

CA 125 IN THE PHYSIOLOGY AND PATHOLOGY
OF THE FEMALE REPRODUCTIVE TRACT;
with particular reference to
the diagnosis of ovarian cancer

M. D. Thesis

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ABSTRACT

This thesis addresses the hypothesis that "the CA 125 tumour associated antigen is of value in the diagnosis of ovarian cancer". The literature concerning CA 125 and diagnostic aspects of ovarian cancer are reviewed in chapter 1 and the research methodology and the characteristics of CA 125 assay systems are described in chapters 2 and 3.

Chapters 4-6 describe studies of the compartmental distribution of CA 125 in the female reproductive tract in physiological and pathological states. The results suggest that CA 125 is a product of normal endometrium, pregnancy endometrium and benign ovarian tumours as well as malignant ovarian tumours, and that there is a physiological rise in serum CA 125 levels during menstruation and early pregnancy. It is concluded that the main factor influencing serum levels of CA 125 is the integrity of the blood:tissue barrier.

Chapter 7 describes a prospective study to evaluate serum CA 125 measurement in the preoperative diagnosis of ovarian cancer. The results indicate that the accuracy of CA 125 measurement is superior to clinical criteria and similar to ultrasound. The highest diagnostic accuracy was achieved by combining CA 125, ultrasound and menopausal status in a risk of malignancy index.

Chapter 8 describes phase 1 of a prospective study of screening for ovarian cancer amongst postmenopausal women. The results indicate that to achieve satisfactory specificity will require a multimodal approach combining CA 125 measurement with either pelvic examination or ultrasonography. Chapter 9 is a report of a phase 2 study of screening for ovarian cancer using the sequential combination of CA 125 and ultrasound. The preliminary results in 20,000 postmenopausal volunteers suggest that the lead time achieved over clinical presentation using this screening protocol is greater than 1 year.

It is concluded that despite limitations of specificity and sensitivity, CA 125 is of value in the preoperative diagnosis of ovarian cancer and may have a role in a multimodal screening protocol for early stage disease.

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This research would not have been initiated without the enthusiastic encouragement of Mr David Oram who provided the framework for the clinical studies and expert guidance in their design.

The work described in this thesis was conducted under the supervision of Professor J. G. Grudzinskas. In addition Professor Grudzinskas provided laboratory facilities, guidance concerning basic scientific and clinical aspects of this research and detailed critical assessment of this manuscript and scientific papers arising from it.

Professor R. C. Bast provided the facilities and materials for studies described in sections 7.1 and 9.2.2.4 during my visit to Duke University. I am grateful for his advice on the conduct of these studies and his comments on the CA 125 literature review (section 1.2).

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PUBLICATIONS

The contents of this thesis have formed the basis for a number of manuscripts listed below:

Chapter 1:

- Jacobs IJ and Bast RC (1989) The CA 125 tumour associated antigen; a review of the literature. Hum Reprod, 4;1-12.
- Jacobs IJ and Oram DH (1988) Screening for Ovarian Cancer. Biomed Pharmacother, 42;589-596.
- Oram DH and Jacobs IJ (1987) Improving the prognosis for ovarian cancer. In; Progress in Obstetrics and Gynaecology, volume 6. Ed. Studd J. Churchill Livingstone, 399-432.

Chapter 4:

- Jacobs IJ Fay TN Stabile I Oram DH Grudzinskas JG (1988) CA 125 distribution in the serum and reproductive tract of pregnant and non pregnant women. Br J Obstet Gynaecol, 95;1190-1194.

Chapter 5:

- Jacobs IJ Fay TN Frost C Oram DH Grudzinskas JG (1989) Serum CA 125 levels during ovulatory and oral contraceptive menstrual cycles and in postmenopausal women. Submitted for publication.

Chapter 6:

- Jacobs IJ Fay TN Stabile I Yovich J Oram DH Grudzinskas JG (1989) Serum levels of CA 125 during the 1st trimester of normal outcome, ectopic and anembryonic pregnancies. Hum Reprod, 5;116-122.

Chapter 7:

- Jacobs IJ Oram DH Fairbanks J Turner J Frost C Grudzinskas JG (1989) Systematic evaluation of CA 125, ultrasonography and clinical criteria to achieve accurate preoperative diagnosis of ovarian cancer. Br J Obstet Gynaecol, in press.

Chapter 8:

- Jacobs IJ Stabile I Bridges J Kemsley P Reynolds C Grudzinskas JG Oram DH (1988). Multimodal Approach to Screening for Ovarian Cancer. Lancet; i, 268-271.

TABLE OF CONTENTS

CHAPTER 1. LITERATURE REVIEW

1.1 INTRODUCTION

1.2 CA 125

1.2.1 The murine monoclonal antibody OC 125

1.2.2 The nature of the CA 125 determinant

1.2.3 Tissue distribution of CA 125 activity

1.2.3.1 Malignancy

1.2.3.2 Non malignant tissues

1.2.4 Serum CA 125 levels in ovarian cancer

1.2.4.1 Preoperative CA 125 levels in ovarian cancer

1.2.4.2 Diagnostic value of preoperative CA 125 levels

1.2.4.3 Prognostic significance of serum CA 125 levels

1.2.4.4 Monitoring disease status by serum CA 125 levels

1.2.4.5 Screening for ovarian cancer by serum CA 125 measurement

1.2.5 Serum CA 125 levels in Endometrial Cancer

1.2.6 Serum CA 125 levels in other malignancies

1.2.7 Serum CA 125 levels in physiological states and benign disease

1.2.7.1 Menstrual cycle

1.2.7.2 Pregnancy

1.2.7.3 Endometriosis

1.2.7.4 Other causes of elevated serum CA 125 levels

1.3 OVARIAN CANCER

1.3.1 General

1.3.2 Screening for Ovarian Cancer

1.3.2.1 Rationale

1.3.2.2 Test criteria

- (a) Specificity**
- (b) Positive Predictive Value**
- (c) Sensitivity**
- (d) Cost Effectiveness**
- (e) Acceptability**

1.3.2.3 Selection of a target population

- (a) Geography**
- (b) Age**
- (c) Reproductive history**
- (d) Family and personal history of malignancy**
- (e) Environmental factors**
- (f) Other possible associations**

1.3.2.4 Potential screening tests

- (a) Vaginal examination**
- (b) Tumour markers**
- (c) Ultrasound**
- (d) Doppler Colour Flow Imaging**
- (e) Other imaging techniques**
- (f) A multimodal approach**

1.3.3 Preoperative diagnosis of Ovarian Cancer

1.3.3.1 Rationale

- (a) Accurate surgical staging**
- (b) Cytoreductive surgery**

1.3.3.2 Diagnostic techniques

- (a) Clinical
- (b) Ultrasound
- (c) Computed Tomography
- (d) Magnetic Resonance Imaging
- (e) Doppler Colour Flow Imaging
- (f) Tumour markers

1.4 PURPOSE OF THIS RESEARCH

CHAPTER 2 METHODOLOGY

2.1 ASSAY PROCEDURES

2.1.1 CA 125

2.1.1.1 Abbott Laboratories/Centocor Radioimmunoassay

2.1.1.2 CIS Radioimmunoassay

2.1.1.3 Abbott Laboratories Enzymeimmunoassay

2.1.2 Tumour Associated Glycoprotein 72 (TAG 72)

2.1.3 CA 15-3

2.1.4 Luteinising Hormone assay

2.1.5 Progesterone assay

2.2 SAMPLE COLLECTION AND STORAGE

2.3 ULTRASOUND SCANS

2.4 STATISTICAL METHODS

CHAPTER 3 CA 125 MEASUREMENT

3.1 COMPARISON OF THREE CA 125 ASSAYS SYSTEMS

3.1.1 Samples and methods

3.1.1.1 Samples

3.1.1.2 Assay procedures

3.1.1.3 Methods

3.1.1.4 Statistical analysis

3.1.2 Results

3.1.2.1 Correlation

3.1.2.2 Sensitivity

3.1.2.3 Intra-assay coefficient of variation

3.2 FURTHER ASSESSMENT OF THE ABBOTT LABORATORIES RADIOIMMUNOASSAY

3.2.1 Samples and methods

3.2.1.1 Samples

3.2.1.2 Assay procedure

3.2.1.3 Method

3.2.2 Results

3.3 DISCUSSION

CHAPTER 4 THE DISTRIBUTION OF CA 125 ACTIVITY
IN THE FEMALE REPRODUCTIVE TRACT

4.1 INTRODUCTION

4.2. SAMPLES AND METHODS

4.2.1 Samples

4.2.2 Methods

4.2.3 Surgical staging

4.2.4 Histological / surgical diagnosis

4.3 RESULTS

4.3.1 Non pregnant patients

4.3.2 Pregnancy

4.3.2.1 1st trimester of pregnancy

4.3.2.2 2nd trimester of pregnancy

4.3.2.3 3rd trimester of pregnancy

4.3.3 Uterine Myomata

4.3.4 Functional ovarian cysts

4.3.5 Benign ovarian tumours

4.3.6 Ovarian cancer

4.3.7 Contralateral ovary

4.4 DISCUSSION

CHAPTER 5 SERUM CA 125 LEVELS DURING OVULATORY AND ORAL
CONTRACEPTIVE MENSTRUAL CYCLES AND IN POSTMENOPAUSAL WOMEN

5.1 INTRODUCTION

5.2 MATERIALS AND METHODS

5.2.1 Subjects

5.2.2 Radioimmunoassays

5.2.3 Statistical analysis

5.3 RESULTS

5.3.1 Ovulatory cycles

5.3.2 Hormonal contraception cycles

5.3.3 Postmenopausal women

5.3.4 Comparison of groups

5.4 DISCUSSION

CHAPTER 6 SERUM LEVELS OF CA 125 DURING THE FIRST TRIMESTER
OF NORMAL PREGNANCY AND IN ECTOPIC AND ANEMBRYONIC PREGNANCIES

6.1 INTRODUCTION

6.2 MATERIALS AND METHODS

6.2.1 Subjects and samples

6.2.2 Radioimmunoassay

6.2.3 Statistical analysis

6.3 RESULTS

6.3.1 Normal pregnancy \ normal outcome

6.3.2 Assisted conception \ normal outcome

6.3.3 Natural conception \ early pregnancy failure

6.4 DISCUSSION

CHAPTER 7 THE ROLE OF CA 125 MEASUREMENT IN THE PREOPERATIVE DIAGNOSIS OF OVARIAN CANCER

7.1 INTRODUCTION

7.2 PATIENTS AND METHODS

7.2.1 Patients

7.2.2 Methods

7.2.2.1 Clinical data

7.2.2.2 Clinical impression

7.2.2.3 Ultrasound scan

7.2.2.4 Radioimmunoassay

7.2.2.5 Histological or surgical diagnosis

7.2.2.6 Statistical analysis

7.3 RESULTS

7.3.1 Characteristics of study population

7.3.2 Individual test criteria

7.3.3 Serum CA 125

7.3.4 CA 125 combined with other tumour associated antigens

7.3.5 Logistic regression analysis of all test criteria

7.3.6 Derivation of the Risk of Malignancy Index (RMI)

7.4 DISCUSSION

CHAPTER 8 THE SPECIFICITY OF CA 125 AS A SCREENING TEST FOR EARLY STAGE OVARIAN CANCER

8.1 INTRODUCTION

8.2 SUBJECTS AND METHODS

8.2.1 Subjects

8.2.2 Methods

8.2.2.1 Vaginal examination

8.2.2.2 CA 125 Radioimmunoassay

8.2.2.3 Ultrasound

8.2.2.4 Follow up

8.2.2.5 TAG 72.3 and CA 15.3

8.2.2.6 Statistical methods

8.3 RESULTS

8.3.1 Study population

8.3.2 Study findings

8.3.3 Follow up

8.3.4 TAG 72.3 and CA 15.3

8.3.5 Specificity

3.4 DISCUSSION

8.4.1 Study population

8.4.2 Study findings

8.4.3 Specificity

8.4.4 Other requirements of a screening test

CHAPTER 9 A PHASE 2 STUDY OF SCREENING FOR OVARIAN CANCER WITH THE COMBINATION OF CA 125 AND ULTRASOUND

9.1 INTRODUCTION

9.2 SUBJECTS AND METHODS

9.2.1 Subjects

9.2.2 Methods

9.2.2.1 CA 125 Radioimmunoassay

9.2.2.2 Ultrasound

9.2.2.3 Follow up

9.3 RESULTS

9.3.1 Study population

9.3.2 Study findings

9.3.3 Years of cancer detected

9.3.4 Test performance

9.4 DISCUSSION

CHAPTER 10 GENERAL DISCUSSION AND CONCLUSIONS

10.1 THE RELATIONSHIP BETWEEN SERUM AND TISSUE LEVELS OF CA 125

10.2 THE PHYSIOLOGICAL SIGNIFICANCE OF CA 125

10.3 PREOPERATIVE DIFFERENTIAL DIAGNOSIS OF OVARIAN CANCER

10.4 THE ROLE OF CA 125 IN SCREENING FOR OVARIAN CANCER

10.5 SUMMARY OF CONCLUSIONS

10.5.1 CA 125 measurement

10.5.2 The distribution of CA 125

10.5.3 Serum CA 125 levels

10.5.4 Preoperative diagnosis of ovarian cancer

10.5.5 Screening for ovarian cancer

10.6 FURTHER RESEARCH IN PROGRESS

10.6.1 Screening for ovarian cancer

10.6.2 Preoperative diagnosis of ovarian cancer

REFERENCES

APPENDIX: THE GYNAECOLOGY CANCER RESEARCH FUND

TABLES

- 1.1. Tumour associated antigens expressed in ovarian cancer.
- 1.2. The proportion of women with ovarian cancer with an elevated preoperative serum CA 125 level in relation to FIGO stage.
- 1.3. Elevation of serum CA 125 (> 35 U/ml) in patients with various histological types of epithelial ovarian cancer.
- 1.4. Sensitivity, specificity and overall predictive accuracy of preoperative serum CA 125 measurement in prediction of findings at 2nd look laparotomy for ovarian cancer.
- 1.5. Proportion of patients with elevated serum CA 125 (> 35 U/ml) in relation to tumour diameter.
- 1.6. Elevation of serum CA 125 (> 35 U/ml) in patients with endometrial cancer in relation to clinical stage of disease.
- 1.7. Elevation of serum CA 125 (> 35 U/ml) in malignancies not involving the reproductive tract.
- 1.8. Serum CA 125 levels in patients with endometriosis in relation to the severity of disease.
- 1.9. Benign disorders associated with elevation of serum CA 125 levels.
- 1.10. Five year survival rates by stage at presentation for epithelial ovarian cancer.
- 3.1. Mean serum CA 125 levels, standard deviation and coefficient of variation of doubling dilutions of ovarian cancer serum assayed 10x in duplicate in 1 run of the ARIA, AEIA and CRIA.

TABLES cont.

- 3.2. Mean values, standard deviation and coefficient of variation of doubling dilutions of ovarian cancer serum assayed 10x in duplicate in 1 run of the ARIA (intraassay variation) and 1x in duplicate in 10 consecutive runs of the ARIA (interassay variation).
- 6.1. Mean log transformed serum CA 125 levels (and 95% confidence limits) by weeks gestation in normal outcome, ectopic and anembryonic pregnancies.
- 7.1. Histological type and FIGO stage of 42 patients with malignant disease.
- 7.2. Statistically significant differences between patients with benign (n=101) and malignant (n=42) pelvic masses.
- 7.3. Sensitivity and specificity of age, menopausal status, clinical impression, ultrasound and CA 125 in the differential diagnosis of benign and malignant masses in 143 patients.
- 7.4. Sensitivity, specificity and predictive accuracy of various upper limits for CA 125, CA 15.3 and TAG 72.3 in distinguishing benign from malignant adnexal masses.
- 7.5. Specificity and sensitivity of combinations of CA 125 and CA 15.3.
- 7.6. Specificity and sensitivity of combinations of CA 125, CA 15.3 and TAG 72.3.
- 7.7. The sensitivity, specificity and the likelihood ratio for malignancy given a positive or negative result for different levels of the Risk of Malignancy Index.
- 8.1. Summary of study results. Note that the false positives to CA 125 did not overlap with those from vaginal examination.

TABLES cont.

- 8.2.** Specificity for ovarian cancer amongst premenopausal women (n=1010) of vaginal examination, serum CA 125 and combinations of these tests with ultrasound scanning.
- 8.3.** Specificity for screening for ovarian cancer amongst apparently healthy postmenopausal women with various combinations of CA 125, TAG 72.3 and CA 15.3.
- 9.1.** Age distribution, expected incidence of ovarian cancer (OPCS 1983) and actual incidence of ovarian amongst the 20,000 volunteers.
- 9.2.** Summary of the performance of CA 125 alone and CA 125 combined sequentially with ultrasound in screening for ovarian cancer amongst 20,000 apparently healthy postmenopausal women.

FIGURES

- 1.1.** The relationship between the specificity of an annual screening programme for ovarian cancer and the number of operative procedures performed for each case of ovarian cancer diagnosed.
- 1.2.** The relationship between test specificity, sensitivity, positive predictive value and the incidence of disease in the screened population.
- 3.1.** Correlation between Abbott Laboratories CA 125 Radioimmunoassay and Abbott Laboratories CA 125 Enzymeimmunoassay.

FIGURES cont.

- 3.2. Correlation between Abbott Laboratories CA 125 Radioimmunoassay and CIS CA 125 Radioimmunoassay.
- 3.3. Correlation between CIS CA 125 Radioimmunoassay and Abbott Laboratories CA 125 Enzymeimmunoassay.
- 3.4. Dilution curves derived from doubling dilutions of pooled ovarian cancer serum for the Abbott Laboratories CA 125 Radioimmunoassay, the Abbott Laboratories CA 125 Enzymeimmunoassay and the CIS CA 125 Radioimmunoassay.
- 4.1. CA 125 activity in matched serum (n=12), endometrium (n=12) and myometrium (n=4) from 12 premenopausal women.
- 4.2. CA 125 activity in matched maternal serum and fetal and maternal tissue extracts from 11 first trimester pregnancies.
- 4.3. CA 125 activity in matched maternal and fetal serum and amniotic fluid from seven second trimester pregnancies.
- 4.4. CA 125 activity in matched maternal and fetal serum and tissue extracts from ten full term pregnancies.
- 4.5. Serum CA 125 levels in samples from patients with uterine fibroids, functional ovarian cysts and benign ovarian tumours.
- 4.6. Serum CA 125 levels in samples obtained from patients with epithelial ovarian cancer by FIGO stage of disease and histological type.
- 4.7. CA 125 activity in serum and cyst fluid from patients with benign and malignant ovarian tumours.

FIGURES cont.

- 4.8. CA 125 activity in serum and ovarian tissue homogenate from patients with benign and malignant ovarian tumours.
- 5.1. Serum CA 125 levels during ovulatory menstrual cycles in 10 individuals.
- 5.2. Mean serum CA 125 levels for individual subjects during menstrual and non menstrual phases of ovulatory menstrual cycles (n=10) and oral contraceptive cycles (n=7) and during a calendar month in postmenopausal women (n=8).
- 6.1. Serum CA 125 levels during the 1st trimester of pregnancies (n=150) achieved following natural conception and with a normal outcome.
- 6.2. Mean and 95% confidence limits around the mean for serum CA 125 at 2 weekly intervals during the 1st trimester of normal pregnancy (assisted and natural conception), anembryonic and ectopic pregnancies.
- 6.3. Serial serum CA 125 levels during 1st trimester pregnancies (n=43) achieved following treatment at an infertility clinic and with a normal outcome.
- 6.4. Peak serum CA 125 levels and gestational age at the time of peak levels in pregnancies (n=43) achieved following treatment at an infertility clinic and with a normal outcome.
- 6.5. Serum CA 125 levels during the 1st trimester of pregnancies achieved following natural conception and resulting in (a) ectopic pregnancies and (b) anembryonic pregnancies.
- 7.1. The likelihood ratio for malignancy of subgroups of patients defined by age, menopausal status, clinical impression score, ultrasound score and serum CA 125 level.

FIGURES cont.

- 7.2. Receiver operator characteristics of various upper limits for age, menopausal status, clinical impression score, ultrasound score, serum CA 125 level and Risk of Malignancy Index (RMI) in the preoperative prediction of the benign or malignant nature of an adnexal mass.
- 7.3. The relationship between menopausal status, ultrasound score, serum CA 125 level and clinical impression score in 139 patients with benign and malignant adnexal masses.
- 7.4. The relationship between serum levels of CA 125, CA 15.3 and TAG 72.3 in 143 patients with an adnexal mass.
- 8.1. Age distribution of 1010 apparently healthy postmenopausal volunteers participating in this study.
- 8.2. Number of years amenorrhoea amongst 1010 apparently healthy postmenopausal volunteers participating in this study.
- 8.3. Serum CA 125 results and centile distribution amongst 1010 apparently healthy postmenopausal women participating in this study.
- 8.4. Serum CA 125 levels at 3, 6, 9 and 12 months after initial screen in relation to initial result in 30 apparently healthy postmenopausal women with initial levels < 30 U/ml and 30 apparently healthy postmenopausal women with initial levels > 30 U/ml.
- 8.5. Relation between upper limit for serum CA 125 and specificity for ovarian cancer in 1010 postmenopausal women.
- 9.1. Distribution of serum CA 125 levels in 20,000 apparently healthy postmenopausal volunteers.

FIGURES cont.

9.2. Summary of study findings. Volunteers have been divided into screen negative and screen positive groups on the basis of their CA 125 level and ultrasound findings. The details of patients with false negative and true positive results are shown.

9.3. Histological findings, FIGO stage, serum CA 125 levels and time course of the diagnostic process in 11 cases of ovarian cancer detected by the screening programme.

9.4. A model of the progress of ovarian cancer from a pre-invasive phase to the development of symptoms of invasive disease.

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Ovarian cancer is responsible for the deaths of more than 4,000 women per year in the United Kingdom, a number greater than the mortality attributable to endometrial and cervical cancers combined. Due to a combination of late presentation and poor response to treatment, the 5 year survival for ovarian cancer is only 30%. Considerable effort has therefore been directed toward the discovery of serum markers for ovarian malignancy which may be of diagnostic value or direct the optimal use of available treatments. A large number of antigens expressed by ovarian cancers have been described and are detected by polyclonal and monoclonal antisera (table 1.1). None of these are tumour specific, but initial reports (Bast et al 1981, 1983) suggested that the CA 125 antigen may be a particularly useful marker for ovarian malignancy. Subsequent work (section 1.2.4) confirmed elevation of serum CA 125 activity in over 80% of women with ovarian cancer and a correlation with the course of the disease.

The studies described in this thesis were undertaken in order to assess the value of CA 125 measurement in the diagnosis of ovarian cancer. In order to provide a basis for design of the prospective studies, preliminary analytical and descriptive studies (chapters 3 - 6) of the nature and distribution of CA 125 activity in physiological states as well as benign and malignant pathology were undertaken. Further studies were then performed to investigate the role of serum CA 125 measurement in screening for early stage ovarian cancer and the preoperative differential diagnosis of the adnexal mass (chapters 7 - 9).

Antigenic markers

1. Oncofetal proteins

Carcinoembryonic antigen	Stall & Martin 1981
Human chorionic gonadotrophin	Donaldson 1980
Alphafetoprotein	Donaldson 1980
Tissue polypeptide antigen	Nakajima et al 1984

2. Antigens defined by polyclonal antisera

OCAA	Bhattacharya & Barlow 1978
OCA	Knauf & Urbach 1978
NB/70K	Knauf & Urbach 1981
OVC-1	Inamura et al 1978
OVC-2	Inamura et al 1978

3. Antigens defined by monoclonal antisera

Source of immunogen in parentheses O=ovarian cancer, E=endometrial cancer, C=colorectal cancer, P=pancreatic cancer, B=breast cancer, M=milk fat globulin, L=laryngeal cancer, Pl=placental alkaline phosphatase.

OC 125 (O)	Bast et al 1981.
ID3 (O)	Bhattacharya et al 1982
MOV-2 (O)	Tagliabue et al 1985
OC 133 (O)	Berkowitz et al 1983
MD 144, MF 61 & MF 116 (O)	Mattes et al 1984
632 (O)	Fleuren et al 1984
OVTL-3 (O)	Poels et al 1986
4F4 & 7A ₁₀ (ov)	Bhattacharya et al 1984
WB12123 (O)	Knauf et al 1986
MH55 & MH94 (E)	Mattes et al 1984
CA 19-9 (C)	Koprowski et al 1979, Charpin et al 1982
DUPAN-2 (P)	Metzgar et al 1982
F36/22 (B)	Papsidero et al 1983, Croghan et al 1984
DF3 (B)	Kufe et al 1984, Sekine et al 1985a,b
B72.3 (B)	Colcher et al 1983, Johnston et al 1985
260FS, 280D11 & 245E7 (B)	Frankel et al 1985, Pirker et al 1985
HMFG1, HMFG2 & AUA1 (M)	Epenetos et al 1982
CA-1 (L)	Ashall et al 1982, Woods et al 1982
NDOG2 (Pl)	Sunderland et al 1984

Enzymes

Placental alkaline phosphatase	Fishman et al 1975
Galactosyltransferase	Bhattacharya et al 1976
beta hexoseaminidase	Chatterjee et al 1982
Amylase	Sirsart et al 1982
Ribonuclease	Sheid et al 1977
5' nucleotidase	Chatterjee et al 1981
Lactic dehydrogenase	Awais 1978
Cystine aminopeptidase	Kalinkov and Buchholz 1980
Alpha-1-fucosidase	Barlow et al 1981

Hormones

Human placental lactogen	Crowther et al 1979
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Miscellaneous

D-dimer of fibrin	Haft et al 1985
Circulating immune complexes	Dodd et al 1985

Table 1.1. Tumour associated antigens expressed in ovarian cancer.

1.2 CA 125

CA 125 is an antigenic determinant on a high molecular weight glycoprotein recognised by a monoclonal antibody (OC 125) which was raised using an ovarian cancer cell line as an immunogen. The function of the glycoprotein expressing CA 125 is unknown and because of its complex nature information about the physical and immunological nature of this antigen is limited. Serum levels of CA 125 were initially reported to be elevated in over 80% of patients with ovarian cancer and to reflect the clinical course of the disease (Bast et al 1983). Subsequently research and clinical interest has concentrated on the role of this antigen in several aspects of the management of ovarian malignancy.

1.2.1 The murine monoclonal antibody OC 125

The OC 125 monoclonal antibody was produced by Bast et al (1981) using a modification of the technique of Kohler and Milstein (1975). BALB/c mice were immunised with a cell line (OVCA 433) established from a patient with a serous papillary cystadenocarcinoma of the ovary. Spleen cells from the mice were subsequently fused with a plasmacytoma cell line. The supernatant from resultant colonies was screened using indirect immunofluorescence by incubation with ovarian cancer cell lines and staining with a combination of fluoresceinated goat anti-mouse IgG and anti-mouse IgM. The most promising clone isolated was designated OC 125 and was selected for reactivity with the OVCA 433 cell line and other epithelial ovarian cancer cell lines

but lack of reactivity with a B lymphocyte cell line from the same individual.

The OC 125 antibody was used in immunohistochemical studies (Kabawat et al 1983a,b) and to develop an immunoradiometric assay for quantitative measurement of the CA 125 determinant (Klug et al 1984). The latter is a "sandwich" assay in which polystyrene beads coated with OC 125 are incubated with the sample to be assayed and with I^{125} radiolabelled OC 125. As multiple CA 125 determinants are associated with each antigen molecule, free determinants on bead bound antigens are detected by binding of the I^{125} labelled OC 125 antibody. No pure preparation of the CA 125 antigen is available and concentration of CA 125 in this assay is therefore expressed in arbitrary units/ml. This assay is commercially available from several sources. The results obtained with different kits are not directly comparable (see chapter 3). Serial monitoring should therefore be performed with kits from a single source.

1.2.2 The nature of the CA 125 determinant

Column chromatography and SDS-polyacrylamide gel electrophoresis followed by Western blotting (Davis et al 1986, O'Brien et al 1986) indicate that CA 125 activity in ovarian cancer serum, amniotic fluid, human milk and supernatant from an ovarian cancer cell line is associated with a moiety of greater than 1,000 kD and a lower molecular weight moiety of 200 to 400 kD. However, Halila (1985) has reported a molecular weight of 130,000 for CA

125 immunoreactive material in both seminal plasma and ovarian cancer serum following gel chromatography (Sephacryl S-300). The latter report did not describe CA 125 activity in the void volume of the column.

The nature of the CA 125 determinant has been investigated after chemical, physical and enzymatic treatment. Hanisch et al (1985) found CA 125 activity to be destroyed by treatment with periodate, mild alkali or neuraminidase. On the basis of these findings it was concluded that the determinant is a sialylated saccharide bound to protein by an alkali labile linkage. However, subsequent work suggests that the CA 125 determinant is proteinaceous in nature. Davis et al (1986) found that concentrations of periodate sufficient to oxidise carbohydrates do not affect CA 125 activity; that heating at 100°C totally destroys CA 125 activity; that exoglycosidase treatment increases CA 125 activity and that CA 125 activity is destroyed by exhaustive protease digestion. Davis et al (1986) have suggested that the apparent sensitivity of CA 125 to periodate and neuraminidase reported by Hanisch et al (1985) was a consequence of the high periodate concentration employed and of heating the antigen to 100°C during neuraminidase treatment.

Although the precise nature of the CA 125 determinant remains unclear, there is agreement that the molecule with which it is associated is a glycoprotein. Using antigen purified from an ovarian cancer cell line, Davis et al (1986) reported a carbohydrate content of 24% on the basis of carbohydrate compositional analysis and buoyant density. If this is

representative of other sources of CA 125, the antigen has a lower carbohydrate content than is typical for mucins such as the other epithelial tumour antigens recognised by monoclonal antibodies (eg 19-9, B72.3, DU-PAN-2 and F36/22). Differences were noted in the buoyant density of the CA 125 antigen isolated from human milk, seminal plasma and an ovarian cancer cell line suggesting slight variability in protein or carbohydrate content.

Davis et al (1986) found the monoclonal antibody 19-9 to be reactive with antigen purified from an ovarian cancer cell line by OC 125 immunoaffinity chromatography suggesting that the CA 125 and 19-9 determinants may be present on the same molecular complex. These findings cannot be attributed to cross specificity between the antigens and the CA 125 determinant. Inhibitory double determinant analyses and immunoblotting studies have clearly shown that the CA 125 determinant itself is distinct from 19-9 and from determinants defined by murine monoclonal antibodies DU-PAN-2, B72.3 and Mov 2 (Lan et al 1987).

1.2.3 Tissue distribution of CA 125 activity

The CA 125 determinant can be identified in fresh frozen sections of many normal and pathological tissues using the OC 125 antibody with an indirect immunoperoxidase technique. Immunohistochemical staining of paraffin embedded material following pronase pretreatment produces a similar pattern of CA 125 expression but does appear to result in a decrease in the proportion of specimens expressing CA 125 (Shishi et al 1985, Koelma et al 1987).

1.2.3.1 Malignancy

Using an indirect immunofluorescence technique Kabawat et al (1983a) screened ovarian tumours for reactivity with OC 125. All benign and borderline and most malignant serous tumours were positive. All mucinous tumours were negative as were Brenner, sex cord and germ cell tumours. In antigen positive tumours CA 125 stained the surface of the cells and there was little or no cytoplasmic staining. No correlation was observed between expression of CA 125 and grade of tumour or stage of disease. In a subsequent report (Kabawat et al 1983b), using an avidin biotin immunoperoxidase technique, it was observed that OC 125 reacted with adenocarcinomas of Mullerian origin whether arising from the endometrium or fallopian tube but only 6 of 60 tumours derived from non coelomic tissues. Further studies indicate that CA 125 can be expressed by mucinous tumours and by a proportion of malignancies arising in the lung, breast, stomach, liver, gall bladder, stomach, pancreas, kidney and large bowel (Dietel et al 1986, Mainguene et al 1986, Nouwen et al 1987).

1.2.3.2 Non malignant tissues

Kabawat et al (1983b) demonstrated expression of the CA 125 antigen in 1st and 2nd trimester fetal tissues derived from the coelomic epithelium (Mullerian ducts, cells lining the fetal peritoneum, pleura and pericardium) and amniotic epithelium, but not cells derived from the fetal ovary or any other fetal tissues. OC 125 also reacted with adult derivatives of coelomic epithelium including epithelial cells from the fallopian tube, endometrium and endocervix. No reactivity was demonstrable with normal ovarian surface epithelium but the CA 125 determinant was

expressed in epithelial cells lining inclusion cysts, papillary excrescences and adhesions where the surface epithelial cells had undergone metaplasia. Reactivity was also found in mesothelial cells lining the adult pleura, pericardium and peritoneum. On the basis of these observations Kabawat et al (1983b) concluded that CA 125 is a differentiation antigen associated with coelomic epithelium and its normal and neoplastic derivatives. The observation that normal adult and fetal ovarian surface epithelium failed to express CA 125 despite the presence of CA 125 in normal mesothelial cells and tumours derived from surface epithelium was interpreted as evidence of a distinct differentiation pathway of ovarian surface epithelium. However, subsequent reports indicate that CA 125 expression can be demonstrated immunohistochemically in some sections of normal ovarian epithelium as well as by epithelia of the pancreas, colon, gall bladder, stomach, lung and kidney (Dietel et al 1986, Nouwen et al 1987, Nouwen et al 1986).

1.2.4 Serum levels in ovarian cancer

1.2.4.1 Preoperative serum CA 125 levels in ovarian cancer

Several studies have reported preoperative serum CA 125 levels in relation to stage and histological type of ovarian malignancy. There is general agreement between these reports that serum CA 125 levels are elevated preoperatively in 80-85% of women with epithelial ovarian cancer (table 1.2). Disease disseminated outside the ovary is associated with elevation of serum CA 125 levels in over 90% of cases (stages II, III and IV). When

confined to ovarian tissue serum CA 125 levels are elevated in 50% of cases.

Although CA 125 was not originally thought to be expressed by mucinous carcinoma of the ovary, subsequent reports indicate elevation of CA 125 levels in the serum of most patients with this histological type (table 1.3). Serum levels of CA 125 are, however, less frequently elevated in mucinous than other histological types of epithelial ovarian cancer. In a study of 36 patients with stage I and II epithelial ovarian carcinoma serum CA 125 levels were found to be greater than 35 U/ml in 18/22 patients with non mucinous tumours and only 4/14 mucinous carcinomas (Zurawski et al 1988a). The authors suggested that the elevation of serum CA 125 observed in association with more advanced stage mucinous carcinomas may be a consequence of metastatic involvement of serosal epithelium. There is little information available concerning grade of tumour and serum CA 125 level. Brioschi et al (1987) found serum levels greater than 35 U/ml in 12/16 grade 1, 14/15 grade 2, 17/19 grade 3 and 5/5 grade 4 cases. Zanaboni et al (1987) reported elevation of serum CA 125 in 6/14 grade 1, 27/29 grade 2 and 30/36 grade 3 cases.

1.2.4.2 Diagnostic value of preoperative serum CA 125 levels

Einhorn et al (1986) measured serum CA 125 levels preoperatively in 100 patients undergoing diagnostic laparotomy for palpable adnexal masses, 23 of whom were subsequently found to have a malignancy. Using an upper limit of 35 U/ml serum CA 125 measurement had a sensitivity for malignant disease of 78%

Author	F. I. G. O. Stage				Total
	I	II	III	IV	
Bast et al (1983)	1/1	2/2	15/16	3/3	21/22
Brioschi et al (1987)	4/13	3/3	29/30	22/23	58/69
Canney et al (1984)	---	---	-----	-----	48/58
Crombach et al (1985a)	3/5	5/6	13/19	10/10	31/40
Cruickshank et al (1987)	3/12	2/3	15/16	10/10	31/42
Fuith et al (1987)	4/6	4/5	16/18	8/10	32/39
Heinonen et al (1985)	0/3	---(9/9 stages II-IV)---			9/12
Kaeseman et al (1986)	----31/46----		----135/153----		166/199
Kivinen et al (1986)	3/3	10/10	15/15	1/1	29/29
Krebs et al* (1986)	3/4	8/8	25/25	7/8	43/45
Li-juan et al (1986)	1/2	3/3	22/23	-----	26/28
Schilthuis et al (1987)	6/8	5/5	20/20	13/13	44/46
Ricolleau et al (1985)	---	---	-----	-----	35/38
Zanaboni et al (1987)	8/15	3/4	29/34	3/4	43/57
Zurawski et al (1988a)	12/24	10/12	-----	-----	--#--
TOTAL	48/96	55/61	199/216	77/82	615/723
	50.0%	90.0%	92.1%	93.9%	85.1%

Table 1.2. The proportion of women with ovarian cancer with an elevated preoperative serum CA 125 level in relation to FIGO stage. (* = upper limit 25 U/ml, remaining studies 35 U/ml, # = not included in total as this was a study of stage I and II disease). Note that totals for each stage do not include studies where stages were combined.

Histological type

	Serous	Mucinous	Endomet -rioid	Clear cell	Undiffer -entiated
Canney et al (1984)	2/37	6/6	4/6	2/4	14/15
Brioschi et al (1987)	36/41	9/13	4/5	2/2	7/8
Crombach et al (1985b)	28/33	2/3	1/2	2/2	2/3
Cruickshank et al (1987)	9/13	5/9	2/2	2/4	12/13
Fuith et al (1987)	26/29	3/4	1/1	---	3/4
Kuzuya et al (1986)	16/16	2/3	3/4	2/2	---
Martoni et al (1986)	23/25	3/3	4/6	3/5	---
Meier et al (1987)	41/42	2/2	2/4	---	7/9
Pansini et al (1986)	8/8	2/2	1/1	---	3/3
Schilthuis et al (1987)	23/24	---	7/8	6/6	6/6
Takahashi et al (1986)	12/12	0/2	---	---	---
Zanaboni et al (1987)	30/37	1/4	10/13	9/11	2/3
TOTAL	254/317	35/51	39/52	28/36	56/64
	80%	69%	75%	78%	88%

Table 1.3. Elevation of serum CA 125 (> 35 U/ml) in patients with various histological types of epithelial ovarian cancer.

(18/22), a specificity of 95% (73/77), a positive predictive value of 82% (18/22) and a predictive accuracy of 91% (91/100). Serum CA 125 levels greater than 65 U/ml were detected in 13/23 patients with malignancy and 1 patient with benign disease (positive predictive value 93%). The results of this study suggested that preoperative CA 125 measurement may be of value in the differential diagnosis of benign and malignant pelvic masses. Vasilev et al (1988) conducted a similar study involving 182 gynaecological patients with pelvic masses but interpretation of their results is difficult as the study population were not consecutive admissions and included a large proportion of women without an adnexal mass. Seventy one patients in this study had uterine fibroids alone, and it is likely that the possibility of ovarian malignancy in most of these cases would be ruled out in routine clinical practice by ultrasound scanning. This study reported a sensitivity for malignant disease of 78% (14/18), a specificity of 78% (128/164), a positive predictive value of 28% (14/50) and a predictive accuracy of 78% (142/182).

Neither of these studies compared the value of preoperative serum CA 125 measurement with clinical data and other diagnostic tests (see chapter 7).

1.2.4.3 Prognostic significance of serum CA 125 levels

Two studies have reported the prognostic significance of serum CA 125 measurement. Cruickshank et al (1987) found no correlation between preoperative serum CA 125 and disease outcome in 41 patients, but follow up duration was short (3-18 months). A collaborative study by the Gynaecological tumour marker group

(GTMG) at 4 centres in West Germany has provided more information on this point. In 202 patients followed up for 2-145 months, (median 73 months) survival was significantly greater for those with a preoperative serum CA 125 < 65 U/ml compared to those with a level > 65 U/ml. No difference in survival was apparent for patients with a preoperative serum CA 125 of 65-500 U/ml compared to patients with a level > 500 U/ml. These findings are consistent with those summarised above in relation to stage of disease and preoperative serum CA 125 level. Patients with early stage disease are less likely to have an elevated serum CA 125 preoperatively and are known to have a better prognosis. The majority of epithelial ovarian malignancies are disseminated at presentation, associated with an elevated serum CA 125 and have a poor outcome. There is little information currently available concerning serum CA 125 in the subtypes of stage I disease and in relation to survival in these stages. Understaging of ovarian malignancy with stage III disease incorrectly classified as stage I is common (Piver et al 1978). It may be the case that such cases are associated with a significantly higher serum CA 125 than is found in true stage I disease.

The GTMG also assessed the prognostic significance of postoperative serum CA 125 in 165 patients. A postoperative serum CA 125 > 65 U/ml was associated with a significantly worse 5 year survival than a postoperative serum CA 125 < 65 U/ml (42% v 5%). In 123 cases analysis of pre and post operative serum CA 125 was possible. Patients with a serum CA 125 < 65 U/ml both pre and postoperatively had a better prognosis than those with a preoperative CA 125 > 65 U/ml falling to < 65 U/ml post-

operatively. Postoperative serum CA 125 was also of prognostic significance in the group of patients with < 2cm residual disease following surgery. In this subgroup women with a CA 125 > 65 U/ml postoperatively all died within 42 months compared to 48% survival at 74 months for women with a CA 125 < 65 U/ml.

The rate of fall of serum CA 125 postoperatively may also be of prognostic significance. Canney et al (1984) measured CA 125 levels in serial serum samples from patients with residual tumour post surgery receiving chemotherapy. CA 125 levels fell with a half life of 22.6 ± 2.2 days in 3 patients with apparently static disease compared to 9.2 ± 4.9 in 12 patients with a good clinical response to chemotherapy. Van der Burg et al (1988) found CA 125 half life to be a prognostic factor for progression rate and time interval to progression of disease in 37 ovarian cancer patients with a pretreatment CA 125 > 60 U/ml receiving cisplatin combination chemotherapy. The median time to progression for 16 patients with a CA 125 half life > 20 days was 11 months compared to 43 months for 21 patients with a half life < 20 days. This possibility will require further investigation.

1.2.4.4 Monitoring disease status by serum CA 125 levels

A considerable number of studies have now reported on the correlation between the course of ovarian cancer and serum CA 125 level. In cases where CA 125 is elevated in preoperative serum samples serum CA 125 levels correlate with the clinical disease status in over 90% of cases. This data alone does not, of course, imply that serum CA 125 measurement is of value in the management of ovarian cancer. In order to be of value in the clinical

context serum CA 125 must provide information more accurately, earlier or more easily than currently available techniques. Some evidence is now available in relation to these criteria.

A persistently rising serum CA 125 level is consistently associated with progression of disease and is frequently evident several months prior to clinical evidence of progression. A doubling of CA 125 outside the normal range of 35 U/ml is a reliable indicator of recurrent disease. The most accurate method of assessing disease status available is second look laparotomy. Several studies have investigated the accuracy of CA 125 measurement as an indicator of disease status using second look findings as a 'gold standard' (table 1.4). An elevated serum CA 125 level prior to second look was in all of these studies an extremely good indicator of the presence of disease. Residual disease was detected in 256/269 patients with an elevated serum CA 125 (positive predictive value 95.2%). However, a serum CA 125 within the normal range did not exclude the presence of disease, (disease was present in 249/523 women with a normal serum CA 125). The overall accuracy of serum CA 125 measurement was 67%. It is worth noting that most of the patients undergoing second look laparotomy in these studies were clinically free of disease. Serum CA 125 level was therefore considerably more sensitive as a detector of disease than clinical assessment. False negative CA 125 results were generally associated with small volume disease reflecting the fact that a minimum tumour volume is necessary to cause elevation of serum CA 125. Serum CA 125 is elevated in only a small proportion of patients with microscopic disease but 60% or more with disease greater than 1-2 cm in diameter (table 1.5).

