Diagnostic accuracy of human epididymis protein 4 (HE4) and cancer antigen 125 (CA125) for screening and follow-up of patients with suspected ovarian cancer in Kenya

A dissertation submitted in partial fulfillment of the requirements for the degree of Master of Medicine in Obstetrics and Gynaecology in the College of Health Sciences, University of Nairobi.

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Declaration and Approval by supervisors

I declare that this research proposal is my original work and has not been published elsewhere or presented for a degree in any other institution.

…….. ……..
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Approval
This proposal has been submitted to the University of Nairobi with our full approval as university supervisors.

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Declaration

I declare that' ***'Diagnostic accuracy of human epididymis protein 4 (HE4) and cancer antigen 125 (CA125) for screening and follow-up of patients with suspected ovarian cancer in Kenya'*** is my own work. All resources and materials I have used or quoted have been indicated and acknowledged by means of reference. I further declare that this dissertation has not been submitted for the award of any other degree or to any university or institution.

Dr. Stephen Mwinga

Signed ……

Date…….

Dedication

This book is dedicated to my mother, Esther Mwinga, my father, Julius Mwinga, my wife, Evelyne, and my children Tendai and Wangari Babu, for their unwavering support to achieve my life goals.

My success is their success.

Certificate of Authenticity

This is to certify that this dissertation is the original work of Dr Stephen Babu Mwinga Master of Medicine student in the Department of Obstetrics and Gynaecology, School of Medicine, University of Nairobi. Registration number H58/64068/2013 (2012 – 2016). The research was carried out in the Gynaecology unit of Kenyatta National Hospital under the supervision of the department of Obstetrics and Gynaecology, School of Medicine, College of Health Sciences, University of Nairobi. It has not been presented to any other university for award of degree.

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May God bless you all.

Abbreviations

AKUHN	Aga Khan University , Nairobi
CA125	Cancer (or carbohydrate) antigen 125
CI	Confidence interval
CT scan	Computerized tomography scan
EOC	Epithelial ovarian cancer
ELISA	Enzyme-linked immuno assay
ERC	Ethics Research Committee
FDA	Food and drug administration
FN	False negative
FP	False positive
HE4	Human epididymis protein 4
KNH	Kenyatta National Hospital
MRI	Magnetic resonance imaging
QA	Quality assurance
QoL	Quality of life
QC	Quality control
REDcap	Research electronic data capture
ROC	Receiver operating characteristic curve
ROMA	Risk of ovarian malignancy algorithm
TN	True negative
TP	True positive
TOM	Tubo-ovarian mass
UoN	University of Nairobi
WAP	Whey acid protein
WFDC2	WAP-type four disulphide core 2

List of figures and tables

Tables

Table of Contents

1.0 Abstract

Introduction

Ovarian cancer is a common cancer in the world and has poor prognosis because it is usually widespread by the time patients are symptomatic. Early diagnosis is often not possible because of the vagueness of symptoms and nonexistence of a screening policy. Cancer antigen 125 (CA125) has been used for a long time to screen for ovarian malignancies and also in follow up after debulking surgery and during chemotherapy. It is elucidated by many benign pelvic pathology. Human epididymis protein 4 (HE4) is a newer marker which is probably superior. It has not been studied in our population.

Research question

What is the individual and combined diagnostic accuracy of HE4 and CA125 for screening patients with suspected malignant ovarian conditions in selected hospitals in Kenya?

Null Hypothesis

There is no difference in effectiveness of HE4 and CA125 as tumour marker for screening for cancer of the ovaries in the Kenyan population.

Objectives

The broad objective was to determine whether HE4 was more effective than CA125 as a tumour marker for screening and follow up patients with ovarian malignancies in Kenyan population.

Specific objectives were to compare specificity and sensitivity, and predictive values of HE4 and CA125, and to determine the best diagnostic cutoff.

Methodology

The study was a case control study. The categorization of cases and controls was be done post-priori after histological diagnosis of ovarian tissue in patients who underwent surgery for tubo-ovarian masses (TOM) detected clinically, sonographically or radiologically. Those with histological diagnosis of ovarian cancer were categorized as cases and benign lesions as controls.

Study sites was Kenyatta National Hospital, Nairobi. Samples were analyzed in Biochemistry laboratory, and for quality control a few in Aga Khan University Hospital, Nairobi. Sample size was 88 patients. Pre-operative serum HE4 and CA125 levels were determined. Intraoperatively the TOM were described, staged and surgically removed.

Variables Independent variables were age, menopausal status, cancer state and stage, parity, age at menarche, age at menopause, history of hormonal contraceptive use. Dependent variables are serum HE4 and CA125 levels, radiological size of tubo-ovarian masses and histological sub type of cases.

Analysis & Results

The mean age was 39.5 (95%CI 25.4-53.6) for benign lesions and 48.9(95%CI 35.5-62.3) for malignant lesions. Mean parity was 1.7 1.7 (95%CI 0-3.1) and 4.7(95%CI 2.5-6.8) for benign and malignant tumours. Common symptoms were abdominal swelling or distention (81.4%) abdominal pain (63%) weight loss (56%), leg swelling (19%) early satiety and bloatedness (22%).There was no significant difference in BMI, and hormonal contraceptive use in the two groups. Lesions size on imaging had a mean of 58.25cm^2 (95%CI 35.22-81.28) for benign and 185.75 cm^2 (95%CI 33.95-337.55) for malignant. Intraoperatively the mean lesion size was 200.65 cm^2 (95%CI 3.99-397.2) for benign and 300 cm^2 (95%CI 115.2-484.77). Histological types were metastatics adenocarcinoma (33%) mostly papillary serous adenocarcinoma, mature teratomas (23%), mucinousa cystadenoma (14%), two patients had poorly differentiated

metastastic tumour with unclear lineage on ultrasound guided biopsy. Other histological types were malignant leydig-sertoli tumours, brenners tumours , simple congenital cyst of Morgagni,.

The mean levels for CA125 was 363.16U/ml(95%CI 189.5-536.8). For malignant tumours the mea value was 769.22U/mL(95%CI 399.7 -1138.74). The mean levels of HE4 was 360pg/dL (95%CI 244.5-475.8) and for benign lesions 72.4pg/dL(48.9-95.8) and for malignant 740pg/dL(95%CI531.7-950.2)Separate sensitivity and specificity for CA125 was 95.12% and 75.5%. for HE4 sensitivity was 57.14% and specificity of 96%. Combined CA125 and HE4 sensitivity and specificity was 97.48% and 62.23%.

Discussion

The levels of CA125 and HE4 in ovarian tumours are both elevated, with higher levels in postmenopausal and malignant lesions. Cut-off levels provided by manufacturer relate well with levels that are required to determine sensitivity and specificity. Sensitivity and specificity levels from our study are similar to levels in other studies.

Conclusion and recommendations

Addition of HE4 to CA125 improved sensitivity of CA125 for screening of ovarian tumours. We recommend that HE4 be added to the test menu for ovarian tumours. We also recommend that additional resources including clinics and theatre to cater for the large number of patients presenting with ovarian masses. We should find a way to expedite histology reporting for these patients.

2.0 Introduction

Ovarian cancer is a common cancer in the world and has the worst prognosis of all gynaecological cancers. (1). It is a common cause of mortality in our gynaecology units as shown by two studies in the two largest referral hospitals in Kenya. (2,3). Ovarian cancer often has dismal prognosis because it is usually widespread by the time patients are symptomatic. (4,5) . Early diagnosis is often not possible because of the vagueness of symptoms and non existence of a screening policy. Several cancer associations do not recommend screening in the general population.(6) . For patients with a high pretest probability of ovarian malignancy, cancer antigen 125 (CA125) has been used for a long time to screen for ovarian malignancies, different authors have cited big differences in the sensitivity and specificity of CA125. There is paucity of local published data on the diagnostic accuracy of CA125 in the African population. The utility of CA125 as a prognostic marker is limited to follow up after debulking surgery and during chemotherapy. Its main drawbacks include its elucidation by non-malignant pelvic pathology including endometriosis, pelvic inflammatory disease, peritonitis and even physiological events like menses. (7). HE4 is a newer marker which is at least as sensitive as and more specific than CA125, although many authors do not agree on the levels of sensitivity and specificity. (6-8) Some authors have combined both tumour markers to improve on the accuracy of ovarian cancer screening. One such is the Risk of Malignancy Algorithm (ROMA) developed by Moore *et al* and which aims to employ the individual advantages of CA125 and HE4 (7, 9). A lot of research about HE4 in the last several years has yielded significant knowledge about the basic science of the molecule. We now know that it is produced by normal tissue in minimal amounts and

over-expressed by ovarian cancer. (10, 11) It is suspected that HE4 may have several advantages lacking from CA125. The improved specificity of HE4 over CA125 can help reduce false positives thereby potentially decreasing unnecessary interventions in patients with suspected ovarian cancer. The performance of HE4 as a screening tool needs to be validated in different populations before being adopted as a routine screening tool for ovarian cancer.

