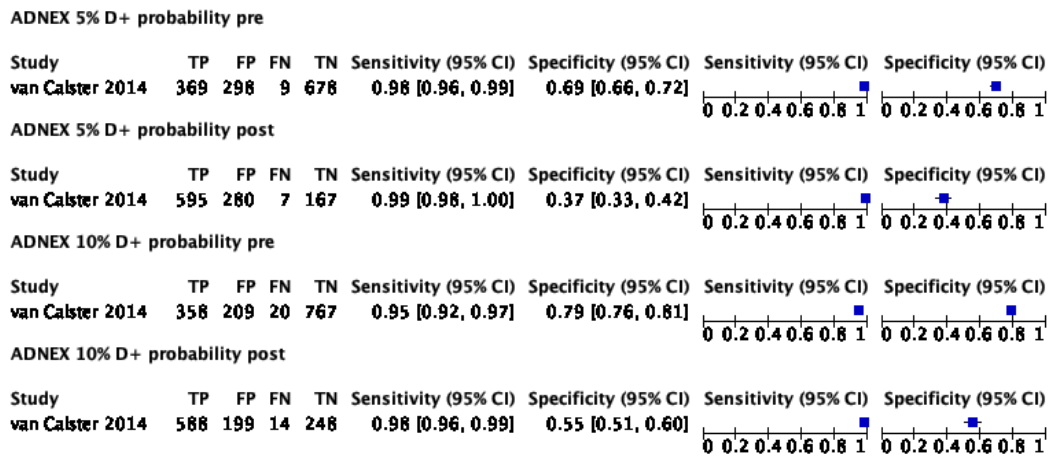


Figure 4. 7: Forest plot of tests: ADNEX at thresholds of 5% and 10% disease probability separately for premenopausal and postmenopausal women.

Comparison of index test accuracy: LR2, ROMA, ACOG v3 CA125 and ACOG v3 OVA1 compared to RMI1 at a fixed specificity of 90%

To maximise data for comparison, studies were included regardless of the test positivity threshold used and an indirect comparison of index tests was undertaken by fitting HSROC curves for premenopausal women and postmenopausal women separately. RMI1 was chosen as the baseline comparator as this is the test combination currently in routine clinical use in the UK. Differences in sensitivity between tests was estimated at fixed specificities of 80% and 90%. Table 4.7 and Figure 4.8 and Figure 4.9 illustrates the results of these comparisons for each version of each index test in premenopausal women and separately in postmenopausal women. These specificity thresholds were chosen in keeping with clinical consensus about an acceptable false positive rate, which is reflected in previous research and RCOG Guidelines. Here results at a fixed specificity of 90% for test versions considered most relevant to current practice are presented (ROMA, LR2, ACOG version 3 (CA125) and ACOG version 3 OVA1). Statistical comparison of the accuracy of ADNEX with RMI1 was not possible as only one ADNEX study was included in this review.

At a fixed specificity of 90%, for every 100 women without an actual diagnosis of ovarian cancer, 90 would correctly be identified as negative by the test whilst 10 would receive a

positive diagnosis of ovarian cancer in error (a false positive result). At this specificity threshold 90 of 100 women would be correctly reassured that they do not have ovarian cancer. A minority, 10 of 100 women, would be referred to secondary care unnecessarily, causing anxiety for the woman concerned as well as further inappropriate testing and risking unnecessary surgery with consequent risk to health and to preservation of fertility.

Comparison of sensitivity at a fixed specificity of 90%: LR2, ROMA, ACOG v3 Ca125 and ACOG v3 OVA1 compared to RMI1 in premenopausal women

At a fixed specificity of 90% RMI1 had a sensitivity of 0.59% (95% CI 0.54 to 0.65) in premenopausal women.

LR2 [0.82% (95% CI 0.79 to 0.86)] and ROMA [0.75% (95% CI 0.69 to 0.80)] were found to have a statistically significantly higher sensitivity compared to RMI1 in premenopausal women (relative DOR 7.33% [95% CI 2.34 to 22.94] p=0.0001 and 3.37% [9% CI 1.22 to 9.28] p=0.02 respectively). Although estimates of sensitivity appeared higher for both ACOG v3 (CA125) [0.73% (95% CI 0.64 to 0.81)] and ACOG v3 (OVA1) [0.82% (95% CI 0.57 to 0.94)] compared to RMI1 this was not statistically significant (relative DOR 2.95% (95% CI 0.91 to 9.61) p=0.07 and 6.73% (95% CI 0.49 to 91.75) p=0.15 respectively).

Based on this analysis, for every 100 premenopausal women with an actual diagnosis of ovarian cancer, 82 (79 to 86) women would test positive and be managed appropriately with LR2, 75 (69 to 80) with ROMA and 59 (54 to 65) with RMI1 whereas 18 (14 to 31) would test negative in error (false negative test result) and their cancer would be missed by LR2, 25 (20 to 31) with ROMA and 41 (35 to 46) with RMI.

Comparison of sensitivity at a fixed specificity of 90%: LR2, ROMA, ACOG v3 Ca125 and ACOG v3 OVA1 compared to RMI1 in postmenopausal women

There was no evidence for statistically significant differences in sensitivity for ROMA, LR2, ACOG v3 (CA125) or ACOG v3 (OVA1) at a fixed specificity of 90%.

Both pre and postmenopausal comparisons of accuracy should be interpreted with some caution as the analysis is limited by the volume of data available and the possible effects of confounding.

Table 4. 7: Comparison of the sensitivity of ROMA, LR2, ACOG V3 (CA125) and ACOG V3 (OVA1) to RMI I at fixed specificities of 80% and 90%.

PREMENOPAUSAL WOMEN								
TEST	**Studies** **N** **D+**	**Diagnostic Odds Ratio (95%CI)**	**ROR (95%CI)**	**P-value**	**Sensitivity at fixed specificity of 80% (95%CI)**	**Difference sensitivity at fixed specificity of 80% compared to RMI I (95% CI)**	**Sensitivity at fixed specificity of 90% (95% CI)**	**Difference sensitivity at fixed specificity of 90% compared to RMI I (95% CI)**
RMI1	6 2990 533	6.9 (4.0, 11.9)	-	-	0.66 (0.57, 0.74)	-	0.59 (0.54, 0.65)	-
ROMA	18 2564 445	23.3 (12.7, 42.5)	3.37 (1.22, 9.28)	0.02	0.80 (0.75, 0.84)	0.14 (0.05, 0.23)	0.75 (0.69, 0.80)	0.16 (0.07, 0.24)
LR2	3 2715 588	50.7 (25.0, 102.8)	7.33 (2.34, 22.94)	0.001	0.86 (0.83, 0.89)	0.20 (0.13, 0.28)	0.82 (0.79, 0.86)	0.23 (0.16, 0.30)
ACOG V3 CA125	3 899 150	20.4 (8.8, 47.2)	2.95 (0.91, 9.61)	0.07	0.78 (0.71, 0.85)	0.13 (0.02, 0.23)	0.73 (0.64, 0.81)	0.14 (0.03, 0.25)
ACOG V3 OVA1	1 235 45	46.5 (4.6, 469)	6.73 (0.49, 91.75)	0.15	0.86 (0.65, 0.95)	0.20 (0.01, 0.38)	0.82 (0.57, 0.94)	0.22 (0.02, 0.43)

Table 4.7 Continued...

TEST	Studies N D+	Diagnostic Odds Ratio (95%CI)	ROR (95% CI)	P-value	Sensitivity at fixed specificity of 80% (95%CI)	Difference sensitivity at fixed specificity of 80% compared to RMI I (95% CI)	Sensitivity at fixed specificity of 90% (95% CI)	Difference sensitivity at fixed specificity of 90% compared to RMI I (95% CI)
RMI1	7 2099 959	21.7 (14.7, 32.1)	-	-	0.84 (0.78, 0.89)	-	0.72 (0.64, 0.79)	-
ROMA	16 2594 1148	38.8 (23.7, 36.5)	1.79 (0.90, 3.55)	0.09	0.90 (0.87, 0.93)	0.06 (-0.01, 0.13)	0.82 (0.73, 0.89)	0.09 (0.00, 0.18)
LR2	3 1861 1008	40.4 (19.1, 85.2)	1.86 (0.82, 4.23)	0.13	0.90 (0.85, 0.94)	0.06 (-0.01, 0.13)	0.82 (0.69, 0.91)	0.10 (0.01, 0.19)
ACOG V3 (CA125)	3 1224 486	20.0 (11.8, 33.9)	0.92 (0.46, 1.85)	0.81	0.83 (0.76, 0.89)	-0.01 (-0.10, 0.08	0.71 (0.56, 0.82)	-0.02 (-0.15, 0.12)
ACOG V3 (OVA1)	1 281 116	14.5 (2.6, 58.9)	0.58 (0.11, 3.05)	0.5	0.76 (0.43, 0.93)	-0.08 (-0.37, 0.21)	0.61 (0.22, 0.90)	-0.11 (-0.50, 0.28)

Notes to table: Results are from fitting HSROC models to all available data taking one threshold from each stud. Where one study reported more than one threshold, the most commonly reported threshold across studies was chosen.

OC: Ovarian Cancer cases. ROR: Relative Diagnostic Odds Ratio.

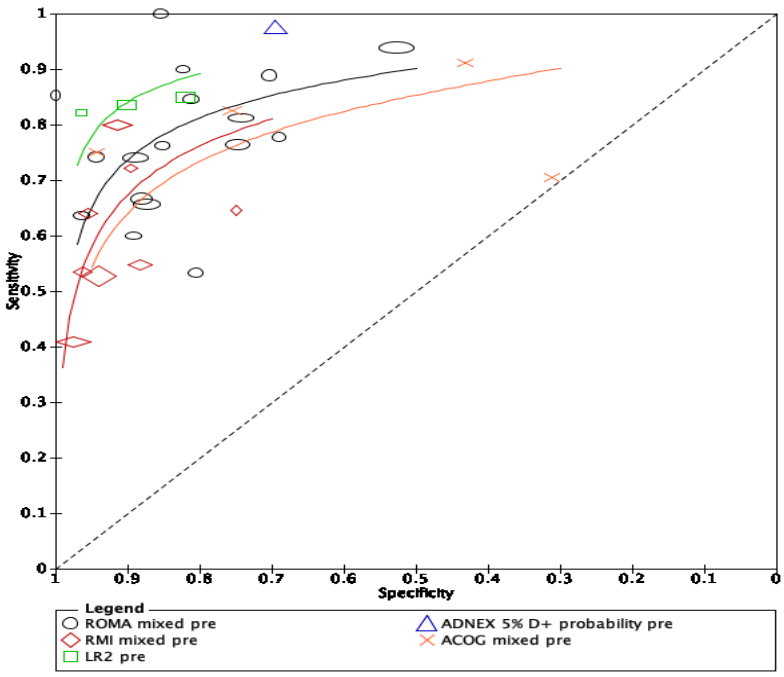


Figure 4. 8:Summary ROC Plot of tests: Premenopausal women: ROMA, RMI1, LR2, ACOG v3CA125 and ACOG v3 OVA1, ADNEX 5% Disease probability.

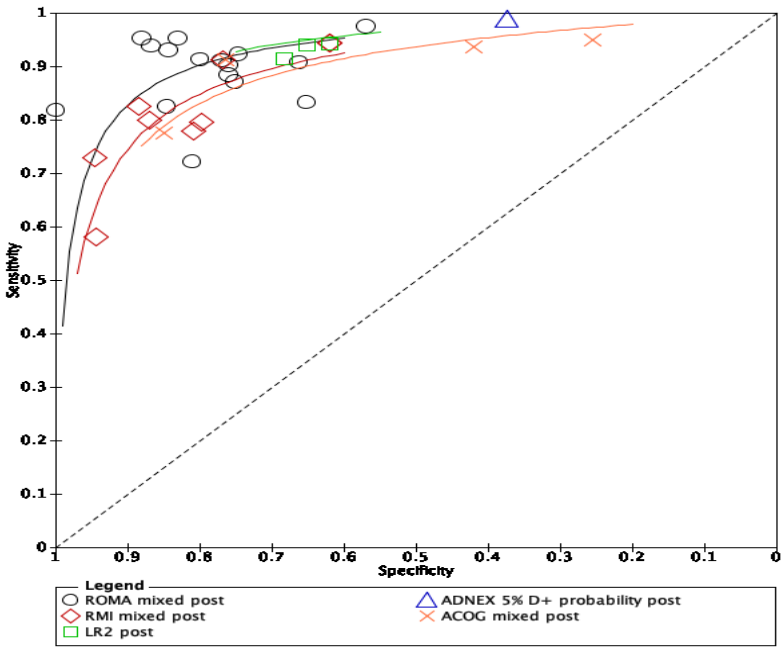


Figure 4. 9: Summary ROC Plot of tests: Postmenopausal women: ROMA, RMI1, LR2, ACOG v3 CA125, ACOG v3 OVA1, ADNEX 5% Disease probability.

4.3.3 Investigation of the effect of classification of borderline tumours on estimates of test accuracy

In current clinical practice, ovarian tumours that are histologically borderline for malignancy are managed as malignant tumours. However, for estimation of test accuracy, disease state needs to be classified as either positive or negative. It was observed that included studies varied in the way borderline tumours were classified: as positive (grouped with malignant tumours) or negative (grouped with benign tumours) for the purposes of accuracy estimation. A comparison of test accuracy estimated by studies excluding borderline tumours from analysis or where the classification of borderline tumours for analysis was unclear, with studies where borderline tumours were classified as positive (grouped with malignant tumours) was performed.

Due to inadequate data this investigation was only possible for ROMA. In premenopausal women 10 ROMA studies either excluded borderline tumours for the purposes of accuracy estimation or the classification of borderline tumours was unclear and in 8 studies borderline tumours were classified as positive (grouped with malignant tumours). In postmenopausal women 9 ROMA studies either excluded borderline tumours for the purposes of accuracy estimation or the classification of borderline tumours was unclear and in 7 studies borderline tumours were classified as positive (grouped with malignant tumours). No evidence was found to suggest that the classification of borderline tumours influenced summary estimates of test accuracy in this review. However, the analysis is limited by the volume of data available and, because this is an indirect comparison, the possible effects of confounding

Table 4. 8: Summary of findings for test combinations in pre and postmenopausal women in secondary care (prevalence ovarian cancer 3% pre and 10% post).

Review question	Symptoms, ultrasound imaging and biochemical markers alone or in combination for the diagnosis of ovarian cancer in women with symptoms suspicious for ovarian cancer					
Setting	Secondary care					
Reference standards	Histology in women who have undergone surgery and clinical follow up (> 6 months) in women with negative index tests results who do not undergo surgery					
Study limitations	Approximately half of included studies were rated as at high risk of bias for the patient selection domain on the basis that sampling was not consecutive and the range of ovarian pathology included was limited. The majority of RMI studies were judged at unclear risk of bias in the index test domain on the basis it was unclear if the index test (including US) was interpreted blind to the reference standard. The majority of studies were judged as unclear applicability concern in the reference standard domain because length of followup for index test -ves (who did not undergo surgery) was not reported. The majority of included studies were judged as at high or unclear applicability in the patient selection domain on the basis that women were either asymptomatic or this was not clear and details of prior tests were not provided.					
Population	Premenopausal women - assume prevalence of ovarian cancer 3%					
Number of studies, premenopausal women, cases of ovarian cancer	A total of 31 studies were included in the comparative analysis. Prevalence in included studies varied between 9% to 28%, (median 19%) in premenopausal women.					
Index Test	Sensitivity (95% CI) at single threshold	Specificity at single threshold (95% CI)	Index test (multiple thresholds)	Sensitivity (95% CI) estimated across multiple thresholds at a fixed specificity of 0.9	Consequences in a hypothetical cohort of 1000 women assuming a specificity of 0.90 and a prevalence of 3%. Number of women who would have their cancer missed (false negatives).	Difference in sensitivity compared to RMI I at a fixed specificity Difference to RMI1 (p value)
RMI1 200	52.2 (45.9, 58.5)	95.4 (92.5, 97.3)	RMI 200 and 250	0.59 (0.54, 0.65)	12 (11 to 14)	----------
LR2	83.6 (78.6, 87.6)	90.4 (81.3, 95.3)	LR2	0.82 (0.79, 0.86)	5 (4 to 6)	p = 0.001
ROMA (13.1(+/-2)	78.3 (71.2, 84.0)	83.3 (78.2, 87.5)	ROMA 7.4 (+/-2);12.5; 13.1 (+/-2)	0.75 (0.69, 0.80)	8 (6 to 9)	p = 0.02
ACOG v3 (CA125)	78.0 (70.4, 84.1)	83.9 (65.1, 92.6)	ACOG v3 (CA125)	0.73 (0.64, 0.81)	8 (6-11)	p = 0.07
ACOG v3 (OVA1)	91.1 (78.8, 97.5)	43.2 (36.0, 50.5)	ACOG v3 (OVA1)	0.82 (0.57, 0.94	5 (2 to 13)	p = 0.15
Population	Postmenopausal women- assume prevalence of ovarian cancer 10%					

Table 4.8 Continued...

Number of studies, postmenopausal women, cases of ovarian cancer	A total of 31 studies were included in the comparative analysis. Prevalence in included studies varied between 40% to 57%, (median 44%) in postmenopausal women.					
Index Test	Sensitivity (95% CI) at single threshold	Specificity (95% CI) at single threshold	Index test	Sensitivity (95% CI) estimated across multiple thresholds at a fixed specificity of 0.9	Consequences in a hypothetical cohort of 1000 women assuming a specificity of 0.90 and a prevalence of 10%. Number of women who would have their cancer missed (false negatives).	Difference in sensitivity compared to RMI1 at a fixed specificity of 0.9 (p value)
LR2	93.9 (92.3, 95.3)	63.9 (60.6, 67.0)	LR2	0.82 (0.69, 0.91)	18 (9 to 31)	p = 0.13
ROMA 27.7 (+/-2)	90.7 (86.4, 93.7)	79.1(74.6, 83.1)	ROMA 25.3 (+/-2); 14.4; 27.7 (+/-2)	0.82 (0.73, 0.89)	18 (11 to 27)	p = 0.09
ACOG v3 (CA125)	84.6 (76.0, 90.5)	78.1 (71.0, 83.9)	ACOG v3 (CA125)	0.71 (0.56, 0.82)	29 (18 to 44)	p = 0.81
ACOG v3 (OVA1)	94.8 (89.1, 98.1)	25.5 (19.0, 32.8)	ACOG v3 (OVA1)	0.61 (0.22, 0.90)	39 (10 to 78	p = 0.76
RMI1 200	75.0 (69.5, 79.8)	90.1(83.1, 94.4)	RMI 200 and 250	0.72 (0.64, 0.79)	28 (21 to 36)	------

Table 4. 9: Summary of findings for test combinations for pre and postmenopausal women in tertiary care (prevalence ovarian cancer 30% pre and postmenopausal).

Review question	Symptoms, ultrasound imaging and biochemical markers alone or in combination for the diagnosis of ovarian cancer in women with symptoms suspicious for ovarian cancer					
Setting	Secondary care					
Reference standards	Histology in women who have undergone surgery and clinical follow up (> 6 months) in women with negative index tests results who do not undergo surgery					
Study limitations	Approximately half of included studies were rated as at high risk of bias for the patient selection domain on the basis that sampling was not consecutive and the range of ovarian pathology included was limited. The majority of RMI studies were judged at unclear risk of bias in the index test domain on the basis it was unclear if the index test (including US) was interpreted blind to the reference standard. The majority of studies were judged as unclear applicability concern in the reference standard domain because length of follow up for index test -ves (who did not undergo surgery) was not reported. The majority of included studies were judged as at high or unclear applicability in the patient selection domain on the basis that women were either asymptomatic or this was not clear and details of prior tests were not provided.					
Population	Premenopausal women - assume prevalence of ovarian cancer 30%					
Number of studies, premenopausal women, cases of ovarian cancer	A total of 31 studies were included in the comparative analysis. Prevalence in included studies varied between 9% to 28%, (median 19%) for premenopausal women.					
Index Test	Sensitivity (95% CI) at single threshold	Specificity (95% CI) at single threshold	Index test	Sensitivity (95% CI) estimated across multiple thresholds at a fixed specificity of 0.9	Consequences in a hypothetical cohort of 1000 women assuming a specificity of 0.90 and a prevalence of 10%	Difference in sensitivity compared to RMI I at a fixed specificity Difference to RMI1 (p value)
LR2	83.6 (78.6, 87.6)	90.4 (81.3, 95.3)	LR2	0.82 (0.79, 0.86)	54 (42 to 63)	p = 0.001
ROMA	78.3 (71.2, 84.0)	83.3 (78.2, 87.5)	ROMA 7.4 (+/-2); 12.5; 13.1 (+/-2)	0.75 (0.69, 0.80)	123 (105 to 138)	p = 0.02
ACOG v3 (CA125)	78.0 (70.4, 84.1)	83.9 (65.1, 92.6)	ACOG v3 (CA125)	0.73 (0.64, 0.81)	81 (57 to 108)	p = 0.07
ACOG v3 (OVA1)	91.1 (78.8, 97.5)	43.2 (36.0, 50.5)	ACOG v3 (OVA1)	0.82 (0.57, 0.94	54 (18, 129)	p = 0.15

Table 4.9 Continued...

RMI1	52.2 (45.9, 58.5)	95.4 (92.5, 97.3)	RMI 200 and 250	0.59 (0.54, 0.65)	123 (105 to 138)	----------
Population	Postmenopausal women - assume prevalence of ovarian cancer 30%					
Number of studies, postmenopausal women, cases of ovarian cancer	A total of 31 studies were included in the comparative analysis. Prevalence in included studies varied between 40% to 57%, (median 44%) in postmenopausal women.					
Index Test	Sensitivity (95% CI) at single threshold	Specificity (95% CI) at single threshold	Index test	Sensitivity (95% CI) estimated across multiple thresholds at a fixed specificity of 0.9	Consequences in a hypothetical cohort of 1000 women assuming a specificity of 0.90 and a prevalence of 10%	Difference in sensitivity compared to RMI I at a fixed specificity Difference to RMI1 (p value)
LR2	93.9 (92.3, 95.3)	63.9 (60.6, 67.0)	LR2	0.82 (0.69, 0.91)	54 (27 to 93)	p = 0.13
ROMA	90.7 (86.4, 93.7)	79.1(74.6, 83.1)	ROMA 25.3 (+/-2); 14.4; 27.7 (+/-2)	0.82 (0.73, 0.89)	54 (33 to 81)	p = 0.09
ACOG v3 (CA125)	84.6 (76.0, 90.5)	78.1 (71.0, 83.9)	ACOG v3 (CA125)	0.71 (0.56, 0.82)	87 (54 to 132)	p = 0.81
ACOG v3 s(OVA1)	94.8 (89.1, 98.1)	25.5 (19.0, 32.8)	ACOG v3 (OVA1)	0.61 (0.22, 0.90)	117 (30 to 234)	p = 0.76
RMI1	75.0 (69.5, 79.8)	90.1(83.1, 94.4)	RMI 200 and 250	0.72 (0.64, 0.79)	84 (63 to 108)	------

CHAPTER 5: RESULTS OF BIOMARKERS REVIEW

5.1 Screening and selection of studies

The original primary searches retrieved a total of 360005 records after de-duplication. After reviewing titles and abstracts, 34968 records were subsequently excluded. Full-text copies of 1037 potentially relevant reports were obtained and screened for inclusion, of which 45 were deemed eligible for inclusion in the biomarker review. Figure 4.1 shows the PRISMA diagram of the study selection process. Studies were excluded if they failed to meet one or more of the specified inclusion criteria with regard to study design, participants, target conditions or ability to derive a 2x2 table. (See Appendix 6 for reasons of exclusion).

The biomarkers included in the review were CA125, HE4 and OVA1. Ninety, nine studies met the original inclusion criteria. The spectrum of ovarian tumour varies with age and it is known that majority of EOC occurs in postmenopausal women. Apart from menopause being a risk factor, this variation in spectrum with different preponderance of histotypes with age affects the estimates of test accuracy due to the potential differential sensitivity of the test to different histotypes. Following our observation of consistent and mostly significant differences in test accuracy, only studies presenting results separately according to menopausal status were included in the synthesis post hoc (n=43). Of the included studies, 30 studies evaluated CA125, 13 studies evaluated HE4 and two studies evaluated OVA1. All the 13 studies estimating the test performance of HE4 also estimated the test performance of CA125 at various thresholds except one study.[138]

5.2 Study characteristics and quality of included studies

5.2.1 Study characteristics of included studies

In terms of pathways, common to all the biomarkers tested: the clinical pathway of patients from presentation to the decision to refer for surgical investigation were not detailed in any included studies. Testing prior to surgical investigation in this patient group will have included clinical history and examination, and may have also included ultrasound imaging or biomarker tests prior to the index test. These tests can be carried out in primary care, by gynaecologists, by gynaecological oncologists or a mixture of both. In clinical practice in the UK, clinical assessment and biomarker testing are most commonly initially performed in primary care. Ultrasound assessment may be initiated by a primary care practitioner but conducted in a secondary care setting. If the primary care practitioner decides to refer a woman for surgical investigation because of a suspicion of ovarian cancer, clinical assessment and ultrasound assessment may be repeated in secondary or tertiary care.

The characteristics of all included studies including the baseline characteristics of participants and the index test are described below under the headings CA125, HE4 and OVA1.

CA125 included studies

The study characteristics for 30 studies reporting the accuracy of CA125 separately in pre and/or postmenopausal women are described in (Table 5.1).

Results for both pre and postmenopausal women were described in 20 studies; only 2/22 studies[214], [137] describing results for premenopausal women did not include results for postmenopausal women. Eight studies included results only for postmenopausal women.[215, 216,217, 218, 219, 196,220,221]

Table 5. 1: Table of study characteristics: CA125 pre and postmenopausal studies.

Author year	Menopausal status	Study criteria and setting	Study design	Participant characteristics	Index test
Bandiera 2011	**pre, post**	**Study criteria:** Not reported **Clinical setting:** Tertiary **Prior test:** Unclear **Exclusions:** BOT excluded. ? Non EOC excluded	**Design:** Unclear **Country:** USA **Centre:** Single centre	**# participants:** 278 **% postmen (n):** 65.8 (183) **# ovarian cancer:** 113 **# benign:** 165 # **borderline:** excluded **Age mean+/-SD:** Pre endo-36.5, benign-41.5, malignant-44.7. post ben- 64, malignant-66.3 **Age median[range]:** Not reported[25-89]	**Prespecified threshold:** Yes-HE4, NO -CA125 **Time interval:** Unclear **Blinded to reference:** Unclear **Blinded to other index:** Not applicable
Benjapipal 2007	**pre, post**	**Study criteria:** Women with ovarian mass scheduled for elective surgery **Clinical setting:** Secondary **Prior test:** Unclear **Exclusions:** Nil	**Design:** Prospective Cross sectional Study **Country:** Thailand Centre: Single centre	# **participants:** 120 % **postmen (n):** 41 (49) # **ovarian cancer:** 59 # **benign:** 61 # **borderline:** Not reported Age mean+/-SD: 46+/-14 **Age median[range]:** Not reported[18-91]	**Prespecified threshold:** Yes **Time interval:** 1 week **Blinded to reference:** Yes **Blinded to other index:** Not applicable
Botsis 1997	**post**	**Study criteria:** Women with suspected pelvic masses **Clinical setting:** Secondary **Prior test:** Unclear Exclusions: Nil	**Design:** Prospective Cross sectional Study **Country:** Greece Centre: Single centre	# **participants in study:** 62 % **postmen (n):** 100 (62) # **ovarian cancer**: 22 # **benign:** 40 # **borderline:** Not reported **Age mean+/-SD:** 54.4 [Not reported] **Age median[range]:** Not reported [47- 78]	**Prespecified threshold:** Yes **Time interval**: median 3 days (range 0-31) **Blinded to reference**: Unclear **Blinded to other index:** Unclear

Table 5.1 Continued...

Chan 2013	**pre, post**	**Study criteria:** Women over 18 years diagnosed with adnexal mass diagnosed by any Imaging method (US, CT or MRI) **Clinical setting:** Unclear **Prior test:** Unclear **Exclusions:** Nil	**Design:** Prospective Cross sectional Study **Country:** Acia- pacific **Centre:** Multicentre	# **participants:** 414 % **postmen (n):** 26%(108 # **ovarian cancer:** 74 # **benign:** 322 # **borderline:** 16 Age mean+/-SD: Not reported **Age median[range]:** Not reported[Not reported]	**Prespecified threshold**: yes **Time interval:** Unclear **Blinded to reference:** Unclear **Blinded to other index:** Unclear
Chen 2015	**pre, post**	**Study criteria:** Women with pelvic masses scheduled for surgery **Clinical setting**: Unclear **Prior test:** Unclear **Exclusions:** Unclear	**Design:** Unclear **Country:** China **Centre:** Single centre	# **participants:** 232 % **postmen (n):** Not reported # **ovarian cancer:** 60 # **benign:** 70 # **borderline:** Not reported **Age mean+/-SD:** benign-33, Malignant-53 **Age median[range]:** Not reported[17-81]	**Prespecified threshold:** Yes **Time interval:** Unclear **Blinded to reference:** Unclear **Blinded to other index:** Unclear
Dowd 1993	**pre, post**	**Study criteria:** Women with CA125 and suspected pelvic mass **Clinical setting:** Secondary **Prior test:** Unclear **Exclusions:** Nil	**Design:** Retrospective Cross sectional Study **Country:** Australia **Centre:** Single centre	# **participants:** 264 % **postmen (n):** 56(143) # **ovarian cancer:** 100 # **benign:** 128 # **borderline:** 36 **Age mean+/-SD**: Benign- 47, Malignant- 54 **Age median[range]:** Not reported[15-89]	**Prespecified threshold:** yes **Time interval:** Unclear **Blinded to reference:** Unclear **Blinded to other index:** Unclear

Table 5.1 Continued...

Franchi 1995	**pre, post**	**Study criteria:** women with suspected pelvic mass on examination/ US **Clinical setting:** Secondary **Prior test:** Unclear **Exclusions:** Nil	**Design:** Unclear **Country:** Italy **Centre:** Single centre	# **participants:** 129 % **postmen (n):** 36 (46) # **ovarian cancer:** 30 # **benign:** 92 # **borderline:** 7 **Age mean+/-SD:** 44 **Age median[range]:** Not reported[12-91]	**Prespecified threshold:** Yes **Time interval:** less than 90 days **Blinded to reference:** Unclear **Blinded to other index:** Not applicable
Fujiwara 2015	**pre, post**	**Study criteria:** Women with adnexal mass diagnosed on Ultrasound/CT/MRI/PET and scheduled for surgery **Clinical setting:** Unclear **Prior test:** Unclear **Exclusions:** Women with previous history of any gynaecological disease and NON EOC excluded	**Design:** Prospective Cross sectional Study **Country:** Japan **Centre:** Multicentre	# **participants:** 221 % **postmen (n):** 40 (90) # **ovarian cancer:** 71 # **benign: 131** # **borderline: 19** Age mean+/-SD: Benign-43, BOT-47, Malignant-54 **Age median[range]:** Not reported[20-79]	**Prespecified threshold:** Yes **Time interval:** less than 42 days **Blinded to reference:** Unclear **Blinded to other index:** Not applicable
Gadducci 1992	**pre, post**	**Study criteria:** Women undergoing laparotomy because of ovarian masses **Clinical setting:** Secondary Prior **test:** Unclear Exclusions: Non EOC excluded	**Design:** Unclear **Country:** Italy **Centre:** Single centre	# **participants in study:** 344 % **postmen (n):** 38 (131) # **ovarian cancer:** 90 # **benign:** 254 # **borderline:** Not reported Age mean+/-SD: Not reported [Not reported] **Age median[range]:** Not reported [Not reported]	**Prespecified threshold:** Yes **Time interval:** Not reported **Blinded to reference:** Unclear **Blinded to other index:** Not applicable

Table 5.1 Continued...

Gadducci 1996	**pre, post**	**Study criteria:** Women with clinical diagnosis of ovarian mass submitted to laparotomy **Clinical setting:** Secondary Prior **test:** Unclear Exclusions: Unclear	**Design:** Unclear **Country:** Italy **Centre:** Single centre	# **participants in study:** 124 % **postmen (n):** 52 (64) # **ovarian cancer:** 56 # **benign:** 65 **# borderline:** Not reported Age mean+/-SD: Not reported **Age median[range]:** malignant: 62 [28-81]; benign: 42 [17-73]	**Prespecified threshold:** Yes **Time interval:** Not reported **Blinded to reference:** Unclear **Blinded to other index:** Unclear
Kuesel 1992	**pre, post**	**Study criteria:** Women with pelvic mass who has undergone laparotomy **Clinical setting:** Secondary Prior **test:** Unclear Exclusions: Unclear	**Design:** Retrospective cross sectional **study Country: Canada Centre:** Single centre	# **participants in study:** 37 % **postmen (n):** 57 (21) # **ovarian cancer:** 13 # **benign:** 24 # **borderline:** Not reported **Age mean+/-SD:** Not reported **Age median[range]:** Not reported [Not reported]	**Prespecified threshold:** No Time **interval**: Not reported **Blinded to reference:** Unclear **Blinded to other index:** Not applicable
Kusnetzoff 1998	**post**	**Study criteria:** patients with clinical or sonographic diagnosis of adnexal masses Clinical setting: Secondary Prior test: Unclear **Exclusions:** Nil	**Design:** Prospective cross sectional study **Country: Argentina Centre:** Single centre	**# participants in study:** 130 % **postmen (n):** 44 (57) # **ovarian cancer:** 39 # **benign:** 91 # **borderline:** 2 (with malignant) **Age mean+/-SD:** All 41.5, pre:34, post:58.3 **Age median[range]:** Not reported [19-82]	**Prespecified threshold:** No **Time interval: 1** week Blinded to **reference:** Unclear Blinded to other index: Unclear

Table 5.1 Continued...