<u>CA 125 level in relation to 2nd look findings</u>						
	CA 125 raised /		CA 125 normal /		Overall	
	Disease present		Disease absent		predictive	
	(sensitivity)		(specificity)		accuracy	
Atack et al (1986)	3/9	33%	8/8	100%	11/17	65%
Berek et al (1986)	12/31	39%	24/24	100%	36/55	65%
Brioschi et al (1987)	17/27	63%	13/13	100%	30/40	75%
Fish et al (1987)	8/12	67%	2/2	100%	10/14	71%
Fuith et al (1987)	7/10	70%	14/14	100%	21/24	88%
Khoo et al (1987)	11/22	50%	19/20	95%	30/42	71%
Krebs* et al (1986)	7/13	54%	18/20	90%	25/33	76%
Halila et al (1988)	16/44	36%	19/22	86%	37/66	56%
Lavin et al (1987)	12/19	63%	9/10	90%	21/29	72%
Meier et al (1987)	12/16	75%	6/6	100%	18/22	82%
Mobus et al (in press)	18/60	30%	51/51	100%	69/111	62%
Mogensen et al (1988)	18/41	44%	10/10	100%	28/51	55%
Niloff et al (1986)	7/25	28%	9/10	90%	16/35	46%
Rome et al (1987)	16/31	42%	18/18	100%	34/49	69%
Rubin et al (1989)	66/84	79%	11/12	92%	77/96	80%
Schwartz et al (1987)	3/9	33%	9/9	100%	12/18	67%
Schilthuis et al (1987)	12/39	31%	19/21	91%	31/60	52%
Zanaboni et al (1987)	11/13	85%	15/17	88%	26/30	87%
TOTAL	256/505	51%	274/287	96%	530/792	67%

Table 1.4. Sensitivity, specificity and overall predictive accuracy of preoperative serum CA 125 measurement in prediction of findings at 2nd look laparotomy for ovarian cancer. (Upper limit 35 U/ml except; * = upper limit 25 U/ml, # = upper limit 65 U/ml).

<u>Tumour size at laparoscopy/laparotomy</u>						
Microscopic	<1cm	<2cm	>1cm	>2cm	>10cm	
Atack et al (1986)	1/4-----	1/3-----	1/2			
Berek et al (1986)	2/7-----	3/13*	6/11*			
Brioschi et al (1987)	3/8-----	14/19				
Canney et (1984)	10/16-----	13/17#	24/24			
Fish et al (1987)	6/7-----	5/6#	15/15			
Halila et al (1988)	2/8-----	2/6-----	12/30			
Khoo et al (1987)	2/6-----	3/7-----	6/9			
Niloff et al (1985)	8/22-----	10/10				
Rome et al (1987)	1/4-----	1/7*	14/20*			
Rubin et al (1989)	10/18---	16/20-----	40/46			
Schilthuis et al (1987)	2/17					
TOTAL	10/46	24/69	37/66	42/68	79/102	39/39
	22%	35%	66%	62%	78%	100%

Table 1.5. Proportion of patients with elevated serum CA 125 (> 35 U/ml) in relation to tumour diameter. (* = >1.5cm or <1.5cm. # = >2cm and <10cm).

A rising serum CA 125 level is therefore extremely reliable evidence for recurrent or progressive disease. On the other hand a CA 125 level falling to or staying within the normal range may be associated with small volume residual disease (in up to 40% of cases) or a tumour not expressing the CA 125 determinant (up to 20% of cases). No study has yet addressed the question of whether earlier detection of recurrent disease by CA 125 monitoring is translated into an improved outlook for the patient.

1.2.4.5 Screening for ovarian cancer by serum CA 125 measurement

This subject is discussed further below (section 1.3.2). Little information is currently available concerning the role of serum CA 125 measurement as a screening test for the early detection of ovarian cancer. It has been suggested by several authors that the occurrence of an elevated serum CA 125 level in a number of benign conditions precludes its use as a screening test for ovarian cancer (Lambert 1987, Schilthuis et al 1987, Meier et al 1987, Fuith et al 1987). As the majority of benign conditions associated with an elevated serum CA 125 occur in the age group with a low incidence of ovarian cancer these limitations of specificity may not be an insuperable problem (see chapter 8).

The sensitivity of serum CA 125 measurement alone or in combination with other tests for preclinical early stage ovarian cancer is unknown. It is unlikely to be higher than the figure of 50% established for clinically diagnosed stage I disease (table 1.2). Retrospective analysis of serum samples stored in the JANUS serum bank has recently provided encouraging evidence that serum CA 125 measurement may be able to detect ovarian malignancy prior

to clinical diagnosis (Zurawski et al 1988b). Serum samples from 105 women who subsequently developed ovarian malignancy (interval 1-143 months) were significantly greater than 323 matched controls. Six of 12 samples collected within 18 months of diagnosis had a serum CA 125 greater than 30 U/ml and 4 of 12 were greater than 65 U/ml. Fourteen of 59 samples collected more than 60 months before diagnosis had a serum CA 125 greater than 30 U/ml.

1.2.5 Serum CA 125 in Endometrial Cancer

Elevated serum CA 125 levels have been reported in endometrial cancer (table 1.6) usually in association with advanced stage disease. Duk et al (1986) found a high correlation between presence of tumour in uterine blood vessels or lymphatics and elevation of serum CA 125. Patients with elevated serum CA 125 and FIGO stage I or II disease usually had extensive metastatic disease in lymphatics or other tissues and were more likely to die of the disease than those patients with a normal serum CA 125 level (6/21 vs 5/73). Endometrial cancer is usually diagnosed at an early stage and the overall prognosis for this disease is good. However, a proportion of apparently early stage patients have extrauterine tumour extension and are at high risk of developing recurrent disease. The observations of Duk et al (1986) suggest that measurement of serum CA 125 levels may provide a biochemical prognostic indicator for endometrial malignancy in addition to the previously established risk factors (histological type, tumour differentiation, myometrial invasion

and vascular invasion). Further evidence to support this possibility has been provided by the recently reported study of Patsner et al (1988). Twenty of 23 patients with clinical stage I or II endometrial cancer who were found to have extrauterine spread of disease during staging laparotomy had elevated preoperative serum CA 125 levels. The positive predictive value of an elevated preoperative serum CA 125 for extrauterine spread of disease in this study was 95%. Berchuck et al (1989) have used an immunohistochemical technique to evaluate the percentage of cancer cells staining for CA 125 and the intensity of staining in sections of 44 endometrial cancers. Expression of CA 125 was significantly higher in tissue from 13 patients found to have metastatic disease on surgical staging than in patients with disease confined to the uterus. Both tissue expression and serum levels of CA 125 may therefore be indicators of metastatic spread of endometrial carcinoma.

1.2.6 CA 125 in other malignancies

Elevation of serum CA 125 in malignancy of the pancreas, stomach, colon and rectum is usually associated with widespread disease involving the peritoneum, liver, lung or bone (Haga et al 1986b), (table 1.7). Serum CA 125 measurement may be of value in the differential diagnosis of pancreatic cancer from chronic pancreatitis. The combination of serum CA 125 with serum CA 19-9 has a sensitivity of up to 97% for pancreatic carcinoma (Haga et al 1986b), whilst available data indicates that serum CA 125 levels are rarely elevated in chronic pancreatitis.

Author	Clinical stage of endometrial cancer		
	I + II	III + IV	All stages
Niloff et al (1984a)	0/11	14/18	14/29
Meier et al (1987)	-----	-----	2/10
Duk et al (1986)	22/100	5/7	27/107
Fuith et al (1987)	-----	-----	13/32
Patsner et al (1988)	21/ 81	8/8	29/89
TOTAL	43/192 22.4%	27/33 81.8%	85/267 31.8%

Table 1.6. Elevation of serum CA 125 (> 35 U/ml) in patients with endometrial cancer in relation to clinical stage of disease.

<u>Malignancy</u>	<u>CA 125 > 35 U/ml</u>		<u>Authors</u>
Breast	33/187	17.6%	1, 3, 5, 7, 8, 10.
Colorectal	35/239	15.1%	1, 2, 5, 6, 10.
Pancreas	113/215	52.6%	1, 4, 6, 9, 10.
Lung	38/129	29.5%	1, 5, 6.
Gastric	25/ 81	30.9%	6, 10.
Biliary tract	11/ 24	45.8%	10.
Liver	25/ 51	49.0%	10.
Oesophageal	2/ 19	10.5%	10.

Table 1.7. Elevation of serum CA 125 (> 35 U/ml) in malignancies not involving the reproductive tract. (1-Bast et al 1983; 2-Canney et al 1984; 3-Crombach and Wurz 1984; 4-Dietel et al 1986; 5-Kawahara et al 1986; 6-Kuzuya et al 1986; 7-Meier et al 1987; 8-Ricolleau et al 1985; 9-Haglund et al 1986; 10-Haga et al 1986b).

1.2.7 CA 125 in physiological states and benign disease

1.2.7.1 Menstrual cycle

Mastropalo et al (1986) reported elevation of serum CA 125 in one patient at the time of menstruation to greater than 300 U/ml. Pittaway et al (1986) reported smaller but significant increases in serum CA 125 at the time of menstruation in 20 infertile patients. Serum levels increased from 13 ± 3 U/ml prior to menstruation to 29 ± 7 U/ml during menstruation in 9 women without endometriosis and from 16 ± 4 U/ml to 54 ± 19 U/ml in 11 women with endometriosis. CA 125 levels during the menstrual cycle in fertile ovulatory women and the relationship of serum CA 125 to gonadal steroids have not been reported (see chapter 5).

1.2.7.2 Pregnancy

Several authors have reported elevation of serum CA 125 during pregnancy (Seki et al 1986, Niloff et al 1984b, Haga et al 1986a, O'Brien et al 1986, Jacobs et al 1988a, see chapter 4). Published data concerning CA 125 levels in pregnancy have reported groups of single samples usually taken more than 6 weeks after the last menstrual period. The pattern of CA 125 variation in normal and abnormal pregnancies is described in chapter 6.

1.2.7.3 Endometriosis

Several studies (Barbieri et al 1986, Giudice et al 1986, Patton et al 1986, Pittaway et al 1986, Takahashi 1987, Fedele 1988a) have established that serum CA 125 levels are elevated in a proportion of patients with endometriosis and that the degree of elevation is related to severity of endometriosis (table 1.8).

Although the serum CA 125 levels associated with endometriosis in these studies were relatively low they may not preclude its use as a diagnostic test for this condition. Pittaway and Douglas (1989) have recently reported a study of 163 women undergoing surgery for pelvic pain. Using a modification of the Centocor CA 125 radioimmunoassay (Pittaway 1989) and an upper limit of 16 U/ml they observed elevations of serum CA 125 in 52%, 86%, 100% and 100% of patients with minimal, mild, moderate and severe endometriosis respectively and only 6% of women without endometriosis. The overall accuracy of this approach was 93% (sensitivity 80%, specificity 94%) and the authors concluded that it was valuable in the differential diagnosis of chronic pelvic pain.

In patients with a diagnosis of endometriosis and an elevated serum CA 125, CA 125 levels do appear to be correlated with the course of the disease. Pittaway et al (1986) found an 84% correlation with clinical course in 44 women with endometriosis (8 untreated, 36 surgical treatment +/- postoperative Danazol). In 6 patients with advanced endometriosis treated surgically (hysterectomy and bilateral oophorectomy) Barbieri (1987) observed a decrease in serum CA 125 from a mean of 85 ± 23 U/ml preoperatively to 16 ± 2.5 U/ml postoperatively. In 10 patients treated with Danazol for 6 months the same author observed a significant reduction in serum CA 125 from 35 ± 16 U/ml to 12 ± 4 U/ml and a correlation with American Fertility Society score as assessed by pre and post treatment laparoscopy. The limitation of serum CA 125 measurement in the follow up of treated endometriosis appears to be similar to the follow up of ovarian

cancer. Whilst an elevated serum CA 125 level is a reliable indicator of persistent disease, a serum CA 125 in the normal range cannot exclude persistent disease (Fedele et al 1988b, Takahashi 1989).

Kaupila et al (1988) followed serum CA 125 levels for 6 months during treatment of endometriosis patients with medical treatment alone or surgical and medical treatment. Medical treatment (Danazol, Medroxyprogesterone Acetate or placebo) was allocated by double blind randomisation and response assessed at 6 months by repeat laparoscopy. Forty patients treated surgically showed a fall in serum CA 125 at 1 month (mean 44.6 ± 56.7 U/ml to 20.6 ± 12.0 U/ml) regardless of postoperative treatment. Although the 48 patients treated medically alone had a significantly improved endometriosis score in both the medroxyprogesterone acetate (MPA) and Danazol groups only those patients treated with Danazol had a significant decrease in serum CA 125. The authors concluded that serum CA 125 levels do not correlate with response to hormonal therapy and that the decrease associated with Danazol treatment is a specific effect of this drug. However, they did not comment on the fact that the mean pretreatment level in the Danazol only treated group was 23 U/ml (decreasing to 9 U/ml), whilst that in the MPA treated group was 17 U/ml (decreasing to 14 U/ml). The failure to observe a significant decrease in serum CA 125 in the MPA treated group may therefore have been related to the relatively low pretreatment CA 125 level in patients in this group. The observations of Kaupila et al (1988) are nevertheless intriguing, particularly in view of the report of Ozasa et al (1988) that mean serum CA 125 levels increased

significantly (19.6 to 30.0 U/ml) within 36 hours in 7 endometriosis patients receiving intramuscular progesterone but not in 5 controls without endometriosis.

1.2.7.4 Other causes of elevated serum CA 125 levels

Serum CA 125 levels may be elevated in a proportion of individuals with several benign disorders in addition to those discussed above (table 1.9). Although serum CA 125 activity in cyst fluid from benign ovarian tumours is extremely high serum CA 125 levels are less than 35 U/ml in most cases. Fleuren et al (1987) detected high levels of CA 125 activity in cyst fluid from mucinous cystadenomas (median 4,950 U/ml range 845-116,000 U/ml) and serous cystadenomas (median 25,700 U/ml range <50-371,000 U/ml). Serum levels of CA 125 are significantly higher in healthy women under 49 years of age than in healthy women over 49 years and healthy men of all ages (Haga et al 1986a). Smoking does not appear to affect serum CA 125 levels (Haga et al 1986a, Green et al 1986).

Author	Upper limit		Stage of Endometriosis				Total
	CA	125 U/ml	I	II	III	IV	
Takahashi et al (1987)	39	-----	1/6	8/8	6/7	15/21	
Barbieri et al (1986)	35	0/23	3/24	----7/13 ^a ----	10/60		
Kaupila et al (1988)	30	9/47	7/25	---11/15 ^a ----	27/87		
Moloney et al (1989)	35	4/25	9/22	3/3	2/2	18/52	
Patton et al (1986)	35	----2/22 [#] ----	----3/15 ^a ----	5/37			
Giudice et al (1986)	35	----	----	----	---	7/8	
Fedele et al (1988a)	35	----2/38 [#] ----	----7/19 ^a ----	9/57			
Pittaway & Favez (1986)	30	1/55	7/37	9/30	7/8	24/130	
	[16*	15/55	25/37	22/30	8/8	70/130]	
TOTAL			14/150	27/114	20/41	15/17	115/452
			9.3%	23.7%	48.8%	88.2%	24.4%
			Stage I + II		Stage III + IV		
			45/324, 13.9%		63/120, 52.5%		

Table 1.8. Serum CA 125 levels in patients with endometriosis in relation to the severity of disease (Staging according to American Fertility Society Criteria). [* Reported in relation to 16 U/ml upper limit, but results for upper limit 30 U/ml calculated for this table and used in total. # Stages I and II combined, ^a Stages III and IV combined].

	<u>CA 125 > 35 U/ml</u>		<u>Authors</u>
<u>Benign ovarian tumours</u>			
Serous	7/ 57	12.3%	4, 7, 9, 10, 11.
Mucinous	3/ 35	8.6%	4, 7, 9, 10, 11.
Benign cystic teratoma	5/ 74	6.8%	4, 7, 9, 10, 11.
Fibroma	1/ 7	14.3%	5, 9, 11.
All benign tumours	31/299	10.4%	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11,
<u>Other benign disorders of the female reproductive tract</u>			
Acute salpingitis	19/ 47	40.4%	11, 12, 13.
Chronic salpingitis	2/ 24	8.3%	11, 14.
Uterine myoma	12/122	9.8%	2, 4, 5, 11, 14, 15.
<u>Disorders of the digestive tract</u>			
All cirrhosis	98/146	67.1%	16, 17, 18, 19.
Cirrhosis + ascites	24/ 24	100.0%	16.
Chronic active hepatitis	3/ 33	9.1%	17, 19.
Acute pancreatitis	20/ 62	32.2%	17, 19, 20.
Chronic pancreatitis	1/ 54	1.9%	19, 20.
<u>Other benign disorders</u>			
Renal failure	7/ 48	14.6%	17, 18.
Diabetes	0/ 10	0.0%	18.

Table 1.9. Benign disorders associated with elevation of serum CA 125 (> 35 U/ml). [Authors; 1-Crombach and Wurz 1984; 2-Einhorn et al 1986; 3-Fleuren et al 1987; 4-Kuzuya et al 1986; 5-Li-juan et al 1986; 6-Meier et al 1987; 7-Pansini et al 1986; 8-Ricolleau et al 1985; 9-Shi et al 1987; 10-Takahashi et al 1986; 11-Vasilev et al 1988; 12-Fuith et al 1987; 13-Halila et al 1986; 14-Barbieri et al 1986; 15-Yabushita et al 1988; 16-Bergmann et al 1986, 1987; 17-Ruibal et al 1984, 1986; 18-Eerdeken et al 1985; 19-Haga et al 1986b; 20-Haglund 1986.]

1.3 OVARIAN CANCER

1.3.1 General

Ovarian cancer is the site of origin for a larger variety of primary tumours than any other organ (Serov et al 1973). Over 90% of primary ovarian malignancies are, however, of epithelial origin (Fox and Langley 1976). The following review encompasses those aspects of the literature on the subject of epithelial ovarian malignancy related to early detection and preoperative diagnosis. Adjuvant therapy options and secondary surgery will not be reviewed in detail.

During the last two decades intensive efforts have been made to improve the surgical and chemotherapeutic management of ovarian cancer and to determine the aetiology of this disease. As a result some significant advances have been made; the importance of thorough surgical staging and debulking surgery has been established, new chemotherapeutic regimes introduced and risk factors for ovarian cancer defined. Although these developments have been encouraging they have not produced a dramatic improvement in ovarian cancer statistics. Whilst the incidence of ovarian cancer has steadily increased during the last 20 years the bleak outlook for the disease has remained largely unchanged. Overall 5 year survival is still only 30%, and cancer of the ovary is now responsible for more than 50% of deaths due to malignancies of the female reproductive tract. Epithelial ovarian cancer remains the greatest challenge in gynaecological oncology.

The only immediately available method for improving ovarian cancer statistics is prophylactic oophorectomy. The available evidence suggests that a significant proportion of ovarian cancers could be or are currently prevented by this approach (Jacobs and Oram 1987). However, the practice of prophylactic oophorectomy by gynaecologists in the United Kingdom is conservative, particularly with regard to premenopausal women (Jacobs and Oram 1989) and this is understandable in view of the potential long term complications of oophorectomy. Two other approaches to improving the prognosis for ovarian cancer are screening to detect early stage disease (section 1.3.2) and accurate preoperative diagnosis to provide a basis for optimal surgical treatment (section 1.3.3).

1.3.2 Screening for Ovarian Cancer

1.3.2.1 Rationale

The ideal screening test for ovarian cancer would detect the disease in a premalignant phase and hence provide a method for prevention of invasive disease. Unfortunately, there is no well defined precancerous lesion of the ovary analogous to cervical dysplasia or atypical endometrial hyperplasia. Even if the existence of such a condition was established the anatomical site of the ovary would make sampling by a non-invasive procedure impossible (cf cervical smear) and its incorporation in a screening programme difficult. Furthermore, there is no convincing data to suggest that benign tumours of the ovary are a premalignant condition. The evidence that benign ovarian cysts

may be the site of malignant transformation is tenuous and based on the documentation of malignant foci in otherwise benign neoplasms and the fact that the average age of women with benign tumours of the ovary is 10 years less than women with malignant tumours. One must conclude that at present there is no basis for screening to detect a precancerous condition of the ovary.

The rationale of screening for early stage ovarian cancer is the well documented observation that 5 year survival for this disease is closely correlated with stage at presentation (table 1.10).

Stage	Percentage incidence (number of patients)	5 year survival (%)
Ia	17.9 (940)	69.7
Ib	4.3 (227)	63.9
Ic	3.0 (157)	50.3
<hr/>		
Total stage I	25.2 (1324)	67.1
<hr/>		
IIa	4.8 (251)	51.8
IIb+c	12.8 (672)	42.4
III	39.5 (2074)	13.3
IV	17.7 (993)	4.1
<hr/>		
Total III + IV	57.2 (3067)	10.4
<hr/>		
Total all stages	100.0 (5254)	30.6
<hr/>		

Table 1.10 Five year survival rates by stage at presentation for epithelial ovarian cancer. (Kottmeier 1982).

Ovarian cancer is characterised by its late and non-specific symptomatology. This feature combined with the inaccessibility of the ovaries to inspection or thorough examination accounts for the fact that 75% of patients have Stage II, III or IV disease at presentation. Whilst 5 year survival for Stage I disease is relatively good, that of women with extra-ovarian disease at presentation is extremely poor.

On the basis of such data, it is reasonable to hypothesise that diagnosis of a greater proportion of cases at Stage I would dramatically improve the overall prognosis for ovarian cancer. This suggestion must be treated with a degree of caution in view of the possibility that an improvement in overall survival figures will be apparent rather than real. Screening by detecting preclinical cases produces lead time, (interval from the screen detected diagnosis until symptomatic diagnosis would have occurred). Survival from the date of detection by screening will therefore be longer than from the date at which clinical presentation would have occurred, even if death occurs at the same time. This problem will have to be addressed during the evaluation of any screening programme for ovarian cancer by performance of a randomised controlled study using mortality (rather than survival) as an end point. Nevertheless, the prognosis for ovarian cancer is so closely related to degree of spread that improved survival through effective early diagnosis is unlikely to be a product of lead time bias alone. There is therefore a theoretical basis for the proposition that an increase in the detection of early stage ovarian cancer will result in an improvement in prognosis for the disease.

The natural history of ovarian malignancy and in particular the length of preclinical phase, (from initiation of malignancy to clinical presentation) are unclear. Even assuming that the rationale for early detection discussed above is valid and that an effective screening test is available, the practicality of a screening programme will depend on the length of the pre-clinical phase. If the preclinical phase is short the screening interval will have to be too frequent to be acceptable in clinical practice.

1.3.2.2 Test criteria

A test for the early detection of ovarian cancer must satisfy the same general requirements as a screening test for any disease process. Consideration of the available information concerning incidence rates and survival data provides a basis for estimating the acceptable limits for these test criteria in the context of screening for ovarian cancer.

(a) **Specificity:** When the diagnostic test for a neoplasm is nearly free of side effects and the cost modest, the requirement for high specificity can be relaxed in order to achieve high sensitivity (eg colposcopy and cervical cancer). In the context of screening for ovarian cancer the consequence of a positive screening test (laparoscopy or laparotomy), is associated with considerable expense and morbidity. High specificity is therefore an essential requirement of any screening test for ovarian cancer. Figure 1.1 illustrates the relationship between test specificity and the number of procedures performed for each case of ovarian cancer diagnosed, on screening a population with an

ovarian cancer incidence of 40/100,000 per year such as women aged 45 years or over in England and Wales (OPCS 1983). An annual screening test for ovarian cancer, even with 100% sensitivity would require 99.6% specificity in order to detect 1 case of ovarian cancer for every 10 operations performed in this population. As is discussed below specificity can vary widely according to the composition of the screened population.

In addition the results of tests for ovarian malignancy may be affected by physiological variables (see chapters 4-6). These are important considerations in selecting a population for screening.

(b) **Positive predictive value:** The positive predictive value of a test is affected by the prevalence of the disease in the screened population as well as the sensitivity and specificity of the test. This criteria is of particular importance to the clinician as it indicates the number of diagnostic tests which must be performed to detect each case of the disease. The definition of acceptable positive predictive value is arbitrary but it is unlikely that less than 10% would be acceptable in the context of screening for ovarian cancer where the diagnostic procedure is laparoscopy or laparotomy. Clinicians are unlikely to employ a test which will result in greater than nine unnecessary procedures for each case of ovarian cancer detected.

As predictive value is influenced by the prevalence of the disease it is dependent on the ratio of ill to non-ill in the investigated group. For a test of given specificity and sensitivity for ovarian cancer the higher the prevalence of the

disease in the screened population the greater the positive predictive value. Recent advances in the epidemiology of ovarian cancer discussed below are of relevance in this context. The combination of test sensitivity and specificity required to achieve a positive predictive value of 10% on screening a population with various incidence rates of ovarian cancer is illustrated in figure 1.2. Clearly a test unsuitable for screening one population of women may be acceptable for a population group with a higher incidence of ovarian cancer.

The level of specificity required to achieve a positive predictive value of 10% could be decreased by screening a population at relatively high risk of developing ovarian cancer, such as women with a family history of the disease. However, such an approach would exclude a large proportion of women destined to develop ovarian cancer from the screening programme. As the most appropriate selection criteria for screening remains age (section 1.3.2.3), a level of specificity of the order of 99.6% is an essential requirement of any screening test for ovarian cancer.

(c) Sensitivity: Unlike specificity the level of sensitivity required of a screening test for ovarian cancer cannot be calculated from available data. It is unclear at what stage in the progress of ovarian malignancy intervention is required, in order to alter the natural history of the disease, rather than to achieve a diagnostic lead time. Since the rationale for screening is provided by survival data for each FIGO stage it is reasonable to require evidence of sensitivity for FIGO stage I or at least stage II disease. Nevertheless, it is theoretically possible that

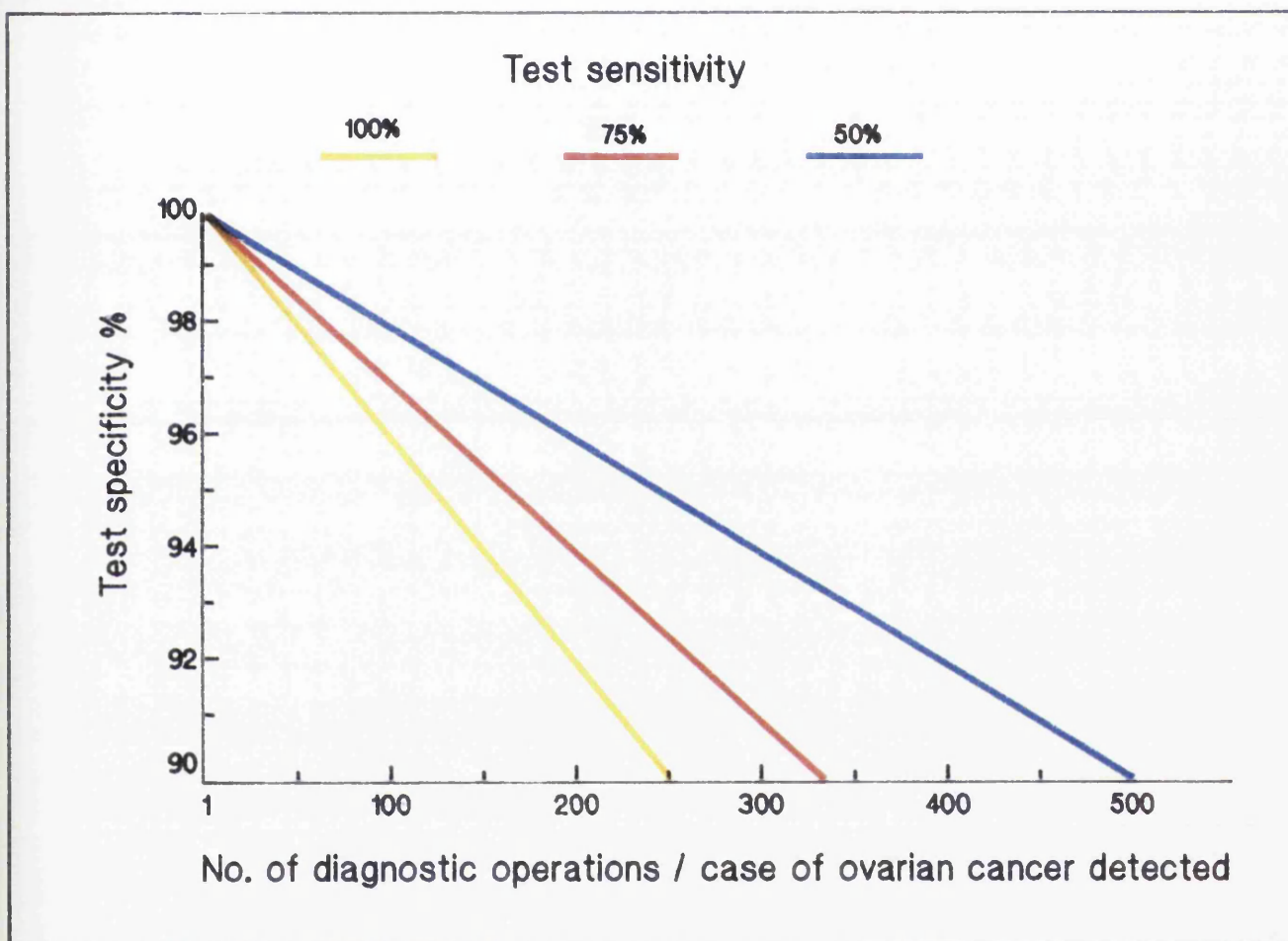


Figure 1.1. The relationship between the specificity of an annual screening programme for ovarian cancer and the number of operative procedures performed for each case of ovarian cancer diagnosed. The relationship is illustrated for test sensitivities of 100%, 75% and 50%. Based on an incidence of 40/100,000 per year in women of 45 years or older in England and Wales (OPCS 1983).

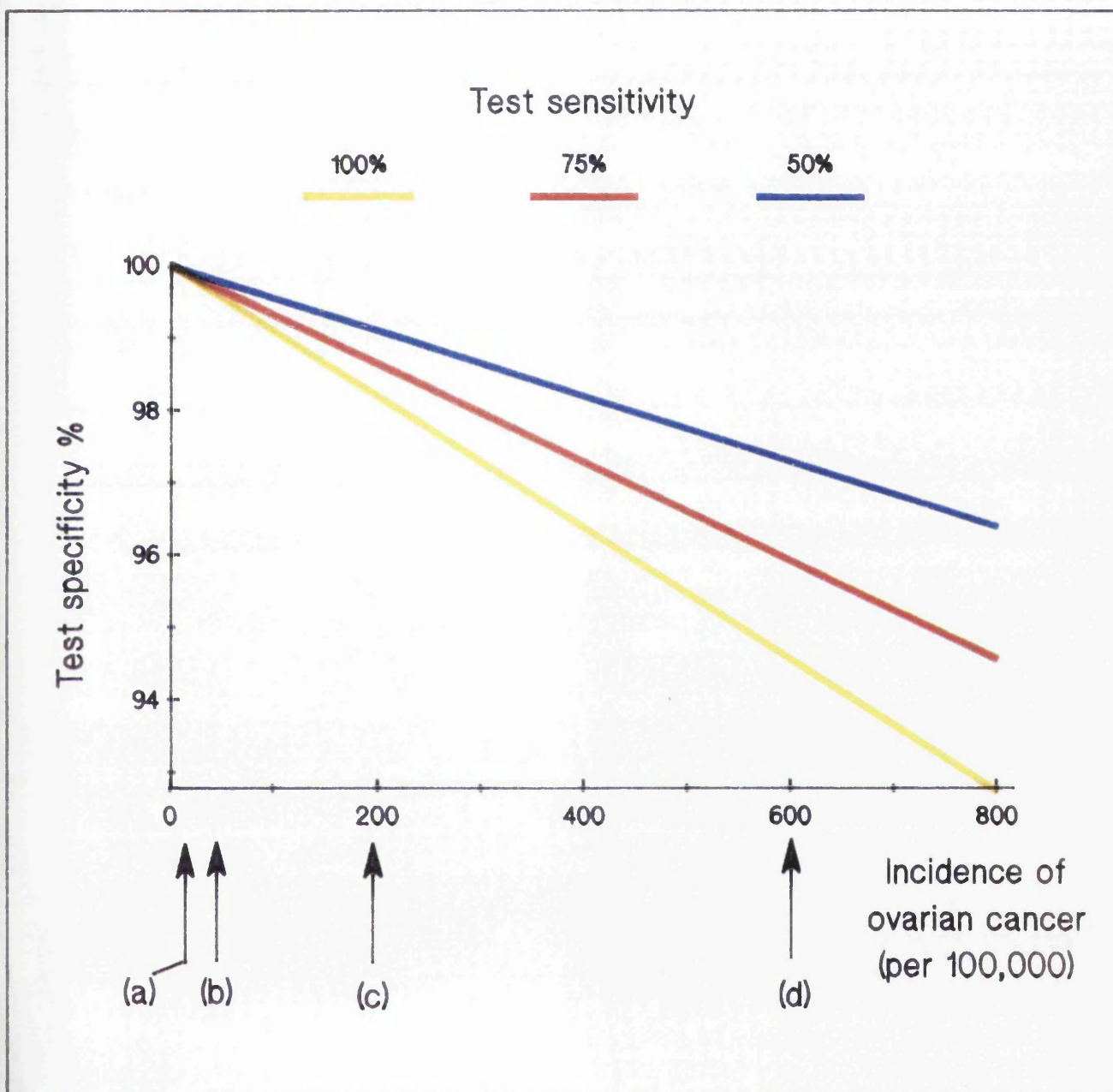


Figure 1.2. The relationship between test specificity, sensitivity, positive predictive value and the incidence of disease in the screened population. The yellow, red and blue lines represent the combinations of test specificity and disease incidence required to achieve a positive predictive value of 10% for tests with sensitivities of 100%, 75% and 50% respectively. The arrows on the x axis show the approximate incidence rates of ovarian cancer in (a) the entire female population, (b) women over 45 years of age, (c) women with a family history of ovarian cancer and (d) women over 45 years of age with a family history of ovarian cancer.

detection of relatively early stage III disease amenable to optimal cytoreduction could contribute to a reduction in mortality.

(d) **Cost Effectiveness:** The cost effectiveness of a screening programme for ovarian cancer would be influenced by several factors including the cost of the screening test, the cost of the diagnostic test and the saving achieved by treatment of early vs late stage disease. Although the cost of these expenses are fixed, the ratio of expenditure to saving will be influenced by the specificity and sensitivity of the test employed and the incidence of the disease in the screened population. Cost effectiveness is therefore another reason for selecting a population with a relatively high incidence of ovarian cancer and for which the test employed has a high specificity.

As the incidence of ovarian cancer is relatively low it is unlikely that a cost benefit will result from ovarian cancer screening. Whether the health benefit is sufficient to merit the cost of screening is a wider question involving health service priorities and political issues.

(e) **Acceptability to target population:** Acceptability of a screening test is a function of the perception of the importance of the test amongst the potential population as well as the nature of the test. Ovarian cancer is an emotive subject and it is likely that a public education programme to encourage participation with screening would be effective. The effectiveness of screening for cervical cancer is limited by the

poor compliance with screening programmes of the social class groups who also have a higher incidence of the disease. This problem will not apply to ovarian cancer as social class is not an important risk factor for this disease.

1.3.2.3 Selection of a target population

Although the aetiology of ovarian malignancy is unclear epidemiological studies have identified risk factors and associations which describe the most susceptible groups of the population.

(a) **Geography:** Ovarian cancer is a disease of western industrialised nations, but even in these countries the incidence was low until this century (Woodruff 1979). Epithelial ovarian cancer is 3 to 5 times more common in industrialised populations than in developing countries (Waterhouse et al 1976) although this may be a reflection in part of differences in registration and reporting. Age adjusted incidence rates range from 14.9 per 100,000 in Sweden to just 2.8 per 100,000 in some areas of Japan whilst intermediate levels are observed in the United Kingdom. Studies of rates among immigrants from developing countries to industrialised areas suggest that country of residence is of greater importance than race (Dunn 1975, Muir and Mectoux 1978). However Japanese, Chinese, Hispanic and black women in the United States of America do have lower incidence rates of ovarian cancer than Caucasian women (Weiss and Peterson 1978) and among Jews living in Israel the rate of ovarian cancer is three times higher for those born in Europe or America compared with Asia and Africa.

(b) **Age:** Age is an important risk factor for all histological types of ovarian cancer. Germ cell tumours and malignant teratomas occur at younger ages than the epithelial tumours, peaking at puberty and in the third decade respectively. However, the various histological types of epithelial ovarian cancer as well as sex cord and mesenchymal tumours have a similar age distribution (Weiss et al 1977) with a low incidence until the fifth decade and a steep increase to a peak incidence in the eighth decade (OPCS 1983). The incidence of all types of ovarian malignancy in England and Wales is 5 per 100,000 per year in women under 45 years of age and 40 per 100,000 per year in women 45 years or older.

(c) **Reproductive history:** There is considerable evidence that events of reproductive life are important factors in the development of epithelial ovarian malignancy. The increase in ovarian cancer incidence over time as well as geographic and racial differences in incidence can probably be accounted for by reproductive factors (Beral et al 1978). Case control studies have consistently revealed that women with ovarian cancer are less likely to have been pregnant than matched controls (Joly et al 1978, Annegers et al 1979, Casagrande et al 1979 and 1983, Risch et al 1983, Weiss et al 1981, Cramer et al 1982a, Rosenberg et al 1982, Hildreth et al 1981, Franceschi et al 1982, La Vecchia et al 1983, Newhouse et al 1977, McGowan et al 1979, Dicker et al 1983). Most of these studies also revealed a significant decrease in risk of developing ovarian cancer amongst oral contraceptive users which was proportional to duration of use. Furthermore, ovarian cancer patients are more likely than

matched controls to have an early menarche or late menopause. These data are consistent with the incessant ovulation hypothesis (Fathalla 1972) which postulates that the trauma of ovulation is responsible for neoplastic change and that any process which suppresses ovulation is protective against ovarian cancer.

(d) Family and personal history of malignancy: There are many case reports of familial aggregation of ovarian cancer. Such an association is supported by several case control studies (Casagrande et al 1979, McGowan et al 1979, Hildreth et al 1981) which indicate that the risk of ovarian cancer is increased up to 18 fold in women whose mother or sister have previously developed the disease. A cancer family syndrome in which ovarian cancer is associated with colon, endometrial, breast and other adenocarcinomas has been described (Lynch and Lynch 1979, Lynch et al 1981). These same malignancies also occur significantly more often as double primaries in the same women. Women with an initial primary of the breast, endometrium or colon have a two to four times greater risk of developing a subsequent ovarian cancer than women of comparable age and race who have not had these malignancies (Schoenberg et al 1969, Schottenfeld et al 1969, Schottenfeld and Berg 1971, Newell et al 1974, Reimer et al 1978, Annegers and Malkasian 1981, Prior and Waterhouse 1981).

(e) Environmental factors: Recent evidence has suggested an association between exposure to asbestos or talc and development of ovarian cancer. Increased rates of ovarian cancer have been reported in women occupationally exposed to asbestos (Acheson et al 1982, Wignall and Fox 1982). Consumer talc is known to be

contaminated with asbestos (Rohl et al 1976) and talc particles have been observed in both normal and neoplastic ovarian tissue (Henderson et al 1971 and 1979). One case-control study revealed a relative risk for development of ovarian cancer of 1.9 among women regularly using talc on sanitary pads or the perineum and a larger risk of 3.4 associated with both of these practices (Cramer et al 1982b).

(f) **Other possible associations:** Three studies suggest that the risk of ovarian cancer is reduced among women who have had a previous hysterectomy with ovarian conservation (Annegers et al 1979, Hildreth et al 1981, Booth 1986). There is some evidence for a number of other risk factors including childhood mumps infection (West 1966, Newhouse et al 1977, Menczer 1979), coffee consumption (Trichopoulos et al 1981) and the use of non contraceptive oestrogens (Weiss et al 1982).

The identification of risk factors for ovarian cancer has obvious relevance to the selection of a population for screening. As was discussed above the relatively low incidence of ovarian malignancy imposes a requirement of extremely high specificity in order to achieve an acceptable positive predictive value in screening for this disease. The ability to identify and screen a group of women with a higher incidence of ovarian cancer would render tests of lower specificity acceptable. Although several risk factors have been identified most are not of immediate practical value. For instance, although family history is a strong risk factor, 95% of women with ovarian cancer do not have a family history of the disease (Hildreth et al 1981). Age

remains the most useful criteria for selecting a population for screening. An annual test with 100% or 60% sensitivity for stage I ovarian cancer would require 99.6% or 99.8% specificity respectively to achieve a positive predictive value of 10% on screening women over 45 years of age. Amongst a group of women with an incidence of ovarian cancer of 800/100,000 (eg those with a strong family history), an annual test with 100% or 60% sensitivity would require 92.7% or 95.7% specificity respectively to achieve the same positive predictive value.

1.3.2.4 Potential screening tests

(a) Vaginal examination: Pelvic examination is generally regarded as lacking sufficient sensitivity to be of value in the early detection of ovarian cancer. The findings of McFarlane et al (1955) are often quoted to support this view. They discovered only 6 ovarian cancers during a total of 18,753 pelvic examinations performed in 1319 women over a 15 year period (1938 - 1952). Only 1 of the 6 women with ovarian cancer survived 5 years (stage at diagnosis was not stated in their report). The incidence of ovarian cancer in this population was apparently low but this is not surprising as follow up was incomplete, (only 537 of the initial 1319 volunteers completed the investigation) and women as young as 30 years were included. It is not possible to reach conclusions concerning either the specificity or sensitivity of pelvic examination from this study.

Some evidence to support the view that vaginal examination lacks sensitivity for the detection of an adnexal mass (either benign

or malignant) was provided by the study of Andolf et al (1986), (see below p.48). Each patient recruited underwent a pelvic examination prior to ultrasonography. Pelvic examination was reported as normal in 18 of 24 benign ovarian cysts, 2 borderline malignancies and one ovarian carcinoma. The size of the benign cysts was not stated but the borderline tumours were 6cm and 4cm in diameter. Although the examinations in this study were performed by 30 different gynaecologists (the scans were performed by 1 technician) and the size of the cysts not stated, these findings do suggest that a significant proportion of benign ovarian cancers are missed by pelvic examination.

(b) **Tumour markers:** The anatomical site and structure of the ovaries renders them inaccessible to direct examination but may make ovarian malignancy particularly suitable for detection by a serum marker. Antigen produced by early stage ovarian cancer may reach the peripheral circulation by two routes. Antigen in cystic or solid area may diffuse into lymphatics or blood vessels in the well vascularised ovarian stroma whilst antigen shed into the peritoneal cavity may reach the thoracic duct and hence venous circulation via diaphragmatic lymphatics.

Serum levels of a large number of substances have been investigated in relation to ovarian cancer. These include enzymes (amylase, placental alkaline phosphatase, galactosyl transferase, lactate dehydrogenase, alpha-1-fucosidase, N-acetyl-glucosamine-transferases), hormones (human placental lactogen, human chorionic gonadotrophin) and metabolic products (ferritin, fibrin degradation products, circulating immune complexes, polyamines)

as well as antigens of unclear biological function recognised by polyclonal and monoclonal antisera (table 1.1). Advances in the technology of antigen purification and radioimmunoassay development during the 1960's and 70's led to production of polyclonal antisera to a number of antigens which have been evaluated in relation to ovarian cancer (carcinoembryonic antigen, alpha-fetoprotein, human chorionic gonadotrophin). During the last decade, the introduction of technology for production of monoclonal antibodies has led to a rapid increase in knowledge about antigens expressed by ovarian malignancy and the production of monoclonal antibodies for their evaluation (CA 19.9, MOV-2, DUPAN2, F36/22, DF3, TAG 72.3, CA 15.3, CA 125). None of these advances have however demonstrated antigens expressed only in ovarian malignancy. All antigens described to date are tumour associated rather than tumour specific and are expressed by other normal and diseased cells. This lack of absolute specificity does not necessarily imply an absolute lack of diagnostic value. Such tumour associated antigens may be valuable in the early detection of ovarian cancer if their sensitivity for early stage ovarian cancer is satisfactory and high specificity can be achieved because either (a) serum levels in patients with early stage ovarian cancer are higher than in women without the disease and there is little or no overlap between the two groups or (b) the overlap between individuals with ovarian cancer and those without the disease selects non-diseased individuals with characteristics which will result in their exclusion by a secondary test.

Stage I ovarian cancer is relatively uncommon and usually diagnosed at surgery. Consequently, there are no large series of pre-operative serum samples from patients with early stage disease and a paucity of information concerning serum levels of tumour markers before treatment in stage I disease. Sufficient material to resolve questions of sensitivity for stage I disease will only be provided by a large scale collaborative study.

It is possible that the combination of a number of tumour markers each with relatively low sensitivity could achieve satisfactory overall sensitivity for stage I ovarian cancer.

