



The ROCA® Test Request Kit Instructions for Use

Date of Issue: JUNE 2015 Date of Latest Revision: DECEMBER 2015.

Product Number: OC2015/01

These Instructions for Use must be read carefully prior to use of the ROCA® Test Request Kit. The accuracy of the ROCA® Test result may be affected if these Instructions for Use are not followed precisely.

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PRECAUTION: The results of the ROCA® Test are not intended to be used alone to diagnose ovarian cancer. The results from the ROCA® Test must be interpreted in conjunction with independent clinical assessment by a qualified consultant. Incorrect use of The ROCA® Test carries the risk of unnecessary testing and surgery. The ROCA® Test is not intended as the sole test to inform the need for surgery

1. Intended Use

For In Vitro Diagnostic Use Only

The ROCA® Test Request Kit is intended to collect the data required for input to the ROCA® Test and provide guidance on interpreting ROCA® Test results. This kit is specifically intended to be used in conjunction with the ROCA® Test.

The ROCA® Test is intended for postmenopausal women between the ages of 50 and 85 or women aged between 35 and 85 with a family history of ovarian and/or breast cancer or a genetic predisposition to ovarian cancer.

2. Summary and Explanation of the ROCA® Test and the ROCA® Test Request Kit

Ovarian cancer is the fifth most common cancer in females in the UK; in 2011, there were 7,116 new cases of ovarian cancer and 4,272 women died of ovarian cancer (1). Ovarian cancer survival rates are higher when the cancer is diagnosed at an earlier stage. Women with stage 1 or 2 ovarian cancer have 5-year survival rates in excess of 70%, while stage 3 ovarian cancer has a 5 year survival rate of 19%, and stage 4 ovarian cancer has a 5 year survival rate of only 4% (1). However, only 30% of ovarian cancers are diagnosed at stages 1 and 2 (1). The incidence of ovarian cancer is higher in women with a family history of ovarian and/or breast cancer, and in carriers of germline mutations in *BRCA1/2* genes or other pre-disposing genes. In women with mutations in *BRCA1/2* genes the lifetime risk of ovarian cancer can be as high as 39% (2). Mutations in the *BRCA1* and *BRCA2* genes occur in an estimated 1 in 325-500 women across the population, and in 1 in 50 women of Ashkenazi Jewish descent (3).

The ROCA® Test Request Kit is intended to be used to collect the data required for input into the ROCA® Test. The ROCA® Test is a computer algorithm that can be used to calculate the probability of a woman having ovarian cancer. The ROCA® Test is intended to be used in women without any symptoms suggestive of ovarian cancer.

The ROCA® Test is the trade name of the Risk of Ovarian Cancer Algorithm (ROCA). The ROCA® Test has been proven to be equivalent to a research algorithm known as ROCA. ROCA has been validated in two prospective clinical validation trials in the UK, namely the UK Collaborative Trial for Ovarian Cancer Screening (UKCTOCS) (4, 5) and the UK Familial Ovarian Cancer Screening Study (UKFOCSS)

(6, 7, 8), the latter in women with a higher than average lifetime risk of ovarian cancer. The published results to date of these trials and a summary of the data showing equivalence between The ROCA® Test and ROCA are summarised in Section 9.

There are two populations of patients who are eligible for the ROCA® Test:

- (a) Women aged between 35 and 85 who meet the criteria designed to estimate a lifetime risk of ovarian cancer of equal to or greater than 10% (denoted herein as $\geq 10\%$) as described in Section 7.3. These patients should be routinely tested using the ROCA® Test on a 4-monthly basis and are recommended to have an annual transvaginal ultrasound (TVUS).
- (b) Women aged between 50 and 85 years who are postmenopausal (defined in Section 7.2) and who are unlikely to be of increased risk of developing ovarian cancer compared to the general population (denoted herein as less than or $< 10\%$ lifetime risk of ovarian cancer). These patients should be routinely tested using the ROCA® Test on an annual basis.

The ROCA® Test reports a numerical score for the risk for ovarian cancer e.g. 1 in 3,500 which is used as an aid to define the next intervention. The smaller the number the greater the risk, for example 1 in 500 is a higher risk than 1 in 1,000. The ROCA® Test scores fall into three categories of risk for ovarian cancer; 'Normal', 'Intermediate' and 'Elevated'. These categories define a guideline for further intervention according to the clinical trial protocols of UKFOCSS clinical trial (6, 7, 8) for women at $\geq 10\%$ lifetime risk of ovarian cancer in category (a) above, and UKCTOCS (4, 5) for women in category (b) above.

The results from the ROCA® Test must be interpreted in conjunction with independent clinical assessment by a suitably qualified consultant and, where performed, the results from a transvaginal ultrasound scan (TVUS) and other clinical investigations. The ROCA® Test is not intended as the sole test to inform the need for surgery.

3. Warnings and Precautions

For In Vitro Diagnostic Use:

- For professional use only.
- Follow these instructions for the ROCA® Test Request Kit. The accuracy of the ROCA® Test result may be affected if these Instructions for Use are not followed precisely.

The ROCA® Test and ROCA® Test Request Kit are intended to be used only by consultant gynaecologists and qualified healthcare professionals working under the direction of the gynaecologist. The clinical decision making for patients after interpretation of results of the ROCA® Test is the sole responsibility of the consultant gynaecologist administering the test. Guidelines are provided are based on the protocols and outcomes of the UKCTOCS (4, 5) and UKFOCSS trials (6, 7, 8), but these recommendations are only a guide and are not a replacement for clinical judgement.

4. The ROCA® Test Request Kit Materials

4.1. Contents

The ROCA® Test Request Kit Instructions for Use (this document),
The ROCA® Test Request Form,

The ROCA® Test Patient Information and Consent form,
Summary Instructions,
Plastic envelope for return of the blood sample and the completed Test Request Form.

The forms listed above, together with the return address for blood samples, are available once the consultant has registered at the ROCA® Test website: www.therocatest.co.uk

4.2 Materials required but not provided

Any standard gel serum separator tube can be used for collecting blood for the ROCA® Test. An appropriate needle, single use blood tube holder, swab and adhesive dressing will also be required.

4.3. Safety Information

The ROCA® Test kit does not contain any biological substances infectious substances or agents which cause disease in humans or animals. Human blood samples taken for this test should be handled as potentially infectious using safe laboratory procedures such as those outlined in Biosafety in Microbiological and Biomedical Laboratories, Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work, or in line with other appropriate biosafety practices.

All unused specimens should be treated as biohazardous waste and disposed of in accordance with local biosafety guidelines. Any items that come into contact with a biological substance i.e. human blood should be treated as biohazardous waste and disposed of as per the local biosafety guidelines.

Blood samples that are being transported must comply with UN3373 packaging regulations. This comprises of 3 layers; the primary receptacle – watertight, leak-proof receptacle containing the specimen packaged with sufficient absorbent material to absorb all fluid in case of any breakage; the secondary packaging – second durable, watertight, leak-proof packaging to enclose and protect the primary receptacle; outer packaging – outer shipping packaging with suitable cushioning material.

All packages containing infectious substances should be clearly marked with the proper shipping name i.e. “Infectious Substance”; the appropriate UN number i.e. “UN3373” and the appropriate warning label.