3.0 Literature review

Prevalence of ovarian cancer

Globally cancer of the ovary is the second most common gynaecological malignancy after cancer of cervix. Annual incidence is estimated at over 200,000 with fatality of more than 120,000(8). It therefore remains the most lethal of all gynaecological cancers largely because it is advanced and widespread by the time of diagnosis(1, 12). In developed countries with good registries, the incidence rate is estimated at 8.8/100,000 women-years(8). In Kenya, together with cancer of the vulva and uterus, it is the fourth commonest cancer in females after cancer of cervix, breast and oesophagus. Here it is the most lethal(3).

Pathophysiology of ovarian cancer

The cause of ovarian cancer is unknown, but is thought to be multi-factorial. It is suspected that the source of malignant cells is distal fallopian tube epithelial cell which pass inflammatory agents from the genital areas to the pelvis. There is mutation of p53 tumour suppressor gene and inactivation of BRCA tumour suppressor genes and

suppression of HE4 genes which are protective. A second genotoxic event by organ specific environmental or ethnic factor leads to gene aberration.

A large hormonal risk profile is evidenced by exposure to reproductive hormones as shown by association with age, menarche, menopause and parity. There also exist geographical and racial differences in the histological types, with a higher incidence of epithelial adeno-carcinoma and clear cell carcinoma in blacks. Having familial history of breast cancer seem to be associated with ovarian cancer, the so called familial ovarian cancer syndrome, and this is thought to be mediated via BRCA1 & 2 genes. (6, 13)

Histology

Cancer of ovary can arise from any of the components of the ovary. More than 90% are epithelial in nature. WHO recognizes 8 subtypes; serous tumours constitute 30-70% and are histologically similar to fallopian tube cancers and are poor prognosis cancers. Mucinous type account for 5-20% and have mucin rich cell. Endometroid tumours make up 10-20% and have endometrial like glandular cells. Clear cell tumours constitute 3-10% and are clear with a glomerular type cells. Undifferentiated type account for 1%. Squamous, transitional type and mixed cell types are uncommon. These are further divided into benign, borderline or malignant subtypes depending on malignant potential and indolent behavior.

Genetics role and malignant characterization

Immuno-histochemical and molecular studies have enabled new insight into the pathogenesis of epithelial cancers and dual characterization of epithelial ovarian cancer into type I and type II, where type I are all major types which exhibit low grade nuclear

and cytoplasmic features. Slow growth and have origin in benign precursor lesions. They exhibit indolent clinical behavior and often present early, confined to one ovary. They are characterized by specific mutation – ERBB2, PTEN, KRAS, BRAF, CTNNB1, PPP2R1A and ARID1A genes. Type II tumours are aggressive cancers which often present late and are characterized by TP53 mutations, HER2/neu and AKT2 oncogenes over-expression. They comprise high grade serous, endometroid and malignant mixed mesodermal (carcinosarcoma) tumours(6)

Diagnostic challenges

Ovarian cancer is often asymptomatic until it is widespread in the abdominal cavity after which it presents with vague, non-specific symptoms. The tumour has a large volume available for enlargement before symptoms become apparent.(12) Most patients are diagnosed with stage III and IV, by which time the prognosis is poor with 5 year survival rates of less than 15%, whereas if caught early survival is better at more than 90%. (14) Early diagnosis is often made coincidentally through Sonography for other medical conditions. There is therefore need for a diagnostic tool that enables early diagnosis which can help reduce morbidity and mortality associated with advanced disease.

Tumour markers

Tumour markers are substances that are produced by tumour cells in larger amounts than normal. They are surrogate markers that can be used to predict risk of new cancers, recurrence, progression or mortality. The value lies in their ability to screen for early cancer, establish diagnosis, estimate prognosis and predict response to therapy. Availability of therapy for a particular cancer is important. Prerequisites for use of

tumour markers are; precise application- screening, risk assessment, prognosis or monitoring of treatment. (15). The last prerequisite is ability to separate patients into two or more groups with different outcomes; and reliable estimation of outcomes into positive and negatives. The ideal tumour markers are produced exclusively by tumours, their levels correlate with tumour burden, are easy to measure without being invasive and can be measured economically. There is no perfect tumour marker and one or more ideal characteristics are not achieved. High sensitivity and specificity makes a tumour marker, and indeed any test, clinically useful for screening of disease and follow-up of treatment. Often, a clinician is interested in the positive and negative predictive value of a test for his or her individual patients. Many tests involve a trade off involving sensitivity and specificity. (16) Clinicians order tests according to pre-test probability determined by local prevalence and their clinical acumen. (17)

CA125

Cancer antigen 125, also known as carbohydrate antigen 125 (CA125) or mucin 16 (MUC16) is a glycoprotein with 22,000 amino acids and is encoded by the MUC16 gene. Mucins are thought to protect the cell from adverse growth conditions by control of signal induction. Tumours use mucin to enhance their growth and metastasis by the anti-adhesive effect of mucin to detach from the tumour and invade surrounding stroma by their adhesive properties. They also use mucin to escape immunological surveillance. MUC16 has a large trans-membrane domain with an N-terminal domain, a tandem repeat domain (both extracellular and highly O-gylcosylated) and a C-terminal domain which contains extracellular components, a trans-membrane portion and a cytoplasmic tail. The

extra-cellular regions undergo proteolytic cleavage and are release into blood.(18, 19) It is currently one of the two compounds approved by the Food and Drug Administration authority (FDA) for screening of ovarian cancer. Its largest drawback is that it is elevated in other pelvic pathology. (14) . It has long been used in the screening and management of ovarian cancer. Its levels correlate well with tumour burden. Its sensitivity is cited as between 41.2% – 83.3% and specificity of ranging from 70-83%. (6, 9) Addition of other tumour markers seems to consistently improve its sensitivity and specificity. (20)

The biggest drawback of CA125 is its elucidation in many physiological and benign pelvic and abdominal conditions including menses, pregnancy, ascites, heart-failure peritonitis, endometriosis and even normal people (1%)(8, 21-23)

HE4

Human epididymis protein 4 (HE4) also known as WAP-type four disulphide core 2 (WFDC2) contains two whey acidic protein domain and a four disulphide bond core. It is encoded by the HE4 gene also known as WAP5, EDDM4 or dj461P17.6 gene. WFDC or WAP signature motif contains 8 cysteines forming four disulphide bonds at the core of the protein and functions as a protease inhibitor. This gene is expressed in pulmonary epithelial cells, epithelium of epididymis and has also been identified in trachea. Its role is unknown but is thought to be related to immune functions as its gene is on chromosome 20q which has immune functions and is also thought to play role in sperm maturation as it is similar in amino acids with extracellular proteinase in mucous sections of genital tract. (24, 25) The protein encoded by this gene is small 10 KDa acidic cysteine rich polypeptide HE4 levels are greatly increased in ovarian cancers and very

little expressed by normal tissue and benign ovarian disease. Yang *et al* demonstrated normal levels of HE4 in women with non-malignant ovarian pathology and raised levels in ovarian cancer. (1) although high tissue levels but low serum levels were reported by Chudecka-Głaz *et al* .(26)

Sensitivity of HE4 is unclear, with some authors quoting lower levels than that of CA125 but most reporting higher levels. There is almost unanimous agreement that it has higher specificity levels than CA125.(6,8). The ROMA algorithm developed by Moore *et al* and other studies conducted comparing utility of the two tumour markers individually versus combined have improved the sensitivity to over 90%, although specificity reduced as a result of CA125. (7, 9, 27) The Risk of malignancy index (RMI) developed by Jacobs *et al* utilizes sonographic findings, CA125 levels and menopausal status and studies reported a sensitivity(28) of 71-88% and specificity of 74- 97%. (23). HE4 is only raised in epithelial cancers of the ovary, which form a vast majority of ovarian malignancies. Its biggest advantage over CA125 is its sensitivity in early EOC during which CA125 levels are not elevated.(29)