(c) **Ultrasound:** The role of ultrasonography in gynaecological practice was transformed by the introduction of real time (in addition to static) scanning. This advance provided a method for visualisation of bowel peristalsis and therefore the differential diagnosis of loops of bowel from ovarian cysts. Ultrasound monitoring of follicular growth in the ovarian cycle is now established practice in most fertility centres.

The use of ultrasonography as a screening test is dependent upon the demonstration of abnormal ovarian morphology or size. Real time ultrasonography was shown to be an accurate method for measurement of ovarian volume by Campbell et al (1982). Subsequently a prospective study involving 5479 women has been undertaken (Bhan et al 1987, Campbell et al 1989). The results have provided preliminary but encouraging evidence of the sensitivity of ultrasound for early stage disease. At the time of the report no interval cases of ovarian cancer had occurred.

Two cases of stage Ia invasive cancer, two stage Ia borderline malignancies, one stage Ib borderline malignancy and four cases of metastatic ovarian disease (from bowel and breast) were detected. The results of the study did not however confirm previous suggestions (Meire et al, 1978) that ovarian morphology as demonstrated by ultrasound was related to histological diagnosis. Unfortunately the specificity of the test in this study was only 97.7% and the positive predictive value only 1.5%. These disappointing findings were largely a consequence of the inability of real time scanning to distinguish benign from malignant ovarian neoplasms or cysts, but false positive results also occurred in patients with no ovarian abnormality. Bhan et al (1987) suggested that at least a proportion of the benign cysts detected had malignant potential and were not false positives. However, as was forcefully pointed out in the discussion of their paper (Fox 1987) and has been mentioned above, there is no evidence that benign tumours of the ovary are pre-malignant.

Andolf et al (1986) scanned 805 women between 40 and 70 years of age attending an obstetrics and gynaecology outpatients clinic in Sweden. 83 patients were recalled for a second scan at which 50 were still positive and 39 subsequently underwent surgery. Two were found to have borderline ovarian malignancies and 1 a stage III endometroid carcinoma. This study involved symptomatic patients referred to an out-patient clinic and must therefore be interpreted with caution in the context of screening an asymptomatic population. Furthermore, 10 patients with 2 positive scans were treated elsewhere and follow up data on the group of 805 patients are not given. These deficiencies would be

expected to decrease the specificity of the test which was only 95.5%. Nevertheless, this study confirms the inability of ultrasound to distinguish benign from malignant disease on the basis of ovarian morphology.

Available data concerning the sensitivity of real time ultrasonography for stage I ovarian cancer is therefore limited but encouraging. On the other hand there is now considerable evidence that the specificity of this technique used alone is not sufficient to justify its use without other confirmatory tests as a screening test in the general population. Demonstration of the premalignant potential of benign ovarian cysts would however provide a role for ultrasound as a preventative technique as well as an early diagnostic test. The possible premalignant potential of benign ovarian cysts is therefore an important and relevant subject. However its investigation will require an extremely large, expensive and long term randomised prospective study.

(d) **Doppler Colour Flow Imaging:** Bourne et al (1989) have recently suggested that transvaginal doppler colour flow imaging of ovarian vasculature may provide a method of improving the specificity of ultrasound screening for ovarian cancer. Using this technique areas of neovascularisation were observed in the ovaries of 7 out of 8 patients with ovarian cancer but were not observed in 30 women with apparently normal ovaries or 9 out of 10 women with benign tumours. It is possible that this approach will be a valuable research tool in the investigation of tumour angiogenesis. It may also have a clinical role in the differential diagnosis of benign and malignant ovarian tumours in

symptomatic patients with an adnexal mass (see section 1.3.3). Unfortunately it is unlikely to be of value in screening asymptomatic women for ovarian cancer as it requires the prior demonstration of ovarian enlargement by real time ultrasonography. In view of the prevailing uncertainty about the natural history of benign ovarian tumours it would not be reasonable to leave a tumour detected by ultrasound in situ even if it has no evidence of neovascularisation.

(e) Other imaging techniques: Computed Tomography and Nuclear Magnetic Resonance scans can be excluded from consideration for a screening programme on the grounds of cost. In addition they share many of the drawbacks of ultrasonography in distinguishing benign from malignant ovarian lesions. Immunoscintigraphy is time consuming, invasive, relatively expensive and at present largely experimental. However, recent advances in the specificity and labelling of monoclonal antibodies used for scanning have raised the possibility that antibody guided imaging may have a role in detecting the benign or malignant nature of a pelvic mass. This technique may therefore be useful as a diagnostic test in patients with a suspected malignancy on a primary screening test such as a tumour marker or ultrasound scan.

(f) A multimodal approach: As the consequences of a false positive result are serious it is logical to consider strategies for improving specificity with minimal sacrifice of sensitivity. This approach may involve the measurement of several tumour markers or the combination of various modalities such as tumour markers, ultrasound and clinical examination (chapters 8 and 9).

1.3.3 Preoperative diagnosis of Ovarian Cancer

1.3.3.1 Rationale

Whilst screening for early stage ovarian cancer remains the subject of research protocols, the best opportunity to influence the natural history of this disease will continue to be at the time of the initial laparotomy. The principles of surgical treatment of ovarian cancer have changed dramatically in recent years but it remains the cornerstone of treatment of established disease. The aims of primary surgery are twofold.

(a) **Accurate surgical staging:** The 5 year survival rates of approximately 70% reported for stage I ovarian cancer (Kottmeier 1982) are inconsistent with the definition of localised disease. Several studies have demonstrated that occult tumour deposits are frequently present on the peritoneum, pelvic or para-aortic lymph nodes, the omentum or the diaphragm (Young et al 1983, Knapp and Friedman 1974, Keetel et al 1974, Piver et al 1978). A consensus has emerged concerning adequate staging of ovarian malignancy (Young et al 1983, Schwartz 1981, Wharton and Herson 1981, Castaldo et al 1981, Averette and Sevin 1982, Piver 1983). Accurate staging results in improvement in survival figures for true stage I disease but does not alter overall survival. Its real significance lies in the identification of stage I and II patients with microscopic or occult residual tumour as it is in these cases that adjuvant therapy is most effective. In addition it eliminates the need to overtreat patients with apparent early stage disease.

(b) **Cytoreductive surgery:** There are a number of theoretical benefits of surgery to leave minimal residual tumour tissue. Several studies have investigated the practical value of this approach. The first (Griffiths et al 1979) demonstrated significantly improved survival in patients with residual tumour less than 15 mm in diameter. The survival curve for patients with large volume disease resected to less than 15 mm was identical to that of patients with disease less than 15 mm in diameter prior to surgery. Subsequent studies have confirmed the survival benefit of optimal cytoreductive surgery (Wharton and Herson 1981, Hacker et al 1983, Chen and Bochner 1985 and Van Lindert et al 1984). The improvement in survival does not seem to be related simply to less invasive and hence more easily resectable tumour. In the studies of Wharton and Herson (1981) and Hacker et al (1983) the survival of patients requiring only hysterectomy, bilateral oophorectomy and infracolic omental resection was no better than that of patients who required extensive debulking procedures. There is also evidence from several non-randomised studies of prolonged disease free survival and increased number of complete responders among patients who commence chemotherapy with small volume residual disease (Griffiths et al 1979, Young et al 1978, Greco et al 1981, Ehrlich et al 1979, Edwards et al 1983).

In spite of the knowledge of the benefits of accurate surgical staging and cytoreductive surgery many patients do not receive appropriate primary surgery. A thorough surgical procedure for ovarian cancer often requires persistent, time consuming and aggressive surgery not feasible during a routine operating list.

Ideally patients with ovarian malignancy should therefore be referred for primary laparotomy by a surgeon with appropriate operative experience. In practice, the diagnosis of ovarian cancer is not usually made preoperatively and consequently inadequate surgical exploration remains commonplace. There is no reliable method currently available for the differential diagnosis of benign pelvic masses and malignant ovarian tumours preoperatively. An accurate method for preoperative diagnosis of ovarian cancer would provide a basis for appropriate referral prior to initial laparotomy and the benefits of thorough surgical staging and cytoreduction.

1.3.3.2 Diagnostic techniques

(a) **Clinical:** The majority of patients with ovarian cancer complain of abdominal pain or swelling at presentation (Morrow 1981) and the tumour is detected by abdominal or pelvic examination. Clinical examination rarely provides a sound basis for the differential diagnosis of benign and malignant pelvic masses unless there is evidence of metastatic disease such as ascites or lymphadenopathy. As discussed in section 1.3.2.3 the incidence of ovarian cancer increases with age and is higher in postmenopausal than premenopausal women. These easily identifiable characteristics provide some indication of the risk of a pelvic mass being malignant in nature.

(b) **Ultrasound:** Real time ultrasonography is an accurate method for determination of the size, location and consistency of a

pelvic mass (Lawson and Albarelli 1977). The accuracy of ultrasound examination at predicting the benign or malignant nature of such a mass is less well established. Several retrospective studies suggest that ultrasound has an accuracy of 87%-91% in differential diagnosis of benign and malignant pelvic lesions (Meire et al 1978, Requard et al 1981, Moyle et al 1983). Patterns suggesting malignancy were irregular solid areas within a tumour mass, septae, ascites and matted bowel loops. In a prospective study involving 312 patients admitted for surgery for a pelvic mass, Herrmann et al (1987) achieved a sensitivity of 75.9%, a specificity of 94.5% and an overall accuracy of 91.0% for differential diagnosis of benign and malignant conditions. Ultrasound scanning in skilled hands would therefore appear to be an effective method for preoperative diagnosis of ovarian cancer. However, a major disadvantage of sonography is its dependence upon the expertise of the operator and the resolution of the scanning device used.

(c) Computed Tomography: Computed Tomography (CT) has several disadvantages when compared to ultrasonography for investigation of patients with suspected pelvic masses. It is relatively expensive, less widely available, exposes the patient to ionising radiation, requires bowel contrast and may require intravenous contrast injection. Although CT is as accurate as ultrasound at establishing the presence of a pelvic mass (Sanders et al 1983) the differential diagnosis of benign and malignant disease is dependent upon demonstration of the same features. Many of these features are demonstrated equally well with ultrasound and internal septations are seen more clearly (Araki et al 1982). The

main advantage of CT in this context is its lack of operator dependence. CT is not therefore a routine investigation for patients with a suspected pelvic mass. Its main role in the management of ovarian malignancy is in assessment of the extent and location of disease.

(d) Magnetic Resonance Imaging: Magnetic Resonance Imaging (MRI) is still undergoing evaluation. MRI is non-invasive and avoids ionising radiation but is relatively time consuming and like CT requires expensive equipment which is not generally available. Preliminary results suggest that MRI may be superior to CT or ultrasound in the identification of malignant change within an ovarian cyst (Powell et al 1987) but this has not been confirmed by a comparative study.

(e) Doppler Colour Flow Imaging: The preliminary report by Bourne et al (1989) discussed in section 1.3.2.4 (d) has provided encouraging evidence that Doppler colour flow imaging of ovarian vasculature may be able to distinguish between benign and malignant ovarian tumours. Prominent areas of vascularisation which were interpreted as representing neovascularisation were seen in association with invasive tumours but not normal ovaries or benign pathology. The applicability of this technique to clinical practice will be limited by the sophisticated equipment required but if the encouraging initial reports are confirmed in larger series it will have an important role in differential diagnosis.

(f) Tumour markers: As all patients with a suspected pelvic mass undergo venepuncture prior to surgery serum measurement of serum levels of tumour markers is an attractive approach to differential diagnosis. The many markers investigated in relation to ovarian cancer have been discussed above (section 1.3.2.4). No individual marker has an established role in differential diagnosis although initial results using serum CA 125 measurement have been encouraging (section 1.2.4.2). Bast et al (1987) have suggested that it may be possible to improve specificity with minimal loss of sensitivity by the use of other tumour markers (such as TAG 72 and CA 15-3) as secondary tests in patients with a raised serum CA 125. This suggestion was based upon the observation that either TAG 72 or CA 15-3 were elevated in 77% of ovarian cancer patients with an elevated CA 125, but only 5% of patients with false positive elevations of CA 125 (Bast et al 1987).

The accuracy of CA 125 in differential diagnosis has not been compared with other diagnostic criteria currently used in clinical practice. A high degree of accuracy may be achievable by combined analysis of clinical data and the results of several diagnostic investigations (chapter 7).

1.4 PURPOSE OF THIS RESEARCH

This research project was initiated in order to assess the hypothesis that "serum CA 125 measurement is of value in the diagnosis of ovarian cancer".

The specific aims of the work were threefold:

(a). To investigate the distribution of CA 125 activity in the female genital tract in physiological states, benign disorders and ovarian cancer.

(b). To determine the value of serum CA 125 measurement alone or in combination with other diagnostic tests in the differential diagnosis of benign and malignant adnexal masses.

(c). To determine the specificity of serum CA 125 measurement alone or in combination with other diagnostic tests in screening for ovarian cancer.

CHAPTER 2

MATERIALS AND METHODS

2.1 ASSAY PROCEDURES

2.1.1 CA 125

The CA 125 determinant is defined by the monoclonal antibody OC 125 (Bast et al 1981). Three commercial immunoassay kits are available for quantitative measurement of CA 125. All are based on the association of multiple CA 125 determinants with each antigen moiety. The antigen is bound simultaneously by OC 125 antibody on a solid phase and by free radio- or enzyme- labelled OC 125. CA 125 concentration is expressed in units per ml with respect to each manufacturer's reference antigenic preparation. A general reference standard is not currently available.

2.1.1.1. Abbott Laboratories/Centocor Radioimmunoassay

Polystyrene beads coated with OC 125 antibody are incubated simultaneously for 18-22 hours at room temperature with 100ul of specimen, standard or control and 100ul of tracer. The tracer consists of I^{125} labelled OC 125. Following incubation, unbound materials are removed by washing each bead three times with 5ml distilled water. Bound radioactivity is determined by counting the beads in a gamma counter. A point to point standard curve is plotted using the results for standards at 5-7, 30, 80, 200 and 500 U/ml and CA 125 concentration in unknowns and controls determined from this standard curve.

2.1.1.2. CIS Radioimmunoassay

The design, principle and procedure of this radioimmunoassay are similar to the Centocor/Abbott Laboratories radioimmunoassay. The solid phase consists of an ELSA fin (trademark of CIS). Recommended incubation time is 16-24 hours and standards are 4-7, 30, 80, 200 and 500 U/ml CA 125.

2.1.1.3 Abbott Laboratories Enzymeimmunoassay

Polystyrene beads coated with OC 125 antibody are incubated simultaneously for 4 hours +/- 5 minutes at 37°C with 100ul of specimen, standard or control and 100ul of tracer. The tracer consists of OC 125 conjugated with horseradish peroxidase. Following incubation unbound material is removed by washing each bead twice with 4-6 ml of distilled water. The beads are then incubated for 30 +/- 1 minute at 15-30°C with o-Phenylenediamine substrate solution which contains hydrogen peroxide. The enzyme reaction is stopped by addition of 1ml of 1N sulphuric acid and the intensity of colour development is read using a spectrophotometer set at 492 nm. A point to point standard curve is plotted using the results for standards at 0, 65, 325 and 650 U/ml and CA 125 concentration in unknowns and controls determined from this standard curve.

When possible, assay kits of the same batch were used in pairs with a single standard curve. Standards were assayed in triplicate and unknown samples in duplicate. Manufacturer's quality control samples were provided with each assay kit and

were included in each assay run. In addition, two "in house" quality controls were prepared by diluting pooled serum from ovarian cancer patients (chapter 3). Aliquots (700ul) of the quality control were stored at -20°C and 3 duplicates included in each CA 125 assay performed.

2.1.2. Tumour Associated Glycoprotein 72 (TAG 72)

A commercially available radioimmunoassay (Centocor TAG 72-4 RIA, Malvern, PA, USA) was used to measure serum levels of tumour associated glycoprotein 72 (TAG 72). This is a solid phase radioimmunoassay in which polystyrene beads coated with cc49 (a mouse monoclonal antibody to TAG 72) are incubated for 4 hours at 37°C with 100ul of specimen, standard or control and 100ul of buffer. At the end of the incubation each bead is washed 3 times with 5ml of distilled water. The beads are then incubated for 18 +/- 2 hours at 2-8°C with ¹²⁵I labelled B72.3 (a mouse monoclonal antibody to TAG 72) and washed again. Bound radioactivity is determined by counting the beads in a gamma counter. A point to point standard curve is plotted using the results for standards at 3, 20, 50 and 100 U/ml (arbitrary units defined by Centocor reference antigen preparation), and TAG 72 concentration in unknowns and controls determined from this standard curve.

2.1.3 CA 15-3

A commercially available radioimmunoassay (Centocor CA 15-3 RIA, Malvern, PA, USA) was used to measure serum levels of carcinoma associated antigen 15-3 (CA 15-3). This is a solid phase

radioimmunoassay utilising 2 monoclonal antibodies 115D8 and DF3 raised against human milk fat globule membrane and a membrane enriched fraction of human breast carcinoma respectively. Polystyrene beads coated with the monoclonal antibody are incubated for 2 hours +/- 5 minutes at room temperature with 200ul of specimen or control diluted 1:51 in zero standard or with 200ul of standard. At the end of the incubation each bead is washed 3 times with 5ml of distilled water. The beads are then incubated for 3 hours +/- 10 minutes at room temperature with ^{125}I labelled monoclonal antibody and washed again. Bound radioactivity is determined by counting the beads in a gamma counter. A point to point standard curve is plotted using the results for standards at 0, 25, 50, 100 and 200 U/ml (arbitrary units defined by Centocor reference antigen preparation), and CA 15-3 concentration in unknowns and controls determined from this standard curve.

2.1.4 Luteinising Hormone Assay

A commercially available radioimmunoassay (Amerlex-M LH RIA, Amersham, UK) was used to measure serum LH. The standards were calibrated against the first International Reference Preparation (1st IRP 68/40). The sensitivity of the assay was 0.75 mIU/ml, the range 1.8 - 150 mIU/ml and the within and between assay coefficients of variation < 3.7% and less < 8.1% respectively.

2.1.5 Progesterone Assay

Serum progesterone estimations were performed by Serono Laboratories (UK) Ltd using a coated tube radioimmunoassay with a sensitivity of 0.1 nmol/L, a range of 0.3 - 250 nmol/L and within and between assay coefficients of variation of < 12%.

2.2 SAMPLE COLLECTION AND STORAGE

The characteristics of the patients and volunteers providing samples for the studies performed for this thesis are described with the results in chapters 3-9.

Cyst fluid, amniotic fluid, and ascitic fluid samples were centrifuged at 3000 rpm for 10 minutes and the supernatant separated, aliquoted and stored at -20°C within 2 hours of collection. Blood samples were allowed to clot at room temperature and then centrifuged at 3000 rpm for 10 minutes. The serum was separated, aliquoted and stored at -20°C within 2 hours of collection.

Tissue samples were washed in 0.5M saline and a representative sample sent for histological examination. Samples were then snap frozen in liquid nitrogen and stored at -20°C. Stored tissue samples were homogenised in phosphate buffered saline pH 7.4 containing 0.05M azide, triton X-100 1% and 175 ug/ml phenyl-methyl-sulphonyl fluoride (50 mg tissue/ml), centrifuged at 10,000 g for 15 minutes at 4°C and the supernatant separated, aliquoted and stored at -20°C.

2.3 ULTRASOUND SCANS

Real time ultrasonography was performed by the abdominal route, using the full bladder technique, by means of a 'Diasonics DS.1' sector scanner with a 3.5 MHz transducer.

2.4 STATISTICAL METHODS

The specific statistical methods employed for data analysis are described within each chapter. Analysis of serum CA 125 results was generally performed using non parametric tests. For analyses assuming a normal distribution and for which a non parametric equivalent is not available, log transformed values for serum CA 125 were used.

CHAPTER 3

CA 125 MEASUREMENT

Measurement of CA 125 activity is by double determinant non competitive assay using the OC 125 monoclonal antibody on a solid phase and as a free labelled tracer (section 2.1.1). CA 125 measurements for studies described in chapters 4-9 was performed using the Abbott Laboratories CA 125 radioimmunoassay. This chapter describes the performance characteristics of the assay and its comparability with other commercial assays for CA 125.

3.1 COMPARISON OF THREE CA 125 ASSAY SYSTEMS

3.1.1 Samples and methods

3.1.1.1 Samples

Peripheral venous blood samples were obtained from a total of 56 individuals: Thirty patients with epithelial ovarian cancer, 9 patients with benign ovarian cysts, 8 women in the 1st trimester of pregnancy and 9 apparently healthy postmenopausal women. These samples were treated as described in section 2.2. In addition samples of peripheral blood from 10 patients with stage III or IV ovarian cancer were treated as described in section 2.2 and the stored serum pooled for use in this study.

3.1.1.2 Assay procedures

The Abbott Laboratories radioimmunoassay (ARIA), CIS radioimmunoassay (CRIA) and Abbott Laboratories enzymeimmunoassay (EIA) were performed as described on section 2.1.1.

3.1.1.3 Methods

(a) Correlation between the assay systems: The samples described above (section 3.1.1.1) were assayed 1x in duplicate in each assay.

(b) Sensitivity, detection limit and intra-assay coefficient of variation: Doubling dilutions of pooled ovarian cancer serum from 1 in 2 to 1 in 512 were assayed 10x in duplicate in each assay.

3.1.1.4 Statistical analysis

The coefficient of linear correlation for pairs of assays was calculated using the Oxstat scientific and statistical data analysis system, version 4.0. The sensitivity of each assay was defined as the highest serial dilution of the pooled ovarian cancer serum which could be distinguished from the next doubling dilution (no overlap of mean \pm 2 standard deviations). The detection limit of each assay was defined as the mean of the zero standard + 2 standard deviations. The intra-assay coefficient of variation was calculated from the results of 10 duplicates of pooled ovarian cancer serum.

3.1.2 Results

3.1.2.1 Correlation between the 3 assay systems

Figures 3.1, 3.2 and 3.3 illustrate the correlation between CA 125 results obtained with the 3 assays in the 56 serum samples assayed 1x in duplicate. The correlation coefficient of the ARIA and CRIA was 0.943 ($p < 0.0001$) and the absolute values obtained with the 2 radioimmunoassays were comparable (regression slope

1.11), (figure 3.2). The correlation coefficients between the radioimmunoassays and the AEIA were also high (AEIA v ARIA, $r = 0.932$, $p < 0.0001$; AEIA v CRIA, $r = 0.918$, $p < 0.0001$). However absolute values obtained using the EIA were lower than those obtained using either of the radioimmunoassays, (regression slopes AEIA v ARIA = 1.53, AEIA v CRIA = 1.76).

3.1.2.2 Sensitivity and Detection Limit

The results obtained from doubling dilutions of pooled ovarian cancer serum for each of the 3 assays are shown in table 3.1 and figure 3.4. The most sensitive assay was the ARIA. Using this assay a 1 in 64 dilution of pooled ovarian cancer serum (mean 25.8 U/ml, SD 2.1 U/ml) could be distinguished from a 1 in 128 dilution (mean 16.1 U/ml, SD 2.0 U/ml). The CRIA and EIA were sufficiently sensitive to distinguish between a 1 in 32 dilution of pooled ovarian cancer serum and a 1 in 64 dilution (CRIA mean 29.5 U/ml, SD 5.9 U/ml v mean 10.7 U/ml, SD 2.9 U/ml; EIA mean 29.0 U/ml, SD 5.1 U/ml v mean 11.9 U/ml, SD 1.1 U/ml). The detection limits of the assays were as follows: EIA 8.7 U/ml, ARIA 8.5 U/ml and CRIA 10.7 U/ml.

3.1.2.3 Intra-assay coefficient of variation

Table 3.1 lists the intra-assay coefficient of variation for each of the 3 assays at dilutions of pooled ovarian cancer serum between 1 in 2 and 1 in 512. The highest dilution of pooled ovarian cancer serum for which the coefficient of variation was less than 10% was 1 in 16 for the EIA (64.5 U/ml), 1 in 32 for the CRIA (29.5 U/ml) and 1 in 64 for the ARIA (25.8 U/ml).

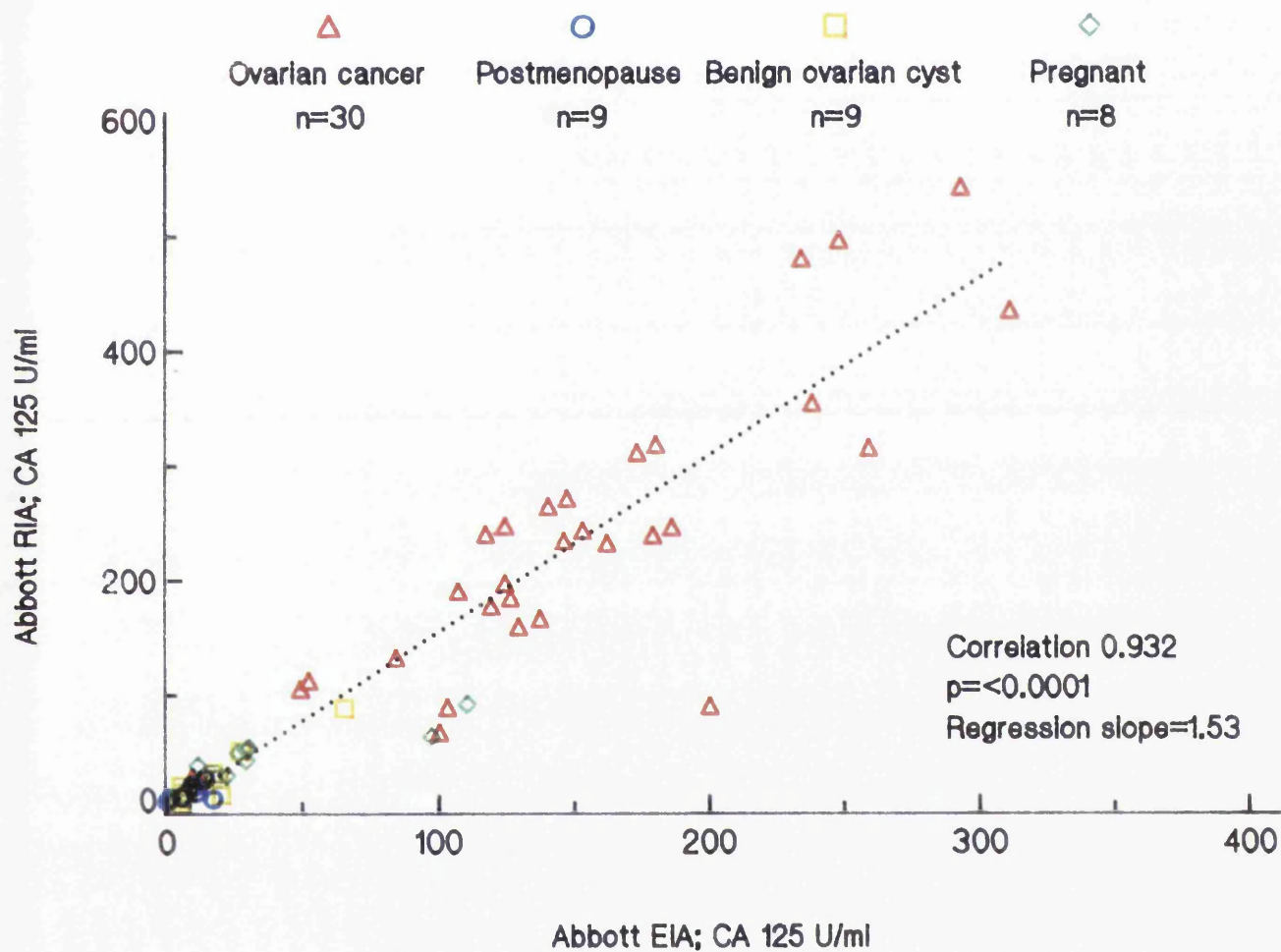


Figure 3.1. Correlation between Abbott Laboratories CA 125 Radioimmunoassay and Abbott Laboratories CA 125 Enzymeimmunoassay in serum samples from ovarian cancer patients (n=30), healthy postmenopausal women (n=9), patients with benign ovarian cysts (n=9) and women in the 1st trimester of pregnancy (n=8).

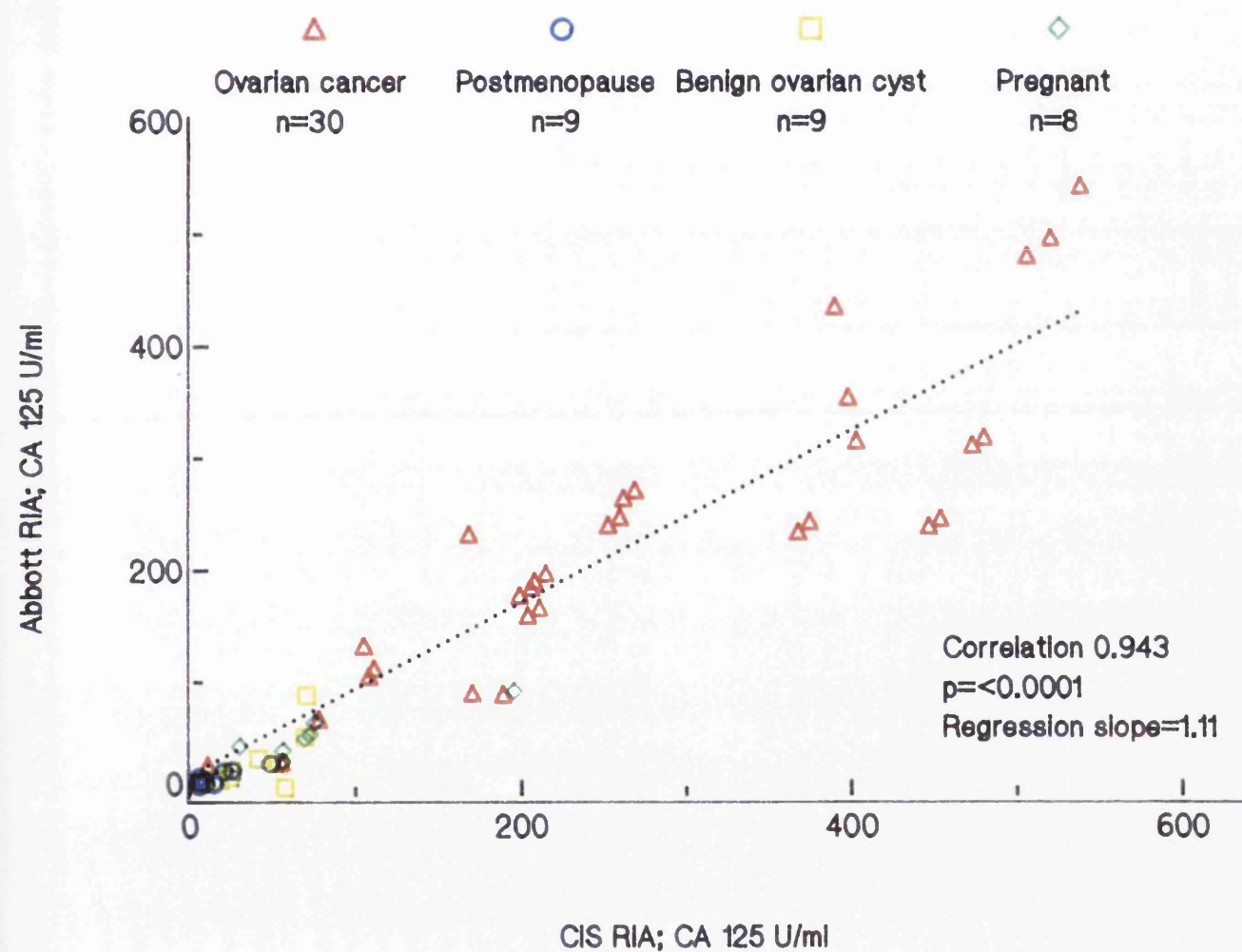


Figure 3.2. Correlation between Abbott Laboratories CA 125 Radioimmunoassay and CIS CA 125 Radioimmunoassay in serum samples from ovarian cancer patients (n=30), healthy postmenopausal women (n=9), patients with benign ovarian cysts (n=9) and women in the 1st trimester of pregnancy (n=8).

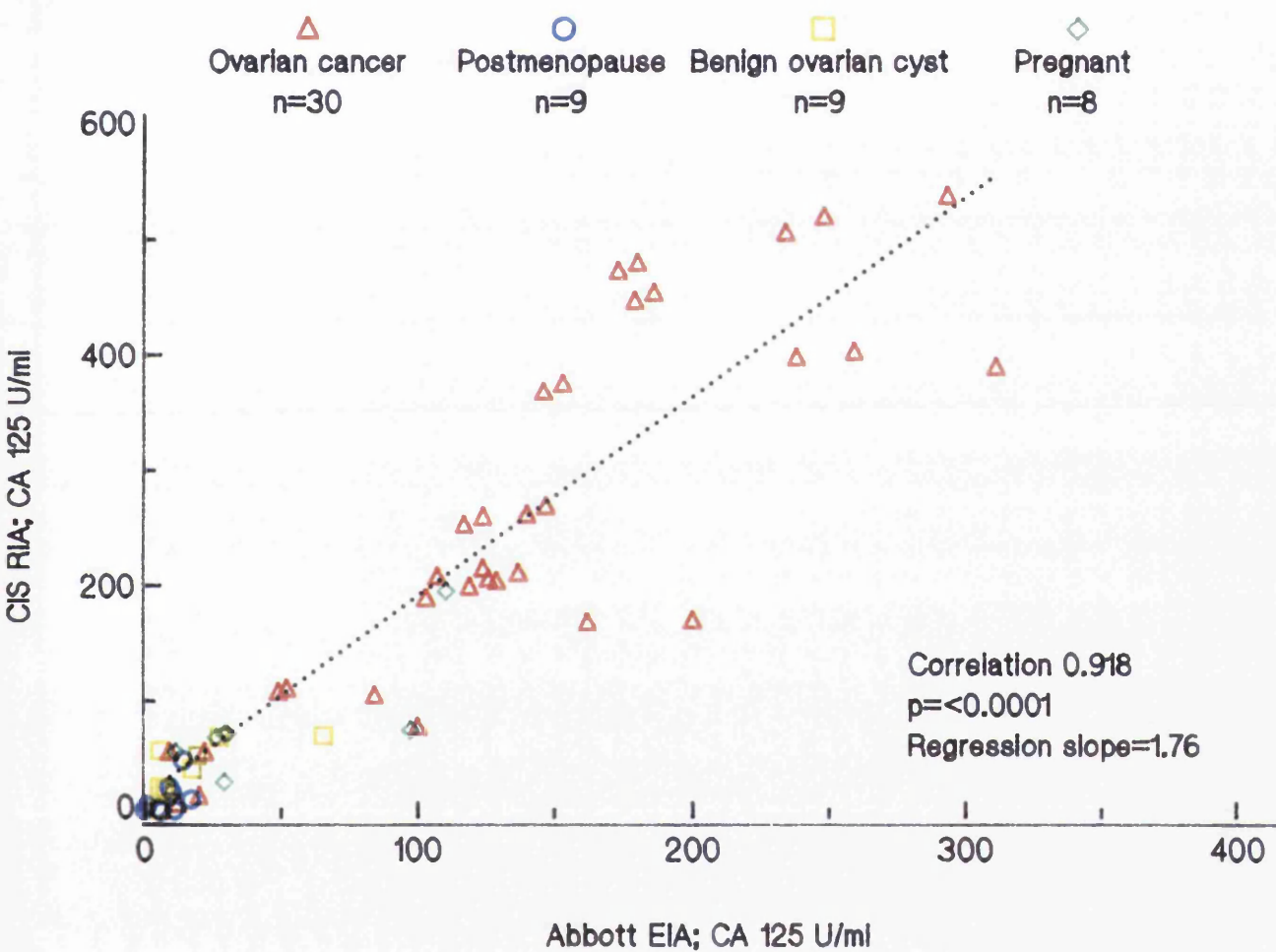


Figure 3.3. Correlation between CIS CA 125 Radioimmunoassay and Abbott Laboratories CA 125 Enzymeimmunoassay in serum samples from ovarian cancer patients (n=30), healthy postmenopausal women (n=9), patients with benign ovarian cysts (n=9) and women in the 1st trimester of pregnancy (n=8).

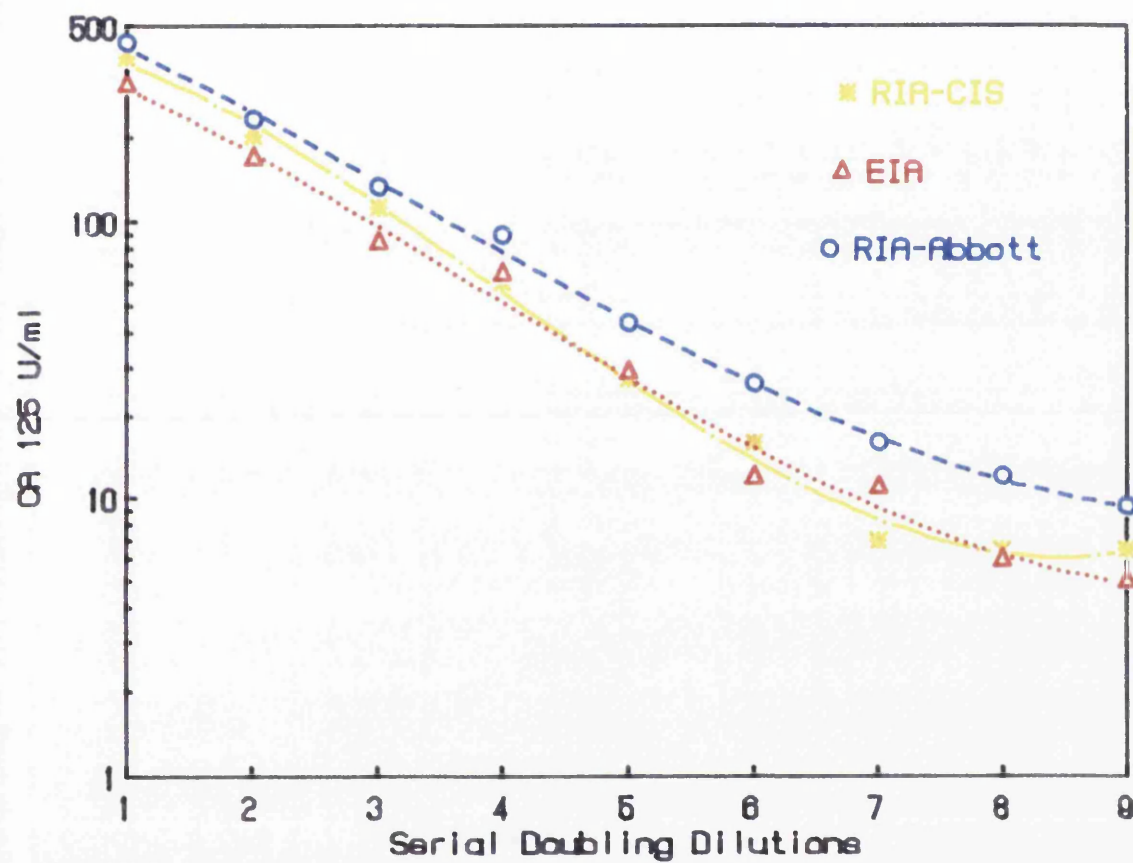


Figure 3.4. Dilution curves derived from doubling dilutions of pooled ovarian cancer serum for the Abbott Laboratories CA 125 Radioimmunoassay, the Abbott Laboratories CA 125 Enzymeimmunoassay and the CIS CA 125 Radioimmunoassay.

		Dilution							
		1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256
Abbott RIA Intraassay	Mean (U/ml)	435	233	132	89	43	26	16	12
	SD (U/ml)	24.6	13.5	7.1	6.3	2.6	2.1	2.0	4.5
	CV (%)	5.7	5.8	5.4	7.1	6.0	8.1	12.4	38.0
CIS RIA Intraassay	Mean (U/ml)	337	169	105	70	30	11	<6.5	<6.5
	SD (U/ml)	24.8	18.0	9.8	8.9	5.9	2.9	--	--
	CV (%)	7.4	10.7	9.3	12.7	20.1	27.1	--	--
Abbott EIA Intraassay	Mean (U/ml)	312	170	84	65	29	12	11	6
	SD (U/ml)	16.4	10.4	4.4	6.8	5.1	1.1	2.7	2.7
	CV (%)	5.3	6.1	5.2	10.6	17.5	9.6	24.9	42.5

Table 3.1. Mean values, standard deviation and coefficient of variation of doubling dilutions of ovarian cancer serum assayed 10x in duplicate in 1 run of the ARIA, AEIA and CRIA.

3.2 FURTHER ASSESSMENT OF THE ABBOTT LABORATORIES CA 125 RADIOIMMUNOASSAY

3.2.1 Samples and methods

3.2.1.1 Samples

(a) Ovarian cancer; Samples of peripheral blood and ascites fluid were obtained from 10 patients with stage III or IV ovarian cancer. (b) Pregnancy; 1st trimester decidua was obtained from 10 women undergoing elective termination of pregnancy. 2nd trimester amniotic fluid was obtained from 7 women at the time of diagnostic fetoscopy. (c) Non pregnant; Endometrium was obtained from 8 women undergoing dilatation and curettage for benign conditions. Tissue samples were homogenised and blood and amniotic fluid samples treated as described in section 2.2. Samples from each source were pooled and stored at -20°C .

3.2.1.2 Assay procedure

The Abbott Laboratories Radioimmunoassay was used as described in section 2.1.1.1.

3.2.1.3 Method

The within and between assay coefficients of variation were determined using serial doubling dilutions of pooled ovarian cancer serum. Each dilution was assayed 10x in duplicate in one assay and 1x in duplicate in 10 consecutive assays. Serial doubling dilutions of pooled samples described in section 3.2.1.1 were assayed in duplicate to assess parallelism.

3.2.2 Results

The within and between assay coefficients of variation for the Abbott Laboratories radioimmunoassay at various points on the standard curve are shown in table 3.2. The highest doubling dilution with an intra-assay coefficient of variation below 10% was 1 in 64 (mean CA 125 25.8 U/ml). The highest doubling dilution with an inter-assay coefficient of variation below 10% was also 1 in 64 (mean CA 125 26.7 U/ml). Pooled samples of ovarian cancer serum, ovarian cancer ascitic fluid, 1st trimester decidua homogenate, 2nd trimester amniotic fluid and endometrial homogenate had parallel dilution curves. Dilutions of ovarian cancer serum with 30 U/ml and 100 U/ml CA 125 activity were prepared on the basis of these results for use as quality controls. Aliquots were stored at minus 20°C and run 3x in duplicate in each CA 125 assay performed during this project.

		Dilution							
		1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256
Abbott RIA Interassay	Mean (U/ml)	442	234	136	87	44	27	15	10
	SD (U/ml)	38.5	14.3	11.2	6.5	4.1	2.5	2.3	5.1
	CV (%)	8.7	6.1	8.2	7.5	9.3	9.3	15.3	49.5
Abbott RIA Intraassay	Mean (U/ml)	435	233	132	89	43	26	16	12
	SD (U/ml)	24.6	13.5	7.1	6.3	2.6	2.1	2.0	4.5
	CV (%)	5.7	5.8	5.4	7.1	6.0	8.1	12.4	38.0

Table 3.2. Mean values, standard deviation and coefficient of variation of doubling dilutions of ovarian cancer serum assayed 10x in duplicate in 1 run of the ARIA (intraassay variation) and 1x in duplicate in 10 consecutive runs of the ARIA (interassay variation).

3.3 DISCUSSION

The results of this study indicate that when the purpose of CA 125 measurement is to monitor serum CA 125 levels over a period of time in ovarian cancer patients, all three assay systems will produce a similiar pattern. The correlation between the assays is good and at levels of CA 125 associated with ovarian cancer the reproducibility satisfactory. There are however differences in absolute CA 125 values between the three assays. For this reason a single assay system should be used for serial monitoring of CA 125 levels in any one individual. In addition there are differences in sensitivity and intra-assay coefficient of variation between the 3 assays at low levels of CA 125 activity. In conditions associated with slight elevation of serum CA 125 levels (eg endometriosis, pregnancy, menstruation) a higher assay sensitivity and better reproducibility in the lower range are required. A radioimmunoassay rather than the enzymeimmunoassay should therefore be selected for these conditions.