5. Principles of the ROCA® Test

ROCA is a Bayesian algorithm co-invented by Professor Ian Jacobs and Dr Steve Skates (9, 10). It was developed by studying the serum Cancer Antigen 125 (CA125) profiles generated over several years from a population of more than 27,000 women, some of whom developed ovarian cancer. CA125 belongs to the family of hybridoma-defined tumour markers and is found in a high percentage of non-mucinous ovarian tumours of epithelial origin (11). Based on understanding the differences and changes in CA125 levels in women developing ovarian cancer compared to those in women who do not have ovarian cancer, ROCA can accurately calculate the probability of a woman having ovarian cancer (4, 5, 7, 8). ROCA works by using an initial risk of a woman having ovarian cancer based on her age, family history of ovarian/breast cancer and/or known genetic pre-disposition to ovarian cancer, e.g. *BRCA1/2* status. This risk is modified based on how closely her CA125 profile matches the profiles seen in healthy women and women with ovarian cancer; the more her profile is like the profile seen in previous ovarian cancer cases, the greater is her estimated risk. By using a woman’s first ROCA result

and subsequently her change in CA125 over time, ROCA is proven to have a high performance for predicting the likelihood of a woman having ovarian cancer (summarised in section 9), and a higher performance than using a single threshold rule e.g. 35 U/ml (5).

6. Procedure for How to Deliver the ROCA® Test using the ROCA® Test Request Kit:

6.1. Initial Consultation

- Counsel the patient on the possible benefits and limitations of the ROCA® Test and provide them with an opportunity to ask any questions.
- Obtain the patient's signature to confirm their understanding and consent on the Patient Information and Consent form (required twice).
- The patient retains the Patient Information and Consent form with one signature page.
- The second signature page of Patient Information and Consent form is retained by the consultant.

6.2. Patient Eligibility

Patient eligibility for the ROCA® Test is determined using Section 7 for:

- Detailed eligibility criteria for the ROCA® Test (Section 7.1).
- Menopausal status (Section 7.2).
- Evidence for $\geq 10\%$ lifetime risk of ovarian cancer (Section 7.3).

At each subsequent consultation for the ROCA® Test, it should be ascertained as to whether any changes in the patient's circumstances (e.g. positive *BRCA1* genetic mutation test or change in menopausal status) that may affect their eligibility or the frequency of testing.

6.3. Completion of the Test Request Form and Taking the Blood Sample

6.3.1 Completing Test Request Form

Note: If all the information on the form is not provided on the Test Request form the delivery of the ROCA® Test result may be delayed.

- Clearly complete and sign the Test Request Form with **ALL** details required.
- If the test request form is completed by a healthcare professional other than the consultant gynaecologist (e.g. a qualified nurse) it is still required that the ordering consultant's name is entered on the Test Request Form.
- Obtain the patient's signature to indicate that they are aware that their personal data and test results are covered by medical confidentiality and data protection legislation.
- Include the ROCA® Test Identifier number on the Test Request Form where it is a patient's second or subsequent ROCA® Test.
- The date and time of blood collection must be added to the Test Request Form **at time of venepuncture (see section 6.3.2)** and Test Request Form must be returned in the envelope provided with the blood sample tube.

6.3.2 Taking of Blood Sample

- A full tube of blood should be collected using any standard gel serum separator tube.
- Do not use a serum collection tube beyond the expiration date of the tube as this may affect the results of the test.

- Clearly complete the label on the blood collection tube with a ball point pen with **patient first name, patient last name and patient date of birth.**
- Do not centrifuge, refrigerate or freeze the blood tube.

6.3.3 Returning the Blood Sample and Test Request Form

- The date and time of blood collection must be added to the Test Request Form **at time of venepuncture** and returned in the envelope provided with the blood sample tube.
- It is critical that the blood is processed within **56 hours** of venepuncture and therefore the accurate time and date of blood collection must be added to the Test Request Form.
- Return the sample at ambient temperature on the same day that the blood is collected. **Note:** Samples received at the testing laboratory later than 56 hours after venepuncture will not be analysed
- Return the Test Request Form and blood sample in the return envelope provided or other suitable packaging to The Doctors' Laboratory, 60 Whitfield St, London W1T 4EU, UK unless alternative arrangements e.g. a courier collection has been agreed with Abcodia Ltd .

6.4. CA125 Testing

The blood sample will be sent to a central laboratory where it will be tested, in accordance with the Roche CA125 II Instructions For Use, to determine the levels of CA125 in the sample. The ROCA® Test uses this result with previous results (where available) to generate the test results.

6.5. Delivery of Results

- ROCA® Test results are sent to the consultant, who is responsible for contacting the patient to deliver the results and discuss any follow up ROCA® Tests and other investigations that may be required.
- The consultant may also wish to inform the patient's general practitioner of the ROCA® Test result if they have consent from the patient to do so (obtained on Patient Information and Consent form).
- If a patient has previously had an 'Intermediate' or 'Elevated' result, but after further investigations the consultant recommends that the patient returns to routine screening, the consultant is required to inform Abcodia so that the patient can be reminded of their next test at the appropriate time by Abcodia.

6.6. Analysis and Follow Up

The consultant is responsible for analysing the ROCA® Test result and determining the most appropriate clinical follow up based on the result and other clinical investigations (section 8 on Interpretation of ROCA® Test results).

6.7 Delivery of Reminders for Future ROCA® Tests

Abcodia will remind the patient of when her next test is due where the previous ROCA® Test result was '**Normal**' or the consultant has notified Abcodia that the patient should be returned to routine screening. The reminder notice will be sent to both the patient and consultant one month before the next ROCA® Test is due.

Where a patient is required to return for follow up ROCA® Tests following an **‘Intermediate’** or **‘Elevated’** result it is the **consultant’s responsibility** to send the patient a reminder notification for their next ROCA® Test.

7. Patient Eligibility and Determination of Menopausal Status and $\geq 10\%$ Lifetime Risk of Ovarian Cancer

7.1 Patient Eligibility

There are two populations of patients who are eligible for the ROCA® Test:

- (a) Women aged between 35 and 85 with a $\geq 10\%$ lifetime risk of ovarian cancer.
- (b) Postmenopausal women between the ages of 50 and 85 at $< 10\%$ lifetime risk of ovarian cancer.

The detailed eligibility criteria for these two populations, as defined by the UKFOCSS and UKCTOCS trials, are as below:

(a) Women aged between 35 and 85 years with an estimated $\geq 10\%$ lifetime risk of ovarian cancer (as defined in Section 7.3).

Patients **NOT** eligible for the ROCA® Test are:

- (a) Women previously treated for an ovarian malignancy.
- (b) Women with a past history of bilateral salpingo-oophorectomy (**Note:** Women who have undergone bilateral oophorectomy, but who still have one or more fallopian tube in situ, are eligible as they may be at increased risk of fallopian tube cancer).
- (c) Women who have had surgery, chemotherapy or radiotherapy for any type of cancer not including premalignant disease within the past 12 months.
- (d) Women who are pregnant are not eligible for the ROCA® Test until 6 weeks after the end of their pregnancy. Neither CA125 nor ultrasound scanning can be used reliably to screen for ovarian cancer during pregnancy.
- (e) Women **under** the age of 35 or **over** the age of 85 with an estimated lifetime risk of ovarian cancer $\geq 10\%$ (as defined in Section 7.3)

(b) Postmenopausal (defined in Section 7.2) women aged between 50 and 85 years at $< 10\%$ lifetime risk of ovarian cancer;

Patients **NOT** eligible for the ROCA® Test are:

- (a) Women previously treated for an ovarian malignancy.
- (b) Women with a history of bilateral oophorectomy.
- (c) Women who have had surgery, chemotherapy or radiotherapy for any type of cancer (not including premalignant disease) within the past 12 months.
- (d) Postmenopausal women (as defined in Section 7.2) who are aged **less** than 50 or **over** 85 years.