Other screening modalities

Ultrasound plays a role in screening of ovarian malignancy especially in determination of physiologic findings, inflammatory processes benign lesions and malignancy. Some authors have reported higher sensitivity and specificity in use of ultrasound than CA125. Larger ovarian masses have been associated with higher malignancy states. Presence of multiplicity of cysts , solid areas and abnormal neo-vascularization seem to correlate well with malignancy. However, there is overlap of presence , spatial distribution and

presence of flow signals in Doppler in benign and malignant ovarian masses, and therefore Doppler evaluation does not discriminate well.(30) Fleisher *et al* reported a sensitivity of 100% and specificity of 82%, negative predictive value of 100% and negative predictive value of 73% using pulsability index, although his sample size was only 45. Sassaone *et al* reported similar values except for negative predictive values reported as 37% with a larger sample size of 143 patients (30)

Combined parameters to score risk

The predictive value of combined tumour markers and ultrasound improves. (12)Two such methods are risk of malignancy index (RMI) and risk of ovarian malignancy algorithm (ROMA). RMI uses a product of ultrasound score, menopausal status, and CA125. Scores of more than 200 are considered high risk. ROMA score uses HE4, CA125 and menopausal status. (27) Anton *et al* in their review found no significant difference in the use of individual tumour markers and the combined scores.(22)

4.0 Problem statement

Cancer antigen 125 (CA125) has not been effective for screening and follow up of patients with epithelial ovarian cancer (EOC) due to its low sensitivity estimated by different authors as between 41.2% – 83.3% and specificity of ranging from 70-83%.(6, 23). Its levels are elevated in many non malignant pelvic conditions including endometriosis, pelvic inflammatory disease, peritonitis and even menses.(23) Besides, it is not produced exclusively by ovaries. It has been used for screening and follow-up of EOC as its levels correlate fairly well with tumour burden. A search for a better tumour marker has revealed human epididymis protein 4 (HE4) which is expressed in very low

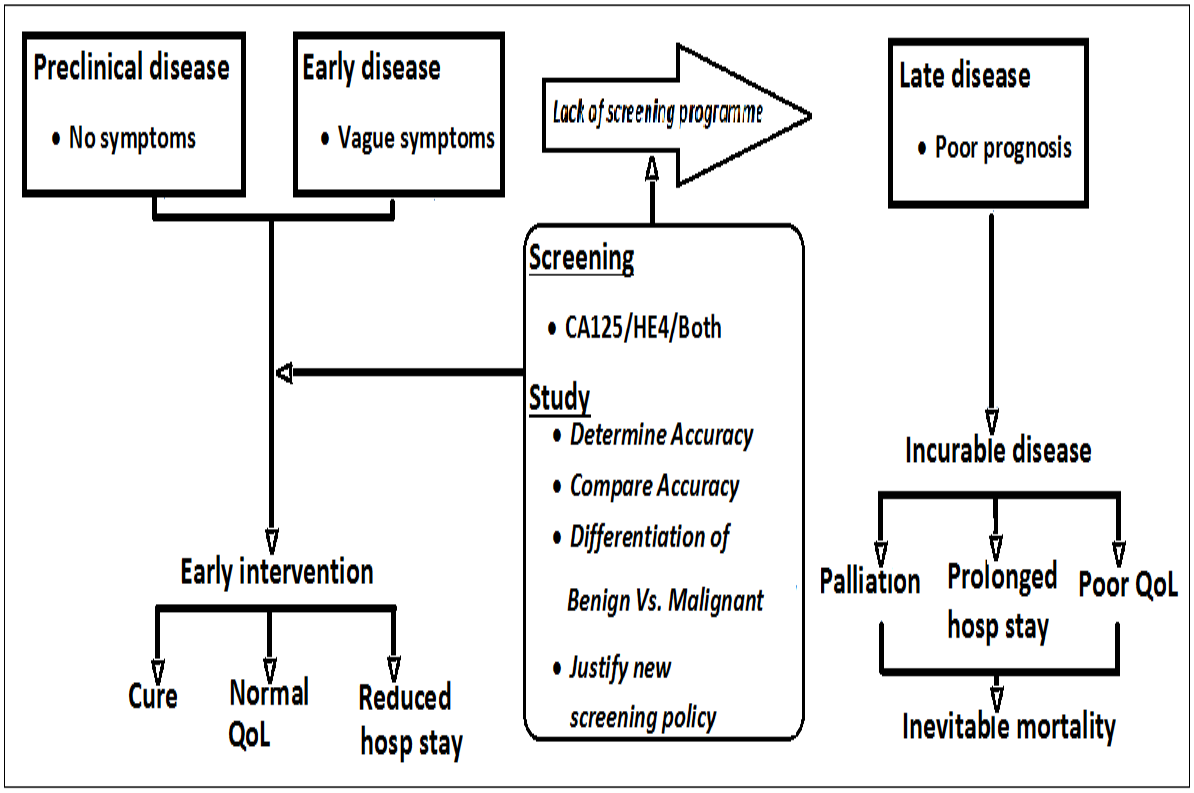
levels by normal ovaries and is over-expressed by ovaries with cancer. HE4 levels can be detected in early cancer in which CA125 levels are normal (31, 32). It has been postulated that HE4 may offer better positive and negative predictive value than CA125 for screening and follow up of ovarian cancer. Currently it is being studied in Scandinavian countries and Far East for predicting treatment failure and prognosis of ovarian cancer. In our population, it has not been studied, hence the need to compare its accuracy for screening and follow up of ovarian cancers, and to establish different cut offs with best diagnostic accuracy for our population.

5.0 Conceptual framework

Preclinical disease often presents with no symptoms. Lack of an accurate screening modality makes the opportunity for pre-emptive intervention rare. Early ovarian cancer presents with vague and non-specific symptoms and signs which can be confused with many conditions. (4, 5)There is lack of clear screening protocols for this stage unless there is a high index of clinical suspicion. Therefore patients present when disease is advanced by which time prognosis is poor. (1) Health workers are left to offer palliative care with increased bed occupancy, prolonged hospital stay and poor quality of life for both patients and health workers and mortality is inevitable. Development of a screening protocol and change in screening policy will enable disease to be picked early during which cure is possible with a shortened hospital stay, improved quality of life and potentially normal life expectancy. The current popular screening tool, CA125 is not sensitive enough to pick early disease. The study examined a newer tool HE4 side by side

and in combination with CA125 on the same population and with same outcomes. (12) Histological examination was used as the gold standard to differentiate benign and malignant disease and also the different subtypes. Inference into diagnostic quality of HE4 vis-à-vis CA125 was made using receiver operator curves (ROC). We shall then examine the best accuracy offered by each marker individually and in combination.

Figure 1: Conceptual framework



6.0 Justification for the Study

Early detection of ovarian cancer is rare because of lack of symptoms, vagueness of early symptoms and lack of screening program. (4, 5) CA125 has moderate sensitivity and specificity for screening for ovarian cancer and is raised in several physiological and non-malignant conditions including menses. It has an inherent inability to pick early disease largely because of its large molecular weight and is therefore not secreted into blood when tumour burden is low. (14, 18, 33) This severely limits its application as an ideal tumour marker as its poor specificity leads to unnecessary invasive procedures being performed on patients with benign conditions. HE4 is probably a superior tumour marker for screening and follow up of ovarian cancer. HE4 has not been studied in our population and therefore its value in screening, diagnosis and follow-up of patients with ovarian neoplasm is unknown. The prevalence of ovarian cancer, as with all diseases, varies in different geographical areas and ethnicities. As a result of this, the predictive value of any test will vary in different populations. We therefore needed to validate its utility in our population. A comparative study on its value in relation to CA125 using the same population and outcomes therefore becomes a valid undertaking. In Europe and Scandinavian countries where it has been studied, different studies have yielded different reference values. So far, no studies have been conducted in Sub Saharan Africa describing experiences with HE4. The gold standard of diagnosis was histological examination was done to determine the malignancy states of the tubo-ovarian masses. This enabled determination of sensitivity, specificity and predictive values of HE4 in relation to CA125.

7.0 Research question

What is the individual and combined diagnostic accuracy of HE4 and CA125 for screening patients with suspected malignant ovarian conditions in selected hospitals in Kenya?

8.0 The Null Hypothesis

There is no difference in diagnostic accuracy of Human epididymis protein 4 (HE4) and cancer antigen 125 (CA125) as tumour marker for screening for cancer of the ovaries in the Kenyan population.

9.0 Objectives

9.1 Broad Objective

To determine the diagnostic accuracy of HE4 and CA125 as a tumour marker for screening of ovarian malignancies in Kenya.

