AN INVESTIGATION OF SEROLOGIAL TUMOUR MARKERS IN EPITHELIAL OVARIAN CANCER

BY

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DEDICATION

This thesis is dedicated, in loving memory,
to my mother.

DECLARATION OF ORIGINALITY

I declare that the work presented herein and the composition of this thesis is my own.

Jane Fisken

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ABSTRACT

Epithelial ovarian cancer (EOC) accounts for over 80% of ovarian carcinomas. More than two-thirds of patients present with metastatic disease resulting in a poor five year survival of 25%. Surgery is the mainstay of treatment; subsequently the majority of patients receive platinum based chemotherapy regimes. Although chemotherapy may improve progression free survival, it has little impact on overall survival.

CA125 has an established role in monitoring response to chemotherapy and providing a lead time to clinical relapse. Its value in prognosis, however, requires clarification. Not all patients express CA125, therefore complementary tumour markers have been intensively sought. The most promising antigenic molecule to date is polymorphic epithelial mucin, against which numerous monoclonal antibodies have been raised. Using several of these antibodies, this thesis investigated the role of the following mucin antigens in EOC; $HMFG_2$, CA153, CA724 and CA199. In addition, tumour-associated trypsin inhibitor (TATI), and p185, the glycoprotein encoded for by the neu (c erb B2/HER 2) oncogene, were evaluated. Double-determinant immunoassay was the most common method of antigen quantitation. An ELISA was developed for $HMFG_2$ "in-house", while the remaining markers were measured using commercial assay kits.

1237 blood samples were collected post-operatively from April 1984 to July 1989 from 250 EOC patients. After retrospective clinical documentation, a database consisting of each patient's case history and serial serum marker levels was developed in collaboration with Unilever Research to perform statistical analyses. CA125 was elevated in a greater proportion of patients with all FIGO stages and histological tumour types than all the other markers. Whilst serum p185 was elevated in very few patients, there were insufficient data (the major restriction being cost) to assess the clinical correlates of CA153, CA199, CA724 and TATI. $HMFG_2$ showed most promise and was therefore evaluated in more detail.

Both CA125 and $HMFG_2$ correlated significantly with tumour burden. CA125 correlated significantly with response to first-line chemotherapy, but could not distinguish complete from partial responders. $HMFG_2$ levels fell in the majority of responders, but did not show a significant correlation. Consequently, both markers had poor sensitivity for microscopic and small volume macroscopic disease at second-look laparotomy. This remains the only accurate method of determining response in patients with no clinically evaluable disease, although its value is questionable. $HMFG_2$ gave similar lead times to relapse as CA125, but in fewer patients. The value of a marker lead time depends ultimately on remaining therapeutic options; only prospective randomised trials will determine the impact pre-clinical "serological diagnosis" of relapse has on outcome.

CA125 and $HMFG_2$ were significant predictors of total and progression free survival after the first cycle and throughout primary chemotherapy. Prognostic information that may influence treatment strategy is desirable as early as possible; in patients with a poor expected outcome continuation of aggressive chemotherapy would not be justified. By dividing the patient group with samples taken after the first cycle of treatment into quartiles on the basis of marker levels, it was possible to identify different prognostic groups. The survival curves for each quartile were not significantly different for $HMFG_2$ but were for CA125. It was possible to divide patients into groups with relatively good, intermediate and poor progression free survival and survival based on CA125 levels at this time. However, CA125 was not independent of performance status in predicting survival. No single parameter will accurately predict outcome in all patients, and although CA125 was an important prognostic factor, it only confirmed information given by several other factors.

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ABBREVIATIONS

αIFN	α-interferon
BSA	bovine serum albumin
BSO	bilateral salpingo-oophorectomy
CA125	cancer antigen 125
CA153	cancer antigen 153
CA199	cancer antigen 199
CA724	cancer antigen 724
cDNA	complemetary deoxyribonucleic acid
CISP/PRED	cisplatin/prednimustine
CPM	counts per minute
CT	computed tomography
CV	coefficient of variation
DNA	deoxyribonucleic acid
ECOG	European Committe for Obstetrics and Gynaecology
EGFR	epidermal growth factor receptor
ELISA	enzyme-linked immunosorbant assay
EOC	epithelial ovarian cancer
EORTC	European Organisation for Research into the Treatment of Cancer
FIGO	Fédération Internationale de Gynécologie et d'Obstétrique
FSH	follicle stimulating hormone
5-FU/P/H/P	5-fluorouracil/cisplatin/hexamethylmelamine/prednimustine
HAMA	human anti-mouse antibody
HCG	human chorionic gonadotrophin
HMFG	human milk fat globule
IRMA	immunoradiometric assay
LH	luteinising hormone
MAb	monoclonal antibody
Mr	molecular weight
PAb	polyclonal antibody

ABBREVIATIONS (continued)

PBS	phosphate buffered saline
PEM	polymorphic epithelial mucin
PSTI	pancreatic secretory trypsin inhibitor
RIA	radiommunossay
RIS	radioimmunoscintigraphy
RNA	ribonucleic acid
SD	standard deviation
SDS	sodium dodecyl sulphate
SE	standard error
TAG	tumour associated glycoprotein
TAH	total abdominal hysterectomy
TATI	tumour associated trypsin inhibitor
TSH	thyroid stimulating hormone
UICC	International Union for the Control of Cancer
UKCCCR	U.K. Commitee for the Coordination of Cancer Research
US	ultrasound

INTRODUCTION

CHAPTER 1

Epithelial Ovarian Cancer (EOC) and its Management

1.1 INTRODUCTION

Epithelial ovarian cancer (EOC) presents one of the most challenging problems to gynaecologists and oncologists. It is the sixth most common cancer in women, accounting for 4% of all female cancers by incidence, and 6% of all female cancer deaths (Deppe and Lawrence, 1988). The incidence is less than that of carcinoma of the uterine cervix and corpus combined, and yet it remains the leading cause of death due to gynaecological malignancy (Booth and Beral, 1985). More than 4000 women die of ovarian cancer each year in Britain.

The insidious onset, together with its propensity for intra-abdominal metastasis, results in approximately two-thirds of women presenting with disseminated disease at the time of initial diagnosis. Once the disease has spread beyond the ovary, the prospect of cure is remote and long-term survival disappointingly poor. Overall, the 5 year survival rate is approximately 30%. Stages I, II, III, and IV have 5 year survival rates in the order of 61%, 40%, 5%, and 3% respectively (Tobias and Griffiths, 1976). Within each stage survival depends upon the tumour grade and histopathological type. The mortality rate has remained unaltered despite advances in surgical and staging techniques and the introduction of cisplatin containing chemotherapeutic regimes. The lack of a reliable screening test for early diagnosis almost certainly contributes to this dismal picture.

Surgery is the mainstay of treatment, with today's procedures among the most involved in gynaecological surgical practice. Complete surgical resection in patients with advanced disease is usually impossible, hence the majority require post-operative therapy with systemic chemotherapy. About 50% of patients with advanced disease respond to first-line chemotherapy, but responses are usually short-lived, and second-line treatments are far less effective and less well tolerated. Thus, most patients enter a cycle of disease remissions and exacerbations involving multiple treatment attempts and failures.

A number of prognostic factors have been identified, including; stage, tumour

grade and histological type, tumour ploidy, age at diagnosis, performance status, and presence of ascites. Virtually all studies, however, have demonstrated that the amount of residual disease left after surgery is the single most important determinant of prognosis (for review, see Webb, 1989).

Post-operative follow-up remains a significant problem for all patients. At present the most effective means of determining true response to therapy in patients deemed clinically free of disease is by recourse to surgery. Numerous reports have questioned whether second-look surgery is of benefit to patients, given that it carries a significant morbidity and mortality rate in subjects already compromised by disease, prior surgery and often intensive chemotherapy. Recently, Luesley *et al.* (1988) have conclusively shown in a prospective randomised trial that second-look surgery has no impact on survival.