Molina 2011	**pre, post**	**Study criteria:** Not reported **Clinical setting:** Unclear **Prior test:** Unclear **Exclusions:** Unclear	**Design:** Retrospective Cross sectional Study **Country:** Spain **Centre:** Single centre	# **participants:** 422 **% postmen (n):** 34(143) # **ovarian cancer:** 111 **# benign:** 285 *benign gyn disease with 137 ovarian cysts # **borderline:** Not reported **Age mean+/-SD:** Not reported **Age median[range]:** Benign gyn disease-40+/- 0.8, Gyn cancer61+/-1.2[17-90]	**Prespecified threshold:** Yes **Time interval:** Unclear **Blinded to reference:** Unclear **Blinded to other index:** Unclear
Partheen 2011	**pre, post**	**Study criteria:** Women with complex cystic mass and suspicious of malignancy undergoing surgery Clinical setting: Tertiary **Prior test:** Unclear **Exclusions:** Solid anpelvic mass were not eligible	**Design:** Prospective Cross sectional Study **Country:** Sweden **Centre:** Single centre	# **participants:** 374 % **postmen (n):** 73.7 (276) # **ovarian cancer:** 108 **# benign:** 215 # **borderline:** 45 Age mean+/-SD: Not reported Age median[range]: Not reported[Not reported]	**Prespecified threshold:** Yes **Time interval:** 1 day **Blinded to reference:** Yes **Blinded to other index:** Unclear
Radosa 2011	**pre, post**	**Study criteria:** women with adnexal mass who subsequently underwent surgery were selected **Clinical setting:** Tertiary **Prior test:** Unclear **Exclusions:** Nil	**Design:** Unclear **Country:** Germany Centre: Single centre	# **participants:** 1362 % postmen (n): 32(442) # **ovarian cancer:** 79 # **benign:** 1259 **# borderline:** 19 **Age mean+/-SD:** 43.3 **Age median[range]:** Not reported[Not reported]	**Prespecified threshold:** Yes **Time interval:** Unclear **Blinded to reference:** Unclear **Blinded to other index:** Unclear

Table 5.1 Continued...

Stiekema 2014	**pre, post**	**Study criteria:** histologically confirmed EOC or benign ovarian disease referred to the institute **Clinical setting:** Tertiary **Prior test:** Unclear **Exclusions:** BOT excluded	**Design:** Retrospective Cross sectional Study **Country:** Netherlands **Centre:** Single centre	# **participants:** 181 % **postmen (n):** &9 (143) # **ovarian cancer:** 147 # **benign:** 34 # **borderline:** Excluded **Age mean+/-SD:** Benign- 47, malignant- 57 **Age median[range]:** Not reported[Not reported]	**Prespecified threshold:** Yes **Time interval:** Unclear **Blinded to reference:** Unclear **Blinded to other index:** Unclear
Strigini 1995	**pre, post**	**Study criteria:** Women undergoing laparotomy for adnexal mass **Clinical setting:** Secondary **Prior test:** Unclear **Exclusions:** Nil	**Design:** Unclear **Country:** Italy **Centre:** Single centre	# **participants:** 109 % **postmen (n):** 31.2 (34) **# ovarian cancer:** 19 # **benign:** 75 # **borderline:** Not reported **Age mean+/-SD:** Not reported **Age median[range]:** 43[18-80]	**Prespecified threshold:** yes **Time interval:** Unclear **Blinded to reference:** Unclear **Blinded to other index:** Unclear
Van Calster 2007	**pre, post**	**Study criteria:** Women with persistent adnexal mass undergoing surgery within 120 days **Clinical setting:** Mixed **Prior test:** Unclear **Exclusions:** Nil	**Design:** Retrospective Cross sectional Study **Country**: Europe **Centre**: Multicentre	# **participants:** 809 % **postmen (n):** 40.4 (431), # **ovarian cancer**: 152 # **benign**: 567 # **borderline**: 52 **Age mean+/-SD:** Not reported **Age median[range]:** Not reported[Not reported]	**Prespecified threshold:** No **Time interval**: 120 days **Blinded to reference:** Unclear **Blinded to other index:** Unclear

Table 5.1 Continued...

Van Gorp 2011	**pre, post**	**Study criteria**: All patients diagnosed with pelvic mass undergoing surgery **Clinical setting**: Unclear **Prior test**: Unclear **Exclusions**: Nil	**Design**: Prospective Cross sectional Study **Country**: Belgium **Centre**: Single centre	# **participants**: 389 % postmen (n): 41.4%(161) # **ovarian cancer**: 161 # **benign**: 228 # **borderline**: Not reported **Age mean+/-SD: Benign**:46.3+/-16 **Malignant:**57.8+/-12.6 Age median[range]: Not reported[Not reported]	**Prespecified threshold**: Yes **Time interval:** 1 day **Blinded to reference:** Unclear **Blinded to other index:** Yes
Holcomb 2011	**pre**	**Study criteria**: Women with adnexal mass undergoing surgery **Clinical setting**: Unclear **Prior test**: Unclear **Exclusions**: postmenopausal women excluded ?non EOC excluded)	**Design**: Prospective Cross sectional Study **Country**: USA **Centre**: Multicentre	# **participants**: 229 % **postmen (n):** 53.6 (265) **# ovarian cancer:** 18 # **benign:** 195 # **borderline:** 16 **Age mean+/-SD:** Not reported **Age median[range]:** Not reported[Not reported]	**Prespecified threshold:** Yes* (but reported for diff cut off than the prespecified) **Time interval:** Less than 30 days **Blinded to reference:** Yes **Blinded to other index:** Not applicable
Anton 2012	**post**	**Study criteria:** Women referred with pelvic masses diagnosed by US or CT or MRI undergoing surgery or image-guided biopsy when they presented with signs of carcinomatosis **Clinical setting:** Secondary **Prior test:** Unclear **Exclusions:** Nil	**Design:** Prospective Cross sectional Study **Country:** Brazil **Centre:** Single centre	# **participants:** 120 % **postmen (n):** 60.8 (73) **# ovarian cancer:** 30 # **benign:** 66 # **borderline:** 17 **Age mean+/-SD:** Benign- 50.7, BOT-56.4, malignant-54.7 **Age median[range]:** Benign- 51, BOT-58, malignant-54[Not reported]	**Prespecified threshold:** Yes **Time interval:** 1 day **Blinded to reference:** Unclear **Blinded to other index:** Unclear

Table 5.1 Continued...

Antonic and Rakar 1995	**post**	**Study criteria:** patients who underwent Doppler and CA125 evaluation of pelvic masses and undergoing surgery **Clinical setting:** Unclear **Prior test:** Unclear Exclusions: Age <35 years excluded	**Design:** Prospective cross sectional study **Country:** Slovenia **Centre**: Single centre	# **participants in study:** 71 % **postmen (n):** 41 (29) # **ovarian cancer:**18 # **benign:** 53 # **borderline:**1 (with malignant) **Age mean+/-SD:** Not reported **Age median[range]:** Not reported [35-82]	**Pre-specified threshold:** No **Time interval:** 1 **week Blinded to reference:** unclear **Blinded to other index:** Unclear
Avsar 2008	**post**	**Study criteria:** Women presenting with adnexal mass scheduled for surgery **Clinical setting:** Secondary **Prior test:** Unclear **Exclusions:** premenopausal women were excluded	**Design:** Unclear **Country:** Turkey **Centre:** Single centre	**# participants:** 46 % **postmen (n):** 100 (46) # **ovarian cancer:** 33 # **benign:** 13 # **borderline:** Not reported **Age mean+/-SD:** 46±8.4 **Age median[range]:** Not reported[Not reported]	**Prespecified threshold**: No **Time interval**: Unclear **Blinded to reference**: Unclear **Blinded to other index:** Unclear
Engelen 2008	**post**	**Study criteria:** Women with pelvic mass scheduled for surgery **Clinical setting:** Secondary **Prior test:** Unclear **Exclusions:** Nil	**Design:** Prospective Cross sectional Study **Country:** Netherlands Centre: Single centre	**# participants:** 138 % **postmen (n):** 51.4 (71) # ovarian cancer: 30 # benign: 88 # borderline: 14 Age mean+/-SD: 51-all, Benign-47, Malignant-61 Age median[range]: [15-87]	**Prespecified threshold:** Yes **Time interval:** Unclear **Blinded to reference:** Unclear Blinded to other index: Unclear
Maggino 1993	**post**	**Study criteria:** Postmenopausal Women with pelvic mass **Clinical setting:** Secondary **Prior test:** Unclear **Exclusions:** premenopausal women excluded	**Design:** Prospective Cross sectional Study **Country:** Italy **Centre:** Single centre	# **participants:** 335 % **postmen (n):** 100 (335) # **ovarian cancer:** 106 # **benign:** 229 # **borderline:** Not reported **Age mean+/-SD:** benign-62, Malignant-63 **Age median[range]:** Not reported[40-91]	**Prespecified threshold:** Yes **Time interval:** Unclear **Blinded to reference:** Unclear **Blinded to other index:** Unclear

Table 5.1 Continued...

Novotny 2012	**post**	**Study criteria:** Women with pelvic abnormalities **Clinical setting:** Secondary **Prior test:** Unclear **Exclusions:** premenopausal women excluded	**Design:** Unclear **Country:** Czech republic **Centre:** Single centre	# **participants:** 256 % **postmen (n):** 100 (256) # **ovarian cancer:** 21 **# benign:** 256 # **borderline:** Not reported **Age mean+/-SD:** Benign-65.28, Malignant-64.37 **Age median[range]:** Benign-64, malignant-63[47-93]	**Prespecified threshold:** No **Time interval:** Unclear **Blinded to reference:** Unclear **Blinded to other index:** Not applicable
Ortiz-Munoz 2014	**post**	**Study criteria:** Women with gynaecological symptoms, diagnosed with primary ovarian cancer **Clinical setting:** Tertiary **Prior test:** Symptoms **Exclusions:** Nil	**Design:** Retrospective Cross sectional Study **Country:** Spain **Centre:** Single centre	# **participants:** 148 % **postmen (n):** 70% (104) # **ovarian cancer:** 29 # **benign:** 119 # **borderline:** not reported **Age mean+/-SD:** not reported **Age median[range]:** Benign premenopausal 39.5 +/- 8.4, postmenopausal 56 +/-11.5. Malignant premenopausal 40.5 +/- 5.8, postmenopausal 57 +/- 9.4[not reported]	**Prespecified threshold:** Yes **Time interval:** 1 day **Blinded to reference:** Unclear **Blinded to other index:** Unclear
Schutter 1994	**post**	**Study criteria:** Women presenting with adnexal mass and undergoing surgery. 199 women were detected on physical examination and the rest on ultrasound **Clinical setting:** Secondary **Prior test:** Unclear Exclusions: <45 years of age were excluded Women with Borderline tumours were excluded	**Design:** Prospective Cross sectional Study **Country:** Netherlands **Centre:** Multicentre	# **participants:** 228 % **postmen (n):** !00 (228) # **ovarian cancer:** 95 # **benign:** 127 # **borderline:** (6) Excluded **Age mean+/-SD:** 63 **Age median[range]:** Not reported[45-88]	**Prespecified threshold:** Yes **Time interval:** Unclear **Blinded to reference:** Unclear **Blinded to other index:** Unclear

Of the 30 studies, 17 were conducted in Europe[216, 217, 222, 223, 224, 219, 193, 196, 194, 192, 165, 221, 137, 225, 226, 139,] 3 in USA,[201, 214, 227, 220] one in China,[202] one in Australia,[228] one in Canada,[229] one in Argentina,[218] one in Brazil,[136] one in Japan,[187] one in Iran,[200] one in Turkey,[215] one in Thailand,[230] and one in Asia-Pacific.[186] Five studies were multicentre studies.[186,187,214, 221, 226] Women were recruited prospectively in less than 50% of the studies (13/29), the rest being retrospective (7) and unclear (9).

CA125 35U/ml (+/-10) was the most commonly used threshold reported in 26 of the included studies. Three of these studies also reported using CA125 at thresholds of 65 (+/-10).[228, 226, 219] Other three studies used CA125 at threshold of 65U/ml (+/-10).[225, 223, 224] One study used CA125 at threshold of 65U/ml (+/-10) for premenopausal women and at threshold of 35U/ml (+/-10) for postmenopausal women.[187]

Spectrum: presentation

All included studies recruited patients undergoing surgical investigation of an adnexal mass. Participants were mostly recruited from secondary care settings (gynaecology units or non-gynaecology departments in the hospital) (17 studies) or a tertiary care setting (specialised gynaecology cancer units) (5 studies). One study recruited its participants from a mixed setting that included both general gynaecology and specialist gynaecology oncology units. None of the included studies recruited participants from primary care setting. These highly selected populations are not representative of unselected, symptomatic women presenting in primary care where testing is initiated because of a suspicion of ovarian cancer.

Spectrum: target condition

Tests such as CA125 and HE4 are less sensitive in non EOC and 25% of the tumours are non EOC in women under 40 years;[4] therefore excluding non EOC tumours can potentially affect test performance and accuracy measures due to the altered disease spectrum. Five studies included only epithelial ovarian cancer (EOC).[201, 200, 187, 214,223] One study excluded solid pelvic masses.[192] Fifteen of the included studies did not report borderline tumours[230, 202, 200, 193, 227, 225, 139, 215, 219, 196, 194, 216, 223, 224, 229] and three excluded borderline tumours.[201, 137, 221]

Spectrum: age and menopausal status

The age of included participants ranged from 12[222] to 93 years old.[196] Information on participant's age by benign and malignant group was reported in 10/30 studies. The mean age for the benign group ranged from 33[202] to 65.3 years[196] and for the malignant group it varied from 51[200] to 64.4 years.[196] Four studies excluded premenopausal women from their study population[215, 219, 196, 221] and one study restricted inclusion to women over 35 years.[220] Postmenopausal women comprised 4100/7771 (52.8%) of the included review study population and across individual studies the proportion ranged from 108/414 (26%)[186] to 143/181 (79%).[137]

HE4 included studies

The study characteristics for 13/22 using HE4 in pre and or postmenopausal women are described in (Table 5.2).

Results for both pre and postmenopausal women were described in 13 studies. Of 12 studies in premenopausal women, all except one study[187] is also included in the 10 studies reporting results for postmenopausal women. Three studies include results from premenopausal women but not from postmenopausal women.[200, 214, 192] One study included results from postmenopausal women only.[187]

Twelve of the included studies used HE4 at threshold of 70pMol/L (+-10). Of the 12 studies, six of them also included HE4 140pMol/L (+-10). The one remaining study used only HE4 140pMol/L (+-10).[193]

Of the 13 studies reporting either pre or postmenopausal results for HE4, six were conducted in Europe,[138,193, 194, 192,137, 139] two in USA,[201, 214] one in China,[202] one in Brazil,[136] one in Iran,[200] one in Japan[187] and one in Asia-Pacific[186]. Three studies were multicentre studies.[214, 186, 187]

Table 5. 2: Table of study characteristics: HE4 pre and postmenopausal studies.

Author year	Menopausal status	Study criteria and setting	Study design	Participant characteristics	Index test
Anton 2012	**pre, post, all**	**Study criteria:** Women referred with pelvic masses diagnosed by US or CT or MRI undergoing surgery or image-guided biopsy when they presented with signs of carcinomatosis **Clinical setting:** Secondary **Prior test:** Unclear **Exclusions:** Nil	**Design:** Prospective Cross sectional Study **Country:** Brazil **Centre:** Single centre	# **participants in study:** 120 % **postmen (n):** 60.8 (73) # **ovarian cancer:** 30 # **benign:** 66 # **borderline:** 17 **Age mean+/-SD:** Benign- 50.7, BOT-56.4, malignant-54.7 **Age median[range]:** Benign- 51, BOT-58, malignant-54[Not reported]	**Prespecified threshold:** Yes **Time interval:** 1 day **Blinded to reference:** Unclear **Blinded to other index:** Unclear
Bandiera 2011	**pre, post, all**	**Study criteria:** Not reported **Clinical setting:** Tertiary **Prior test:** Unclear **Exclusions:** BOT excluded. ? Non EOC excluded	**Design:** Unclear **Country:** USA **Centre:** Single centre	# **participants in study:** 278 % **postmen (n):** 65.8 (183) # **ovarian cancer:** 113 # **benign:** 165 # **borderline:** excluded **Age mean+/-SD:** Pre endo-36.5, benign-41.5, malignant-44.7. post ben- 64, malignant-66.3 **Age median[range]:** Not reported[25-89]	**Prespecified threshold:** Yes - HE4, No - CA125 **Time interval:** Unclear **Blinded to reference:** Unclear **Blinded to other index:** Not applicable
Chen 2015	**pre, post, all**	**Study criteria:** Women with pelvic masses scheduled for surgery **Clinical setting:** Unclear **Prior test:** Unclear **Exclusions:** Unclear	**Design:** Unclear **Country:** China **Centre:** Single centre	# **participants in study:** 232 % **postmen (n):** Not reported # **ovarian cancer:** 60 # **benign:** 70 # **borderline:** Not reported Age mean+/-SD: benign-33, Malignant-53 **Age median[range]:** Not reported[17-81]	**Prespecified threshold:** Yes **Time interval:** Unclear **Blinded to reference:** Unclear **Blinded to other index:** Unclear

Table 5.2 Continued...

Karlsen 2012	**pre, post, all**	**Study criteria:** Women admitted to surgery for pelvic mass or pelvic pain potentially caused by malignant disease or endometriosis **Clinical setting:** Secondary **Prior test:** Unclear **Exclusions:** Nil	**Design:** Prospective Cross sectional Study **Country:** Denmark **Centre:** Single centre	# **participants in study:** 1218 % **postmen (n):** 51(621) # **ovarian cancer:** 261 # **benign:** 809 # **borderline:** 79 **Age mean+/-SD:** Not reported **Age median[range]:** 51[16-90]	**Prespecified threshold:** Yes **Time interval:** 2 weeks **Blinded to reference:** Unclear **Blinded to other index:** Unclear
Ortiz-Munoz 2014	**pre, post, all**	**Study criteria:** Women with gynaecological symptoms, diagnosed with primary ovarian cancer **Clinical setting:** Tertiary **Prior test:** Symptoms **Exclusions**: Nil	**Design:** Retrospective Cross sectional Study **Country:** Spain **Centre:** Single centre	# **participants in study:** 148 % **postmen (n):** 70% (104) # **ovarian cancer:** 29 # **benign:** 119 # **borderline:** not reported **Age mean+/-SD:** not reported **Age median[range]:** Benign premenopausal 39.5 +/- 8.4, postmenopausal 56 +/-11.5. Malignant premenopausal 40.5 +/- 5.8, postmenopausal 57 +/- 9.4[not reported]	**Prespecified threshold:** Yes **Time interval:** 1 day **Blinded to reference:** Unclear **Blinded to other index:** Unclear
Stiekema 2014	**pre, post, all**	**Study criteria**: histologically confirmed EOC or benign ovarian disease referred to the institute **Clinical setting:** Tertiary **Prior test:** Unclear **Exclusions:** BOT excluded	**Design:** Retrospective Cross sectional Study **Country:** Netherlands **Centre:** Single centre	# **participants in study:** 181 % **postmen (n):** &9 (143) # **ovarian cancer:** 147 # **benign:** 34 # **borderline:** Excluded Age mean+/-SD: Benign- 47, malignant- 57 **Age median[range]:** Not reported[Not reported]	**Prespecified threshold:** Yes **Time interval:** Unclear **Blinded to reference:** Unclear **Blinded to other index:** Unclear

Table 5.2 Continued...

Van Gorp 2011	**pre, post, all**	**Study criteria:** All patients diagnosed with pelvic mass undergoing surgery **Clinical setting:** Unclear **Prior test:** Unclear **Exclusions**: Nil	**Design:** Prospective Cross sectional Study **Country:** Belgium **Centre:** Single centre	# **participants in study:** 389 % **postmen (n):** 41.4%(161) # **ovarian cancer:** 161 # **benign:** 228 # **borderline:** Not reported Age mean+/-SD: Benign:46.3+/-16 Malignant:57.8+/-12.6 Age median[range]: Not reported[Not reported]	**Prespecified threshold:** Yes **Time interval: 1** day **Blinded to reference:** Unclear **Blinded to other index:** Yes
Molina 2011	**pre, post**	**Study criteria:** Not reported **Clinical setting:** Unclear **Prior test:** Unclear **Exclusions**: Unclear	**Design:** Retrospective Cross sectional Study **Country:** Spain **Centre:** Single centre	# **participants in study:** 422 % **postmen (n):** 34(143) # **ovarian cancer:** 111 # **benign**: 285 *benign gyn disease with 137 ovarian cysts # **borderline:** Not reported Age mean+/-SD: Not reported **Age median[range]:** Benign gyn disease-40+/- 0.8, Gyn cancer61+/-1.2[17-90]	**Prespecified threshold:** Yes **Time interval:** Unclear **Blinded to reference:** Unclear **Blinded to other index:** Unclear
Chan 2013	**pre, all**	**Study criteria:** Women over 18 years diagnosed with adnexal mass diagnosed by any Imaging method (US, CT or MRI) **Clinical setting:** Unclear **Prior test:** Unclear **Exclusions:** Nil	**Design:** Prospective Cross sectional Study **Country:** Asia- pacific **Centre:** Multicentre	# **participants in study:** 414 % **postmen (n):** 26%(108 # **ovarian cancer:** 74 # **benign:** 322 # **borderline:** 16 **Age mean+/-SD:** Not reported **Age median[range]:** Not reported[Not reported]	**Prespecified threshold:** yes **Time interval:** Unclear **Blinded to reference:** Unclear **Blinded to other index:** Unclear

Table 5.2 Continued...

Farzaneh 2014	**pre, all**	**Study criteria:** Women with adnexal mass undergoing surgery **Clinical setting:** Secondary **Prior test:** Unclear **Exclusions:** Non epithelial Ovarian cancer was excluded	**Design:** Prospective Cross sectional Study **Country:** Iran **Centre:** Single centre	# **participants in study:** 100 % **postmen (n):** 31(31) # **ovarian cancer:** 43 # **benign:** 56 # **borderline:** Not reported **Age mean+/-SD:** 44+/-16 **Age median[range]:** Not reported[Not reported]	**Prespecified threshold:** No **Time interval:** Unclear **Blinded to reference:** Unclear **Blinded to other index:** Unclear
Holcomb 2011	**pre**	**Study criteria:** Women with adnexal mass undergoing surgery **Clinical setting:** Unclear **Prior test:** Unclear **Exclusions:** postmenopausal women excluded ?non EOC excluded)	**Design:** Prospective Cross sectional Study **Country:** USA **Centre:** Multicentre	# **participants in study:** 229 % **postmen (n):** 53.6 (265) # **ovarian cancer:** 18 # **benign:** 195 # **borderline:** 16 **Age mean+/-SD:** Not reported **Age median[range]:** Not reported[Not reported]	**Prespecified threshold:** Yes* (but reported for diff cut off than the prespecified) **Time interval:** Less than 30 days **Blinded to reference:** Yes **Blinded to other index:** Not applicable
Partheen 2011	**Pre**	**Study criteria:** Women with complex cystic mass and suspicious of malignancy undergoing surgery Clinical setting: Tertiary Prior test: Unclear **Exclusions**: Solid anpelvic mass were not eligible	**Design:** Prospective Cross sectional Study Country: Sweden Centre: Single centre	**# participants in study:** 374 % **postmen (n):** 73.7 (276) # **ovarian cancer:** 108 # benign: 215 # borderline: 45 Age mean+/-SD: Not reported **Age median[range]:** Not reported[Not reported]	**Prespecified threshold:** Yes **Time interval:** 1 day **Blinded to reference:** Yes **Blinded to other index:** Unclear
Fujiwara 2015	**post**	**Study criteria:** Women with adnexal mass diagnosed on Ultrasond/CT/MRI/PET and scheduled for surgery **Clinical setting:** Unclear **Prior test:** Unclear **Exclusions:** Women with previous history of any gynaecological disease and NON EOC excluded	**Design:** Prospective Cross sectional Study **Country:** Japan **Centre:** Multicentre	# **participants:** 221 % **postmen (n):** 40 (90) # **ovarian cancer:** 71 # **benign:** 131 # **borderline:** 19 **Age mean+/-SD:** Benign-43, BOT-47, Malignant-54 **Age median[range]:** Not reported[20-79]	**Prespecified threshold:** Yes **Time interval:** less than 42 days **Blinded to reference:** Unclear **Blinded to other index:** Not applicable

Spectrum: presentation

All included studies recruited patients undergoing surgical investigation of an adnexal mass. Participants were recruited from either secondary care setting – 3 studies (gynaecology units or non-gynaecology departments in the hospital) or tertiary care setting – 4 studies (specialised gynaecology cancer units) and 6 unclear if it was secondary or tertiary care setting. None of the included studies recruited participants from primary care setting.

Spectrum: target condition

Four studies included only epithelial ovarian cancer (EOC).[201, 200, 214, 187] One study excluded solid pelvic masses.[192] Five of the included studies did not report borderline tumours[202, 194, 139, 193, 200] and two excluded borderline tumours.[201, 137]

Spectrum: age and menopausal status

The age of included participants ranged from 16[138] to 90 years old.[138, 193] The mean age for the benign group ranged from 33[202] to 52.8 years[201] and for the malignant group it varied from 53[202] (Chen 2015) to 57.8 years[139] as reported in six studies. One study excluded postmenopausal women from the study population[214]. The proportion of postmenopausal women in the included studies that report both pre and postmenopausal women varied from 108/414 (26%)[186] to 143/181 (79%)[137]. Overall the postmenopausal women were 2189/4326 and represented 50.6% of the included study population.

OVA1 included studies

The study characteristics of two included studies using OVA1 in pre and postmenopausal women separately[188, 205] are described in Table 5.3

Table 5. 3: Table of study characteristics: OVA1.

Marker	Author year	Menopausal status	Study criteria and setting	Study design	Participant characteristics	Index test
OVA1	**Grenache 2015**	Pre, post	**Study criteria:** Women with abnormal adnexal mass detected on physical examination and Imaging Included Ultrasound, CT or MRI) followed by surgery **Clinical setting:** Unclear **Prior test:** Unclear **Exclusions:** Unclear	**Design:** Cross sectional study based on two previous clinical trials **Country:** USA **Centre:** Multicentre	# **participants:** 146 % **postmen (n):** 52 (76) # **ovarian cancer:** 19 # **benign:** 115 # **borderline:** 7 **Age mean+/-SD:** 52 **Age median[range]:** Not reported[18-89]	**Prespecified threshold:** Yes **Time interval:** Unclear **Blinded to reference:** Unclear **Blinded to other index:** Unclear
OVA1	**Bristow 2013**	Pre, post	**Study criteria:** Women undergoing surgery within 3 months of a documented pelvic mass on imaging (CT, US or MRI) **Clinical setting:** Mixed **Prior test:** Unclear **Exclusions:** Nil	**Design:** Prospective cross sectional study **Country:** USA **Centre:** Multicentre	# **participants:** 490 % **postmen (n):** 43.9 (217) # **ovarian cancer:** 65 # **benign:** 402 # **borderline:** 17 **Age mean+/-SD:** 48.6 (SD 14) **Age median[range]:** 48[18-87]	**Prespecified threshold:** Yes Time interval: less than or equal to 3 months Blinded to reference: Unclear Blinded to other index: Unclear

Both studies were conducted in USA at multiple centres.

Spectrum: presentation

Both OVA1 studies recruited patients undergoing surgical investigation of adnexal masses and recruited participants from centres that included both general gynaecology and specialist gynaecology oncology units.[205]

Spectrum: target conditions

Both studies reported a range of ovarian pathology including non EOC and borderline tumours.

Spectrum: age and menopausal status

The age of the participants in these studies ranged from 18-89 years old. Both studies presented results for pre and postmenopausal women separately. The proportion of postmenopausal women was 76/146 (52%)[188] and 505/1024 (44%).[205]

Only one study was identified that presented results for the biomarkers HCG, LDH and AFP. However, this study only reported results using a combination of all these biomarkers with CA125, so these results were not eligible to be included in this review, but were considered for the review of combination tests.

5.2.2 Methodological quality of included studies

The methodological quality for CA125, HE4 and OVA1 for studies reporting separately results for pre and postmenopausal women are summarised in (Figure 5.1, Figure 5.2, Figure 5.3) and graphs (Appendix 13 and 14). The data was extracted using a QUADAS-2 criteria proforma tailored to the review topic (Appendix 3) focusing on four domains of methodological quality: patient selection; index test; reference standard; and flow and timing. Tailoring of QUADAS-2 to the clinical topic is already explained in detail in the test combination review chapter.

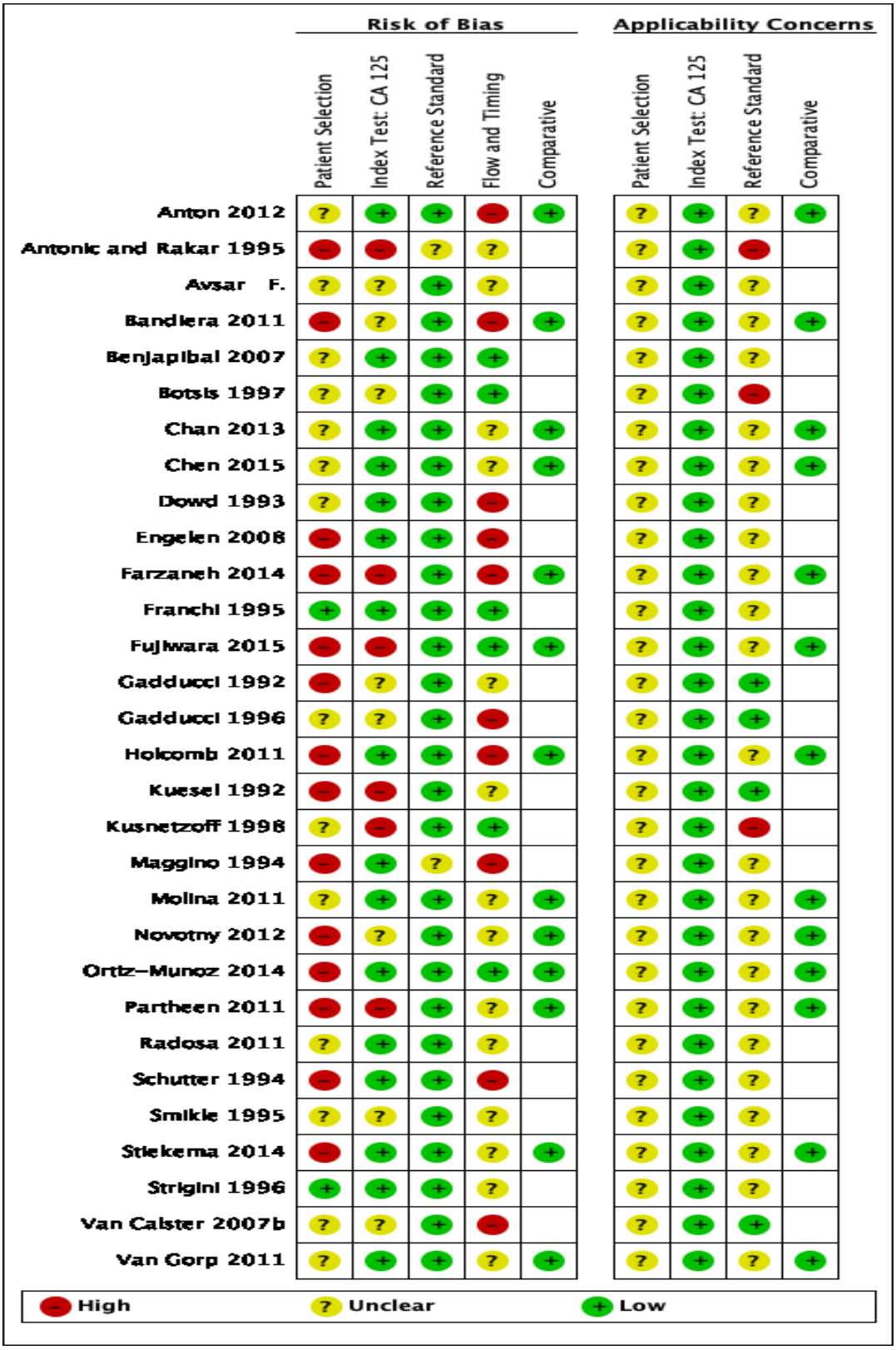


Figure 5. 1: CA125 QUADAS-2: Pre and postmenopausal studies bias applicability concerns summary.

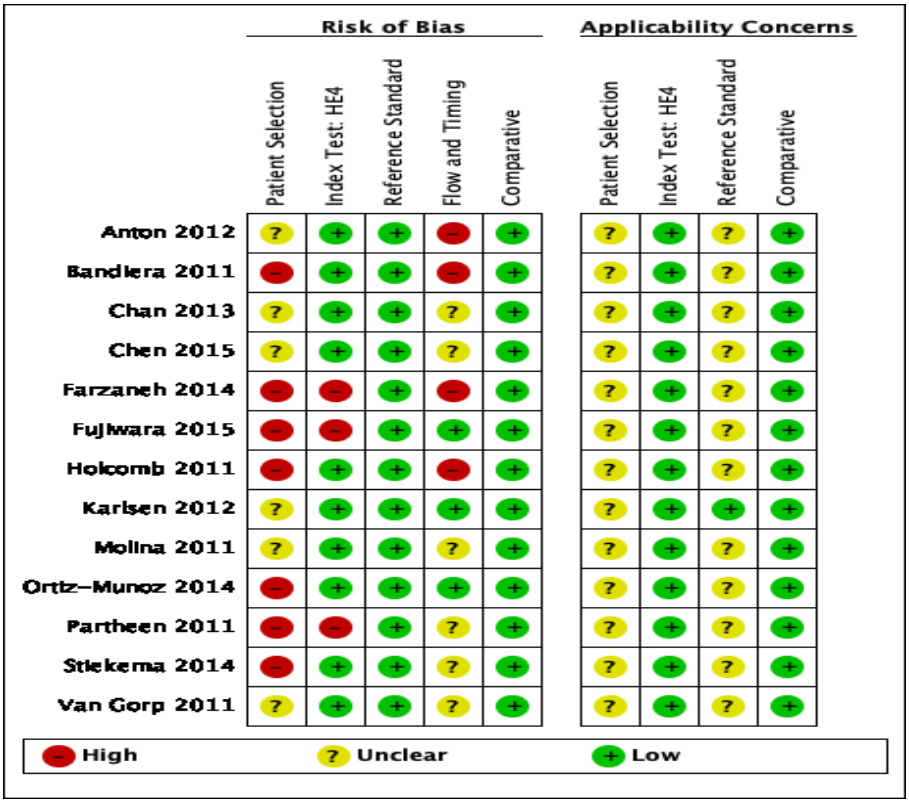


Figure 5. 2: HE4 QUADAS-2: Pre and postmenopausal studies risk of bias and applicability concerns summary.