9.2 Specific Objectives

1. To determine the specificity and sensitivity of HE4 and CA125 individually and in combination for screening and follow-up of ovarian cancer
2. To compare the positive and negative predictive values of HE4 and CA125 individually and in combination for screening of ovarian cancer
3. To correlate levels of HE4 and CA125 with histological cell types
4. To determine the HE4 and CA125 cut-offs with best accuracy for diagnosing epithelial ovarian cancer

10. Methodology

10.1 Research design

The study was a case control study.

Justification for design

This design was selected as it offered the best ability to compare an existing screening modality with a new modality, and also separated those with disease and those without. Further, this design enabled the investigators to safely test a new test (HE4) with the gold standard. This enabled study of both tests without confounders. Both tests were offered to all participants so ensure that no patient was disadvantaged. The design enabled calculation of sensitivity, specificity and predictive values.

Categorization of cases and controls

The categorization of cases and controls was done post-priori after histological diagnosis of ovarian tissue in patients undergoing surgery for tubo-ovarian masses (TOM). (8, 23).

Recruitment

Patients were recruited from outpatient clinics presenting or referred with tubo-ovarian masses detected clinically, sonographically or radiologically. They were subjected to the current standard abdomino-pelvic physical examination and hospital-specific imaging modality i.e. sonographical and/or magnetic resonance imaging (MRI) or computed tomography (CT scan). We were alive to the fact that some patients may have had difficulties accessing more complicated and costly imaging modalities, although in the ideal all patients would have been subjected to similar imaging. The standard test which is CA125 was carried out and HE4 was also be done. Histological diagnosis was carried

out to confirm or exclude malignancy. Those with histological diagnosis of ovarian cancer were categorized as cases and benign lesions as controls. Our entry point was patients with tubo-ovarian masses (TOM) diagnosed by clinically, radiologically or intra operatively by laparotomy or laparoscopy. Pre-operative serum HE4 and CA125 levels were determined. Intraoperatively the TOM was described and staged and surgically removed and examined histologically to determine state of malignancy. For those found malignant, appropriate referral (where applicable) for chemotherapy was instituted. We used the numbers we recruited to determine the cut off values for malignancy for our population and compare these values with what the manufacturers of the kit give as reference values.

10.2 Study sites and settings

10.2.1 Study sites

10.2.1.1 Points of recruitment

Patients were recruited from outpatient clinics, gynaecological wards and theatres of Kenyatta National Hospital (KNH)

10.2.1.2 Set up of hospital

KNH

In KNH Gynaecology clinics are held weekly on Tuesday, Wednesday and Thursday. On Fridays there is a specialized gynaecology oncology clinic. Theatre days are on Thursdays. A total of 100 patients are seen weekly. There is a ward for acute gynaecology patients and another for non-emergency cases.

Blood and tissue analysis

1. Samples were analyzed in Biochemistry laboratory, department of pathology, Kenyatta National Hospital
2. Tissues were examined in the Histopathology departments of University of Nairobi.

Justification for study site

Kenyatta National Hospital is the largest teaching and referral hospital in East and Central Africa. It is also one of the two public hospitals which have specialist oncologists. For this reason many patients are referred to KNH for suspected oncologists and we are likely to get most of our sample size from KNH.

10.3. Study population

Patients attending Gynaecological outpatient clinics in Kenyatta National Hospital (KNH)

10.4. Laboratory evaluation

Serum level of HE4 and CA125 were assayed using ELISA kits on Cobas 4800 platform (Roche Diagnostics Corporation, IN, USA) located in the KNH Biochemistry laboratory, and as a Quality assurance, we ran 20% of the samples on Architect i1000SR platform (Abbott Laboratories, IL, USA) at the department of Biochemistry, Pathology, Aga Khan University Hospital, Nairobi.

10.5 Inclusion and exclusion criteria

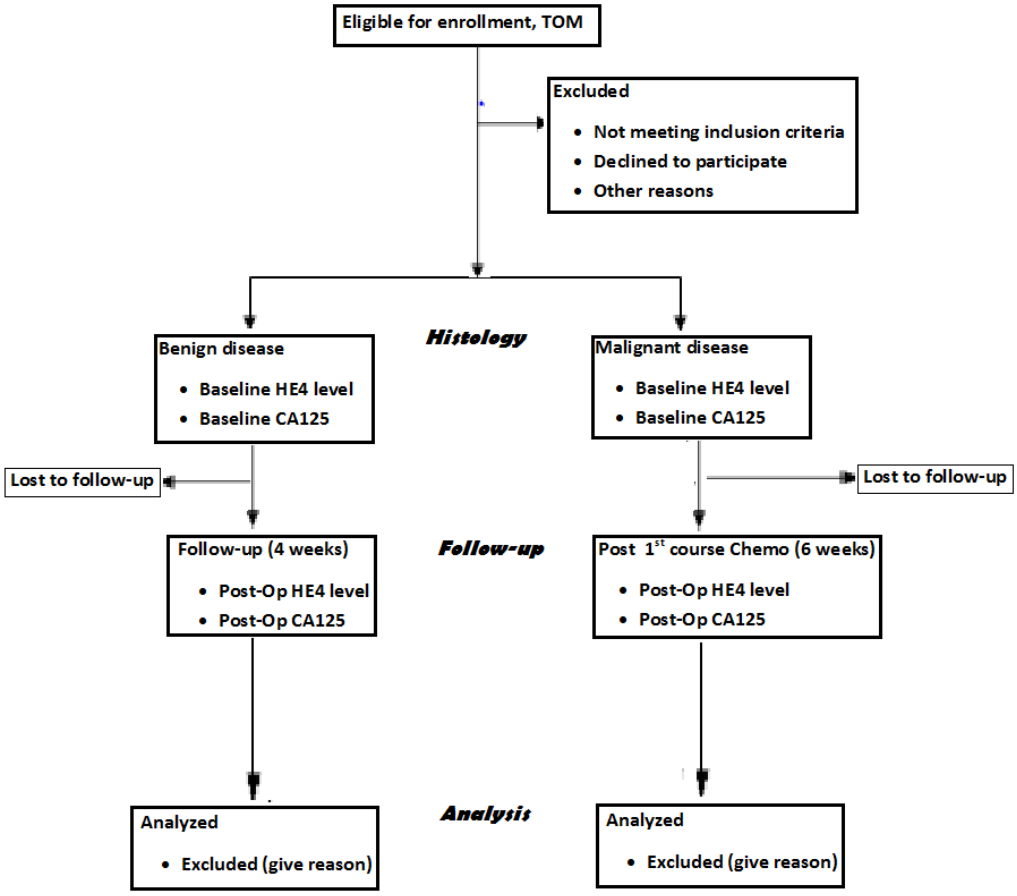
Inclusion criteria

1. Patients with tubo-ovarian or adnexal masses
2. Not on chemotherapy
3. Willingness to participate

Exclusion criteria

1. Inability to sign consent or unwillingness to participate
2. Intrauterine pregnancy
3. Confirmed multiple malignancies

Figure 2: Recruit flow chart



10.6 Sample size calculation

The study sample size was determined using the formula described by Buderer N in 1996 which incorporates the prevalence of disease into sample size calculation for sensitivity and specificity. (34)

W= the maximum clinically acceptable width of 95% confidence interval (CI).

SN = value of the expected sensitivity of HE4 (90%), CA125 (90%)

SP = value of the expected specificity of HE4 (80%), CA125 (80%)

Prevalence of disease among the tubo-ovarian masses cases estimated at 40%.(35)

Number with disease, TP+FN

TP+FN = Z_{α}^{2} is the value from the standard normal table with α being Type I error rate (1.96).

Calculation using sensitivity

$$TP+FN = Z_{\alpha/2}^{2} \frac{SN(1-SN)}{W^{2}}$$

$$= 1.96^{2} \times \frac{0.9(1-0.9)}{0.01^{2}}$$

$$N1 = \frac{TP+FN}{P}$$

=87 cases

Calculation using specificity

$$FP+TN = Z_{\alpha/2}^{2} \frac{SP(1-SP)}{W2}$$

$$= 1.96 \times \frac{0.8(1-0.8)}{(1-0.8)}$$

$$N2 = \frac{FP+TN}{(1-P)}$$

= 88 cases

Where

TP= True positives
FN= False negatives
P=Prevalence
CI= Confidence interval

N2 was larger than N1. We therefore used N2.

10.7 Sampling method

All patients who were eligible and willing to be recruited

10.8 Methods of Recruitment

The principal investigator, study assistants and colleagues recruited participants from clinics, accident and emergency department and wards. All patients with TOM larger than 8cm were offered the chance for recruitment.