Traditionally, oncologists have focused on the dynamics of treatment and disease response, with increased remission rates, extension of the disease free interval and survival being their ultimate goals. Management of advanced disease remains a significant problem, and while earlier diagnosis may be critical in reducing the mortality rate this has yet to be proven experimentally. The ability to recognize and treat persistent residual or recurrent disease effectively will undoubtedly have more immediate impact on survival. Until recently the quality of life for these patients has rarely been examined. Perhaps equally important in determining which patients will benefit from chemotherapy is the identification of those who do not stand to benefit in terms of improved disease free survival and survival, and the subsequent saving or re-allocation of resources gained from a reduction in ineffective therapy. More emphasis should then be placed on palliation and improving the quality of life of these patients.

This chapter will examine the problems that ovarian cancer and its management using conventional methods of treatment presents. Finally, it will introduce the areas where new technological developments might aid or improve upon existing strategies.

1.2 EPIDEMIOLOGY

1.2.1 Incidence

EOC accounts for 90% of all primary malignant ovarian cancer (Fox, 1985) and has an average incidence of 15 new cases per 100,000 of the female population per year (Piver, 1987). It has been estimated that 1 in 70 women are likely to develop EOC during their lifetime (Cutler and Young, 1975), compared to 1 in 12 for breast cancer. The incidence increases with age, peaking at 40 years for benign disease and 55-60 years for malignant ovarian disease. This has led to the suggestion that benign disease may be a precursor to malignant disease (Anderson, 1990). However, the evidence is largely circumstantial and anecdotal (Scully, 1982). The true proportion that progress in this manner is unknown.

1.2.2 Geographical distribution

EOC is a disease of Western industrialized society, like the majority of degenerative illnesses. Japan, however, has the lowest incidence in the world (Waterhouse, 1976). Environmental factors seem to be more important than ethnic, although to date there are no clues as to whether diet or other environmental agents may be carcinogenic for the ovary.

1.2.3 Genetic factors

Three types of ovarian cancer may arise: sporadic, where no family history is seen through two generations; familial, where two or more first degree relatives have, or have had, the disease (Lynch type II), and; hereditary, where the familial pattern shows an autosomal dominant trait (Lynch type I). Family history, recognised as the most easily identifiable and greatest of risk factors, accounts for about 5% of all cases (Ponder *et al.*, 1990). Estimates of the relative risk vary from 3-fold (Lynch *et al.*, 1990, Ponder *et al.*, 1990) to 20-fold (Franceschi *et al.*, 1982). A preliminary report of the U.K. Office of Population

Census and Surveys study shows the relative risk of death is substantially increased for a first degree relative when the index case is diagnosed before 50 years of age (Ponder *et al.*, 1990). The most common familial occurrence of ovarian cancer is in association with breast cancer (Lynch *et al.*, 1978). Ovarian cancer is also linked to carcinoma of the endometrium and colon. The short interval between diagnosis or simultaneous occurrence suggests a common aetiology.

Hereditary ovarian cancer, characterised by early age at onset, a high incidence of bilaterality, and vertical transmission, has been estimated to account for 5-10% of all cases (Lynch *et al.*, 1990). The increased risk is believed to be due to the inheritance of a single autosomal dominant gene; which has yet to be proven by demonstration of linkage between ovarian cancer and a known genetic marker. Strong ethical pressure exists to perform prophylactic oophorectomy on such women; however, this is no guarantee of total protection against future development of intraperitoneal carcinomatosis. Tobacman *et al.* (1982) described 28 women from 16 ovarian cancer prone families who had prophylactic oophorectomy, three of whom later developed intraperitoneal carcinomatosis. They concluded that EOC may arise from any tissue derived from coelomic epithelium. Chen *et al.* (1985) later reported the case of a 38 year old woman who developed peritoneal carcinomatosis three years after oophorectomy because of a strong family history. Retrospective pathological assessment of her resected ovaries revealed occult foci of malignant cells. A third possible explanation for the development of peritoneal carcinomatosis after prophylactic oophorectomy could be that fragments of ovarian tissue had been left behind at operation. Lynch *et al.* (1990) offer the hypothesis that a subset of patients in hereditary ovarian cancer families may manifest a so-called "familial peritoneal ovarian carcinomatosis syndrome" in whom prophylactic oophorectomy would be ineffective.

Until 1970 there were only five cases of familial ovarian cancer reported in the literature (Piver *et al.*, 1982). By 1985, the Familial Ovarian Cancer Registry established in 1981 (Piver *et al.*, 1984) had accumulated over 100 cases. The

registry at King's College Hospital, London, now has over 300 ovarian cancer families (Bourne,T., personal communication). Studies on such families may throw more light on the biological nature and natural history of the disease. Perhaps most importantly they may help to determine the gene(s) involved in the transmission of hereditary ovarian cancer, thus enabling the definition of a high risk group suitable for targeting surveillance and management strategies. To date, experimental evidence does not lead strongly to any particular chromosomal location, although there is much interest in the tumour suppressor gene, p53, found on chromosome 17 (Eccles *et al.*, 1990).

Lynch *et al.* (1990) were the first to report prophylactic oophorectomy in two patients, from breast/ovarian cancer families, who both had markedly elevated levels of the tumour marker CA125 which returned to normal post-operatively. Histological examination revealed ovarian cancer in both women, demonstrating the possibility of using CA125 to screen ovarian cancer prone families (see chapter 3, section 3.5.2, p 74).

1.2.4 Reproductive factors

Many studies have reported the association of ovarian cancer with reproductive and menstrual history and the use of exogenous hormones. Beral (1987) noted an inverse correlation between ovarian cancer mortality and family size; such that low death rates were seen in Spain, Bulgaria, and Japan where large families are common, and high death rates were seen in Sweden and Denmark where family sizes are small. It has been consistently reported that nulliparous women are at an increased risk of developing the disease compared with parous women, the risk declining progressively with the number of children a woman has (Booth, 1986).

Fathalla (1971) first introduced the idea of "incessant ovulation", and hence trauma to the ovary as a promoting factor in ovarian carcinogenesis. This has been supported by other investigators (Casagrande *et al.*, 1979, La Vecchia *et al.*, 1983). Events which shorten the number of ovulations or suppress them,

have been shown to have a protective effect; late menarche, early menopause, first pregnancy before the age of 25, and use of oral contraceptives.

In 1977, Newhouse *et al.* reported that oral contraceptive use was associated with a reduced risk of ovarian cancer. Since then, a decline in incidence of EOC and functional cysts amongst users has been detected (Vessey *et al.*, 1987, Booth *et al.*, 1989). Booth *et al.* (1989) have estimated that 28 laparotomies per 100,000 women are avoided each year as a consequence of pill use. Other than prophylactic oophorectomy in women from ovarian cancer prone families, this is the only clear example of ovarian cancer prevention to date. The Oxford Family Planning Association study showed a 50% decrease in risk amongst those who have ever taken oral contraceptives, consistent with the results of recent U.S. case-control studies (The Centers for Disease Control, Cancer and Steroid Hormone Study, 1983). More data is required to confirm or refute the findings that protection increases with increasing duration of use and persists after stopping (Vessey *et al.*, 1987).

A reduction in risk has also been seen in women who have had a previous hysterectomy with ovarian conservation (Booth, 1986). Post-menopausal oestrogen use for symptom relief has been associated with increased risk in America (Hoover *et al.*, 1977), and in Britain, although hormone replacement therapy is less prevalent in the U.K. (Beral, 1987).

1.2.5 Dietary and other environmental factors

It has been suggested that diets typical of Western society, with a high consumption of animal fats, may be important in the aetiology of ovarian cancer. Byers *et al.* (1983) conducted a systematic survey of the dietary habits of women with ovarian cancer and found no clear association with consumption of fats, protein, vitamin C, alcohol or tea, but did suggest a high fibre intake and a diet rich in vitamin A might afford some protection. There is, however, no consensus as to what dietary measures should be taken to avoid or reduce the risk of EOC.

No epidemiological or experimental evidence exists to incriminate viruses in ovarian neoplasia, although the mumps virus was suspected due to its gonadotrophic properties (Cramer *et al.*, 1983a). Known carcinogens which come into contact with the ovaries through the pelvis or vagina, such as ionizing irradiation, asbestos and talc, may increase the risk, although the evidence is controversial (Beral, 1987).