7.2. Menopausal Status

Prior to using the ROCA Test, postmenopausal status needs to be assessed. A patient is deemed postmenopausal if she has had either:

- (a) Over 12 months amenorrhoea following a natural menopause; or
- (b) Over 12 months of hormone replacement therapy commenced for menopausal symptoms; or
- (c) Over 12 months since onset of menopausal symptoms in women who have had a hysterectomy with retention of one or both ovaries or who use the Mirena IUCD.

On the condition that the above criteria are met, it is not necessary to determine Follicle-Stimulation Hormone and Luteinising Hormone (FSH/LH) levels in women using the ROCA® Test. Where there is any doubt (eg. women who do not report menopausal symptoms but have ceased menstruation through use of Mirena IUCD or hysterectomy) measuring serum LH and FSH may be helpful.

For patients aged between 35 and 85 who have an estimated $\geq 10\%$ lifetime risk of ovarian cancer (as defined in Section 7.3) menopausal status needs to be determined by the consultant when **every** blood sample for use in the ROCA® Test is taken while the patient is still premenopausal, or if there is any doubt on menopausal status until the age of 55 years. For the premenopausal population the ROCA® Test result takes into account the higher normal baseline levels and fluctuations of CA125 and the ROCA® Test will perform with higher accuracy when menopausal status is defined correctly. If there is any doubt in menopausal status, and in the absence of results from testing for FSH/LH, the patient should be classified as **postmenopausal**.

7.3 Determining if a Patient has an Equal to or Greater than 10% Lifetime Risk of Ovarian Cancer

At a patient's first consultation for The ROCA® Test it should be determined whether they have an estimated $\geq 10\%$ lifetime risk of ovarian cancer. At each subsequent consultation it should be ascertained whether there are any changes in the patient's circumstances which might affect their lifetime risk of ovarian cancer. It is the requesting consultant's responsibility to ensure the correct determination of a patient's ovarian cancer lifetime risk. The accuracy of the ROCA® Test result may be affected if this is not carried out correctly. If either the consultant or patient has any doubts as to the likelihood of an estimated $\geq 10\%$ risk of ovarian cancer, it is recommended that the patient should be referred to a clinical geneticist or genetic counsellor for further evaluation, in order to clarify their level of risk.

Note: That for families with Ashkenazi Jewish ethnicity a history of tubal or primary peritoneal cancers may be considered equivalent to ovarian cancers. Breast cancer only families with Ashkenazi Jewish ethnicity negative on full *BRCA1* and *BRCA2* screening **do not** have a $\geq 10\%$ lifetime risk of ovarian cancer.

The flowchart in Figure 1 below is used in conjunction with Questions 1-5 in Table 1 to determine if a woman has an estimated $\geq 10\%$ lifetime risk of ovarian cancer. The outcome should be used as follows:

- If the patient is determined to be at an estimated $\geq 10\%$ lifetime risk of ovarian cancer it is recommended to follow the interpretation of results in the guidelines in section 8.1 **only** if she

meets the ROCA® Test eligibility criteria in section 7.1. The consultant could also consider recommending the patient to a clinical genetics service.

- If a patient is determined not to be at higher risk of ovarian cancer compared to the general population (<10% lifetime risk) then it is recommended to follow the guidelines in section 8.2 **only** if she is aged between 50 and 85 years, is postmenopausal and meets the eligibility criteria in Section 7.1.

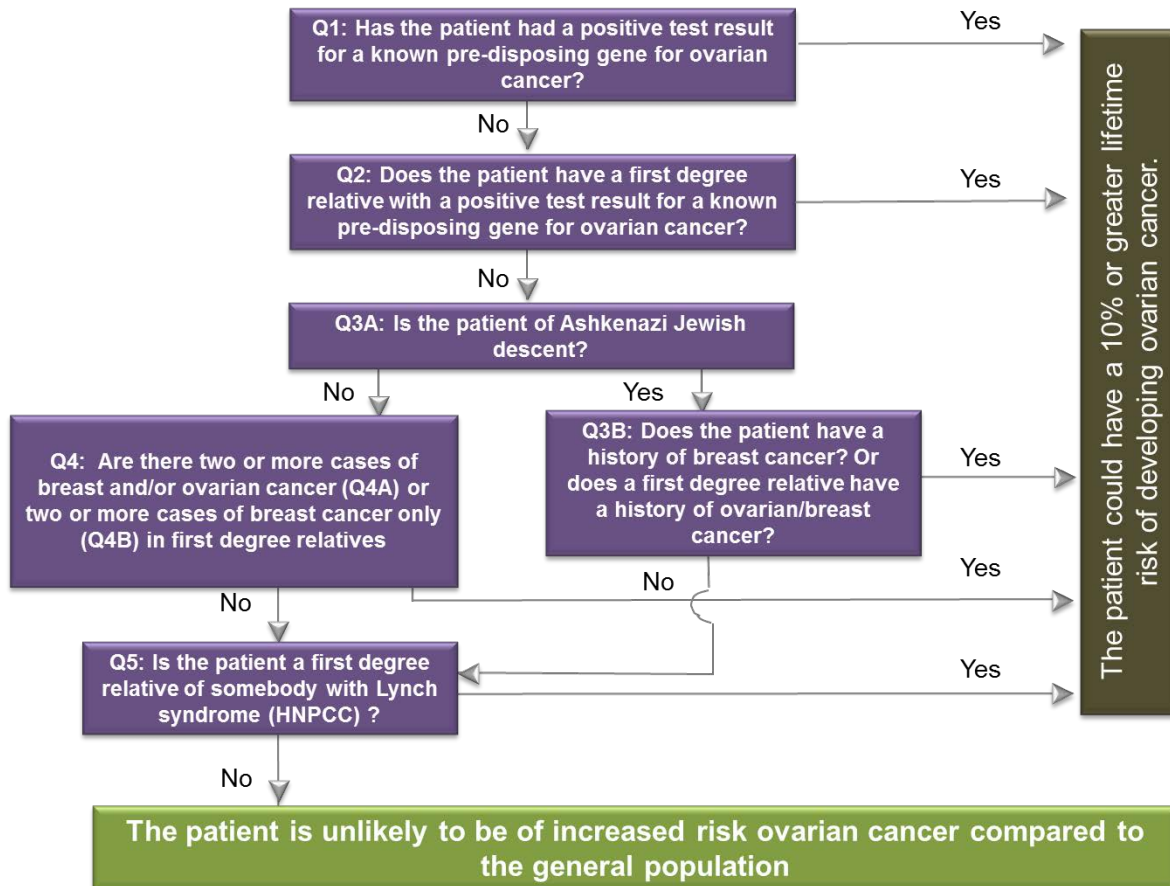


Figure 1. Flowchart for determining if a patient has an estimated $\geq 10\%$ lifetime risk of ovarian cancer

Table 1. Questions for the flowchart in Figure 3 for determining if a patient has an estimated $\geq 10\%$ lifetime risk of ovarian cancer.