10.9 Variables

10.9.1 Independent variables

Age, menopausal status, cancer state and stage, parity, age at menarche, age at menopause, history of hormonal contraceptive use

10.9.2 Dependent variables

Serum HE4 and CA125 levels, radiological size of tubo-ovarian masses, stage of tumours, histological sub type of cases and grade of malignancies,

10.10 Research instruments

A form containing biodata, and cancer staging data, levels of HE4 and CA125 developed by the Principal investigator was used to abstract data. The form contained biodata, HIV status, smoking, alcohol intake, contraception and referral status. We did not test for HIV status or any other test, but we recorded the status if known. Clinical symptoms and investigations were also be captured in the form. Surgical details were captured to enable staging of the tumours. Histopathological description of biopsy was entered to determine malignancy states of the tumours. This was key in determining sensitivity, specificity and predictive values were assayed using ***ELISA kits on Cobas 4800 platform*** (Roche Diagnostics Corporation, IN, USA) located in the KNH Biochemistry laboratory,

Quality assurance, we ran 20% of the samples on ***Architect i1000SR platform*** (Abbott Laboratories, IL, USA) at the department of Biochemistry, Pathology, Aga Khan University Hospital, Nairobi.

Manufacturer's instructions on sample collection, processing and analysis were followed and appropriate quality control (QC) and quality assurance (QA) protocols followed. (36) Levels of HE4 and CA125 were then correlated with histological diagnosis to determine the levels vis-á-vis disease states

10.11 Data collection

Data collection was carried out manually using data abstraction form by the principal investigator and trained research assistants.

10.12 Research assistants qualifications and training and roles

Clinical officers (physician assistants with a 3 year diploma level training), nursing officer (BScN and Diploma KRCN) and laboratory technician (Diploma- laboratory medicine) were recruited from KNH and trained in a 1 day seminar. Training covered data collection and back up with simulations. The assistants were tasked with triage of participants for suitability of recruitment and biodata recording. They would then inform the principal investigators who will then assess and recruit the participants.

10.13 Data analysis

Data were entered into an open access data-base, Microsoft Access (Windows Corporation, Redmond, WA, USA) and protected using a password. Analysis was done using Microsoft Excel (Windows Corporation, Redmond, WA, USA) and Stata

(StataCorp LP, TX, USA). The prevalence of ovarian cancer was calculated as the proportion of cases with ovarian cancer on histology/ number of patients with tubo-ovarian masses who underwent surgery for removal expressed as a percentage. Histology was considered as the gold standard test and was therefore used to determine the correct disease status. HE4 was the new test that was being evaluated. ROC (receiver operating characteristic) curve was used to evaluate the diagnostic performance (sensitivity and specificity) of HE4 at various cut off points. A 2 x 2 table was used to compare the performance of the new test to the gold standard test. All histology results that were negative for ovarian malignancy were considered true negatives (TN). Histology results which were positive for ovarian malignancy were considered true positives (TP).

HE4 specificity was calculated as follows: TN/TN + FP. Sensitivity was calculated as TP/ FN + TP. Positive predictive value was calculated as follows: TP/TP+FP. Negative predictive values was calculated as TN/TN+FN(16, 17, 34)

10.14 Ethical considerations.

Ethical approval was obtained from Ethical research committee (ERC) of Kenyatta National Hospital and University of Nairobi. Informed signed consent was sought from patients. They were informed of their right to refuse to participate and their quality of care would not be diminished by refusal to participate. They were also informed of their right to withdraw from the study at any point without giving a reason of doing so. It was impossible to collect data devoid of personal identifiers, but personal identifiers were removed at analysis. Data were protected using a secure password. Data results will only be shared with the hospital, university and ministry of health for the purposes of research,

education and policy formulation according to the laid down regulations. HE4, CA125 and histology were sponsored by KNH and were therefore free of charge to the patients. Histology results for KNH patients will be fast tracked for study participants. We declare that this may have influenced patients to participate in the study.

11. Results

We recruited a total of 90 patients from the period of September 2015 to April 2016. Of these, 79 were suitable for inclusion in the study. 70 were schedule for theatre. 10 underwent ultrasound guided biopsy in view of their frail condition which was judged to be unsuitable for explorative laparotomy. All patients had blood taken for CA125 and HE4. Tissue samples were sent to histopathology department for tissue diagnosis.

Table 1: socio-demographic characteristics of patients by histological classification

		Tumour type		
		Benign	Malignant	P values
Mean Age(yrs)		39.5 (95%CI 25.4-53.6)	48.9(95%CI 35.5-62.3)	0.28
Parity		1.7 (0-3.1)	4.7(2.5-6.8)	0.0092
Age at Menarche (yrs)		15.9 (14.0-17.7)	15.3 14.0-16.6)	0.72
Referral from another hospital		62.5%	88%	
Post Menopausal status		37.5	62.5	0.28
BMI		21.77(16.72-26.8)	20.71(17.97-23.45)	0.689
Hormonal contraceptives use	Yes	41.7	58.3	0.73
	No	60	40	0.52
Lesion size	Image	58.25 (35.22-81.28)	185.75(33.95-337.55)	0.01
	Surgery	200.65(3.99-397.2)	300(115.2-484.77)	0.403

Socio-demographics of patients

Table 1 shows socio-demographic characteristics of the participants. The youngest participant was aged 16 years, the oldest was aged 73. Mean age was 44.9 years (95%CI 38.1- 51.7). The highest parity was 10, and several patients were nulliparous. The mean parity was 3.2 (95%CI 2.2-4.2). The mean last delivery was 17.3 years go (95% CI 11.3-23.4) with a range of 3 months to 49 years from date of recruitment.) . Just over half of the participants were menopausal. For the postmenopausal women, the mean age at menopause was 52.1 years (95%CI 49.52-54.7). The mean last normal menstrual period was 4.1 years, (95%CI 1.8 – 6.4) .The average age at menarche was 15.6 years (95%CI 14.88-16.24 . Majority (73%) of participants were negative, but 15% did not know their HIV status. Interestingly, one of the participants reported use of nicotine, which she did for a very brief period in her experimental teenage year. A few (22%) reported that they consumed alcohol socially, one or two units per week. Mean weight of participants was 49.9 kgs (95%CI 45.7-54.0), and average height was 151.2 cm (95%CI 148.2-154.2), although this represented weight after loss attributed to illness. Just over half, 54%, reported use of hormonal contraceptives, the most popular was DMPA (25%) followed by OCPs (16%) IUCD was used by 16%. Twelve percent had history of use of more than one method at different periods. The average duration of use of hormonal methods was 5.56 years. Majority of patients (64%) were referred from other hospitals, generally in the Nairobi metropolis. Mbagathi hospital referring the highest number (19%)

.

Symptoms

The most common symptoms were abdominal swelling or distention (81.4%), abdominal pain (63%), weight loss (56%) others were leg swelling (19%), early satiety and bloatedness (22%), vomiting 15%. Others were dyspepsia, night sweats constipation, periumbilical ulceration (sister Mary Josephs node) and vaginal bleeding accounting for less than 10%

Imaging modalities

Most patients had access to ultrasound. CT scan was done by a third, and a few did MRI in addition to either ultrasound and/or CT scan. The mean area (some images only reported lesions in 2 dimensions, so we considered the 2 larger dimensions) was 120.1 cm^2 (95%CI 70-170 cm^2). There was a general predilection of disease to occur on the right (48%) vis a vis the left (28%), the rest (20%) had widespread disease including periumbilical ulcerated nodes (4%). Most lesions had thick walls (55%), a few had thin (22%), and for 22 % the wall were not described lesions were further described as complex, mixed or multilocular in 26% and simple, cystic or unilocular in 13%. Half had presence of fluid reported on imaging.

Intraoperative findings

The mean lesions size was 258.83cm2(95%CI 70-170.3), which was more than double the mean size found on imaging of 120cm2 (95%CI 70-170.2). This was perhaps likely due to the lag between imaging and operation hence disease progression.

There was a predilection of disease to occur on the right side, with 55%% of all lesions in the right adnexal region left sided lesions were seen in 22% and the rest had widespread

disease. Peritoneum was found thickened or nodular in 44%. Uterus was found involved in only a small proportion (28%), tubes were affected in 33% where affected had nodules. Nodes were described in only 16%. It is not clear if they were missed or were not affected. Mature teratomas presenting with hair, sebum and bony material were described in 23% of the lesions.