Although several protective factors have been identified, many are not of immediate practical relevance. Insufficient aetiological factors are known to allow primary prevention through their control. For reviews of the epidemiology of ovarian cancer see Piver (1987) and Beral (1987).

1.3 SYMPTOMS

1.3.1 Early disease

Early symptoms of EOC are vague. Most women experience no symptoms or mild symptoms unless a pathological accident occurs, such as torsion, intracystic haemorrhage, rupture or infection. Insidious signs such as vague abdominal discomfort, dyspepsia, flatulence, bloating and other gastrointestinal disturbances may occur (Barber, 1984). All of these gastrointestinal symptoms are non-specific and may precede diagnosis by several months.

1.3.2 Advanced disease

Symptoms of advanced disease include; abdominal distention due to increasing tumour mass, and/or accumulation of ascites, abdominal pain due to progressive compression of surrounding pelvic structures, dyspepsia, and vaginal bleeding (Hudson and Curling, 1985). Barber (1984) proposed a triad of suspicious features, including age greater than 35 years, persistent unexplained gastrointestinal symptoms, and a history of ovarian imbalance and malfunction, which should alert a physician to the possibility of ovarian cancer.

1.4 PATHOGENESIS

The ovary appears deceptively innocuous on microscopic examination, yet it has extraordinary metaplastic capabilities. Tumours derived from ovarian tissue may mimic virtually any other tissue in the female genital tract (Fox, 1985). This results from retention of the pluri-potentiality of the Müllerian duct by adult ovarian serosa. Ovarian tumours therefore commonly resemble tumours of the fallopian tube, uterus, and cervix (Fox, 1985).

Epithelial ovarian tumours are thought to arise in one of several ways from ovarian serosa: from direct malignant change in the serosa, from prior formation of a serosal inclusion cyst which represents a pinched-off invagination of the surface epithelium, or from malignant change in a benign cyst which has evolved from a serosal inclusion cyst (Anderson, 1990). In the experience of Fox (1990), "*malignant change is rarely, if ever, seen either in inclusion cysts or in serous cystadenomas, occurs with extreme infrequency in mucinous cystadenomas, but does occur with some frequency in endometriotic cysts*".

Cramer and Welch (1983b) attempted to unite all known aetiological factors to explain the mechanism(s) underlying ovarian cancer pathogenesis, but the histogenesis of EOC remains obscure. This will have important implications for screening programmes - until more is known about the natural history of ovarian cancer, the benefits of detecting cysts cannot be determined.

1.5 CLASSIFICATION

DiSaia and Creaseman (1985) classified ovarian tumours according to the tissue of origin, illustrating the great diversity of ovarian tumour types. Thus, ovarian tumours may be derived primarily from coelomic epithelium, germ cells, specialized gonadal stroma, non-specific mesenchyme, and from metastatic tumours of the breast, endometrium, gastrointestinal tract (Krukenberg tumours) and lymphomas.

Different tumour types occur with different frequencies in distinctive age ranges, (see table 1.1), and each has different prognostic significance. Ovarian tumours derived from coelomic epithelium predominate.

Table 1.1 ***Frequency of histogenetic tumour type with age***

Tumour derived from:	0-20 years	20-50 years	>50 years
Coelomic epithelium	29 %	71 %	81 %
Germ cells	59 %	14 %	6 %
Specialized gonadal stroma	8 %	5 %	4 %
Non-specific mesenchyme	4 %	10 %	9 %

taken from DiSaia and Creaseman (1984).

The World Health Organisation (WHO) classification scheme (see table 1.2 overleaf) extends DiSaia and Creaseman's criteria to incorporate not only cell type, but also the degree of malignancy and architectural growth characteristics (exophytic or growing on the surface, and endophytic or growing into a cyst). A given tumour may show varying combinations of these characteristics. For a full description of the macroscopic and microscopic appearances of ovarian tumours see Fox (1985).

1.6 TUMOUR GRADE

Each tumour is graded according to how closely its cells resemble their normal counterparts. Four grades are recognised; well differentiated (grade 1), moderately differentiated (grade 2), poorly differentiated (grade 3), and undifferentiated (grade 4) in descending order of resemblance to their tissue of origin. More than one grade may exist in the same tumour. Tumour grade is one of the most important prognostic factors, with outlook worsening with progressive loss of cell differentiation.

Table 1.2 ***WHO classification of EOC***

Histological type	Variety	
A Serous tumours	**1. Benign**	(a) Cystadenoma and papillary cystadenoma
		(b) Surface papilloma
		(c) Adenofibroma and cystadenofibroma
	2. Borderline	(a) Cystadenoma and papillary cystadenoma
		(b) Surface papilloma
		(c) Adenofibroma and cystadenofibroma
	3. Malignant	(a) Adenocarcinoma, papillary adenocarcinoma and papillary cystadenocarcinoma
		(b) Surface papillary carcinoma
		(c) Adenofibroma and cystadenofibroma
B Mucinous tumours	**1. Benign**	(a) Cystadenoma and papillary cystadenoma
		(b) Surface papilloma
		(c) Adenofibroma and cystadenofibroma
	2. Borderline	(a) Cystadenoma and papillary cystadenoma
		(b) Surface papilloma
		(c) Adenofibroma and cystadenofibroma
	3. Malignant	(a) Adenocarcinoma and cystadenocarcinoma
		(b) Adenofibroma and cystadenofibroma
C Endometrioid tumours	**1. Benign**	(a) Adenoma and cystadenoma
		(b) Adenofibroma and cystadenofibroma
	2. Borderline	(a) Adenoma and cystadenoma
		(b) Adenofibroma and cystadenofibroma
	3. Malignant	(a) Carcinoma (i) Adenocarcinoma
		(ii) Adenoacanthoma
		(iii) Adenofibroma and cystadenofibroma
		(b) Endometrioid stromal sarcomas
		(c) Mixed mesodermal tumours
D Mesonephroid (clear cell) tumours	**1. Benign**	(a) Adenofibroma
	2. Borderline	
	3. Malignant	(a) Carcinoma and adenocarcinoma
E Brenner tumours	**1. Benign**	
	2. Borderline	
	3. Malignant	
F Mixed epithelial tumours	**1. Benign**	
	2. Borderline	
	3. Malignant	
G Undifferentiated tumours		
H Unclassified tumours		

see Serov *et al.* (1983).

1.7 TUMOUR SPREAD

Ovarian cancer tends to remain within the peritoneal cavity throughout its course, causing death by progressive inanition due to intestinal tract

obstruction. Intra-peritoneal implantation and contiguous growth are the most common modes of spread, although lymphatic and haematogenous dissemination may occur. There is often contiguous growth to the adjacent bowel, pelvic peritoneum, bladder, rectosigmoid caecum, ileum and omentum. Ovarian cancer, however, usually invades superficially and mucosal involvement of the bladder and bowel is rare.

The spread pattern of ovarian cancer is primarily based on the flow of cells within abdominal or ascitic fluid, and on the lymphatic drainage of the ovary. Obstruction of diaphragmatic lymphatics by tumour cells contributes to the development of ascites by impairing peritoneal lymphatic drainage, while transdiaphragmatic spread to the lymph nodes on the thoracic surface of the diaphragm leads to development of pleural effusion. Right pleural effusion is frequently the first extraperitoneal manifestation. Spread is often more extensive than expected, even when only the pelvic area seems to be grossly involved. In the past, understaging was a frequently reported and serious problem that resulted in selection of inappropriate therapy (Young, 1983).

1.8 FIGO STAGING

Staging of EOC is based on clinical and surgical findings, and is a prognostic factor of major importance. The internationally recognised FIGO (Fédération Internationale de Gynécologie et d'Obstétrique) staging system is shown in table 1.3 overleaf.