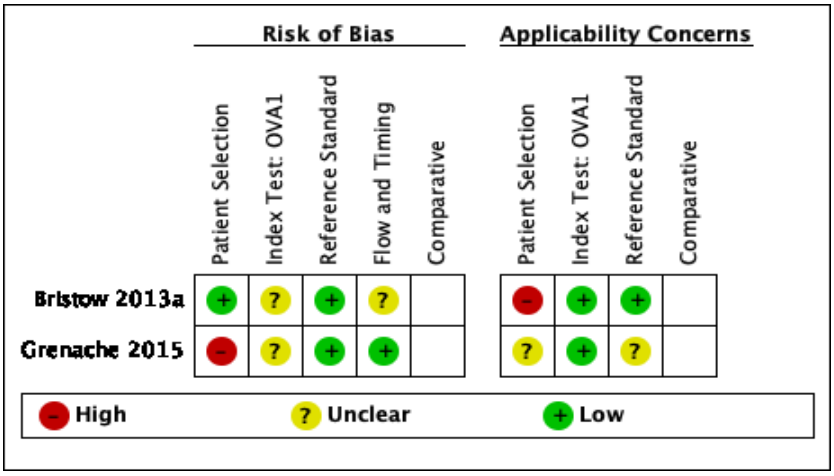


Figure 5. 3: OVA1 QUADAS-2: Pre and postmenopausal studies risk of bias and applicability concerns summary.

Methodological quality of studies including CA125

The prevalence of ovarian cancer in the studies was high, indicating an advanced position of the study in the patient pathway. The median prevalence of ovarian cancer in premenopausal women was 22% (IQR 11% to 31%, range 3 to 81%). The median prevalence of ovarian cancer in postmenopausal women was 46% (IQR 32% to 58%, range 8 to 71%).

QUADAS data for the included pre and postmenopausal studies (30) only is shown in Figure 5.1 and appendix 13

Patients were recruited prospectively in 14 studies[136, 186, 187, 192, 200, 214, 216, 217, 220, 230, 219, 221, 139, 218] retrospectively in 7 studies [229, 137, 193, 194, 226-228] and recruitment was unclear in 9 studies.[201, 202, 215, 222-224, 165, 196, 225] 21 studies used a cross sectional study design, where in 9 studies the study was unclear.[223, 224 165, 196, 201, 202, 215, 222]

Risk of bias

For the patient selection domain all the studies were judged as unclear or high risk of bias.[222, 225] For studies rated as unclear or high risk of bias this was most often because of a lack of an explicit mention or absence of consecutive sampling in combination with a limited range of tumour types and benign pathology presented and lack of clarity on prior testing.

For the index test domain nearly 50% of the studies (14/30) were judged as unclear or high risk of bias, the most common reason for was lack of clarity regarding prespecified threshold for test positivity

For the reference standard domain all studies were judges as low risk of bias.

In the flow and timing domain only 9/30 studies were judged at low risk; the commonest reason for unclear or high risk of bias was lack of clarity of the interval between the conduct of the index test and the reference standard whereas those studies (12) judged at high risk of bias in this domain did not include all patients in their analysis.

Applicability concerns

Participant selection, all studies were judged as unclear risk due to lack of clarity on patients presenting symptomatology. The prevalence of ovarian cancer in the studies was high, indicating an advanced position of the study in the patient pathway. The median prevalence of ovarian cancer in premenopausal women was 22% (IQR 11% to 31%, range 3 to 81%, 19 studies). The median prevalence of ovarian cancer in postmenopausal women was 46% (IQR 32% to 58%, range 8 to 71%, 22 studies).

All studies were judged as low applicability concern for the index test domain because the index test is objective and not dependent on operator skill.

For the reference standard domain 4/30 studies[223, 224, 226, 229] were judged as low applicability concern and 3 as high applicability concern[216, 218, 220] as it was unclear how borderline tumours had been handled for the paper's analysis.

Methodological quality of studies including HE4

The prevalence of ovarian cancer in the studies was high, indicating an advanced position of the study in the patient pathway. The median prevalence of ovarian cancer in these premenopausal women was 25% (IQR 13% to 30%, range 8 to 81%). The median prevalence of ovarian cancer in these postmenopausal women was 50% (IQR 45% to 59%, range 18 to 81%).

QUADAS-2 data was for 13 HE4 studies, is shown in Figure 5.2 and Appendix 14.

Risk of bias

Patients were recruited prospectively in 8 studies,[136, 138, 139, 186, 187, 192, 200, 214] retrospectively in 3 studies[137, 193, 194] and recruitment was unclear in 2 studies.[201, 202] 11 studies used a cross sectional study design, and for 2 studies the study design was unclear.[201, 202]

For the patient selection domain, all studies had inappropriate exclusions as summarised in the table of study characteristics (Table 5.2) and were judged as unclear or high risk of bias for patient selection

10/13 studies were judged as low risk of bias for the index test domain. 3 studies[187, 192, 200] were judged as high risk as the threshold was not specified.

All the included studies were judged as low risk of bias for the reference standard domain as only surgical patients were included in all the included studies.

For the flow and timing domain 6 studies were judged at high risk of bias because not all patients were included in the analysis.

Applicability concerns

No studies were judged as having a low applicability concern for the participant selection domain due to the fact that study participants did not obviously represent symptomatic women presenting in primary care. The prevalence of ovarian cancer in the studies was high, indicating an advanced position of patients in the clinical pathway. The median prevalence of ovarian cancer in premenopausal women was 25% (IQR 13% to 30%, range 8 to 81%, 12 studies). The median prevalence of ovarian cancer in postmenopausal women was 50% (IQR 45% to 59%, range 18 to 81%) from 10 studies.

All the studies were judged as low applicability concern for the index test domain because the index test is objective and not dependent on operator skill. For the reference standard domain all studies except 1[138] were judged as having an unclear applicability concern as it was unclear how borderline tumours had been handled for the paper's analysis. (Figure 5.2)

Methodological quality of studies including OVA1

Prevalence of ovarian cancer in the 2 studies was 17% [93] and 18%.[188]

Both OVA1 studies presented test accuracy data separately for pre and postmenopausal women. Figure 5.3 is the QUADAS-2 plot for these studies.

Risk of bias

One study[188] was at high risk of bias in the patient selection domain as patients were not recruited randomly/consecutively and the other unclear as it was unclear if there was consecutive recruitment of patients. In both studies patients were recruited prospectively. One study used a cross sectional design[93] and in the other study accuracy data was obtained from

clinical trial data.[188] Risk of bias was low for the index test domain for both studies due to objectivity of standardised serum testing.

Applicability concerns

Both studies were judged as having as unclear applicability concern for the participant selection domain due to the fact that study participants did not obviously represent symptomatic women presenting in primary care. Applicability concerns were low for the index test domain for both studies due to objectivity of standardised serum testing. Both studies grouped borderline results with malignant and were therefore classified as low applicability concern in the reference standard domain.

For CA125, HE4, and OVA1 results were examined at commonly used and where these existed clinically relevant test thresholds. Results of test performance compared at these thresholds demonstrate the effect of test threshold on test performance (e.g. Figure 5.4, Figure 5.5).

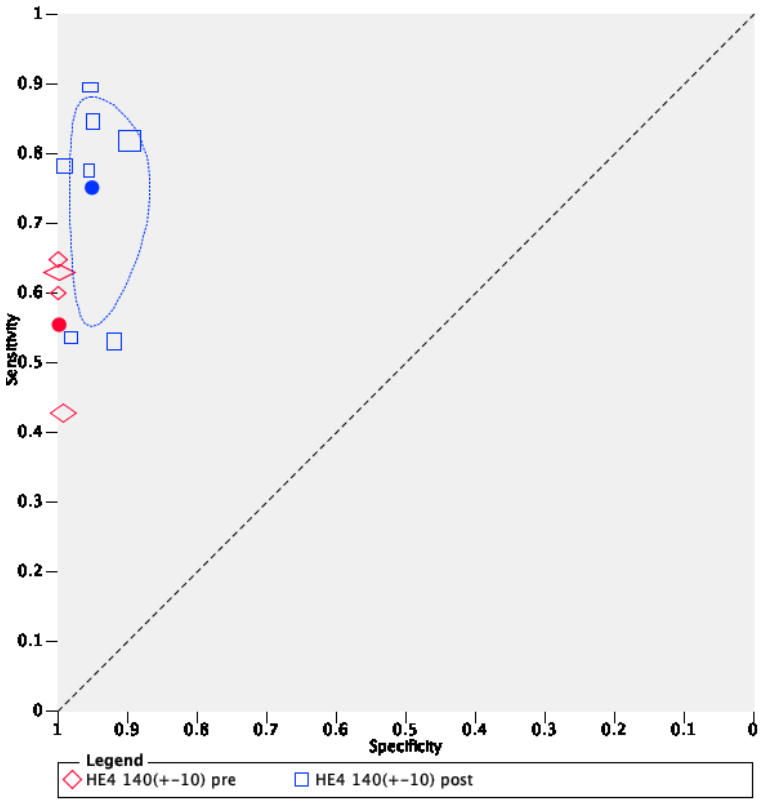


Figure 5. 4: Summary ROC Plot of tests: HE4 140 (+-10) pMol/L for pre and postmenopausal women.

5.3 Test positivity thresholds used in included studies

For CA125, the thresholds investigated were 35U/ml (RCOG recommended for postmenopausal women), 65U/ml (modified ACOG) and 200U/ ml (recommended by RCOG for premenopausal women). In addition, results within a 5U range of the test thresholds 35U/ml (30-40U/ml) and 65U/ml (60-70U/ml) were investigated.

For HE4 there is no consensus on the clinically agreed threshold cut off for test positivity. Therefore, thresholds recommended by the manufacture and most commonly reported in the literature (70pMol/L and 140pMol/L) were analysed. In addition, two analyses including results within a 10U range of these test thresholds (60-80pMol/L and 130-150pMol/L) were conducted. Results of all test positivity thresholds are reported in Table 5.9, forest plot in Appendix 24 and 25.

Well documented differences in spectrum in pre compared to postmenopausal women exist which means that differences in the percentage of postmenopausal women across studies is a source of between study heterogeneity; the most accurate test in premenopausal women might not be the most accurate test in postmenopausal women. The observed effect of menopausal status on test performance is shown in Tables 5.5 and Table 5.7 (e.g. Figure 5.4, Figure 5.5, Figure 5.6 and Figure 5.7). A significant difference in test performance was demonstrated for pre compared to postmenopausal women. Therefore, the results are presented separately for premenopausal and postmenopausal women, based on the 43 studies presenting results stratified by menopausal status

In addition to threshold and menopausal status, additional analyses were conducted to investigate the effects on test accuracy of management of borderline tumours in individual study analyses and comparison of tests based on direct (within study) comparisons and indirect (across study) comparisons (see Appendix 15, Table 5.5 and 5.7). Within study comparisons are more valid than between study comparisons as they are less prone to confounding resulting from variation in population characteristics, index test conduct and study design. The management of borderline tumours in included studies was variable. In this review grouping of borderline tumours with malignant tumours was considered an appropriate analysis strategy reflecting management of borderline tumours in clinical practice. Studies excluding borderline tumours from analysis were considered clinically inappropriate and in some studies

management of borderline tumours for analyses was unclear. For CA125, only at a threshold of 35 U/ml there was sufficient evidence to give estimates of sensitivity and specificity separately in pre and postmenopausal women for studies using clinically appropriate and inappropriate or unclear grouping of borderline results (see Appendix 15). This analysis demonstrated that estimates of sensitivity in CA125 35U/ml studies using appropriate grouping of borderline tumours were lower than in studies using inappropriate grouping or where the analysis strategy was unclear. Estimates of sensitivity and specificity for other tests and thresholds are therefore likely to be overestimates, and this should be kept in mind when interpreting the results.

5.3.1 Accuracy of CA125

Diagnostic accuracy was investigated at three commonly used thresholds of 35U/ml, 65U/ml and 200U/ml with two sensitivity analyses including results within a 5U range of the test thresholds (30- 40U/ml and 60-70U/ml). Average sensitivity and specificity values for thresholds within a 5 U range were unchanged compared to analysis based on exact threshold values (refer to table illustrating sensitivity and specificity at both point thresholds and threshold ranges). The discussion below is therefore focussed on results at two threshold ranges of 30U/ml (+/-5) and 65U/ml (+/-5). Table 5.4 presents diagnostic accuracy results for CA125 at each prespecified test threshold by menopausal status. The table reports the number of studies, the total number of women and women with ovarian cancer contributing to each summary estimate of diagnostic accuracy.

Table 5. 4: Test performance: CA125 at common thresholds.

Menopausal status	Test	Threshold	Number studies (N total, N OC)	Sensitivity (95% CI)	Specificity (95% CI)
Pre	CA125	35	15 (2514, 376)	80 (72 to 86)	66 (60 to 72)
Post	CA125	35	16 (2526, 993)	84 (79 to 87)	83 (81 to 85)
Pre	CA125	35ish	17 (3042, 473)	78 (71 to 84)	66 (61 to 71)
Post	CA125	35ish	21 (3303, 1259)	84 (80 to 87)	83 (81 to 84)
Pre	CA125	35ish	7	71 (58 to 82)	70 (62 to 77)
Post	CA125	35ish	9	78 (74 to 82)	82 (80 to 84)
Pre	CA125	65	3 (641, 135)	NA	NA
Post	CA125	65	4 (876, 368)	NA	NA
Pre	CA125	65ish	4 (763, 165)	60 (49 to 71)	85 (79 to 89)
Post	CA125	65ish	4 (876, 368)	73 (68 to 77)	92 (89 to 94)
Pre	CA125	200	1 (445, 86)	29 (20 to 40)	98 (96 to 99)
Post	CA125	200	1 (364, 156)	54 (46 to 62)	97 (94 to 99)

Table 5. 5: Test performance: CA125 comparisons.

Menopausal status	Test 1 (Threshold)	Test 2 (Threshold)	Number studies (N total, N OC)	Sensitivity (95% CI)	Specificity (95% CI)	Notes
Post- pre	CA125 35ish	CA125 35ish	102 (17 pre and 21 post, 64 all)	5.0 (-2.6 to 12.7)	16.4 (10.4 to 22.5)	Univariate, 1 RE are zero. Estimates and 95% CI within 2% of single models
Post- pre	CA125 35ish	CA125 35ish	15	5.5 (-2.9 to 14.0)	15.5 (9.2 to 21.8)	Direct studies
Post- pre	CA125 65ish	CA125 65ish	32 (4 pre and 4 post, 24 all)	13.0 (1.0 to 24.9)	6.8 (1.3 to 12.3)	Univariate, 2 RE are zero
Post- pre	CA125 65ish	CA125 65ish	3	18.6 (7.4 to 29.8)	4.6 (0.3 to 8.8)	Direct studies
Pre (35i[SM1] sh minus 65ish)	CA125 65ish	CA125 35ish	21	18.1 (4.0 to 32.2)	-18.5 (-25.9 to -11.2)	Bivariate
Pre (35ish minus 65ish)	CA125 65ish	CA125 35ish	2 (566,129)	13.5 (0.1 to 26.8)	-19.5 (-24.8 to -14.1)	Direct studies
Post (35ish minus 65ish)	CA125 65ish	CA125 35ish	25	10.3 (4.6 to 16.0)	-9.3 (-12.2 to -6.4)	Univariate, 2 zero RE estimates, all within 1% of single models
Post (35ish minus 65ish)	CA125 65ish	CA125 35ish	3	7.9 (1.8 to 14.0)	-11.3 (-15.5 to -7.0)	Direct studies

CA125: Threshold 30U/ml to 40U/ml

Figure 5.5 presents study results for pre and postmenopausal women with CA125 at a threshold of within 5U/ml of 35U/ml in ROC space. The corresponding forest plot is included in the Appendix 16.

For premenopausal women in studies with appropriate grouping of borderline tumours (borderline tumours grouped with malignant tumours), the average sensitivity was 71% (95% CI 58 to 82) with an average specificity was 70% (95% CI 62 to 77), based on 9 studies (Appendix 15). In premenopausal women for all studies sensitivity was higher [78% (95% CI 71 to 84)] and specificity lower [(66% (95% CI 61 to 71) (Appendix 15).

For postmenopausal women in studies with appropriate grouping of borderline tumours the average sensitivity was 78% (95% CI 74 to 82) with an average specificity of 82% (95% CI 80 to 84), based on 9 studies (Appendix 15). In postmenopausal women for all studies sensitivity was higher [84% (95% CI 80 to 87)] and specificity lower [83% (95% CI 81 to 84)] (Appendix 15).

Results in postmenopausal women are less heterogeneous than results in premenopausal women, and heterogeneity in sensitivity is greater in both pre and postmenopausal women when all studies are included compared to estimates based on sub groups of studies using appropriate grouping of borderline tumours in study analyses (see ROC plots Figure 5.5 and Forest plots Appendix 16 referred to above).

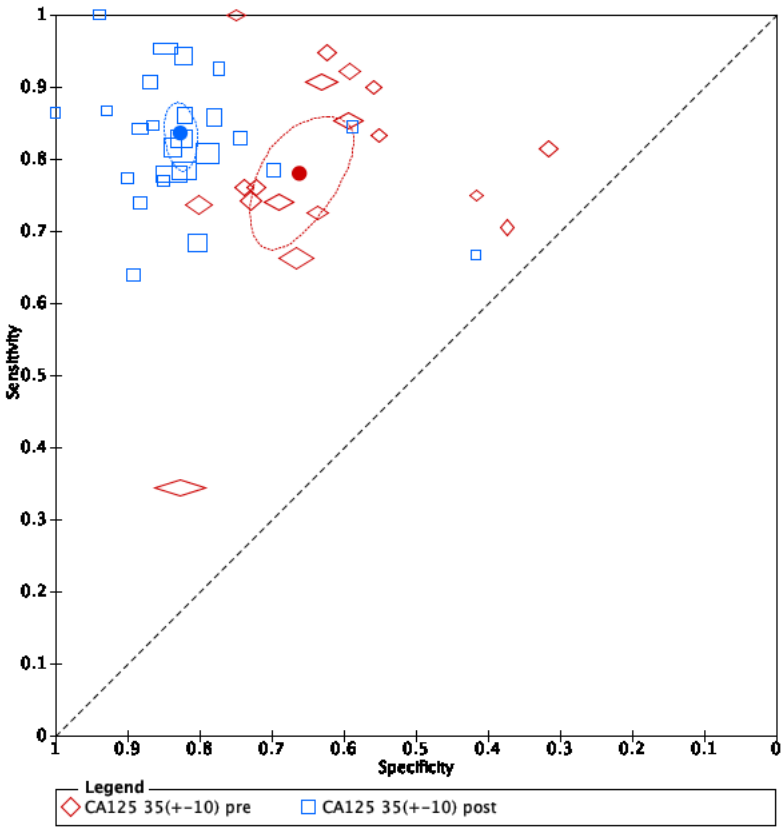


Figure 5. 5: Summary ROC Plot of tests: CA125 35(+-10) U/ml for pre and postmenopausal women.

CA125: Threshold 60U/ml to 70U/ml

Figure 5.6 presents study results for pre and postmenopausal women with CA125 at a threshold of within 5U/ml of 65U/ml in ROC space. The corresponding forest plot is included in the Appendix 17.

At a threshold of within 5U/ml of 65U/ml, in premenopausal women the average sensitivity was 60% (95% CI 49 to 71) with an average specificity of 85% (95% CI 79 to 89), based on 4 studies with 763 women, 165 with ovarian cancer (Table 5.4). Confidence intervals for summary estimates are wide as meta-analysis is based on only 4 studies; individual study results for sensitivity range from (95% CI 49 to 71) 50% to 73% and specificity results vary from 75% to 90%.

For postmenopausal women the average sensitivity was 73% (95% CI 68 to 77) with an average specificity of 92% (95% CI 89 to 94), based on 4 studies with 876 women, 368 with ovarian cancer (Table 5.4).

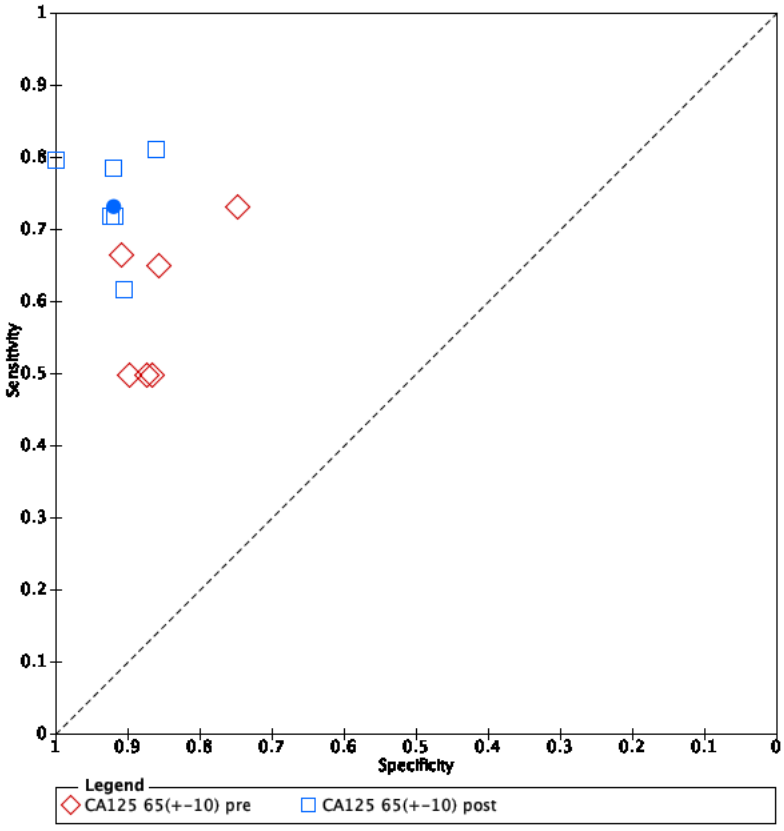


Figure 5. 6: Summary ROC Plot of tests: CA125 65(+-10) U/ml for pre and postmenopausal women.

CA125: Threshold 200U/ml

Only 1 study reported results for CA125 at a threshold of 200U/ml for pre and postmenopausal results (Table 5.4). For premenopausal women sensitivity was 29% (95% CI 20 to 40) and specificity was 98% (95% CI 96 to 99). For postmenopausal women sensitivity was 54% (95% CI 46 to 62) and specificity was 97% (95% CI 94 to 99). This study undertook appropriate grouping of borderline tumours in the analysis, is at low risk of bias and therefore estimates of sensitivity are unlikely to be overestimated.

5.3.2 Accuracy of HE4

Average sensitivity and specificity values for thresholds within a 10pMol/L range of 70pMol/L and 140pMol/L (60-80pMol/L and 130-150pMol/L) were unchanged compared to analysis based on an exact threshold values (see Table 5.6 illustrating sensitivity and specificity at both point thresholds and thresholds ranges). The discussion below is therefore focussed on results at two threshold ranges of 70pMol/L (+/-10) and 140pMol/L (+/-10). Table 5.6 presents diagnostic accuracy results for HE4 at each prespecified test threshold by menopausal status. The table reports the number of studies, the total number of women and women with ovarian cancer contributing to each summary estimate of diagnostic accuracy.

Table 5. 6: Test performance: HE4 at common thresholds.

Menopausal status	Test	Threshold	Number studies (N total, N OC)	Sensitivity (95% CI)	Specificity (95% CI)
Pre	HE4	70	7 (1467, 225)	73 (67 to 79)	91 (86 to 94)
Post	HE4	70	3 (417, 268)	85 (76 to 91)	77 (48 to 93)
Pre	HE4	70ish	11 (1718, 297)	74 (68 to 79)	91 (87 to 94)
Post	HE4	70ish	5 (601, 328)	84 (76 to 89)	78 (63 to 88)
Pre	HE4	140	2 (112, 30)	NA	NA
Post	HE4	140	5 (927, 392)	77 (66 to 84)	96 (91 to 98)
Pre	HE4	150	2 (437, 69)	?	?
Post	HE4	150	2 (348, 203)	?	?
Pre	HE4	140ish	4 (549, 99)	55 (43 to 67)	100 (98 to 100)
Post	HE4	140ish	7 (1275, 595)	75 (64 to 83)	95 (91 to 97)

Table 5. 7: Test performance: HE4 comparisons.

Menopausal status	Test 1 (Threshold)	Test 2 (Threshold)	Number studies (N total, N OC)	Difference in Sensitivity (95% CI) (Test1-Test2)	Difference in Specificity (95% CI) (Test1-Test2)	Notes
Post- Pre	HE4 70ish	HE4 70ish	16 (11 pre, 5 post)	10.2 (1.4 to 19.0)	-14.0 (-26.8 to -1.1)	Univariate, all studies
Post- Pre	HE4 70ish	HE4 70ish	4	13.2 (2.1 to 24.3)	-11.5 (-27.6 to 4.7)	Univariate, direct studies only *RE at zero for some variables, but sens and spec within 2% of bivariate above for pre
Post- Pre	HE4 140ish	HE4 140ish	20	18.9 (3.0 to 34.8)	-4.8 (-7.8 to -1.9)	Bivariate
Post- Pre	HE4 140ish	HE4 140ish	8	21.5 (3.1 to 39.8)	-5.7 (-8.7 to -2.8)	Direct only Univariate. Estimates within 1% of single test values
Pre	HE4 70ish	HE4 140ish	12	18.9 (5.7 to 32.1)	-8.0 (-11.7 to -4.3)	Bivariate, 1 RE of zero. All values within 1% estimates and 2% 95% CI from single tests
Table 5.7 Continued...			3 (296, 72)	23.0 (3.0 to 43.0)	-7.1 (-10.7 to -3.6)	Direct only, univariate
Post	HE4 70ish	HE4 140ish	10 (5 70ish, 7 140ish, 2 overlap)	9.4 (-2.3 to 21.1)	-17.2 (-29.9 to -4.6)	Univariate, all values within 1% estimates and 2% 95% CI from single tests
Post	HE4 70ish	HE4 140ish	2 (309, 138)	7.4 (-22.5 to 37.3)	-15.3 (-25.7 to -4.9)	Direct only, univariate

HE4: Threshold 60 pMol/L to 80 pMol/L

Figure 5.7 presents individual study results for pre and postmenopausal women with HE4 at a threshold of within +/- 10pMol/L of 70pMol/L in ROC space. The corresponding forest plot is included in the Appendix 18.

For premenopausal women the average sensitivity was 74% (95% CI 68 to 79) with an average specificity of 91% (95% CI 87 to 94), based on 11 studies with 1718 women, 297 with ovarian cancer, at a threshold of 60-80pMol/L. These estimates of sensitivity may be inflated by grouping of borderline results (Figure 5.8 and corresponding forest plot Appendix 22).

For postmenopausal women the average sensitivity was 84% (95% CI 76 to 89) with an average specificity of 78% (95% CI 63 to 88) based on 5 studies with 601 women, 328 with ovarian cancer, for a threshold of 60-80pMol/L. The 95% CI for specificity is wide (78% specificity, 95% CI 63 to 88; Table 2), indicating the high heterogeneity in these studies. These estimates of sensitivity may be inflated by grouping of borderline results. (e.g. Figure 5.9 and corresponding forest plot Appendix 23).

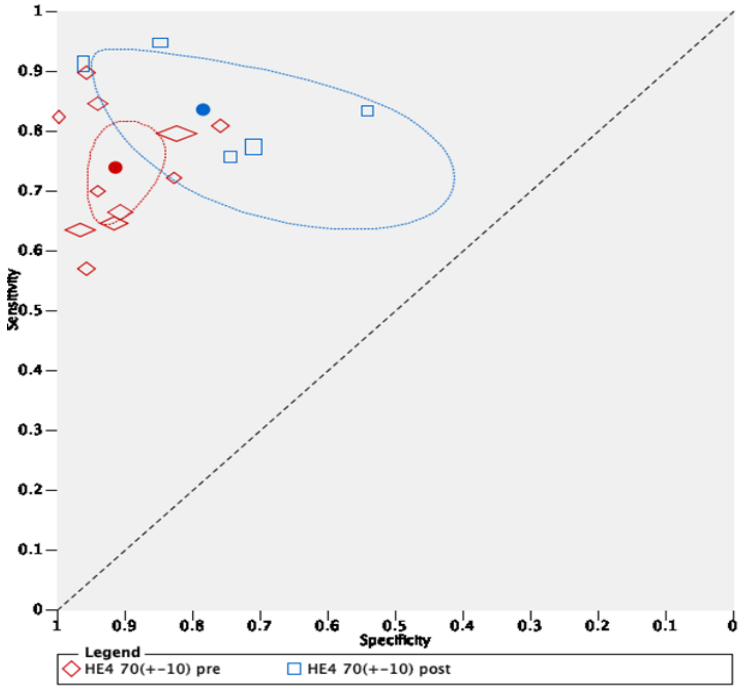


Figure 5. 7: Summary ROC Plot of tests: 11 HE4 70(+-10) pMol/L for pre and postmenopausal women.

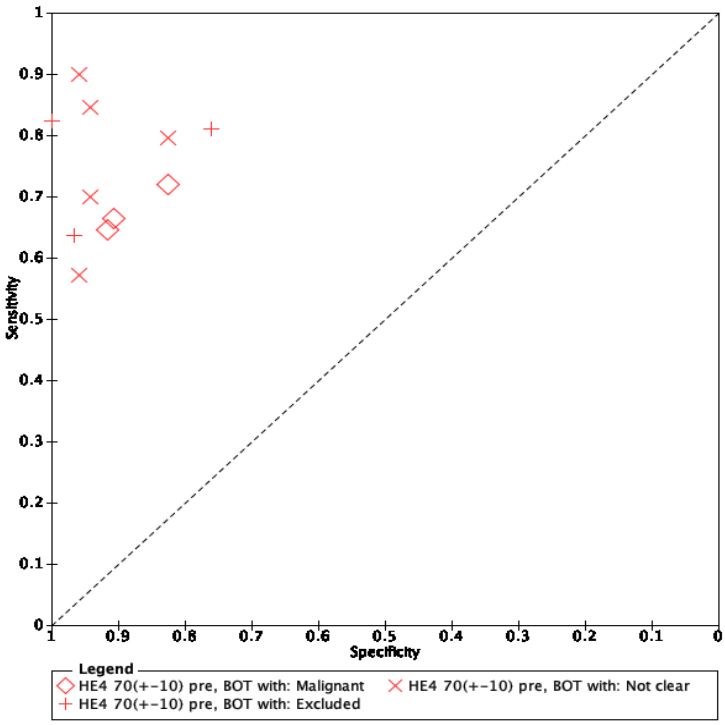


Figure 5. 8: Summary ROC Plot of 11 HE4 70(+-10) BOT for premenopausal women.

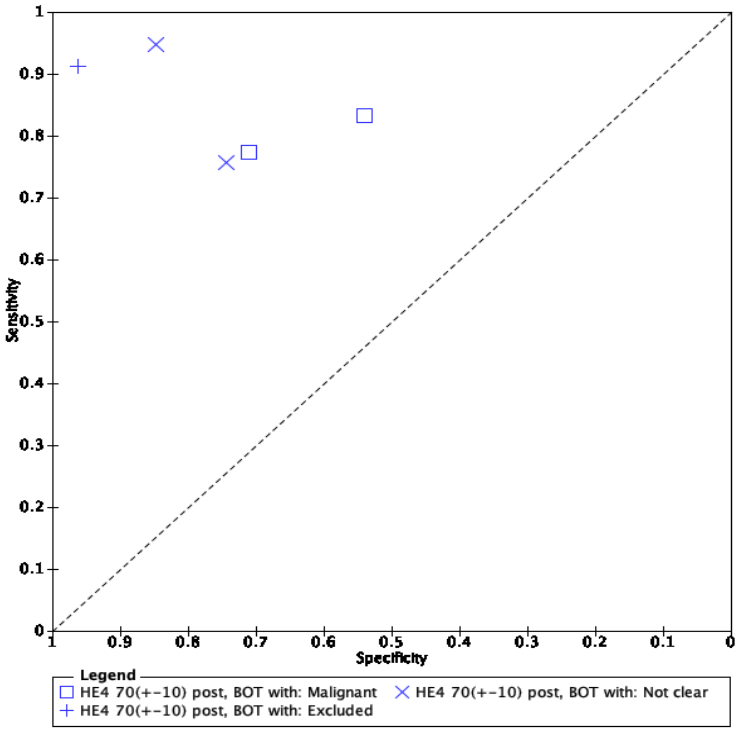


Figure 5. 9: Summary ROC Plot of 12 HE4 70(+-10) BOT for postmenopausal women.

HE4: Threshold 130pMol/L to 150pMol/L

Figure 5.10 presents individual study results for pre and postmenopausal women with HE4 at a threshold of within +/- 10pMol/L of 70pMol/L in ROC space. The corresponding forest plot is included in the Appendix 19.