Histology

Table 2: Histological types and tumour marker levels

	No	(%)	CA125 (mean levels)	HE4 (mean levels)
Metastatic adenocarcinoma	20	25%	1168	1036.8
Mature teratomas	17	21.3%	29.81	24.66
Papillary serous adenocarcinoma	16	12.5%	109.4	205.7
Mucinous cystadenorcinoma	10	6.3%	77.81	139.45
Infectious lesions	5	5%	52.81	61.22
Endometroid	4	5%	62.82	57.22
Ectopic pregnancy *	4	5%	66.42	124.18
Metastatic tumour of Unclear lineage	2	2.5%	626.78	678
Congenital cysts	2	2.5%	67.73	273.65

*pregnancy test negative

Metastatic adenocarcinoma was the most common diagnosis representing 25 %. papillary serous adenocarcinoma was the most common subtype representing 12.5%. Others were mature teratomas 21.3%, mucinous cystadenomas 6.3%. Two patients had malignancies which were described as poorly differentiated metastatic tumours with unclear lineage. These were ultrasound guided biopsies. A small proportion of samples

(5%) were products of conception, which had tested negative for pregnancy detection test, and had therefore presented diagnostic dilemma for doctors. Five patients had no features of malignancy intra-operatively. Two had congenital cysts of Morgagni. One 16 year old standard 8 pupil had infective frozen abdomen likely from severe or recurrent pelvic infections, which was described as 'widespread metastatic disease with pelvic adenopathy' on MRI. Four had hydrosalpinx which were confused for tubo-ovarian masses. Classification of tumours into benign and malignant was done post priori after histology results. Cases were those with malignant classification, and controls were benign.

Tumour markers

CA 125

The mean CA125 levels for the entire population was 363.16 U/mL (95%CI 189.5-536.8). For the cases, the mean values for CA125 was 769.22 u/mL (95%CI 399.7-1138.74). These values were noted higher with the epithelial tumours especially serous adenocarcinomas. Benign or low tumours presented with mean value of 56.36 (95% 49.4-63.3). which is in keeping with other studies .Manufacturer of the CA125 kit give reference values of 0- 35 for normal and 36-70 for benign conditions.

HE4

The mean values for entire population was 360 pg/dL (95%CI 244.5-475.8) Cases had mean of 740.9 (95%CI531.7-950.2) and controls had mean of 72.4(95%CI 48.9-95.8). References values provided by the manufacturer of the kit were 0-35pmol/L for premenopausal and upto 140 pmol/L for postmenopausal women.

Table 3: Sensitivity, specificity, likelihood ratios and predictive values of CA125 and HE4

	CA125		**HE4**		**Combined CA125 +HE4**
		95%CI		**95%CI**	
Sensitivity	94.12%	80.32-99.28%	57.14%	18.41-90.10%	97.47%
Specificity	66.67%	51.05-80.00%	93.33%	81.73-98.60%	62.23%
Positive predictive ratio	68.09%	52.88-80.91%	57.14%	18.41-90.10%	63.24%
Negative predictive ratio	93.75%	79.19-99.23	93.33%	81.73-98.60%	97.36%

Sensitivity, specificity and predictive values

In our study, sensitivity of CA125 was 94.12% (95%CI 80.32-99.28) and that of HE4 was 57.14(95%CI 18.41-98.60) which was the same as other series. Positive Predictive values were 68% and 57.14% for CA125 and HE4 respectively. Negative predictive values were 93.75% and 93.33% for CA125 and HE4. Combined in parallel, the sensitivity increased to 97.48% and as expected specificity reduced to 62.23%.

12. Discussion

For CA125, we found sensitivity of 94.12% and specificity of 66.67%. This sensitivity is higher than what Hamed *et al* and Karlen found in their work, but the specificity was similar (6,9). HE4 sensitivity was found at 57.14% and specificity of 93.33%. When combined , sensitivity was increased to 97.47% , although specificity reduced to 62.23%. Steffensen *et al* found similar findings.(12)

Positive predictive ratio for CA125 was 68.09% and negative predictive ratio of 93.75%. HE4 had positive predictive ratio of 57.14% and a negative predictive value of 93.33%. When combined, positive predictive ratio was 63.24% but the negative predictive ratio of 97.36% which was higher than the individual values. Although the values for positive predictive ratio was lower in our study, we found a higher value than what Steffensen *et al* reported of 95%. (12)

Table 2 shows tumour levels for the different histological types. Adenocarcinoma had the highest levels of CA125 and HE4 at mean of 1168U/mL and 1026.8 pg/dL. This was followed by metastatic carcinoma of unclear lineage (CA125 mean 626.78U/mL and HE4 678pg/dL . Benign masses mature teratomas had a low levels of CA 125 at 29.8 U/mL and HE4 of 24.66.pg/dL. We had similar findings to Anto et al. (22) who found higher levels in epithelial tumours than other subtypes.

***Socio**-demographics of participants*

The mean age of participants was 44.9 years. In the cases the mean age was higher at 48.9 years compared to 39.5 years in the controls. More benign lesions were found in the premenopausal group compared to post menopausal group which had a higher proportion

of malignant lesions. Higher parity was associated with higher proportions of malignancy, with the mean parity of 4.7 in the malignancy group. Although it is not clear if this is an association rather than an independent risk factor. Other workers describe high parity as an independent risk factor lending credence to the theory of incessant ovulation as an important risk factor for cancer of ovary. A few participants reported social use of alcohol, mostly quantified as " one or two drinks a week". We find it odd that only one participant reported negligible use of nicotine. It is therefore hard to study effects of these recreational substances as risk factors.

Conclusion and recommendation

CA125 as a tumour marker has an important role in screening and more importantly in follow-up of cancer especially if levels are significantly raised before commencement of therapy. HE4 levels start to rise early, even in pre-clinical disease. It is however not yet available commercially in our setting. Its levels corresponded very well in patients with epithelial cancers which represents the majority of ovarian cancers. When combined, sensitivity improved especially in postmenopausal women. From our ROC, the cut-off of 35u/ml and HE4 cut-off of 70pg/ml as given by manufacturers of the kits is sensible to use. We therefore recommend that the values are used for our setting. We recommend that HE4 be introduced in addition to CA125 for screening and possibly follow up of ovarian cancers.

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APPENDICES

Appendix I: Consent Form

Introduction and consent
My name is_____and we are conducting a study to compare the clinical utility of human epididymis protein 4 (HE4) and cancer antigen 125 (CA125) in screening and follow up of cancer of ovaries in Kenya. We shall conduct this study in Kenyatta National Hospital, St Mary's Mission Hospital and Machakos Level 5 Hospital.
Study purpose
The purpose of the study is to validate the current method of screening for cancer of ovary (CA125) and compare it to a newer method (HE4) in order to determine if we should change how we screen.
Benefits
There is no monetary or financial benefit you derive from the study. You will however get a free HE4 and CA 125 test. Your participation may be important in coming up with interventions to improve the wellbeing of patients with cancer of the ovary in this region and Kenya at large.
Risks
There are no additional risks to you for participation. We shall collect a blood sample (20ml) to run HE4 and CA125 and any other test for cancer of ovary, which is the norm in patients with a pelvic mass.
Confidentiality
This interview is private and confidential. The blood tests and histology that will be done will be used for your care and therefore results will bear your name. However during data entry and analysis, your identification will be anonymized and your name will not be disclosed or used. The information you provide shall be used for the purpose of the study and any other test for cancer. You can also skip any questions that you do not want to answer.
Extra costs and time
You will not incur any extra cost by Participating in the study. This interview will take about 15 minutes.
Voluntariness of participation
Your participation in this study is voluntary. If you decide not to participate, you will not be penalized. Also, you can change your mind during the study and choose not to participate.
Storage and future use of blood sample
The blood sample drawn will be stored and may be used for future studies with permission from appropriate ethics committees.

Client consent check-off
May I begin the interview now?

If client responds "yes," the interviewer should sign and date the statement below and continue with the interview.
I certify that I have read the above statement and that the client has agreed to the interview. I also certify that any information the client discloses will remain confidential.

Signed: _____ Date: _____

If respondent says "no," the interviewer should sign and date the statement below and move on to another respondent.
I certify that I have read the above statement and that the client did not agree to be interviewed.

Signed: _____ Date: _____

Consent certificate

I _____ from _____

Declare that I've read and understood the informed consent form and willingly participate in the study. I understand that it is voluntary and there are no direct monetary benefits to me.