The results of other investigators are in accord with the findings described in this chapter. Pittaway (1988) observed a good correlation between the CA 125 radioimmunoassay purchased from Centocor (which is identical to the ARIA described here) and the Abbott Laboratories enzymeimmunoassay. The enzymeimmunoassay was less sensitive and he concluded that the radioimmunoassay should be used for measuring relatively low concentrations of CA 125 in conditions such as endometriosis. Subsequently Pittaway (1989) has proposed a modification of the standard curve incorporating additional standards at 2.8, 11.1, 20.6 and 45.6

U/ml to improve reproducibility at CA 125 levels in the lower range. A recent report (Fisken et al 1989) described similar correlation coefficients between the 3 assay systems to those described here (ARIA v CRIA 0.93; ARIA v AEIA 0.85; CRIA v AEIA 0.86). The radioimmunoassay systems were superior to the enzymeimmunoassay in terms of reproducibility and sensitivity. Fisken et al (1989) also noted that although the correlation between the assay systems was good the slope of the correlation was not 1.0 and confirmed the observation that there is discrepancy between absolute values in U/ml between the 3 assays.

For the purposes of the studies described in this thesis it was necessary to achieve acceptable reproducibility in the lower range of the assay. The ARIA achieved higher sensitivity than the CRIA or EIA and was therefore an appropriate choice. Parallel dilution curves were obtained using the ARIA with ovarian cancer serum, non-pregnant endometrium, 1st trimester decidua and amniotic fluid suggesting immunochemical identity of CA 125 activity from these sources.

For financial reasons it was not possible to determine the inter-assay coefficient of variation for the CRIA or EIA in this study. A multicentre study to assess long term variations in assay performance and inter-laboratory variations would be valuable. It may then be possible to establish an inter-laboratory quality control arrangement.

CHAPTER 4

THE DISTRIBUTION OF CA 125 ACTIVITY
IN THE FEMALE REPRODUCTIVE TRACT

4.1 INTRODUCTION

Previous serological and immunohistochemical studies have documented elevation of serum CA 125 and tissue expression of CA 125 in a number of benign disorders and physiological conditions as well as in malignancy. Levels of CA 125 activity in tissue and fluids in these conditions have not however been quantified. The work described in this chapter was undertaken in order to investigate the relationship between levels of CA 125 activity in serum and various tissue and fluid compartments.

The studies described in section 1.2.7.3 have established that serum CA 125 levels are elevated in endometriosis and are related to the severity of the disease. There is evidence that a number of other benign pelvic disorders are associated with an elevated serum CA 125 level in a small proportion of cases (table 1.9). By virtue of their prevalence, site and space occupying nature the benign disorders most likely to be a source of confusion in the diagnosis of ovarian cancer are fibroids and benign ovarian cysts or solid tumours. Sections 4.3.3 - 4.3.5 describe CA 125 levels in serum samples obtained from patients with these conditions. Section 4.3.6 describes serum CA 125 levels in serum samples obtained from a series of patients with epithelial ovarian cancer.

4.2. SAMPLES AND METHODS

4.2.1 Samples

- (a) Proliferative (n=7) and secretory (n=5) endometrium were obtained from premenopausal women undergoing dilatation and curettage or hysterectomy for benign conditions, who had not taken synthetic sex hormones in the 4 weeks prior to operation. Venous blood was obtained from the same women prior to operation. Myometrium was obtained from the four patients undergoing a hysterectomy.
- (b) Matched samples of decidua (n=10), trophoblast (n=9), fetal membranes (n=9) and maternal venous blood were obtained from 11 women undergoing elective termination of pregnancy at 8-13 weeks gestation (according to menstrual data).
- (c) Maternal and fetal blood samples were obtained at the time of 2nd trimester diagnostic fetoscopy from 7 retrospectively defined normal pregnancies (18-21 weeks gestation).
- (d) Matched samples of decidua (n=8), trophoblast (n=9), amniotic fluid (n=8), cord blood (n=8) and maternal venous blood (n=8) were obtained at term from 10 women who underwent a normal vaginal delivery or an elective caesarean section at term following an uneventful pregnancy.
- (e) Peripheral venous blood samples were obtained from patients (n=37) with a clinical diagnosis of uterine fibroids confirmed by ultrasound or laparoscopy and no evidence of other pathology.
- (f) Peripheral venous blood and/or cyst fluid were obtained from women (n=12) with functional ovarian cysts at the time of

laparotomy or laparoscopy.

- (g) Peripheral venous blood, cyst fluid and/or tumour tissue were obtained at the time of laparotomy from women (n=81) with benign ovarian tumours. Where possible a tissue sample was obtained from the apparently normal contralateral ovary.
- (h) Peripheral venous blood was obtained preoperatively from women (n=65) undergoing surgery at the London Hospital and subsequently found to have epithelial ovarian cancer. A further 15 serum samples obtained preoperatively from patients subsequently found to have FIGO stage I or II epithelial ovarian cancer were provided by Mr Derek Cruickshank (Department of Obstetrics and Gynaecology, Aberdeen). Peripheral venous blood, cyst fluid and/or tumour tissue were obtained at laparotomy from women (n=15) undergoing surgery at the London Hospital. Where possible a tissue sample was obtained from the apparently normal contralateral ovary.

4.2.2 Methods

Decidua, trophoblast and membranes were obtained by dissection under naked eye observation as described by Rutanen et al (1986a and 1986b) and representative samples sent for histological examination to confirm tissue type. All tissue samples were homogenised and stored as described in section 2.2. Cyst fluid, amniotic fluid and blood samples were centrifuged and stored as described in section 2.2. CA 125 activity was determined by radioimmunoassay as described in section 2.1.1.1.

4.2.3 Surgical staging

All patients included in section 4.2.1 (h) of this study underwent a thorough surgical staging procedure including peritoneal fluid cytology, hysterectomy, bilateral salpingo-oophorectomy and omentectomy. In all cases the large and small bowel, stomach, liver, spleen, diaphragm paracolic gutters and peritoneal surfaces were carefully inspected and suspicious areas biopsied or resected. In patients with apparent stage I or II disease para-aortic node sampling was performed.

4.2.4 Histological / surgical diagnosis

The diagnosis of a benign or malignant ovarian neoplasm was in all cases confirmed by reference to the histological report. The diagnosis of functional ovarian cysts was usually made at surgery (laparoscopy or laparotomy) at which no specimen was obtained and could not therefore be confirmed histologically. Three women were pregnant (1st trimester) at the time of diagnosis of a benign cyst.

4.3 RESULTS

4.3.1 Non pregnant patients

Serum CA 125 activity in all samples from apparently healthy non-pregnant women were less than 35 U/ml (median 19.3 U/ml, range 7.2-27.0 U/ml), (figure 4.1). CA 125 levels in homogenates of non pregnant endometrium ranged from 100.9 U/100 mg to 3,341 U/100 mg

(median 388.0 U/100mg) and were higher than in myometrium (median 32.1 U/100mg, range 14.2-49.9 U/100mg), ($p < 0.01$). There was no significant difference apparent between CA 125 activity measured in homogenates of proliferative and secretory endometrium.

4.3.2 Pregnancy

4.3.2.1 1st trimester of pregnancy

Serum CA 125 levels exceeded 35 U/ml in 8 of 11 women (median 53.6 U/ml, range 15.6-268.3 U/ml), (figure 4.2). Tissue levels of CA 125 in 1st trimester decidua (median 4547 U/100mg, range 340.4-20851 U/100mg) were greater than in fetal membranes (median 122.6 U/100mg, range 15.0-611.5 U/100mg), ($p < 0.01$) and trophoblast (median 60.5 U/100mg, range 14.6-720 U/100mg), ($p < 0.01$).

4.3.2.2 2nd trimester of pregnancy

Maternal serum levels in the 2nd trimester pregnancies did not exceed 35 U/ml (median 18.5 U/ml, range 12.0-25.1 U/ml), (figure 4.3). Fetal serum CA 125 levels were consistently less than 10 U/ml. By contrast, CA 125 levels in 2nd trimester amniotic fluid samples were two orders of magnitude higher (median 4825 U/ml, range 3200-9,300 U/ml).

4.3.2.3 3rd trimester of pregnancy

Maternal serum CA 125 levels were less than 35 U/ml in 7 of 8 samples (median 19.2 U/ml, range 16.8-43.8 U/ml), (figure 4.4). Fetal serum levels were all less than 20 U/ml and less than maternal serum levels ($p < 0.01$). CA 125 activity in decidua (median 116.5 U/100mg, range 32.7-449.9 U/100mg) was less than in

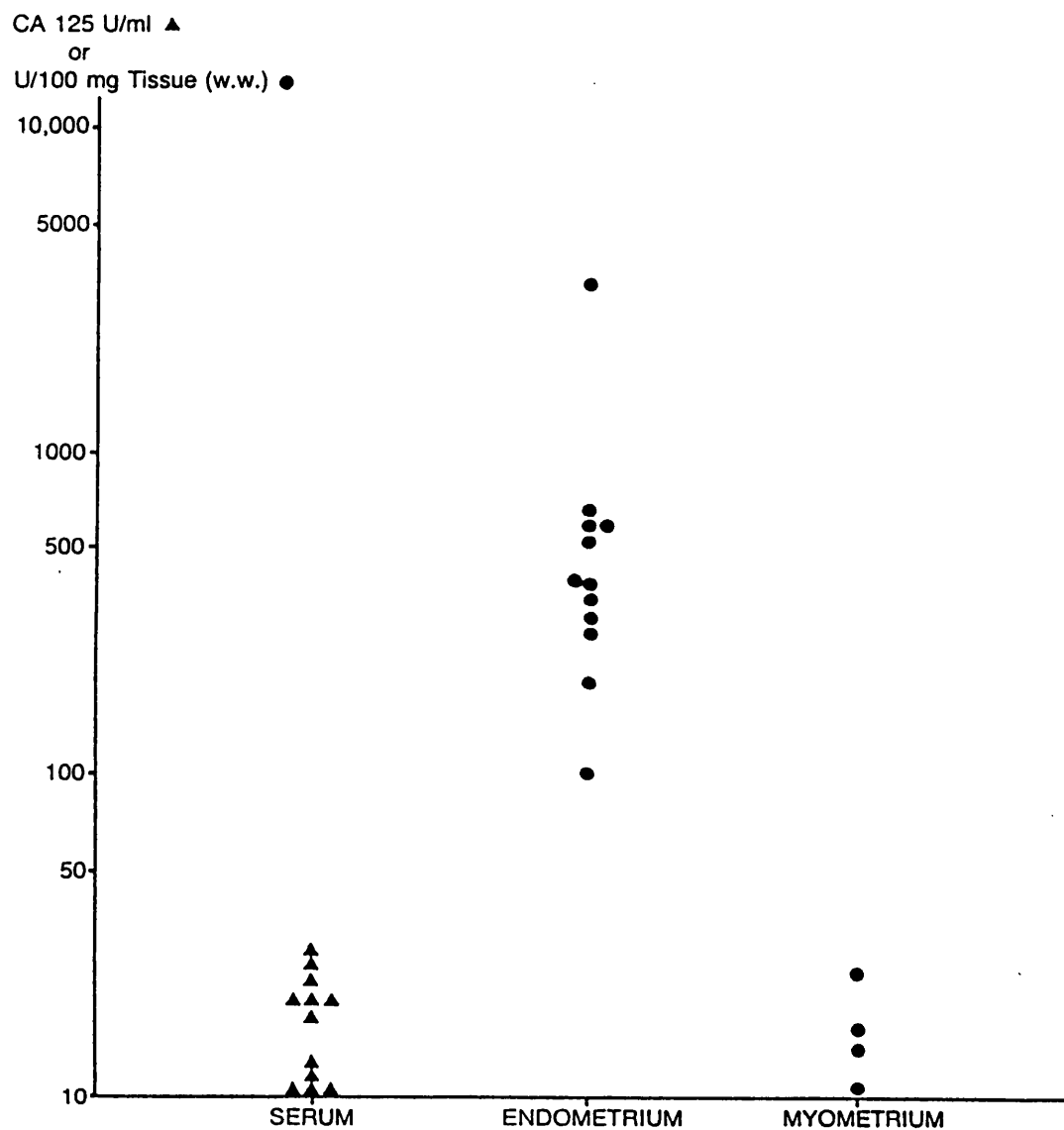


Figure 4.1. CA 125 activity in matched serum (n=12), endometrium (n=12) and myometrium (n=4) from 12 premenopausal women.

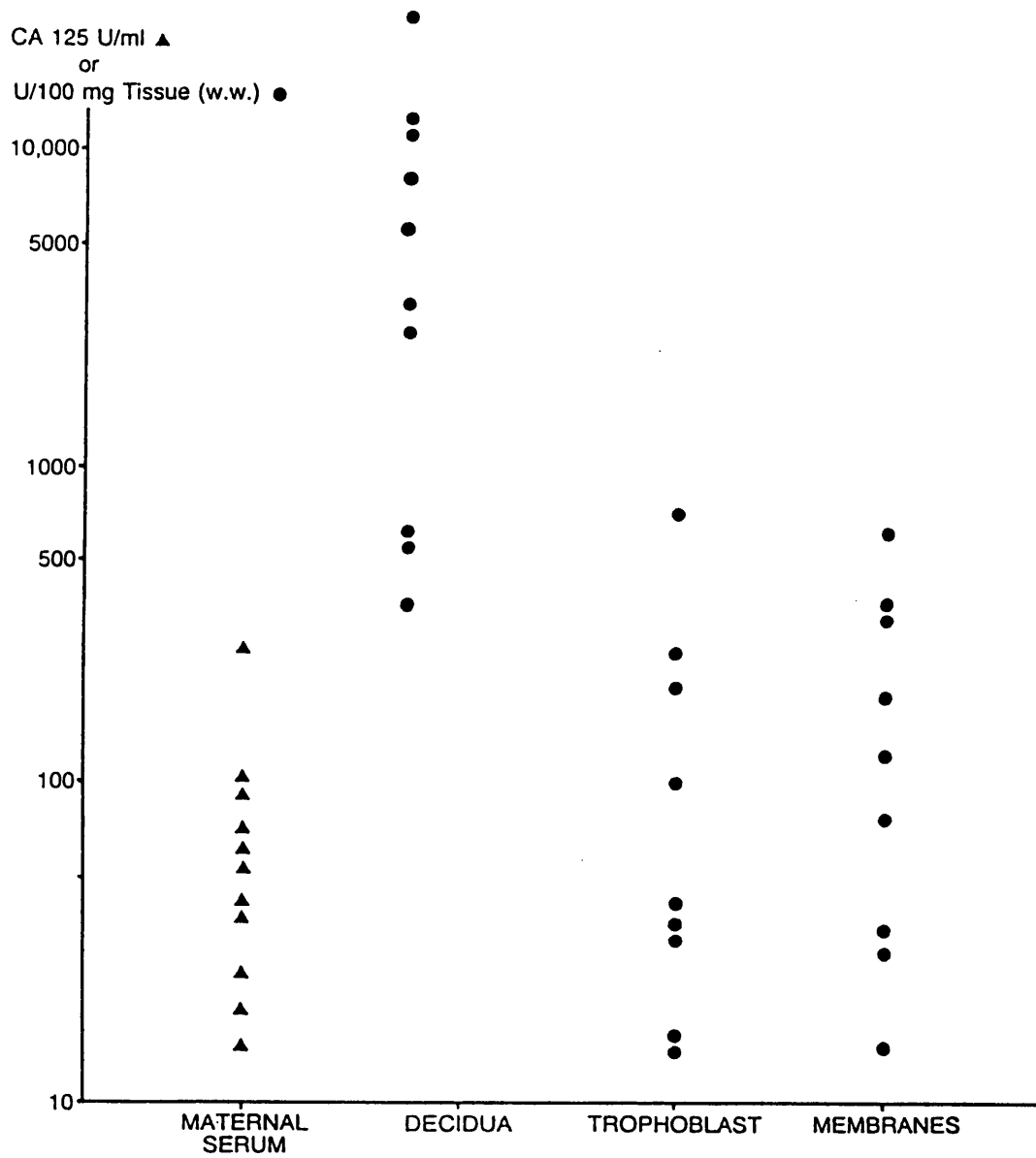


Figure 4.2. CA 125 activity in matched maternal serum and fetal and maternal tissue extracts from 11 first trimester pregnancies.

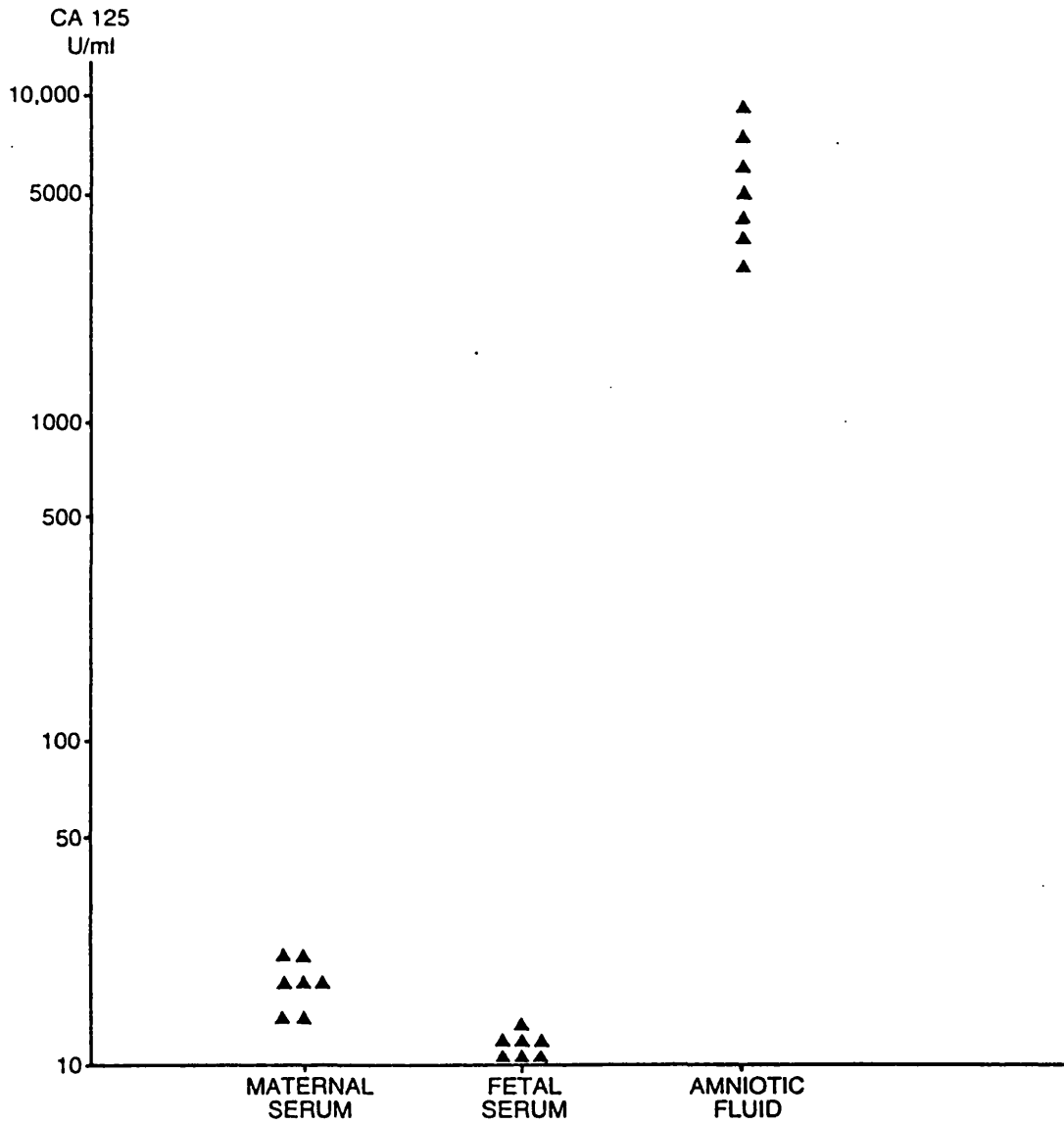


Figure 4.3. CA 125 activity in matched maternal and fetal serum and amniotic fluid from seven second trimester pregnancies.

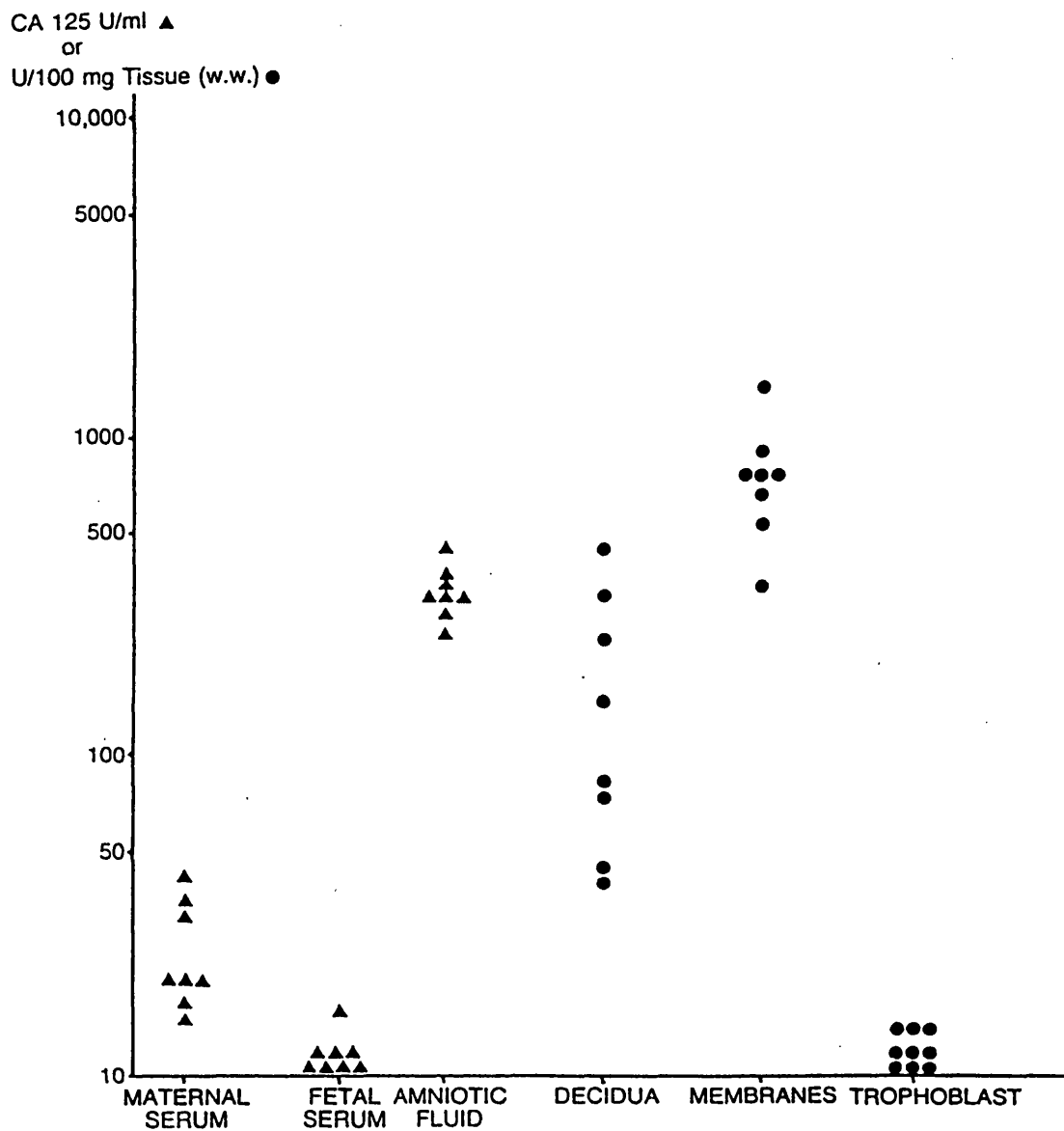


Figure 4.4. CA 125 activity in matched maternal and fetal serum and tissue extracts from ten full term pregnancies.

fetal membranes (median 757.4 U/100mg, range 399-1452 U/100mg), ($p < 0.01$), whilst all trophoblastic specimens obtained at term contained < 10 U/100 mg CA 125 activity. CA 125 activity in amniotic fluid was 10 fold greater than in maternal serum (median 315.5 U/ml, range 313-463 U/ml).

The highest tissue levels of CA 125 activity were detected in 1st trimester decidual homogenate and were greater than in non pregnant endometrium ($p < 0.01$) and term decidua ($p < 0.01$). Serum CA 125 levels were higher in specimens obtained during the 1st trimester of pregnancy than in the 2nd trimester ($p < 0.01$), 3rd trimester ($p < 0.05$) or from non-pregnant women ($p < 0.05$). CA 125 activity in amniotic fluid was higher in the second trimester than at term ($p < 0.002$).

4.3.3 Uterine Myomata

Serum CA 125 levels were > 35 U/ml in 2/18 premenopausal women with uterine fibroids and 0/19 postmenopausal women with uterine fibroids (figure 4.5).

4.3.4 Functional ovarian cysts

All 9 patients with follicular ovarian cysts and all 3 with corpus luteum cysts had serum CA 125 levels less than 35 U/ml (median 15.2 U/ml, range < 7 -27.6 U/ml). Cyst fluid levels of CA 125 in 8 samples from follicular cysts were < 7 U/ml in all but 1 case with a level of 21,970 U/ml (figure 4.5).

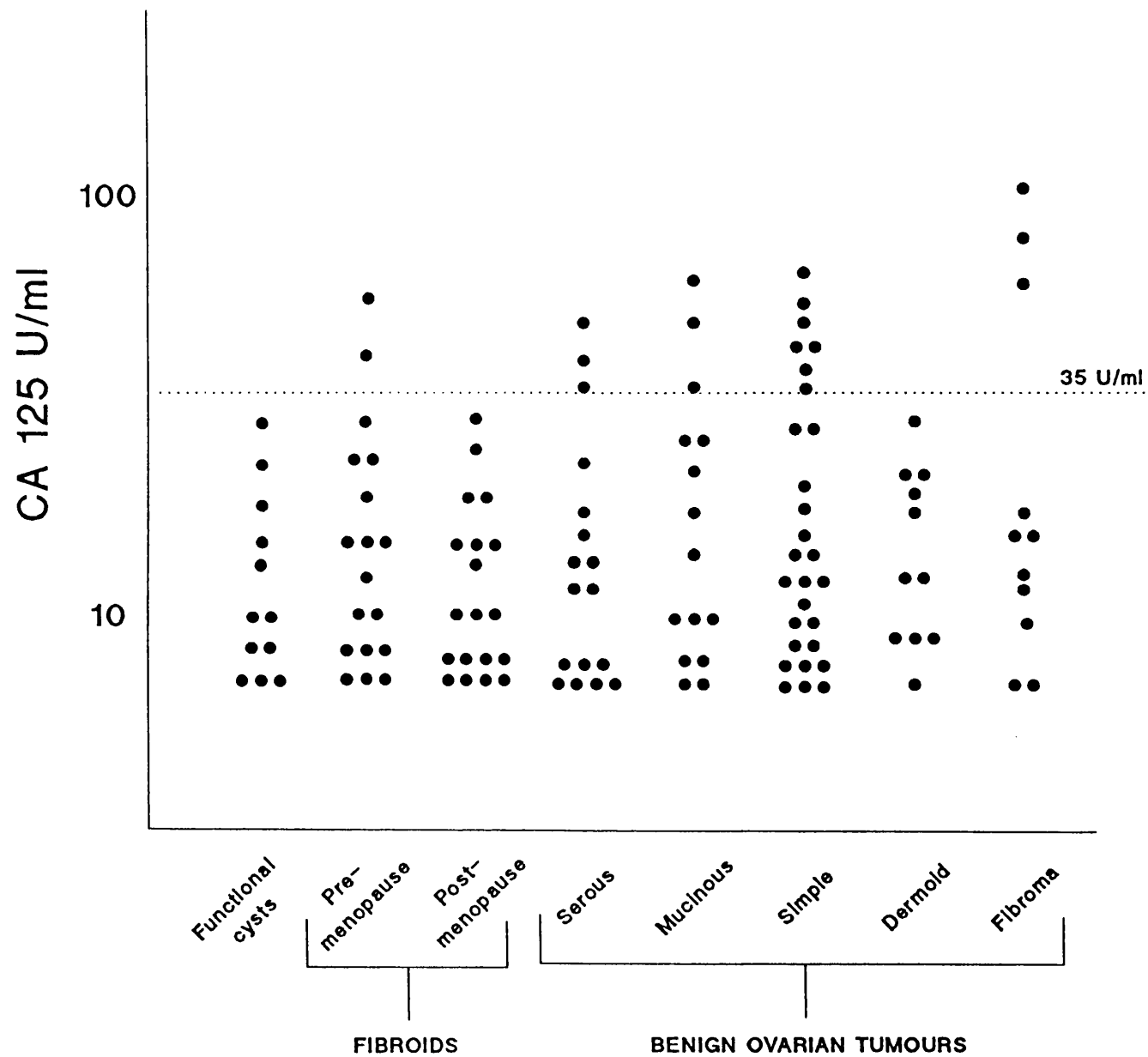


Figure 4.5. Serum CA 125 levels in samples from patients with uterine fibroids, functional ovarian cysts and benign ovarian tumours.

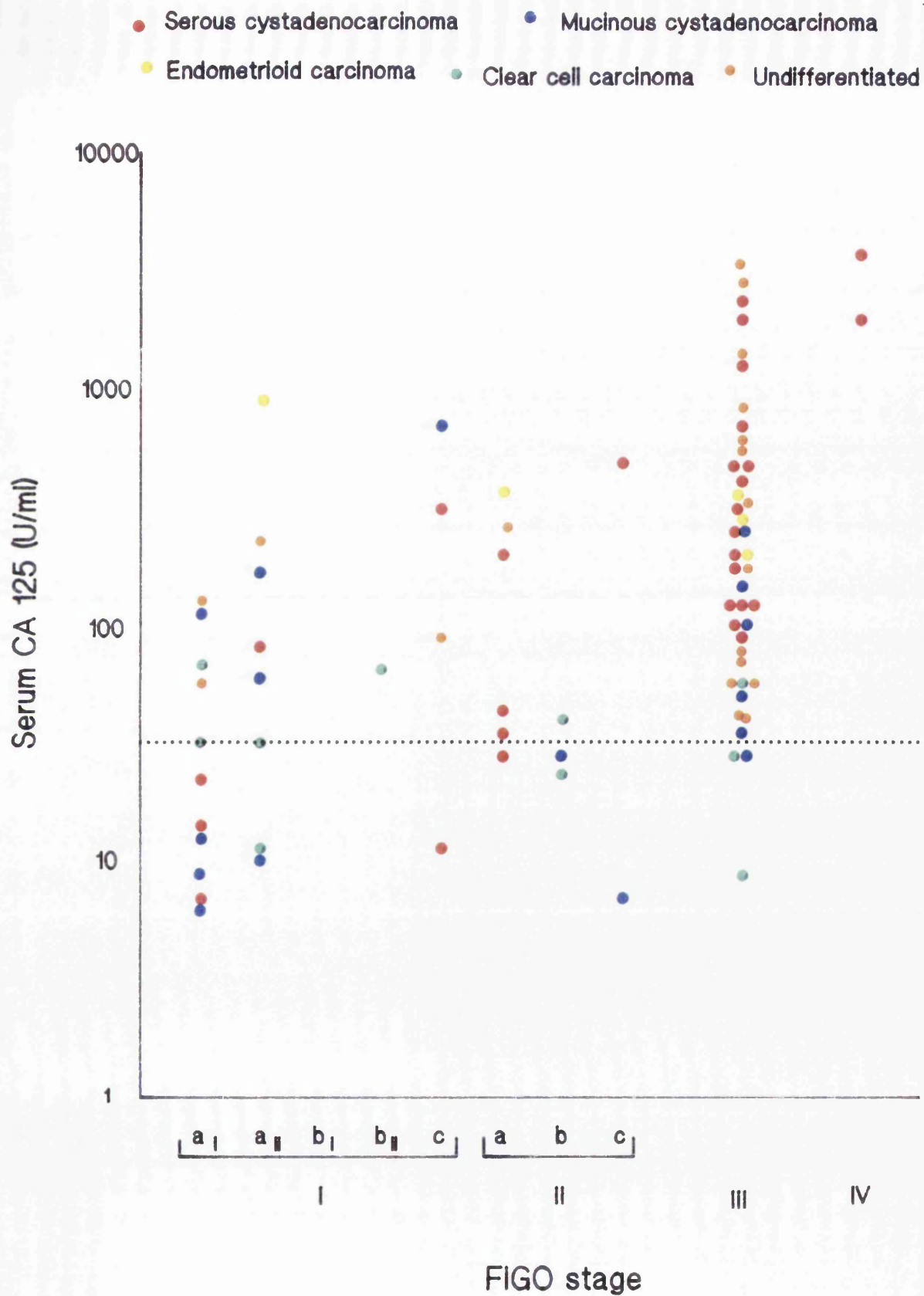


Figure 4.6. Serum CA 125 levels in samples obtained from patients with epithelial ovarian cancer by FIGO stage of disease and histological type.

4.3.5 Benign ovarian tumours

Serum samples were obtained from 81 women with benign ovarian tumours. Matched samples of cyst fluid were obtained in 23 cases with cystic tumours. Matched tissue samples were obtained in 16 cases. Overall serum CA 125 levels were greater than 35 U/ml in 16/82 serum samples from patients with benign tumours. Three of these tumours were detected during the 1st trimester of pregnancy. Analysis by histological type revealed elevation of CA 125 > 35 U/ml in 3/17 serous cystadenomas, 3/15 mucinous cystadenomas, 0/11 benign cystic teratomas, 3/11 fibromas (1 pregnant) and 7/28 benign simple cysts (2 pregnant), (figure 4.5). Serum CA 125 levels ranged from < 7 - 113.4 U/ml (median 14.5 U/ml) and were significantly less than cyst fluid levels (range < 7 - 151,900 U/ml, median 5,970 U/ml). Tissue levels ranged from 8.1 - 20,680 U/100mg (median 41.2 U/100mg).

4.3.6 Ovarian cancer

Figure 4.6 illustrates serum CA 125 levels in ovarian cancer patients according to FIGO stage and histological type. Serum CA 125 levels exceeded 30 U/ml in 62/79 cases (78.5%). Twenty four patients were found to have FIGO stage I disease of which 14 had serum CA 125 levels greater than 30 U/ml. Elevations in stage Iai (median 24 U/ml, range 7-125 U/ml) and Iaii disease (median 73 U/ml, range 10-950 U/ml) were modest. Serum CA 125 levels exceeded 100 U/ml in 2/11 patients with stage Iai disease and 3/8 with stage Iaii disease. Of the 4 patients with stage Ic disease 3 had serum CA 125 levels greater than 30 U/ml and 2 greater than

100 U/ml. Eleven patients had FIGO stage II disease of which 7 had serum CA 125 levels greater than 30 U/ml and 4 greater than 100 U/ml (median 42 U/ml, range 7-513 U/ml). FIGO stage III disease was found in 42 patients and was associated with a serum CA 125 level greater than 30 U/ml in 39/42 cases. The median value in stage III disease was 202 U/ml (range 8 - 3,200 U/ml). Elevated serum CA 125 levels were observed in patients with all histological types of epithelial ovarian cancer.

Tissue samples were obtained from 10 cases of ovarian cancer and cyst fluid from 12 cases. Tissue levels ranged from 1,466-138,000 U/100mg (median 12,790 U/100mg) and cyst fluid levels from 80.3 - > 2,589,000 U/ml (median 7,930 U/ml).

Figures 4.7 and 4.8 show serum levels of CA 125 for benign and malignant tumours in relation to cyst fluid and tissue CA 125 activity respectively. The high tissue and cyst fluid levels in malignant tumours were generally associated with elevated serum CA 125 levels. By contrast serum levels associated with the presence of benign tumours were low, despite high cyst fluid and tissue CA 125 activity in a number of cases. CA 125 activity was significantly greater in tissue from ovarian cancers than in benign ovarian tumours. No significant difference was detected between cyst fluid CA 125 activity in the benign and malignant tumours.

4.3.7 Contralateral ovary

Tissue samples of the contralateral histologically normal ovary were obtained from 2 patients with benign tumours and 2 patients with ovarian cancer. Patients with tissue levels of 11.3 U/100mg in a benign fibroma and 13.2 U/100mg in a benign mucinous cystadenoma had CA 125 activity of 872 U/100mg and < 7 U/100mg respectively in the contralateral ovary. Patients with tissue levels of 11,310 U/100mg in an undifferentiated adenocarcinoma and 8,050 U/100mg in a serous papillary borderline malignancy had CA 125 activity of 55.7 U/100mg and 87.3 U/100mg respectively in the contralateral ovary.

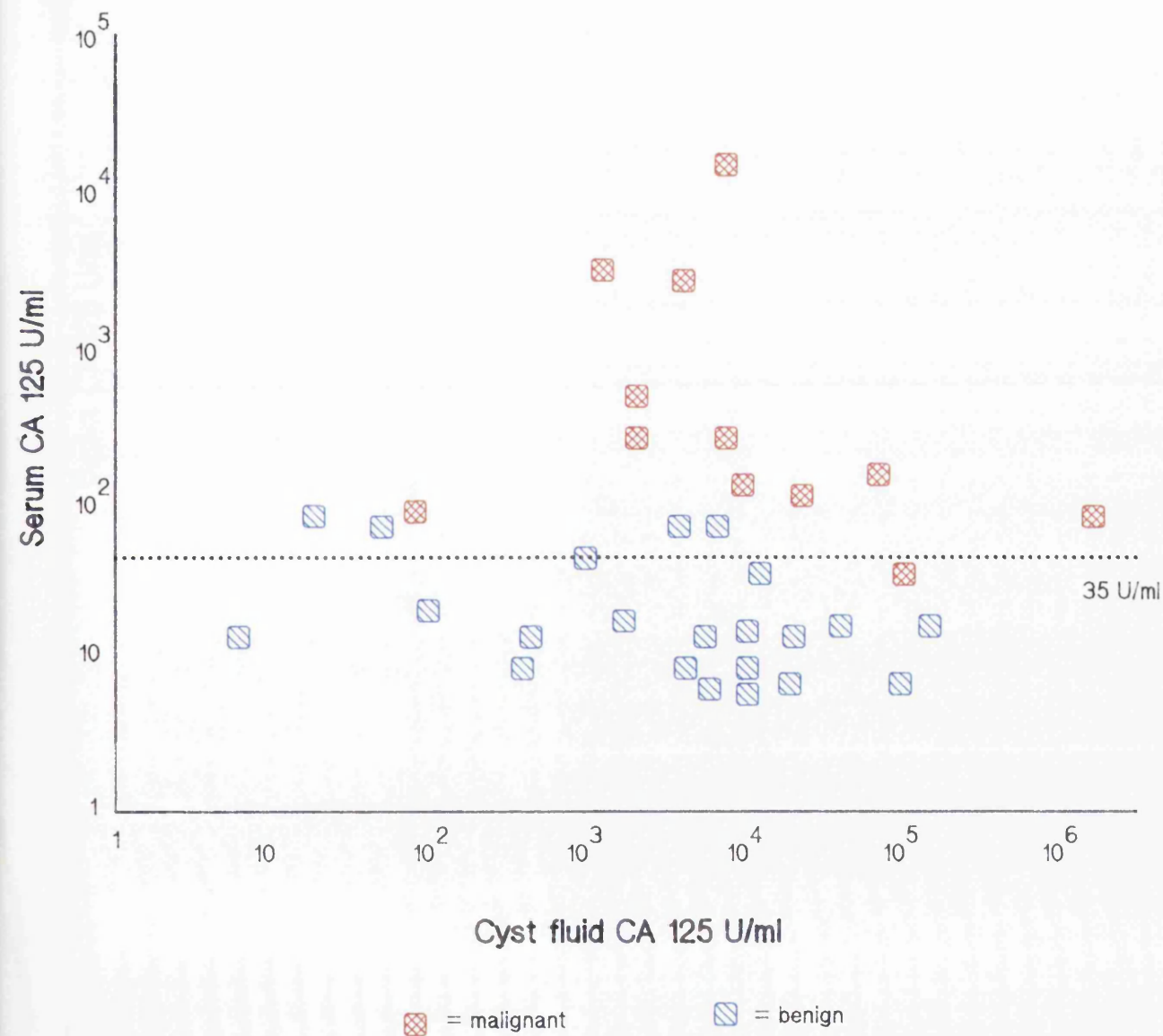


Figure 4.7. CA 125 activity in serum and cyst fluid from patients with benign and malignant ovarian tumours.

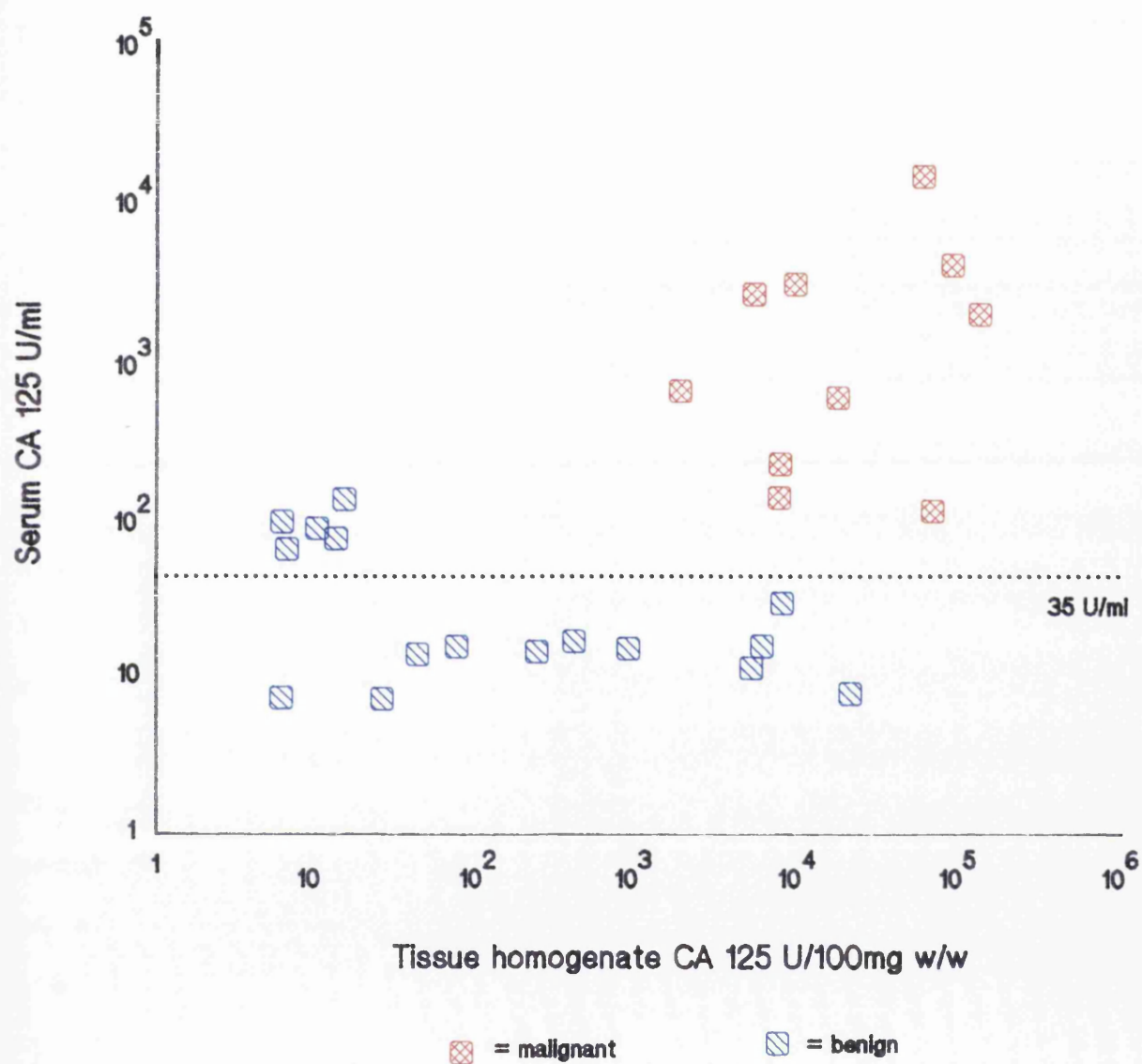


Figure 4.8. CA 125 activity in serum and ovarian tissue homogenate from patients with benign and malignant ovarian tumours.