For premenopausal women using HE4 thresholds between 130-150pMol/L, the average sensitivity was 55% (95% CI 43 to 67) with an average specificity of 100% (95% CI 98 to 100), based on 4 studies with 549 women, 99 with ovarian cancer (Table 5.6). The estimates of sensitivity may be inflated by grouping of borderline results; the one study with appropriate grouping of borderline results had a sensitivity of 43% compared to 60%, 63% and 65% in the other three studies where borderline results were excluded or grouping was unclear

For postmenopausal women using HE4 thresholds between 130-150pMol/L, the average sensitivity was 75% (95% CI 64 to 83) with an average specificity of 95% (95% CI 91 to 97) based on 7 studies with 1275 women, 595 with ovarian cancer (Table 5.6). Estimates of sensitivity may be inflated by grouping of borderline results, as the one study with correct grouping of borderline results had a sensitivity of 53% compared to values ranging from 78% to 89% in the other 6 studies where borderline results were excluded or grouping was unclear. For postmenopausal women the 95% CI ellipse for specificity covers roughly 40% of ROC space indicating the high heterogeneity in these studies.

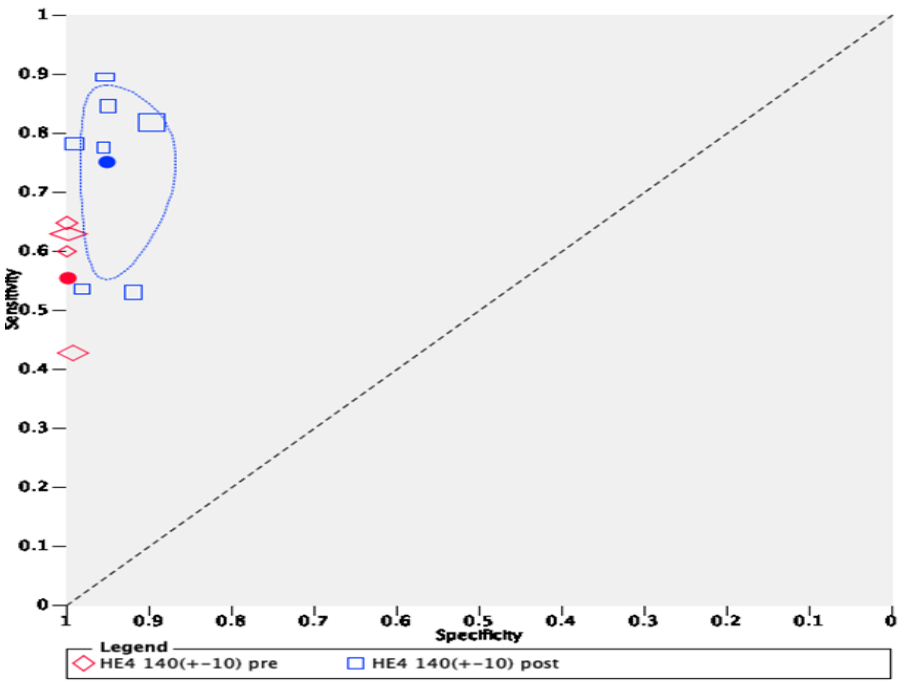


Figure 5. 10: Summary ROC Plot of tests: HE4 140pmol/L for premenopausal and postmenopausal women.

5.3.3 Accuracy of OVA1

Only 2 studies reported results for OVA1 at a threshold of 5 for premenopausal women and 4.4 for postmenopausal women. Both studies appropriately grouped borderline tumours with malignant for analysis (Table 5.8). The sensitivity for premenopausal women was 94% (95 CI 80 to 99) and specificity was 62% (95% CI 57 to 67). The sensitivity for postmenopausal women was 93% (95 CI 85 to 97) and specificity was 41 (95% CI 35 to 48).

Table 5. 8: Test performance of OVA1

Menopausal status	Test	Threshold	Number studies (N total, N OC)	Sensitivity (95% CI)	Specificity (95% CI)
Pre	OVA1	5	2	94 (80 to 99)	62 (57 to 67)
Post	OVA1	4.4	2	93 (85 to 97)	41 35 to 48)

5.4 Heterogeneity analysis

Heterogeneity analyses were conducted for menopausal status, thresholds of test positivity and appropriate BOT grouping (BOT combined with malignant tumours vs BOT combined with benign or unclear grouping for analyses).

The following planned heterogeneity analyses were not performed due to insufficient studies with differences in the relevant study characteristics or with these study characteristics reported:

-Generalist (primary care/community/family practice) versus specialist setting (cancer unit/cancer centre/gynaecological oncology)

-Histological subtype of EOC vs Non EOC

-Reference standard QUADAS-2 domain: High/unclear risk of bias versus low risk of bias in the reference standard domain where low risk of bias is a minimum of 12 months follow-up for study participants not receiving surgery initially following a negative index test result.

5.4.1 Effect of menopausal status on test performance

The importance of using menopausal status when interpreting test results are demonstrated for HE4 and CA125 at all thresholds examined.

At a threshold of 30-40U/ml based on 15 direct within study comparisons, specificity of CA 125 was higher in postmenopausal women compared to premenopausal women by 15.5% (95% CI 9.2 to 21.8) (Table 5.5, Figure 5.5). Specificity results were more heterogeneous for premenopausal women (range 32% to 83%) than for postmenopausal women (59% to 87%) (see Forest plots in Appendix16).

At a threshold of 60-70U/ml based on 3 direct comparison studies, for postmenopausal women sensitivity was significantly higher by 18.6% (95% CI 7.4 to 29.8) and specificity was significantly higher by 4.6% (95% CI 0.3 to 8.8) than for premenopausal women's (Table 5.5, Figure 5.6). Study results were heterogeneous, particularly for sensitivity.

For the single CA125 study reporting accuracy at a threshold of 200U/ml sensitivity was statistically higher in postmenopausal women (25% higher) based on non-overlapping 95% confidence intervals, but specificity was within 1% of the estimate for premenopausal women (Table 5.5).

For HE4, diagnostic test accuracy was statistically different between pre and postmenopausal women for both sensitivity and specificity, based on both direct and indirect test comparisons (Table 5.7). At a threshold of 60-80pMol/L based on direct comparisons, for postmenopausal women sensitivity was higher by 13.2% (95% CI 2.1 to 24.3) and specificity was lower by 11.5% (95% CI 4.7 to 27.6) compared to premenopausal women (Table 5.7, Figure 5.7). At a threshold of 130 to 150 pMol/L based on direct comparisons, for postmenopausal women sensitivity was higher by 21.5% (95% CI 3.1 to 39.8) and specificity was lower by 5.7% (95% CI 2.8 to -8.7) than for premenopausal women (Table 5.7, Figure 5.10).

5.4.2 Effect of test threshold on test performance

For both HE4 and CA125 tests, statistically different test performance was found when comparing the most commonly used test thresholds: for HE4 60-70pMol/L compared to 130-150pMol/L and for CA125 30-40U/ml compared to 60–70U/ml. This was based on both direct and indirect test comparisons.

For, CA125 in both pre and postmenopausal women, both sensitivity and specificity were significantly different when thresholds between 30-40U/ml and 60-70U/ml were compared. For CA125 in premenopausal women, sensitivity was significantly higher using a threshold of 30-40U/ml by 18.1% (95% CI 4.0 to 32.2) and specificity was significantly lower by 18.5% (95% CI -11.2 to -25.9) compared to a threshold of 60-70U/ml, based on 21 indirect and direct comparison studies (Table 5.5, Figure 5.5 compared to Figure 5.6). A similar result was found based on 2 direct comparison studies (Table 5.5). For CA125 in postmenopausal women, sensitivity was significantly higher using a threshold of between 30-40U/ml by 10.3% (95% CI 4.6 to 16.0) and specificity was significantly lower by 9.3% (95% CI -6.4 to -12.2) compared to a threshold of 60–70U/ml, based on 25 indirect and direct comparison studies (Table 5.5, Figure 5.5 compared to Figure 5.6). A similar result was found based on 3 direct comparison studies (Table 5.5). For both pre and postmenopausal women the optimum threshold will depend on whether the increase in sensitivity achieved with a threshold of 30-40U/ml is worth

the decrease in specificity compared to 60-70U/ml. This in turn is dependent on the absolute magnitude of test errors determined by the prevalence of ovarian cancer in the testing setting.

At a prevalence of 0.23% typical of a primary care presentation, CA125 at a threshold of 60-70U/ml may be the preferred test as the higher sensitivity of CA125 at 30-40U/ml would on average identify no additional women with ovarian cancer, but result in 185 fewer women with a correct true negative result (185 more women with false positive result). However, at a point in the clinical pathway with a prevalence of 10%, an average additional 18 women with true positive results might be considered to outweigh the disadvantages of an average of 167 more women with false positive results. Table 5.10 presents results for four prevalence scenarios.

For HE4 in premenopausal women, at thresholds between 60-80pMol/L sensitivity was higher by 23.0% (95% CI 3.0 to 43.0) and specificity was lower by 7.1% (95%CI -3.6 to - 10.7) compared to a threshold of 130-150pMol/L (Table 5.7) based on 3 direct comparison studies. A similar result was found based on 12 indirect and direct comparison studies (Table 5.7, Figure 5.7 compared to Figure 5.10). For HE4 in postmenopausal women specificity was significantly lower (17.2% (95% CI 4.6 to 29.9) at a threshold of 60-80pMol/L compared to a threshold of 130-150pMol/L based on 10 indirect comparison studies. Sensitivity was higher but not significantly (9.4% (95% CI -2.3 to 21.1) (Table 5.7, Figure 5.7 and Figure 5.10). A similar result was found based on 2 direct comparison studies (Table 5.7). In postmenopausal women HE4 at a threshold of 130-150pMol/L is therefore preferred as it offers a higher specificity (no significant difference in sensitivity). In premenopausal women the evidence is less clear as an increase in sensitivity at a threshold of 60-70pMol/L has to be balanced against a decrease in specificity compared to a threshold of 130-150pMol/L. The magnitude of test errors determined by the prevalence of ovarian cancer in the testing setting will determine whether the increase in sensitivity is worth the trade off of a decrease in specificity.

Table 5. 9: Summary of findings: HE4, CA125 and OVA1 at common thresholds.

Test	Test threshold	Menopausal status	Dir/ All/ BOT	N Studies (N Participants,DP)	Sensitivity (95%	Specificity(95% CI)		Prevalence 0.23%	Prevalence 4%	Prevalence 10%	Prevalence 30%
HE4	70ish	Pre	All	11 (1718, 297)	74 (68 to 79)	91 (87 to 94)	True postives	2 (2 to 2)	30 (27 to 32)	74 (68 to 79)	222 (204 to 237)
							False negatives	1 (0 to 1)	10 (8 to 13)	26 (21 to 32)	78 (63 to 96)
							False positives	90 (60 to 130)	86 (58 to 125)	81 (54 to 117)	63 (42 to 91)
HE4	70ish	post	All	6 (601, 328)	84 (76 to 89)	78 (63 to 88)	True positives	2 (2 to 2)	34 (30 to 36)	84 (76 to 89)	252 (228 to 267)
							False negatives	0 (0 to 1)	6 (4 to 10)	16 (11 to 24)	48 (33 to 72)
							False positives	219 (120 to 369)	211 (115 to 355)	198 (108 to 333)	154 (84 to 259)
							True negatives	778 (629 to 878)	749 (605 to 845)	702 (567 to 792)	546 (441 to 616)
HE4	140ish	Pre	All	4 (549, 99)	55 (43 to 67)	100 (98 to 100)	True positives	1 (1 to 2)	22 (17 to 27)	55 (43 to 67)	165 (129 to 201)
							False negatives	1 (1 to 1)	18 (13 to 23)	45 (33 to 57)	135 (99 to 171)
							False positives	0 (0 to 20)	0 (0 to 19)	0 (0 to 18)	0 (0 to 14)
							True negatives	998 (978 to 998)	960 (941 to 960)	900 (882 to 900)	700 (686 to 700)
HE4	140ish	post	all	7 (1275, 595)	75 (64 to 83)	95 (91 to 97)	True positives	2 (1 to 2)	30 (26 to 33)	75 (64 to 83)	225 (192 to 249)
							False negatives	1 (0 to 1)	10 (7 to 14)	25 (17 to 36)	75 (51 to 108)
							False positives	50 (30 to 90)	48 (29 to 86)	45 (27 to 81)	35 (21 to 63)
							True negatives	948 (908 to 968)	912 (874 to 931)	855 (819 to 873)	665 (637 to 679)
CA125	35ish	Pre	BOT	7 (,)	71 (58 to 82)	70 (62 to 77)	True positives	2 (1 to 2)	28 (23 to 33)	71 (58 to 82)	213 (174 to 246)
							False negatives	1 (0 to 1)	12 (7 to 17)	29 (18 to 42)	87 (54 to 126)
							False positives	299 (229 to 379)	288 (221 to 365)	270 (207 to 342)	210 (161 to 266)
							True negatives	698 (619 to 768)	672 (595 to 739)	630 (558 to 693)	490 (434 to 539)
CA125	35ish	post	BOT	9 (,)	78 (74 to 82)	82 (80 to 84)	True positives	2 (2 to 2)	31 (30 to 33)	78 (74 to 82)	234 (222 to 246)
							False negatives	1 (0 to 1)	9 (7 to 10)	22 (18 to 26)	66 (54 to 78)
							False positives	180 (160 to 200)	173 (154 to 192)	162 (144 to 180)	126 (112 to 140)
							True negatives	818 (798 to 838)	787 (768 to 806)	738 (720 to 756)	574 (560 to 588)
CA125	65ish	Pre	All	4 (763, 165)	60 (49 to 71)	85 (79 to 89)	True positives	1 (1 to 2)	24 (20 to 28)	60 (49 to 71)	180 (147 to 213)
							False negatives	1 (1 to 1)	16 (12 to 20)	40 (29 to 51)	120 (87 to 153)
							False positives	150 (110 to 210)	144 (106 to 202)	135 (99 to 189)	105 (77 to 147)

Table 5.9 Continued...

CA125	65ish	Post	all	4 (876, 368)	73 (68 to 77)	92 (89 to 94)	True	positives	2	(2	to	2)	29	(27	to	31)	73	(68	to	77)	219	(204	to	231)
							False	negatives	1	(1	to	1)	11	(9	to	13)	27	(23	to	32)	81	(69	to	96)
							False	positives	80	(60	to	110)	77	(58	to	106)	72	(54	to	99)	56	(42	to	77)
							True negatives		918 (888 to 938)				883 (854 to 902)				828 (801 to 846)				644 (623 to 658)			
CA125	200	pre	All	1 (445, 86)	29 (20 to 40)	98 (96 to 99)	True	positives	1	(0	to	1)	12	(8	to	16)	29	(20	to	40)	87	(60	to	120)
							False	negatives	2	(1	to	2)	28	(24	to	32)	71	(60	to	80)	213	(180	to	240)
							False	positives	20	(10	to	40)	19	(10	to	38)	18	(9	to	36)	14	(7	to	28)
							True negatives		978 (958 to 988)				941 (922 to 950)				882 (864 to 891)				686 (672 to 693)			
1212	200	Post	All	1 (364, 156)	54 (46 to 62)	97 (94 to 99)	True	positives	1	(1	to	1)	22	(18	to	25)	54	(46	to	62)	162	(138	to	186)
							False	negatives	1	(1	to	1)	18	(15	to	22)	46	(38	to	54)	138	(114	to	162)
							False	positives	30	(10	to	60)	29	(10	to	58)	27	(9	to	54)	21	(7	to	42)
							True negatives		968 (938 to 988)				931 (902 to 950)				873 (846 to 891)				679 (658 to 693)			
OVA1	5	pre	All	2 (,)	94 (80 to 99)	62 (57 to 67)	True	positives	2	(2	to	2)	38	(32	to	40)	94	(80	to	99)	282	(240	to	297)
							False	negatives	0	(0	to	0)	2	(0	to	8)	6	(1	to	20)	18	(3	to	60)
							False	positives	379	(329	to	429)	365	(317	to	413)	342	(297	to	387)	266	(231	to	301)
							True negatives		619 (569 to 668)				595 (547 to 643)				558 (513 to 603)				434 (399 to 469)			
OVA1	4.4	Post	All	2 (,)	93 (85 to 97)	41 (35 to 48)	True	positives	2	(2	to	2)	37	(34	to	39)	93	(85	to	97)	279	(255	to	291)
							False	negatives	0	(0	to	0)	3	(1	to	6)	7	(3	to	15)	21	(9	to	45)
							False	positives	589	(519	to	649)	566	(499	to	624)	531	(468	to	585)	413	(364	to	455)
							True negatives		409 (349 to 479)				394 (336 to 461)				369 (315 to 432)				287 (245 to 336)			

5.4.3 Effect of borderline test results on test performance

For CA125 between thresholds of 30 U/ml to 40 U/ml in premenopausal women, there are 17 studies, 7 of which grouped borderline results appropriately for analysis (Figure 5.11) and for postmenopausal women 21 studies, 9 of which grouped borderline results appropriately for (Figure 5.12). From visual inspection of (Figure 5.11 compared to Figure 5.12) and a sensitivity analysis restricted to studies grouping borderline tumours appropriately, studies grouping borderline tumours appropriately have a lower sensitivity but this was statistically significant only for postmenopausal women. Formal heterogeneity tests of these two subgroups found a statistically significant lower average sensitivity in the low ROB group (-10.2%; 95% CI -15.3 to -5.1 (Appendix 15), but there was no statistical difference in specificities.

Investigation of the effects of borderline tumour categorisation could not be carried out for CA125 at thresholds between 60 and 70 or for HE4 due to lack of studies see Figure 5.8 and Figure 5.9.

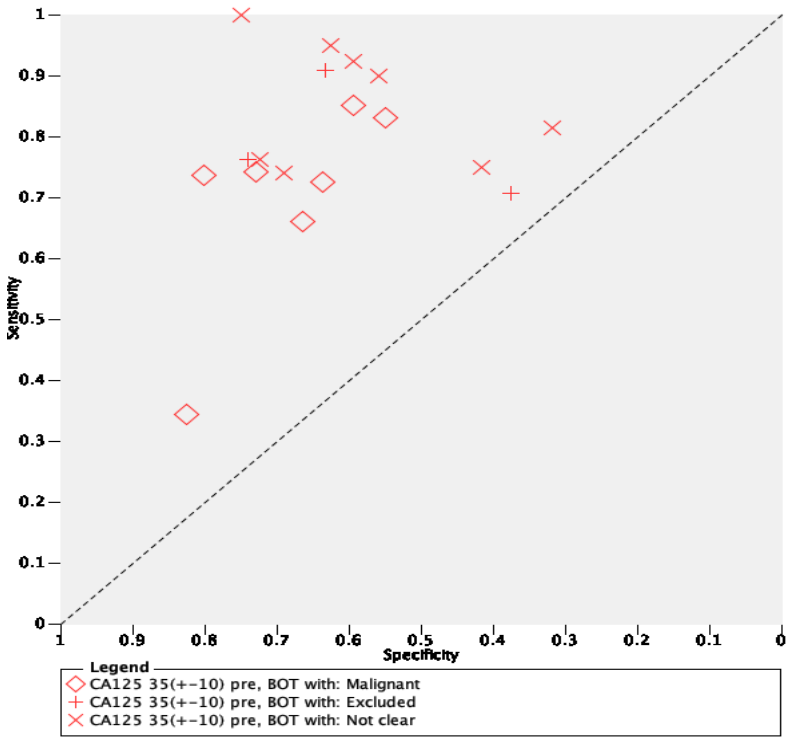


Figure 5. 11: Summary ROC Plot of 29 CA125 35(+-10) BOT for premenopausal women.

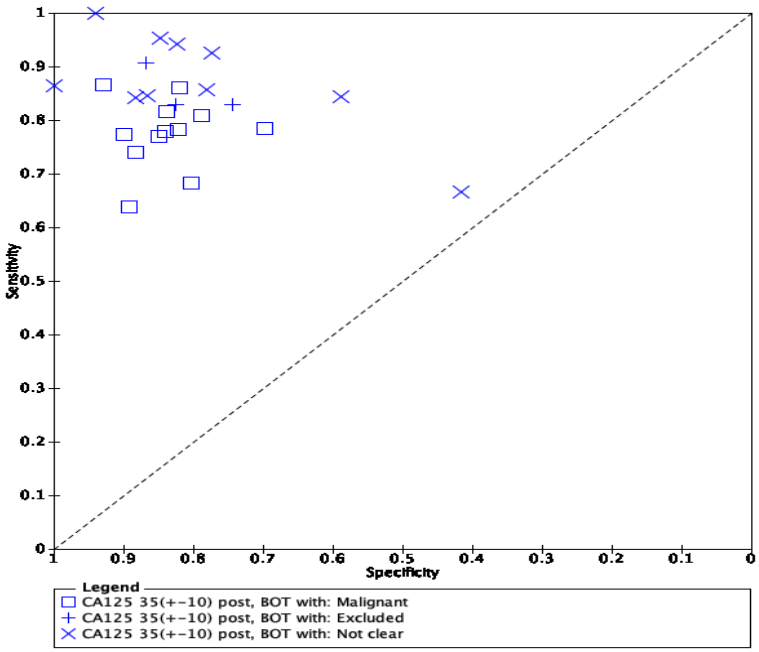


Figure 5. 12: Summary ROC Plot of CA125 35(+-10) BOT for postmenopausal women.

5.4.4 Other planned sensitivity analysis

Planned sensitivity analysis including only studies with low concern about applicability in the patient selection domain of QUADAS-2 compared to studies with high or unclear applicability concern was not conducted, as all studies had a rating of unclear applicability in this domain for both HE4 and CA125.

5.5 CA125 and HE4: comparison of test performance

For the comparison of CA125 and HE4 at different thresholds results from direct comparisons and those studies using an appropriate grouping of borderline tumours were preferred. Comparisons are based on all available studies where this was not available.

For interpretation of comparisons, one can conclude that there is a clear advantage of one test over another if one of the following is observed:

(i) both the difference in sensitivity and specificity are statistically higher for one test, so the test with the higher sensitivity and specificity would be preferred

(ii) the difference in sensitivity is not statistically different between tests but specificity is statistically higher for one test, so the test with the higher specificity is preferred

(iii) difference in specificity is not statistically different between tests but sensitivity is statistically higher for one test, so the test with the higher sensitivity is preferred.

Advantage of one test over another is not supported by the evidence if the following is observed:

(iv)There is no statistically significant difference between the sensitivity and specificity of the two tests

If there is a statistically significant difference in both the sensitivity and specificity between the two tests, with an increase in sensitivity combined with a decrease in specificity, or vice versa then interpretation of the advantages and disadvantages of tests depend on the consequences of false positive and false negative test results and the prevalence of disease. Prevalence of disease determines the absolute number of true positive and false positive test results.

Table 5.10 demonstrates differences in test performance between HE4 and CA125 at different thresholds. Table 5.11 provides a summary of those tests and thresholds where a test comparison results in opposite directions for the difference in sensitivity and specificity and illustrates the average difference in the absolute numbers of women who would have a true positive and true negative test result between the two tests, at four hypothetical prevalences of ovarian cancer representing different points in the clinical pathway. Prevalence increases along a clinical pathway as prior tests filter out causes of false positive test results.

Table 5. 10: Test performance: Comparison HE4 and CA125.

Menopausal status	Test 1 (Threshold)	Test 2 (Threshold)	Number studies (N total women, N OC)[a]	Difference in Sensitivity (95% CI)	Difference in Specificity (95% CI)	Notes
Pre **(CA125- HE4)**	HE4 70ish	CA125 35ish	18 (17 CA125, 11 HE4, 10 of both) (3621, 522 OC)	4 (-4.8 to 13.0)	-25.2 (-31.6 to -18.8)	*note Radosa 2011 outlier not omitted as otherwise single test accuracy varies from these estimates
Pre	HE4 70ish	CA125 35ish	10 each (1139, 248)	9.9 (1.0 to 18.7)	-26.8 (-33.4 to -20.1)	Direct only
Pre	HE4 70ish	CA125 35ish	6	12.8 (0.3 to 25.3)	-23.7 (-37.5 to -10.0)	Direct with BOT=1 only
Post	HE4 70ish	CA125 35ish	22 (21 CA125, 4 HE4, 4 of both) (3442,1372)	-2.2 (-8.1 to 3,8)	4.4 (-2.8 to 11.6)	Univariate HE4 same estimates, slightly different 95% CI to single CA125 - 2% less sens estimate, spec same
Post	HE4 70ish	CA125 35ish	4 each (462, 215)	-1.5 (-11.2 to 8.2)	12.2 (0.28 to 24.0)	Direct only
Post	HE4 70ish	CA125 35ish	2	-3.3 (-16.8 to 10.2)	20.5 (7.3 to 33.7)	Direct with BOT=1 only
Pre	HE4 70ish	CA125 65ish	15 (2481, 462)	-14.0 (-27.7 to 0.3)	-6.8 (-13.0 to -0.3)	All CA125 v.similar-1% differnce on 95% CI HE4 – same

Table 5.10 Continued...

Pre	HE4 70ish	CA125 65ish	0	NA	NA	Direct only
Pre	HE4 70ish	CA125 65ish	5	-11.4 (-25.8 to 3.1)	-3.6 (-8.1 to 9.1)	All BOT1 studies
Post	HE4 70ish	CA125 65ish	9 (1477, 696)	-11.3 (-19.4 to -3.3)	14.1 (1.6 to 26.7)	All CA125 same as other estimates HE4 only 1% difference in 95% CI
Post	HE4 70ish	CA125 65ish	0	NA	NA	Direct only
Post	HE4 70ish	CA125 65ish	5	-5.2 (-13.1 to 2.7)	27.1 (15.3 to 39.0)	All BOT1 studies[SM1]
Pre G3	HE4 140ish	CA125 35ish	17 (3042, 473)	23.0 (9.4 to 36.7)	-33.7 (-39.5 to -27.8)	All CA125 similar,2% diff in 95% CI HE4 same
Pre	HE4 140ish	CA125 35ish	4 (549, 99)	25.5 (9.1 to 41.9)	-30.6 (-39.4 to -21.7)	Direct Estimates slightly different from all study estimates - 3%ish different
Pre	HE4 140ish	CA125 35ish	8 (only 1 HE4 study)	28.1 (9.3 to 47.0)	-28.7 (-36.3 to -21.2)	BOT1 studies
Post	HE4 140ish	CA125 35ish	22 (3785, 1462)	12.6 (4.7 to 20.5)	-11.0 (-13.8 to -8.2)	All
Post	HE4 140ish	CA125 35ish	6 (793, 392)	14.9 (2.9 to 27.0)	-12.4 (-16.7 to -8.1)	Direct

Table 5.10 continued...

Post	HE4 140ish	CA125 35ish	10 (only 1 HE4 study)	25.0 (15.2 to 34.8)	-9.9 (-16.1 to -3.7)	BOT1 studies
Pre	HE4 140ish	CA125 65ish	8 (1312, 264)	3.7 (-13.9 to 21.4)	-15.2 (-20.3 to -10.0)	All
Pre	HE4 140ish	CA125 65ish	0	NA	NA	Direct
Pre	HE4 140ish	CA125 65ish	3 (2 CA125, 1 HE4)	12.7 (-5.7 to 31.1)	-12.1 (-15.5 to -8.7)	BOT1 studies
Post	HE4 140ish	CA125 65ish	11 (2151, 963)	-1.4 (-8.1 to 5.4|)	-1.3 (-4.3 to 1.7)	All
Post	HE4 140ish	CA125 65ish	0	NA	NA	Direct
Post	HE4 140ish	CA125 65ish	4 (1 HE4 205, 119; 3 CA125 842, 355)	20.6 (10.5 to 30.7)	0.1 (-6.1 to 6.4)	BOT1 studies

5.5.1 Test performance in premenopausal women

In premenopausal women comparison of test performance was possible between CA125 and HE4. There were no direct comparisons of OVA1 with CA125 or HE4. A statistical comparison of OVA1 with CA125 or HE4 using meta-regression was not possible with only 2 OVA1 studies.

A) One comparison demonstrates a clear advantage of one test over another: When HE4 at a threshold of 130-150pMol/L is compared to CA125 at a threshold of 60-70U/ml there is no significant difference in sensitivity but CA125 has a statistically significant lower specificity (-12.1, 95% CI -15.5 to -8.7). HE4 at a threshold of 130-150pMol/L is preferred over CA125 at a threshold of 60-70U/ml.

B) One comparison demonstrates no significant difference between tests: When CA125 at a threshold of 60-70U/ml is compared to HE4 at a threshold of 60-80pmol/L no significant difference in sensitivity or specificity is observed.

C) For three comparisons there is a statistically significant difference in both the sensitivity and specificity between the two tests, with an increase in sensitivity combined with a decrease in specificity, or vice versa. The choice between tests will therefore depend on a trade off in the absolute numbers of test errors which in turn will be determined by the prevalence of ovarian cancer in the tested population:

1) When CA125 at a threshold of 30-40 U/ml is compared to CA125 at a threshold of 60-70U/ml. CA125 30-40U/ml has a sensitivity of 71% (95% CI 58 to 82) which is higher than the sensitivity of CA125 60-70U/ml by 18.1% (95% CI 4.0 to 32.2). However, CA125 30-40U/ml has a specificity of 70% (95% CI 62 to 77) which is lower than CA125 60–70U/ml by 18.5% (95% CI -25.9 to -11.2). At a prevalence of 0.23% that is closer to represent a primary care population CA125 at a threshold of 60-70U/ml would be the preferred test. The higher sensitivity of CA125 at 30-40U/ml would on average identify no additional women with ovarian cancer, but result in 185 more women with a false positive result. However, at a point in the clinical pathway with a prevalence of 10%, an average additional 18 women with true positive results would be identified by CA125 at 30-40U/ml which might be considered to outweigh the disadvantages of an average of 167 more women with false positive results compared to CA125 60–70U/ml.

2) When CA125 at a threshold of 30-40U/ml is compared to HE4 at a threshold of 60-80p/Mol/L CA125 30-40U/ml has a sensitivity which is significantly higher, 12.8% more (95% CI 0.3 to 25.3) than HE4 at a threshold of 60-80p/Mol/L but a specificity of 70% (95% CI 62 to 77) which is 23.7% (95% CI -37.5 to -10.0) lower than HE4 at a threshold of 60-80pMol/L. At a prevalence of 0.23% typical of a primary care population HE4 at a threshold of 60-80pMol/L would be the preferred test. HE4 60-80pMol/L has a sensitivity of 74% (95% CI 68 to 79) and a specificity of 91% (95% CI 87 to 94). The higher sensitivity of CA125 at a threshold of 30-40U/ml would on average identify no additional women with ovarian cancer but 236 more women would receive a false positive result. In contrast at a point in the clinical pathway with a prevalence of 10%, an average additional 13 women with ovarian cancer who would be identified (true positive results) might be considered to outweigh the disadvantages of an average of 213 more women with false positive results. Table 5.11 presents results for four prevalence scenarios.

3) When CA125 at a threshold of 30-40U/ml is compared to HE4 at a threshold of 130-150pMol/L, CA125 30- 40U/ml has a sensitivity (95% CI 58 to 82) which is 25.5% (95% CI 9.1 to 41.9) higher than HE4 130-150pMol/L but a specificity of 71% (95% CI 58 to 82) which is 30.6% (95% CI -21.7 to -39.4) lower than HE4 130-150pMol/L. At a prevalence of 0.23% typical of a primary care presentation HE4 at a threshold of 130-150pMol/L would be the preferred test. On average the higher sensitivity of CA125 at 30-40U/ml would identify one additional woman with ovarian cancer, but 308 more women would receive a false positive result. In contrast at a point in the clinical pathway with a prevalence of 10%, an average additional 26 women with ovarian cancer who would be identified (true positive results) might be considered to outweigh the disadvantages of an average of 278 more women with false positive results.

In premenopausal women, based on consideration of A), B) and C) above and Table 5.9, HE4 at a threshold of 60-80pMol/L is the preferred test in settings with prevalence typical of primary care populations. HE4 at a threshold of 130-150pMol/L is the preferred test in test settings with prevalence typical of specialist settings.

5.5.2 Test performance in postmenopausal women

In postmenopausal women comparison of test performance was possible between CA125 and HE4. There were no direct comparisons of OVA1 with CA125 or HE4. A statistical comparison of OVA1 with CA125 or HE4 using meta-regression was not possible with only 2 OVA1 studies.

A) Two comparisons demonstrate clear advantage of one test over another:

1) CA125 30-40U/ml has a higher specificity than HE4 60-80pMol/L.

When CA125 at a threshold of 30-40U/ml is compared to HE4 at a threshold of 60-80pMol/L there is no statistically significant difference in sensitivity. CA125 30-40U/ml has a specificity of 82% (95% CI 80 to 84) based on direct comparison studies which is statistically significantly 12.2% (95% CI 0.28 to 24.0) higher compared to HE4 60-80pMol/L.

2) CA125 at 60-70U/ml has a higher specificity than HE4 60-80pMol/L.

When CA125 at a threshold of 60-70U/ml is compared to HE4 at a threshold of 60-80pMol/L evidence for any difference in sensitivity is inconsistent. However, CA125 60-70U/ml has a specificity of 92% (95% CI 89 to 94 based on indirect comparison studies which is 14.1% (95 % CI 1.6 to 26.7) higher than HE4 60-80pMol/L.

B) One comparison demonstrates weak evidence for one test being preferred over another.

CA125 60-70U/ml may have a higher sensitivity compared to HE4 130-150pMol/L.

When CA125 at a threshold of 60-70U/ml is compared to HE4 at a threshold of 130-150pMol/L, there is no evidence for a difference in specificity but based on small number of high quality CA125 has a sensitivity of 73% (95% CI 68 to 77) which is 20.6% (95% CI 10.5 to 30.7) higher compared to HE4 130-150pMol/L.

C) For one comparison there is a statistically significant higher sensitivity for one test at a particular threshold but the specificity is lower and therefore the choice between tests depends on the trade off in the absolute numbers of test errors which in turn will be determined by the prevalence of ovarian cancer in the tested population.

CA125 at a threshold of 30-40U/ml compared to HE4 at a threshold of 130-150pMol/L sensitivity is 15% (95% CI 3 to 27.0) higher but a specificity 12% (95% CI -8 to -17) lower.