Signed: _____ Date: _____

In case of any queries or concerns contact the following:

1. Principal investigator
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3. UON/KNH ethics and Research Committee
   P. O. Box 19676 Code 00202
   Nairobi.
   Tel. (254-020) 2726300-9 Ext 44355
   E-mail: uonknh_erc@uonbi.ac.ke

4. Fomu ya kutoa idhini kwa utafiti

Kuanzishwa na ridhaa
Jina langu is_____.Tunafanya utafiti ili kulinganisha utumizi wa antijeni ya human pididymis protini 4 (HE4) na antijeni ya kansa 125 (CA125) kwa saratani ya ovari Kenya. Utafiti huu utafanywa katika Hospitali ya Taifa ya Kenyatta, St Marys Mission Hospital na Machakos Hospitali.

Madhumuni ya utafiti
Madhumuni ya utafiti ni kuhalalisha njia ya sasa ya uchunguzi kwa kansa ya ovari (CA125) na kulinganisha kwa njia mpya (HE4) ili kujua kama tunapaswa kubadili jinsi tunapima saratani hii.
Faida
Hautafaidika ki fedha kwa kuhusika kwa utafiti huu, walakin utapata vipimi vya bure. Ushiriki wako utaboresha ustawi wa wagonjwa na saratani ya ovari nchini
Madhara
Hakuna madthara ya kushiriki. Tutakusanya sampuli za damu (20ml) kuendesha HE4 na CA125 na mtihani yoyote nyingine kwa ajili ya saratani ya ovari, ambayo ni ya kawaida kwa wagonjwa wa aina hii.
Usiri
Mahojiano haya ni binafsi na siri. Vipimo vya damu na Histologia vitatumika kwa matibabu yako na yatkuwa na jina lako. Hata hivyo wakati wa kuingia matokeo na uchambuzi, utambulisho wako utafichwa na jina lako halitafunuliwa au kutumika. Habari kutoa zitatumika kwa madhumuni ya utafiti na mtihani mwingine yeyote kwa kansa. Unaweza pia ruka maswali yoyote ambayo wewe hutataka kujibu.
Gharama za ziada na wakati
Hutagharamika kifedha kwa kushiriki. Mahojiano haya yatachukua muda wa dakika 15.
Ushiriki wa hiari
Ushiriki wako katika utafiti huu ni hiari. Ukiamua kutoshiriki, huwezi kupewa adhabu. Pia, unaweza kubadili akili yako wakati wa utafiti na kuchagua kuto kushiriki.
Kuhifadhi na matumizi ya baadaye ya sampuli za damu
Sampuli za damu inayotolewa itahifadhiwa na labda kutumika kwa ajili ya masomo ya baadaye kwa ruhusa ya kamati za maadili sahihi.

Ridhaa ya mteja kuangalia
Naomba kuanza mahojiano sasa?

Kama mteja anajibu "**ndiyo**" mhojaji inapaswa kusaini na tarehe kauli chini na kuendelea na mahojiano. Ninathibitisha kwamba Nimesoma maelezo ya hapo juu na kwamba mteja amekubali mahojiano. Mimi pia kuthibitisha kwamba taarifa yoyote mteja atafafanua itabaki kubaki siri.

Saini: _____ Tarehe: _____

Kama mteja amedinda kushiriki, mhojaji inapaswa kusaini na tarehe kauli chini na kuendelea na kujibu mwingine.
Ninathibitisha kwamba Nimesoma maelezo ya hapo juu na kwamba mteja hakukubaliana na kuhojiwa.
Saini: _____ Tarehe: _____

Cheti cha Ridhaa

Mimi _____ kutoka _____

Kutangaza kwamba nimepata kusoma na kuelewa fomu ya ridhaa na kwa hiari nitashiriki katika utafiti. Naelewa kuwa ni hiari na hakuna fedha wala faida moja kwa moja kwangu.

Saini : _____ Tarehe: _____

Kwa maswali yoyote au wasiwasi wasiliana yafuatayo;

1. mpelelezi Mkuu

Dk Stephen Mwinga
S.L.P 22232-00100 Nairobi
Simu ya rununu: 0721226680
Barua pepe: guka49@yahoo.com

2. Kiongozi msimamizi
Profesa Koigi Kamau
Idara ya Uzazi & Gynaecologia, Chuo kikuu cha Nairobi
Simu ya rununu: 0722714402
Barua pepe: koigikamau@kenyaweb.com

3. UON / KNH maadili na Kamati ya Utafiti
P. O. Box 19676 - 00202
Nairobi.
Simu: (020) 2726300-9 Ext 44355
Barua pepe: uonknh_erc@uonbi.ac.ke

Appendix Ii: Abstraction Form

Serial No.	Hospital	Date of recruitment

Age	Parity	Gravidity	Last delivery	LNMP
Age at Menarche	Age at menopause	HIV + / - Unknown	Smoking pack years	Alcohol Unit years
Weight(kg)	Height(Meters)	Hormonal Contraceptive Type/Duration / Last use		
Was patient referred from another hospital? ☐ Yes ☐ No				Where?

Symptoms	Description (if any)	Duration	
1.			
2.			
3.			
4.			

Investigations

Imaging	Lesion description by Imaging
☐ Ultrasound ☐ CT scan ☐ MRI ☐ Others (specify)	Size (cm)…….. Anatomical Site(s) ……. Sides ☐Unilateral ☐Bilateral ☐ N/A Septae ☐Nil ☐Unilocular ☐Multilocular ☐ N/A Walls ☐ Thin ☐Thick ☐N/A Fluid type ☐ Absent ☐Serous ☐Blood ☐ Mixed ☐N/A Others (specify)

Laboratory				
Complete blood counts	☐ Yes ☐ No	HB……g/dl	WBC.../mm3	Plts…../mm3
Urea	☐ Yes ☐ No	……µmol/l		
Creatinine	☐ Yes ☐ No	…...mmol/l		

Others (specify)				

Surgery	Date	
Intra operative description of mass	Size	
	Site	
	One ovary/ both ovaries	
	Peritoneum	
	Uterus	
	Tubes	
	Nodes	
	Others	

Others

Histology
Description & Grade

Pathologist ……..……..Centre……Date ……

	Levels	Date done
HE4 levels		
CA125 levels		
Other tumour markers (Specify)		

Filled by ……Date ……/……../2015

Appendix III: Work plan

Months	**Sep**	**Oct**	**Nov**	**Dec**	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**
Proposal development										
Proposal presentation										
Ethical approval (KNH/UoN ERC)										
Training of study team & pilot of tools										
Data Collection										
Analysis of data										
Analyses/ manuscript writing										
Publication										

Appendix IV: Budget

Item	Unit price KES	Units	Costs (KES)	Remarks
Laboratory				
HE4 test	1000	88	88,000	Kits donated by Phillips Healthcare Technologies limited and Abbott Laboratories South Africa (PTY) Ltd and KNH Research and programs
CA125	1000	88	88,000	KNH Research and programs
Bench charges	1000	88	88,000	Waived by AKUH
Gross pathology				
Histology	1000	88	88000	Sponsored by KNH Research and programs
Research assistants				
Clinical officer and nursing assistants	1500	3 * 30	135,000	One assistants for each hospital i.e. KNH, Machakos and St. Mary's for 3 months
Lab technologist	1000	30	30,000	
Office costs				
Telephone	5000	1	5,000	Sponsored by KNH Research and programs
Internet	5000	1	5,000	
Stationery	5000	1	5,000	
Manuscript				
Printing	5000	1	5,000	
Binding	3000	1	3,000	
Publishing	30000	1	30,000	
Total			**565,000**	

Appendix v: Interpretation of results

Interpretation of CA 125 result

STUDY TITLE: ……..
PARTICIPANT ID: …….
AGE: ……..
DATE OF SAMPLE COLLECTION:……..

CLINICAL HISTORY:

RESULT:…… **REFERENCE INTERVAL: 0-35 U/mL***

*This test was performed using a CA 125 II assay on the Architect 1000SR analyser. The reference interval value was derived from a study where 94.4% of the healthy female subjects had CA 125 II assay values at or below 35.0 U/mL

Interpretive comment
Patients with confirmed ovarian carcinoma may have pretreatment CA 125 assay values in the same range as healthy individuals. Elevations in circulating OC 125 defined antigen may be observed in patients with non-malignant disease. For these reasons, a CA 125 assay value, regardless of level, should not be interpreted as absolute evidence for the presence or absence of malignant disease. The CA 125 assay value should be used in
conjunction with information available from clinical evaluation and other diagnostic procedures. Changes observed in serial CA 125 assay values when monitoring ovarian cancer patients should be evaluated in conjunction with other clinical methods used for monitoring ovarian cancer patients.