Table 1.3 ***FIGO staging of EOC***

Stage	Features
I	Growth limited to the ovaries
Ia	Growth limited to one ovary; no ascites; no tumour on the external surface; capsule intact
Ib	Growth limited to both ovaries; no ascites; no tumour on the external surface; capsule intact
Ic	Tumour either stage Ia or Ib but with tumour on the surface of one or both ovaries; or with capsule ruptured; or with ascites present containing malignant cells or with positive peritoneal washings
II	Growth involving one or both ovaries with pelvic extension
IIa	Extension and/or metastases to the uterus and/or tubes
IIb	Extension to other pelvic tissues
IIc	Tumour either stage IIa or IIb, but with tumour on surface of one or both ovaries; or with capsule(s) ruptured; or with ascites present containing malignant cells or with positive peritoneal washings
III	Tumour involving one or both ovaries with peritoneal implants outside the pelvis and/or positive retroperitoneal or inguinal nodes; superficial liver metastasis equals stage III; tumour limited to true pelvis but with histologically proven malignant extension to small bowel or omentum
IIIa	Tumour grossly limited to the true pelvis with negative nodes but with histologically confirmed microscopic seeding of abdominal peritoneal surfaces
IIIb	Tumour of one or both ovaries with histologically confirmed implants of abdominal peritoneal surfaces none exceeding 2cm in diameter; nodes negative
IIIc	Abdominal implants greater than 2cm on diameter and/or positive retroperitoneal or inguinal nodes
IV	Growth involving one or both ovaries with distant metastases; if pleural effusion present, there must be positive cytology to allot a case to stage IV; parenchymal liver metastasis equals stage IV

Staging Announcement, 1986.

1.9 MANAGEMENT

1.9.1 Pre-operative work-up

Diagnosis of malignancy ultimately rests with histological examination by the pathologist of biopsy material taken at surgery. It is important that a woman with suspected ovarian carcinoma should be referred to a specialist centre for adequately planned optimal surgical debulking and staging to maximise her chances of long-term survival.

A pre-operative work-up of the patient is vital and should include the following:-

1. Complete history and physical examination
2. Papanicoloau (Pap) smear of cervix
3. Complete blood count
4. Renal function tests
5. Liver function tests
6. β-hCG, AFP and CA125 serum assay for subsequent monitoring
7. Chest radiograph
8. Intravenous pyelogram to rule out ureteral obstruction and define mass
9. Proctosigmoidoscopy
10. Barium enema, upper gastrointestinal and small bowel series to identify primary bowel cancer or impending bowel obstruction secondary to ovarian cancer

The last three procedures are more often performed in America (Deppe and Lawrence, 1988). Ultrasound, computed tomography and lymphangiography cannot characterize tissue less than 0.5cm in diameter, but are useful to confirm clinical impression. None of these techniques can replace exploratory laparotomy as the definitive staging technique.

1.9.2 Primary surgery

Surgery is the cornerstone of management of EOC. It has three major roles. The first is to establish a diagnosis, by determining the type and grade of the tumour and the stage of the disease. The second role is therapeutic, by performing cytoreduction and allowing provision of intraperitoneal access for further therapy. Lastly, surgery has a palliative role in reducing tumour bulk and relieving obstruction. The need for subsequent therapy and its selection is determined by the findings and success of ablative surgery. Although many prognostic factors have been identified, the only one the gynaecologist can influence is the amount of post-operative residual disease, making adequate surgical debulking the most important facet of patient management. There is,

however, controversy concerning the precise influence surgery has on survival, as will be seen later.

Laparotomy entails opening the abdomen with a midline vertical incision curved around the umbilicus, and conducting a thorough exploration of the entire abdominal cavity. All pelvic organs are inspected, suspicious sites biopsied and frozen sections sent for histopathological examination. Ascites is aspirated if present, if not, washings are taken with saline from the cul-de-sac, lateral and paracolic gutters and subdiaphragmatic areas, most common sites of early metastasis. Detailed accounts of surgical procedures in ovarian cancer may be found in Griffiths (1986), and Hacker (1989).

After primary laparotomy, the volume, location, measurement, and number of residual masses should be accurately recorded to enable response to future therapy to be determined. The diameter of the largest residual mass has been adopted as the best measurement, as attempts to estimate the volume of disease and percentage debulk proved futile. Precise measurements are impossible because of the multiplicity of deposits. Several studies have established the upper limit for optimal debulking to be in the region 1-2cm residual disease. The extent of residual disease has an impact on response to therapy, time to progression and findings at second-look surgery, which ultimately affect survival.

1.9.3 Primary surgery of early disease

Complete surgical resection is a relatively simple procedure when the disease is confined to the ovaries. Patients with early stage disease (FIGO stages I and II) are usually managed by total abdominal hysterectomy (TAH) and bilateral salpingo-oophorectomy (BSO) because of the high incidence of spread to the endometrium and bilaterality. The issue of ovarian conservation in young nulliparous women remains unsettled, however, it is considered reasonable to preserve childbearing capacity in women with stage 1a low grade or mucinous tumours (Griffiths, 1986).

The importance of accurate staging has been emphasised in several studies. Young *et al.* (1983) reported results of restaging laparotomy in 100 women with apparent stage Ia-IIb disease inclusive referred to member institutes of the Ovarian Cancer Study Group (set up in 1976 under the auspices of the National Cancer Institute), in which 31 patients were "upstaged", 77% of those to stage III. There was a strong association between the percentage of patients "upstaged" and tumour grade, 46% with grade 3, 34% with grade 2, and 16% with grade 1 tumours were "upstaged". It has since become apparent that accurate surgical staging is reflected in a marked improvement in survival within both stages I and II (Griffiths, 1986).

1.9.4 Primary surgery of advanced disease

The preferred operations are TAH, BSO, and, commonly, resection of the greater omentum. Bowel resection or partial bladder resection with ureter re-implantation is often necessary. However, complete surgical resection in patients with advanced disease is usually impossible. The options remaining include biopsy only, limited resection of primary tumour, or aggressive cytoreduction or "debulking". Optimal debulking is theoretically feasible in 85% of patients, however, Hacker (1989) reported that this is only being achieved in 31-48% of patients, reflecting the difficulties in making a pre-operative diagnosis and scepticism regarding the need for extensive surgery.

At the beginning of the century, a number of gynaecological surgeons were advocating complete removal of the primary tumour which, Miegs suggested, would render the remaining tumour more susceptible to radiotherapy (Miegs, 1934). In 1968, Munnell advocated a "maximal surgical effort", and this was first quantitated by Griffiths *et al.* in 1975. In a retrospective study from the Boston Hospital for women, Griffiths *et al.* (1975) showed that survival in a group of patients who had residual metastatic disease resected to less than 1.5cm in diameter was no different to that in a group of patients in whom the largest metastatic lesions were less than 1.5cm at the outset. Using multivariate analysis, the authors found survival of patients who underwent optimal

cytoreduction to be independent of the extent of spread, organ involvement, grade or operation performed (Griffiths *et al.*, 1975).

Hacker *et al.* (1983) found that the survival of patients, after debulking to less than 1.5cm, in whom initial metastases were greater than 10cm was similar to those who had suboptimal debulking, suggesting that excision of large tumour masses failed to alter the prognosis. Heintz *et al.* (1988), however, showed that survival could be improved by up to 12 months by aggressive resection of large masses and subsequent intensive cisplatin combination chemotherapy. The precise influence of surgical cytoreduction on survival is unclear. It is most plausible that patients in whom optimal debulking was achieved had a favourable prognosis in any case. The issue of the relative significance of tumour resection versus tumour resectability has been raised by several authors (Heintz *et al*, 1988, Zanaboni *et al*, 1988, and McDermott *et al*, 1988) and is presently being addressed by the Gynaecological Oncology Group.

In a rare report of its kind, Blyth and Wahl (1982) showed that, contrary to opinion, an aggressive surgical approach actually improved the quality of life of patients. Webb (1989) has shown that the morbidity rate of such an approach is acceptibly low, the most common post-operative complication is pneumonia and this occurs in a small percentage of patients. Hence, current philosophy dictates as aggressive an approach as possible without creating an inordinate morbidity and mortality, despite controversy over the value of such an approach in patients with advanced disease.