At a prevalence of 0.23% typical of a primary care presentation the higher sensitivity of CA125 at a threshold of 30-40U/ml would not result in any additional ovarian cancers detected. However, the lower specificity of CA125 at a threshold of 30-40U/ml would result in 124 (81-167) more postmenopausal women with false positive test results.

At a higher prevalence typical of specialist settings, the higher sensitivity of CA125 at a threshold of 30-40U/ml would identify an additional 6 (1 - 11) women with ovarian cancer. However, an additional 119 (78 - 160) would have a false positive result.

In postmenopausal women, based on consideration of A), B) and C) above and Table 5.11, HE4 at a threshold of 130-150pMol/L is the preferred test in testing settings with prevalence typical of primary care populations. CA125 at a threshold of 30-40U/ml is the preferred test in test settings with prevalence typical of specialist settings.

Table 5. 11: Summary of findings: Difference between HE4 and CA125 at common thresholds

						Prevalence 0.23%		Prevalence 3%		Prevalence 10%		Prevalence 30%	
Test 1	Test 2	Menopausal status	N Studies (N, DP)	Difference sensitivity (95% CI)	Difference specificity (95% CI)[SM1]	Mean diff in TP (95% CI)	Mean diff in TN (95% CI)	Mean diff in TP (95% CI)	Mean diff in TN (95% CI)	Mean diff in TP (95% CI)	Mean diff in TN (95% CI)	Mean diff in TP (95% CI)	Mean diff in TN (95% CI)
HE4 70ish	HE4 140ish	Pre	3 (296, 72)	23 (3 to 43)	-7 (-4 to -11)	1 (0 to 1) fewer TP with HE4 at 140ish	71 (36 to 107) more TN with HE4 at 140ish	7 (1 to 13) fewer TP with HE4 at 140ish	69 (35 to 104) more TN with HE4 at 140ish	23 (3 to 43) fewer TP with HE4 at 140ish	64 (32 to 96) more TN with HE4 at 140ish	69 (9 to 129) fewer TP with HE4 at 140ish	50 (25 to 75) more TN with HE4 at 140ish
HE4 70ish	CA125 35ish	Pre	6 (0, 0)	13 (0 to 25)	-24 (-10 to -38)	0 (0 to 1) more TP with CA125 at 35ish	236 (100 to 374) fewer TN with CA125 at 35ish	4 (0 to 8) more TP with CA125 at 35ish	230 (97 to 364) fewer TN with CA125 at 35ish	13 (0 to 25) more TP with CA125 at 35ish	213 (90 to 338) fewer TN with CA125 at 35ish	38 (1 to 76) more TP with CA125 at 35ish	166 (70 to 263) fewer TN with CA125 at 35ish
HE4 140ish	CA125 35ish	Pre	4 (549, 99)	26 (9 to 42)	-31 (-22 to -39)	1 (0 to 1) more TP with CA125 at 35ish	308 (217 to 393) fewer TN with CA125 at 35ish	8 (3 to 13) more TP with CA125 at 35ish	300 (210 to 382) fewer TN with CA125 at 35ish	26 (9 to 42) more TP with CA125 at 35ish	278 (195 to 355) fewer TN with CA125 at 35ish	77 (27 to 126) more TP with CA125 at 35ish	216 (152 to 276) fewer TN with CA125 at 35ish
CA125 35ish	CA125 65ish	Pre	22 (0, 0)	20 (7 to 32)	-19 (-12 to -26)	0 (0 to 1) fewer TP with CA125 at 65ish	190 (-124 to -255) more TN with CA125 at 65ish	6 (2 to 10) fewer TP with CA125 at 65ish	184 (120 to 248) more TN with CA125 at 65ish	20 (7 to 32) fewer TP with CA125 at 65ish	171 (112 to 230) more TN with CA125 at 65ish	59 (21 to 97) fewer TP with CA125 at 65ish	133 (87 to 179) more TN with CA125 at 65ish
HE4 70ish	HE4 140ish	Post	10 (0, 0)	9 (-2 to 21)	-17 (-5 to -30)	0 (0 to 0) fewer TP with HE4 at 140ish	172 (46 to 298) more TN with HE4 at 140ish	3 (1 to 6) fewer TP with HE4 at 140ish	167 (45 to 290) more TN with HE4 at 140ish	9 (2 to 21) fewer TP with HE4 at 140ish	155 (41 to 269) more TN with HE4 at 140ish	28 (7 to 63) fewer TP with HE4 at 140ish	120 (32 to 209) more TN with HE4 at 140ish

HE4 70ish	CA125 35ish	Post	4 (462, 215)	-2 (-11 to 8)	12 (24 to 0)	0 (0 to 0) more TP with CA125 at 35ish	122 (239 to 3) fewer TN with CA125 at 35ish	0 (3 to 2) more TP with CA125 at 35ish	118 (233 to 3) fewer TN with CA125 at 35ish	2 (-11 to 8) more TP with CA125 at 35ish	110 (216 to 3) fewer TN with CA125 at 35ish	5 (34 to 25) more TP with CA125 at 35ish	85 (168 to 2) fewer TN with CA125 at 35ish
HE4 70ish	CA125 65ish	Post	10 (1477, 696)	-10 (-18 to 2)	14 (26 to 1)	0 (0 to 0) fewer TP with CA125 at 65ish	135 (259 to 9) more TN with CA125 at 65ish	3 (5 to 1) fewer TP with CA125 at 65ish	131 (250 to 9) more TN with CA125 at 65ish	10 (18 to 2) fewer TP with CA125 at 65ish	122 (234 to 8) more TN with CA125 at 65ish	30 (53 to 7) fewer TP with CA125 at 65ish	95 (182 to 6) more TN with CA125 at 65ish
HE4 140ish	CA125 35ish	Post	6 (793, 392)	15 (3 to 27)	-12 (-8 to -17)	0 (0 to 1) more TP with CA125 at 35ish	124 (81 to 167) fewer TN with CA125 at 35ish	4 (1 to 8) more TP with CA125 at 35ish	120 (79 to 162) fewer TN with CA125 at 35ish	15 (3 to 27) more TP with CA125 at 35ish	112 (73 to 150) fewer TN with CA125 at 35ish	45 (9 to 81) more TP with CA125 at 35ish	87 (57 to 117) fewer TN with CA125 at 35ish
CA125 65ish	CA125 35ish	Post	3 (842, 355)	8 (2 to 14)	-11 (-7 to -16)	0 (0 to 0) more TP with CA125 at 35ish	113 (70 to 155) fewer TN with CA125 at 35ish	2 (1 to 4) more TP with CA125 at 35ish	110 (68 to 150) fewer TN with CA125 at 35ish	8 (2 to 14) more TP with CA125 at 35ish	102 (63 to 140) fewer TN with CA125 at 35ish	24 (5 to 42) more TP with CA125 at 35ish	79 (49 to 109) fewer TN with CA125 at 35ish

5.6 Summary of results

The robustness and applicability of the results are limited by the heterogeneity between studies especially the lack of representation of the entire spectrum of disease by including only EOCs or excluding BOTs in some included studies and these are identified and downgraded in quality assessment. Bearing these caveats in mind the following recommendations are suggested.

5.6.1 Thresholds CA125

For CA125, both sensitivity (higher) and specificity (lower) were significantly different when threshold of 30-40U/ml was compared to threshold cut off 60-70U/ml in both pre and postmenopausal women. Prevalence will therefore determine whether increase in sensitivity is worth decrease in specificity.

5.6.2 Thresholds HE4

HE4 at a threshold of around 130-150pMol/L is preferred in postmenopausal women because of increased specificity. In premenopausal women, however, there is again a trade off between sensitivity and specificity increased sensitivity at a threshold of 60-70pMol/L has to be balanced against a decrease in specificity when compared to a threshold of 130-150pMol/L with prevalence being the determining factor.

5.6.3 Test comparisons

In premenopausal women, HE4 at a threshold of 60-80pMol/L is the preferred test in settings with prevalence typical of primary care populations. HE4 at a threshold of 130-150pMol/L is the preferred test in test settings with prevalence typical of specialist settings.

In postmenopausal women, HE4 at a threshold of 130-150pMol/L is the preferred test in settings with prevalence typical of primary care populations. CA125 at a threshold of 30-40U/ml is the preferred test in test settings with prevalence typical of specialist settings.

CHAPTER 6: DISCUSSION OF BIOMARKERS AND COMBINATION REVIEW

I present below, an overarching discussion of results from both the biomarkers and the combination test systematic reviews. In this section, I aim to discuss the common methodological challenges, pitfalls, data gaps across both reviews. I will also summarise interpretation of results, applicability across different prevalence rates of the target condition, OC, clinical and research implications and discuss recommendations.

6.1 Biomarkers review

6.1.1 Comparison of accuracy of CA125, HE4 and OVA1

Women with symptoms suspicious of ovarian cancer will present to primary care ideally or to emergency service for investigation and management. When the women present to the primary care in UK and have symptoms suspicious of OC, Nice guidelines recommend CA125 and referral for investigation to secondary care or tertiary care.

Biomarkers are unlikely to be used on their own independently in secondary or tertiary care for making management decisions. Their most likely use is perhaps as a triage test in low prevalence in primary care to guide referral. However, the real risk of missing cancers has to be balanced against the risk of causing anxiety, effect on the quality of life for the family, the pressure on resources and patient wishes. Biomarkers are a more objective test in comparison to ultrasound or combination testing with ultrasound as a component. In addition to the ease of use, cost and the lack of the need for special training for implementation make them attractive as stand-alone tests in a low prevalence testing i.e. primary care for triaging women with an index of suspicion of OC to further testing and management. Hence, a decision based on diagnostic test performance in low prevalence settings seems most of value in assessing the utility of biomarkers.

For biomarkers, I compared the accuracy of CA125, HE4 and OVA1. OVA1 results could not be used in a meta-analysis due to limited number of available studies as this is a relatively new

biomarker. Therefore, I compared the accuracy of CA125 and HE4 at commonly used clinical thresholds for test positivity for biomarkers; in pre and postmenopausal women, considering appropriate BOT categorisation (BOT with malignant compared to BOT combined with benign or unclear categorisation for analyses) using either direct comparison of tests (both tests done in in the same patient cohort) or indirect comparisons (tests done in different patient cohort). The results are also presented in the context of prevalence and the effect of threshold and accuracy on test performance based on hypothetical prevalence at different points in the clinical pathway as the patient progresses through the triage at different clinical settings - primary care, emergency services, secondary and tertiary.

The comparison of HE4 (threshold cut off of 60-80pMol/L and 130-150pMol/L) against CA125 (30-40U/ml and 60-70U/ml) for detection of OC shows the test performance is significantly different for pre and postmenopausal women both in direct and indirect comparison studies.

Studies that report an appropriate grouping of results of borderline tumours with malignant tumours were few but showed statistically significant differences in premenopausal women for CA125 cut off of 30-40U/ml to studies where BOT categorisation was unclear or excluded. This may be partially influenced by the included studies with unclear BOT categorisation that may have included some or all studies that have grouped BOTs appropriately but unclear due to unclear reporting.

When the thresholds are compared for pre and postmenopausal women, CA125 as expected display an increase in specificity and drop in sensitivity with increasing threshold Table (see Table 5.10). When the sensitivity and specificity of the test at different test positivity threshold cut offs were compared in hypothetically constructed 4 different prevalence settings to represent the spectrum in clinical care setting the expected trade offs were seen and therefore the magnitude of test errors (prevalence) will determine whether increase in sensitivity is worth the trade off with decrease in specificity.

HE4 at a test positivity threshold of 130-150pMol/L is recommended in postmenopausal women as test positivity threshold cut off of 60-80pMol/L showed a significantly lower specificity by 17.2% (95% CI 4.6 to 29.9) with similar sensitivity. However, in premenopausal women, the evidence is less clear as it again shows trade offs between sensitivity and specificity at different threshold test positivity cut offs; an increase in sensitivity at a threshold of 60-

80pMol/L has to be balanced against a decrease in specificity compared to a threshold of 130-150pMol/L to determine if the increase in sensitivity is worth the drop in specificity with prevalence being the arbitrary factor.

When CA125 and HE4 are compared at different thresholds, there were tests that showed i) a clear advantage of one test over the other (HE4 at a threshold of 130-150pMol/L is compared to CA125 at a threshold of 60-70U/ml) ii) no significant difference between tests (CA125 at a threshold of 60-70U/ml is compared to HE4 at a threshold of 60-80pmol/L) iii) there is a statistically significant difference in both the sensitivity and specificity between the two tests, with an increase in sensitivity combined with a decrease in specificity, or vice versa (all the other comparisons except the two mentioned above). Based on these considerations, 1) In premenopausal women HE4 at a threshold of 60-80pMol/L and 130-150pMol/L is the preferred test in settings with prevalence that resembles primary care populations and specialist settings respectively 2) In postmenopausal women, HE4 at a threshold of 130-150pMol/L is the preferred test in testing settings with prevalence typical of primary care populations and CA125 at a threshold of 30-40U/ml is the preferred test in test settings with prevalence typical of specialist settings. (Table 5.11).

Studies that report an appropriate grouping of borderline results with malignant were few a trend was observed (in CA 125 biomarker studies) for higher average estimates of sensitivity from studies that either excluded borderline tumours from analysis or where the classification of borderline tumours for analysis was unclear compared to studies where borderline tumours were classified as positive (grouped with malignant). This difference was significant for post-menopausal women.

6.1.2 Applicability of findings to the review question

The above results have to be interpreted carefully as comparisons are based on a small number of comparison studies. In addition, all the tests were conducted in high prevalence settings and the mathematical construct of test behaviour in low prevalence settings may not hold up in real life testing. The comparison studies at different threshold cut offs do not show any particular test where the accuracy outperforms the other to make a hard recommendation. The comparisons show the expected trade-off between sensitivity and specificity and in the context of prevalence may aid the clinicians and patients to guide the decisions within the context.

Table 5.11 presents results for four prevalence scenarios. Magnitude of test errors (prevalence) and or the significance and implications of false positives or false negatives or will determine whether increase in sensitivity is worth decrease in specificity.

Therefore, bearing the limitations discussed, I believe, HE4 is recommended at threshold test positive cut off of 60-80pMol/L for premenopausal women and 130-150pMol/L for postmenopausal women in low prevalence settings.

6.2 Combination review

6.2.1 Comparison of the accuracy of RMI1, ROMA, LR2, ACOG v3 (CA125) and ACOG v3 (OVA1)

In the UK, women with a suspected adnexal mass and with either an abnormal CA125 or US examination are referred for investigation to secondary care where an RMI1 is performed. Therefore, the performance of ROMA, LR2, ACOG v3 (CA125) and ACOG v3 (OVA1) is investigated relative to RMI1. A statistical comparison between ADNEX and other tests was not possible because only one ADNEX study was included in this review.

Due to variation in test positivity thresholds, a comparison of accuracy with reference to a fixed specificity of 90% was undertaken; this was chosen in keeping with a clinically acceptable false positive rate reflected in previous research and RCOG Guidelines. In premenopausal women, at a fixed specificity of 90%, LR2 and ROMA were found to have a statistically significantly higher sensitivity compared to RMI1. ACOG v3 (CA125) and ACOG v3 (OVA1) were observed to have a lower sensitivity compared to RMI1 at a fixed specificity of 90% but this was not statistically significant.

6.2.2 Applicability of findings to the review question

Translating these findings to estimates from an on-going trial of the prevalence of ovarian cancer in postmenopausal women in secondary care of 10% [231].

For a hypothetical cohort of 1000 premenopausal women in secondary care, assuming a prevalence of ovarian cancer of 10% (100 women have ovarian cancer and 900 women do not) a fixed test specificity of 90% would result in 90 false positives.

The results of the analyses suggest that in premenopausal women 18 (14 to 21) would have their cancer missed (false negatives) if tested with LR2, leading to a delay in diagnosis, morbidity and possibly mortality, 25 (20 to 31) would have their ovarian cancer missed by ROMA, 41 (35 to 46) by RMI, 27 (19 to 36) by ACOG v3 (CA125) and 18 (6 to 43) by ACOG vs OVA1.

In postmenopausal women there was no evidence for statistically significant differences in sensitivity for ROMA, LR2, ACOG v3 (CA125) or ACOG v3 (OVA1) at a fixed specificity of 90%. The results of the analyses suggest that in postmenopausal women 18 (9 to 31) would have their cancer missed if tested with LR2, 18 (11 to 27) would have their ovarian cancer missed if tested by ROMA, 28 (21 to 36) by RMI, 29 (18 to 44) by ACOG v3 (CA125) and 39 (10 to 78) by ACOG v3 OVA1.

Corresponding estimates adopting a baseline prevalence of ovarian cancer of 30%, (representative of a tertiary care setting) with a fixed specificity of 90% are illustrated in the Summary of Findings (Table 4.8 and 4.9).

It is important to note that in practice women and clinicians may be willing to accept a reduction in specificity to 90% to achieve a higher sensitivity and that this is likely to be influenced by factors such as baseline risk (prevalence) of ovarian cancer which will be dependent on healthcare setting and menopausal status and the adverse consequences of unnecessary investigation and treatment, for example loss of fertility.

6.3 Strengths and weaknesses of the review

6.3.1 Strengths

The strengths of this review are based on the thorough approach to the search, data extraction and data analysis and presentation of results and summaries of findings, all of which aimed at providing the best estimates of test accuracy relevant for clinical practice.

This is a comprehensive review of test combinations for the diagnosis of ovarian cancer. Although literature searches were completed in 2015 this review remains the most up to date comprehensive review to my knowledge. Sensitive search strategies were used to capture relevant literature regardless of country of publication, publication status (published or unpublished), language or clinical setting (primary care or specialist care (secondary and tertiary). A comprehensive systematic search including a search for studies performed in primary care (family practice). No studies were identified of the accuracy of biomarkers to diagnose ovarian cancer in a primary care setting and this is identified as a gap in knowledge. The studies were analysed and test performance compared both by separate patient group (postmenopausal and premenopausal women) and by clinically relevant test thresholds in each patient group. This sought to identify the accuracy of each test that would have not only the most relevance test but the most useful threshold in clinical practice.

Novel features of this review include systematic investigation of the effects of menopausal status and classification of borderline tumours on estimates of test accuracy and statistical comparison of test combinations relevant to clinical practice at the time of writing. An attempt was made to mitigate against heterogeneity by restricting the analysis to primary tumours of adnexal origin and where this was not possible or unclear in studies with mixed primary, recurrent and metastatic disease, this was reflected in downgrading of quality assessment.

Previous reviews have not recognised the importance of separate analysis of pre and postmenopausal women at the same time as ensuring meta-analysis is adequately analysed for the test thresholds with different test performance. The test accuracy results were investigated for a range of thresholds for each biomarker using both numerical values (+/-10) and +/-2 standard deviations; results of both approaches were similar demonstrating the robustness of the analysis. Finally, the effect of head to head comparison of tests and appropriate categorisation of borderline tumours on test accuracy was undertaken by analysing results using only studies that included direct comparison and studies including and excluding appropriate BOTs categorisation respectively.

6.3.2 Weaknesses

Due to time and resource constraints non English Language studies were not considered for data extraction and analyses. The impact of this omission on study findings is unknown. A

further weakness of the review is the fact that the search strategy is now 4 years old. I am aware of more recent primary publications that may have an impact on the conclusions of this review, in particular publication of several papers investigating the accuracy of ADNEX. In this review, only one ADNEX study was identified and therefore it was not possible to include this test in statistical comparisons of test accuracy. Factors to consider regarding the importance of this omission include the fact that variables in the ADNEX model overlap considerably with components in the test combination LR2, which outperformed RMI in premenopausal women in the comparative analysis. However, variables included in the ADNEX model include whether US examination is being performed in secondary or tertiary care settings which suggests estimates of accuracy will have limited applicability in primary care settings.

The majority of the limitations of this review originate from deficiencies in the primary evidence base. Lack of data and poor reporting precluded quality assessment and investigation of potential important sources of variation in test accuracy estimates. These potential sources included clinical setting (primary versus specialist), target condition (in particular primary versus recurrent and metastatic disease) and cancer histological subtype and stage; included studies were very mixed with respect to the range of ovarian pathology included with some for example restricting to epithelial ovarian cancer whilst others included metastatic disease to the ovaries in the definition of disease positive which could not be disaggregated for the purposes of analysis. Lack of disaggregation in primary studies was most marked when considering pre and postmenopausal subgroups separately.

6.4 Applicability of findings to the review question

This review question aimed to answer the question of the accuracy of test combinations (symptoms and signs, imaging and biomarkers) for women with symptoms suspicious for ovarian cancer. In the UK, the National Institute for Health and Care Excellence (NICE)[115] and the Royal College of Obstetrician and Gynaecologists (RCOG)[232] recommend women with suspicious symptoms presenting in primary care should receive additional investigations with CA125 and if raised, a transvaginal ultrasound (TVS) scan should also be performed prior to referral to secondary care. Once in secondary care either RMI1 or ultrasound examination using International Ovarian Tumour Analysis (IOTA) criteria are used to determine if onward referral to tertiary care (gynaecological oncologist) is required for further management.[232]

The American College of Obstetrics and Gynecology (ACOG)[24] recommends TVS as the initial test of choice if physical examination suggests the presence of an adnexal mass. Subsequent referral to tertiary care is recommended in the presence of any one of risk factors (family history), abnormal findings on imaging, a raised CA125, a raised OVA1 or a raised ROMA test.

In the UK therefore, the presence of suspicious symptoms is a trigger for further investigation which includes testing with biomarkers and US in primary care and the use of combination tests in secondary care. The majority of included studies were judged as at high or unclear applicability to the review question on the basis that women were either asymptomatic or it was not clear if they were symptomatic at the point of index test use. Further no studies of the accuracy of test combinations to diagnose ovarian cancer in a generalist setting were identified. The majority of included studies have a prevalence of ovarian cancer that is in keeping with tertiary hospitals. Prevalence estimates varied in different reviews based on the studies; in premenopausal women median prevalence was CA125 studies - 22% (range 3% -18%) HE4 studies - 25% (range 8% - 81%) combination studies - 19% (range 9%- 28%) and postmenopausal women CA125 studies - 46% (range 8% -71%) HE4 studies - 50% (range 18% - 81%) combination studies - 44% (range 40%- 57%) which is higher than would be expected for either premenopausal or post-menopausal women indicating advanced position in the patient pathway. Estimates from the literature estimate the prevalence of ovarian cancer in women with symptoms was 0.23% in primary care.[83, 233] Test accuracy estimates from this review are therefore unlikely to be applicable to generalist settings where the prevalence of ovarian cancer is lower and the spectrum of the tested population more heterogeneous. With the exception of one study[138] all included women had a confirmed adnexal mass at the point of testing. This single study was identified as an outlier, with the lowest estimated specificity (53%) and highest sensitivity (94%). Early in the ovarian cancer testing pathway it would be expected that test specificity would be lower, particularly in premenopausal women, reflecting a more diverse population in terms of comorbidity (for example endometriosis and functional benign tumours) and normal physiological processes such as the menstrual cycle, which are causes of false positive test results and a lower test specificity. Thus, in generalist settings the relationship between sensitivity and specificity and menopausal status observed in this review may be reversed. The implication is that estimates of the accuracy of index tests in this review are likely to be applicable to specialist settings (secondary and tertiary care) but are unlikely to be applicable to women without a confirmed adnexal mass, i.e. in primary care settings.

With the exception of ROMA, all combination test included in this review included an US imaging component, particularly the LR2 and ADNEX models. The applicability of the review findings to practice will therefore be influenced by the skill of the US operator. With the exception of one LR2 study[203] all other studies were judged as at high or unclear risk of bias on the basis that sonographers were specialists or their level of skill was not reported; therefore it cannot be assumed that the performance of RMI, LR2, ACOG v3 (CA125), ACOG v3 (OVA1) or ADNEX could be replicated by non specialist sonographers as would be the case for investigations initiated in primary care or secondary care settings.

A further concern regarding the applicability of this review's findings is the fact that in the majority of studies borderline tumours were either excluded or it was unclear how they were classified for estimation of test accuracy (excluded, classified as malignant or classified as benign). Borderline ovarian tumours account for an estimated 15% of ovarian tumours[234] and excluding them from estimation of test accuracy will artificially alter the spectrum of disease compared to that occurring in practice. No evidence was found in the combination review to demonstrate that management of borderline tumours significantly affected estimates of accuracy. However, this investigation is limited by the volume of data available, lack of clarity in reporting and the possible effects of confounding. In a similar investigation undertaken for borderline tumours in the biochemical markers for the diagnosis of ovarian cancer, a trend was observed (in CA125 biomarker studies- larger dataset,) for higher average estimates of sensitivity from studies that either excluded borderline tumours from analysis or where the classification of borderline tumours for analysis was unclear compared to studies where borderline tumours were classified as positive (grouped with malignant). This difference was significant for postmenopausal women. It is therefore possible that estimates of sensitivity may be overestimated in this review.

6.5 Conclusions

6.5.1 Implications for practice

This review has demonstrated that menopausal status causes consistent and significant changes in disease spectrum which is reflected in differences in test performance for women presenting with an adnexal mass. The implications of this finding for practice is that the utility of tests for

diagnosing ovarian cancer should be considered separately in premenopausal and postmenopausal women.

For biomarkers, in premenopausal women, HE4 at a threshold of 60-80pMol/L and 130-150pMol/L is the preferred test in test settings with prevalence representative of primary care and specialist settings respectively; In postmenopausal women, HE4 at a threshold of 130-150pMol/L and CA125 at a threshold of 30-40U/ml is the preferred test in test settings with prevalence representative of primary care and specialist settings respectively.

The other advantages the biomarkers offer as a triage test in the primary care setting are they are less expensive (HE4 and CA125) less invasive, easier to do, less uncomfortable for patients, technically less challenging, and more objectively interpreted.

The implications of the findings from both systematic reviews to practice for premenopausal women presenting with an adnexal mass suspicious for ovarian cancer, LR2 or ROMA are the test combinations of choice to determine future management. Current Guidelines recommending RMI as a diagnostic or triage test in premenopausal women in secondary care settings should be changed to recommend LR2 or ROMA. Similarly, CA125 threshold of 200U/ml as recommended by the RCOG guidance is based on a single study and has a performance of sensitivity of 29% and specificity of 98% in premenopausal women. Given the extremely limited data on which this recommendation is based, this review recommends that this threshold should not be used as a cut off as a triage tool for referral from secondary to tertiary care. Premenopausal patients, being investigated with adnexal masses in secondary care would be more accurately triaged into tertiary care with the use of combination tests such as LR2 or ROMA.

For postmenopausal women presenting with an adnexal mass in specialist settings, no test combination demonstrated statistically significant performance differently to RMI.

The single ADNEX study could not be included in the statistical comparison of tests and I, therefore have to acknowledge the uncertainty about the relative performance of ADNEX.

Choice of which combination test (LR2 or ROMA) should replace RMI in practice in premenopausal women in secondary care will require consideration of relative costs and the feasibility of introducing the test. The adoption of ROMA does not rely on availability of

ultrasound expertise but would require investment in laboratory facilities for processing HE4 tests. No evidence was identified to support recommendation of a specific ROMA threshold for use in clinical practice beyond the ranges used in included studies: between 7.4 to 13.1 in premenopausal women and 14.4 to 27.7 in postmenopausal women. The adoption of LR2 into routine practice would require investment in sonographer training.

The implications of this for a test used in primary/Secondary care would be that 90% (900) women would be referred to specialist care appropriately. Inappropriate referral cause anxiety for the woman concerned as well as further inappropriate testing and possible unnecessary investigations. In addition, false positive referrals to secondary care divert resources from those women who actually have malignancy. The added implications of this for a test used in specialist care is that unnecessary false positive results may receive extensive surgery with consequent risk to health and to preservation of fertility for premenopausal women.

The implications of these findings for women presenting in generalist settings, early on in the diagnostic pathway, is less clear. Participants in included studies had a confirmed adnexal mass and the presence of symptoms at the time of testing was mostly not reported. The median prevalence of OC in the different reviews was upwards of 19% and as high as 50%. Included participants are therefore likely to represent a referred population rather than a population in whom referral is being considered. The comparative accuracy of tests observed here may also not be stable when transferred to non-specialist settings.

6.5.2 Implications for research

The priority for research at this time is to update this review to consider more recent publications (after 2015), in particular, to include ADNEX in a comparative analysis of accuracy. However, it should be noted that the ADNEX model includes a variable for the setting in which the model is applied (oncology versus other hospitals) and so whilst its comparison may be robust to the setting in which included studies have been conducted, the model would not be applicable to non-specialist settings in its current form.

Due to variation in thresholds reported in included studies and in the absence of evidence for clinically significant threshold effects for individual tests the approach to comparative analysis for combination tests was to fix specificity at a clinically applicable threshold of 90%. However, the difference in test performance in premenopausal compared to postmenopausal

women demonstrated in this review raises the possibility that using different tests in pre and postmenopausal women may be necessary to optimise diagnostic utility. In premenopausal women avoiding unnecessary surgery and preserving fertility by maximising specificity and minimising false positives may be considered relatively more important than in postmenopausal women. Primary research investigating the performance of tests at clinically agreed thresholds, in a clinically suspected population and separately in pre and postmenopausal women would facilitate exploration of thresholds and tests specific to the menopausal status of women being tested. Indeed, the results from a longitudinal test accuracy study to validate risk scores in symptomatic women Refining Ovarian Cancer Test accuracy Scores[231] is awaited. Primary studies should in future clearly report the occurrence of tumours found to be borderline at histology. Separate classification of these tumour types will ensure test accuracy research can be used flexibility as knowledge advances about the malignant potential of such tumours and their most effective management.

This review has demonstrated the lack of research on the accuracy of tests early in the ovarian cancer testing pathway: the first clinical encounter of symptomatic women in whom ovarian cancer is being considered as a differential diagnosis. The review also identifies that most studies have a high prevalence of cancer, suggesting the impact of prior testing and specialist care settings. The diagnostic utility of tests in generalist settings could be evaluated on the basis of current NICE Guidelines (NG 12) ; taking into account the financial and clinical costs of broadening the recommendations, the guideline development group agreed to use a 3% PPV threshold value to underpin recommendations for suspected cancer pathway referrals and urgent direct access investigations. Future studies performed earlier in the ovarian cancer diagnostic pathway should also take care to report aspects of setting that will have a bearing on test performance such as healthcare setting (for example primary care or rapid access hospital clinic), presenting signs and symptoms and details of test conduct such as the skill of those eliciting symptoms, signs and conducting and interpreting imaging tests. In populations such as these that are more heterogeneous the use of rigorous clinical follow up as a reference standard in index test negative cases should be pursued.

CHAPTER 7: SUMMARY OF THESIS

7.1 Background

This thesis aimed to identify the best diagnostic tests for ovarian cancer through narrative review of reviews and systematic reviews. An initial scoping search identified a large volume of literature including many systematic reviews. Thus, faced with the fundamental question as to whether the planned systematic reviews were justified, I conducted a review of reviews to check if the research question had been previously answered and to assess the quality of the evidence. I found that published systematic reviews were of variable quality and scope and the answer lacking in clarity, both for accuracy of tests and applicability to clinical practice. Therefore, I conducted a further 2 systematic reviews to evaluate the accuracy, applicability and currency of biomarkers alone and combination testing in the diagnosis of ovarian cancer in symptomatic women. The search and methodology employed for these reviews are common. This section summarises the main findings and discusses the interpretation of results for clinical practice and research including the Quantity and quality of evidence.

7.2 Review of existing reviews

The Review of reviews includes 20 publications reporting 23 separate systematic reviews. Studies that evaluated tests that are currently used clinically or FDA approved for use in the initial diagnosis and management of ovarian cancer were included. Two reviews investigated the accuracy of symptoms (both individual and in combination), 27 investigated the accuracy of a range of biomarkers (CA125, HE4, OVA1) and 35 investigated the accuracy of test combinations (RMI1-4, ROMA, ACOG, LR1 and LR2).

7.2.1 Quality assessment

The review of existing reviews found methodological blind spots in; Question formulation with missing data on many key variables including: presentation, prior testing, menopausal status, test positivity thresholds of index test, target condition and reference standard leading to a lack of objectivity and transparency in inclusion of studies. Heterogeneity of ovarian cancer is a key

consideration for DTA and can affect test accuracy and recommendations. For e.g. defining ovarian cancer or benign disease by pathology type, symptomatic or asymptomatic are essential while considering inclusion criteria to enable selection of right studies for meta-analysis, comparison of tests, sensitivity analysis and make apt recommendations. The quality assessment also did not provide clarity or details on items pertaining to risk of bias and applicability (ROB and ROA). The applicability of the reviews could not be assessed due to lack of information regarding clinical presentation and prior testing in primary studies and its relevance to accuracy, with only one included review commenting on this key aspect. None of the reviews presented all the relevant information on PppITR. Quality assessment was unclear in 75% of reviews and hierarchical models was not used in 75% of reviews; important sources of heterogeneity are not incorporated, all resulting in poor applicability to clinical practice.

7.2.2 Reporting as assessed by PRISMA DTA checklist

All the reviews did not meet the reporting standard. Key elements of methodology such as selection criteria, study selection, data extraction or planned syntheses were lacking and where available lacked clarity; for e.g. even though the index test and the reference standard were the best reported items in the majority of the reviews, threshold of tests was not clarified. The methods described for meta-analyses often did not clarify the criteria for studies being pooled and no detail on how differences in threshold, histology and reference standard would be handled in analyses. Similar deficiencies were identified in the reporting of results. The lack of details in reporting precludes any assessment or comment regarding any study specific tailoring of the QUADAS tool that may have been undertaken to appropriately assess all aspects of ROB/ROA. All but 1 included reviews reported on accuracy of tests but stratified results by menopausal status/ BOTs/ stage or additional analyses investigating heterogeneity were reported in less than 25% of the studies.