SIGNED:…….. **DATE**……

CLINICAL PATHOLOGIST

Interpretation of HE4 result

STUDY TITLE: ……
PARTICIPANT ID: …….
AGE: ……..
DATE OF SAMPLE COLLECTION:……

CLINICAL HISTORY:

RESULT:……

REFERENCE INTERVAL: Premenopausal (0-70 pmol/L), Post menopausal (0-140 pmol/L)

Interpretive comment
The level of HE4 cannot be used as absolute evidence for the presence or absence of malignant disease. The HE4 results should be used in conjunction with other clinical data; *e.g.*, symptoms, medical history, clinical and radiological findings. If the HE4 results are inconsistent with clinical evidence, additional testing is suggested to confirm the result. Patients with confirmed ovarian cancer may have HE 4 assay values in the same range as healthy women. Certain histological types of ovarian cancer (*e.g.*, mucinous or germ cell tumours) rarely express HE4, therefore the use of the ARCHITECT HE4 assay is not recommended for monitoring of patients with known mucinous or germ cell ovarian cancer. Conversely, elevated levels of HE4 antigen may be present in individuals with non-malignant disease.

SIGNED:…….. **DATE**……

CLINICAL PATHOLOGIST

Appendix vii. Hospital approvals

ST. MARY'S MISSION HOSPITAL, NAIROBI
P.O. BOX 3409 – 00506 Nyayo Stadium, Nairobi
Tel: 0208014924, 0721545640
Email: info@smmh.co.ke
Website: www.smmh.co.ke

14th August 2015

Dr Stephen Mwinga
Senior House Officer,
Department of Obs & Gyn
KNH/ UON
NAIROBI

Dear Dr. Stephen Mwinga

RE: STUDY TTLED "DIAGNOSTIC ACCURACY OF HUMAN EPIDIDYMIS PROTEIN 4 (HE4) AND CANCER ANTIGEN 125 (CA125) FOR SCREENING AND FOLLOW-UP OF PATIENTS WITH SUSPECTED OVARIAN CANCER IN KENYA"

We acknowledge receipt of your research proposal to carry out the above study and note your application to carry out part of the study at St. Mary's Mission Hospital.

We have reviewed your proposal and found the study to be current, relevant and appropriate. We too feel it will be very informative and beneficial to the many cancer patients who present to us for care. We are willing to participate in the study and to allow you to use our site for the study.

From your proposal we note the following:-

1. You have not included the letter of approval from your department. We require this.
2. You state you are awaiting ERC clearance. We require the approval before commitment.
3. You are looking at "TOMs" but it is not clear from your proposal how you intend to handle the "TOMs" found at surgery to be obviously non neoplastic e.g ectopics, abscesses etc that are not neoplastic. Will they be excluded or will they be included as benign masses. Will these introduce bias? You need to tighten the methodology more.
4. You state you will train Clinical Officers as research assistant. Could you clarify if you plan to train our hospital Clinical Officers or you will come with them from elsewhere.
5. The study involves collection of blood samples for analysis at AKUH but you have not quite explained how this is to be done, by who, and how the lab results get back to the patients file to assist in their care.
6. None of our hospital gynecologist are included as part of your study team yet we will be heavily involved in pre, intra and post op care of these patients. I do the bulk of gyn care at our hospital. I would want to be included as part of the study team and in the final write up.

We ask that you look into the above issues to assist with final approval of your study

Yours sincerely,

Dr. Konya W.P MBChB, MMED (Obs/Gyn)
CHAIR, ETHICS AND RESEARCH COMMITTEE
ST. MARY'S MISSION HOSPITAL, LANGATA

ST. MARY'S MISSION HOSPITAL
P. O. Box 3409 - 00506
NAIROBI

GOVERNMENT OF MACHAKOS

MACHAKOS HOSPITAL

A LEVEL 5 REFERRAL FACILITY

P.O. Box 19 - 90100
MACHAKOS.
Tel. No.2021685, 20 24141/2,3 2020260,20 21325
Fax : 044-2021979
Email-machakoshospital@yahoo.com

Medical Superintendent's Office
P.O. Box 1223 – 90100
MACHAKOS.
Tel. No. 044-2021979
Fax : 044-2021979
Email-machakoshospital@yahoo.com

REF: J.10BVOL.IV/977
Your Ref: **E/A VOL/II/132**

10th September 2015

Dr Stephen Mwinga

H58/64068/2013
Senior House Officer
KNH/ UON, Department of Obstetrics & Gynaecology

REQUEST TO CARRY OUT STUDY IN MACHAKOS HOSPITAL

As a follow up to the letter dated 10th September 2015 on the above subject, kindly note, the Training committee received your request to carry out study '***Diagnostic Accuracy of human epididymis protein 4 (HE4) and Cancer antigen 125 (CA125) for screening and follow-up of patients with suspected ovarian cancer in Kenya'***.

We have no objections to the study in our institution. However the final approval will be granted once we receive a certificate of clearance from KNH/UoN Ethics Research committee. Kindly furnish us with the clearance for final approval.

For: Medical Superintendent
P. O. Box 1223-90100,
MACHAKOS
MACHAKOS HOSPITAL
LEVEL 5 REFERRAL FACILITY

Dr Munga Edgar
Psychiatrist and Chair Training Committee
For: Medical superintendent
MACHAKOS LEVEL 5 HOSPITAL

KENYATTA NATIONAL HOSPITAL
P.O. BOX 20723, 00202 Nairobi

Tel.: 2726300/2726450/2726550
Fax: 2725272
Email: knhadmin@knh.or.ke

Ref: KNH/RH/16/VOL.II

Date: 29th June, 2015

Head of Research and Programs,
KENYATTA NATIONAL HOSPITAL

RE: REQUEST FOR GRANT FOR DR. STEPHEN MWINGA, REGISTRATION NO. H58/64068/2013

The above named is a senior house officer registered at the department of obstetrics and gynaecology pursuing Master of Medicine (MMed) in Obstetrics and Gynaecology.

The ERC has approved his study titled '***Diagnostic Accuracy of human epididymis protein 4 (HE4) and Cancer antigen 125 (CA125) for screening and follow-up of patients with suspected ovarian cancer in Kenya'***

Currently the burden of cancer is on the rise in Kenya. Kenyatta National Hospital (KNH) as the apical refrerral centre continues to bear the brunt of this burden with an ever increasing bed occupancy dedicated to cancer at the expense of other illnesses. The study aims to validate the current modalities for screening suspected ovarian cancer (CA125) and study the accuracy of a newer marker (HE4). The main part of the study will be carried out in KNH.

The department therefore forwards his request for consideration.

DR JOHN ONG'ECH
ASSISANT DIRECTOR
REPRODUCTIVE HEALTH DEPARTMENT

Appendix viii. St Mary's reply

9th September 2015.

Chairman,
Ethics and Research Committee,
St. Mary's Mission Hospital, Langata.

Dear Sir,

RE: RESPONSE TO CONCERNS ABOUT STUDY APPROVAL

I am in receipt of your letter date 14th August. I am grateful for your consideration and I wish to respond to issues raised:

1. I received department approval on 10th April 2014, and I attach a copy of the same.
2. I applied to ERC (**P421/06/2015**) I have received revisions and corrections which I have resubmitted. I will forward the clearance as soon as I get it.
3. 'TOM' My study seeks to study the positive and negative predictive values of both HE4 and CA125 for any TOM. Lesions that carry no risk of malignancy at the point of diagnosis such as obvious ectopic pregnancies or obvious pelvic abscess will be excluded. Lesions which carry a risk of malignancy but which will be found to be benign at histology will be included in the study as true negatives and will help in calculations of sensitivity and specificity. This classification will be *post priori.* Similarly, lesions which present with diagnostic challenges will be included.
4. I will recruit and train a clinical officer from each of the participating hospitals.
5. Blood collection will be collected by the study assistant as per protocol and submitted to AKUH where analysis will be done and results sent back to hospital to enable decision making. The turnaround time will be one week to ensure patients are not disadvantaged.
6. The hospital gynaecologist being in teaching position will be included in study team in a supervisory role and will be part of final publication. I have already reached three which is the maximum number of 'formal' supervisors allowed by the department.

I will be happy to address any other queries.

Yours truly,

Dr Stephen Mwinga
H58/64068/2013
Senior House Officer
KNH/ UON, Department of Obstetrics & Gynaecology