4.4 DISCUSSION

Previous studies have described a significant increase in maternal serum CA 125 levels during the first trimester of pregnancy compared with the non pregnant state or later in pregnancy and high levels of CA 125 in amniotic fluid (Niloff et al 1984b, Halila et al 1986, Seki et al 1986, O'Brien et al 1986). The results described in section 4.3.1 and 4.3.2 are consistent with these previous observations, but in addition demonstrate high levels of CA 125 activity in normal endometrium and first trimester decidua. Recent reports using both immunohistochemical and immunoradiometric techniques have confirmed that high levels of CA 125 activity are detectable in normal endometrium, decidua and amniotic fluid (Barbati et al 1989, Kobayashi et al 1989a, 1989b, Crombach et al 1989, Scharl et al 1989).

The origin of CA 125 activity in pregnancy has not yet been established. Although a fetal origin has been suggested (Halila et al 1986, Seki et al 1986), O'Brien et al (1986) detected only low levels of the antigen in cord blood and neonatal urine specimens. Our findings of low levels of CA 125 in fetal blood in both the 2nd trimester and at term (figures 4.5 and 4.6), are in accord with the reports of Barbati et al (1989) and Kobayashi et al (1989a), and do not support a fetal origin for CA 125. The high levels of CA 125 activity seen in 1st trimester decidua in association with increased maternal serum levels raise the possibility that the source of CA 125 activity in pregnancy is the decidualised endometrium. This suggestion is supported by

Bischof et al (1986) who have reported a reduction in CA 125 content of tissue and media in cycloheximide treated in vitro cultures of 1st trimester decidua compared to controls. Further circumstantial evidence of a decidual origin for CA 125 is the striking similarity between the compartmental distribution of CA 125 and that of placental protein 14 (synonyms: progestagen dependent endometrial protein, alpha uterine protein, pregnancy associated endometrial alpha 2 globulin), a protein of endometrial/decidual origin (Bell 1986). The highest serum and decidual tissue levels of both CA 125 and PP14 have been observed in the 1st trimester whilst the highest amniotic fluid levels were seen in the 2nd trimester (Fay et al 1988).

Barbati et al (1989) and Kobayashi et al (1989a) have suggested that amnion epithelial cells may be the source of the high levels of CA 125 activity in amniotic fluid. This hypothesis was based upon immunohistochemical evidence of CA 125 in amnion and the correlation between CA 125 levels in cytosolic extracts of amnion and amniotic fluid levels at various gestational ages. However, as the levels of CA 125 activity are extremely high in decidua it is equally possible that the decidua secretes CA 125 into the amniotic cavity through the chorion and amnion. All authors are agreed that amniotic fluid levels of CA 125 activity decrease with increasing gestational age. Whether this finding reflects decreased production, metabolic breakdown or a dilutional factor is unclear.

The high levels of CA 125 activity detected in endometrium here are also consistent with the hypothesis that CA 125 is a product

of the normal endometrium/decidua. Further evidence for the endometrial origin of CA 125 is the observation of high CA 125 activity in endometrial and cervical mucus (de Bruijn et al 1986, Crombach et al 1989). The high endometrial levels of CA 125 in women with low serum CA 125 activity suggest that CA 125 is not normally secreted into the peripheral circulation and that there may be a barrier to CA 125 between the normal endometrium and the circulation. The reason for elevation of serum CA 125 levels at the time of menstruation in a small group of women is unclear, although tubal reflux with absorption of CA 125 via the peritoneum has been suggested (Mastropaolo et al 1986).

The serum CA 125 levels documented in patients with fibroids, benign ovarian cysts and ovarian cancer were consistent with data reviewed in chapter 1 (tables 1.2 and 1.9). Elevated levels were observed in a small but significant proportion of patients with benign ovarian tumours. The degree of elevation was modest and only exceeded 100 U/ml in a single case. Such cases do however represent a diagnostic dilemma; they are also likely to be reported as abnormal by imaging techniques such as ultrasound scanning and cannot therefore be readily distinguished from the cases of malignant ovarian disease associated with serum CA 125 levels of 30 U/ml to 100 U/ml.

The literature summarised in table 1.2 was in the main reported as the proportion of cases in each stage with serum CA 125 levels greater than the cut-off of 35 U/ml used by Bast et al (1983). The results for the 79 ovarian cancer patients in section 4.2.2.6 were consistent with the literature. Of the small series of cases

with stage I disease 54% (13/24) had a serum CA 125 level greater than 35 U/ml confirming the limited sensitivity of serum CA 125 measurement for the earliest FIGO stage. Consideration of the actual levels of CA 125 as represented in figure 4.8, rather than the proportion greater than an arbitrary cut-off reveals a trend of increasing levels with FIGO stage. Serum CA 125 levels in stage III disease were 1-2 orders of magnitude greater than in stage I disease.

The high tissue and cyst fluid levels of CA 125 in both benign and malignant ovarian disease provide an interesting contrast to the serum levels documented in these conditions. If ovarian tumours are the primary source of serum CA 125 activity in ovarian cancer the difference in serum levels between benign and malignant disease must be related to differences in the nature of these conditions. A plausible explanation is that the intact basement membrane of benign ovarian tumours provides an effective barrier between tumour producing cells and the circulation (Fleuren et al 1987). The high molecular weight of CA 125 would prevent diffusion through the basement membrane into the circulation. The slight elevation of serum CA 125 in a small proportion of cases of benign tumours could be explained either by limited diffusion down a concentration gradient or by damage to the basement membrane by neoplastic but non invasive growth. The high serum CA 125 levels in malignant disease could be a result of invasive destruction of the basement membrane releasing relatively large amounts of CA 125 activity into the circulation. This model would also explain the association of low serum CA 125 levels with high tissue levels in normal endometrium and of

moderately elevated serum levels with high tissue levels in early pregnancy decidua. During the normal menstrual cycle the basement membrane of the endometrium provides a barrier to the high molecular weight moiety expressing the CA 125 epitope. During early pregnancy there is an increase in tissue CA 125 activity but in addition disruption of the basement membrane by the processes of implantation and placentation with consequent release of antigen into the circulation. A parallel can also be drawn between the CA 125 activity in the cyst fluid of benign tumours and in amniotic fluid. Antigen shed by tumour cells or decidual cells is able to diffuse into cyst fluid or amniotic fluid without reaching the circulation and high levels of CA 125 are found in both.

CHAPTER 5

SERUM CA 125 LEVELS DURING OVULATORY
AND ORAL CONTRACEPTIVE MENSTRUAL CYCLES
AND IN POSTMENOPAUSAL WOMEN

5.1 INTRODUCTION

Previous studies have revealed elevated serum CA 125 levels in premenopausal compared to postmenopausal women (Haga et al 1986a, Zurawski et al 1987). The observation of an increase in serum CA 125 levels during menstruation in infertile women with and without endometriosis (Mastropaolo et al 1986, Pittaway and Favez 1987) has suggested that the higher serum levels of CA 125 observed in premenopausal women may be related to menstruation. However, CA 125 levels during the menstrual cycle of women with a normal gynaecological history have not been described and the relationship of serum CA 125 to other events of the cycle is unclear. This chapter describes studies on cyclical variation of serum CA 125 in healthy premenopausal and postmenopausal women.

5.2 MATERIALS AND METHODS

5.2.1 Subjects

(a) Twelve apparently healthy premenopausal women of median age 27 years (range 22 to 43 years), with a history of regular menstruation (cycle length 25 - 36 days) and not using an intrauterine contraceptive device or hormonal contraception were recruited for daily or alternate daily venepuncture during a single menstrual cycle (195 samples). Ovulation was confirmed retrospectively in 10 cycles by the detection of a serum LH peak

> 40 mIU/l in association with a rise in luteal phase progesterone to greater than 22 nmol/L or (in the absence of mid cycle LH measurements), a luteal phase progesterone > 40 nmol/L for 4 consecutive days.

(b) Seven apparently healthy premenopausal women of median age 25 years (range 20 to 31 years) using the combined oral contraceptive pill (Ethinyloestradiol 30ug, levonorgestrel 150ug or 250ug) for greater than 6 months, but not taking any other medication and with no history of a gynaecological disorder were recruited for daily or alternate daily venepuncture during a single menstrual cycle (129 samples).

(c) Eight apparently healthy postmenopausal women of median age 56 years (range 52 to 63 years) and median duration of amenorrhoea of 6 years (range 2-14), not receiving hormone replacement therapy, were recruited for daily or alternate daily venepuncture for a single calendar month (138 samples). Women taking medication of any kind and/or with a history of oophorectomy or hysterectomy were excluded.

5.2.2 Radioimmunoassays

Peripheral venous blood samples were collected and stored as described in section 2.3 and the CA 125, LH and progesterone radioimmunoassays performed as described in sections 2.1.1.1, 2.1.4 and 2.1.5 respectively.

5.2.3 Statistical analysis

All cycles in premenopausal women were related to the first day of menstruation (day 1). As serum CA 125 is approximately log normally distributed (Jacobs et al 1988a) data analysis was performed using log-transformed values for serum CA 125. The 95% confidence limits (CI) for mean values of log serum CA 125 were calculated. Within each group analysis of variance was used to identify the factors related to serum CA 125 levels. Conservative tests were adopted when necessary to take into account the repeated measures aspect of the design. In estimating the effect of factors possibly significant in the analysis of variance, average CA 125 levels in individuals in relation to variations of the particular factor (eg menstruation or its absence) were compared using a matched 't' test to avoid problems associated with repeated measures. In comparing the groups of individuals unmatched 't' tests using averaged values (within person) were used for the same reason.

5.3 RESULTS

5.3.1 Ovulatory cycles

The serum CA 125 levels in 10 ovulatory subjects are shown in figure 5.1. The highest serum CA 125 levels in individual volunteers ranged from 16.6 U/ml to 37.7 U/ml (median 25.5 U/ml) and occurred during menstruation in 9/10 cycles and during the luteal phase in the remaining cycle. Analysis of variance of log transformed serum CA 125 values by individual, day or week of cycle and occurrence of menstruation revealed significant differences between individuals ($p < 0.0001$) and at the time of menstruation ($p < 0.0001$). No significant additional variation was accounted for by day or week of the cycle. A matched t-test revealed that the mean difference between individual mean levels during menstruation (20.9 U/ml, CI 16.4-26.7 U/ml) and non menstrual portions of the cycle (12.7 U/ml, CI 10.7-15.2) was 63% (CI 36-95% increase), ($p < 0.01$), figure 5.2.

5.3.2 Hormonal contraception cycles

The highest serum CA 125 levels in individual volunteers ranged from 7.0 U/ml to 38.9 U/ml (median 14.2 U/ml). Analysis of variance by individual, day or week of the cycle and occurrence of menstruation revealed significant differences in mean serum CA 125 levels between individuals ($p < 0.0001$), but not in relation to the day or week of the cycle or menstruation. There was no significant difference between individual mean levels during menstruation (9.8 U/ml, CI 7.7 - 12.5 U/ml) and non menstrual portions of the cycle (10.4 U/ml, CI 8.2 - 13.4 U/ml), (figure 5.2).

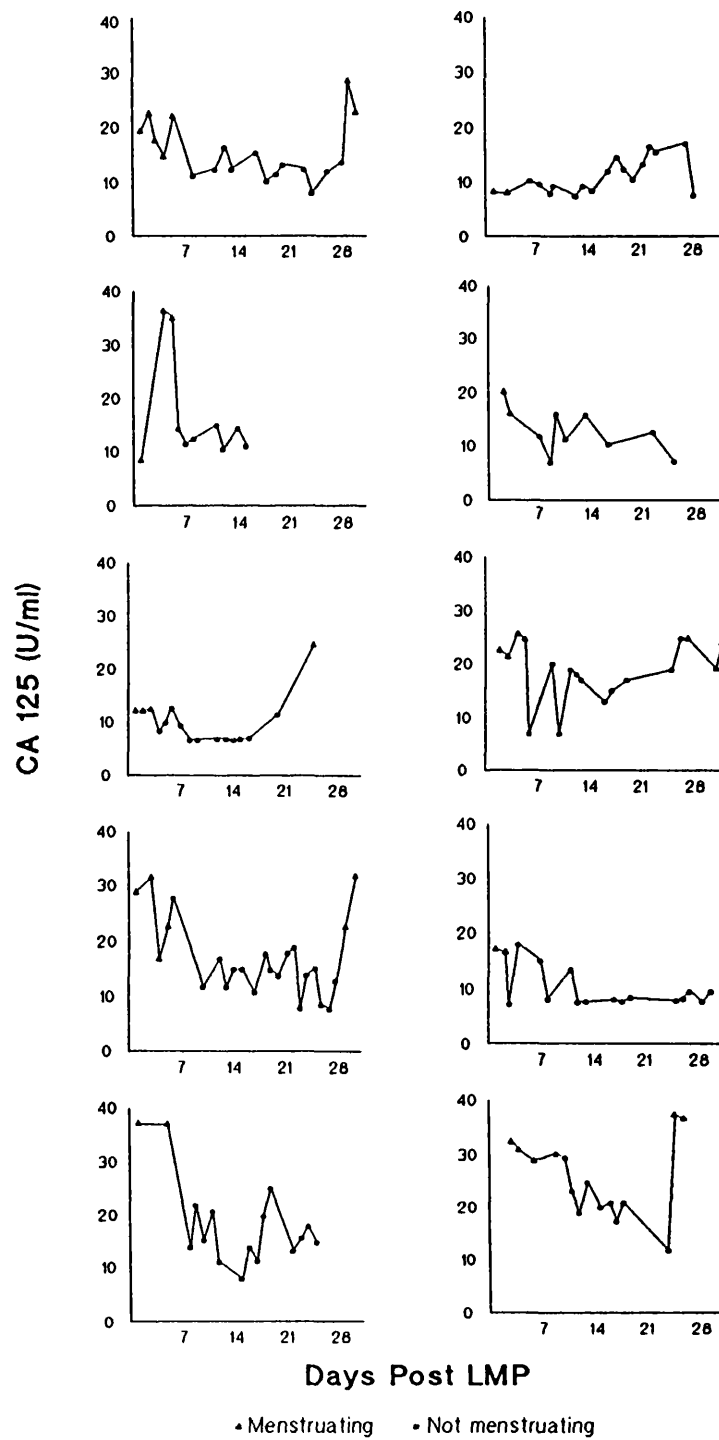


Figure 5.1. Serum CA 125 levels during ovulatory menstrual cycles in 10 individuals.

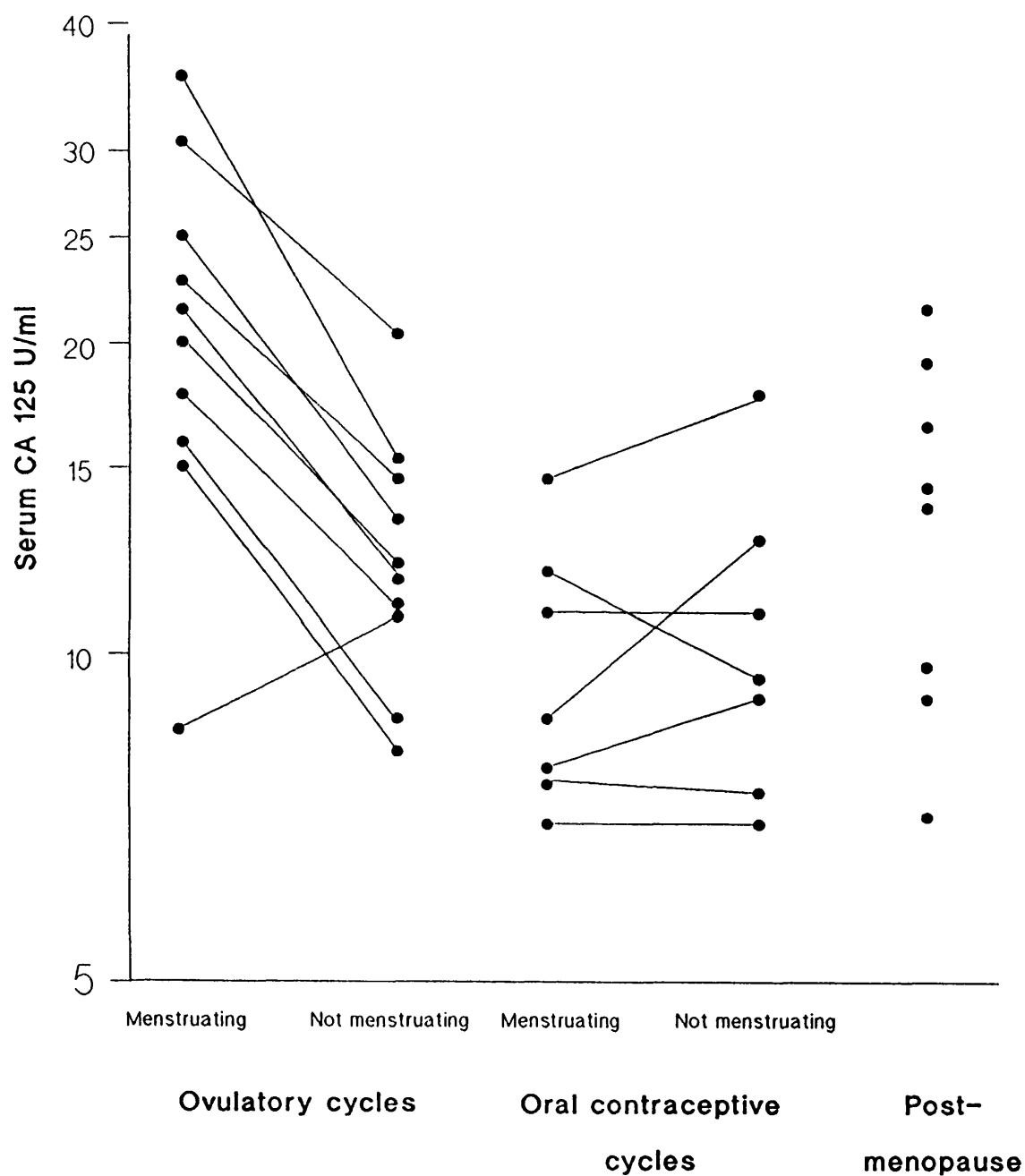


Figure 5.2. Mean serum CA 125 levels for individual subjects during menstrual and non menstrual phases of ovulatory menstrual cycles (n=10) and oral contraceptive cycles (n=7) and during a calendar month in postmenopausal women (n=8).

5.3.3 Postmenopausal women

Peak serum CA 125 levels in samples obtained from 8 postmenopausal women ranged from 9 U/ml to 29 U/ml (median 19.5 U/ml). It was not possible to date samples from a standard reference point in this group; day 1 was therefore arbitrarily designated as the first day of sampling. Analysis of variance revealed significant differences between individuals ($p < 0.0001$).

5.3.4 Comparison of groups

No significant difference was detected between mean levels throughout the sampling period in individuals from the ovulatory cycle (14.2 U/ml, CI 12.0-16.8 U/ml), hormonal contraception cycle (10.2 U/ml, CI 8.1-13.2 U/ml) or postmenopausal groups (13.4 U/ml, CI 10.2-17.6 U/ml). However mean levels during menstruation in the ovulatory subjects were higher than mean levels throughout the sampling period in the hormonal contraception group or the postmenopausal group ($p < 0.01$, figure 5.2).

5.4 DISCUSSION

The results of this study indicate that serum CA 125 levels are elevated during the menstrual phase of ovulatory cycles. This rise was not detected in women taking the combined oral contraceptive pill. These observations are consistent with the elevated serum CA 125 levels in premenopausal compared to postmenopausal women, seen in earlier cross sectional studies (Haga et al 1986a, Zurawski et al 1987). The elevations observed in association with menstruation are sufficient to account for

the differences between serum CA 125 levels in pre and postmenopausal women. The degree of elevation of serum CA 125 levels observed during the menstrual phase of ovulatory cycles in healthy women was consistent with the observations of Takahashi et al (1988) and Masahashi et al (1988). These authors reported significantly greater elevations of serum CA 125 during menstruation in women with endometriosis and have suggested that serum CA 125 measurement during menstruation may be of diagnostic value.

The elevation of serum CA 125 associated with menstruation is modest compared to the serum CA 125 levels observed in malignancy and does not have any implications for the use of the CA 125 radioimmunoassay in monitoring established ovarian cancer. However, these observations may be of importance in the context of applying the CA 125 radioimmunoassay to screening for early stage ovarian cancer. As the incidence of ovarian cancer is relatively low, high specificity is essential in order to achieve an acceptable positive predictive value when screening for this disease (Jacobs et al 1988a). Our findings suggest that it may be possible to apply the same normal range to healthy, non pregnant premenopausal women as to postmenopausal women, as long as sampling does not occur during menstruation.

The mechanism of serum CA 125 elevation during menstruation is unclear. High levels of CA 125 activity are detectable in both proliferative and secretory endometrium (Jacobs et al 1988b) as well as endometrial and cervical mucus (de Bruijn et al 1986). Scharl et al (1989) have recently reported that the staining

pattern of the endometrium varies according to the phase of the menstrual cycle. Expression was confined to the luminal surface in the early proliferative phase, in cytoplasmic granules during the mid and late proliferative phases and in the secretions of endometrial glands as well as on the luminal surface in the secretory phase. However, the increase in serum levels during menstruation may not be a consequence of increased tissue CA 125 synthesis or activity. It has been suggested that the increased serum CA 125 levels observed during menstruation are a consequence of retrograde menstruation with absorption of CA 125 activity via the peritoneum (Mastropaolo 1986). Serial sampling of individuals pre and post tubal ligation would be valuable in the investigation of this hypothesis. An alternative explanation is that the moiety expressing CA 125 is unable to cross the normal blood/tissue barrier but reaches the circulation during menstruation when this barrier is disrupted. The occurrence of a process which in some way alters normal tissue barriers in relation to tissues known to produce CA 125, is a common factor in most physiological and pathological events associated with elevation of serum CA 125 levels (Jacobs and Bast 1989). Such events occur in malignancy, pregnancy, menstruation, endometriosis, pelvic inflammatory disease and benign ovarian tumours.

CHAPTER 6

SERUM LEVELS OF CA 125 DURING THE FIRST TRIMESTER OF NORMAL PREGNANCY AND IN ECTOPIC AND ANEMBRYONIC PREGNANCIES

6.1 INTRODUCTION

The function of the glycoprotein expressing CA 125 remains unclear but the distribution of the antigen suggests that it may have a physiological role. The compartmental distribution of CA 125 in the reproductive tract of healthy pregnant and non pregnant women (Jacobs et al 1988b, chapter 4) suggests that CA 125 is a product of normal endometrium and decidua. In addition the highest serum and tissue levels of CA 125 were observed during the first trimester of pregnancy. This chapter describes clinical studies which were performed in order to observe the variation of serum CA 125 during early pregnancy and some of its complications.

6.2 MATERIALS AND METHODS

6.2.1 Subjects and samples

(a) Natural conception / normal outcome: Single serum samples were obtained from 149 women in the 1st trimester of pregnancy (4-13 weeks gestation) following natural conception and with a retrospectively defined normal outcome. Gestational age was calculated retrospectively from the first day of the last menstrual period or by ultrasound assessment of gestational age in the first trimester in women with irregular menstruation.

(b) Assisted conception / normal outcome: Serum samples were obtained at approximately weekly intervals during the first trimester of pregnancy, from women achieving pregnancy following successful treatment for infertility at the Pivet Medical Centre, Perth, Western Australia. A total of 369 serum samples were obtained from 43 singleton pregnancies with a normal outcome. Ovulation was achieved spontaneously (n=6) or induced with clomiphene citrate (n=37). Fertilisation was achieved by artificial insemination by husband or donor (n=11), in vitro fertilisation (n=8) or following intercourse (n=24). All conception cycles were monitored at regular intervals and the date of ovulation documented by ultrasound scanning or the identification of the LH surge. Gestational age is expressed as interval after the last menstrual period, and calculated on the assumption that the date of ovulation was day 14 of the menstrual cycle, or by ultrasonic assessment of gestational age in the first trimester in women with irregular menstruation.

(c) Natural conception / early pregnancy failure: Serum samples were obtained from patients with ectopic pregnancies (n=38) and anembryonic pregnancies (n=78) following natural conception. Gestational age was calculated retrospectively from the first day of the last menstrual period. Anembryonic pregnancy was defined as an empty gestational sac of volume greater than 2.5mls visualised by real time ultrasonography via the abdominal route.

6.2.2 Radioimmunoassay

Peripheral venous blood samples were collected and stored as described in section 2.3 and the CA 125 and LH radioimmunoassays performed as described in sections 2.1.1.1 and 2.1.4 respectively.

6.2.3 Statistical analysis

All data analysis was performed using log-transformed values for serum CA 125 and the Statistical Package for the Social Sciences. In groups (a) and (c), mean (\pm standard error) of log CA 125 levels were calculated for gestational ages 2-3, 4-5, 6-7, 8-9, 10-11 and 12-13 weeks, and compared using analysis of variance. In group (b), for which serial samples were available, mean CA 125 for each patient in each time period was first computed and then a group mean (\pm standard error) calculated for comparison with the results from groups (a) and (c).

6.3 RESULTS

6.3.1 Normal pregnancy \ normal outcome

Serum CA 125 levels in women in the natural conception normal outcome group, between 4 and 13 weeks post menstruation, are shown in figure 6.1. High and low serum CA 125 levels were apparent during the 1st trimester of pregnancy and analysis of variance confirmed significant variation with gestational age ($p < 0.0001$), the mean log transformed serum CA 125 levels being highest at 6-7 weeks gestation, and were greater than at 4-5 weeks, 10-11 weeks or 12-13 weeks gestation, (figure 6.2, table 6.1).

6.3.2 Assisted conception \ normal outcome

Serial serum CA 125 levels for each pregnancy monitored are shown in figure 6.3. A rise in serum CA 125 levels to at least twice the non pregnant level was observed in all but one of the 43 pregnancies. Peak serum CA 125 levels ranged from < 7 U/ml to 1,398.0 U/ml (median 48.8 U/ml) and occurred at a median gestation of 45 days, (figure 6.4). The pattern of mean log serum CA 125 levels by gestational age was not significantly different to that observed in group (a), (figure 6.2, table 6.1).

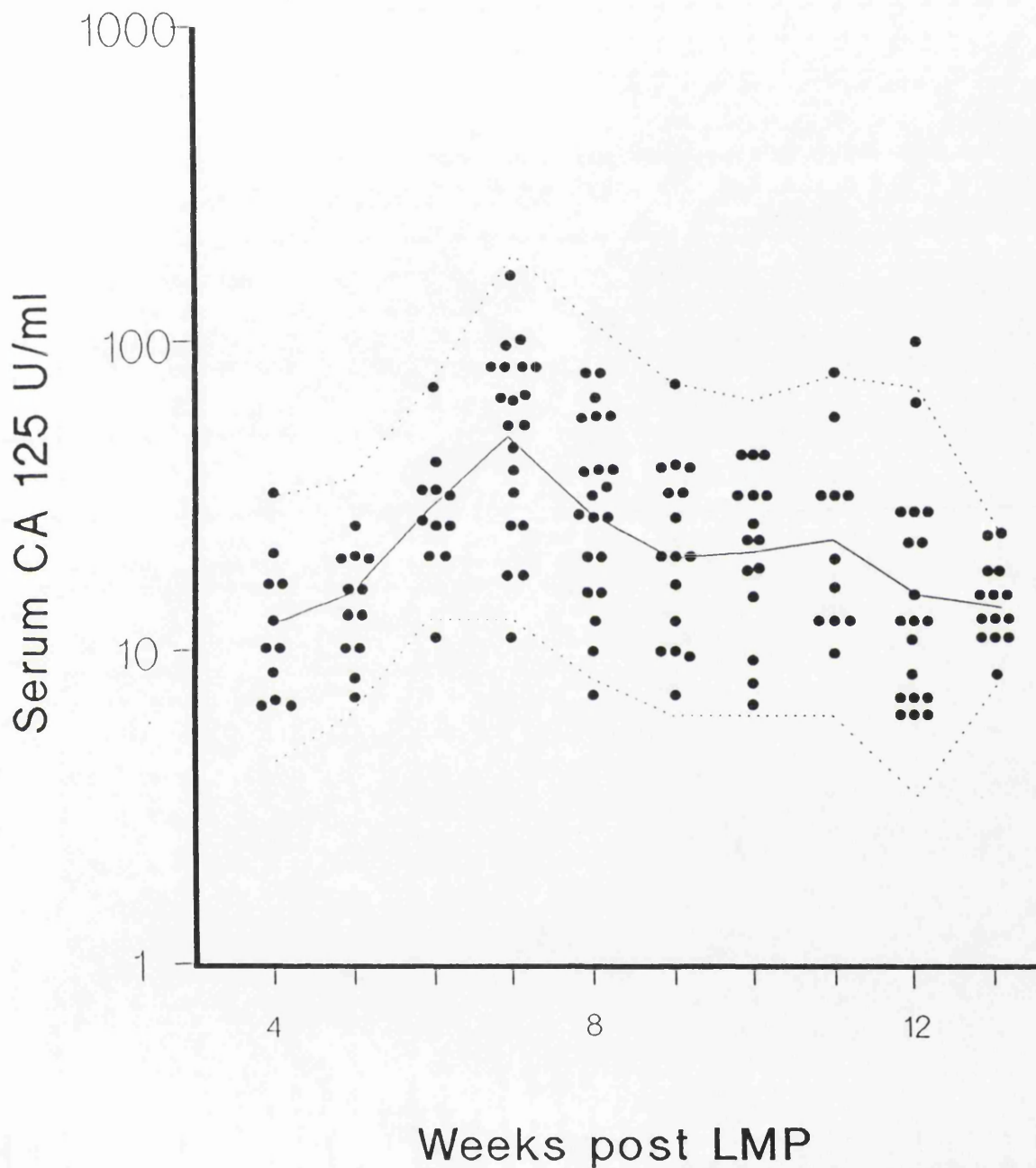


Figure 6.1. Serum CA 125 levels during the 1st trimester of pregnancies (n=150) achieved following natural conception and with a normal outcome. (Solid line = mean level. Dotted lines = ± 2 standard deviations from mean).

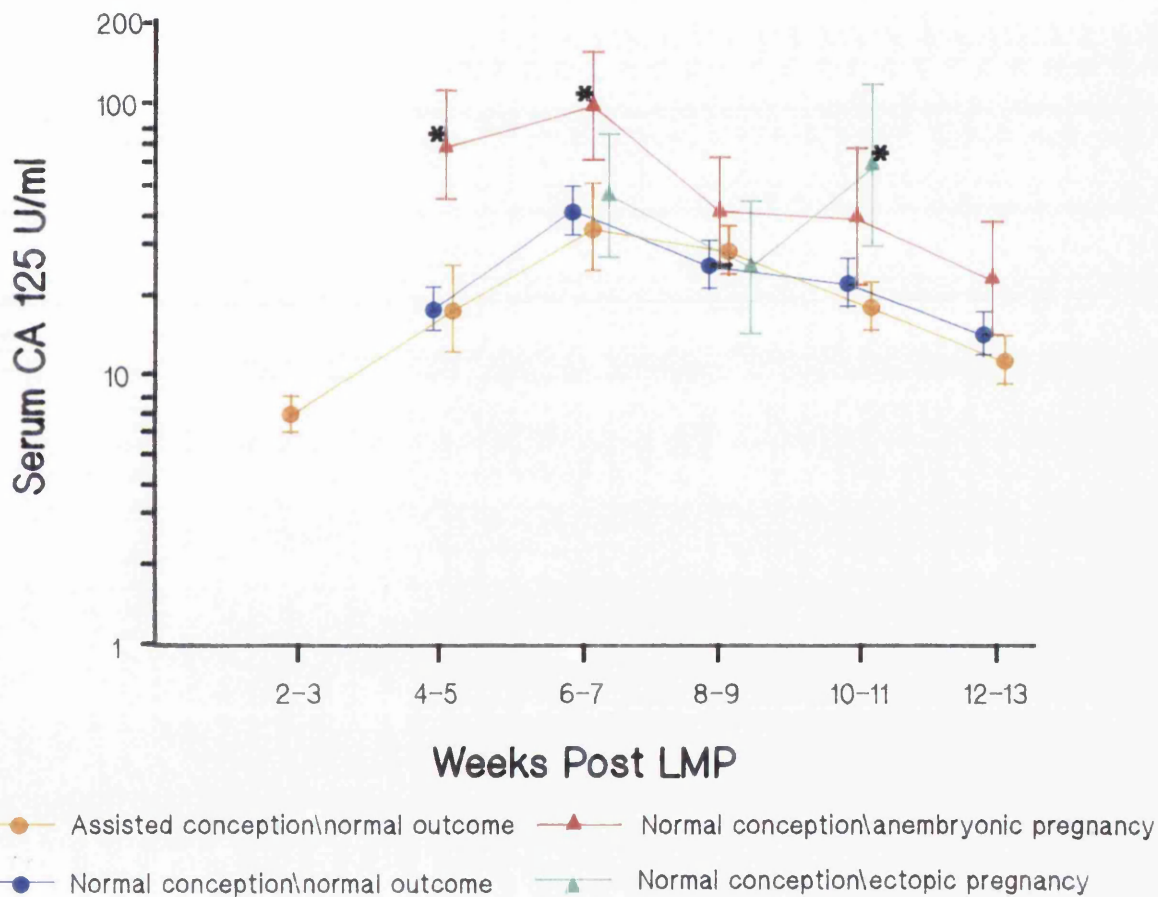


Figure 6.2. Mean and 95% confidence limits around the mean for serum CA 125 at 2 weekly intervals during the 1st trimester of normal outcome (assisted and natural conception), anembryonic and ectopic pregnancies. (* = mean serum CA 125 level significantly greater than pregnancies with a normal outcome).

Weeks post LMP	Normal conception normal outcome	Assisted conception normal outcome	Normal conception ectopic pregnancy	Normal conception anembryonic pregnancy
4-5 weeks	13.3 (10.9-16.1)	18.3 (13.0-25.9)	————	72.6 (46.2-114.0)
6-7 weeks	40.1 (31.7-50.7)	36.5 (25.6-52.0)	46.7 (27.5-79.3)	99.1 (64.0-153.5)
8-9 weeks	25.6 (20.5-31.9)	29.5 (23.2-37.5)	27.2 (15.2-48.5)	40.8 (25.4-65.6)
10-11 weeks	21.7 (17.1-27.6)	16.9 (13.1-21.7)	63.1 (31.0-125.9)	39.1 (22.1-69.2)
12-13 weeks	14.3 (12.1-16.7)	11.7 (9.6-14.3)	————	22.8 (15.0-34.7)

Table 6.1. Mean log transformed CA 125 levels (U/ml) and 95% confidence limits by weeks gestation in normal outcome, ectopic and anembryonic pregnancies.

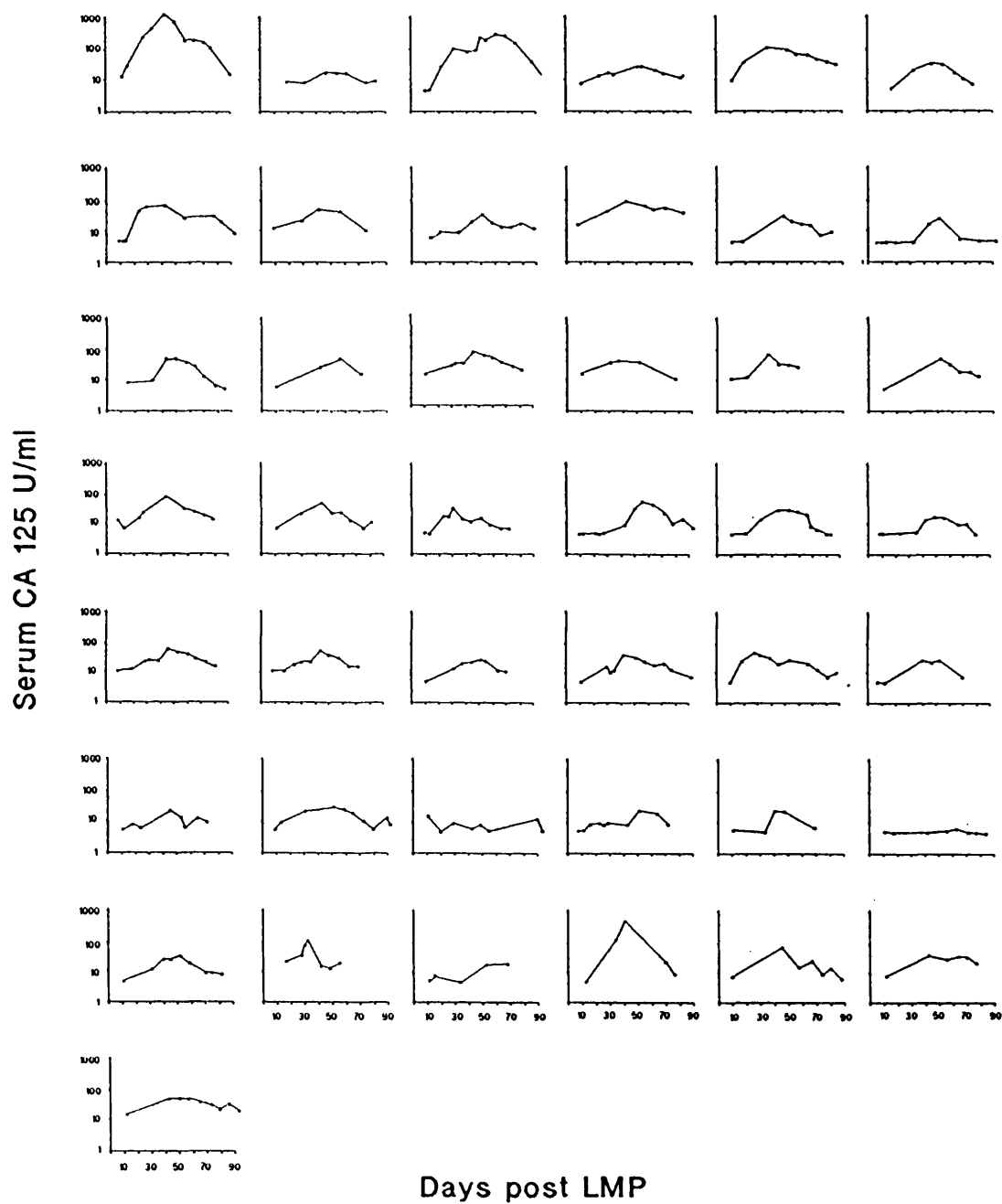


Figure 6.3. Serial serum CA 125 levels during 1st trimester pregnancies (n=43) achieved following treatment at an infertility clinic and with a normal outcome.

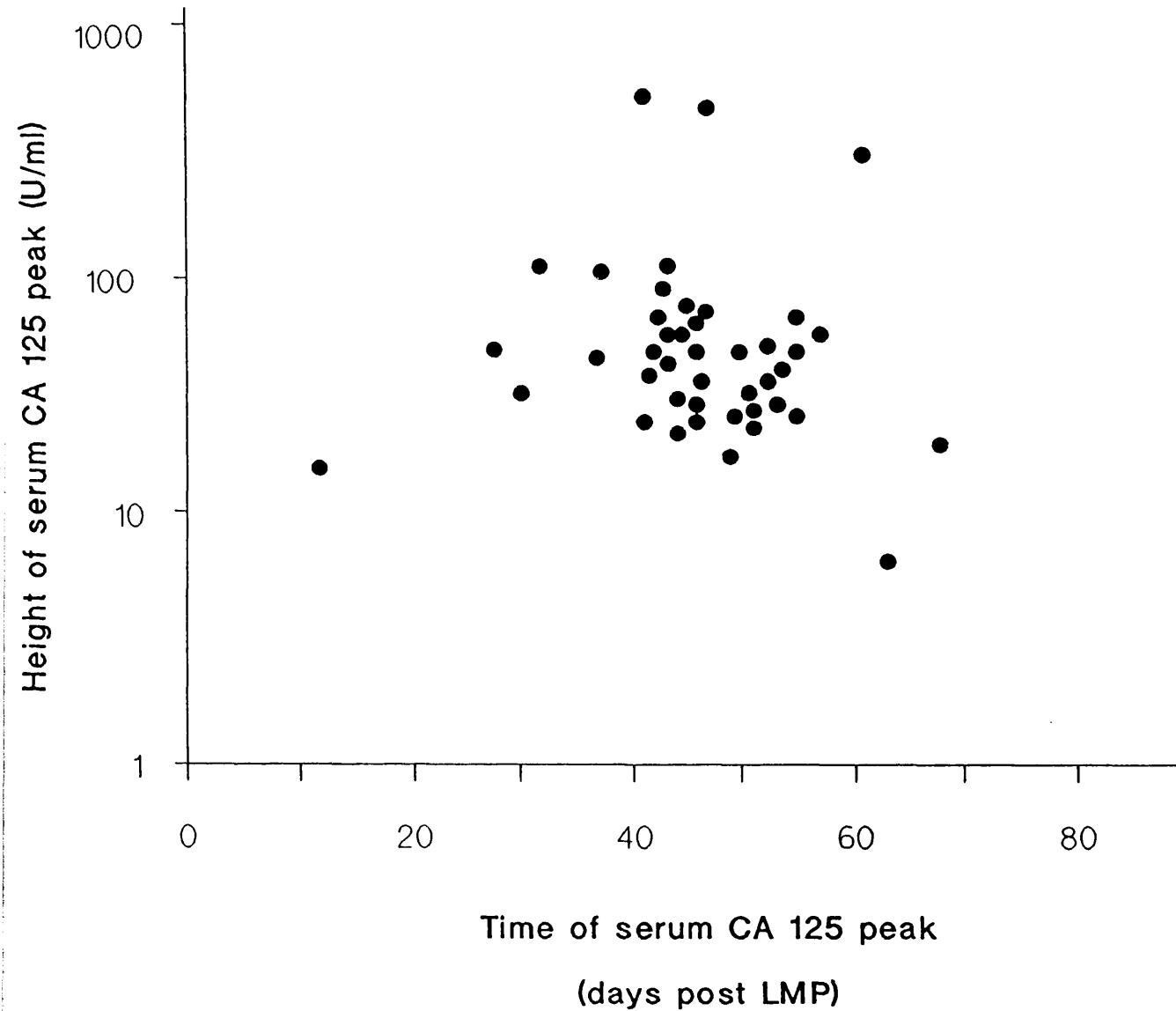


Figure 6.4. Peak serum CA 125 levels and gestational age at the time of peak levels in pregnancies (n=43) achieved following treatment at an infertility clinic and with a normal outcome.

6.3.3 Natural conception \ early pregnancy failure

Figure 6.5 illustrates serum CA 125 levels in patients with ectopic and anembryonic pregnancies, superimposed on the range of serum CA 125 in the normal conception / normal outcome pregnancies. Analysis of variance indicated significant variation in log serum CA 125 by week of gestation in patients with anembryonic pregnancies ($p = 0.0106$) but not in patients with ectopic pregnancies ($p = 0.17$). Significant differences were observed between serum CA 125 levels in the normal pregnancy outcome groups and both ectopic pregnancy ($p=0.006$) and anembryonic pregnancy ($p < 0.0001$) groups. Mean log transformed CA 125 values were higher in the ectopic pregnancy group at 10-11 weeks gestation than in the normal pregnancy outcome groups (both assisted and natural conception), (figure 6.5b, table 6.1). Mean log transformed CA 125 values were higher in the anembryonic pregnancy group at 4-5 weeks and 6-7 weeks than in the normal pregnancy outcome groups, (both assisted and natural conception), (figure 6.5a, table 6.1). From 4-8 weeks gestation 24 of 39 samples from anembryonic pregnancies were more than 2 standard deviations above the mean for normal outcome pregnancies, whilst from 8-12 weeks 35 of 39 were within 2 standard deviations of the mean.