7.2.3 Conclusion

In conclusion, my assessment of the existing reviews i) did not identify any health care models that compared all the tests head to head as stratified by menopausal status ii) identified major limitations in the existing reviews where differentiated results for pre and postmenopausal results were available, iii) did not address prevalence and spectrum of ovarian cancer in the populations studied. iv) Showed lack of clarity on inclusion, prevalence and the accuracy of

tests for BOTS; potentially affecting the accuracy of the overall results, as well as limiting clinical applicability for use in management which is a significant limitation v) Needs improved reporting of results using PRISMA DTA checklist vi) In my opinion, cannot be used to inform clinical practice.

7.3 Biomarkers and combination review

The biomarker and combination reviews identified common methodological issues in included primary studies. For both reviews, clinical pathways of patients from presentation to decision for surgery intervention were not detailed in any included studies and the majority of the studies only included women undergoing surgery for adnexal mass.

7.3.1 Quality of included primary studies

The majority of included studies showed unclear or high ROB and ROA due to issues with i) patient selection as inadequate information on symptoms ii) reference standard when information regarding inclusion of BOTs were unclear iii) flow and timing domain with lack of clarity of interval between the conduct of the index test and the reference standard and verification bias as studies only included women undergoing surgery. All studies for biomarkers were considered low ROB due to inherent objectivity of interpretation unlike studies incorporating ultrasound (RMI, LR2, ACOG and variations, ADNEX) where there is potential for confirmation bias i.e. knowledge about disease status affecting index test interpretation. Appropriate categorisation of BOTs, menopausal status and direct vs indirect comparison of tests in studies were considered when analyses were conducted.

7.3.2 Results of the meta-analyses

Results of the meta-analysis of the combination review

The meta-analyses finds i) a difference in the accuracy of diagnostic tests by menopausal status. Broadly, tests demonstrate greater sensitivity and lower specificity in postmenopausal women across all versions of index tests at all thresholds identified in the review ii) LR2 or ROMA demonstrate the best sensitivity at fixed specificity of 90% in premenopausal women. No test combination performance showed statistically significant difference compared to RMI in

postmenopausal women iii) Analysis of accuracy for tests in BOTs was only possible for ROMA and showed no difference in test accuracy iv) No significant accuracy difference by threshold was seen for ROMA or RMI1 v) Limited reporting on expertise of the operator in studies incorporating USS.

Statistical comparison between ADNEX and any test was not possible because only one ADNEX study was included in this review. Prevalence estimates in premenopausal women varied between 9% and 28%, median 19% and in postmenopausal women 40% to 57%, median 44% which represents a very highly selected population which represents tertiary care setting. Applicability to clinical practice is limited because of lack of investigation of important sources of heterogeneity and lack of primary studies in settings of lower prevalence, i.e. unselected populations

Results of the meta-analyses of the biomarker review

Meta-analyses were performed comparing different thresholds within each test for pre and postmenopausal women with appropriate BOT categorisation when enough studies were available and also for direct and indirect comparison.

The meta-analyses found difference in the accuracy of diagnostic tests by menopausal status.

1) For CA125 prevalence will determine the preferred test as sensitivity (higher) and specificity (lower) were significantly different between threshold cut off of 30-40U/ml and 60-70U/ml in both pre and postmenopausal women. The prevalence of the test setting will ordain if the increase in sensitivity is worth the decrease in specificity. 2) HE4 at a threshold of around 130-150pMol/ is recommended in postmenopausal women because of increased specificity. 3) In premenopausal women, there is again a trade off between sensitivity and specificity between test positivity cut off threshold for 60-70 pMol/L and 130-150pMol/L. Therefore, increased sensitivity at a threshold of 60-70 pMol/L has to be balanced against a decrease in specificity at a threshold of 130-150pMol/L with prevalence at the point of testing guiding the choice of threshold used. 4) Between HE4 is and CA125, HE4 is recommended in premenopausal women; i) HE4 at a threshold of 60-80pMol/L and 130-150pMol/L is the recommended test when the prevalence at the point of testing represents a typical primary care populations and specialist settings respectively. 5) In postmenopausal women, i) HE4 at a threshold of 130-150pMol/L is the preferred test in settings with prevalence typical of primary care populations

ii) CA125 at a threshold of 30-40U/ml is the preferred test in test settings with prevalence typical of specialist settings. 6) Analysis of accuracy for tests in BOTs was only possible for CA125 threshold of 30-40U/ml and shows statistically significant difference in postmenopausal women.

7.4 Implications

7.4.1 Implications of review of existing reviews

The narrative review of reviews influenced the search date and strategy of the systematic reviews on biomarkers and combinations. The deficiencies and limitations identified during the review of reviews helped tailor the quality assessment tool to provide information that is transparent and provide context to testing. The lack of clinical applicability of the data in the existing reviews informed the analyses on menopausal status, test thresholds, BOT categorisation and the plan for heterogeneity analyses for the systematic reviews.

7.4.2 Clinical Implications of combination and biomarker review

The review identifies no studies investigating the accuracy of biomarkers to diagnose primary ovarian cancer in a primary care setting.

For Premenopausal women presenting with an adnexal mass suspicious for ovarian cancer, LR2 or ROMA are the test combinations of choice to determine future management. RMI as a diagnostic or triage test in premenopausal women in secondary care settings should be replaced by LR2 or ROMA. This analyses showed no statistical differences between diagnostic tests in comparison to RMI in postmenopausal women presenting with an adnexal mass in specialist settings. As all the included studies were conducted in tertiary or secondary settings, implications of these findings for women presenting in generalist settings, early on in the diagnostic pathway, is less clear.

In prevalence representative of primary care setting, HE4 is recommended at threshold test positive cut off of 60-80pMol/L for premenopausal women and 130-150pMol/L for postmenopausal women. In prevalence representative of secondary care setting, HE4 at test

positive cut off of 60- 80pMol/L is recommended for premenopausal women and CA125 at threshold test positive cut off 30-40U/ml for postmenopausal women.

However, one must be mindful while interpreting these recommendations, i) they are based on studies which were all conducted in secondary care or tertiary care setting with varying prevalence ii) they are based on limited number of studies and iii) recommendations are based on a mathematical construct that likely represent average prevalence in primary and secondary care settings

7.4.3 Recommendations for future research

Studies based in lower prevalence and in the primary care setting are required to enable recommendations for primary care to improve triaging and resource utilisation. Primary research investigating the performance of tests in a clinically suspected population, at clinically agreed thresholds and reporting results separately in pre and postmenopausal women would facilitate exploration of thresholds and tests specific to the menopausal status and tailor recommendations. This would also be a benchmark for new tests discovered in the future to determine utility and role. The clinical presentation and setting of recruitment be clearly reported to determine relevant clinical utility and applicability to define the role of the test as triage, replacement or add on. The Information on clinical pathway needs to be reported to enable judging the results in context and pool studies appropriately for meta-analyses and reduce the impact of heterogeneity (symptomatic women/ prior testing/ clinical setting of the test). The expertise of the sonographers where ultrasound is a component of the test combination should be reported to enable applicability and implications on training and resource. Primary research investigating the performance of tests in a clinically suspected population, at clinically agreed thresholds and reporting results separately in pre and postmenopausal women would facilitate exploration of thresholds and tests specific to the menopausal status and tailor recommendations. Given what we now know regarding the biological behaviour primary studies should in future clearly report the histological details of tumours especially borderline tumours and early stage to enable patient tailored decisions and drive future goals for research. Test accuracy studies should report on all women undergoing testing (test negative patients and patients managed conservatively) using follow-up as the reference standard to avoid verification bias and enable patient counselling for testing and risk of false negative in low risk patients.

7.5 Strength and weakness

7.5.1 Strength and weakness of review of existing reviews

To my knowledge, this is the only review of reviews evaluating the currency and applicability of the existing diagnostic test accuracy reviews in ovarian cancer. It is also the first and at the time of writing, the only review that has assessed the reporting quality of the existing DTAs on ovarian cancer using the newly published PRISMA DTA checklist. The search is comprehensive and has evaluated all the tests in routine clinical practice. Non-English reviews are not included and double data screening and evaluation was not undertaken due to lack of resources. Random samples of review of reviews were double data evaluated as a pilot sample for consistency of understanding and use.

7.5.2 Strengths and weakness of biomarker and combination review

Comparison of all test combinations relevant to routine practise was undertaken. Based on the knowledge and understanding gained from review of existing reviews, gaps were identified and efforts made to tailor the review to answer the gaps on knowledge and make the review clinically relevant and limit the impact of heterogeneity. Systematic evaluation of effect of menopausal status, threshold effects and systematic evaluation of impact of categorisation of BOTs were undertaken to tailor recommendations and improve clinical utility. In the summary of findings tables, likely numbers of women with test results for biomarkers HE4 and CA125 at different test thresholds for different prevalence of ovarian cancer representing different steps in the clinical pathway of diagnosis are provided to demonstrate the clinical applicability and accuracy of test in different settings.

There was a significant interval between the search date and review results and this will be addressed by publishing an update soon. Foreign and unpublished literatures were excluded. Some of the objectives could not be achieved due to limitations in the primary studies.

7.6 Conclusion

In conclusion, my thesis finds key methodological issues in literature that preclude clear recommendations for diagnostic testing in women with adnexal masses. With the caveat of these limitations, I recommend i) Threshold test positivity of 60-80pMol/L and 130-150pMol/L for HE4 in pre and postmenopausal women for low prevalence setting ii) ROMA or LR2 in premenopausal women to replace RMI in secondary/tertiary setting. For postmenopausal women, I am unable to make similar recommendations. Further research is needed to overcome the issues identified.

APPENDIX

Appendix 1 **: Biomarkers Search strategy.**

Database: Ovid MEDLINE(R) 1946 to April Week 3 2015

1 exp Ovarian Neoplasms/di
2 exp Adnexal Diseases/di
3 ((ovar$ or adnexal or fallopian or peritoneal$ or pelvic) adj3 (cancer$ or carcinoma$ or malignan$ or mass or masses or cyst or cysts or neoplas$ or tumour$ or tumor$)).tw.
4 ((borderline or border line) adj4 ovar$).tw.
5 exp Fallopian Tube Neoplasms/di
6 exp Peritoneal Neoplasms/di
7 exp Pelvic Neoplasms/di
8 ((epithelial or germ cell) adj5 ovar$).tw.
9 or/1-8
10 exp Tumor Markers, Biological/
11 exp Biological Markers/
12 Proteomics/
13 Genetic Markers/
14 Metabolomics/
15 multiplex$.tw.
16 multivariate.tw.
17 (CA125 or CA-125 or HE4 or OVA 1 or OVA1 or HCG or LDH or AFP or CEA).tw.
18 CA-125 Antigen/
19 Chorionic Gonadotropin/
20 L-Lactate Dehydrogenase/
21 alpha-Fetoproteins/
22 Carcinoembryonic Antigen/
23 or/10-22
24 9 and 23
25 limit 24 to (humans and yr="2009-2015")

Appendix 2 **: Data extraction form**

Please enter your initials (NR, RC)

STUDY IDENTIFICATION AND STUDY TYPE

Study ID (number allocated in included studies PDFs and 'notes' field in reference manager)	Comments
Study authors (surname and year) Country in which study conducted Study design Please choose from: - 'Prospective' cross-sectional test accuracy study **(P CS)** - 'Retrospective' cross-sectional test accuracy study **(R CS)** - Case-control test accuracy study **(CC)** - Comparison of the accuracy of tests or testing strategies in 2 different populations (e.g. a randomised trial of tests sor testing strategies) **(Between-person comparison - BPC)** - Within-person comparison of test accuracy **(WPC)** - Unclear **(U)**	

PATIENT SELECTION DETAIL

For studies comparing two index tests or testing strategies in different patient populations complete details for each patient population (copy and paste table if necessary)

FOR NON-COMPARATIVE STUDIES OR WITHIN-PERSON TEST COMPARISONS (WPC) assessing the accuracy of one index test or one index testing strategy **describe methods of participant selection as reported** (cut and paste from paper if possible)		**Comments**
Include total number of study participants		
FOR BETWEEN-PERSON COMPARATIVE STUDIES (BPC) of two index tests or testing strategies in different patient populations **describe methods of participant selection receiving each index test or testing strategy as reported**		
(cut and paste from paper if possible)		
Include total number of study participants receiving each test or testing strategy		
Clinical setting mentioned	**Y/N/U**	
If yes	**Primary/community**	
	Secondary/hospital/cancer unit/cancer centre	
	NR	
Risk factors such as age, menopause, family history, BRCA status, other cancers mentioned in the study		

INCLUDED PATIENT CHARACTERISTICS DETAIL

For studies comparing two index tests or testing strategies in different patient populations complete details for each patient population (copy and paste table if necessary)

FOR NON-COMPARATIVE STUDIES OR WITHIN-PERSON TEST COMPARISONS (WPC) describe characteristics of included patients **as reported (cut and paste from paper if possible)**		Comments
FOR BETWEEN-PERSON COMPARATIVE STUDIES (BPC) **of two index tests or testing strategies in different patient populations** describe characteristics of participants receiving each index test or testing strategy as reported **(cut and paste from paper if possible)**		
Age as reported or not reported ('NR') *(delete options as necessary)*	**- Age range:** **- Age mean (SD):**	
Menopausal status (n/%)	pre	Post
Prior test(s)	**Symptoms** **Signs** **Biomarker/s**	
Histology	**Number (%)**	
Benign	**Number (%)**	
Endometriosis		
Others		
Tumours of low malignant potential (LMP/borderline)	**Number (%)**	
Malignant	**Number (%)**	
I	**Number (%)**	
II	**Number (%)**	

III	**Number (%)**
IV	**Number (%)**

PATIENT SELECTION RISK OF BIAS

PATIENT SELECTION	
A. Risk of bias	
Describe methods of patient selection:	
a) Was a consecutive or random sample of patients enrolled?	Yes/No/Unclear
b) Was a case-control design avoided?	Yes/No/Unclear
c) Did the study avoid inappropriate exclusions?	Yes/No/Unclear
a) include all ages and regardless of menopausal status or justify restrictions	
b) include all stages of ovarian cancer.	
c) include co-morbidities such as infertility and endometriosis	
Could the selection of patients have introduced bias?	**RISK: LOW/HIGH/UNCLEAR**
If a) and b) and c) 'YES' = low risk of bias	
If a) or b) or c) 'No' = high risk of bias	
If a) or b) or c) 'Unclear' = unclear risk of bias	

B. Concerns regarding applicability	
Describe included patients (prior testing, presentation, intended use of index test and setting):	
Is there concern that the included patients do not match the review question? ***a) Patients all symptomatic OR symptomatic and asymptomatic can be disaggregated*** ***b) Prior tests primary care: self reported symptoms*** ***c) Prior tests secondary care: self reported symptoms OR self reported symptoms PLUS one or more of biochemical markers and ultrasound***	**CONCERN: LOW/HIGH/UNCLEAR** **Low - a) and b) and C) Yes** **High - a) or b) or C) No** **Unclear - a) or b) or C) Unclear**

INDEX TEST(S) DETAILS

For studies comparing two index tests or testing strategies in different patient populations complete details for each index test or testing strategy (copy and paste table if necessary)

INDEX TEST(S)	Test (note the type of symptom, biomarker, ultrasound variable)	Test threshold (what constitutes abnormal test symptoms, signs, biomarkers or USS: threshold value or fixed value of abnormality	Threshold value or fixed value of abnormality	Clinical setting in which index test performed	If operator was blinded to previous test (s) result	Describe what prior tests information was available to those interpreting index test	Detail about conduct of index test that might be a source of heterogeneity (e.g. experience of operator (ultrasound, symptoms), type of technology (biomarkers)) - For test combinations test order and rule for combining tests
	Symptoms (list)	Yes No NR	(Number of symptoms and time element)	Primary/community/family practice Secondary: hospital/cancer unit/cancer centre/gyn oncologist NR/U/Mixed	Yes No NR		
	Signs	Yes No NR		Primary/community/family practice Secondary: hospital/cancer unit/cancer centre/gyn oncologist NR/U/Mixed	Yes No NR		
	Biomarkers	Yes No NR		Primary/community/family practice Secondary: hospital/cancer unit/cancer centre/gyn oncologist NR/U/Mixed	Yes No NR		
	USS	Yes No NR		Primary/community/family practice Secondary: hospital/cancer unit/cancer centre/gyn oncologist NR/U/Mixed	Yes No NR		
	Combination	Yes No NR		Primary/community/family practice Secondary: hospital/cancer unit/cancer centre/gyn oncologist NR/U/Mixed	Yes No NR		

If a **combination of tests (a testing strategy) was used for each participant** please detail:	**Combination:**	**Comments**
- What combination of tests?	**Order:**	
- The order in which tests were performed?	**Rule for combining tests:**	
- The rule for combining test results:		
E.g. + and + = surgery		
E.g. + and - = surgery		
E.g. + and - = no surgery		
E.g. – and - = no surgery		
Etc		
or not reported **('NR')**		

INDEX TEST(S)

(If more than one index test was used, please complete for each test).

A1. Risk of bias (symptoms)	
Describe the index test and how it was conducted and interpreted:	
a) Was the index test or testing strategy result interpreted without knowledge of the results of the reference standard?	Yes/No/Unclear
b) If a threshold was used, was it prespecified?	Yes/No/Unclear
Could the conduct or interpretation of the index test have introduced bias?	**RISK: LOW/HIGH/UNCLEAR** **High - a) or b) No** **Low- a) and b) Yes** **Unclear - a) or b) Unclear**

A2. Risk of bias (ultrasound**)**	
Describe the index test and how it was conducted and interpreted:	
a) Was the index test or testing strategy result interpreted without knowledge of the results of the reference standard?	Yes/No/Unclear
b) If a threshold was used, was it prespecified?	Yes/No/Unclear
Could the conduct or interpretation of the index test have introduced bias?	**RISK: LOW/HIGH/UNCLEAR** **High - a) or b) No** **Low - a) and b) Yes** **Unclear - a) or b) Unclear**

A3. Risk of bias (Biomarkers) rule different because objective test in comparison to US and symptom elicitation	
Describe the index test and how it was conducted and interpreted:	
a) Was the index test or testing strategy result interpreted without knowledge of the results of the reference standard?	Yes/No/Unclear
b) If a threshold was used, was it prespecified?	Yes/No/Unclear
Could the conduct or interpretation of the index test have introduced bias?	**RISK: LOW/HIGH/UNCLEAR** **High - b) No or (a) and b)) No** **Low - a) and b) Yes** **Unclear - a) or b) Unclear**

A4. Risk of bias (within-study combination)	
Describe the index test and how it was conducted and interpreted:	
a) Was the index test or testing strategy result interpreted without knowledge of the results of the reference standard?	Yes/No/Unclear
b) If a threshold was used, was it prespecified?	Yes/No/Unclear
c) i) Were symptoms/signs interpreted without knowledge of ultrasound or biomarkers; ii) was ultrasound interpreted without knowledge of biomarkers	Yes/No/Unclear
Could the conduct or interpretation of the index test have introduced bias?	**RISK: LOW/HIGH/UNCLEAR** **High - a) or b) or c) No** **Low - a) and b) or c)Yes** **Unclear - a) or b) or c) Unclear**

B. Concerns regarding applicability	
a) Is the skill of person performing US and eliciting symptoms detailed? (level of training and/or experience)	**Yes/No/Unclear/NA**
b) Was US performed in all patients by non-specialised sonographers	**Yes/No/Unclear**
c) Was US performed with knowledge of symptoms/signs/biomarkers	**Yes/No/Unclear**
Is there concern that the index test, its conduct or interpretation differ from the review question?	**CONCERN: LOW/HIGH/UNCLEAR** **High - a) or b) or C) No** **Low - a) and b) or c)Yes** **Unclear - a) or b) Unclear**

REFERENCE STANDARD AND TARGET CONDITION DETAIL

REFERENCE STANDARD

Surgery (%) Follow-up (%) and length of follow-up

TARGET CONDITION

TARGET CONDITION	**Epithelial ovarian cancer (EOC)**	Number (%)	**Comments**
Target conditions are ovarian cancer (see list of different histology of ovarian cancer)	Serous		
	Mucinous		
	Endometrioid		
	Clear		
	Germ cell tumours		
	Stromal cell tumours		
	LMP		
	Others (metastasis)		

REFERENCE STANDARD RISK OF BIAS

A. Risk of bias

Describe the reference standard and how it was conducted and interpreted:	
a) Is the reference standard likely to correctly classify the target condition? Index test +ve: Histology following laparoscopy or laparotomy	**Yes/No/Unclear**
b) Is the reference standard likely to correctly classify the target condition? Index test –ve: Yes - if a minimum follow-up period of greater than 12 months is included as required to assess whether the target condition is present No - if a minimum 12-month follow-up period is absent Unclear - if no information on follow-up period is included	**Yes/No/Unclear**
Could the reference standard, its conduct or its interpretation have introduced bias	**RISK: LOW/HIGH/UNCLEAR** **High - a) or b) No** **Low - a) and b) Yes** **Unclear - a) or b) Unclear**

DOMAIN 3: REFERENCE STANDARD (continued)

B. Concerns regarding applicability

Is there concern that the target condition as defined by the reference standard does not match the review question?	**CONCERN: Yes/No/Unclear**
Yes - ovarian cancer, borderline and metastatic disease are not differentiated (and cannot be for analysis) No - ovarian cancer, borderline and metastatic disease can be differentiated for analysis Unclear - unclear if ovarian cancer, borderline and metastatic disease have been disaggregated	

DOMAIN 4: FLOW AND TIMING

A. Risk of bias	
Describe any patients who did not receive the index test(s) and/or reference standard or who were excluded from the 2 x 2 table (refer to study flow diagram):	
Describe the time interval and any interventions between index test(s) and reference standard:	
a) Was there less than 3 months interval between application of each index test and application of the reference standard?	**Yes/No/Unclear**
b) Did all patients receive a reference standard?	**Yes/No/Unclear**
c) Did all index test -ve patients receive the same reference standard?	**Yes/No/Unclear**
d) Were all patients who underwent testing included in the analysis?	**Yes/No/Unclear**
Could the conduct or interpretation of reference standard have introduced bias?	**RISK: LOW/HIGH/UNCLEAR** **LOW - a) and b) and c) and d) - Yes** **HIGH - a) and b) and c) and d) - No** **UNCLEAR - a) and b) and c) and d) – Unclear**

COMPARATIVE DOMAIN (if applicable)

A. Risk of bias	
Describe the selection process for participants to receive one or other index test or index testing strategy	
Describe the time interval and any interventions between index test(s) for within-person test comparisons	
a) *For studies comparing two or more index tests or testing strategies in **different** patient populations were the selection criteria for participants receiving one or other index test or testing strategy the same?*	***Yes/No/Unclear/NA***
b) *For within-study comparisons of index tests:* *- was the interval between application of each index test < 3 months*	***Yes/No/Unclear/NA***
c) *For within-study comparisons of individual index tests:* *- were index tests interpreted blind to the results of other index test results*	***Yes/No/Unclear/NA***
Could the conduct of the comparative study have introduced bias? LOW - a) OR (b) and c)) - Yes HIGH - a) OR (b) and c)) - No UNCLEAR - a) OR (b) or c)) – Unclear	**RISK: LOW/HIGH/UNCLEAR**
B. Concerns regarding applicability	
Describe included patients (prior testing, presentation, intended use of index test and setting):	
Is there concern that included patients have been selected in a different way to participants in non-comparative studies *Low - No* *High - Yes* *Unclear – Unclear*	**CONCERN: LOW/HIGH/UNCLEAR**

RISK OF BIAS FOR MULTIVARIABLE DIAGNOSTIC MODELLING STUDIES (if applicable)

1. Participant selection	**DEV**	***Yes/No/Unclear***	**VAL**	***Yes/No/Unclear***
a) Were appropriate data sources used, e.g. cohort, RCT or nested case-control study data?	**DEV**	*Yes/No/Unclear*		
b) Were participants enrolled at a similar state of health, or were predictors considered to account for differences?	**DEV**	*Yes/No/Unclear*	**VAL**	*Yes/No/Unclear*
Could the selection of patients have introduced bias? **HIGH**: a) OR a) and b) - YES **LOW**: a) OR a) and b) - NO **UNCLEAR**: a) OR a) and b) – UNCLEAR		**HIGH/LOW/ UNCLEAR**		**HIGH/LOW/ UNCLEAR**
3. Predictors	**DEV**	***Yes/No/Unclear***	**VAL**	***Yes/No/Unclear***
a) Were predictors defined and assessed in a similar way for all participants?	**DEV**	*Yes/No/Unclear*		
b) Are all predictors available at the time the model is intended to be used	**DEV**	*Yes/No/Unclear*	**VAL**	*Yes/No/Unclear*
c) Were all relevant predictors analysed?: ***No*** if symptoms only; ***No*** if US index test only; ***No*** if combination of index tests (symptoms, US and biomarkers) but miss out US OR Symptom OR FDA approved biomarkers	**DEV**	*Yes/No/Unclear*	**VAL**	*Yes/No/Unclear*
Could the definition, measurement or analysis of predictors introduced bias? **HIGH**: a) OR b) OR c) - YES **LOW**: a) OR b) OR c) - NO **UNCLEAR**: a) OR b) OR c) – UNCLEAR	**DEV**	**HIGH/LOW/ UNCLEAR**	**VAL**	**HIGH/LOW/ UNCLEAR**
3. ANALYSIS	**DEV**	***Yes/No/Unclear***	**VAL**	***Yes/No/Unclear***
a) Were there a reasonable number of outcome events?	**DEV**	*Yes/No/Unclear*		
b) Were there a reasonable number of outcome events?			**VAL**	*Yes/No/Unclear*
c) Were non-binary predictors handled appropriately?	**DEV**	*Yes/No/Unclear*	**VAL**	*Yes/No/Unclear*
d) Was selection of predictors based on univariable analysis avoided?	**DEV**	*Yes/No/Unclear*		
e) Do predictors and their assigned weights in the final model correspond to the results from multivariable analysis?	**DEV**	*Yes/No/Unclear*		
f) For the model or any simplified score, were relevant performance measures evaluated, e.g. calibration, discrimination, (re)classification and net benefit?	**DEV**	*Yes/No/Unclear*	**VAL**	*Yes/No/Unclear*
g) Was the model recalibrated or was it likely (based on the evidence presented, e.g. calibration plot) that recalibration was not needed?	**DEV**	*Yes/No/Unclear*	**VAL**	*Yes/No/Unclear*
h) Was model validation undertaken in individuals other than those in the model development (external validation)?			**VAL**	*Yes/No/Unclear*
Could the analysis strategy have introduced bias? **HIGH**: a) OR b) OR c) OR d) OR e) OR f) OR g) OR h) - YES **LOW**: a) OR b) OR c) OR d) OR e) OR f) OR g) OR h) - NO **UNCLEAR**: a) OR b) OR c) OR d) OR e) OR f) OR g) OR h) - UNCLEAR	**DEV**	**HIGH/LOW/ UNCLEAR**	**VAL**	**HIGH/LOW/ UNCLEAR**

TEST ACCURACY DATA

If reported please complete the following 2 x 2 contingency table. For studies investigating the accuracy of more than one index test or testing strategy please complete a 2 x 2 table for each test/testing strategy (cut and paste table as necessary). Imaging test results will be dichotomous.

LOWEST LEVEL OF AGGREGATION:

Fill in data as available.

	REFERENCE STANDARD (ovarian cancer)	REFERENCE STANDARD (borderline)	REFERENCE STANDARD (benign)	
INDEX TEST/TESTING STRATEGY +ve for ovarian cancer				**TOTAL INDEX TEST +ve**
INDEX TEST/TESTING STRATEGY +ve for borderline				**TOTAL INDEX TEST +ve**
INDEX TEST/TESTING STRATEGY +ve for benign				**TOTAL INDEX TEST –ve**
	DISEASE +ve	**TOTAL borderline**	**DISEASE -ve**	**TOTAL 'N'**
Aggregation borderline +ve	**TOTAL DISEASE +ve**		**TOTAL DISEASE -ve**	**TOTAL 'N'**
Aggregation borderline –ve	**TOTAL DISEASE +ve**	**TOTAL DISEASE -ve**		**TOTAL 'N'**

INSERT ANOTHER MORE DETAILED TABLE WITH SUB-CATEGORIES OF OVARIAN CANCER FOR LOW GRADE AND HIGH GRADE, TYPE 1 AND TYPE 2, EARLY-STAGE AND LATE-STAGE

	REFERENCE STANDARD (early-stage)	REFERENCE STANDARD (advanced-stage)	
INDEX TEST/TESTING STRATEGY +ve (early-stage)			**TOTAL INDEX TEST +ve**
INDEX TEST/TESTING STRATEGY +ve (late-stage)			**TOTAL INDEX TEST –ve**
	DISEASE +ve	**DISEASE -ve**	**TOTAL 'N'**

	REFERENCE (Type 1)	STANDARD	REFERENCE (Type 2)	STANDARD	
INDEX TEST/TESTING STRATEGY +ve (Type 1)					**TOTAL INDEX TEST +ve**
INDEX TEST/TESTING STRATEGY +ve (Type 2)					**TOTAL INDEX TEST -ve**
	DISEASE +ve		**DISEASE -ve**		**TOTAL 'N'**

Appendix 3 **: QUADAS-2.**

PATIENT SELECTION RISK OF BIAS

PATIENT SELECTION	
A. Risk of bias	
Describe methods of patient selection:	
a) Was a consecutive or random sample of patients enrolled?	Yes/No/Unclear
b) Was a case-control design avoided?	Yes/No/Unclear
c) Did the study avoid inappropriate exclusions?	Yes/No/Unclear
a) include all ages and regardless of menopausal status or justify restrictions	
b) include all stages of ovarian cancer	
c) include co-morbidities such as infertility and endometriosis	
Could the selection of patients have introduced bias?	**RISK: LOW/HIGH/UNCLEAR**
If a) and b) and c) 'YES' = low risk of bias	
If a) or b) or c) 'No' = high risk of bias	
If a) or b) or c) 'Unclear' = unclear risk of bias	

B. Concerns regarding applicability	
Describe included patients (prior testing, presentation, intended use of index test and setting):	
Is there concern that the included patients do not match the review question?	**CONCERN: LOW/HIGH/UNCLEAR**
a) Patients all symptomatic OR symptomatic and asymptomatic can be disaggregated	**Low - a) and b) and C) Yes**
b) Prior tests primary care: self reported symptoms	**High - a) or b) or C) No**
c) Prior tests secondary care: self reported symptoms OR self reported symptoms PLUS one or more of biochemical markers and ultrasound	**Unclear - a) or b) or C) Unclear**

INDEX TEST(S)

(If more than one index test was used, please complete for each test).

A1. Risk of bias (symptoms)

Describe the index test and how it was conducted and interpreted:	
a) Was the index test or testing strategy result interpreted without knowledge of the results of the reference standard?	Yes/No/Unclear
b) If a threshold was used, was it prespecified?	Yes/No/Unclear
Could the conduct or interpretation of the index test have introduced bias?	**RISK: LOW/HIGH/UNCLEAR** **High - a) or b) No** **Low - a) and b) Yes** **Unclear - a) or b) Unclear**

A2. Risk of bias (ultrasound)

Describe the index test and how it was conducted and interpreted:

a) Was the index test or testing strategy result interpreted without knowledge of the results of the reference standard?

Yes/No/Unclear

b) If a threshold was used, was it prespecified?

Yes/No/Unclear

Could the conduct or interpretation of the index test have introduced bias?