1.9.5 Interval debulking

Unfortunately, the biological aggressiveness and manner of spread renders some tumours unamenable to debulking. Abdominal carcinomatosis presents a formidable problem to the surgeon, pelvic debulking should be possible if this can be overcome. Haematogenous dissemination resulting in parenchymal organ involvement often renders the patient inoperable. In some cases, secondary debulking following several courses of chemotherapy may be

possible. According to Griffiths (1986) this is based on the erroneous belief that chemotherapy can render inoperable tumours operable.

Secondary debulking is, however, a more difficult technical procedure. Berek *et al.* (1983) were able to perform optimal cytoreduction in 69% of 39 patients at primary operation and only 39% of 32 patients at secondary operation. It is only feasible when the patient is able to continue with intensive chemotherapy after primary surgery (Griffiths *et al.*, 1979). Survival of patients debulked this way is much poorer than those debulked at primary surgery. Lawton *et al.* (1990) achieved macroscopic tumour clearance in 24% of 108 patients after three or five cycles of cisplatin therapy. Median progression free survival in the debulked group was 17 months compared to nine months in those who were not debulked, while 88% of 16 patients debulked at primary laparotomy remained in complete remission after 36 months of follow-up.

1.9.6 Post-operative therapy

The majority of EOC patients require post-operative treatment with chemotherapy. Treatment is selected on the basis of the extent of disease or stage, size and location of residual tumour, presence of ascites and general condition of the patient. Until the early 1950s, surgery was considered the optimal treatment, followed by radiotherapy if there was omental involvement or when the patient relapsed. This was the only approach possible before the advent of chemotherapy, however, survival rates were very poor. Whilst excellent results have been achieved in patients with minimal residual disease after surgery in recent years, in general there is a lack of reliable data with which to assess the contribution to survival or palliation from most management approaches. Prospective randomised controlled trials provide the best opportunity to define clearly the value of a treatment strategy.

1.9.7 Radiotherapy - current status

There have been very few well designed prospective randomised trials with

careful stratification of known important prognostic factors to assess the value of radiotherapy in EOC. Usually radiotherapy is reserved for women with early stage disease or those with minimal residual post-operative disease, accounting for about one third of all patients. This is because the dosage required to destroy a mass >2cm diameter exceeds normal tissue tolerance.

Schray *et al.*(1983) treated 53 advanced EOC patients with <2cm residual or recurrent disease with whole abdominal salvage radiotherapy. No benefit was seen in patients who had disease >2cm prior to chemotherapy or macroscopic disease at second-look laparotomy. In patients with minimal residual disease, radiotherapy is the one adjuvant method following surgery which has been shown to improve long-term survival consistent with cure. Indeed, it has been shown to double the proportion of patients cured when compared to surgery alone (Bush 1984). Optimal use of radiotherapy yields a 10% improvement in long-term survival for the total patient population. There is no evidence that combination chemotherapy achieves the same survival advantage.

In summary, post-operative radiotherapy is regarded as being valuable: in patients with stage I and II disease and no macroscopic residual disease; to a whole abdominopelvic volume (because of the manner of spread); in other rare tumours such as dysgerminoma; and so long as careful attention is paid to dosimetry, technique and patient positioning. It is not indicated: in patients with stages Ia(i) and Ib(i); as a pre-operative therapy or; as a pelvic only volume as a radical therapy (Spooner, 1986). The role of radiocolloid installation into the peritoneal cavity remains to be more clearly defined.

1.9.8 Chemotherapy

Chemotherapy for ovarian cancer was first introduced in 1952. Triethyl-melamine (TEM), a nitrogen mustard derivative, produced a favourable response in 30% of patients (Sykes *et al.*, 1955). Subsequently, other alkylating agents were tested and by the end of the 1950s chemotherapy emerged as an effective palliative treatment. Alkylating agents remained the major

chemotherapeutic agents for a decade, melphalan being the most commonly used due to its reliable activity. No single agent proved superior to another. However, only 50% of patients responded to these agents and the majority of those soon relapsed. This prompted the evaluation of new cytotoxic agents as second-line therapy. Responses after alkylating agents failed were rare, although second-line hexamethylmelamine and cisplatin showed sufficient clinical activity to encourage their use as first-line treatments. Other non-alkylating agents that have demonstrated activity included doxorubicin, 5-fluorouracil and methotrexate.

The high relapse rate, poor response rate to second-line treatment and encouraging activity of certain agents, coupled with the fact that different agents had different sites of activity at the cellular level and different mechanisms of toxicity encouraged the development of combination chemotherapy - an approach that was working with Hodgkin's disease and acute leukaemia in children.

One of the first non-alkylating agents tested was 5-fluorouracil, which proved disappointing on its own but was initially promising in combination with actinomycin D and cyclophosphamide as ACFUCY. This combination gave a 38% response rate (complete and partial) in 47 patients who had failed to respond to first-line melphalan therapy (Smith and Rutledge, 1970). The purpose of this and other similar studies was to demonstrate the potential activity and evaluate the toxicity of the combination prior to use in a randomised controlled trial against conventional first-line therapy. One of the first such trials, reported by Smith *et al.*(1972) compared melphalan to the same ACFUCY regime, and found similar response rates (42% and 45% respectively). The early observation by Smith and Rutledge was not substantiated later when more precise response criteria were applied, and had to be abandoned due to the very high toxicity and high number of treatment related deaths in patients given second-line ACFUCY.

Promising responses were later seen with other combinations. Young *et al.*

(1978) reported a 75% first-line response rate to hexamethylmelamine, methotrexate, 5-fluorouracil, and cyclophosphamide compared to 55% in patients who received melphalan. Delgado *et al.* (1979) reported an 89% response rate to hexamethylmelamine, 5-fluorouracil, and cyclophosphamide compared to 58% using melphalan. Although increased response rates were found, no associated increase in survival was seen.

Of many trials that have been performed, few have shown a statistically significant survival advantage of combination over single agent therapy (Young *et al.*, 1978, and Trope, 1981). There has, however, been a trend in favour of platinum containing regimes. The important contribution of cisplatin to combination chemotherapy was shown in three studies, from the Mayo Clinic (Decker *et al.*, 1982), the Netherlands Cancer Institute (Neijt *et al.*, 1984), and the Gynaecological Oncology Group (Omura *et al.*, 1986). Response rates of 60-80% can be expected in patients with advanced disease, of which about 25% will be complete responses. The introduction of platinum containing regimes has, despite their increased activity, still not clarified the situation with regards to improvement in survival (Lawton and Blackledge, 1986). Although certain subgroups of patients appear to benefit in the short-term, long-term benefit is unclear. Superior response rates do not necessarily lead to improved survival.

Few studies report the duration of response. Schwartz and Smith (1980) reported disease recurrence with a mean time to relapse of one year in 12% of 58 patients who each had a pathologically documented complete response. Gershenson *et al.*(1985) report a relapse rate of 30% within five years of complete response. Several studies have confirmed the importance of prognostic factors in determining outcomes, especially the amount of residual disease after surgery (Griffiths *et al.*, 1975, Klein *et al.*, 1985, and Swenerton *et al.*, 1985).

A major problem in treating patients with chemotherapy is the development of drug resistance. Cisplatin is also limited by its toxicity, which commonly

necessitates dose reduction or withdrawal. Other platinum analogues, carboplatin and iproplatin, have similar activity but less toxicity. Evidence is accumulating that resistance is less likely to develop by intensifying initial therapy (Malpas, 1989). The use of "up-front" or initial high-dose therapy is still in the early stages clinically. Kaye (1990) has contended that "up-front" therapy might improve the quality of life for some patients simply by shortening the time spent on therapy.

Alternatively, intraperitoneal (i.p.) chemotherapy may overcome this problem and also that of systemic toxicity by enabling local dose intensification. It has potential in patients with microscopic disease, and disease less than 2cm remaining after surgery (Blackledge *et al.*, 1990). Several requirements must be met by candidate drugs, including: absence of local peritoneal toxicity, a steep dose-response relationship, low peritoneal permeability, capability of direct tumour kill, and rapid plasma clearance. Clinical trials have been performed in small patient groups using 5-fluorouracil, methotrexate, doxorubicin, melphalan, mitomycin C, and cisplatin (Brenner, 1986). A randomised controlled trial by the EORTC (European Organisation for Research into the Treatment of Cancer) Gynaecological Oncology Group is currently underway to assess the effectiveness of i.p. chemotherapy in maintaining pathological complete response.