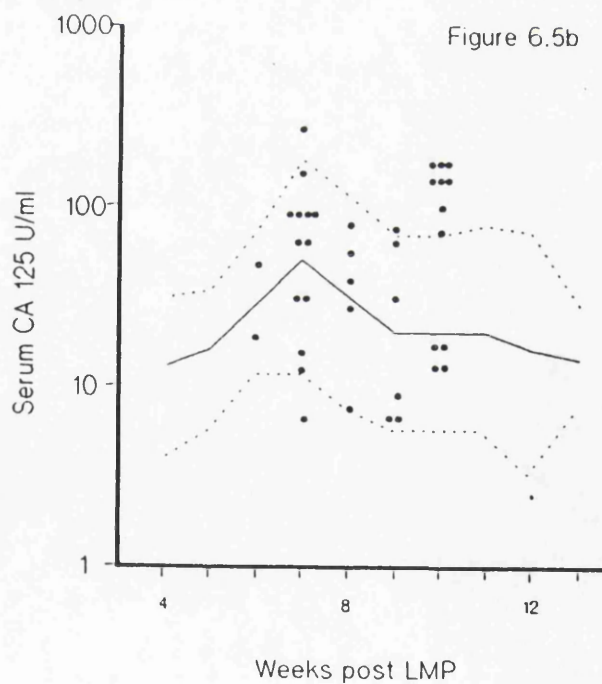
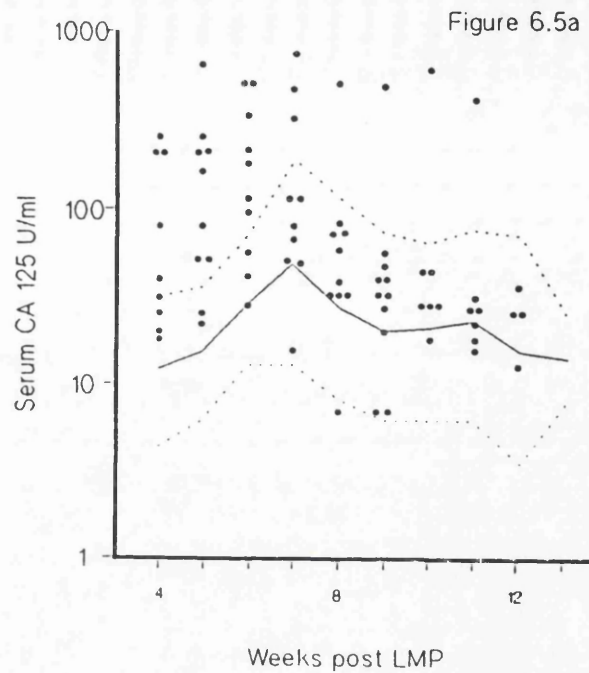


Figure 6.5. Serum CA 125 levels during the 1st trimester of pregnancies achieved following natural conception and resulting in (a) anembryonic pregnancies (n=78) and (b) ectopic pregnancies (n=38). (Solid line = mean level for normal outcome pregnancies. Dotted lines = ± 2 standard deviations from mean for normal outcome pregnancies).

6.4 DISCUSSION

This study has demonstrated a consistent pattern of a rise and fall in serum CA 125 levels during the first trimester of pregnancies with a retrospectively defined normal outcome. The findings are in accord with earlier reports, in which elevations of serum CA 125 levels during the first trimester in comparison to the latter weeks of pregnancy or the non pregnant state were observed (Seki et al 1986, Niloff et al 1984b, Haga et al 1986a, O'Brien et al 1986, Jacobs et al 1988a). With the exception of 8 cases reported by Seki et al (1986) these studies have been based on observations from single samples obtained more than 6 weeks after the last menstrual period. Consequently, high serum CA 125 levels appeared to occur in a relatively small proportion of pregnancies. The findings described in this chapter suggest that this interpretation was incorrect. A transient elevation of serum CA 125 levels in the 1st trimester is consistently observed in assisted conception pregnancies (figure 6.3). Although serial samples were not obtained from natural conception normal outcome pregnancies it is likely that the same pattern occurs in these pregnancies given the similarity in mean levels of serum CA 125 during the first trimester in assisted conception and natural conception pregnancies (figure 6.2).

The physiological basis for elevation of serum CA 125 levels during the first trimester is unclear. As described in chapter 4 high levels of CA 125 are detectable in aqueous extracts of 1st trimester decidua compared to non pregnant endometrium or decidua obtained at term. The increase in serum levels may therefore

simply represent diffusion of the CA 125 moiety down an increased tissue:blood concentration gradient. Quirk et al (1987) have hypothesised that during early pregnancy there is leakage of CA 125 from the gestation sac to the peritoneal cavity via the fallopian tubes from where it is absorbed into the circulation. As the gestation sac enlarges, and the decidua capsularis fuses with the decidua parietalis, they suggest that this route of drainage is obstructed and hence serum CA 125 levels fall toward the end of the 1st trimester. Alternatively CA 125 may be released from decidual cells into the circulation as a result of the disruption to tissue planes in early pregnancy during the processes of implantation and placentation. Disruption to blood tissue barriers could equally explain the rise in serum CA 125 levels during and immediately following delivery which has recently been reported (Itahashi 1988, Kobayashi 1989a).

The role of the moiety expressing CA 125 and the controlling mechanisms of the increased tissue production of CA 125 observed in early pregnancy are obscure. Evidence from 3 studies of CA 125 expression in vitro suggests that CA 125 production may be a regulated cellular process which can be modified by synthetic steroids and other drugs. Bischof et al (1986) demonstrated inhibition of CA 125 production in monolayer culture of human endometrial stromal cells by medroxyprogesterone. This inhibition could be blocked by oestradiol or the progesterone antagonist RU486. Ishiwata et al (1986) found that treatment of four ovarian cancer cell lines with the dibutyryl derivative of cyclic AMP stimulated CA 125 release. Most recently, Karlan et al (1988) have demonstrated inhibition of release of CA 125 from OVCA 433

cells by treatment with dexamethasone. The inhibition was concentration dependent and specific to glucocorticoids. These reports suggest that the observed rise in serum and tissue CA 125 activity may be related to the complex endocrinological events of early pregnancy.

This study was not designed to assess the diagnostic value of serum CA 125 measurement during early pregnancy but has provided some information about two complications of early pregnancy. First, the elevation of serum CA 125 observed in assisted conception pregnancies was transient and variable in its timing, duration and magnitude (figure 6.4). The 'normal range' for serum CA 125 is consequently wide. Secondly, although differences were observed between mean serum CA 125 levels in anembryonic and ectopic pregnancies compared to those with a normal outcome, there was considerable overlap in the range of values in the various groups. For these reasons it is unlikely that serum CA 125 measurements during early pregnancy will be of clinical value.

The high levels of CA 125 observed in anembryonic pregnancies are intriguing and suggest that the presence of the fetus suppresses the release of CA 125 into the circulation. The rise in serum levels of CA 125 in ectopic pregnancies suggests that decidual trophoblast interaction is not essential for release of CA 125 into the maternal circulation. These observations are in contrast to serum levels of proteins of trophoblastic origin such as hCG PAPPa and SP1 which are depressed in women with anembryonic pregnancies (Jacobs et al 1988c, Stabile et al 1989).

CHAPTER 7

THE ROLE OF CA 125 MEASUREMENT IN
THE PREOPERATIVE DIAGNOSIS OF OVARIAN CANCER

7.1 INTRODUCTION

The greatest opportunity to influence the natural history of ovarian cancer occurs at the time of the initial laparotomy. The aims of surgical management at this time are to accurately determine the extent of disease and to reduce residual tumour volume to a minimum (Griffiths 1987, Hacker 1987). In spite of the knowledge of the benefits of accurate surgical staging and cytoreductive surgery, many patients do not receive appropriate surgery at the time of surgical diagnosis (Young et al 1983). Adequate treatment at primary laparotomy is generally considered to require persistent, time consuming and aggressive surgery often not feasible during a routine operating list. Ideally, patients with ovarian malignancy should therefore be referred for primary laparotomy to a surgeon with appropriate operative experience and resources. In practice, the diagnosis of ovarian cancer is often difficult to make preoperatively and inadequate surgical exploration by junior and/or inexperienced surgeons is a regular occurrence. Reliable methods for preoperative diagnosis of ovarian cancer would provide a rational basis for referral prior to diagnostic laparotomy. Patients with ovarian cancer could thereby be ensured of the benefits of thorough surgical staging and cytoreduction by an experienced surgeon.

This study was undertaken in order to assess the value of serum CA 125 measurement in preoperative diagnosis of ovarian cancer and to compare it with diagnostic criteria commonly used in current clinical practice. In addition the role of 2 other tumour associated antigens (CA 15.3 and TAG 72.3) was assessed.

7.2 PATIENTS AND METHODS

7.2.1 Patients

One hundred and forty three patients admitted consecutively to the Gynaecology Department at The London Hospital for elective surgical investigation of an adnexal mass were recruited for this study after informed consent.

7.2.2 Methods

7.2.2.1 Clinical data

A standardised computer data sheet recording socio-demographic characteristics, past medical history, the history of the current condition, and findings on physical examination was completed.

7.2.2.2 Clinical impression

Overall clinical impression of the benign or malignant nature of the pelvic mass was assessed in each case by one individual (IJ) on the basis of all the information obtained preoperatively by the clinical team. This assessment was made with access to the clinical history and physical examination and the results of any investigations performed preoperatively (eg. haematology, biochemistry, chest X-ray, ultrasound, intravenous urogram) but without knowledge of the serum CA 125 level. Clinical impression was scored on a scale of 1-5, (1=benign, 2=probably benign, 3=uncertain, 4=probably malignant, 5=malignant).

7.2.2.3 Ultrasound scan

An ultrasound scan was performed as part of routine preoperative assessment on all but 4 patients (1 with malignant disease, 3 with benign disease) by the staff of the Radiology department at The London Hospital. All of the scans were performed via the abdominal route with the full bladder technique, using a Diasonics DS.1 sector scanner with a 3.5 MHz transducer. Ultrasound reports were scored 1 point for each of the following characteristics; (i) multilocular cyst (ii) evidence of solid areas (iii) evidence of metastases (iv) presence of ascites (v) bilateral lesions.

7.2.2.4 Radioimmunoassay

Peripheral venous blood samples were collected and stored as described in section 2.2 and the CA 125, TAG 72.3 and CA 15.3 radioimmunoassays performed as described in sections 2.1.1.1, 2.1.2 and 2.1.3 respectively.

7.2.2.5 Histological or surgical diagnosis

When a surgical specimen was sent for pathological examination a copy of the report was obtained. In cases where a specimen was not sent for histological examination, the surgical diagnosis was assumed to be correct (eg. follicular cysts).

7.2.2.6 Statistical analysis

Postmenopausal status is defined as greater than one year of amenorrhoea or age over 50 years for women who had previously undergone a hysterectomy (n=11). Comparison between patients with

benign and malignant disease was performed using the Student's t-test for age, the Chi-square test for menopausal status and the Mann Whitney U test for ultrasound score and clinical impression. The Student's t-test was used to compare log transformed values of serum CA 125, serum CA 15.3 and serum TAG 72.3 in patients with benign and malignant disease. Sensitivity is defined as the percentage of patients with malignant disease who had a positive test result. Specificity is defined as the percentage of patients with benign disease who had a negative test result. The sensitivity and specificity of each criteria at various action lines was calculated together with the exact confidence intervals from the binomial distribution.

The likelihood ratio (LR) of malignancy for a test result is defined as the odds for malignancy in patients with the test result, divided by the odds for malignancy in the entire population (the prior odds). The LR is therefore independent of the prevalence of malignant disease in the particular population studied, and can be generalised to predict risks of malignancy in other populations, by multiplying the LR by the prior odds for malignancy in that population. The LR for test results within specified limits were calculated and confidence intervals for the LR were estimated using a technique analogous to that used for relative risks in case control studies (Gart and Zweifel 1967, Breslow and Day 1980). Symmetric confidence intervals for the log LR were calculated by adding 0.5 to the numerators and denominators used to calculate the sensitivity and false positive rate of the test, taking logs to obtain an estimate of the log LR

and calculating Taylor series confidence intervals (Kleinbaun et al 1982).

Stepwise logistic regression was performed using the BMDP statistical package (program PLR). The 4 patients who did not have a preoperative ultrasound scan were excluded from the analysis. In performing the analysis clinical impression score was omitted because of its subjective nature. Logistic regression analysis provided a formula for calculation of the likelihood ratio associated with a patient's menopausal status, ultrasound score and serum CA 125 level. This formula was simplified without loss of diagnostic precision to provide a Risk of Malignancy Index (RMI) suitable for use in clinical practice.

7.3 RESULTS

7.3.1 Characteristics of study population

Benign gynaecological conditions including benign ovarian tumours (70), functional ovarian cysts (11), endometriosis (6), fibroids (6), pelvic adhesions (4) and fimbrial cysts (2) were diagnosed in 99 patients, no abnormality having been detected in 2 patients. The histological diagnosis and FIGO stage in the 42 patients found to have malignant disease is summarised in table 7.1. Primary invasive epithelial ovarian malignancies were found in 36 women and were most commonly stage III and of serous or undifferentiated histological type.

7.3.2 Individual test criteria

Statistically significant differences between the benign and malignant groups were observed for age, menopausal status, clinical impression score, ultrasound score, serum CA 125, serum CA 15.3 and serum TAG 72.3 (table 7.2). There was no significant association between other features of the history, presentation or examination and the presence of benign or malignant disease.

The likelihood ratio for malignancy associated with individual test results are shown in figure 7.1. Subgroups of patients with a likelihood ratio (LR) for malignancy greater than the entire study population (LR 1.0) were defined by age greater than 60

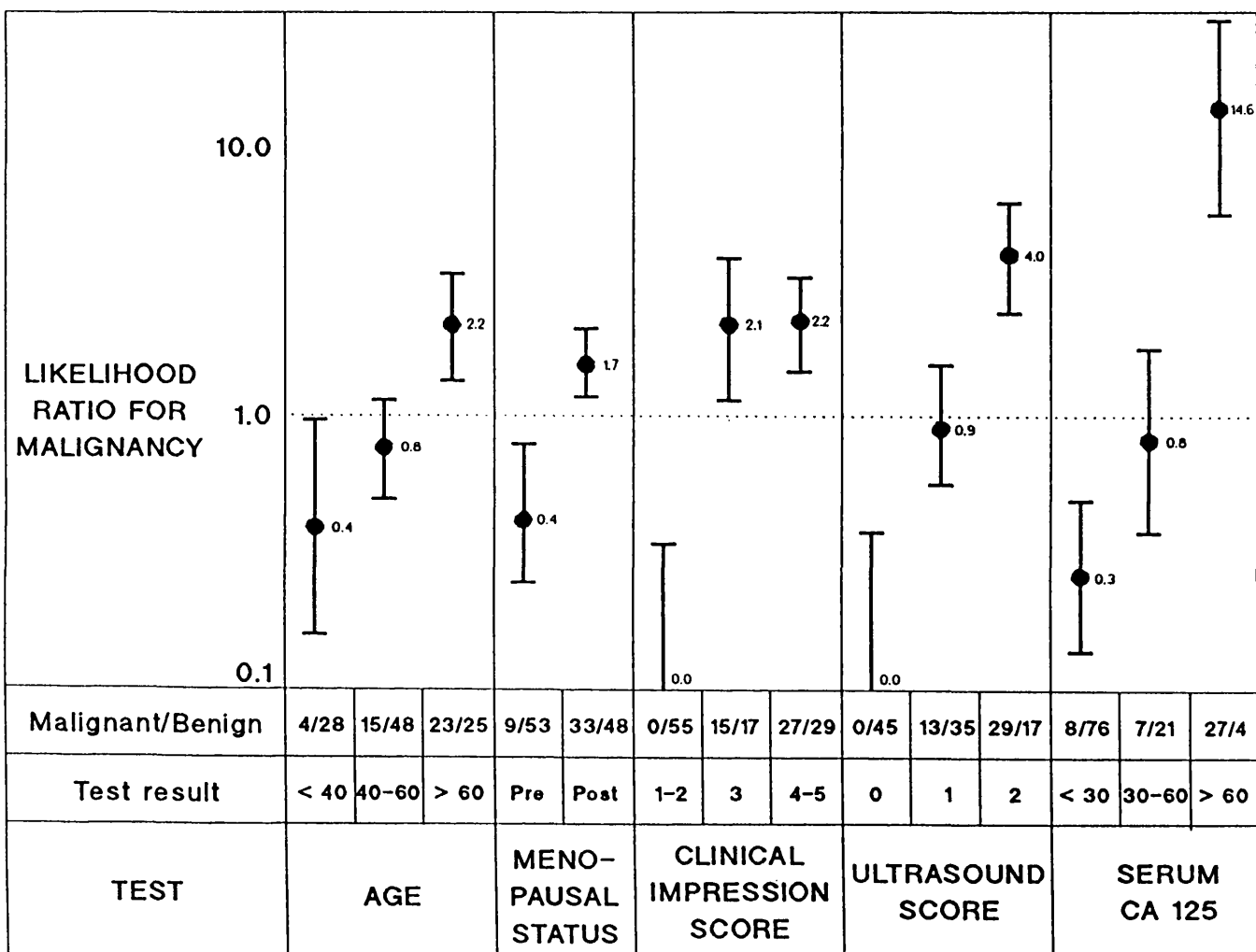


Figure 7.1. The likelihood ratio for malignancy of subgroups of patients defined by age, menopausal status, clinical impression score, ultrasound score and serum CA 125 level.

HISTOLOGICAL TYPE	FIGO STAGE				TOTAL
	I	II	III	IV	
Serous cystadenocarcinoma	1	0	10	1	12
Mucinous cystadenocarcinoma	2	0	4	0	6
Endometrioid adenocarcinoma	1	1	2	0	4
Clear cell carcinoma	1	1	2	0	4
Undifferentiated carcinoma	1	1	8	0	10
Borderline malignancy	4	0	0	0	4
Dysgerminoma	1	0	0	0	1
Metastatic bowel adenocarcinoma	-	-	-	-	1

Table 7.1. Histological type and FIGO stage of 42 patients with malignant disease.

TEST	BENIGN n=101	MALIGNANT n=42	Significance level
Age	Mean 48.8years SD 14.3 years	Mean 59.0 years SD 11.8 years	Students t-test $p < 0.001$
Postmenopausal	48.0%	80.5%	Chi square test $p < 0.001$
Clinical impression	Median 2 points Range 1-4 points	Median 4 points Range 3-5 points	Mann Whitney U $p < 0.0001$
Ultrasound	* Median 1 point Range 0-2 points	#Median 2 points Range 1-2 points	Mann Whitney U $p < 0.0001$
Serum CA 125	Mean 17.5 U/ml CI 4.3-70.2 U/ml	Median 122.0 U/ml CI 6.1-3,394.8 U/ml	Mann Whitney U $p < 0.0001$

Table 7.2. Statistically significant differences between patients with benign (n=101) and malignant (n=42) pelvic masses. (* n=98, # n=41. CI = 95% confidence intervals).

years (LR 2.21), postmenopausal status (LR 1.65), a clinical impression score of 3 (LR 2.12) or more (LR 2.24), an ultrasound score of 2 (LR 4.10), a serum CA 125 of 60 U/ml or more (LR 16.22), a serum CA 15.3 of 60 U/ml or more (LR 7.8) and a serum TAG 72.3 of 10 U/ml or more (LR 24.1).

The sensitivity and specificity for individual criteria at various upper limits are listed in table 7.3 and illustrated in figure 7.2. A sensitivity and specificity greater than 70% were achieved only by serum CA 125 (upper limit between 30 U/ml and 40 U/ml) and ultrasound (score of 2). A further 3 criteria achieved a sensitivity and specificity greater than 70% and 50% respectively (age 50 years, postmenopausal status and a clinical impression score of 3).

7.3.3 Serum CA 125

Serum CA 125 results in relation to histological or surgical diagnosis are illustrated in figure 7.3. Twenty one patients with benign disease had serum CA 125 levels greater than 30 U/ml but less than 60 U/ml. This was most frequently observed in patients with benign ovarian cysts but also occurred in association with endometriosis (n=4) and functional cysts (n=2). A further 3 patients with benign ovarian cysts had serum CA 125 levels between 60 U/ml and 90 U/ml. Only 1 patient with benign disease (an ovarian fibroma discovered during the 1st trimester of pregnancy) had a serum CA 125 level exceeding 90 U/ml. A serum CA

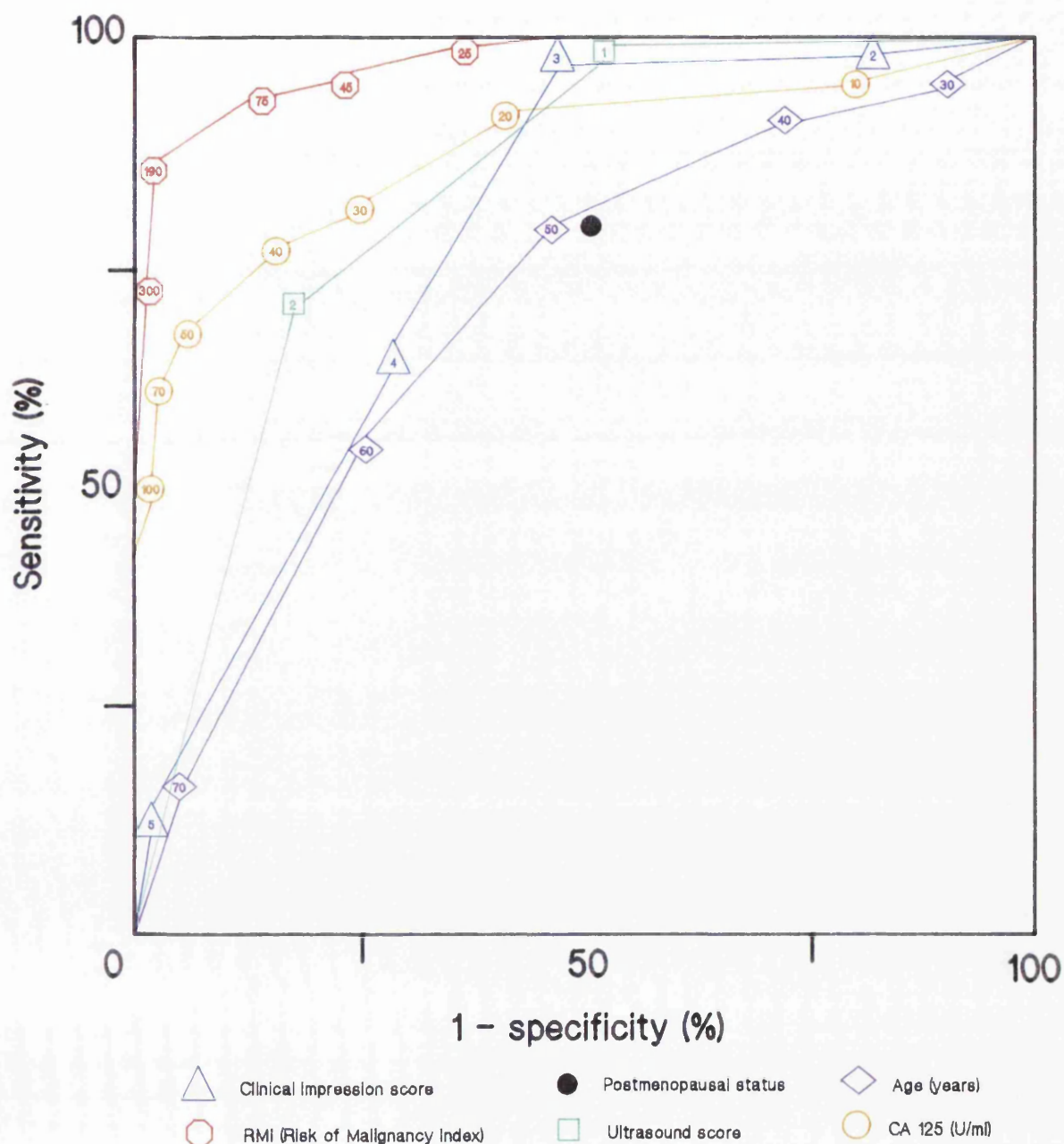


Figure 7.2. Receiver operator characteristics of various upper limits for age, menopausal status, clinical impression score, ultrasound score, serum CA 125 level and Risk of Malignancy Index (RMI) in the preoperative prediction of the benign or malignant nature of an adnexal mass.

TEST	Action line	Sensitivity % (95% confidence limits)	Specificity % (95% confidence limits)
AGE (years)	40	90.5 (77.4-97.3)	27.7 (19.5-37.9)
	50	78.6 (63.2-89.7)	53.5 (43.7-64.0)
	60	54.8 (38.7-70.2)	75.2 (65.3-83.1)
	70	16.7 (7.0-31.4)	96.0 (90.1-98.9)
MENOPAUSAL STATUS	Post-menopausal	78.6 (63.2-89.7)	52.5 (42.3-62.6)
CLINICAL IMPRESSION SCORE	3	100.0 (91.6-100.0)	54.5 (44.2-64.5)
	4	64.3 (48.0-78.5)	71.3 (61.4-79.9)
	5	9.5 (2.7-22.6)	100.0 (96.4-100.0)
ULTRASOUND SCORE	1	100.0 (91.4-100.0)	46.9 (36.8-57.3)
	2	70.7 (54.5-83.9)	82.7 (73.7-89.6)
SERUM CA 125 (U/ml)	30	81.0 (65.9-91.4)	75.2 (65.5-83.3)
	50	66.7 (50.5-80.4)	94.1 (87.5-97.9)
	70	61.9 (45.6-76.4)	98.0 (93.0-99.8)
	90	57.1 (41.0-72.3)	99.0 (94.6-100.0)

Table 7.3. Sensitivity and specificity of age, menopausal status, clinical impression, ultrasound and CA 125 in the differential diagnosis of benign and malignant masses in 143 patients. (Ultrasound data includes only 139 patients).

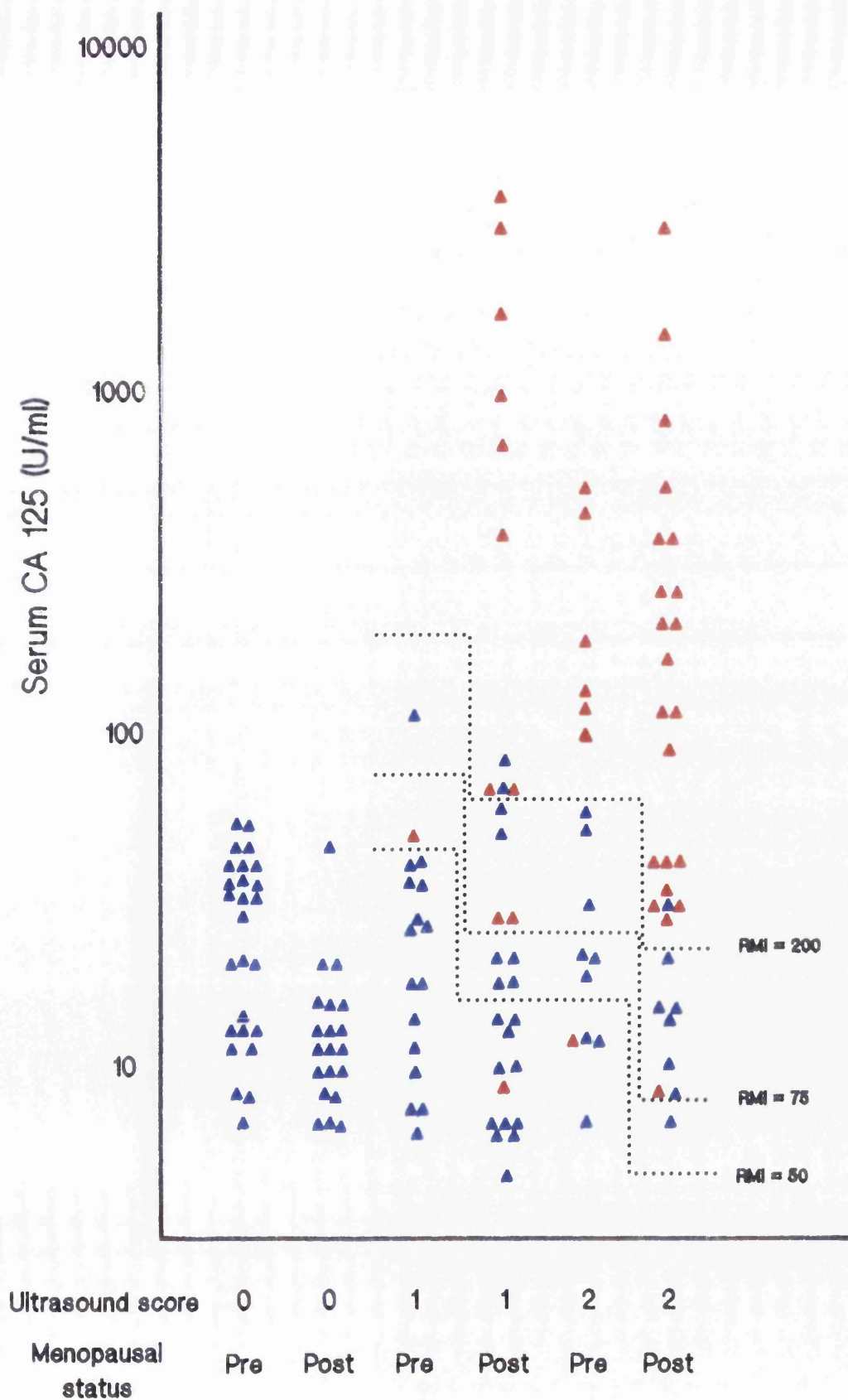


Figure 7.3. The relationship between menopausal status, ultrasound score, serum CA 125 level and clinical impression score in 139 patients with benign (open symbols) and malignant (closed solid symbols) adnexal masses. Dotted lines represent Risk of Malignancy Index (RMI) levels of 50, 75 and 200.

125 level greater than 60 U/ml was therefore associated with a high likelihood ratio for malignancy (LR 16.22). Conversely, a serum CA 125 level less than 30 U/ml was associated with a low likelihood ratio for malignancy (LR 0.25), but it did not exclude the presence of malignancy. Eight patients with malignant disease had a serum CA 125 of less than 30 U/ml. Two had clear cell carcinomas (both stage III), 2 mucinous cystadenocarcinomas (stage Ia and stage III), 1 a dysgerminoma, 1 metastatic colonic adenocarcinoma and 2 borderline ovarian malignancies (both stage Ia). A further 7 patients with malignant disease had serum CA 125 levels between 30 U/ml and 60 U/ml.

7.3.4 CA 125 combined with other tumour associated antigens

The relationship between serum levels of CA 125, CA 15.3 and TAG 72.3 is illustrated in figure 7.4 and the specificity and sensitivity of the individual antigens at various upper limits is shown in table 7.4. Table 7.5 lists the specificity and sensitivity of various combinations of CA 125 and CA 15.3. In this analysis patients were classified as positive if their serum level of either marker exceeded the specified upper limits. This combination of both tumour markers achieved a higher sensitivity but lower specificity than CA 125 alone. Of the 22 patients with malignant disease and a serum CA 15.3 greater than 30 U/ml, 19 also had a serum CA 125 greater than 30 U/ml, 15 greater than 50 U/ml and 14 greater than 70 U/ml. Addition of TAG 72.3 did not alter this analysis as all patients with an elevated TAG 72.3 (> 10 U/ml) also had either an elevated serum CA 125 or CA 15.3.

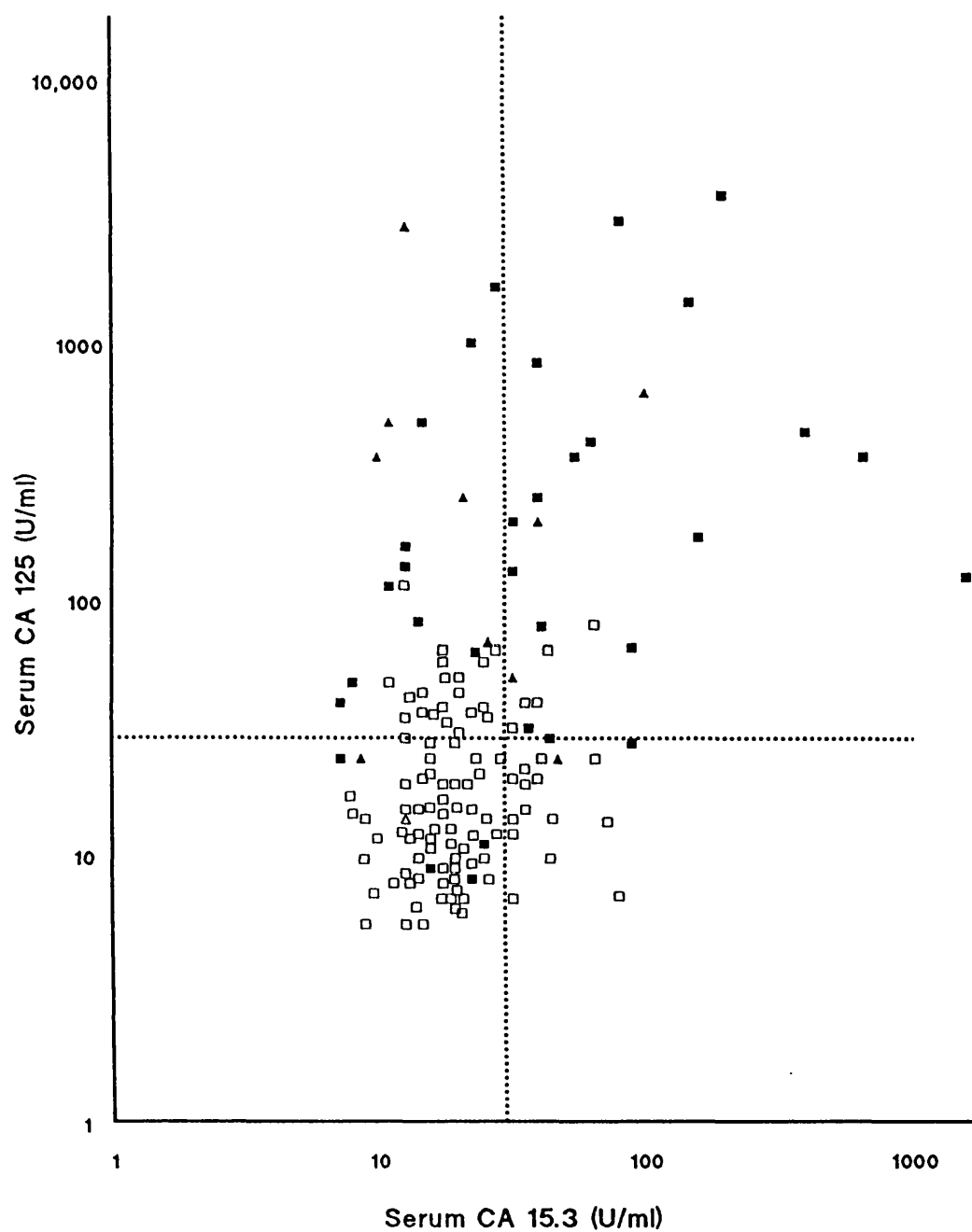


Figure 7.4. The relationship between serum levels of CA 125, CA 15.3 and TAG 72.3 in 143 patients with an adnexal mass. (Open symbols = benign, closed symbols = malignancy, triangle = TAG 72.3 > 10 U/ml, square = TAG 72.3 < 10 U/ml).

Antigen	Upper limit	Sensitivity	Specificity	Accuracy
CA 125	20 U/ml	92.6	59.4	69.2
CA 125	30 U/ml	81.0	75.2	76.9
CA 125	50 U/ml	66.7	94.1	86.0
CA 125	70 U/ml	61.9	98.0	87.4
CA 15.3	20 U/ml	69.0	59.4	62.2
CA 15.3	30 U/ml	52.3	79.2	71.3
CA 15.3	50 U/ml	31.0	96.0	76.9
CA 15.3	90 U/ml	21.4	100.0	76.9
TAG 72.3	10 U/ml	23.8	99.0	76.9
TAG 72.3	20 U/ml	14.3	100.0	74.8
TAG 72.3	50 U/ml	9.5	100.0	73.4

Table 7.4. Sensitivity, specificity and predictive accuracy of various upper limits for CA 125, CA 15.3 and TAG 72.3 in distinguishing benign from malignant adnexal masses.

CA 125 (U/ml)	CA 15.3 (U/ml)			
	>20	>30	>50	>90
> 20	37.6/97.6	48.5/92.9	53.5/92.7	55.4/92.9
> 30	46.5/92.9	62.4/88.1	73.3/83.3	75.2/83.3
> 50	57.4/88.1	75.2/81.0	91.1/71.4	94.1/69.0
> 70	60.4/88.1	81.2/80.9	94.1/69.0	98.0/61.9

Table 7.5. Specificity and sensitivity of combinations of CA 125 and CA 15.3. Cases were classified as malignant if the serum level of either antigen exceeded the specified upper limit. (Specificity/Sensitivity).

CA 125 (U/ml)	CA 15.3 (U/ml)	TAG 72.3 (U/ml)	Specificity (%)	Sensitivity (%)
> 20	> 30	> 10	89.1	69.0
> 30	> 30	> 10	93.1	66.7
> 50	> 30	> 10	98.0	50.0
> 70	> 30	> 10	100.0	45.2
.....				
> 20	> 20	> 10	81.2	71.4
> 30	> 20	> 10	90.1	69.0
> 50	> 20	> 10	95.0	64.3
> 70	> 20	> 10	99.0	57.1

Table 7.6. Specificity and sensitivity of combinations of CA 125, CA 15.3 and TAG 72.3. Cases were classified as malignant if the serum level of any 2 antigens exceeded the specified upper limit.

Table 7.6 lists the results of a second analysis in which patients were classified as positive if the serum level of any 2 of CA 125, CA 15.3 or TAG 72.3 exceeded the specified upper limits of normal. The highest accuracy (85.3%) was achieved using upper limits of 30 U/ml for both CA 125 and CA 15.3. This combined a sensitivity of 66.7% with a specificity of 93.1%.

7.3.5 Logistic regression analysis of all test criteria

Stepwise logistic regression analysis was performed with the results for age, menopausal status, ultrasound, CA 125, CA 15.3 and TAG 72.3. This analysis revealed that menopausal status, ultrasound score and serum CA 125 level were all significantly ($p < 0.01$) and independently related to the likelihood ratio for malignancy. No significant improvement in the logistic regression formula was achieved by the addition of age, serum CA 15.3 or serum TAG 72.3 nor were any interaction terms significant. The Risk of Malignancy Index (RMI) was derived from the logistic regression analysis (as described below in section 7.3.6) and was defined as follows:

$$RMI = U \times M \times \text{serum CA 125}$$

where;

U = 0 for ultrasound score of 0
 = 1 for ultrasound score of 1
 = 3 for ultrasound score of 2-5

M = 1 if premenopausal
 = 3 if postmenopausal

Examples: (1) A premenopausal patient with a serum CA 125 level of 40 U/ml and 2 ultrasound features of malignancy would have an RMI score of 120 ($U = 3$, $M = 1$, $CA\ 125 = 40$). (2) A postmenopausal patient with the same ultrasound and CA 125 results would have an RMI of score 360 ($U = 3$, $M = 3$, $CA\ 125 = 40$). (3) All patients with no ultrasound features of malignancy would have an RMI score of zero regardless of their menopausal status or serum CA 125 result ($U = 0$).

The performance of the RMI at various upper limits is illustrated in figure 7.2 and can be seen to combine higher levels of specificity and sensitivity than were achieved by individual criteria or combinations of tumour associated antigens. The relationship between menopausal status, ultrasound score and serum CA 125 level is illustrated in figure 7.3. Superimposed on figure 7.3 are action lines defined by RMI levels which achieved useful combinations of specificity and sensitivity. Table 7.7 shows the sensitivity, specificity and the likelihood ratio for malignancy given a positive result for different levels of the RMI. An RMI of 200 achieved a sensitivity of 85.4% (CI, 70.8%-94.4%) and a specificity of 96.9% (CI, 91.3%-99.4%). The likelihood ratio for malignancy of patients predicted to have benign and malignant disease by an RMI of 200 was 0.15 and 42.1 respectively, representing a 280 fold difference in the odds for malignancy.

RMI Score	Sensitivity	Specificity	Likelihood ratio for	
	% (95% CI)	% (95% CI)	maligancy if result is positive	negative
25	100.0 (91.4-100.0)	62.2 (51.9-71.8)	2.7	0.00
50	95.1 (83.5-99.4)	76.5 (66.9-84.5)	4.1	0.06
75	92.7 (80.1-98.5)	84.7 (76.0-91.2)	6.1	0.09
100	85.4 (70.8-94.4)	87.8 (79.6-93.5)	7.0	0.17
150	85.4 (70.8-94.4)	93.9 (87.2-97.7)	14.0	0.16
200	85.4 (70.8-94.4)	96.9 (91.3-99.4)	42.1	0.15
250	78.0 (62.4-89.4)	99.0 (94.5-100.0)	76.9	0.22

Table 7.7. The sensitivity, specificity and the likelihood ratio for malignancy given a positive or negative result for different levels of the Risk of Malignancy Index.

7.3.6 Derivation of the Risk of Malignancy Index (RMI)

The model for the log odds ratio (OR) provided by stepwise logistic regression analysis was:

$$\text{Log (OR)} = a + [b \times \log (\text{CA 125})] + [c \times \text{US}] + [d \times \text{MS}] \quad (1)$$

where;

US = ultrasound score	MS = menopausal score
a = -10.46	b = 1.71 (SE = 0.41)
c = 1.77 (SE = 0.69)	d = 1.86 (SE = 0.76)

Attempts were made to improve the fit of this model by incorporating squared terms (to test for curvature) and various interaction terms (to test for independence of risk factors) but no variable was associated with risk not explained by CA 125, ultrasound score and menopausal status at the 1% level. Equation (1) was converted to a formula for the odds ratio by raising to the power e and rearranging:

$$\text{OR} = 1.68 \times 10^{-4} \times (U \times M \times \text{CA 125})^{1.71} \quad (2)$$

where;

U = 0	if ultrasound score is 0	M = 1	if premenopausal
= 1	if ultrasound score is 1	M = 2.97	if postmenopausal
= 2.82	if ultrasound score is 2		

Formula 2 was converted to a formula for the likelihood ratio (LR) in the population by dividing by the prior odds ratio in the population (42/101). A rearrangement gave the following:

$$\text{LR} = (U \times M \times \text{CA 125} / 96.3)^{1.71} \quad (3)$$

This exact formula for the likelihood ratio was simplified to provide the Risk of Malignancy index (RMI). The performance of the RMI in differentiating between benign and malignant disease was identical to the true LR given by formula (3), (ie at any chosen specificity their sensitivities were equal).

7.4 DISCUSSION

The aim of differential diagnosis of the nature of an adnexal mass, is the identification of patients with ovarian cancer preoperatively, so that arrangements can be made for surgical staging and cytoreduction by an experienced surgeon. In current clinical practice the risk of malignancy is assessed preoperatively with reference to all available clinical information and the results of diagnostic tests. A clinical impression score was therefore incorporated in this study in order to provide a baseline to compare with other potential diagnostic criteria. All information routinely available to the clinician preoperatively was considered in the assessment, (the serum CA 125 result was not routinely available and therefore not considered). The results of this study suggest that a significant improvement in the accuracy of preoperative diagnosis could be achieved by the systematic evaluation of individual criteria or combinations of criteria. Although the performance of the clinical impression score was better than age or menopausal status alone it was inferior to ultrasound or CA 125 alone and to these tests combined with menopausal status in the Risk of Malignancy Index. The clinical impression score was more reliable as an indicator of benign disease than of malignant disease. All 55 patients classified as 'probably benign' or 'benign' were found to have benign disorders whereas only 27 of 56 patients classified as 'probably malignant' or 'malignant' were found to have malignant disease (figure 7.1).

The diagnostic performance of serum CA 125 measurement in this study was comparable to previous reports (Einhorn et al 1986, Vasilev et al 1988, Malkasian et al 1988, Patsner et al 1988, Di-Xia et al 1988). As would be anticipated from data described in previous chapters CA 125 measurement suffered from limitations of both sensitivity and specificity. A proportion of patients with benign ovarian cysts and endometriosis had moderately elevated CA 125 levels whilst some patients with malignancy had low or only slightly elevated levels. However, high CA 125 levels (> 90 U/ml) were almost entirely restricted to malignancy. The one exception involved a patient with a benign cyst during pregnancy. The possibility that this elevation of serum CA 125 was due to pregnancy rather than malignancy could therefore have been considered preoperatively (see chapter 5). CA 125 estimation was a reliable predictor of the presence of malignant disease in the group of patients with high serum CA 125 levels, (figure 7.2). Although patients with a serum CA 125 level greater than 60 U/ml were at particularly high risk of malignancy (figure 7.1) the sensitivity of an action line of 60 U/ml or more was relatively low (table 7.3). The optimal upper limit for CA 125 in preoperative differential diagnosis was 30 U/ml (sensitivity 81.0%, specificity 75.2%).