RISK: LOW/HIGH/UNCLEAR

High - a) or b) No

Low - a) and b) Yes

Unclear - a) or b) Unclear

A3. Risk of bias (biomarkers) rule different because objective test in comparison to US and symptom elicitation

Describe the index test and how it was conducted and interpreted:	
a) Was the index test or testing strategy result interpreted without knowledge of the results of the reference standard?	Yes/No/Unclear
b) If a threshold was used, was it prespecified?	Yes/No/Unclear
Could the conduct or interpretation of the index test have introduced bias?	**RISK: LOW/HIGH/UNCLEAR** **High - a) or b) No** **Low - a) and b) Yes** **Unclear - a) or b) Unclear**

B. Concerns regarding applicability	
a) Is the skill of person performing US and eliciting symptoms detailed? (level of training and/or experience)	**Yes/No/Unclear/NA**
b) Was US performed in all patients by non-specialised sonographers	**Yes/No/Unclear**
c) Was US performed with knowledge of symptoms/signs/biomarkers	**Yes/No/Unclear**
Is there concern that the index test, its conduct or interpretation differ from the review question?	**CONCERN: LOW/HIGH/UNCLEAR** **High - a) or b) or C)No** **Low - a) and b) or c)Yes** **Unclear - a) or b) Unclear**

REFERENCE STANDARD RISK OF BIAS

A. Risk of bias	
Describe the reference standard and how it was conducted and interpreted:	
a) Is the reference standard likely to correctly classify the target condition? Index test +ve: Histology following laparoscopy or laparotomy	**Yes/No/Unclear**
b) Is the reference standard likely to correctly classify the target condition? Index test –ve: Yes - if a minimum follow-up period of greater than 12 months is included as required to assess whether the target condition is present No - if a minimum 12-month follow-up period is absent Unclear - if no information on follow-up period is included	**Yes/No/Unclear**
Could the reference standard, its conduct or its interpretation have introduced bias	**RISK: LOW/HIGH/UNCLEAR** **High - a) or b) No** **Low - a) and b) Yes** **Unclear - a) or b) Unclear**

DOMAIN 3: REFERENCE STANDARD (continued)

B. Concerns regarding applicability	
Is there concern that the target condition as defined by the reference standard does not match the review question?	**CONCERN: Yes/No/Unclear**
Yes - ovarian cancer, borderline and metastatic disease are not differentiated (and cannot be for analysis)	
No - ovarian cancer, borderline and metastatic disease can be differentiated for analysis	
Unclear - unclear if ovarian cancer, borderline and metastatic disease have been disaggregated	

DOMAIN 4: FLOW AND TIMING

A. Risk of bias	
Describe any patients who did not receive the index test(s) and/or reference standard or who were excluded from the 2 x 2 table (refer to study flow diagram):	
Describe the time interval and any interventions between index test(s) and reference standard:	
a) Was there less than 3 months interval between application of each index test and application of the reference standard?	**Yes/No/Unclear**
b) Did all patients receive a reference standard?	**Yes/No/Unclear**
c) Did all index test -ve patients receive the same reference standard?	**Yes/No/Unclear**
d) Were all patients who underwent testing included in the analysis?	**Yes/No/Unclear**
Could the conduct or interpretation of reference standard have introduced bias?	**RISK: LOW/HIGH/UNCLEAR** **LOW - a) and b) and c) and d) - Yes** **HIGH - a) and b) and c) and d) - No** **UNCLEAR - a) and b) and c) and d) – Unclear**

COMPARATIVE DOMAIN (if applicable)

A. Risk of bias	
Describe the selection process for participants to receive one or other index test or index testing strategy	
Describe the time interval and any interventions between index test(s) for within-person test comparisons	
a) *For studies comparing two or more index tests or testing strategies in **different** patient populations were the selection criteria for participants receiving one or other index test or testing strategy the same?*	***Yes/No/Unclear/NA***
b) *For within-study comparisons of index tests:* *- was the interval between application of each index test < 3 months*	***Yes/No/Unclear/NA***
c) *For within-study comparisons of individual index tests:* *- were index tests interpreted blind to the results of other index test results*	***Yes/No/Unclear/NA***
Could the conduct of the comparative study have introduced bias? LOW - a) OR (b) and c))- Yes HIGH - a) OR (b) and c)) - No UNCLEAR - a) OR (b) or c)) – Unclear	**RISK: LOW/HIGH/UNCLEAR**
B. Concerns regarding applicability	
Describe included patients (prior testing, presentation, intended use of index test and setting):	
Is there concern that included patients have been selected in a different way to participants in non-comparative studies *Low - No* *High - Yes* *Unclear - Unclear*	**CONCERN: LOW/HIGH/UNCLEAR**

RISK OF BIAS FOR MULTIVARIABLE DIAGNOSTIC MODELLING STUDIES (if applicable)

1. Participant selection	DEV	*Yes/No/Unclear*	VAL	*Yes/No/Unclear*
a) Were appropriate data sources used, e.g. cohort, RCT or nested case-control study data?	**DEV**	*Yes/No/Unclear*		
b) Were participants enrolled at a similar state of health, or were predictors considered to account for differences?	**DEV**	*Yes/No/Unclear*	**VAL**	*Yes/No/Unclear*
Could the selection of patients have introduced bias? **HIGH**: a) OR a) and b) - YES **LOW**: a) OR a) and b) - NO **UNCLEAR**: a) OR a) and b) – UNCLEAR		**HIGH/LOW/ UNCLEAR**		**HIGH/LOW/ UNCLEAR**
3. Predictors	**DEV**	***Yes/No/Unclear***	**VAL**	***Yes/No/Unclear***
a) Were predictors defined and assessed in a similar way for all participants?	**DEV**	*Yes/No/Unclear*		
b) Are all predictors available at the time the model is intended to be used	**DEV**	*Yes/No/Unclear*	**VAL**	*Yes/No/Unclear*
c) Were all relevant predictors analysed?: ***No*** if symptoms only; ***No*** if US index test only; ***No*** if combination of index tests (symptoms, US and biomarkers) but miss out US OR Symptom OR FDA approved biomarkers	**DEV**	*Yes/No/Unclear*	**VAL**	*Yes/No/Unclear*
Could the definition, measurement or analysis of predictors introduced bias? **HIGH**: a) OR b) OR c) - YES **LOW**: a) OR b) OR c) - NO **UNCLEAR**: a) OR b) OR c) – UNCLEAR	**DEV**	**HIGH/LOW/ UNCLEAR**	**VAL**	**HIGH/LOW/ UNCLEAR**
3. ANALYSIS	**DEV**	***Yes/No/Unclear***	**VAL**	***Yes/No/Unclear***
a) Were there a reasonable number of outcome events?	**DEV**	*Yes/No/Unclear*		
b) Were there a reasonable number of outcome events?			**VAL**	*Yes/No/Unclear*
c) Were non-binary predictors handled appropriately?	**DEV**	*Yes/No/Unclear*	**VAL**	*Yes/No/Unclear*
d) Was selection of predictors based on univariable analysis avoided?	**DEV**	*Yes/No/Unclear*		
e) Do predictors and their assigned weights in the final model correspond to the results from multivariable analysis?	**DEV**	*Yes/No/Unclear*		
f) For the model or any simplified score, were relevant performance measures evaluated, e.g. calibration, discrimination, (re)classification and net benefit?	**DEV**	*Yes/No/Unclear*	**VAL**	*Yes/No/Unclear*
g) Was the model recalibrated or was it likely (based on the evidence presented, e.g. calibration plot) that recalibration was not needed?	**DEV**	*Yes/No/Unclear*	**VAL**	*Yes/No/Unclear*
h) Was model validation undertaken in individuals other than those in the model development (external validation)?			**VAL**	*Yes/No/Unclear*
Could the analysis strategy have introduced bias? **HIGH**: a) OR b) OR c) OR d) OR e) OR f) OR g) OR h) - YES **LOW**: a) OR b) OR c) OR d) OR e) OR f) OR g) OR h) - NO **UNCLEAR**: a) OR b) OR c) OR d) OR e) OR f) OR g) OR h) – UNCLEAR	**DEV**	**HIGH/LOW/ UNCLEAR**	**VAL**	**HIGH/LOW/ UNCLEAR**

Appendix 4 **: Review reporting: Title, Introduction and Methods.**

Author and publication date	Title	Introduction			Methods (Yes-Y, No-N, Limited-L)									
		Rationale	**Clinical Back**	**Obj**	**Eligibility criteria**	**Info sources**	**Search strategy**	**Study selection**	**Data collection**	**Data extraction definitions**	**RoB & Appl**	**Accuracy measures (all per patient)**	**Planned Synthesis**	**Meta-analysis**
E.R. Myers / Feb 2006	N	Y	Y	Y	L	L	Y	Y	L	L	Y	Y	L	Y
P. Geomini /Feb 2009	Y	Y	L	L	L	L	L	Y	N	L	Y	Y	Y	Y
L.R. Medeiros/ Feb 2009	Y	Y	N	Y	L	L	L	Y	L	L	Y	Y	L	Y
NICE April /2011 (a)sym	N	Y	L	Y	L	L	Y	Y	L	L	Y	Y	L	N
NICE April /2011 (b) bio	N	Y	L	Y	Y	L	Y	Y	L	L	Y	Y	N	Y
NICE April /2011 (c)combi	N	Y	L	Y	L	L	Y	N (meta analysis selected 1 SR by Geomini and 1 additional updated study	Summary of Geomini; No meta analyses	Summary Limited	N	N	N	N
S.Yu/ Feb 2012	N (only meta analysis in title)	Y	N	L	L	L	L	Y	L	L	Y (but not reported in results)	Y	N	Y
J.E. Dodge/ Apr 2012	Y	Y	N	L	L	L	L	Y	N	L	Y	L	N	L
F. Li / June 2012	Y	Y	N	L	L	Y	L	Y	L	L	Y	Y	N	Y
L. Wu/ Sept 2012	Y	Y	N	L	L	L	L	Y	N	L	Y	Y	Y	Y
J. Lin/ Nov 2012	Y	Y	N	L	L	L	L	Y	L	N	L	Y	N	L
J. Lin/ Dec 2012	Y	Y	N	L	L	Y	N	L	L	L	L	Y	N	L
S.Ferraro/ Feb 2013	Y	Y	N	L	L	L	L	Y	L	L	Y	Y	N	L
Z. Yang/ Jul 2013	N (only meta analysis)	Y	N	L	L	Y	L	Y	N	L	Y	Y	N	L
J. Lin/ Dec 2013	Y	Y	N	L	L	L	L	Y	L	N	L	Y	N	L
J. Kaijser / Dec 2013	Y	Y	Y	L	L	Y	L	Y	L	Y	Y	L	L	Y
J.Wang/ Mar 2014	N(only metanalysis	Y	N	L	L	L	L	Y	L	L	Y	Y	L	Y
S. Zhen /Apr 2014	No (only metanalysis	Y	N	L	L	L	L	L	N	L	Y	Y	L	L
X.Y. Liao / Nov 2014	No (only metanalysis)	Y	N	L	L	L	L	Y	N	L	N	Y	N	L
A.C.L. Macedo / Sept 2014	Y	Y	N	L	Ls(all but Study design reported)	L	L	Y	L	L	Y	Y	N	Y
M. Stukan / Feb 2015	N	Y	L	L	L	L	L	N	N	L	Y	N	N	N

M.Ebell / Mar 2016	Y	Y	N	L	L	L	L	L	L	L	Y	Y	N	L
E.M.J. Meys/ May 2016	Y	Y	N	L	L	Y	L	Y	L	Y	Y	L	L	Y

Notes:

Title: Title acknowledges report as a SR; Objectives: (Y, N, Limited) was based on all, none or some of the following criteria being reported: Population, Index tests, target condition; etc

Appendix 5 **: Review reporting: Results and Discussion.**

Author and publication date	Title	Results (Yes-Y, No-N, Limited- L)						Discussion			Review funding
		Study selection	**Study charac**	**RoB & Appl**	**Results individual studies**	**Synthesis across studies**	**Additional analysis**	**Summary of evidence**	**Limitations**	**Conclusions**	
E.R. Myers / Feb 2006	N	L	Y	L	L	Y	Y,	Y	Y	Y	Y
P. Geomini / Feb 2009	Y	Y	L	L	N	Y	Y	L	N	L	N
L.R.Medeiros/ Feb 2009	Y	Y	L	Y	Y	Y	Y	Y	Y	N	N
NICE April /2011 (a)Symptoms	N	L	L	L	Y (only forest plot)	Y (as range)	N	L	Y	L	Y
NICE April /2011 (b)Biomarkers	N	L	Y	Y	Y	Y	N	Y	Y	L	Y
NICE April /2011 Combi	N	Summary of Geomini		N							Y
S. Yu / 2012	N	L	L	N (results not reported)	Y	Y	L	L	N	L	Y
J. E. Dodge/ Apr 2012	Y	N	L	N	L	L	N	Y	Y	L	N
F. Li / June 2012	Y	Y	L	Y	L	Y	L	Y	Y	N	N
L.Wu/ Sept 2012	Y	L	L	Y	L	Y	L	Y	L	Y	N
J.Lin/ Nov 2012	Y	Y	L	N	L	Y	N	L	L	N	N
J.Lin/ Dec 2012 (details for Index test only reported for HE4)	Y	N	Yes for HE4, No for CA125 and ROMA	N	Yes only for HE4, No for CA125 and ROMA	Yes for all	Limited for HE4, No for CA125 and ROMA	L	L	L	Y
S.Ferraro/ Feb 2013	Y	L	L	Y	L	Y	N	Y	Y	Y	N
Z. Yang/ Jul 2013	N	Y	N	Y	L	Y	N	L	Y	L	Y

J.Lin/ Dec 2013 (details for Index test only reported for HE4)	Y	Y	Yes for HE4, No for CA125 and ROMA	N	Yes only for HE4, No for CA125 and ROMA	Yes for all	Limited for HE4, No for CA125 and ROMA	L	L	L	Y
J. Kaijser / Dec 2013	Y	Y	L	Y	Y	Y	L	Y	Y	Y	Y
J.Wang / Mar 2014	N	Y	L	N	L	Y	L	L	Y	L	N
S. Zhen /Apr 2014	N	L	L	L	L	Y	L	Y	L	L	Y
X. Y. Liao / Nov 2014	N	L	L	N	L	Y	N	L	N	N	N
A.C.L. Macedo / Sept 2014	Y	Y	L	Y	Y	Y	L	Y	L	L	N
M. Stukan / Feb 2015	N	L	L	L	L	NA	NA	L	N	L	N
M.Ebell / Mar 2016	Y	Y	L	L	L	Y	N	Y	L	L	N
E.M.J. Meys/ May 2016	Y	L	L	Y	L	Y	L	Y	Y	Y	Y

Appendix 6 : List of excluded studies with reasons for exclusions.

List of excluded studies with reasons for exclusions (Reasons in order of frequency of studies excluded) (737 studies excluded)

No 2x2 table (N=253 studies excluded)

1. Ovarian cancer: screening, treatment, and followup. NIH Consensus Statement. 1994;12(3):1-30.

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3. CA-125 remains best predictor of ovarian cancer. Contemporary OB/GYN. 2011;56(5):16.

4. Clinical use of tumor markers in China. Tumor Biology. 2014;35:S11.

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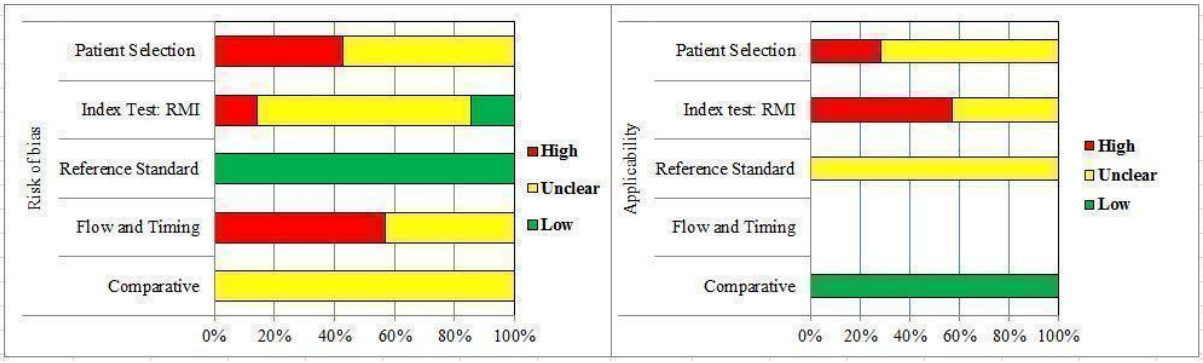
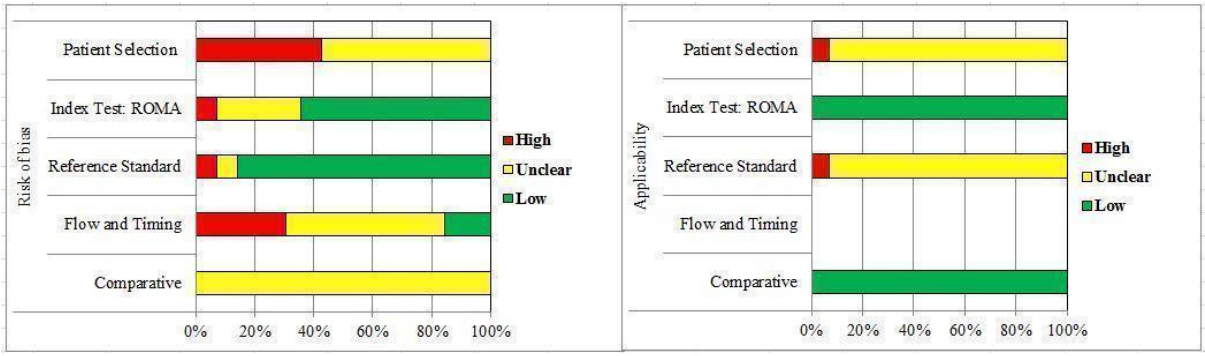
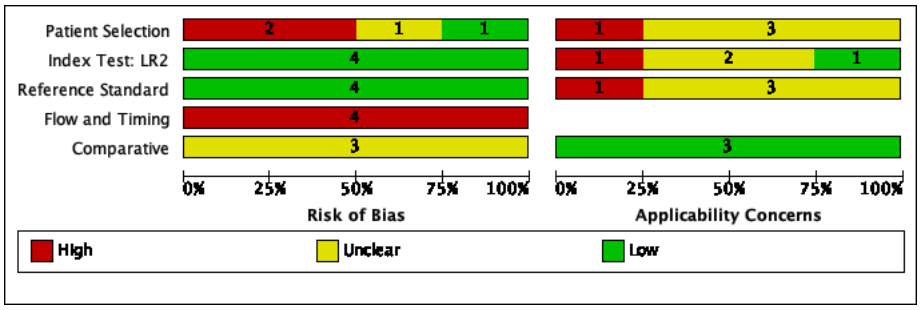
Appendix 7 **: Risk of bias and applicability concerns for RMI1 studies reporting results separately for pre and postmenopausal women.**



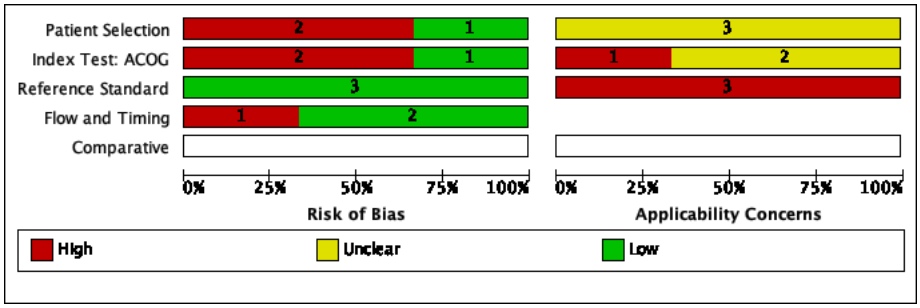
Appendix 8 **: Risk of bias and applicability concerns for ROMA studies reporting results separately for pre and postmenopausal women.**



Appendix 9 **: Risk of bias and applicability concerns graph for LR2 studies reporting results separately for pre and postmenopausal women.**



Appendix 10 **: Risk of bias and applicability concerns for ACOG v3 CA125 and ACOG v3 OVA1 studies reporting results separately for pre and postmenopausal women.**



Appendix 11 **: Comparison of test accuracy of LR2, RMI I, RMI III, ACOG V1 and ACOG V3 (CA125) at fixed thresholds between pre and postmenopausal women.**

Test	Studies N, D+	Analysis Method	Sensitivity	Specificity	Ratio of Sens odds (p value)	Ratio of Spec odds (p value)
LR2 pre	3, 2715, 588	Un	83.6 (78.6, 87.6)	90.4 (81.3, 95.3)	3.02 (2.04, 4.49) (P<0.0001)	0.21 (0.11, 0.43) (P<0.0001)
LR2 post	3, 1861, 1008	Un	93.9 (92.3, 95.3)	63.9 (60.6, 67.0)		
RMI1 200 pre	5, 2634, 502	Un	52.2 (45.9, 58.5)	95.4 (92.5, 97.3)	2.71 (1.92, 3.83) (P<0.0001)	0.42 (0.19, 0.94) (P=0.03)
RMI1 200 post	5, 1879, 862	Un	75.0 (69.5, 79.8)	90.1 (83.1, 94.4)		
RMI1 250 pre	1, 356, 31	Bp	54.8 (36.0, 72.7)	88.3 (84.3, 91.6)	3.88 (1.61, 9.34) (P=0.003)	0.52 (0.30, 0.90) (P=0.02)
RMI1 250 post	2, 220, 97	Un	82.5 (73.6, 88.8)	79.7 (71.6, 85.9)		
RMI3 200 pre	2, 606, 84	Un	73.6 (61.3, 83.1)	91.1 (87.3, 94.0)	2.15 (0.42, 10.9) (P=0.36)	0.15 (0.09, 0.23) (P<0.0001)
RMI3 200 post	2, 604, 314,	Un	85.8 (58.3, 96.3)	61.7 (56.0, 67.1)		
ACOG V1 pre	3, 716, 154	Un	69.4 (59.3, 78.0)	60.5 (36.1, 80.6)	4.64 (2.32, 9.27) (P=0.001)	0.73 (0.26, 2.10) (P=0.265)
ACOG V1 post	3, 1301, 585	Un	91.4 (86.3, 94.7)	53.0 (43.9, 61.9)		
ACOG V3 (CA125) pre	3, 899, 150	Un	78.0 (70.4, 84.1)	83.9 (65.1, 92.6)	1.54 (0.77, 3.12) (P=0.23)	0.76 (0.28, 2.04) (P=0.59)
ACOG V3 (CA125) post	3, 1224, 486	Un	84.6 (76.0, 90.5)	78.1 (71.0, 83.9)		

Notes to table: Analysis method: bp = single study estimate. uv=univariate analysis. bv=bivariate analysis. D+ = Primary ovarian cancer plus borderline ovarian tumours (where borderline tumours included in primary study analysis) minus metastatic disease (where this could be disaggregated from primary ovarian cancer in primary study analysis).

Appendix 12 **: Summary Estimates of Sensitivity and Specificity for all tests, all versions, all test positivity thresholds separately for premenopausal, postmenopausal and pre and postmenopausal mixed populations.**

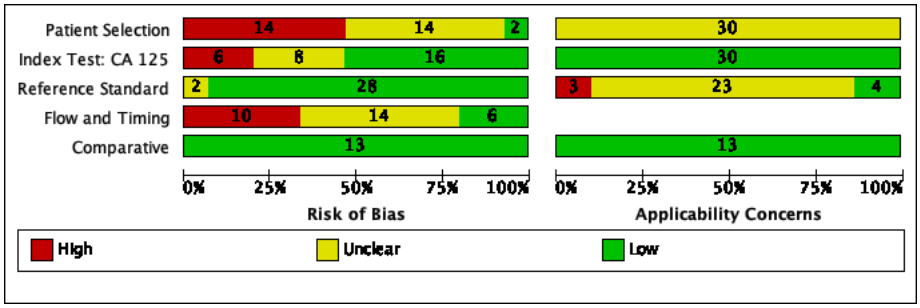
	PREMENOPAUSAL			POSTMENOPAUSAL			PRE AND POSTMENOPAUSAL COMB		
-Test Version -Threshold	**Studies** **N** **D+** **Analysis**	**Sensitivity (95% CI)**	**Specificity (95% CI)**	**Studies** **N** **OC cases** **Analysis**	**Sensitivity (95% CI)**	**Specificity (95% CI)**	**Studies** **N** **OC cases** **Analysis**	**Sensitivity (95% CI)**	**Specificity (95% CI)**
ADNEX 3% post test probability	1 1354 378 bp	97.9 (95.9-98.9	56.6 (53.4-59.6)	1 1049 602 bp	99.5 (98.6-99.8)	25.1 (21.3-29.3)	1 2403 980 bp	98.9 (97.8, 99.5)	46.6 (44.0, 49.2)
ADNEX 5% post test probability	1 1354 378 bp	97.6 (95.5-98.7)	69.5 (66.5-72.3)	1 1049 602 bp	98.8 (97.6-99.4)	37.4 (33.0-41.9)	1 2403 980 bp	98.4 (97.2, 9.2)	59.4 (56.8, 61.9)
ADNEX 10% post test probability	1 1354 378 bp	94.7 (92.0-96.6)	78.6 (75.9-81.1)	1 1049 602 bp	97.7 (96.1-98.6)	55.5 (50.9-60.0)	1 2403 980 bp	96.4 (94.8, 97.7)	71.3 (68.9, 73.7)
ADNEX 15% post test probability	1 1354 378 bp	90.5 (87.1-93.0)	83.4 (80.9-85.6)	1 1049 602 bp	96.5 (94.7-97.7)	63.5 (59.0-67.9)	1 2403 980 bp	94.2 (92.1, 95.8)	77.2 (95.0, 79.4)
LR2 10% post test probability	3 2715 558 un	83.6 (78.6, 87.6)	90.4 (81.3, 95.3)	3 1861 1008 un	93.9 (92.3, 95.3)	63.9 (60.6, 67.0)	4 4888 1728 Un	90.3 (87.5, 92.6)	81.5 (74.7, 86.8)
RMI1 200	5 2634 502	52.2 (45.9, 58.5)	95.4 (92.5, 97.3)	5 1879 862 un	75.0 (69.5, 79.8)	90.1 (83.1, 94.4)	31 12335 3688 bv	73.1 (68.6, 77.2)	91.9 (89.4, 93.8)
Appendix 12 Continued...									
250	356 31	(36.0, 72.7)	(84.3, 91.6)	2 220 97	82.5 (73.6, 88.8)	79.7 (71.6, 85.9)	14 4818 1452	71.5 (67.7, 75.1)	92.2 (88.4, 94.9)

	bp			un			bv		
RMI2 200	-	-	-	-	-	-	19 4989 1328 Bv	76.7 (69.9, 82.4)	85.2 (81.6, 88.2)
RMI2 250	-	-	-	-	-	-	6 911 256 Bv	71.5 (61.8, 79.6)	89.7 (86.1, 92.4)
RMI3 200	2 606 84 un	73.6 (61.3, 83.1)	91.1 (87.3, 94.0)	2 604 314 un	85.8 (58.3, 96.3)	61.7 (56.0, 67.1)	19 5945 1761 bv	75.9 (68.8, 81.9)	84.7 (80.0, 88.5)
RMI3 250	-	-	-	-	-	-	5 1683 436 Bv	78.4 (67.4, 86.5)	89.6 (86.3, 92.2)
RMI4 400	-	-	-	-	-	-	3 506 92 un	82.6 (73.5, 89.1)	89.1 (20.8, 73.2)
RMI4 450	-	-	-	-	-	-	6 1971 462 Bv	92.2 (90.2, 93.9)	
ROMA 12.5 pre 14.4 post	3 302 68 un	63.5 (51.0, 74.4)	89.3 (80.8, 94.3)	3 299 177 un	88.0 (80.6, 92.8)	68.3 (57.4, 77.5)	3 601 245 un	80.2 (72.0, 86.5)	82.6 (76.9, 87.2)
ROMA 13.1 pre 27.7 post	5 855 100 un	75.8 (66.1, 83.5)	80.8 (72.5, 87.0)	5 760 314 bv	87.8 (82.2, 94.3)	76.9 (72.7, 80.7)	5 1615 414 Bv	86.4 (81.1, 90.4)	79.5 (73.5, 84.5)
ROMA 7.4 pre 25.3 post	3 975 107 un	84.1 (63.4, 94.2)	75.6 (55.6, 88.5)	3 767 339 bn	95.3 (90.5, 97.7)	77.5 (59.4, 89.0)	5 2194 673 bv	92.1 (86.8, 95.4)	73.3 (56.9, 85.1)

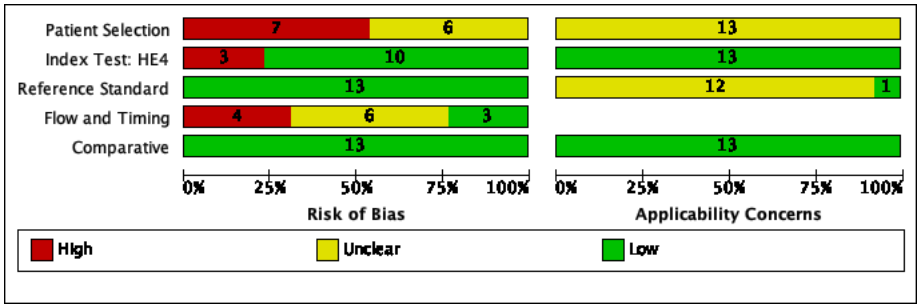
ROMA 7.4(+-2) pre 25.3(+-2) post	6 1269 173 bv	82.6 (71.6, 89.9)	80.0 (68.4, 88.1)	7 1396 544 bv	92.2 (87.4, 95.2)	81.8 (71.8, 88.8)	-	-	-
ROMA 13.1(+-2) pre 27.7 (+-2) post	13 1487 307 bv	78.3 (71.2, 84.0)	83.3 (78.2, 87.5)	9 1497 610 bv	90.7 (86.4, 93.7)	79.1 (74.6, 83.1)	-	-	-
ACOG V1	3 716 154 un	69.4 (59.3, 78.0)	60.5 (36.1, 80.6)	3 1301 585 un	91.4 (86.3, 94.7)	53.0 (43.9, 61.9)	2 1180 428 un	83.5 (73.1, 90.4)	52.5 (31.4, 72.7)
ACOG V2	1 240 48 bp	85.4 (72.2,93.9)	60.0 (52.6, 66.9)	1 597 263 bp	93.2 (89.4, 95.9)	59.9 (54.4, 65.2)	-	-	-
ACOG V3 CA125	3 899 150 un	78.0 (70.4, 84.1)	83.9 (65.1, 92.6)	3 1224 486 un	84.6 (76.0, 90.5)	78.1 (71.0, 83.9)	2 1286 325 un	79.4 (74.6, 83.4)	75.4 (69.1, 80.8)
ACOG V3 OVA1	1 235 45 bp	91.1 (78.8, 97.5)	43.2 (36.0, 50.5)	1 281 116 bp	94.8 (89.1, 98.1)	25.5 (19.0, 32.8)	1 516 161 bp	93.8 (88.9, 97.0)	34.9 (30.0, 40.1)

Notes to table: Analyses here have been undertaken using the bivariate model to estimate average sensitivity and specificity for each test at each specified threshold. Where possible estimates have been made separately for premenopausal, postmenopausal and pre and postmenopausal mixed groups. Analysis method: bp = single study estimate. uv=univariate analysis. bv=bivariate anlaysis. D+ = Primary ovarian cancer and borderline ovarian tumours (where borderline tumours included in primary study analysis). ACOG threshold: any one component test positive, overall test positive.