1.9.9 Alternative therapy

Approximately two-thirds of ovarian epithelial tumours have functional oestrogen and/or progesterone receptors (Leake and Owens, 1990). Endocrine therapy is usually reserved for palliation of patients with advanced disease refractory to chemotherapy as primary response rates are poor (Deppe and Lawrence, 1988). Biological response modifiers, such as interferons, monoclonal antibodies, tumour necrosis factor, are thought to act by modifying host behaviour to the tumour (Mihich *et al.*, 1986). Several have been tested with chemotherapy although the results are inconclusive.

Several of the monoclonal antibodies used in this thesis to evaluate serum tumour-associated antigens have also been used therapeutically. Radiolabelled monoclonal antibodies are currently undergoing clinical trials in an attempt to target tumour cells bearing the appropriate tumour-associated antigen. Thus, it is hoped that this new anti-cancer treatment will be more effective by reducing the toxicity associated with conventional chemotherapy and allowing dose intensification. One of the major problems with this type of treatment at present is host production of human anti-mouse antibodies "HAMAs" which prevent effective repeat administration of antibody. Production of "humanised" monolconal antibodies is expected to circumvent this problem. This area will be described in more detail in chapter 3 (section 3.2.6, p 63).

1.9.10 Second-look surgery

Second-look laparotomy was first introduced in 1948 by Wangensteen to diagnose early recurrence in asymptomatic colorectal carcinoma patients (Wangensteen *et al.*, 1949). Rutledge and Burns first used it in 1966 in ovarian cancer patients to assess disease status following chemotherapy. Second-look laparotomy may be defined as the surgical re-exploration of patients who have completed a planned course of therapy after a definitive primary surgical procedure, who have no clinical or radiographical evidence of disease. The aim of second-look surgery is therefore to determine tumour status and plan future treatment if necessary. The pre-operative work-up is similar to that of primary surgery, and the procedure is essentially similar in its execution and aims. The surgeon must examine previous sites of documented disease and make every effort to rule out the presence of residual disease after operation. Thus, second-look operation is a meticulous search for persistent disease and should be made by the gynaecologist familiar with the history, mode of spread and findings at primary laparotomy. Not all patients however will be eligible for the procedure. It is mainly performed for one of the following reasons: to determine whether non-palpable disease is responding to chemotherapy and to detect early recurrence; when the patient develops an isolated resectable recurrence; when initial surgery is limited and significant tumour regression with post-

operative therapy renders the patient operable; and when initial laparotomy findings are unclear and staging is incomplete.

Second-look laparoscopy is a useful diagnostic test for determining the presence of resectable, unresectable or diffuse disease following chemotherapy, but has a high false negative rate (20-75%) and is therefore not an alternative to second-look laparotomy (Malviya and Deppe, 1988).

The timing of second-look operation is variable and must be individualised, although most are performed between six months and one year after the first operation when the patient has completed their first course of chemotherapy. Optimal timing depends on the extent of residual disease after primary laparotomy and the number of courses chemotherapy the patient has had.

1.9.11 Second-look outcome

The outcomes, or extent of disease present, at second-look laparotomy are traditionally classified as: **negative**, when no histologic or cytologic evidence of disease is found, in such circumstances the patient is said to have a pathological complete response; **microscopic**, when no tumour is identified by the surgeon but cytologic washings or biopsies demonstrate persistent disease; **macroscopic**, when persistent disease is identified grossly by the surgeon and confirmed by the pathologist; or deemed an inadequate procedure, when surgical exploration is incomplete due to surgical or anaesthetic mishap, or if extensive inoperable adhesions are present.

1.10 EVALUATION OF RESPONSE TO THERAPY

Evaluation of response to therapy has been severely hampered by the difficulty in measuring tumour response, by using varying response criteria and by a general lack of reporting of survival outcomes. It is performed using both objective and subjective means (Miller *et al.*, 1981).

1.10.1 Endpoint evaluation

It is important to consider at what point treatment outcome is to be documented. Ultimately, the success of a treatment regimen may be defined using a number of different endpoint criteria. These include: the percentage of patients with evaluable disease who respond to treatment; the findings at second-look surgery; the length of time until recurrence/relapse (disease free survival) or disease progression/relapse (progression free survival); and the overall survival of a group of patients.

Negative second-look laparotomy is a frequently reported endpoint. Although it confers survival advantage the risk of disease recurrence after a negative second-look laparotomy is uncertain. Also, the majority of patients selected for second-look laparotomy are clinically disease free and therefore represent a better prognostic group. As mentioned earlier, Gershenson *et al.* (1985) reported that up to 30% of patients with negative second-look laparotomy relapse within a five year period.

1.10.2 Objective response criteria

Objective evaluation includes determination of tumour size, change in size, change in number of lesions, and duration of response since the start of therapy. Objective disease assessment is conventionally determined with the aid of radiographical scanning techniques; ultrasound scanning and computed tomography, and second-look surgical procedures; laparoscopy and laparotomy. Information gained using scanning techniques is not always unequivocal and is often subject to wide variability in interpretation.

At each consultation, usually monthly at the outset of treatment (although scans are performed less frequently), any change is evaluated. Solid tumours are measured in centimetres and dimensions consist of the largest diameter and perpendicular diameter at the widest portion of the tumour. Responses have been defined by the International Union for the Control of Cancer (UICC),

shown in table 1.4, below.

Table 1.4 ***UICC response criteria***

UICC Response	Definition
Cure	The achievement of disease free survival that extends without treatment, beyond the period of risk of relapse.
Complete remission (CR)	The disappearance of all clinical evidence of active tumour for a minimum of six weeks. Cytologic washings taken at second-look must be consistently negative. A pathologic CR is when no histologic or cytologic evidence of disease is found at laparotomy.
Partial remission (PR)	A 50% or greater reduction in the sum of diameters of all measured lesions, with no new tumour manifestations appearing during therapy.
Stable disease (SD)	A steady state or a response less than PR or progression. There is no appearance of any new lesion and no worsening of symptoms.
Progressive disease (PD)	The unequivocal increase of at least 25% in the size of any measurable lesion or the appearance of new lesions.
Relapse	The appearance of new lesions or reapppearance of old lesions in patients who have been in CR. For patients who have been in PR, relapse is defined as an increase of 50% or more in the sum of products of the diameters of all measured lesions over that which was obtained at the time of maximum tumour regression.
Remission duration	The duration of response since the start of treatment (expressed in days, weeks or months) until renewed increase in tumour size is more than 25% of the product of two diameters, measured on two consultations during therapy.

UICC, 1988.

1.10.3 Subjective response criteria

Subjective evaluation includes: determination of performance status using internationally agreed nominal scales; changes in weight, appetite, pain, specific organ symptoms; changes in initially pathological laboratory parameters of kidney function, liver function, other organ functions, erythrocyte sedimentation rate, and specific biochemical parameters of the disease; and evaluation of treatment toxicities based on leucocyte and platelet counts, and haemoglobin level. Other symptoms include nausea, vomiting, stomatitis, and alopecia.

Examples of two commonly used scales, the ECOG (European Committee on Obstetrics and Gynaecology) performance status and toxicity are shown below.

ECOG Performance status

0 Able to carry on normal activity.

1 Patient able to live at home with tolerable tumour manifestations.

2 Patient with disabling tumour manifestations, but less than 50% of time in bed.

3 Patient severely disabled and more than 50% of time in bed, but able to stand up.

4 Patient very sick and bedridden.

5 Dead.

Toxicity

0-No symptoms, **1**-Mild, **2**-Moderate, **3**-Severe, **4**-Life threatening

1.11 ALTERNATIVE METHODS OF DETERMINING RESPONSE

Most gynaecological oncologists would like to see second-look surgery replaced with a less traumatic but equally accurate, non-invasive or less invasive method of determining response to chemotherapy and disease status. Luesley *et al.* (1988) have shown in a prospective randomised trial that second-look surgery has no impact on overall survival, however, it is true that certain patients will benefit from the procedure. It would be useful before operating to be able to determine which patients will benefit, and thus save a number of unnecessary operations. Chambers *et al.* (1988) argue that second-look surgery should be limited to experimental protocol situations in which precise measurement of tumour size may be important. Not many surgeons would disagree with this.