The diagnostic performance of CA 125 was only marginally improved by combination with the results for CA 15.3 and TAG 72.3. The rationale for combining these antigens was provided by a study of 50 serum samples from patients with an elevated serum CA 125 level and benign disease. TAG 72.3 and CA 15-3 were only elevated in 6% and 2% respectively of these samples (Bast et al 1987). As CA 15-3

and TAG 72.3 levels were elevated in 60% and 47% respectively of serum samples from ovarian cancer patients, it was hypothesised that the improvement in specificity on combined measurement with CA 125 would be greater than the sacrifice of sensitivity. Unfortunately, the trade off between specificity and sensitivity on combining these 3 antigens did not result in a net benefit in diagnostic performance. This may not be the case using other combinations of antigens.

The increase in the incidence of malignant ovarian tumours with increasing age and after the menopause is well documented and was confirmed in this study. In contrast benign disorders were equally distributed between pre and post menopausal women (figure 7.1). Consequently, although action lines of age 50 years and postmenopausal status achieved relatively high sensitivity their clinical value as individual tests was limited by low specificity (table 7.3, figure 7.2).

The ultrasound findings in this study confirm previous reports that although features such as multiloculation, solid areas, ascites and bilaterality are more frequently associated with malignant masses, they may be identified in benign disease (Herrmann et al 1987, Moyle et al 1983, Koyabashi 1976, Meire et al 1978, Deland et al 1979), (figure 7.3). Although an ultrasound score of 2 was associated with a high risk of malignancy, the clinical value of ultrasound as an individual test was limited by the observation of features of malignancy in 52 patients with benign disease. The main value of ultrasound was like clinical impression, in the reliable exclusion of malignant disease. The

absence of ultrasound evidence of solid areas, multiloculation, bilaterality, ascites or metastatic disease was uniformly associated with the presence of benign disease. The ultrasound score was therefore complementary to the serum CA 125 result which provided a reliable indicator of the presence of malignancy in patients with high levels.

Stepwise logistic regression analysis incorporating menopausal status, ultrasound score and serum CA 125 level provided the most valuable approach to improving diagnostic precision. The likelihood ratio and logistic regression analysis are independent of the ratio of benign to malignant disorders in the study population. The Risk of Malignancy Index (RMI), which was derived from the logistic regression formula, can therefore be generalised to populations with a prevalence of benign and malignant disease other than that observed in the London Hospital population. The RMI provides a quantitative assessment of the risk of malignancy based on menopausal status, ultrasound score and serum CA 125 level and can be used to discriminate between benign and malignant disease in the same way as other variables. The performance of the RMI in discriminating patients with benign and malignant disease was slightly inferior to the best possible discrimination based on menopausal status, ultrasound score and serum CA 125. However, because of its association with the likelihood ratio, the RMI is more likely to be robust and applicable to other populations than a rule based on the best possible discrimination.

Previous studies have noted that discrimination between benign and malignant disease may be improved by combined consideration of CA 125 and menopausal status (Malkasian et al 1988), CA 125 and other tumour markers (Yedema et al 1988, Knauf and Bast 1988) or CA 125 and ultrasound (Gadducci et al 1988). However, a systematic basis for combined consideration of these criteria has not been defined. The RMI provides a simple diagnostic index, suitable for use in clinical practice, which defines the optimal combination of diagnostic criteria. The ability of the RMI to distinguish between benign and malignant disease reflects the complementarity of ultrasound findings and serum CA 125 levels. Few patients with malignant disease and an elevated serum CA 125 level had low ultrasound scores, whilst most patients with benign disease and an elevated serum CA 125 level had ultrasound scores of 0 or 1 (figure 7.3). It was therefore possible to improve specificity with minimal sacrifice of sensitivity. Further refinement of the RMI to achieve greater diagnostic accuracy may be possible using additional tumour markers and by the application of Doppler blood flow imaging to ovarian tumours (Bourne et al 1989).

Various upper limits for the RMI combined higher levels of sensitivity and specificity than individual criteria alone (figure 7.2). The action line selected for clinical decision making will depend upon the balance between sensitivity and specificity appropriate to local resources. A low action line (eg RMI=50) may be preferable when limitations on referral for specialist care are minimal. Where the availability of specialist care is limited because of distance or resources, some degree of sacrifice in sensitivity to achieve high levels of specificity may be

appropriate. The action line can then be set at a higher RMI level (eg 75 or 200). A clinician selecting patients for specialist management in this way would expect to correctly classify approximately 90% of patients with a benign or malignant adnexal mass preoperatively.

The available evidence suggests that the outlook for ovarian cancer is influenced by the quality of the primary surgical procedure. Application of the RMI in clinical practice would provide a rational basis for specialist referral of patients with malignant disease prior to diagnostic surgery. A larger proportion of ovarian cancer patients would as a result undergo optimal staging and cytoreduction. Whether the introduction of the RMI into clinical practice will actually be translated into an improvement in prognosis for ovarian cancer patients is the subject of ongoing research.

CHAPTER 8

**THE SPECIFICITY OF CA 125 AS A
SCREENING TEST FOR EARLY STAGE OVARIAN CANCER**

8.1 INTRODUCTION

Serum CA 125 levels are elevated in a variety of physiological states, benign disorders and malignant conditions apart from ovarian cancer. In view of this apparent lack of specificity some authors have rejected the possibility that serum CA 125 measurement may have a role as a screening test for ovarian cancer. The results of the studies in chapter 4 and careful review of the literature suggest that such conclusions may not be justified. The incidence of ovarian cancer is low until the 5th decade and there is a steep rise in incidence after the menopause. These observations define a relatively high risk population which forms the logical target population for an ovarian cancer screening programme. As the majority of physiological states and benign disorders associated with an elevated serum CA 125 only occur in premenopausal women or are clinically apparent (eg liver failure), CA 125 measurement may achieve satisfactory specificity when used to screen postmenopausal women. In addition it may be possible to achieve high levels of specificity with relatively little sacrifice of sensitivity by the combination of serum CA 125 measurement with other diagnostic tests.

This study was performed to determine the specificity of serum CA 125 measurement alone and in combination with other diagnostic procedures as a screening test for ovarian cancer in apparently healthy postmenopausal women.

8.2 SUBJECTS AND METHODS

8.2.1 Subjects

Women over 45 years of age and with greater than 12 months amenorrhoea were invited to volunteer for the study by the following methods: (a) articles describing the project in the local and national press. (b) leaflets distributed by the occupational health departments of a number of companies collaborating with the project. Women wishing to participate were able to contact the screening clinic by telephone, post or through their company occupational health department.

The following eligibility criteria were strictly applied to women volunteering to participate.

- (1) Forty five years of age or older.
- (2) A history of more than 1 years amenorrhoea.
- (3) No past history of ovarian cancer.
- (4) No past history of bilateral oophorectomy.
- (5) Resident in the United Kingdom.
- (6) Not currently known to have active malignancy of any type. A malignancy last treated more than 1 year before volunteering and with no evidence of recurrence did not exclude participation.

Once eligibility was established appointments were sent by post along with a detailed fact sheet describing the study, the consent form and data sheet. Appointments were made for at least 3 months after initial contact with each volunteer in order to

avoid the screening clinic being used as a general practice surgery. If volunteers were found to be ineligible when they attended the clinic they were excluded at that time.

8.2.2 Methods

An ovarian cancer screening clinic was established in the Academic Unit of Obstetrics and Gynaecology at The London Hospital (Ethical Committee approval EC 903) and all volunteers were seen in this clinic. After giving informed consent volunteers completed a questionnaire and underwent a vaginal examination and venepuncture for CA 125 radioimmunoassay.

8.2.2.1 Vaginal examination

An abnormal examination was defined as a palpable pelvic mass of any size which could be clinically distinguished as being separate from the uterus and gastrointestinal tract.

8.2.2.2 CA 125 Radioimmunoassay

Blood samples were separated and stored as described in section 2.2. CA 125 radioimmunoassay was performed within 4 weeks of sample storage as described in section 2.1.1.1. A serum CA 125 level of 30 U/ml or more was defined as abnormal.

If both vaginal examination and serum CA 125 level were normal, volunteers were informed by post within 6 weeks of their initial visit. If either the vaginal examination or the serum CA 125 level were abnormal, volunteers were recalled for a real time ultrasound scan.

8.2.2.3 Ultrasound

Real time ultrasonography was performed via the abdominal route by a gynaecologist with 2 years ultrasound experience or a consultant radiologist, using a Dasonics DS.1 sector scanner with a 3.5MHz transducer. Ovarian diameter was measured in 3 dimensions and ovarian volume calculated using the formula for an ovoid as described by Campbell et al (1982). A volume greater than 8.8mls was defined as abnormal. If the result of the ultrasound scan was abnormal the patient was advised to consult her general practitioner who was informed independently of the scan report. Subsequent referral to a gynaecologist for further management was arranged by the general practitioner. Women with a normal ultrasound scan were followed up at 3 monthly intervals for one year with repeat serum CA 125 measurement and ultrasound examination at each visit. In addition a group of 30 women with an initial CA 125 less than 30 U/ml on initial sampling were randomly recruited for 3 monthly venepuncture for 1 year.

8.2.2.4 Follow up

All volunteers were followed up at 1 year after their initial visit by postal questionnaire. If no reply was received within 1 month further efforts to complete follow up were made by telephone, recorded delivery letters, through the next of kin and via the general practitioner. If a volunteer indicated that she had required medical attention since the initial visit details were obtained from the hospital or general practice involved.

8.2.2.5 TAG 72.3 and CA 15.3

Serum samples from volunteers with an initial CA 125 level greater than 20 U/ml were assayed retrospectively for TAG 72 and CA 15.3. Measurement was performed by radioimmunoassay as described in sections 2.1.2 and 2.1.3 respectively. Upper limits of 30 U/ml and 10 U/ml were assigned for CA 15.3 and TAG 72.3 respectively.

8.2.2.6 Statistical methods

The specificity of a single test was defined as the number of individuals with a negative test result in whom ovarian cancer did not develop within one year of the test (true negatives) divided by the total number of individuals in whom ovarian cancer did not develop within one year of the test. The specificity of a combination of tests was defined as the number of individuals with a negative test result to any one of the tests in whom ovarian cancer did not develop within 1 year of the test (true negatives) divided by the total number of individuals in whom ovarian cancer did not develop within one year of the test. A chi-squared test with Yates' correction was used for comparison of specificity. Exact confidence intervals from the binomial distribution were used to calculate confidence intervals for specificity.

8.3 RESULTS

8.3.1 Study population

During the 2 months following the initial request for volunteers over 2,000 offers to participate were received. Appointments were sent to the first 1050 women who appeared to fulfill the eligibility criteria. Thirty three women did not keep their appointment to attend the screening clinic. A further 7 were found to be ineligible on attendance (3 active malignancy, 4 premenopausal). A total of 1010 women were therefore recruited to the study over a 6 month period. The age distribution of the study population is shown in figure 8.1. The median age was 54.0 years (range 45-83 years). The number of years amenorrhoea reported by these women ranged from 1 to 33 years with a median of 4.9 years (figure 8.2). The mean number of pregnancies beyond 28 weeks in the past obstetric history of this population was 1.99 (range 0-9). Two hundred and twenty eight (22.6%) of the study population had undergone hysterectomy with conservation of one or both ovaries. Forty five women had a past history of malignancy (of which 18 had been treated for cancer of the breast), but at the date of recruitment had no evidence of recurrent disease.

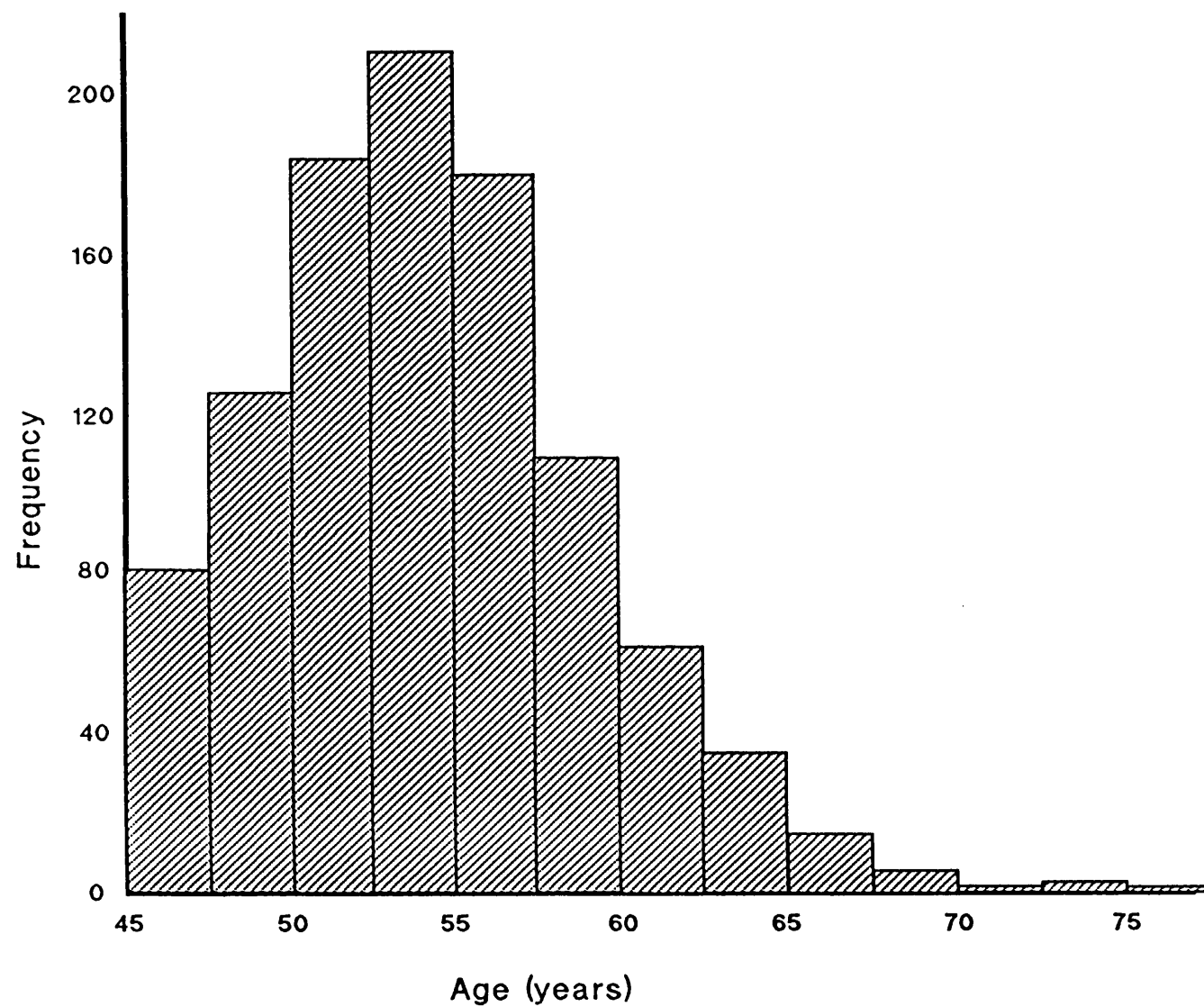


Figure 8.1. Age distribution of 1010 apparently healthy postmenopausal volunteers participating in this study.

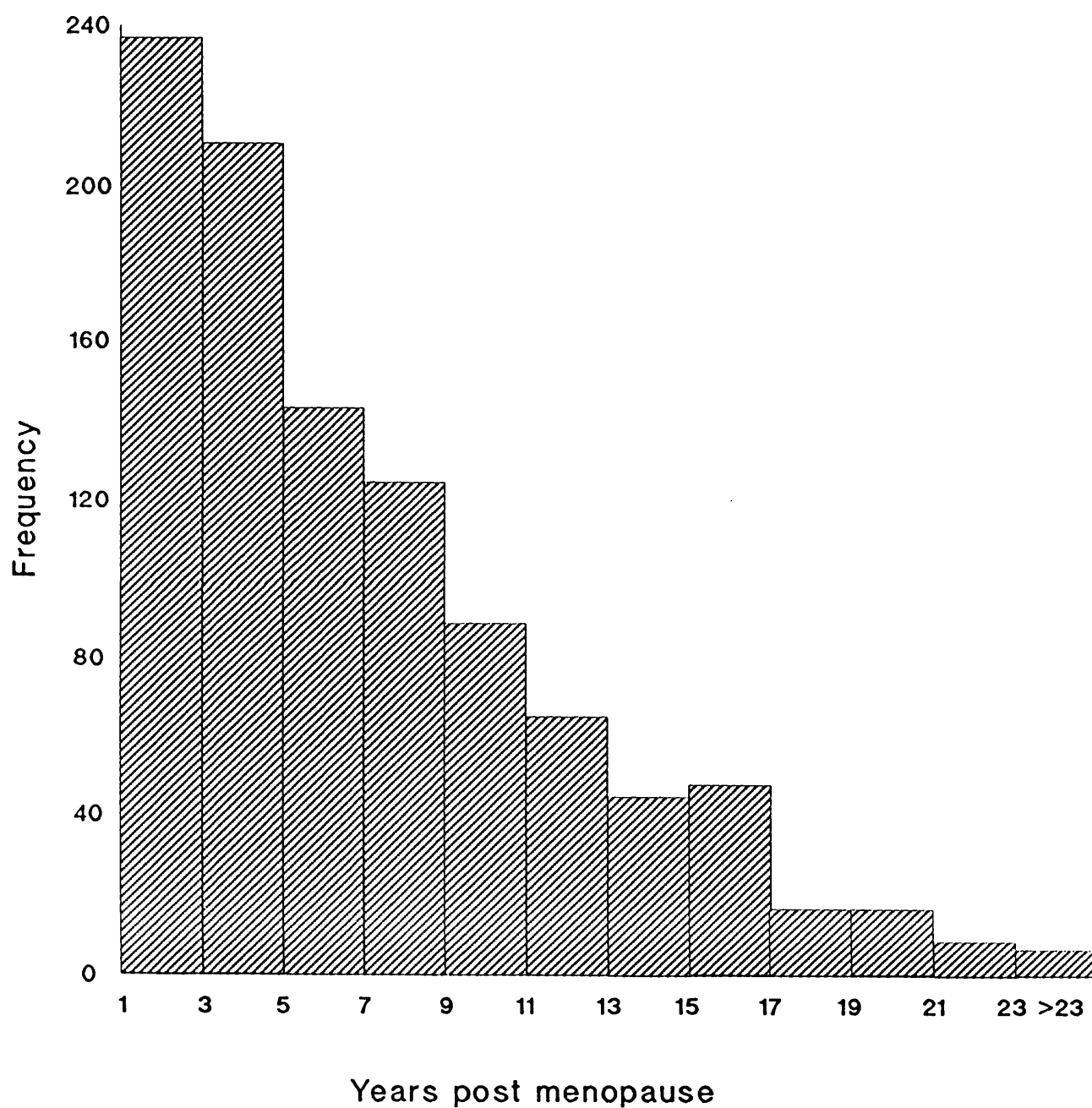


Figure 8.2. Number of years amenorrhoea amongst 1010 apparently healthy postmenopausal volunteers participating in this study.

8.3.2 Study findings

The serum CA 125 results for the 1010 women in the study, and their centile distribution are shown in Figure 8.3. Four women had serum CA 125 levels greater than 50 U/ml (60, 64, 101 and 112 U/ml). There was no correlation between serum CA 125 level and age, number of years amenorrhoea, parity, previous hysterectomy or past history of malignancy.

On the basis of the vaginal examination findings and serum CA 125 level the population has been divided into 4 groups (table 8.1):

Group I. CA 125 > 30 U/ml and vaginal examination abnormal (n=1)

A 55 year old nulliparous women was apparently healthy and asymptomatic on attending the study clinic and had no personal or family history of malignancy. Her serum CA 125 level was 32 U/ml and vaginal examination revealed a 10 cm diameter pelvic mass. Ultrasound examination demonstrated a multilocular pelvic mass measuring 10cm x 9cm x 9cm with solid elements. At laparotomy this mass was found to arise from the left ovary. A thorough surgical stage and histological examination identified the tumour as a stage Ia clear cell carcinoma of the left ovary. 11 days post-operatively her CA 125 level had decreased to 11 U/ml and 3 months postoperatively was 12 U/ml. There were no false positive results in this group.

Group II. CA 125 > 30 U/ml and vaginal examination normal (n=30)

Serum CA 125 levels ranged from 30 U/ml to 112 U/ml. There was no statistically significant difference between this group and the rest of the study population with respect to age, parity, years of amenorrhoea, history of previous hysterectomy or past history of malignancy.

Twenty-eight of these women had normal ultrasound scans and have subsequently been followed up at three monthly intervals for at least 12 months. During follow up, repeat ovarian ultrasound findings have remained normal and there has been no evidence of conditions which may have accounted for their elevated serum CA 125 levels. Serum CA 125 levels in these 28 women remained greater than 30 U/ml in 16 at 3 months, in 12 at 6 months, in 10 at 9 months and in 7 at 12 months.

The other two women in this group had abnormal ultrasound scans. One of these women (serum CA 125 60 U/ml), was thought to have a left ovarian cyst of volume 13ml on ultrasound scan and at surgery was found to have a fimbrial cyst. Ultrasound examination of the second woman (serum CA 125 32.7 U/ml) suggested the presence of a 75ml right ovarian cyst. At laparotomy both ovaries appeared normal but dense adhesions were noted tethering a distended caecum to the right adnexa and pelvic side wall which appeared to be related to her previous hysterectomy and ovarian cystectomy.

Group III. CA 125 < 30 U/ml and vaginal examination abnormal
(n=27)

Seventeen women had normal ovarian volume measurements on ultrasound scan. In 8 of these 17 women ultrasound findings were consistent with the diagnosis of fibroids ranging from 3cm to 6cm in diameter. In the remaining 9 cases with a normal scan following abnormal vaginal examination, there was no apparent explanation for this discrepancy.

The remaining ten women had abnormal ultrasound scan findings. Six were found to have benign ovarian cysts at laparotomy and one a fimbrial cyst. The volume of these cysts as measured by ultrasound ranged from 75mls to 600 mls. Two women were found to have no pelvic abnormality at laparoscopy. In both cases the apparent abnormality on ultrasound scan was of small volume (13ml and 19ml). One woman with an ultrasound report of a unilocular 75ml cyst of the right side of the pelvis has declined surgery and is apparently well one year later.

Group IV. CA 125 < 30 U/ml and vaginal examination normal
(n=952)

Nine hundred and fifty two women had a normal examination and a normal serum CA 125 level.

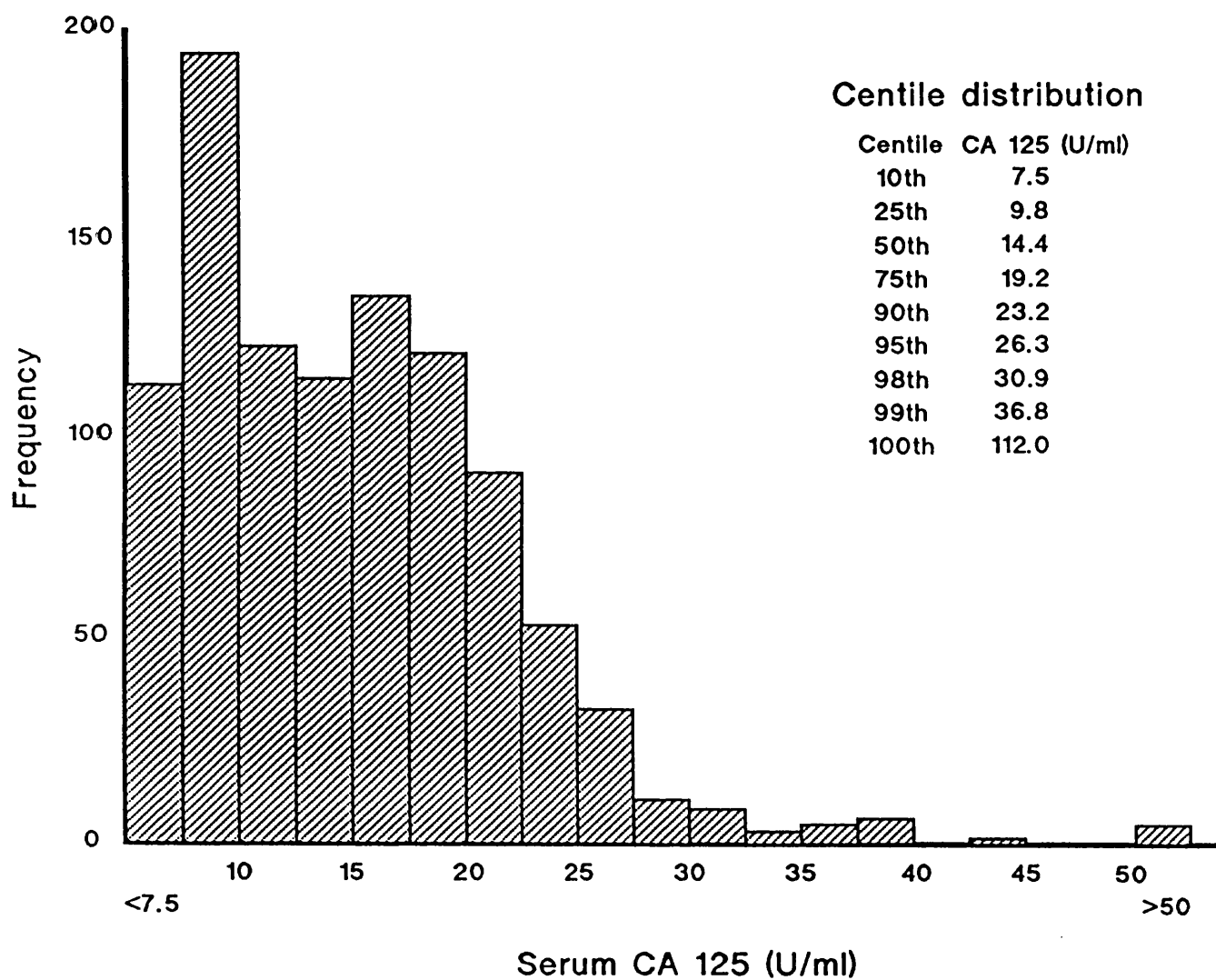


Figure 8.3. Serum CA 125 results and centile distribution amongst 1010 apparently healthy postmenopausal women participating in this study.

	CA 125 positive	CA 125 negative
Vaginal examination positive	Group I: n=1 (true positive) Ultrasound +ve 1 Ultrasound -ve 0	Group III: n=27 (false positive) Ultrasound +ve 10 Ultrasound -ve 17
Vaginal examination negative	Group II: n=30 (false negative) Ultrasound +ve 2 Ultrasound -ve 28	Group IV: n=952 (true negative) Ultrasonography not performed

Table 8.1. Summary of study results. Note that the false positives to CA 125 did not overlap with those from vaginal examination.

8.3.3 Follow up

All 1010 volunteers were followed up at between 12 and 18 months after their initial visit. No cases of ovarian cancer were documented in groups II, III or IV.

The results of serial 3 monthly CA 125 measurement are illustrated in figure 8.4. During follow up serum CA 125 levels in women with initially elevated levels fell toward the normal range. In 30 women with an initial CA 125 over 30 U/ml there was a decrease in mean CA 125 levels at each 3 monthly follow up. CA 125 levels in these women remained elevated above 30 U/ml in 16 women at 3 months, 12 at 6 months, 10 at 9 months and 7 at 12 months. Five women had an initial CA 125 greater than 50 U/ml. CA 125 levels had declined over the follow up period in 4 of these 5 patients. The remaining patient had a persistently elevated serum CA 125 (110 U/ml, 120 U/ml and 102 U/ml at 3, 6 and 9 months respectively) and 3 monthly ultrasound scans consistently revealed a thickened endometrial interface. This patient underwent curettage 12 months after initial recruitment and histological examination revealed endometrial hyperplasia without atypia. At 15 and 18 month follow up her serum CA 125 levels were 21 U/ml and 18 U/ml respectively. The other 29 women with an initial CA 125 > 30 U/ml were apparently well with no evidence of malignancy at 12 month follow up.

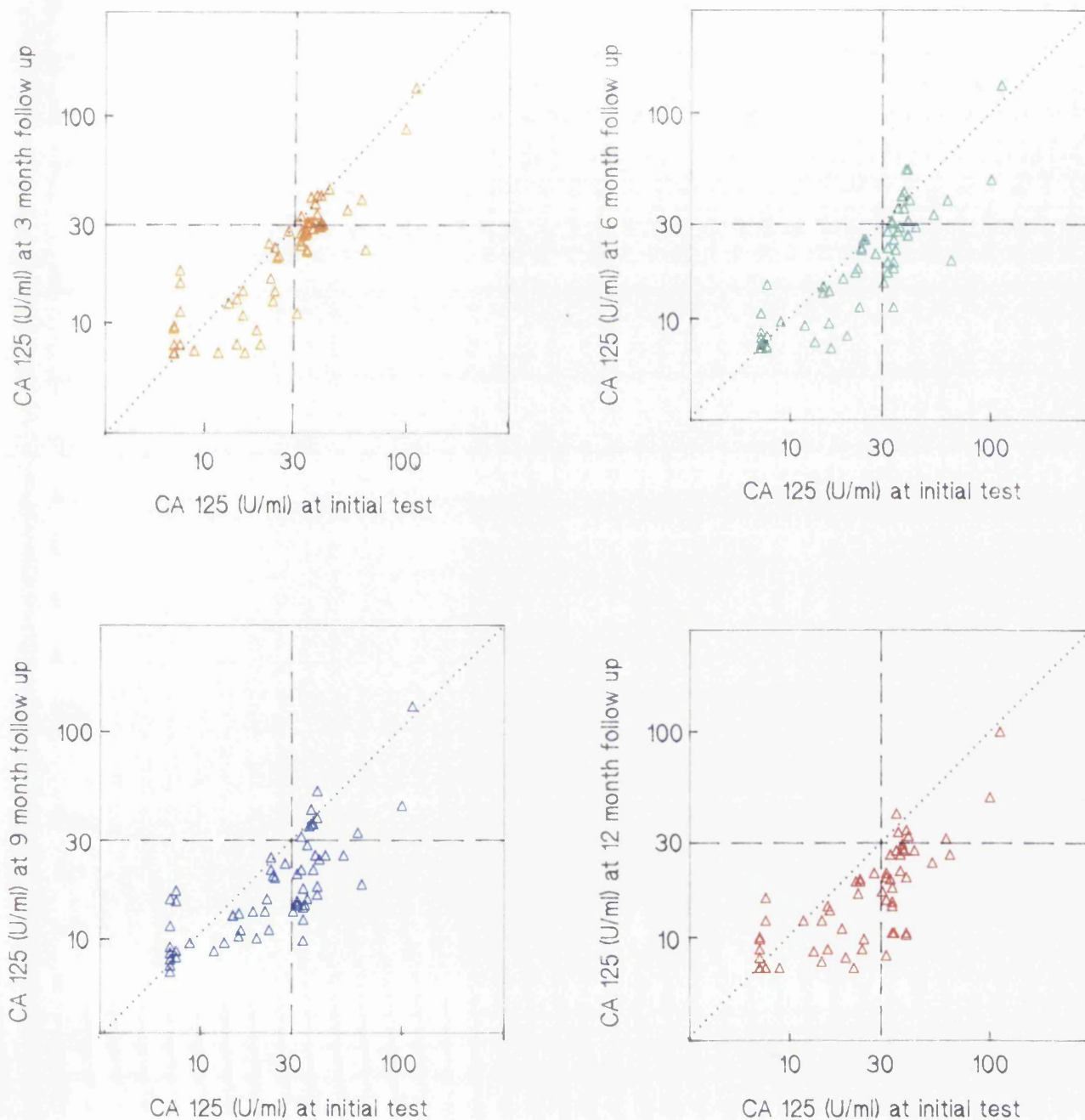


Figure 8.4. Serum CA 125 levels at 3, 6, 9 and 12 months after initial screen in relation to initial result in 30 apparently healthy postmenopausal women with initial levels < 30 U/ml and 30 apparently healthy postmenopausal women with initial levels > 30 U/ml.

8.3.4 TAG 72.3 and CA 15.3

Of the 1010 volunteers 217 had a serum CA 125 above 20 U/ml. The volunteer found to have stage I ovarian cancer had TAG 72.3 and CA 15.3 results of 3.4 U/ml and 35.2 U/ml respectively. Of the other 216, CA 15.3 levels were above 30 U/ml in 66/216 and TAG 72.3 above 10 U/ml in 2/216.

8.3.5 Specificity

The specificity of the tests used in this study alone and in combination is shown in table 8.2. The specificity of serum CA 125 measurement for ovarian cancer using an upper limit of 30 U/ml was 97.0%. Figure 8.5 illustrates the relationship between the upper limit of normal adopted for serum CA 125 and the specificity for ovarian cancer in the 1010 women recruited to this study.

Several combinations and interpretations of tests were assessed in order to improve overall specificity (table 8.3). Raising the upper limit for CA 125 increased specificity to 99.5% at 50 U/ml and 99.7% at 70 U/ml but brought the one patient with ovarian cancer within the normal range. Definition of a positive test as a serum CA 125 level greater than 30 U/ml at both the initial test and at 3 month follow up achieved a specificity of 98.4%.

<u>Test</u>	<u>Specificity</u>	95% confidence limits
CA 125	97.0%	95.8% - 98.0%
VE	97.3%	96.2% - 98.3%
VE & USS	99.0%	98.1% - 99.5%
CA 125 & USS	99.8%	99.3% - 99.9%
CA 125 & VE	100.0%	99.6% -100.0%
CA 125, VE & USS	100.0%	99.6% -100.0%

Table 8.2. Specificity for ovarian cancer amongst premenopausal women (n=1010) of vaginal examination, serum CA 125 and combinations of these tests with ultrasound scanning.

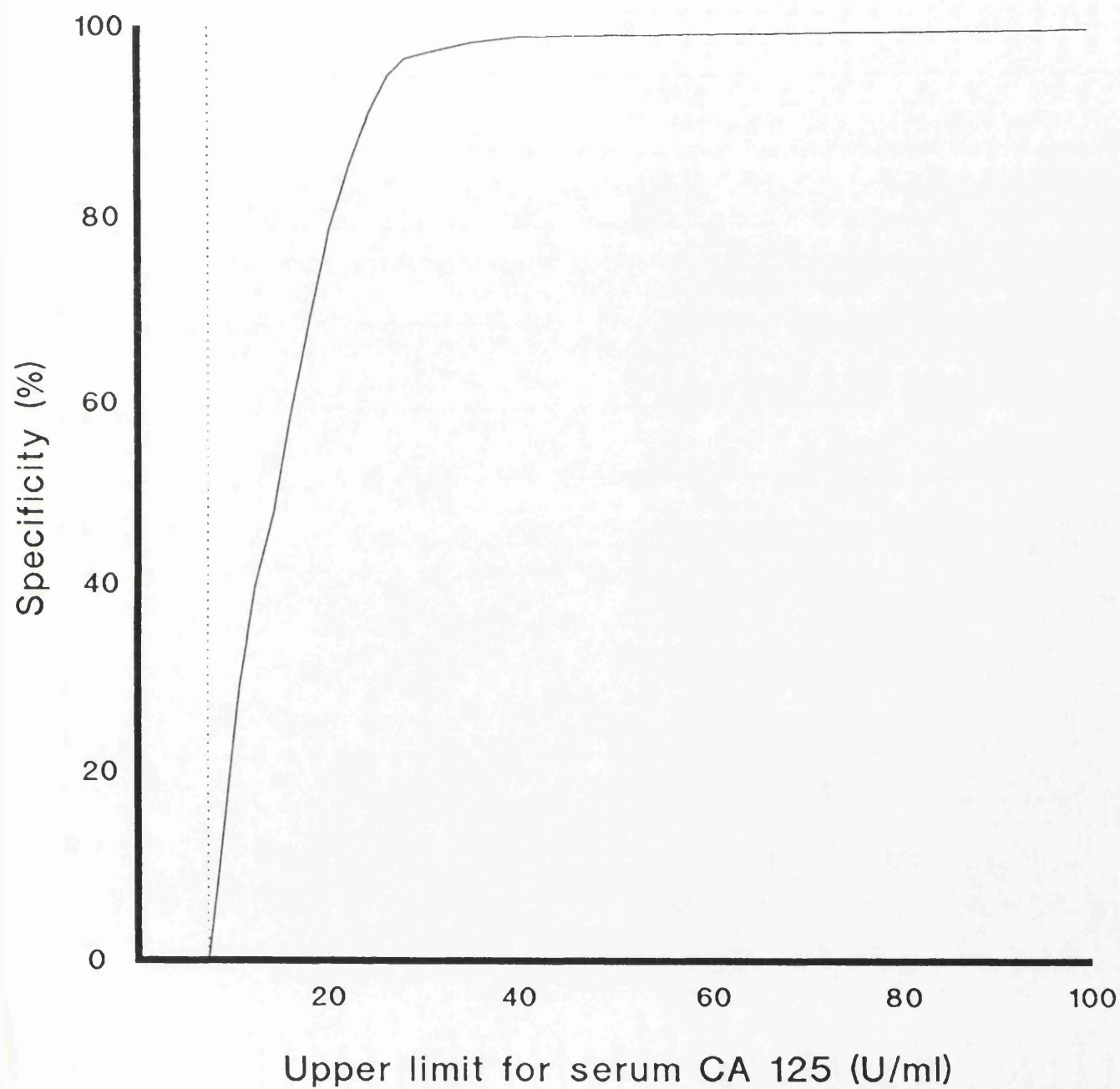


Figure 8.5. Relation between upper limit for serum CA 125 and specificity for ovarian cancer in 1010 postmenopausal women.

Initial CA 125	Specificity			
	A	B	C	D
CA 125 > 20 U/ml	78.6%	-----	93.5%	-----
CA 125 > 30 U/ml	97.0%	98.4%	98.9%	99.6%
CA 125 > 50 U/ml	99.5%	99.6%	99.9%	100.0%
CA 125 > 70 U/ml	99.7%	99.8%	100.0%	100.0%

A = CA 125 alone, single point estimation.

B = A + CA 125 above 30 U/ml on 2nd estimation at 3 months.

C = A + either CA 15.3 > 30 U/ml or TAG 72.3 > 10 U/ml.

D = All conditions in A, B and C must be satisfied.

Table 8.3. Specificity for screening for ovarian cancer amongst apparently healthy postmenopausal women with various combinations of CA 125, TAG 72.3 and CA 15.3.

The use of ultrasound as a secondary investigation to CA 125 increased the overall specificity to 99.8%. The combination of serum CA 125 measurement and vaginal examination yielded one true positive result and no false positives (specificity 100%). Vaginal examination alone achieved a specificity of 97.3% which was increased to 99.0% by combination with ultrasound. The specificity of ultrasound examination amongst the group of women with a serum CA 125 > 30 U/ml was greater than amongst the group of women with an abnormal vaginal examination (93.3% versus 63.0%, $\chi^2=6.6$, $p=0.014$).

Definition of a positive result as an elevated serum CA 125 in combination with either a CA 15.3 > 30 U/ml or a TAG 72.3 > 10 U/ml achieved a specificity of 98.9% (table 8.3). Neither of the patients with a CA 125 > 30 U/ml and a false positive ultrasound had elevated levels of CA 15.3 or TAG 72.3. If a serum CA 125 > 30 U/ml at initial and 3 month test was required in addition to either a CA 15.3 > 30 U/ml or a TAG 72.3 > 10 U/ml test specificity was increased further to 99.6%.

8.4 DISCUSSION

8.4.1 Study population

The response to requests for volunteers for this project was large and immediate reflecting the current widespread interest in screening and preventative medicine. The complete compliance with recall for ultrasound and follow up suggests that the study design was acceptable to volunteers, although the high motivation of this self selected study group is acknowledged.

8.4.2 Study findings

A total of 11 women with benign disorders or no abnormality underwent laparoscopy or laparotomy. Two of these false positive results were attributable to the combination of CA 125 and ultrasound. It is unlikely that the false positive result due to the presence of a fimbrial cyst could have been avoided. However, it is possible that false positive results due to pelvic adhesions involving fluid filled loops of bowel could be prevented by the performance of a repeat scan before recommending diagnostic surgery. The other nine false positive results were attributable to the combination of vaginal examination and ultrasound. It is possible that surgery could have been avoided in the 2 cases found to have no pelvic abnormality at laparoscopy, by serial scanning of what appeared to be small cysts over a period of time. In the remaining 7 cases the ultrasound impression of a cystic pelvic structure was correct

but all were benign (6 benign ovarian cysts and 1 fimbrial cyst). All of these cysts appeared unilocular, unilateral and without solid areas on ultrasound. It may therefore have been possible to classify them as benign on the basis of criteria such as those used in chapter 7. However, as the malignant potential of benign ovarian cysts is unclear it would not be justifiable to simply observe such pathology once detected and the management of these cases would not have been altered. A high false positive rate due to benign disease is therefore an inevitable consequence of screening with tests such as ultrasound and vaginal examination which reflect ovarian enlargement and cyst formation. Such tests will yield positive results in the presence of both benign and malignant cysts. Serum CA 125 levels although to some extent affected by ovarian size, reflect a different aspect of ovarian function and are only elevated in a small proportion of benign ovarian tumours. This explains the finding that ultrasound has a higher specificity when used amongst women with a raised CA 125 than women with an abnormal vaginal examination. In the absence of convincing evidence that benign ovarian cysts have premalignant potential, vaginal examination and ultrasound scanning either alone or in combination are not acceptable as screening tests for ovarian cancer.

8.4.3 Specificity

The requirement of high specificity in screening for ovarian cancer has been discussed previously (chapter 1). In particular it was noted that at current incidence rates an annual screening test for ovarian cancer, even with 100% sensitivity would require 99.6% specificity in order to achieve a positive predictive value of 10% (ie. 9 false positive tests for each case of ovarian cancer identified). As even a small fall in specificity would produce a relatively large decrease in the predictive value and the consequence of a positive screening test is surgical intervention, extremely high specificity is an essential requirement of any screening test for ovarian cancer. It was anticipated in the design of the prospective study that none of the three tests used alone would have acceptable specificity for ovarian cancer. This was confirmed for CA 125 and vaginal examination (table 8.2) and has previously been demonstrated for ultrasound (Goswamy 1987, Bhan et al 1987, Andolf et al 1986). The study was therefore designed to achieve high specificity by combining the tests and it was cost effective to employ the most expensive test (ultrasound) on a secondary basis. This strategy made possible a comparison of the specificity of all possible combinations except ultrasound alone. Serial CA 125 measurement and retrospective analysis of TAG 72.3 and CA 15.3 provided information concerning other possible approaches to improving specificity.

The relationship between the upper limit of normal for CA 125 and the specificity of serum CA 125 measurement is shown in figure 8.5. The curve suggests that the most appropriate choice of upper limit for CA 125 as a screening test for ovarian cancer is between 23 U/ml and 30 U/ml. Above this range relatively little increase in specificity is gained for an increment in CA 125 (and hence potential sacrifice in sensitivity), whilst below this there is a considerable loss of specificity for a given incremental decrease in CA 125. The specificity of CA 125 alone using an upper limit between 23 U/ml and 30 U/ml was only 90-97%. To achieve adequate specificity using CA 125 measurement alone as a screening test would require an upper limit of at least 60 U/ml with consequent loss of sensitivity. Addition of a requirement for two CA 125 levels > 30 U/ml at initial visit and 3 month follow up would only increase the specificity to 98.4%. In order to achieve adequate specificity a secondary diagnostic test for women with a raised CA 125 is required. This role could be fulfilled by combination with vaginal examination (specificity 100%) or ultrasound (specificity 99.8%). The use of two other tumour markers assessed in this context (CA 15.3 and TAG 72.3) did produce an increase in specificity but only to 98.9%. The rather complicated requirement of two CA 125 results >30 U/ml at initial visit and 3 month follow along with elevation of either TAG 72.3 or CA 15.3 did however achieve a specificity of 99.6%.

Recent reports suggest that a proportion of false positive serum CA 125 results may be due to the production of human antibodies against murine immunoglobulins by some individuals. Mogensen and

Moller (1988) found that addition of murine serum to the human serum sample assayed, eliminated elevations of serum CA 125 in 3 of 6 healthy individuals but not in 3 patients with ovarian cancer. Klug et al (1988) characterised the false positive CA 125 activity in the serum of a healthy individual with apparent serum CA 125 levels varying between 150 U/ml and 450 U/ml. On the basis of immunoaffinity, size chromatography, western blotting and murine antibody blocking studies they concluded that the false positive activity was due to a specific human IgM antibody to the murine monoclonal antibody OC125. The relevance of these observations to screening a large population of apparently healthy individuals is clear. Recognition of the false positive results arising from this phenomena may provide a method for improving the specificity of serum CA 125 measurement without any sacrifice of sensitivity for ovarian cancer.