<p>N.B. For all these criteria, the patient must either be a mutation-carrier, or be a first degree relative of a mutation carrier or cancer-affected individual. Abbreviations: FDR = First Degree Relatives (mother, sister, daughter, brother); MBC = Male Breast Cancer; Proband = patient in consultation.</p>		
Q1	<p>Has the patient had a positive test result for a known pre-disposing gene for ovarian cancer?</p>	<p>Ovarian cancer predisposing genes are <i>BRCA1</i>, <i>BRCA2</i>, <i>MLH1</i>, <i>MSH2</i>, <i>MSH6</i>, <i>PMS1</i> and <i>PMS2</i>.</p>

<p>Q2</p>	<p>Does the patient have a first degree relative who has been genetically tested and found to have a mutation one of the ovarian cancer predisposing genes?</p>	<p>Ovarian cancer predisposing genes are <i>BRCA1</i>, <i>BRCA2</i>, <i>MLH1</i>, <i>MSH2</i>, <i>MSH6</i>, <i>PMS1</i> and <i>PMS2</i>.</p>
<p>Q3A</p>	<p>Is the patient of Ashkenazi Jewish descent*?</p>	<p>If 'Yes' go to Q3B</p>
<p>Q3B</p>	<p>Does the patient have Ashkenazi Jewish ethnicity and any one of the following: (History of tubal or primary peritoneal cancers may be considered equivalent to ovarian cancers). *Breast cancer only families in these categories negative on full <i>BRCA1</i> and <i>BRCA2</i> screening do not have a $\geq 10\%$ lifetime risk of ovarian cancer.</p>	<p>1, Breast cancer (<40 years) or bilateral breast cancer (first cancer <50 years) in proband, irrespective of family history of cancer; 2, Breast cancer in proband (<50 years) and one FDR with breast cancer (<50 years) or ovarian cancer (any age) or MBC (any age); 3, Breast cancer in proband (<60 years) and one FDR with breast cancer (<40 years) or ovarian cancer (any age) or MBC (any age); 4, One FDR with ovarian cancer (<50 years), 5, FDR with breast and ovarian cancer in the same woman (any age); 6, Two FDR with breast cancer (<40 years); 7, Two MBC (<60 years) in the family and proband is a FDR of one of them.</p>
<p>Q4A</p>	<p>Families with ovarian or ovarian & breast cancer.</p>	<p>Any one of the following criteria are met: 1, ≥ 2 individuals with ovarian cancer who are FDR; 2, One ovarian cancer and 1 breast cancer <50 years who are FDR; 3, One ovarian cancer and 2 breast cancers <60 years who are FDR; 4, Breast cancer in proband (≤ 45 years) and mother with both breast and ovarian cancer (in the same person); 5, Breast cancer in proband (≤ 40 years) and sister with both breast and ovarian cancer (in the same person). Criteria 1, 2, and 3 above can be modified where paternal transmission is occurring i.e. families where affected relatives are related by second degree through an unaffected intervening male relative and there is an affected sister are eligible.</p>
<p>Q4B</p>	<p>Families with only breast cancer* *Families in these categories negative on full <i>BRCA1</i> and <i>BRCA2</i> screening do not have a $\geq 10\%$ lifetime risk of ovarian cancer.</p>	<p>Any one of the following criteria are met: 1, ≥ 4 breast cancers; 2, 3 breast cancers related by FDR a. one ≤ 30 years, or b. all ≤ 40 years, or c. one MBC and one bilateral breast cancer; 3, Breast cancer in proband ≤ 50 years and a. breast cancer in mother (age of onset being ≤ 30 years in one and ≤ 50 years in the other), or</p>

		b. bilateral breast cancer in mother (≤ 40 years onset)); c. one MBC and one bilateral breast cancer; 4, Two MBC (one < 40 years) in the family and proband is a FDR of one of them.
Q5	Families with Lynch syndrome (HNPCC)	The family contains ≥ 3 individuals with a HNPCC related cancer (colorectal, endometrial, small bowel, uretic and renal pelvic cancers) who are FDR and ≥ 1 case is diagnosed before 50 years and the cancers affect ≥ 1 generation.

8. Interpretation of ROCA® Test Results

8.1 Interpretation of ROCA® Test Results for Patients Aged between 35 and 85 with an Estimated $\geq 10\%$ Lifetime Risk of Ovarian Cancer.

The guidelines for patients aged between 35 and 85 years with an estimated $\geq 10\%$ lifetime risk of ovarian cancer, as implemented in the UKFOCSS prospective clinical trial (7, 8), are shown in Figure 2. The ROCA® Test result categories for these patients are summarised in Table 2 below.

Risk-reducing bilateral salpingo-oophorectomy is currently the only clinically proven way of preventing ovarian and fallopian tube cancer and is the current recommendation for women at high risk of ovarian cancer (12).

An unsatisfactory scan is defined as a TVUS where the ovaries have not been visualised because of a poor view of the pelvis due to any cause, such as bowel gas. As ovarian appearance varies with different aspects of the ovarian cycle in premenopausal women, where possible, scans should be scheduled for the early follicular phase (day 3-6 of the cycle).

Patients who become pregnant during their screening with the ROCA® Test are no longer eligible for the test because pregnancy affects CA125 levels and ovarian appearance on ultrasound scanning, making these tests unreliable in screening for ovarian cancer during pregnancy. Screening of patients with the ROCA® Test can recommence six weeks after the end of the pregnancy.