Appendix 13 **: CA125 Risk of bias and applicability concerns graph: review authors' judgements about each domain presented as percentages across included studies.**



Appendix 14 **: HE4 Risk of bias and applicability concerns graph: review authors' judgements about each domain presented as percentages across included studies.**



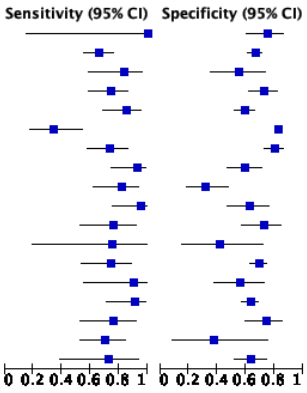
Appendix 15 : **Test performance: CA125 treatment of borderline results.**

Menopausal status	Test	Threshold	Number studies (N total, N OC)	Sensitivity (95% CI)	Specificity (95% CI)	Notes
Pre all studies	CA125	35ish	17	78 (71 to 84)	66 (61 to 71)	Bivariate, same metandi
Post all studies	CA125	35ish	21	84 (80 to 87)	83 (81 to 84)	Bivariate, same metandi
Pre BOT1 only	CA125	35ish	7	71 (58 to 82)	70 (62 to 77)	Bivariate agrees with metandi
Post BOT1 only	CA125	35ish	9	78 (74 to 82)	82 (80 to 84)	Bivariate agrees with metandi
BOT1-BOT2/3 Pre	CA125	35ish	17	-11.1 (-24.5 to 2.4)	7.3 (-3.1 to 17.8)	Bivariate
BOT1-BOT2/3 Post	CA125	35ish	21	-10.2 (-15.3 to -5.1)	-1.4 (-4.7 to 1.9)	univariable

Appendix 16 : **CA125 35 (+/- 10) pre post Forest plot.**

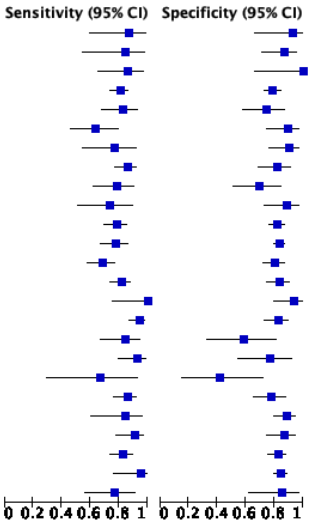
CA125 35(+-10) pre

Study	TP	FP	FN	TN	Threshold (pmol/L)	BOT with	Sensitivity (95% CI)	Specificity (95% CI)
Smikle 1995	2	12	0	36		Not clear	1.00 [0.16, 1.00]	0.75 [0.60, 0.86]
Van Calster 2007b	57	120	29	239	30.0	Malignant	0.66 [0.55, 0.76]	0.67 [0.61, 0.71]
Anton 2012	15	13	3	16	35.0	Malignant	0.83 [0.59, 0.96]	0.55 [0.36, 0.74]
Dowd 1993	32	21	11	57	35.0	Malignant	0.74 [0.59, 0.86]	0.73 [0.62, 0.82]
Holcomb 2011	29	79	5	116	35.0	Malignant	0.85 [0.69, 0.95]	0.59 [0.52, 0.66]
Radosa 2011	10	147	19	704	35.0	Malignant	0.34 [0.18, 0.54]	0.83 [0.80, 0.85]
Van Gorp 2011	31	28	11	114	35.0	Malignant	0.74 [0.58, 0.86]	0.80 [0.73, 0.86]
Bandiera 2011	24	28	2	41	35.0	Not clear	0.92 [0.75, 0.99]	0.59 [0.47, 0.71]
Benjapibal 2007	22	30	5	14	35.0	Not clear	0.81 [0.62, 0.94]	0.32 [0.19, 0.48]
Chen 2015	19	18	1	30	35.0	Not clear	0.95 [0.75, 1.00]	0.63 [0.47, 0.76]
Farzaneh 2014	16	13	5	34	35.0	Not clear	0.76 [0.53, 0.92]	0.72 [0.57, 0.84]
Kuesel 1992	3	7	1	5	35.0	Not clear	0.75 [0.19, 0.99]	0.42 [0.15, 0.72]
Molina 2011	20	70	7	156	35.0	Not clear	0.74 [0.54, 0.89]	0.69 [0.63, 0.75]
Ortiz-Munoz 2014	9	15	1	19	35.0	Not clear	0.90 [0.55, 1.00]	0.56 [0.38, 0.73]
Chan 2013	20	99	2	170	35.0	Excluded	0.91 [0.71, 0.99]	0.63 [0.57, 0.69]
Partheen 2011	16	13	5	37	35.0	Excluded	0.76 [0.53, 0.92]	0.74 [0.60, 0.85]
Stiekema 2014	24	5	10	3	35.0	Excluded	0.71 [0.53, 0.85]	0.38 [0.09, 0.76]
Franchi 1995	8	26	3	46	40.0	Malignant	0.73 [0.39, 0.94]	0.64 [0.52, 0.75]



CA125 35(+-10) post

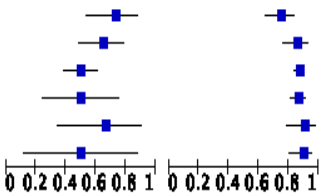
Study	TP	FP	FN	TN	Threshold (pmol/L)	BOT with	Sensitivity (95% CI)	Specificity (95% CI)
Antonic and Rakar 1995	13	1	2	13		Malignant	0.87 [0.60, 0.98]	0.93 [0.66, 1.00]
Smikle 1995	11	5	2	32		Not clear	0.85 [0.55, 0.98]	0.86 [0.71, 0.95]
Farzaneh 2014	19	0	3	9	25.0	Not clear	0.86 [0.65, 0.97]	1.00 [0.66, 1.00]
Van Calster 2007b	126	44	30	164	30.0	Malignant	0.81 [0.74, 0.87]	0.79 [0.73, 0.84]
Fujiwara 2015	34	10	7	29	31.5	Excluded	0.83 [0.68, 0.93]	0.74 [0.58, 0.87]
Anton 2012	23	4	13	33	35.0	Malignant	0.64 [0.46, 0.79]	0.89 [0.75, 0.97]
Botsis 1997	17	4	5	36	35.0	Malignant	0.77 [0.55, 0.92]	0.90 [0.76, 0.97]
Dowd 1993	80	9	13	41	35.0	Malignant	0.86 [0.77, 0.92]	0.82 [0.69, 0.91]
Engelen 2008	29	10	8	23	35.0	Malignant	0.78 [0.62, 0.90]	0.70 [0.51, 0.84]
Kusnetzoff 1998	17	4	6	30	35.0	Malignant	0.74 [0.52, 0.90]	0.88 [0.73, 0.97]
Maggino 1994	83	41	23	188	35.0	Malignant	0.78 [0.69, 0.86]	0.82 [0.77, 0.87]
Radosa 2011	60	65	17	339	35.0	Malignant	0.78 [0.67, 0.87]	0.84 [0.80, 0.87]
Schutter 1994	69	25	32	102	35.0	Malignant	0.68 [0.58, 0.77]	0.80 [0.72, 0.87]
Van Gorp 2011	97	14	22	72	35.0	Malignant	0.82 [0.73, 0.88]	0.84 [0.74, 0.91]
Avsar F.	13	2	0	31	35.0	Not clear	1.00 [0.75, 1.00]	0.94 [0.80, 0.99]
Bandiera 2011	82	17	5	79	35.0	Not clear	0.94 [0.87, 0.98]	0.82 [0.73, 0.89]
Benjapibal 2007	27	7	5	10	35.0	Not clear	0.84 [0.67, 0.95]	0.59 [0.33, 0.82]
Chen 2015	37	5	3	17	35.0	Not clear	0.93 [0.80, 0.98]	0.77 [0.55, 0.92]
Kuesel 1992	6	7	3	5	35.0	Not clear	0.67 [0.30, 0.93]	0.42 [0.15, 0.72]
Molina 2011	72	13	12	46	35.0	Not clear	0.86 [0.76, 0.92]	0.78 [0.65, 0.88]
Ortiz-Munoz 2014	16	10	3	75	35.0	Not clear	0.84 [0.60, 0.97]	0.88 [0.79, 0.94]
Chan 2013	39	7	4	46	35.0	Excluded	0.91 [0.78, 0.97]	0.87 [0.75, 0.95]
Partheen 2011	77	29	16	136	35.0	Excluded	0.83 [0.74, 0.90]	0.82 [0.76, 0.88]
Novotny 2012	20	36	1	199	36.0	Not clear	0.95 [0.76, 1.00]	0.85 [0.79, 0.89]
Franchi 1995	20	3	6	17	40.0	Malignant	0.77 [0.56, 0.91]	0.85 [0.62, 0.97]



Appendix 17 **: CA125 65 (+/- 10) pre post Forest plot.**

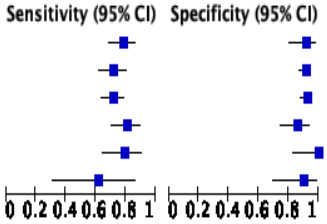
CA125 65(+-10) pre

Study	TP	FP	FN	TN	Threshold (pmol/L)	BOT with	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Fujiwara 2015	22	23	8	69	62.1	Excluded	0.73 [0.54, 0.88]	0.75 [0.65, 0.83]		
Dowd 1993	28	11	15	67	65.0	Malignant	0.65 [0.49, 0.79]	0.86 [0.76, 0.93]		
Van Calster 2007b	43	45	43	314	65.0	Malignant	0.50 [0.39, 0.61]	0.87 [0.84, 0.91]		
Gadducci 1992	8	26	8	171	65.0	Not clear	0.50 [0.25, 0.75]	0.87 [0.81, 0.91]		
Gadducci 1996	8	4	4	41	65.0	Not clear	0.67 [0.35, 0.90]	0.91 [0.79, 0.98]		
Strigini 1996	3	7	3	62	65.0	Not clear	0.50 [0.12, 0.88]	0.90 [0.80, 0.96]		



CA125 65(+-10) post

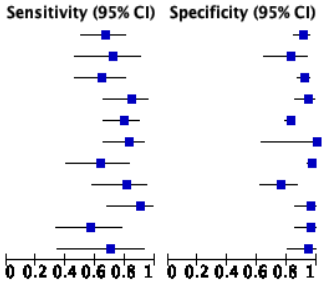
Study	TP	FP	FN	TN	Threshold (pmol/L)	BOT with	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Dowd 1993	73	4	20	46	65.0	Malignant	0.78 [0.69, 0.86]	0.92 [0.81, 0.98]		
Maggino 1994	76	19	30	210	65.0	Malignant	0.72 [0.62, 0.80]	0.92 [0.87, 0.95]		
Van Calster 2007b	112	16	44	192	65.0	Malignant	0.72 [0.64, 0.79]	0.92 [0.88, 0.96]		
Gadducci 1992	60	8	14	49	65.0	Not clear	0.81 [0.70, 0.89]	0.86 [0.74, 0.94]		
Gadducci 1996	35	0	9	20	65.0	Not clear	0.80 [0.65, 0.90]	1.00 [0.83, 1.00]		
Strigini 1996	8	2	5	19	65.0	Not clear	0.62 [0.32, 0.86]	0.90 [0.70, 0.99]		



Appendix 18 **: HE4 70 (+/- 10) pre/post Forest plot.**

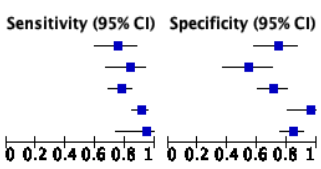
HE4 70(+-10) pre

Study	TP	FP	FN	TN	Threshold (pmol/L)	BOT with	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Van Gorp 2011	28	13	14	129	70.0	Malignant	0.67 [0.50, 0.80]	0.91 [0.85, 0.95]		
Anton 2012	13	5	5	24	70.0	Malignant	0.72 [0.47, 0.90]	0.83 [0.64, 0.94]		
Holcomb 2011	22	16	12	179	70.0	Malignant	0.65 [0.46, 0.80]	0.92 [0.87, 0.95]		
Bandiera 2011	22	4	4	65	70.0	Not clear	0.85 [0.65, 0.96]	0.94 [0.86, 0.98]		
Karlsen 2012	39	93	10	437	70.0	Not clear	0.80 [0.66, 0.90]	0.82 [0.79, 0.86]		
Stiekema 2014	28	0	6	8	70.0	Excluded	0.82 [0.65, 0.93]	1.00 [0.63, 1.00]		
Chan 2013	14	9	8	260	70.0	Excluded	0.64 [0.41, 0.83]	0.97 [0.94, 0.98]		
Partheen 2011	17	12	4	38	71.8	Excluded	0.81 [0.58, 0.95]	0.76 [0.62, 0.87]		
Chen 2015	18	2	2	46	72.3	Not clear	0.90 [0.68, 0.99]	0.96 [0.86, 0.99]		
Farzaneh 2014	12	2	9	45	75.0	Not clear	0.57 [0.34, 0.78]	0.96 [0.85, 0.99]		
Ortiz-Munoz 2014	7	2	3	32	77.0	Not clear	0.70 [0.35, 0.93]	0.94 [0.80, 0.99]		

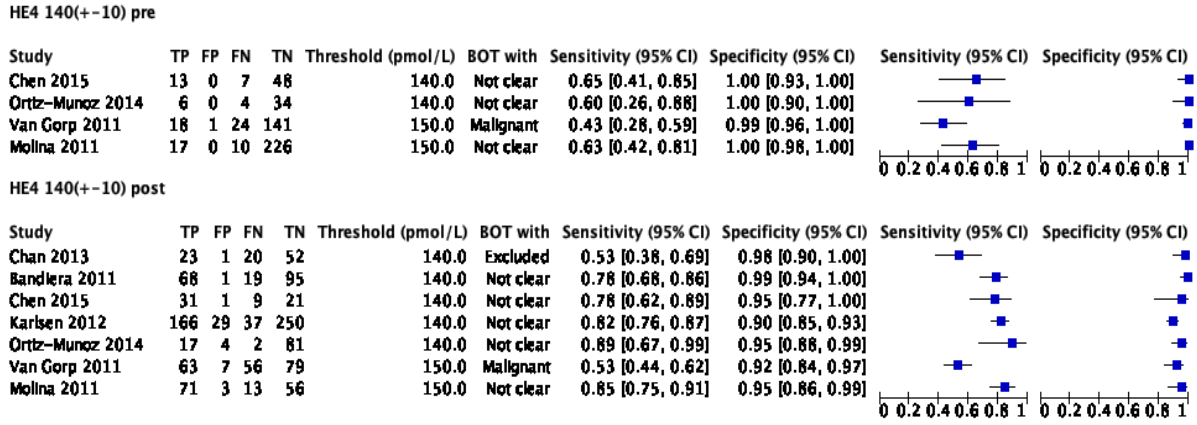


HE4 70(+-10) post

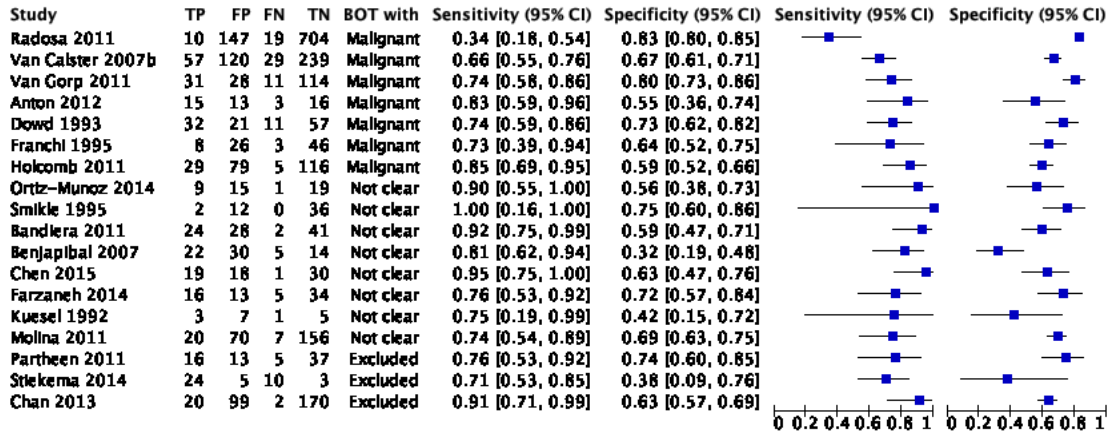
Study	TP	FP	FN	TN	Threshold (pmol/L)	BOT with	Sensitivity (95% CI)	Specificity (95% CI)	Sensitivity (95% CI)	Specificity (95% CI)
Fujiwara 2015	31	10	10	29	63.3	Not clear	0.76 [0.60, 0.88]	0.74 [0.58, 0.87]		
Anton 2012	30	17	6	20	70.0	Malignant	0.83 [0.67, 0.94]	0.54 [0.37, 0.71]		
Van Gorp 2011	92	25	27	61	70.0	Malignant	0.77 [0.69, 0.84]	0.71 [0.60, 0.80]		
Stiekema 2014	103	1	10	25	70.0	Excluded	0.91 [0.84, 0.96]	0.96 [0.80, 1.00]		
Ortiz-Munoz 2014	18	13	1	72	77.0	Not clear	0.95 [0.74, 1.00]	0.85 [0.75, 0.92]		



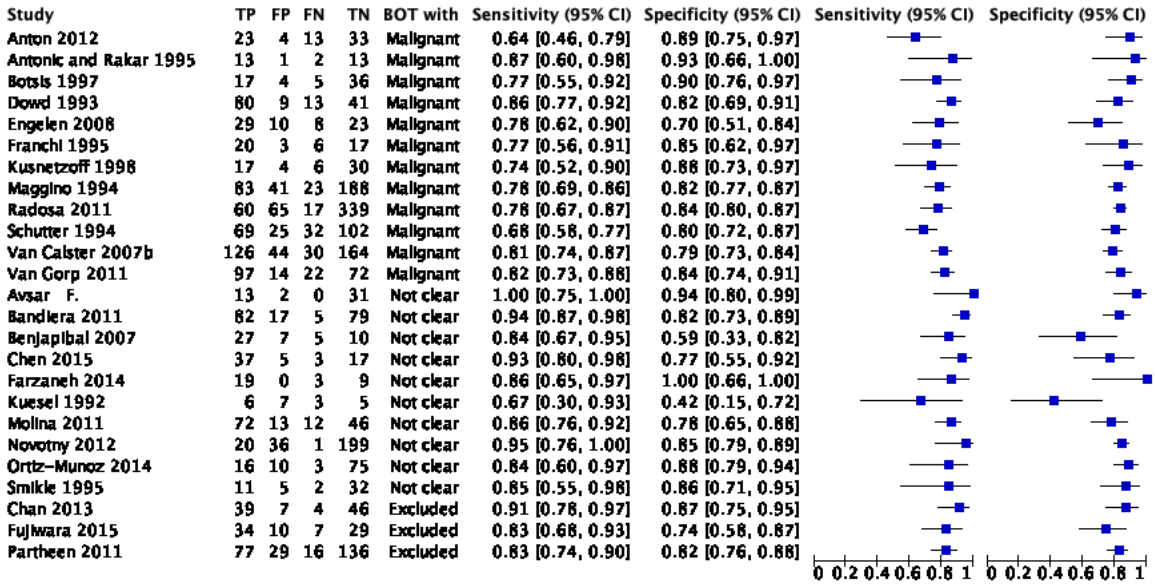
Appendix 19 **: HE4 140 (+/- 10) pre post Forest plot.**



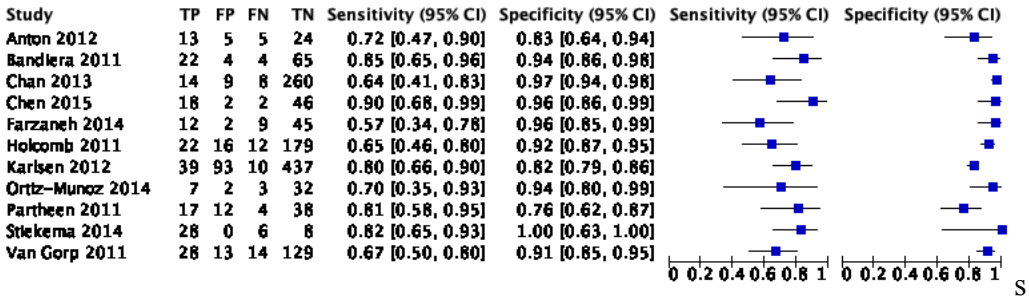
Appendix 20 **: CA125 35 (+/- 10) pre BOT Forest plot.**



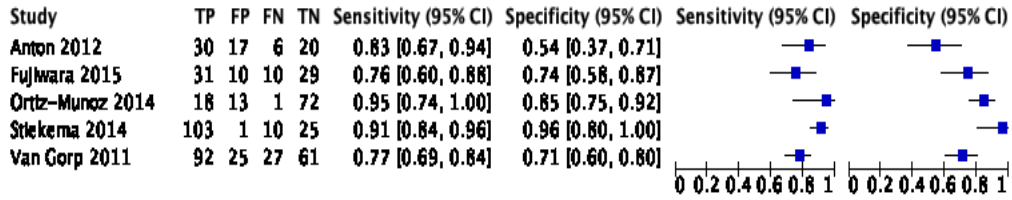
Appendix 21 **: CA125 65 (+/- 10) post BOT Forest plot.**



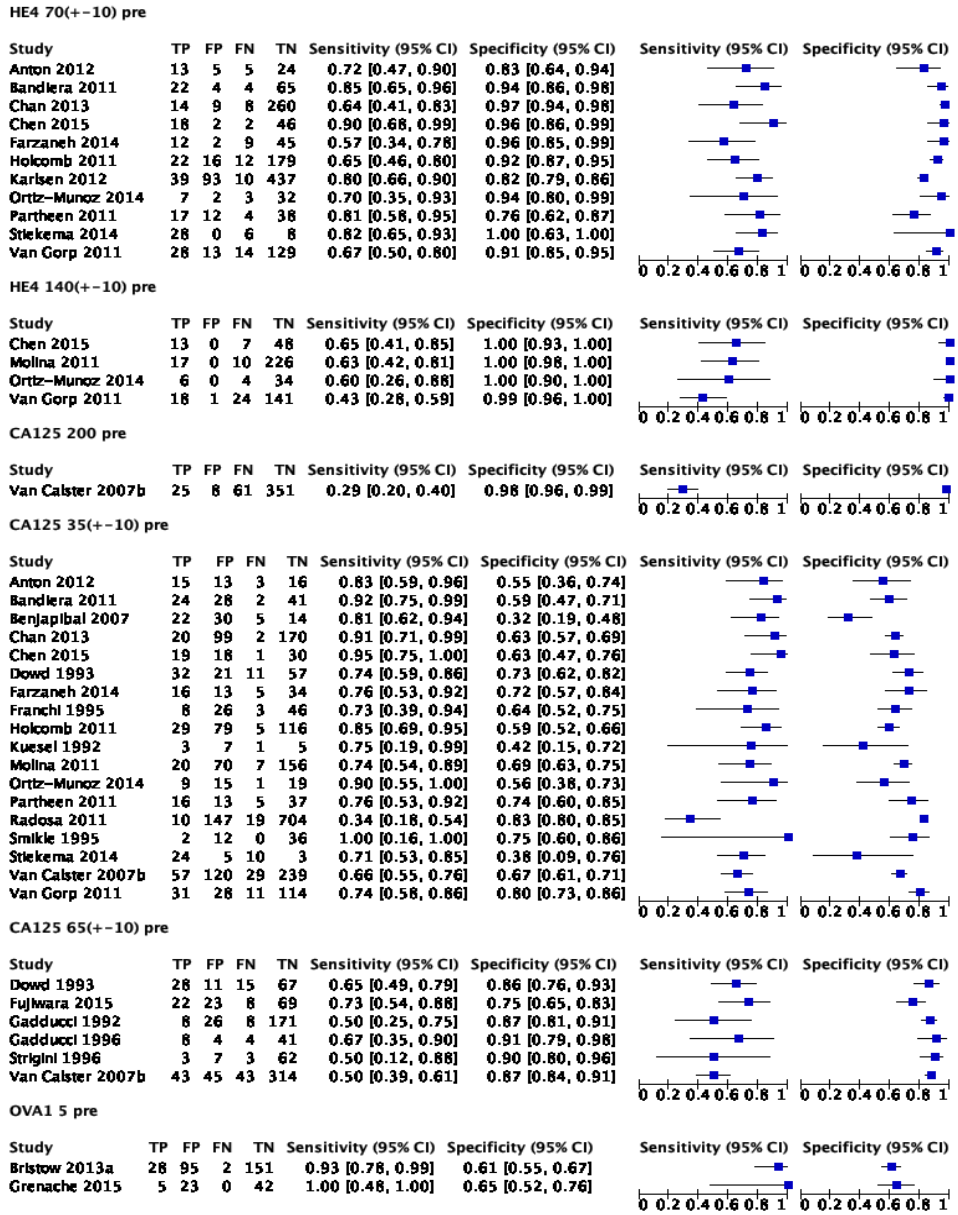
Appendix 22 **: HE4 70 (+/- 10) pre BOT Forest plot.**



Appendix 23 **: HE4 70 (+/- 10) post BOT Forest plot.**



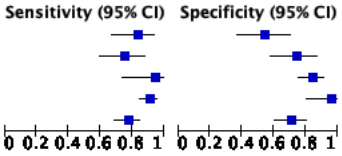
Appendix 24 : CA125, HE4 and OVA1- All tests Pre Forest plot.



Appendix 25 **: CA125, HE4 and OVA1- All tests post Forest plot.**

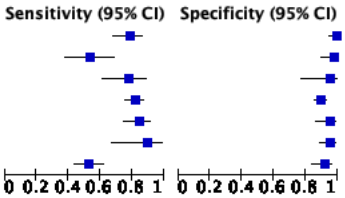
HE4 70(+-10) post

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Anton 2012	30	17	6	20	0.83 [0.67, 0.94]	0.54 [0.37, 0.71]
Fujiwara 2015	31	10	10	29	0.76 [0.60, 0.88]	0.74 [0.58, 0.87]
Ortiz-Munoz 2014	18	13	1	72	0.95 [0.74, 1.00]	0.85 [0.75, 0.92]
Stiekema 2014	103	1	10	25	0.91 [0.84, 0.96]	0.96 [0.80, 1.00]
Van Gorp 2011	92	25	27	61	0.77 [0.69, 0.84]	0.71 [0.60, 0.80]



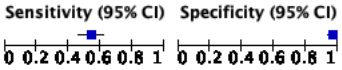
HE4 140(+-10) post

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Bandiera 2011	68	1	19	95	0.78 [0.68, 0.86]	0.99 [0.94, 1.00]
Chan 2013	23	1	20	52	0.53 [0.38, 0.69]	0.98 [0.90, 1.00]
Chen 2015	31	1	9	21	0.78 [0.62, 0.89]	0.95 [0.77, 1.00]
Karlsen 2012	166	29	37	250	0.82 [0.76, 0.87]	0.90 [0.85, 0.93]
Molina 2011	71	3	13	56	0.85 [0.75, 0.91]	0.95 [0.86, 0.99]
Ortiz-Munoz 2014	17	4	2	81	0.89 [0.67, 0.99]	0.95 [0.88, 0.99]
Van Gorp 2011	63	7	56	79	0.53 [0.44, 0.62]	0.92 [0.84, 0.97]



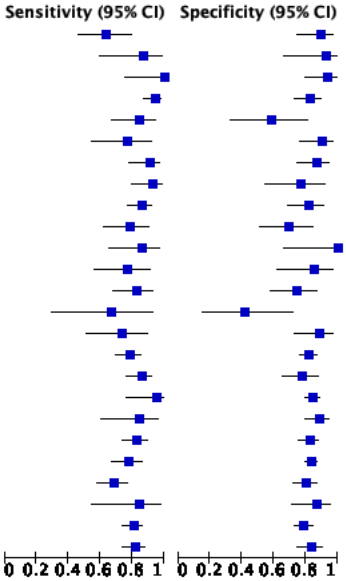
CA125 200 post

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Van Calster 2007b	85	6	71	202	0.54 [0.46, 0.62]	0.97 [0.94, 0.99]



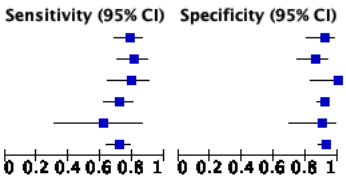
CA125 35(+-10) post

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Anton 2012	23	4	13	33	0.64 [0.46, 0.79]	0.89 [0.75, 0.97]
Antonic and Rakar 1995	13	1	2	13	0.87 [0.60, 0.98]	0.93 [0.66, 1.00]
Avsar F.	13	2	0	31	1.00 [0.75, 1.00]	0.94 [0.80, 0.99]
Bandiera 2011	82	17	5	79	0.94 [0.87, 0.98]	0.82 [0.73, 0.89]
Benjapibal 2007	27	7	5	10	0.84 [0.67, 0.95]	0.59 [0.33, 0.82]
Botsis 1997	17	4	5	36	0.77 [0.55, 0.92]	0.90 [0.76, 0.97]
Chan 2013	39	7	4	46	0.91 [0.78, 0.97]	0.87 [0.75, 0.95]
Chen 2015	37	5	3	17	0.93 [0.80, 0.98]	0.77 [0.55, 0.92]
Dowd 1993	80	9	13	41	0.86 [0.77, 0.92]	0.82 [0.69, 0.91]
Engelen 2008	29	10	8	23	0.78 [0.62, 0.90]	0.70 [0.51, 0.84]
Farzaneh 2014	19	0	3	9	0.86 [0.65, 0.97]	1.00 [0.66, 1.00]
Franchi 1995	20	3	6	17	0.77 [0.56, 0.91]	0.85 [0.62, 0.97]
Fujiwara 2015	34	10	7	29	0.83 [0.68, 0.93]	0.74 [0.58, 0.87]
Kuesel 1992	6	7	3	5	0.67 [0.30, 0.93]	0.42 [0.15, 0.72]
Kusnetzoff 1998	17	4	6	30	0.74 [0.52, 0.90]	0.88 [0.73, 0.97]
Maggino 1994	83	41	23	188	0.78 [0.69, 0.86]	0.82 [0.77, 0.87]
Molina 2011	72	13	12	46	0.86 [0.76, 0.92]	0.78 [0.65, 0.88]
Novotny 2012	20	36	1	199	0.95 [0.76, 1.00]	0.85 [0.79, 0.89]
Ortiz-Munoz 2014	16	10	3	75	0.84 [0.60, 0.97]	0.88 [0.79, 0.94]
Partheen 2011	77	29	16	136	0.83 [0.74, 0.90]	0.82 [0.76, 0.88]
Radosa 2011	60	65	17	339	0.78 [0.67, 0.87]	0.84 [0.80, 0.87]
Schutter 1994	69	25	32	102	0.68 [0.58, 0.77]	0.80 [0.72, 0.87]
Smikle 1995	11	5	2	32	0.85 [0.55, 0.98]	0.86 [0.71, 0.95]
Van Calster 2007b	126	44	30	164	0.81 [0.74, 0.87]	0.79 [0.73, 0.84]
Van Gorp 2011	97	14	22	72	0.82 [0.73, 0.88]	0.84 [0.74, 0.91]



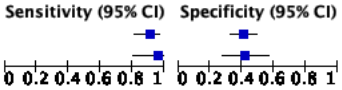
CA125 65(+-10) post

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Dowd 1993	73	4	20	46	0.78 [0.69, 0.86]	0.92 [0.81, 0.98]
Gadducci 1992	60	8	14	49	0.81 [0.70, 0.89]	0.86 [0.74, 0.94]
Gadducci 1996	35	0	9	20	0.80 [0.65, 0.90]	1.00 [0.83, 1.00]
Maggino 1994	76	19	30	210	0.72 [0.62, 0.80]	0.92 [0.87, 0.95]
Strigini 1996	8	2	5	19	0.62 [0.32, 0.86]	0.90 [0.70, 0.99]
Van Calster 2007b	112	16	44	192	0.72 [0.64, 0.79]	0.92 [0.88, 0.96]



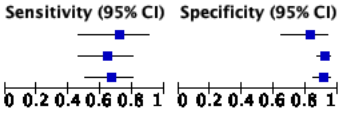
OVA1 4.4 post

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Bristow 2013a	53	92	5	64	0.91 [0.81, 0.97]	0.41 [0.33, 0.49]
Grenache 2015	25	29	1	21	0.96 [0.80, 1.00]	0.42 [0.28, 0.57]



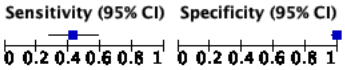
HE4 70(+−10) pre

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Anton 2012	13	5	5	24	0.72 [0.47, 0.90]	0.83 [0.64, 0.94]
Holcomb 2011	22	16	12	179	0.65 [0.46, 0.80]	0.92 [0.87, 0.95]
Van Gorp 2011	28	13	14	129	0.67 [0.50, 0.80]	0.91 [0.85, 0.95]



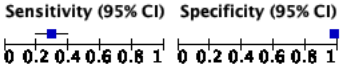
HE4 140(+−10) pre

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Van Gorp 2011	18	1	24	141	0.43 [0.28, 0.59]	0.99 [0.96, 1.00]



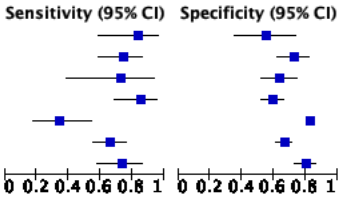
CA125 200 pre

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Van Calster 2007b	25	8	61	351	0.29 [0.20, 0.40]	0.98 [0.96, 0.99]



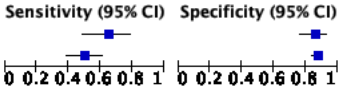
CA125 35(+−10) pre

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Anton 2012	15	13	3	16	0.83 [0.59, 0.96]	0.55 [0.36, 0.74]
Dowd 1993	32	21	11	57	0.74 [0.59, 0.86]	0.73 [0.62, 0.82]
Franchi 1995	8	26	3	46	0.73 [0.39, 0.94]	0.64 [0.52, 0.75]
Holcomb 2011	29	79	5	116	0.85 [0.69, 0.95]	0.59 [0.52, 0.66]
Radosa 2011	10	147	19	704	0.34 [0.18, 0.54]	0.83 [0.80, 0.85]
Van Calster 2007b	57	120	29	239	0.66 [0.55, 0.76]	0.67 [0.61, 0.71]
Van Gorp 2011	31	28	11	114	0.74 [0.58, 0.86]	0.80 [0.73, 0.86]



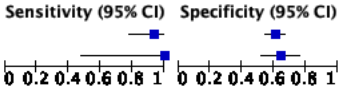
CA125 65(+−10) pre

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Dowd 1993	28	11	15	67	0.65 [0.49, 0.79]	0.86 [0.76, 0.93]
Van Calster 2007b	43	45	43	314	0.50 [0.39, 0.61]	0.87 [0.84, 0.91]



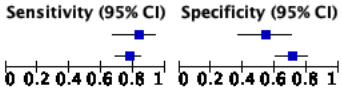
OVA1 5 pre

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Bristow 2013a	28	95	2	151	0.93 [0.78, 0.99]	0.61 [0.55, 0.67]
Grenache 2015	5	23	0	42	1.00 [0.48, 1.00]	0.65 [0.52, 0.76]



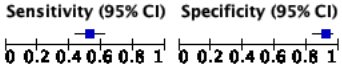
HE4 70(+-10) post

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Anton 2012	30	17	6	20	0.83 [0.67, 0.94]	0.54 [0.37, 0.71]
Van Gorp 2011	92	25	27	61	0.77 [0.69, 0.84]	0.71 [0.60, 0.80]



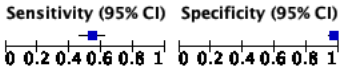
HE4 140(+-10) post

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Van Gorp 2011	63	7	56	79	0.53 [0.44, 0.62]	0.92 [0.84, 0.97]



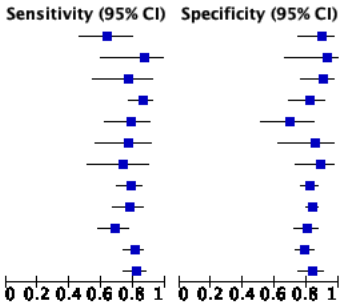
CA125 200 post

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Van Calster 2007b	85	6	71	202	0.54 [0.46, 0.62]	0.97 [0.94, 0.99]



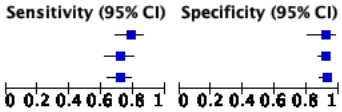
CA125 35(+-10) post

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Anton 2012	23	4	13	33	0.64 [0.46, 0.79]	0.89 [0.75, 0.97]
Antonic and Rakar 1995	13	1	2	13	0.87 [0.60, 0.98]	0.93 [0.66, 1.00]
Botsis 1997	17	4	5	36	0.77 [0.55, 0.92]	0.90 [0.76, 0.97]
Dowd 1993	80	9	13	41	0.86 [0.77, 0.92]	0.82 [0.69, 0.91]
Engelen 2008	29	10	8	23	0.78 [0.62, 0.90]	0.70 [0.51, 0.84]
Franchi 1995	20	3	6	17	0.77 [0.56, 0.91]	0.85 [0.62, 0.97]
Kusnetzoff 1998	17	4	6	30	0.74 [0.52, 0.90]	0.88 [0.73, 0.97]
Maggino 1994	83	41	23	188	0.78 [0.69, 0.86]	0.82 [0.77, 0.87]
Radosa 2011	60	65	17	339	0.78 [0.67, 0.87]	0.84 [0.80, 0.87]
Schutter 1994	69	25	32	102	0.68 [0.58, 0.77]	0.80 [0.72, 0.87]
Van Calster 2007b	126	44	30	164	0.81 [0.74, 0.87]	0.79 [0.73, 0.84]
Van Gorp 2011	97	14	22	72	0.82 [0.73, 0.88]	0.84 [0.74, 0.91]



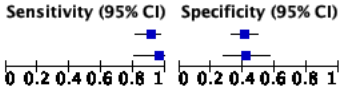
CA125 65(+-10) post

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Dowd 1993	73	4	20	46	0.78 [0.69, 0.86]	0.92 [0.81, 0.98]
Maggino 1994	76	19	30	210	0.72 [0.62, 0.80]	0.92 [0.87, 0.95]
Van Calster 2007b	112	16	44	192	0.72 [0.64, 0.79]	0.92 [0.88, 0.96]



OVA1 4.4 post

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Bristow 2013a	53	92	5	64	0.91 [0.81, 0.97]	0.41 [0.33, 0.49]
Grenache 2015	25	29	1	21	0.96 [0.80, 1.00]	0.42 [0.28, 0.57]



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