8.4.4 Other requirements of a screening test

In addition to the requirement for high specificity the demonstration that a screening programme for ovarian cancer is of value will require evidence of sensitivity for early stage disease, of cost effectiveness and ultimately of a significant reduction in mortality in a randomised controlled study. No test which is currently available has demonstrated sufficient specificity to be considered potentially valuable when used on its own. Until screening techniques with greater individual specificity are developed, further investigation of screening for ovarian cancer amongst postmenopausal women should concentrate on

the combinations of tests which have been shown in this study to achieve acceptable specificity. Although the combination of serum CA 125 measurement with vaginal examination achieved a specificity of 100%, the value of this approach is likely to be limited by the lack of sensitivity of vaginal examination. The combination of serum CA 125 measurement with ultrasound examination offers the most hope of a specific and sensitive screening test for the early detection of ovarian cancer.

CHAPTER 9

A PHASE 2 STUDY OF SCREENING FOR OVARIAN CANCER WITH THE COMBINATION OF CA 125 AND ULTRASOUND

INTRODUCTION

The initial study of screening for ovarian cancer (Chapter 8) indicated that acceptable levels of specificity for the detection or exclusion of ovarian cancer can be achieved on screening a population of apparently healthy postmenopausal women with a combination of CA 125 and ultrasound. On the basis of these findings a phase 2 study was commenced to further evaluate this screening protocol. The aims of the phase 2 study were threefold: First, to obtain an estimate of the lead time in diagnosis achieved by detecting ovarian cancer with this screening protocol; secondly, to assess the sensitivity of the screening protocol for preclinical ovarian cancer in postmenopausal women; and thirdly, to investigate the appropriate screening interval for ovarian cancer using this screening protocol. This chapter is an interim report of the results of the initial screen in the first 20,000 women recruited for the phase 2 study. As recall and follow up for the 2nd phase study is continuing the results presented here relate in the main to the first aim stated above.

9.2 SUBJECTS AND METHODS

9.2.1 Subjects

Women over 45 years of age and with greater than 12 months amenorrhoea were invited to volunteer for the study by the following methods:

- (a) Articles describing the project in the national press.
- (b) Leaflets distributed by the occupational health departments of companies collaborating with the project.
- (c) Postal invitations from the age-sex register of 40 general practices from England, Scotland and Wales collaborating with the project.

The eligibility criteria were the same as described in section 8.2.1 for the phase 1 study. Once eligibility was established appointments were sent by post along with a detailed fact sheet describing the study, the consent form and data sheet. Appointments were made for at least 3 months after initial contact with each volunteer in order to avoid the screening project being used as an alternative to a general practice surgery. If volunteers were found to be ineligible when they attended for their registration visit they were excluded at that time.

Volunteers in group (a) attended the Ovarian Cancer Screening Clinic at the London Hospital for their registration visit. For volunteers in groups (b) and (c) the registration visit was arranged at their place of work or general practitioners surgery respectively.

9.2.2 Methods

After giving informed consent volunteers completed a questionnaire and underwent venepuncture for CA 125 radioimmunoassay (London Hospital Ethics Committee approval EC 903).

9.2.2.1 CA 125 Radioimmunoassay

Blood samples were separated and stored as described in section 2.2. The CA 125 radioimmunoassay was performed within 4 weeks of sample storage as described in section 2.1.1.1. A serum CA 125 level of 30 U/ml or more was defined as abnormal.

If the serum CA 125 level was normal, volunteers were informed by post within 6 weeks of their registration visit. If the serum CA 125 level was abnormal, volunteers were recalled for a real time ultrasound scan.

9.2.2.2 Ultrasound

Real time ultrasonography was performed via the abdominal route either; (a) at the London Hospital by a gynaecologist with 2 years ultrasound experience or a consultant radiologist or (b) by a consultant radiologist at another hospital collaborating with the project. The measurements performed were as described in section 8.2.2.3. Ultrasound findings were classified as follows:

- (i) Abnormal; ovarian volume greater than 8.8 ml.
- (ii) Equivocal; ovarian volume less than or equal to 8.8 ml but abnormal ovarian morphology.
- (iii) Normal; ovarian volume less than or equal to 8.8 ml.

If the result of the ultrasound scan was abnormal the patient was advised to consult her general practitioner who was informed independently of the scan report. Subsequent referral to a gynaecologist for further management was arranged by the general practitioner. Women with a normal ultrasound scan were followed up at 3 monthly intervals with repeat serum CA 125 measurements. In this group repeat ultrasound scans were performed if serum CA 125 levels doubled between consecutive samples or remained greater than 100 U/ml in two consecutive samples. Women with an equivocal ultrasound report attended at 3 monthly intervals for a repeat ultrasound scan and serum CA 125 estimation for up to 1 year. If during follow up a repeat ultrasound scan was classified as normal or abnormal further management was as described above for volunteers with an initially normal or abnormal scan.

9.2.2.3 Follow up

At the registration visit volunteers were randomly allocated to attend for further screening visits at intervals of 1 year, 2 years or 3 years after their registration visit. If a volunteer had required medical attention since the registration visit details were obtained from the hospital or general practice involved. The screening procedure on follow up remained the same as at the registration visit described in sections 9.2.2.1 and 9.2.2.2.

9.3 RESULTS

9.3.1 Study population

The first 20,000 volunteers were recruited over a 2 year period. 7,000 attended directly at the London Hospital, 4,000 participated at work whilst the remaining 9,000 attended their general practitioners surgery. The age distribution of the study population is shown in table 9.1. The median age was 56 years (range 45-85 years). The number of years amenorrhoea reported by these women ranged from 1 to 43 years with a median of 6.2 years. The mean number of pregnancies beyond 28 weeks in the past obstetric history of this population was 2.2 (range 0-14). Four thousand four hundred and fifty (22.8%) of the study population had undergone hysterectomy with conservation of one or both ovaries. Eight hundred and ninety two women had a past history of malignancy (of which 403 had been treated for cancer of the breast), but at the date of recruitment had no evidence of recurrent disease.

9.3.2 Study findings

The distribution of CA 125 results in the study population is summarised in figure 9.1. On the basis of the results of their initial screening visit the population has been divided into 3 groups (figure 9.2).

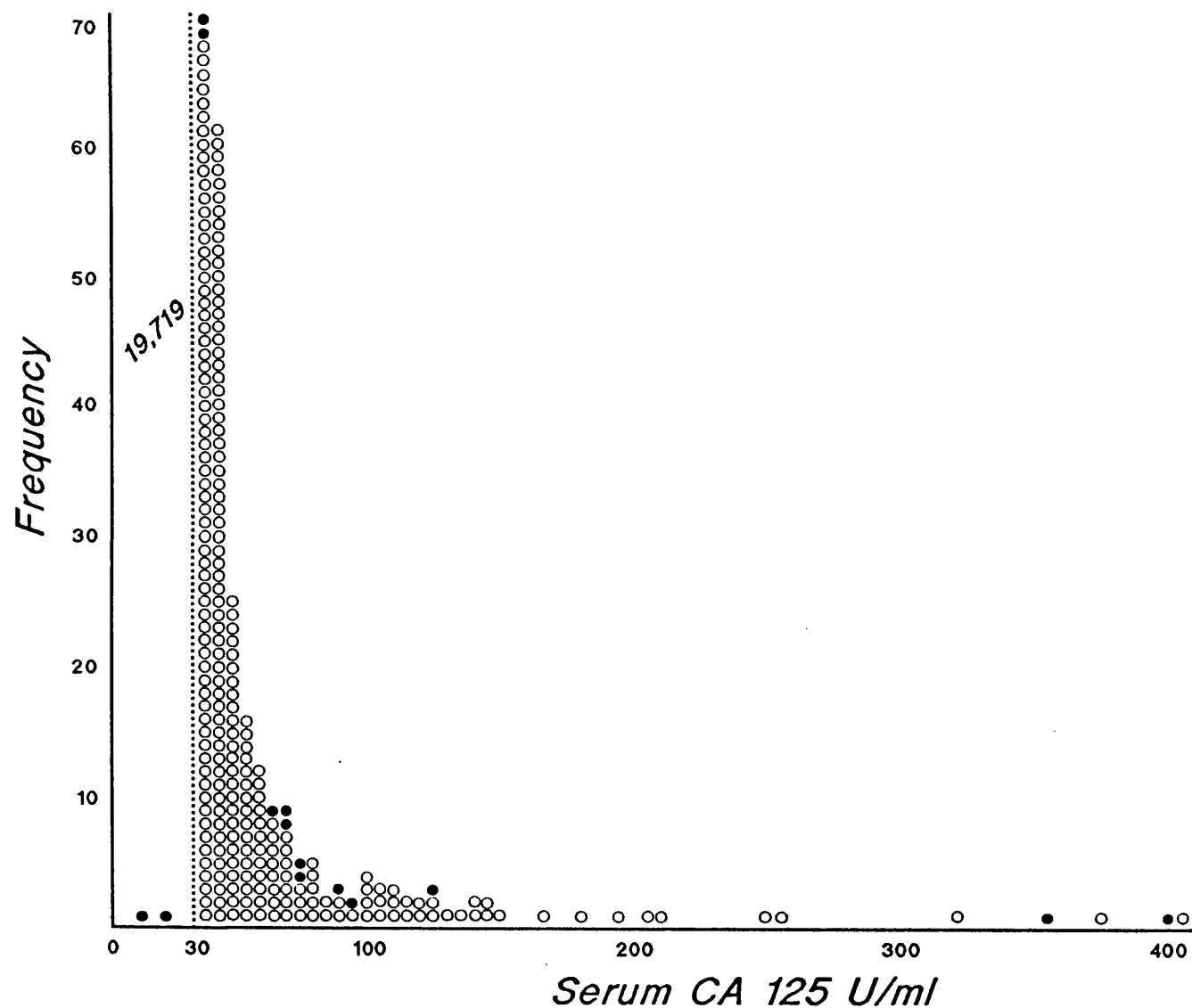


Figure 9.1. Distribution of serum CA 125 levels in 20,000 apparently healthy postmenopausal volunteers. Volunteers with a serum CA 125 level < 30 U/ml not diagnosed as having ovarian cancer at the time of this report are not represented by individual symbols ($n = 19,717$). Solid symbols = volunteers with a diagnosis of ovarian cancer ($n = 14$). Open symbols = volunteers with serum CA 125 > 30 U/ml not diagnosed as having ovarian cancer ($n = 269$).

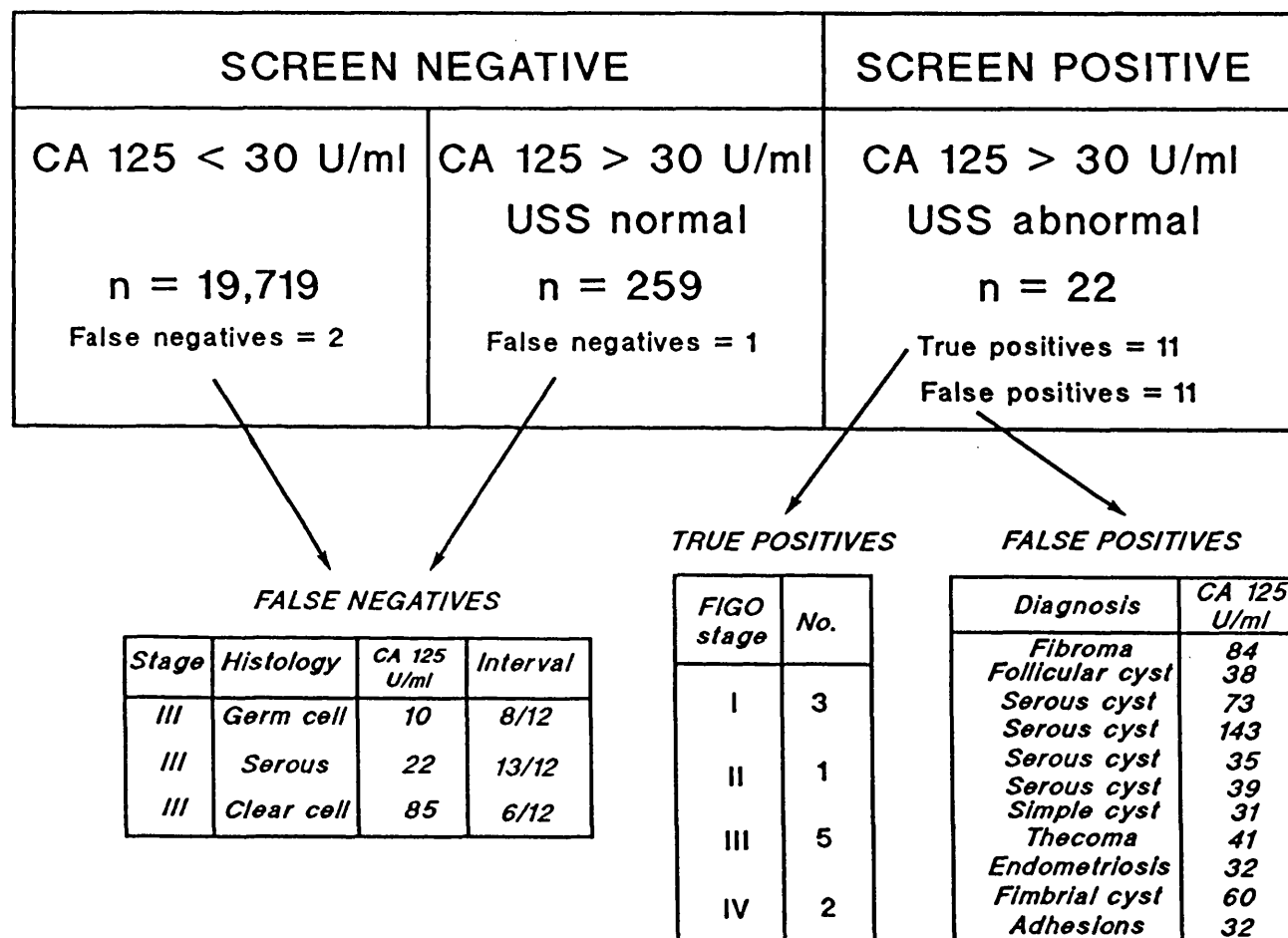


Figure 9.2. Summary of study findings. Volunteers have been divided into screen negative and screen positive groups on the basis of their CA 125 level and ultrasound findings. The details of patients with false negative and true positive results are shown.

Group I. Screen negative: CA 125 < 30 U/ml (n=19,719)

Of the 20,000 volunteers 19,719 had serum CA 125 levels less than 30 U/ml. At the time of this report a subsequent diagnosis of ovarian cancer had been made in 2 of these patients. A diagnosis of stage III germ cell tumour was made 8 months after the registration visit of a volunteer with an initial serum CA 125 level of 10 U/ml. A diagnosis of stage III serous cystadenocarcinoma was made 13 months after the registration visit of a volunteer with an initial serum CA 125 level of 22 U/ml.

Group II. Screen negative: CA 125 > 30 U/ml (n=259)

The ultrasound findings were normal in 257 volunteers with a CA 125 level > 30 U/ml. Two other volunteers with a serum CA 125 level > 30 U/ml declined to attend for an ultrasound scan and have therefore been classified as screen negative. One of these volunteers with an initial serum CA 125 level of 85 U/ml presented with stage III clear cell carcinoma 6 months after her registration visit.

Group III. Screen positive (n=22)

Laparoscopy or laparotomy was performed on 22 volunteers with an elevated serum CA 125 level and either abnormal ultrasound findings or equivocal findings which became abnormal on follow up at 3, 6 or 9 months. Eleven of these volunteers had benign pelvic pathology (figure 9.2). The remaining 11 screen positive volunteers were found to have invasive epithelial ovarian cancer at laparotomy. Details of the 11 true positive cases are summarised in figure 9.3.

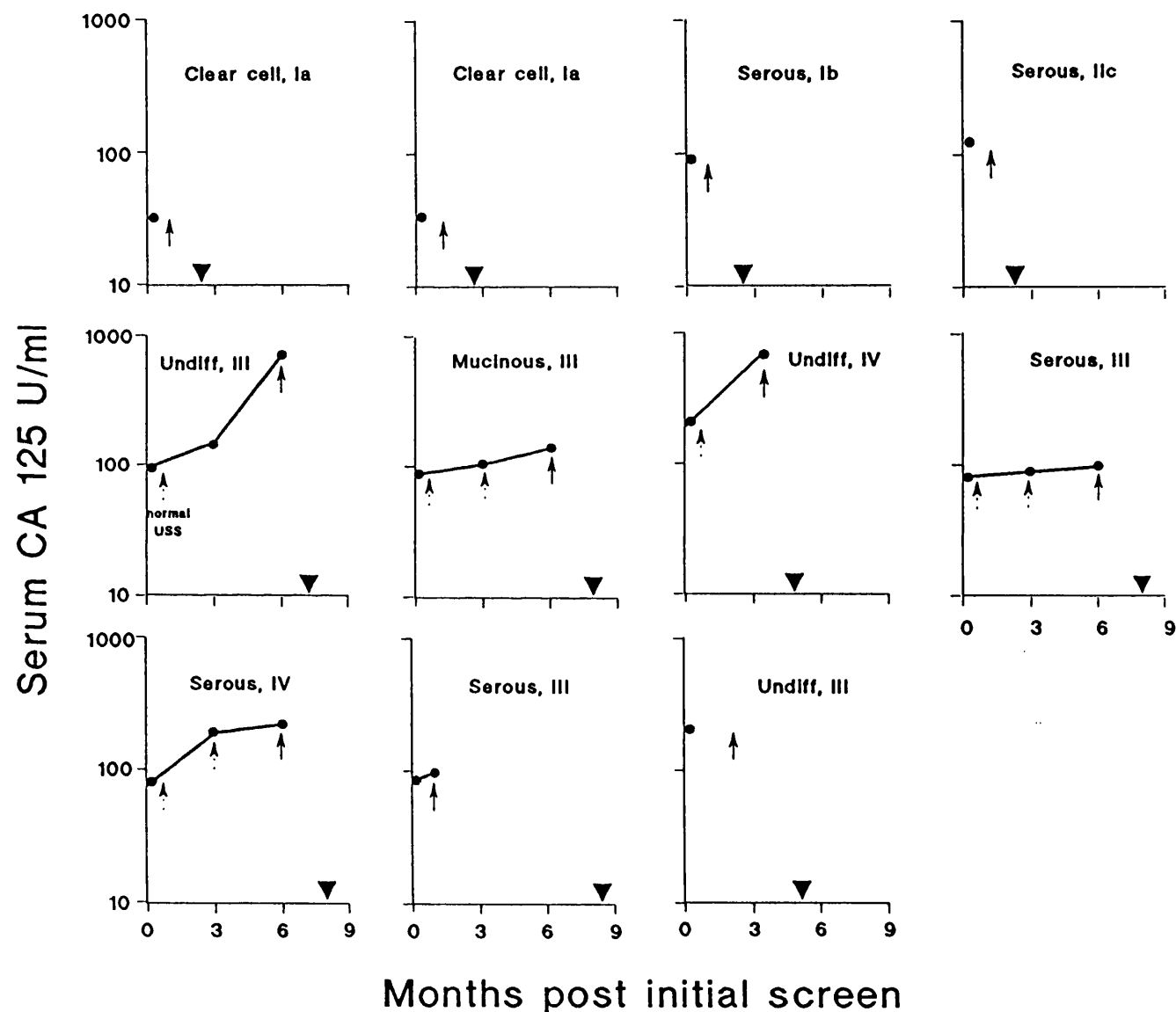


Figure 9.3. Histological findings, FIGO stage, serum CA 125 levels and time course of the diagnostic process in 11 cases of ovarian cancer detected by the screening programme. (Dotted arrow = ultrasound findings equivocal, solid arrow = ultrasound findings abnormal, triangle = time of surgical diagnosis).

9.3.3 Years of cancer detected

The expected incidence of ovarian cancer for the study population can be estimated from OPCS cancer incidence data (OPCS 1983), table 9.1. Table 9.1 also lists the number of cases of ovarian cancer actually detected in each age group. A total of 11 cases were detected by the screening programme in a population with an expected incidence of 7.3 cases per year.

9.3.4 Test performance

Table 9.2 summarises the performance of the screening programme at the time of this report. The sequential combination of CA 125 and ultrasound achieved a specificity of 99.95%, a positive predictive value of 50.0% and an apparent sensitivity (with follow up still in progress), of 78.6%. The comparable figures for CA 125 alone were 98.65%, 14.6% and 85.7%.

Age Group (years)	No. of Volunteers	Incidence of ovarian cancer Rate/100,000*	Expected cases/year	Actual cases
45-49	2,308	20.5	0.47	1
50-54	5,818	33.0	1.92	2
55-59	6,459	36.4	2.35	4
60-64	3,224	46.7	1.51	2
65-69	1,526	47.7	0.73	1
70-74	467	46.7	0.22	1
75-79	115	47.9	0.06	0
80-84	81	48.7	0.04	0
> 84	2	43.0	0.00	0
Total	20,000	----	7.30	11

Table 9.1. Age distribution, expected incidence of ovarian cancer (OPCS 1983*) and actual incidence of ovarian amongst the 20,000 volunteers.

	CA 125 & Ultrasound		CA 125 alone	
	Positive result	Negative result	Positive result	Negative result
Ovarian Cancer present	11 (a)	3 (b)	12 (a)	2 (b)
Ovarian Cancer absent	11 (c)	19,975 (d)	270 (c)	19,716 (d)
Specificity $d / (d + c)$	99.95%		98.65%	
Detection rate $a / (a + b)$	78.6%		85.7%	
Positive predictive value $a / (a + c)$	50.0%		14.6%	

Table 9.2. Summary of the performance of CA 125 alone and CA 125 combined sequentially with ultrasound in screening for ovarian cancer amongst 20,000 apparently healthy postmenopausal women.

9.4 DISCUSSION

The results described in this chapter represent a preliminary report of the initial prevalence screen for the phase 2 study. Follow up and attendance for subsequent screens are currently underway and will provide further information about the screening programme on completion. These preliminary results have confirmed the high specificity and positive predictive value of the screening protocol as well as providing a basis for estimating the lead time achieved compared to clinical presentation.

Figure 9.4 is a model of the progress of ovarian cancer from a preinvasive phase (A) to the development of symptoms of invasive disease (E). Between these extremes are phases B and C during which the cancer is invasive but screening test negative and positive respectively. The model assumes that the screening test is negative in the preinvasive phase and incorporates a separate pathway (D) for cases in which the screening test remains negative at the time of clinical presentation. For CA 125 pathway D would account for approximately 15% of patients (see chapter 1, table 1.2).

The expected incidence of symptomatic invasive ovarian cancer in phase E for the study population is approximately 7.3 per year (table 9.1). If the proposed model is valid the incidence of symptomatic invasive ovarian cancer in phase E must approximate to the incidence of disease in phase A, in phase B and in phase C and phase D combined. Furthermore all ovarian cancers detected by the screening programme must be in phase C at the time of

detection as symptomatic volunteers were excluded at registration and volunteers in other phases (A, B and D) would be screen negative. The length of time spent in phase C can be therefore be estimated from the results of this study. The expected number of incident cases entering phase C is estimated by the expected incidence for phase E (7.3 cases/year) minus the expected incidence for phase D (0.15×7.3 cases/year) = 6.2 cases/year. The length of time spent in phase C is therefore estimated by the actual number of cases detected (11) divided by the number of cases expected each year (6.2). The results of this study suggest that the length of time spent in phase C is approximately 1.8 years ($11/6.2$).

The validity of this estimate of the length of time in phase C is dependent the assumption that the age related incidence of ovarian cancer in the study population is similiar to the national incidence for each age group provided by OPCS data. It is likely that women with a family history of ovarian cancer were over represented in the study population compared to the general population as they have a particularly strong motivation to volunteer. As family history is a well established risk factor for ovarian cancer this factor would tend towards an overestimate of the duration of phase C. Conversely, the proportion of women with a previous hysterectomy was higher than would be expected in the general population. This factor would tend toward an underestimate of the duration of phase C as available evidence (Booth 1986, Hildreth 1981, Annegers 1979) suggests that there is a reduction in risk of ovarian cancer amongst women who have had a previous hysterectomy with ovarian conservation.

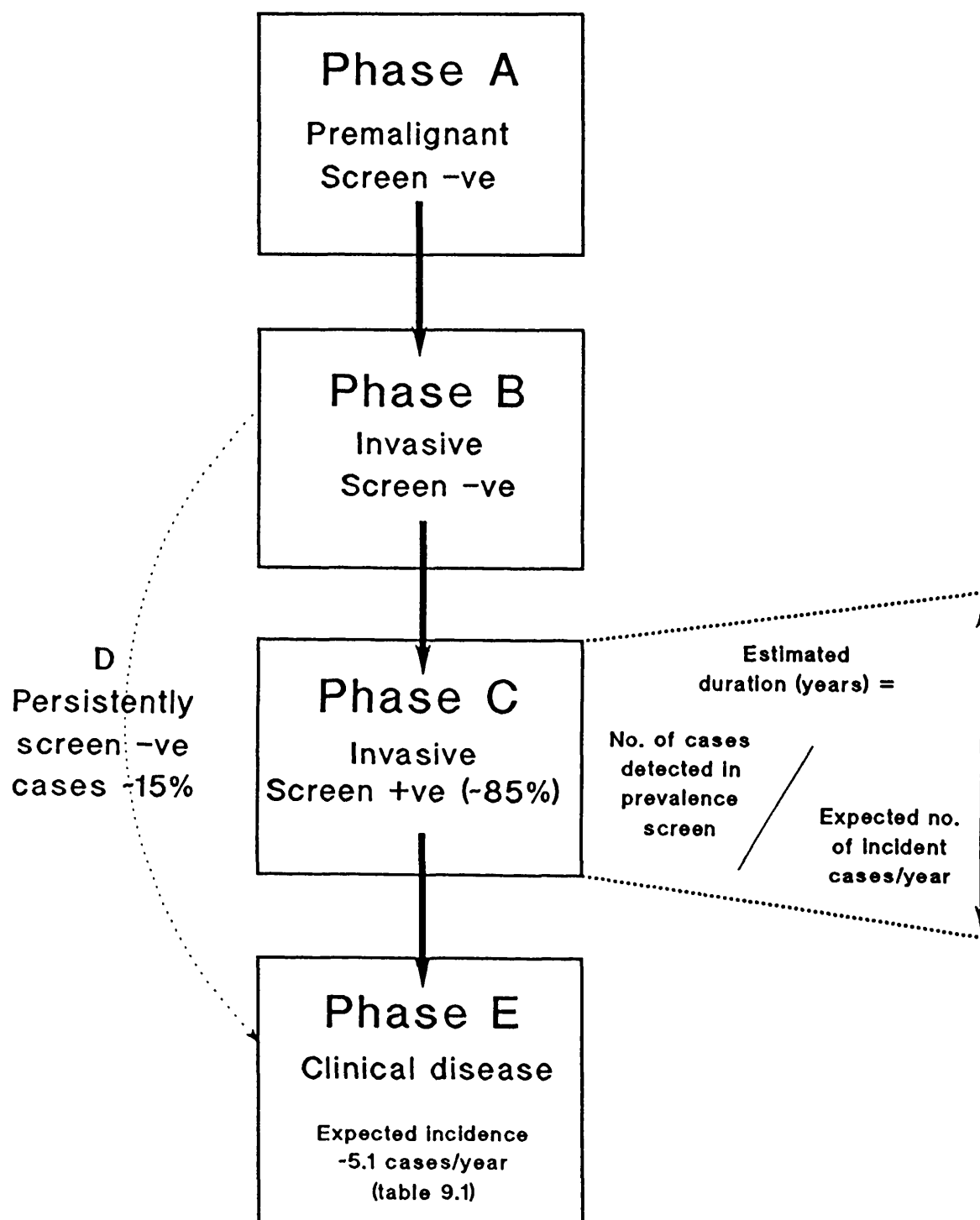


Figure 9.4. A model of the progress of ovarian cancer from a preinvasive phase to the development of symptoms of invasive disease.

The estimate obtained from this study is slightly less than that suggested by the only other source of data for estimating the duration of phase C. In the JANUS study (Zurawski et al 1988b) sera taken from 39,300 healthy volunteers was stored and tested for CA 125 after ovarian cancer had presented clinically in 105 women. The incidence of ovarian cancer in this population over a 12 year follow up period was 8.8 cases/year and 25 of the 105 volunteers who subsequently developed ovarian cancer had an initial CA 125 > 30 U/ml. The estimated length of time spent in phase C calculated as described above was therefore 3.3 years [$25 / 8.8 - (8.8 \times 0.15)$]. The directly comparable figure from our own study would be 1.9 years ($12/6.2$), (as for direct comparability the volunteer with a CA 125 of 85 U/ml who declined to return for an ultrasound scan would be classified as screen positive).

Only 4 of the 11 cases detected to date in the phase 2 study had stage I or II ovarian cancer. This finding may in part reflect the fact that the available results are the findings of a prevalence screen. The remaining 7 cases with advanced stage disease may represent cases which have spent a relatively long period of time in phase C prior to the prevalence screen. If so, follow up screening of the same population should detect a larger proportion of early stage disease than the prevalence screen. Cases detected at follow up screens will have been in phase C for a period of time limited by the screening interval.

CHAPTER 10

GENERAL DISCUSSION AND CONCLUSIONS

10.1 THE RELATIONSHIP BETWEEN SERUM AND TISSUE LEVELS OF CA 125

Normal serum CA 125 levels are found in a proportion of epithelial ovarian malignancies due to lack of expression of the antigen. A second group of epithelial ovarian malignancies (largely of early stage), are not associated with elevation of serum CA 125 levels despite producing high tissue levels of the antigen. The phenomenon of high tissue CA 125 levels in association with normal serum levels is not confined to early stage malignancy. As documented in chapter 4, benign ovarian tumours, normal endometrium and 2nd trimester amniotic fluid contain high levels of CA 125 activity but are in the majority of cases associated with serum CA 125 levels less than 35 U/ml. It is clear that elevation of serum CA 125 is not only related to production of the CA 125 antigen. The specificity of CA 125 activity for ovarian cancer at tissue level is extremely poor, whilst serum measurement has relatively high specificity for ovarian cancer. The clinical value of serum CA 125 measurement appears to be related more closely to factors influencing release of the antigen into the circulation than to its synthesis.

The common factor in all physiological and pathological events associated with elevation of serum CA 125 levels is the occurrence of a process which in some way alters normal tissue barriers in relation to tissues known to produce CA 125.

Such events may occur in malignancy, pregnancy, menstruation, endometriosis, pelvic inflammatory disease and benign tumours. It is not surprising therefore that elevation of serum CA 125 is most consistently associated with invasive, widely spread malignancy and less commonly with early stage, localised disease. When increased CA 125 synthesis is not usually associated with alteration in tissue barriers, an increase in serum levels is uncommon (eg benign ovarian tumours). It is likely that the intact capsule of most benign ovarian tumours is an effective barrier to the high molecular weight moiety expressing CA 125 antigen. When increased tissue production of CA 125 coincides with changes in surface barriers (eg decidua in the first trimester of pregnancy) a rise in serum CA 125 is usual. As there is no evidence of increased endometrial production of CA 125 at the time of menstruation it would seem that serum CA 125 levels may also rise due to alteration in tissue barriers alone without any increase in synthesis, although this phenomena may be attributable due to retrograde menstruation. The one clear exception to this pattern is elevation of serum CA 125 in liver disease. Normal liver does not express CA 125 (Eerderkens 1985) and peritoneal irritation cannot explain serum CA 125 elevation in cirrhotic patients without ascites and jaundice. A plausible explanation is that CA 125 is metabolised in the liver and that elevated CA 125 levels in cirrhotic patients are a consequence of liver failure (Ruibal 1986).

The hypothesis that elevation of serum levels of CA 125 is dependent upon an alteration in tissue barriers has important implications for the use of CA 125 measurement as a screening test for early stage ovarian cancer. If this hypothesis is valid it follows that the malignant process will only be associated with an elevation of serum CA 125 levels when it has caused a degree of disruption of tissue barriers. This process may occur either by the process of invasion itself or by precipitating an inflammatory response. Such events probably occur relatively late in the process of carcinogenesis but may occur relatively early on the time scale of clinical staging. As screening must be performed at intervals, which could not in practice be less than 1 year, the crucial point then becomes the time lapse from the initial disruption of tissue barriers to the initiation of metastatic spread. If metastatic spread follows closely on disruption of tissue barriers, screening with CA 125 is unlikely to reduce mortality. This limitation will apply even if serum CA 125 measurement has high sensitivity for early stage disease, since the opportunity to 'catch' the disease at an early stage will arise infrequently. If the time lapse to metastatic spread is months or years a reduction in overall mortality may be achieved by interval screening. Unfortunately it may be the case that the tumours with a poor prognosis progress rapidly from tissue disruption to metastasis whilst only those with a relatively good prognosis have a longer time span and are amenable to screening.

10.2 THE PHYSIOLOGICAL SIGNIFICANCE OF CA 125

The CA 125 antigen in common with all other tumour markers described to date is tumour associated rather than tumour specific. Although the OC 125 antibody was raised using an ovarian cancer antigen as an immunogen the CA 125 epitope which it recognises is expressed by a number of normal tissues and in various benign pathological conditions. The pattern of CA 125 expression on normal tissues supports the suggestion of Kabawat et al (1983b) that CA 125 is a differentiation antigen associated with coelomic epithelium and its derivatives.

The function of the moiety expressing CA 125 in normal tissues remains obscure. However, the observations here raise the possibility that it may be involved in the function of normal endometrium and decidua. Recent studies of the secretory activity of the endometrium suggest that it should not be considered a passive end organ responding cyclically to ovarian steroids. There is now good evidence that the human endometrium is capable of synthesising a number of hormones and proteins. These secretory products may be of importance in regulating endometrial differentiation during the menstrual cycle and in the complex interactions between the endometrium and trophoblastic cells during pregnancy. The distribution of CA 125 in the reproductive tract and its serum profile during the menstrual cycle and in

pregnancy are similar to that of PP14, a protein of endometrial origin. PP14 (synonyms; endometrial protein 15 - Bell et al 1985a, pregnancy associated endometrial alpha 2 globulin - Bell et al 1985b, alpha uterine protein - Sutcliffe et al 1980, progestagen dependent endometrial protein - Joshi et al 1980) is the major secretory product of the endometrium during the latter half of the menstrual cycle and the 1st trimester of pregnancy. Peak tissue levels of both PP14 and CA 125 occur in early pregnancy endometrium and peripheral serum levels reflect this profile with a peak occurring at 8 weeks gestation. Peak amniotic fluid levels of both PP14 and CA 125 occur in the 2nd trimester of pregnancy. The similarity in compartmental distribution of CA 125 and PP14 is consistent with an endometrial origin of CA 125 activity.

It is likely that many of the controlling mechanisms of endometrial function act locally at a paracrine level. As discussed above (section 10.1) CA 125 activity in serum may reflect alterations in the blood:tissue barrier rather than functional interactions. The physiological significance of variations in CA 125 activity in the complex endocrinological events of the menstrual cycle and early pregnancy is more likely therefore to be revealed at a cellular level than by the study of serum variations. In vitro studies of endometrium and decidua may provide a valuable tool for investigation of the synthesis and function of CA 125.

10.3 PREOPERATIVE DIFFERENTIAL DIAGNOSIS OF OVARIAN CANCER

The data described in chapter 7 confirm the value of serum CA 125 measurement in the preoperative assessment of patients with an adnexal mass. When considered in isolation the performance of a single serum CA 125 estimation was superior to clinical criteria and equivalent to that of real time ultrasonography. The majority of patients with ovarian malignancy had serum CA 125 levels considerably higher than that of a healthy population. Elevated serum CA 125 levels were also observed in a proportion of patients with benign disease but were of a moderate degree. Patients with benign disease rarely had serum CA 125 levels greater than 60 U/ml, and only exceptionally greater than 100 U/ml. A preoperative CA 125 of 60 U/ml or more should therefore be regarded as indicative of a high likelihood of malignancy.

The value of serum CA 125 estimation in the differential diagnosis of ovarian cancer was maximised when it was considered in relation to other diagnostic criteria. Malignant disease was always associated with at least one ultrasound feature of malignancy. The absence of any ultrasound features of malignancy was therefore a reliable indicator of benign disease even in patients with a moderately elevated serum CA 125 level. The additional value of considering menopausal status was not surprising in view of the predominance of benign and physiological causes of an elevated serum CA 125 in premenopausal as opposed to postmenopausal women. The Risk of Malignancy Index

(RMI) combines these 3 criteria in a formula which is sufficiently simple to be routinely applied in clinical practice. The RMI provides a reproducible method for achieving accurate preoperative diagnosis of the benign or malignant nature of ovarian disease in over 90% of cases.

There is good evidence that an optimal primary surgical procedure for ovarian cancer is associated with a relatively good prognosis and response to chemotherapy. There is also an increasing acceptance in the United Kingdom of the need for subspecialisation in gynaecological oncology. Against this background it is desirable and realistic to use a reliable diagnostic index such as the RMI, to ensure that whenever possible patients with ovarian cancer undergo primary surgery by a surgeon experienced in the management of the disease. This may involve referral within a department or hospital, or referral to a specialist centre. Accurate preoperative diagnosis would provide a number of other clinical benefits. First, it would enable medical and nursing staff to provide appropriate preoperative advice and psychological preparation. Secondly, patients likely to require extensive surgery involving bowel could receive suitable bowel preparation. Thirdly, it may be possible to avoid a midline incision in the group of patients at particularly low risk of malignancy.

10.4 THE ROLE OF CA 125 IN SCREENING FOR OVARIAN CANCER

The report of the Janus Serum Bank (Zurawski et al 1988b) indicates that elevated serum CA 125 levels may occur several years prior to the diagnosis of ovarian malignancy. These findings confirmed the potential role of CA 125 measurement in screening for ovarian cancer. However, the data also confirmed the evidence of chapter 4 of this thesis and other reports (table 1.2) that elevations of CA 125 in early stage ovarian cancer are modest. To achieve high sensitivity for early stage disease will therefore require the definition of a positive screening result at low levels of CA 125 activity. The lower limit for the definition of a positive CA 125 result is determined by two considerations.

The first consideration is that of assay reproducibility and is dependent upon the performance characteristics of the CA 125 radioimmunoassay (chapter 3). The lowest serum CA 125 levels with intra- and inter-assay coefficients of variation less than 10% were 25.8 U/ml and 25.3 U/ml respectively. The minimum definition of a positive screening result, compatible with adequate quality control using currently available assay systems will therefore be 25-30 U/ml.

The second consideration is the inevitable sacrifice of specificity inherent in lowering the definition of a positive result in order to maximise sensitivity. As previously discussed (1.3.2 and 8.4.3) the level of specificity required to achieve an

acceptable positive predictive value on screening a population of women aged 45 years or older on an annual basis is 99.6%. The data described in chapter 8 indicate that in order to achieve this level of specificity using CA 125 alone would require an upper limit of 80 U/ml and considerable sacrifice of sensitivity. In order to achieve adequate specificity whilst maximising sensitivity required the incorporation of CA 125 measurement as one step in a multimodal screening programme. The most hopeful approach would seem to be CA 125 measurement combined with real time ultrasonography.

The preliminary results of phase 2 of the ovarian cancer screening project have confirmed the high specificity of the combination of CA 125 and ultrasound. In addition the findings in the initial prevalence screen of 20,000 volunteers suggest that the interval between the development of screen detectable disease and clinical evidence of disease is approximately 1.8 years. Interval follow up screens for the phase 2 study are continuing to establish the sensitivity of the screening protocol for preclinical ovarian cancer as well as the relationship between screening interval and the ratio of early to late stage cancers detected. If the results are sufficiently encouraging a randomised controlled study will be performed to assess the impact of the screening protocol on mortality from ovarian cancer.

10.5 SUMMARY OF CONCLUSIONS

10.5.1 CA 125 measurement

- (a) The most sensitive assay system for CA 125 measurement is the Abbott Laboratories radioimmunoassay.
- (b) Although the correlation between the 3 available assays for CA 125 is good, the absolute values in U/ml are not directly comparable.

10.5.2 The distribution of CA 125

- (a) CA 125 activity is not specific to the malignant process. It may also be associated with normal tissues in physiological states and with benign pathological conditions.
- (b) The compartmental distribution of CA 125 in the reproductive tract suggests an endometrial\decidual origin of CA 125 activity.
- (c) A common factor associated with conditions in which serum CA 125 levels are elevated is the disruption of normal tissue barriers.

10.5.3 Serum CA 125 levels

- (a) A consistent pattern of rise and fall of serum CA 125 levels is associated with the menstrual cycle and the first trimester of pregnancy.
- (b) There are differences in the pattern of variation of serum CA 125 during the first trimester of normal outcome, ectopic and anembryonic pregnancies.

- (c) Elevations of serum CA 125 in early stage ovarian cancer are quantitatively small and are observed in approximately 50% of cases of clinically apparent stage I disease.

10.5.4 Preoperative differential diagnosis of ovarian cancer

- (a) Serum CA 125 measurement is of clinical value in the preoperative differential diagnosis of patients with benign and malignant adnexal masses.
- (b) The value of CA 125 in differential diagnosis is maximised by its incorporation in a Risk of Malignancy Index with ultrasound findings and menopausal status.

10.5.5 Screening for ovarian cancer

- (a) No individual screening test currently available has demonstrated clinically acceptable levels of specificity on screening for ovarian cancer amongst apparently healthy postmenopausal women.
- (b) Acceptable levels of specificity on screening for ovarian cancer can be achieved by the combination of CA 125 with either ultrasound or pelvic examination.
- (c) The interval between the development of screen positive ovarian cancer and clinical evidence of disease is approximately 1.8 years for the screening combination of CA 125 and ultrasound.

10.6 FURTHER RESEARCH IN PROGRESS

10.6.1 Screening for ovarian cancer

Phase 2 of the ovarian cancer screening project is continuing in order to provide further information concerning the sensitivity of the screening protocol for preclinical ovarian cancer and the appropriate screening interval. The current investigations include an assessment of the acceptability and cost of the screening protocol as well as recall and follow up of volunteers. If the results justify further investigation a randomised controlled study will be initiated amongst a group of volunteers at relatively high risk of ovarian cancer, selected on the basis of family history.

10.6.2 Preoperative diagnosis of ovarian cancer

A prospective study is currently underway to assess the performance and validity of the Risk of Malignancy Index amongst another group of patients undergoing investigation for an adnexal mass. Efforts are being made to improve the accuracy of the RMI by incorporating additional tumour markers (including placental alkaline phosphatase, HMFG2 and NB70K), as well as the selective use of sophisticated imaging techniques (NMR, radio-immunoscintigraphy and doppler blood flow measurement).



APPENDIX:

THE GYNAECOLOGY CANCER RESEARCH FUND

THE GYNAECOLOGY CANCER RESEARCH FUND

The Gynaecology Cancer Research Fund (GCRF) is a registered charity (no. 292053) which was founded by the author of this thesis in June 1985. The aim of the Fund is "to raise funds for research into and the treatment of gynaecological malignancy" (Gynaecology Cancer Research Fund Constitution 1985). As the establishment of the GCRF provided the foundation for the studies described in this thesis, brief details of the charity are provided below.

Foundation

The research programme described in this thesis was conceived in 1985. It was apparent that the ovarian cancer screening project would require funding on a scale which would not initially be available from the major grant giving bodies. The GCRF was founded on June 12th 1985 in order to raise funds for the proposed investigations.

Organisation

The GCRF is directed by an executive Committee consisting of a chairman (Dr I Jacobs), secretary (Sister S Sharp), treasurer (Mr D Oram) and committee member (Professor JG Grudzinskas). In 1988 a fund raising committee was established (chairman Mrs K Blincoe).

Income

From June 1985-1988 the GCRF received donations from over 250 UK companies and 50 charitable trusts. An increasing proportion of the Funds income is derived from individuals donations and sponsored events.

Expenditure

The GCRF provided most of the financial support required to initiate the studies described above. This support included laboratory consumables, the CA 125 radioimmunoassay, computer software, freezers for sample storage and the salaries of a research sister and research secretary. Subsequently the ovarian cancer screening project has received grants from the British Medical Association (TP Gunton Award), the Royal College of Obstetricians and Gynaecologists (Eden Fellowship) and the Birthright Trust. The GCRF remains the largest single contributor to the ovarian cancer screening project.

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