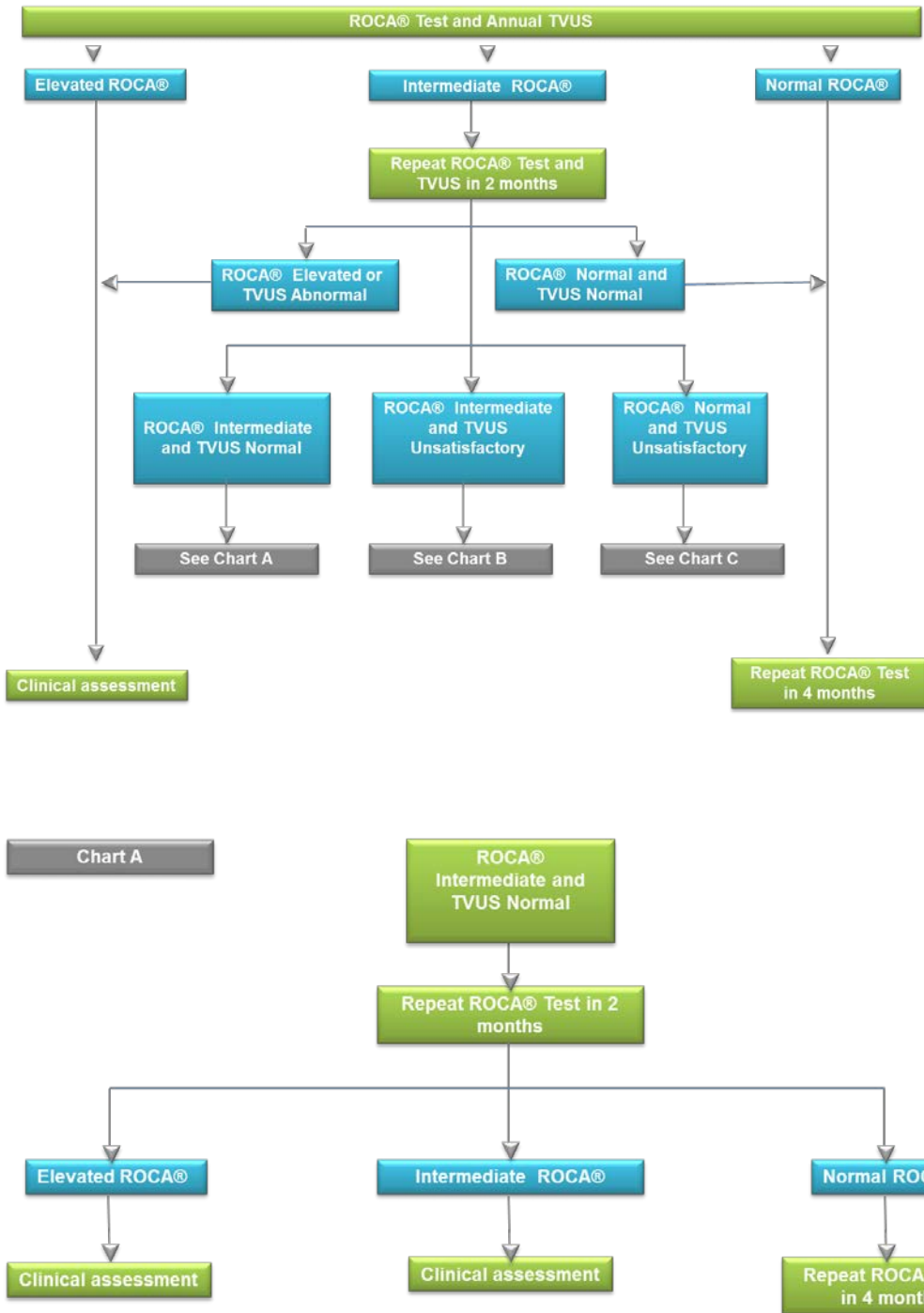


Figure 2. Including charts A-D. The recommended guidelines for patients with a $\geq 10\%$ lifetime risk of ovarian cancer aged between 35 and 85 years as implemented in the UKFOCSS trial (7, 8).

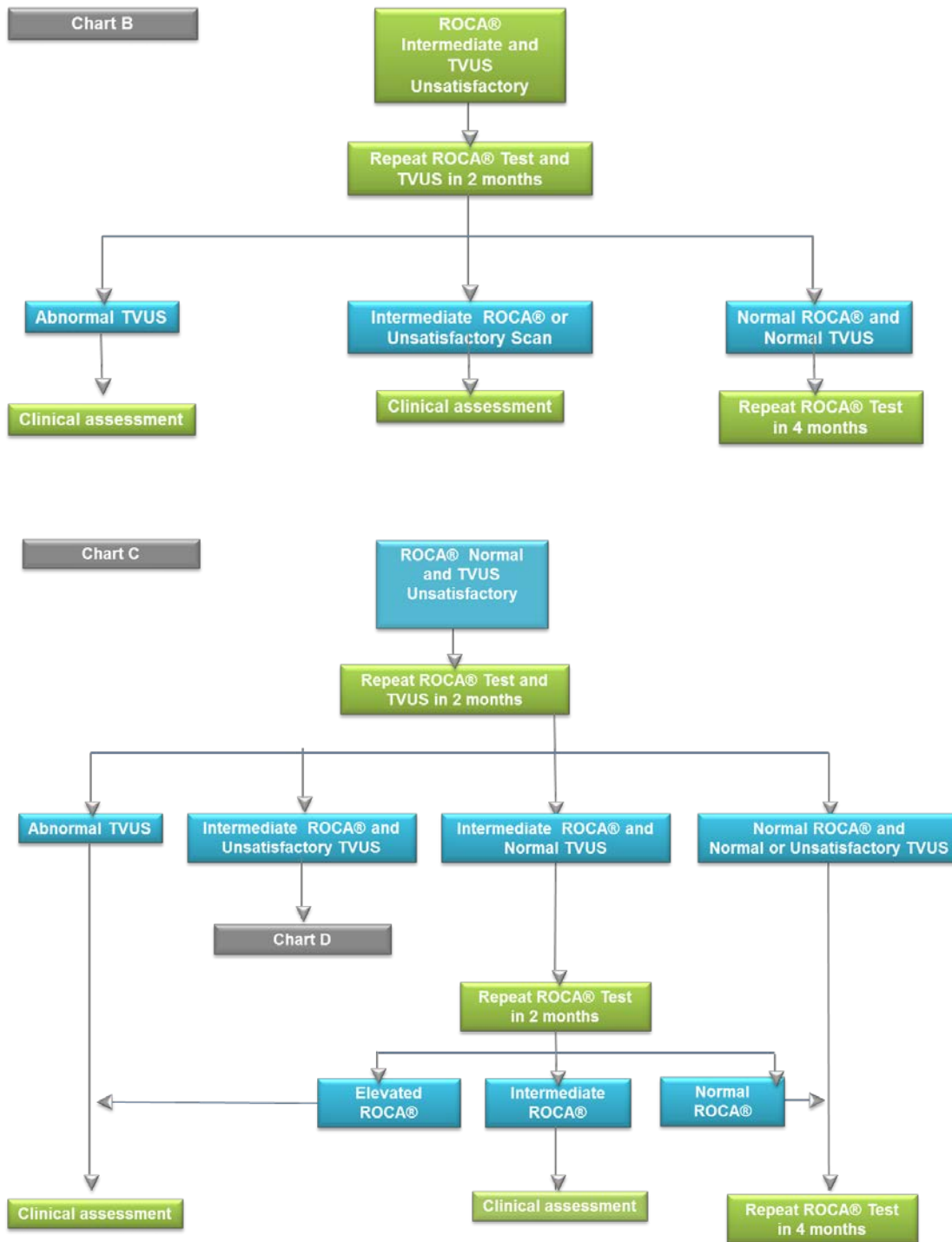


Figure 2 (continued) including charts A-D. The recommended guidelines for patients with a $\geq 10\%$ lifetime risk of ovarian cancer aged between 35 and 85 years as implemented in the UKFOCSS trial (7, 8).

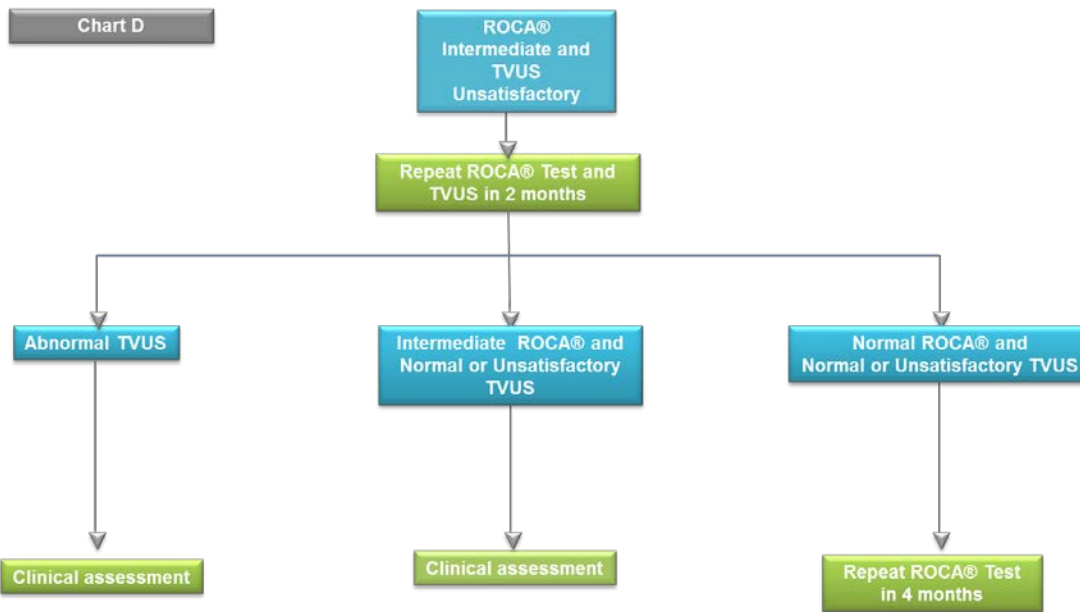


Figure 2 (continued) including charts A-D. The recommended guidelines for patients with a $\geq 10\%$ lifetime risk of ovarian cancer aged between 35 and 85 years as implemented in the UKFOCSS trial (7, 8).