Several possible alternatives to second-look laparotomy have been investigated, such as ultrasound scanning, computed tomography, radioimmunoscintigraphy, magnetic resonance imaging, and tumour marker assay, but none

as yet has proved an acceptable reliable alternative (Malviya and Deppe, 1988). In the absence of second-look laparotomy, all available information, objective and subjective together with clinical examination, is used to form a complete picture of the disease status of a patient.

Aside from the difficulties in determining response to therapy, another major difficulty in managing ovarian cancer patients with currently available chemotherapy is deciding to stop or change treatment when drug resistance develops, or to re-initiate treatment for progressive disease at a stage when the patient may still respond. Most patients with stage III or IV disease will eventually fail primary chemotherapy. Second-line treatment is far less successful in patients who have already failed the most efficacious therapy.

The toxicity of chemotherapy mandates that it is terminated when it is clear that the patient has become resistant to it or has not responded at all, and is unlikely to gain benefit from further treatment. It is well known, however, that patient satisfaction bears little correlation with objective tumour response (Cody and Slevin, 1989). Thus, if response to treatment can be accurately determined, the question of how to procede is not so simple given the currently available options and patient desire for active therapy.

The need for a more rational approach to the selection of patients for phase II trials is well recognised, and the work of Blackledge *et al.* (1989) represents a welcome attempt to move in this direction. Prognostic factors that may aid treatment decisions are sought intensively. In this respect, tumour markers (see chapters 2 and 3) may help guide such clinical decision making. This thesis investigates the role of several putative serological ovarian tumour markers in monitoring response to therapy and aiding decision making in the management of patients with epithelial ovarian cancer.

CHAPTER 2

Tumour Markers

2.1 INTRODUCTION

Malignant transformation is accompanied by genotypic changes, resulting in a diversity of phenotypic differences between normal and malignant cells (Sidebottom, 1987). The major difficulty facing tumour immunologists is not so much finding these differences, but determining their significance and identifying ways of exploiting them to detect and destroy cancerous cells (see chapter 3).

The search for tumour-associated antigens specific to ovarian cancer dates back to 1956 (Witebsky *et al.*, 1956). Over 100 putative ovarian tumour-associated antigens, encompassing a wide variety of molecules, have since been reported in the literature. Most of these do not normally elicit an immune response, hence the term "marker" is more appropriate, and will be used throughout this thesis. Tumour markers are of enormous interest because they may help refine diagnosis and rationalise therapy. Unfortunately, no absolutely tumour-specific marker has been discovered for ovarian cancer, or any spontaneous human malignancy, except for the special case of idiotypic determinants of lymphoid malignancy (Glennie and Stevenson, 1987).

Not long after the development of the hybridoma technique by Köhler and Milstein (1975), enabling better definition of tumour markers, doubt regarding the existence of "tumour specific" markers emerged (Old, 1981). Woodruff (1990) has argued more recently that "*It is not surprising that a particular marker which is present in a normal progenitor cell should be conserved during transformation and be present in all neoplastic cells which are descendents of this progenitor. It would, however, be remarkable if all the neoplastic cells carrying such a marker also carried a unique marker of a kind found only in neoplastic cells*". The very fact that changes in a few genes, among the hundred thousand or so genes present in a mammalian cell genome, are sufficient to induce transformation implies that the occurrence of proteins with tumour specificity must be extremely rare if they exist at all (see section 2.2.1). Nevertheless, a distinguishable difference in relative

concentration between normal and malignant cells affords a tumour-associated antigen-bearing molecule marker potential. Although none is known to be unique to a particular malignancy, some aims of tumour immunologists have been realised (see chapter 3).

This chapter will give an overview of different types of tumour markers before considering the requirements of an ideal marker. Ovarian tumour markers discovered to date will be reviewed and, finally, the development, pattern of expression and characteristics of tumour markers investigated in this thesis will be examined in detail.

2.2 TYPES OF TUMOUR MARKER

Tumour markers are usually substances such as hormones, enzymes, glycoproteins and proteins, which may be found on the tumour cell surface and/or secreted into the circulation (Malkin, 1987). Daar and Lennox (1987) have divided tumour markers into the six categories shown in table 2.1.

Table 2.1 ***Tumour marker classification***

Type of tumour marker	Features
1. Normal differentiation antigens	These may be: appropriate to the differentiation stage of the cell in which malignant transformation occurs; not appropriate to this stage, but normally expressed at other stages in the same tissue or other tissue, or; appropriate to the stage of differentiation, but only found on dividing cells.
2. Major histocompatability antigens	These may be: appropriate i.e. like those on normal tissue of the individual in which the tumour arises, or; inappropriate i.e. like those not normally expressed and indicative of new specificities.
3. Viral antigens	These may: belong to the transforming virus (for virally induced tumours), or; be expressed by endogenous viral genes.
4. Oncogene antigens	
5. Oncofoetal antigens	
6. Unusual glycoprotein and glycolipid antigens	

The majority of ovarian tumour markers investigated to date belong to the last three categories, although none of these categories are exclusive. The last three types of markers will therefore be described, for a full description of the first three categories see Daar and Lennox (1987).

2.2.1 Oncogene antigens

In 1911, Rous correctly hypothesised that an infectious agent was involved in the transmission of sarcomas in chickens infected with cell free extracts of tumours (Rous, 1911). The causal agents were identified much later as RNA retroviruses. Huebner and Todaro (1969) first proposed the existence of viral oncogenes, and suggested that they could be integrated into the host's genome and remain dormant or activate causing cancer. Viruses were later shown to have acquired their oncogenes from normal mammalian cells they had infected.

All multicellular eukaryotes have genes which very closely resemble viral oncogenes. Clues to the functions of these cellular proto-oncogenes have come from studies of proteins encoded by structurally similar viral oncogenes. Four classes were originally identified; tyrosine protein kinases (class I), GTP binding proteins (class II), growth factors (class III) and nuclear proteins (class IV) (Daar and Lennox, 1987). Other classification schemes exist, e.g. based on the cellular location - nuclear or cytoplasmic (Weinberg, 1989). Cellular proto-oncogenes do not cause cancer, but may become transforming when activated by one of the following mechanisms: point mutation in the proto-oncogene; attachment of strong promoters/ enhancers to the proto-oncogene; promoter/enhancer insertion near the proto-oncogene; rearrangement of the proto-oncogene in the genome; defined chromosomal translocation of the proto-oncogene, or; amplification of the proto-oncogene (Teich, 1988).

Three broad phases have been identified in the evolution of a tumour; initiation, promotion, and progression, although the exact number of steps incurred in the development of any tumour is unknown. Knudson (1985) hypothesised that two

collaborating oncogenes must be activated in order to initiate transformation (the "two-hit" hypothesis). Not all oncogenes fit this model however; activation of a single oncogene may also cause transformation, e.g. Ha-ras transfection of NIH/3T3 cells is sufficient to cause transformation (Weinberg, 1989).

Oncogenes and their products are potentially tumour markers of exquisite specificity for the transformed state. It would be useful to identify cell surface antigens specifically associated with this state especially if such markers were directly linked with the mechanism responsible for transformation. While the oncogene paradigm developed over the past decade provides a powerful explanation of cancer at the molecular level it is far from complete. Hereditary predisposition to cancer is a well documented phenomenon. Tumorigenesis often involves loss of growth regulatory genes or anti-oncogenes. An anti-oncogene or tumour suppressor gene may be defined as a gene whose repression, inactivation, dysfunction or loss results in cell transformation (Rayter *et al.*, 1989). Currently there is much interest in one such gene, p53 found on chromosome 17, which has been found to be deleted in a high proportion of ovarian carcinomas (Eccles *et al.*, 1990).