Table 2. The ROCA® Test result categories for patients with a $\geq 10\%$ lifetime risk of ovarian cancer aged between 35 and 85 as implemented in the UKFOCSS trial (7, 8).

Pre-menopausal Patient ROCA® Test Result	Postmenopausal Patient ROCA® Test Result	Category
< 1 in 1,500	< 1 in 1000	Normal
1 in 1,500 to 1 in 5	1 in 1000 to 1 in 5	Intermediate
> 1 in 5	> 1 in 5	Elevated

The following guidelines are recommended based on the UKFOCSS trial protocol for each result category:

- If a patient receives a **‘Normal’** result it is recommended that the patient has the ROCA® Test routinely every 4 months in conjunction with an annual TVUS.
- If a patient has an **‘Intermediate’** result the consultant should contact the patient to discuss the result and remind her within 2 months to attend for another ROCA® Test and TVUS. The recommended actions from the outcomes of the repeat ROCA® Tests and TVUS for the **‘Intermediate’** results are shown in detail in Figure 2, charts A to D. If a patient has three consecutive **‘Intermediate’** results, then she should be referred for clinical assessment as deemed necessary by the consultant.

- If a patient has an '**Elevated**' result the consultant should contact the patient to discuss her result and it is recommended the patient is referred immediately for clinical assessment and TVUS.

The clinical assessment should include ruling out other causes of CA125 elevation which include: colitis, chronic active hepatitis, cirrhosis, renal disease with serum creatinine >2.0 mg/dL, systemic lupus erythematosus, sarcoidosis, acute pancreatitis, diverticulitis, endometriosis, polyarteritis nodosa, Sjögren's syndrome, pericarditis, rheumatoid arthritis and osteoarthritis.

Expected Results:

The expected percentages of results in each category for the ROCA® Test for patients aged between 35 and 85 with a ≥10% lifetime risk of ovarian cancer, when used in conjunction with TVUS and clinical expertise are shown in Table 3. The figures are based on the UKFOCSS trial outcome (13).

Table 3. Expected percentage of results in each category for the ROCA® Test based on the UKFOCSS Trial (13).

Category	% of Patients Expected to Receive Result
Normal	87.56 %
Intermediate	12.38 %
Elevated	0.06 %

8.2 Interpretation of ROCA® Test Results for Postmenopausal Patients Aged between 50 and 85 with a <10% Lifetime Risk of Ovarian Cancer

The recommended guidelines for postmenopausal patients aged 50 to 85 with a <10% lifetime risk of ovarian cancer as implemented in the UKCTOCS trial (5) are shown in Figure 3 below. The ROCA® Test result categories for these patients are summarised in Table 4 below.

An unsatisfactory scan is defined as a TVUS where the ovaries have not been visualised because of a poor view of the pelvis due to any cause such as bowel gas.

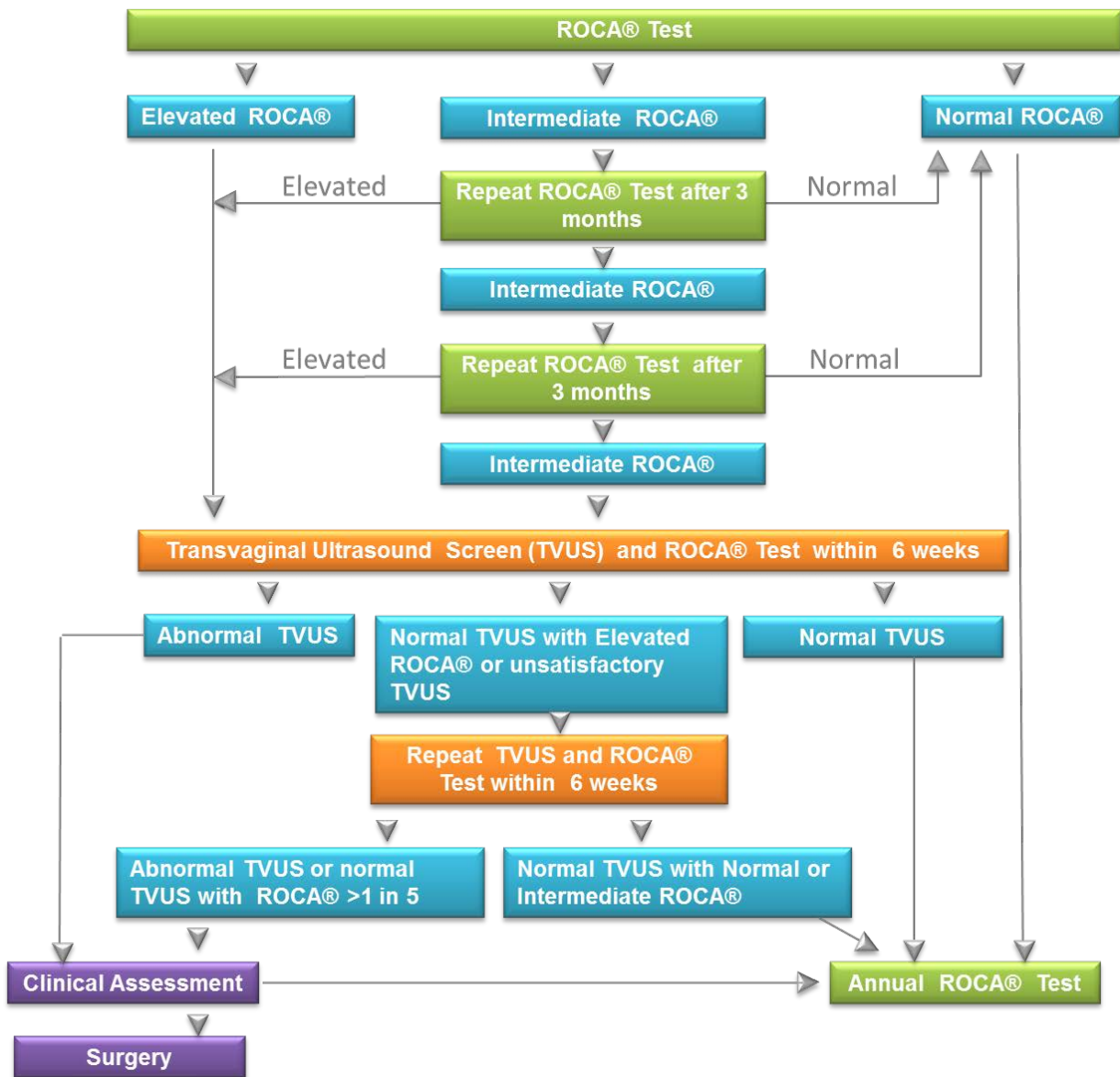


Figure 3. The ROCA® Test guidelines for postmenopausal patients aged between 50 and 85 years with a <10% lifetime risk of ovarian cancer as implemented in the UKTOCS trial (4, 5).

Table 4. The ROCA® Test result categories summary for postmenopausal women aged between 50 and 85 years with a <10% lifetime risk of ovarian cancer.

ROCA® Test Result	Category
< 1 in 3,500	Normal
≥ 1 in 3,500 and < 1 in 1,000	Intermediate
≥ 1 in 1,000	Elevated

The following guidelines are recommended based on the UKTOCS trial protocol for each result category:

- If a patient receives a **‘Normal’** result of < 1 in 3,500 it is recommended to have annual routine testing with the ROCA® Test.

- If a patient has an **'Intermediate'** result equal to or greater 1 in 3,500 and less than 1 in 1,000 the consultant should contact the patient to discuss her result. The patient should also be reminded to attend for another ROCA® Test 3 months after the **'Intermediate'** result. If the repeat ROCA® Test is **'Normal'** then the patient should return to annual testing. If the repeat ROCA® Test is **'Elevated'** then see the **'Elevated'** category for recommended actions. If the patient has three consecutive **'Intermediate'** results it is recommended she is referred for TVUS and a further ROCA® Test within 6 weeks. The recommended actions subsequent to this TVUS and further ROCA® Test are shown in Figure 3. Either a return to annual screening or clinical assessment are recommended depending on the results of the TVUS and further ROCA® Test.
- If a patient has an **'Elevated'** result of 1 in 1,000 or greater the consultant should contact her to discuss the result, refer her for TVUS and a repeat ROCA® Test is recommended within 6 weeks. If the repeat ROCA® Test is **'Normal'** and the TVUS is also normal then the patient returns to annual screening. If repeat ROCA® Test is **'Normal'** and the TVUS is abnormal this triggers a clinical assessment by the consultant to determine if surgery is recommended (Figure 3). If the repeat ROCA® Test result is **'Elevated'** and the TVUS is normal or unsatisfactory then a further repeat ROCA® Test and TVUS are recommended within 6 weeks. (Figure 3). If after the first elevated test result or after two intermediate repeat tests, a patient has a normal TVUS and a ROCA® Test result of a > 1 in 5 then this is highly indicative of the presence of ovarian cancer (5). It is recommended then that the patient is referred immediately for clinical assessment, which may include a CT scan of chest and abdomen and mammography.

The clinical assessment should include ruling out other causes of CA125 elevation which include: colitis, chronic active hepatitis, cirrhosis, renal disease with serum creatinine > 2.0 mg/dL, systemic lupus erythematosus, sarcoidosis, acute pancreatitis, diverticulitis, endometriosis, polyarteritis nodosa, Sjögren's syndrome, pericarditis, rheumatoid arthritis and osteoarthritis.

Expected results:

The expected percentage of results in each category for the ROCA® Test for postmenopausal women aged between 50 and 85 years with a <10% lifetime risk of ovarian cancer when the ROCA® Test is used in conjunction with TVUS and clinical expertise are shown in Table 5. These figures are based on the UKCTOCS trial incidence screening outcome (5).

Table 5 Expected percentage of results in each category for the ROCA® Test based on UKCTOCS trial outcome (5).

ROCA® Test Result Category	% of Patients Expected to Receive Result
Normal	90.0%
Intermediate	8.5%
Elevated	1.5%

9. Performance Characteristics

9.1 Introduction

The Risk of Ovarian Cancer Algorithm (ROCA) has been validated in a number of studies in the UK and US, in particular the United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS) (4, 5) and the United Kingdom Familial Ovarian Cancer Screening Study (UKFOCSS) (6, 7, 8).

UKCTOCS is a randomized, controlled clinical trial that started in 2001 and recruited 202,638 postmenopausal women aged between 50 and 74. The study evaluated the Risk of Ovarian Cancer Algorithm in a Multimodal Screening (MMS) strategy using ROCA as the first line test combined with TVUS as the means to further evaluate 'Elevated' ROCA scores. MMS was compared to the performance of (i) annual TVUS alone, and (ii) no screening. The UKCTOCS trial results published to date are described below (4, 5) and final outcome on survival and mortality will report in 2015. ROCA is also being evaluated in a one arm prospective trial in the United States in postmenopausal women, using the same MMS strategy as UKCTOCS (14).

ROCA was evaluated in The United Kingdom Familial Ovarian Cancer Screening Study (UKFOCSS) in women who have an estimated $\geq 10\%$ lifetime risk of developing ovarian cancer, due to a family history of ovarian and/or breast cancer or a genetic risk such as *BRCA1* or *BRCA2* gene mutations, Lynch syndrome or women of Ashkenazi (Eastern European) Jewish descent with specific familial cancer histories (6, 7, 8). UKFOCSS Phase 2 recruited 4,351 women aged between 35 and 84 who were screened for on average 3.2 years per woman with a median 3.75 year follow up. The results of the UKFOCSS trial are described in detail below.

9.2 UKCTOCS Trial

Between 2001 and 2005, a total of 202,638 postmenopausal women aged 50 to 74 years were randomly assigned in the UKCTOCS trial in a 2:1:1 ratio to three groups: no treatment (control; n=101,359); annual CA125 screening interpreted using the ROCA with TVUS as a second line test (MMS, n=50,640); or annual screening with TVUS alone (n=50,639). Women with abnormal ROCA or TVUS results had repeat tests. Women with persistent abnormality on repeat screens underwent clinical evaluation and, where appropriate, surgery.

In 2009, the results of the prevalence screen for UKCTOCS were published (4). The results of this initial screen for the detection of all primary ovarian and tubal cancers (ICD10 codes C56 and C57.0) were sensitivity of 89.4%, specificity of 99.8%, and a positive-predictive value (PPV) of 43.3% for MMS. Overall 8.7% of the women in the MMS group required a repeat test and 0.3% women required clinical evaluation. Surgery was performed on 0.2% women in the MMS group and 45 primary ovarian and tubal cancers were detected.

In 2015, the results of the incidence screening for UKCTOCS were published, analysing 296,911 incidence screens performed for 46,237 volunteers from the start of the trial in June 2002 until the last screen in December 2011 (5). The median number of incidence screens was seven. The performance characteristics of the MMS incidence screening for primary ovarian and tubal cancers are shown in Table 6 below. The sensitivity, specificity, and positive-predictive values for detection of primary invasive ovarian and tubal cancers were 85.8%, 99.8% and 20.8% respectively. 0.2% of screens resulted in women having screen positive surgery. Primary ovarian/tubal malignancies were

detected in 154 of the 640 women having surgery (24.1%) and 411 had normal or benign pathology, with the remaining 45 having non-ovarian malignant neoplasms. Of the screen detected invasive epithelial ovarian/tubal cancers 82% were Type II (poor prognosis histological subtype).

The data was also used to compare the performance of ROCA with what would have been achieved with using a fixed cut off value for CA125. Using fixed CA125 cut-offs at last annual screen of >35, >30 and >22U/mL would have identified 41.3%, 48.4% and 66.5% of invasive epithelial ovarian/tubal cancers respectively, compared to the 85.8% detected using ROCA in conjunction with in MMS with TVUS and clinical assessment (5). The sensitivity and specificity of ROCA alone (i.e. not in combination with TVUS and clinical assessment) was analysed and was found to be 87.1% and 87.6% respectively.

Table 6. Performance characteristics for detection of malignant ovarian, fallopian tube and primary peritoneal neoplasms (ICD10 codes C56 C57.0 and C48.2) in the UKCTOCS Incidence screen (5). CI = Confidence Interval.

Total	MMS Incidence Screening (5)
Number of women screen years	296,911
Number of surgeries	640
Primary invasive epithelial ovarian and fallopian tube malignancies within one year of screen (ICD10 C56,C57.0)	
Screen positives	133
Screen negatives	22
Sensitivity	85.8%
95% CI	79.3– 90.9
Specificity	99.8%
95% CI	99.8-99.8
Positive-predictive value	20.8%
95% CI	17.7-24.1
Number of operations per screen positive	4.8
Primary invasive epithelial ovarian , tubal and primary peritoneal malignancies within one year of screen (ICD10 C56,C57.0 & C48.2)	
Screen positives	145
Screen negatives	25
Sensitivity	85.3%
95% CI	79.1- 90.3
Specificity	99.8%
95% CI	99.8-99.8
Positive-predictive value	22.7%
95% CI	19.5-26.1
Number of operations per screen positive	4.4

9.3 UKFOCSS Trial Phase 2

The UKFOCSS trial aimed to investigate screening with ROCA as an approach for women at high risk of ovarian/fallopian tube cancer to facilitate delaying risk-reducing salpingo-oophorectomy (RRSO) in order to complete a family or delay surgical menopause. Between June 2007 and March 2012, 4,531

women at an estimated $\geq 10\%$ lifetime risk of ovarian and fallopian tube cancers were recruited to the UKFOCSS Phase 2 trial (7, 8). The women were screened at 42 UK centres for 14,263 women screen years with an average of 3.2 years per woman. The median age was 45.5 years (range 34 - 85 years with a third aged over 50 years). Screening comprised 4-monthly CA125 tests analysed by ROCA, adjusted for menopausal status. TVUS was annual in those with 'Normal' algorithm results, but was triggered sooner if results were not 'Normal'. Women with suspicious scan and/or algorithm results were referred for consideration of surgical intervention. There were 18 incident ovarian and fallopian tube cancers diagnosed. Six of the 18 were occult cancers from RRSO. No symptomatic interval cancers occurred. Therefore, 100% of the ovarian and fallopian tube cancers that were not found at RRSO were detected by ROCA. Sensitivity for detection of incident ovarian and fallopian tube cancers was 100% (CI 74-100%) if occult cancers were classified as true positives and 67% (41-87%) if they were classified as false negatives. PPV and negative predictive value (NPV) of incidence screening were 13% (7 - 22%) and 100% (99 - 100%) respectively. 42% incident screen-detected ovarian and fallopian tube cancers were stage I/II ($p=0.69$). 92% of incident screen-detected cancers on Phase 2 were completely cytoreduced and no woman was sub-optimally cytoreduced during UKFOCSS Phase 2.

9.4 The ROCA® Test and ROCA Equivalence Study

The ROCA® Test is the trade name of the Risk of Ovarian Cancer Algorithm (ROCA) that has been assessed in clinical trials in the UK. The ROCA® Test was developed as medical device software under the EC IVD Directive (98/79/EC) and in accordance with quality system standards EN ISO 13485 (Medical devices — Quality Management Systems — Requirements for Regulatory Purposes), and IEC 62304 (Medical Device Software – software life cycle processes).

As a part of the project an automated suite of tests was developed and run to ensure that the results derived from the ROCA® Test were equivalent to ROCA. This testing demonstrated equivalence between the results from the ROCA® Test and ROCA. ROCA and the ROCA® test both function in exactly the same way. A woman's initial risk of having ovarian cancer is based on published age adjusted ovarian cancer incidence rates observed in the general population and these risks are held as a table within the ROCA® Test. This population based risk is adjusted according to the modified CA125 values from 25,000 women, some of whom developed ovarian cancer, and these modified CA125 values are stored as a table within ROCA and the ROCA® Test.

ROCA and the ROCA® Test use the woman's actual CA125 results together with her age, menopausal status and whether she has a greater than 10% lifetime risk to calculate her personal risk of having ovarian cancer. This risk is expressed as a number between 0 and 1.

The automated test suite ran 3 tests:

- 1) Checked that for each age that the initial risk in both ROCA and the ROCA® Test had identical values.
- 2) Compares the transformed CA125 values for 25,000 women (note that there are multiple values for women) were identical in ROCA and the ROCA® Test.
- 3) For 10 'dummy' patients that the personal risk calculated by ROCA and the ROCA® Test were identical to 15 decimal places.

All tests passed successfully with no errors reported.

10. Limitations

- The ROCA® Test is not intended as the sole test to determine whether a patient should proceed to surgery for ovarian cancer. The ROCA® Test is intended to inform a qualified consultant about the need for TVUS and other clinical assessment as deemed necessary.
- Risk-reducing bilateral salpingo-oophorectomy is the only way of preventing ovarian and fallopian tube cancer and is the current recommendation for women at high risk of ovarian cancer.
- In the UKCTOCS trial ROCA used in multimodal screening had a sensitivity of 85.8% for detection of ovarian and fallopian tube cancer in postmenopausal women (5) and therefore did not detect approximately 15% of women with ovarian cancers. A patient who is using the ROCA® Test should be encouraged to visit her GP or her consultant should she develop the currently recognised symptoms of ovarian cancer.
- The PPV of ROCA when used in conjunction with TVUS in a multimodal screening mode in the UKCTOCS trial was 20.8% for detection of ovarian and fallopian tube cancer in postmenopausal women aged 50 to 85 (5). Therefore, in 4 out of 5 patients in this group referred for surgery other less serious abnormalities were found. In a number of patients who undergo surgery no abnormality was found at all.
- The PPV of ROCA in the UKFOCSS trial for patients aged between 35 and 85 at $\geq 10\%$ lifetime risk of ovarian cancer was 13% (7, 8). Therefore, in 7 out of 8 women referred for surgery other less serious abnormalities were found.
- The ROCA® Test has not been validated in the following populations:
 - (a) Women previously treated for an ovarian malignancy.
 - (b) Women with a history of bilateral oophorectomy, and in the case of women with $\geq 10\%$ lifetime risk of ovarian cancer, those with bilateral oophorectomy and both fallopian tubes removed.
 - (c) Women currently being treated with chemotherapy for cancer or recurrent cancer in the last 12 months.
 - (d) Postmenopausal women with a $< 10\%$ lifetime risk of ovarian cancer aged under 50 and over 85 years of age.
 - (e) Women with a lifetime risk of ovarian cancer of $\geq 10\%$ but aged under 35 years,
 - (f) Pregnant women.
- Not all patients with ovarian cancer who are identified by the ROCA® Test will have early disease. Even though the cancer is detected before any apparent symptoms, the disease may still be advanced. The ROCA® Test may therefore not make a difference to the type of treatment received by these patients or the eventual outcome of the treatment.
- Because the ROCA® Test is used in conjunction with TVUS to triage patients for clinical assessment, consultants using the test are required to be competent in interpreting pelvic ultrasound imaging reports.

- Other causes of CA125 elevation which include colitis, chronic active hepatitis, cirrhosis, renal disease with serum creatinine > 2.0 mg/dL, systemic lupus erythematosus, sarcoidosis, acute pancreatitis, diverticulitis, endometriosis, polyarteritis nodosa, Sjögren's syndrome, pericarditis, rheumatoid arthritis and osteoarthritis.
- The serum tested for CA125 is not subjected to microbial contamination testing. Contaminated samples may give erroneous results.

11. Disclaimer

The performance data presented in Section 9 (Performance Characteristics) of these Instructions for Use were obtained using the procedures indicated herein. Any change or modification of the procedure not recommended in these Instructions for Use may affect the results of the ROCA® Test, in which event Abcodia disclaims all warranties expressed, implied or statutory including the implied warranty of merchantability and fitness for use and excludes all liability in respect of such to the fullest extent permitted by law.

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In order to obtain technical assistance contact: 0845 474 0002 (cost of the call is a maximum of 4p per minute from a BT landline).

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