2.2.2 Oncofoetal antigens

During the development of multicellular organisms, cells divide and differentiate to become functional organ systems. After they have acquired the metabolic pathways for performing their specialized functions they undergo no further change. In the process of differentiation cells not only acquire new specificities, functions and morphological features but also new antigenic characteristics. Differentiation is widely accepted to be under genetic control, although it is uncertain whether the establishment and maintenance of all specialization generally involves irreversible genetic changes. Normally, embryonic cells produce a series of gene products which are replaced after birth by an adult set of gene products, and are not expressed again in large amounts in adulthood.

The multistep nature of carcinogenesis (Bishop, 1987, and Weinberg, 1989) originating in loss of genetic control and manifesting in multiple phenotypic changes has already been mentioned in the previous section. These changes are not infrequently pleiotropic, following from a single structurally or functionally altered gene. Such gene alterations often result in derepression of normally silent foetal genes and re-expression of their products. Malignant cells are closely related to foetal states of differentiation in that they are less well differentiated than their normal adult counterparts. This relationship, between malignancy and the embryonic state, has been recognised for nearly a century but only became established on a molecular basis with the discovery of α-foetoprotein (AFP) in 1963 by Abelev *et al.*, and carcinoembryonic antigen (CEA) two years later (Gold and Freedman, 1965).

2.2.3 Unusual glycoprotein and glycolipid antigens

Tumour cell surfaces have many altered carbohydrate structures, for review see Feizi (1985). Such changes may have a considerable impact on cellular recognition and communication. Mucus glycoproteins or mucins, of interest in this thesis, which contain greater than 50% carbohydrate by weight are among the most promising tumour markers to date. Other cell surface glycoproteins and glycolipids also contain a high proportion of glycans. These may contain 1-20 carbohydrate residues and can vary between tissues and within a tissue giving rise to extensive heterogeneity. At least three types exist; containing terminal fucose, sialic acid, or sulfate residues, the first being neutral and the last two being acidic. Often more than one type of glycan is present in the same mucin molecule. Addition of glycans during post-translational processing which gives protection against proteolytic degradation, increases the apparent molecular weight of the molecule on SDS polyacrylamide gel electrophoresis.

Change in the amounts of glycosyl-transferases and pool size of substrates may alter post-translational processing of nascent proteins causing fairly major changes in carbohydrate structures. Consequently, changes may occur in an

antigenic determinant's structure and/or accessibility. Monoclonal antibodies originally raised against specific carbohydrate determinants may no longer recognise them after such changes have occurred. Apart from the use of monoclonal antibodies and lectins, other biochemical techniques have been used to detect such changes in antigen expression (Sidebottom, 1987). The presence of alterations in the carbohydrate composition of mucins provides a unique opportunity to study biochemical alterations in cell surface carbohydrate composition during carcinogenesis. For review of the synthesis and characterisation of mucins see Hilkens (1988).

2.3 THE IDEAL TUMOUR MARKER

The ideal properties for a tumour marker depend ultimately on its intended application. Immunohistochemical detection, radioimmuno-scintigraphy, or imaging, and immunotherapy require markers which are readily detectable on the tumour cell surface, whilst serum markers are essential for non-invasive diagnosis and monitoring. These applications will be dealt with in detail in chapter 3.

2.3.1 Ideal properties of a serum tumour marker

The advantage of measuring secreted markers compared to the physical measurement of a mass is the representation of *viable* tumour burden given by a tumour cell product, rather than total tumour burden as imaged radiologically. Daar and Lennox (1987) have identified several ideal properties of a serum tumour marker. The ideal serum tumour marker should:-

1. be easy and inexpensive to measure.
2. be specific to and commonly associated with the tumour in question.
3. positively correlate with the tumour volume.
4. reflect the current disease status and change as the status of the tumour changes over time.
5. precede and predict recurrences before they become clinically detectable.

6. have stable levels which are not subject to wide fluctuations.

In addition, a serum tumour marker assay shoud be reproducible and the antibodies should not cross-react with determinants found on other molecules. It is extremely rare for a tumour marker to conform to all or even most of the above criteria. Despite this, however, a few markers do have a well proven place in clinical disease management. No discussion of tumour marker ideals is complete without a mention of HCG and choriocarcinoma as an example. Measurement of HCG, which is closest to the ideal serum marker, has had a major impact on the clinical management and survival rates of choriocarcinoma, described in the following section.

2.3.2 Human chorionic gonadotrophin

HCG is a glycoprotein composed of two subunits, α (molecular weight 14,900 D) which is almost identical to the α subunits of thyroid stimulating hormone (TSH), follicle stimulating hormone (FSH), and luteinising hormone (LH); and β (molecular weight 23,000 D) which is similar to the β chain of other glycoprotein hormones, except for the carboxy-terminal sequence which renders it antigenically distinct. Early assays of HCG employed polyclonal antibodies directed against the intact molecule and were unable to discriminate between HCG and LH. Vaitukaitis *et al.* (1972) were the first to develop an assay using a polyclonal antibody directed against the β subunit, enabling the more specific detection of β HCG. Many monoclonal antibody assays to β HCG have been developed since.

HCG is normally secreted by the placental syncytiotrophoblast to maintain corpus luteum function and preserve progesterone secretion during the early stages of gestation. It can be detected as early as five days after conception, and reaches a peak at 8-10 weeks of pregnancy. Serum levels are found elevated in the presence of germ cell tumours and gestational trophoblastic malignancy (Bagshawe, 1978). Apart from pregnancy testing, β HCG assay is primarily used in the diagnosis and monitoring of these tumours. The use of β HCG measurement to diagnose and monitor treatment of choriocarcinoma

following evacuation of hydatidiform mole presents one of the more precise and satisfying features of cancer management, described below.

2.3.3 Management of choriocarcinoma with β HCG

Choriocarcinoma develops after evacuation of benign trophoblastic disease, hydatidiform mole, in 5-10% of cases. It is among the most chemosensitive of human solid tumours, responding to a variety of agents, particularly methotrexate, which is the usual first-line treatment. The malignant form may develop months or years after molar pregnancy, and for this reason women treated for this condition are followed up for at least a year by serial serum β HCG measurement as part of a national screening programme (see chapter 3, section 3.4, p65).

The criteria for active therapy include: high β HCG levels more than four weeks after evacuation; progressively increasing β HCG values at any time after evacuation, and; histological evidence of choriocarcinoma or evidence of metastases (WHO, 1983). Patients may be divided into two prognostic groups depending on levels of β HCG; those in the low risk group receive single agent therapy, while those in the high risk group receive combination chemotherapy. Serial measurements are used to monitor response to therapy and to follow-up patients after treatment; as β HCG is highly specific, increasing levels are indicative of relapse (Rustin, 1987). Adoption of β HCG serial measurement has contributed to a substantial reduction in mortality in patients with non-metastatic disease.

In summary, β HCG is ideal for monitoring choriocarcinoma because there is a close linear relationship between β HCG and the number of choriocarcinoma cells, and β HCG assay can detect as few as 10^5 cells. In addition, choriocarcinoma is a particularly chemosensitive tumour. It must be emphasized, however, that the success of β HCG in management of this disease owes most to the existence of a well defined high risk group with a relatively high disease prevalence.

2.4 OVARIAN TUMOUR MARKERS

The vital need for tumour markers that will facilitate earlier accurate diagnosis, monitoring, and development of more specific, less toxic therapy for women with EOC was demonstrated in chapter 1. Bhattacharya *et al.* (1985a) identified four major groups of molecules as potential markers for ovarian malignancy: tumour associated antigens, oncofoetal antigens, carcinoplacental glycoproteins and, complex proteins or glycoproteins with enzymic activity. These correspond roughly to the last two groups subsequently described by Daar and Lennox (1987). Confusion may result from the different classification schemes used by different authors; categorisation is difficult and is not absolute as there is extensive overlap between categories.

Ovarian tumour markers have been identified using a variety of techniques. Many were initially identified after raising antibodies to ovarian tumour tissue extracts or other samples from patients with EOC, while other markers were first associated with non-ovarian tumours, and were subsequently found to be expressed in a high proportion of EOC patients. Table 2.2 lists ovarian tumour markers reported in the literature to date. This list is by no means exhaustive, new putative markers appear in the literature on an almost monthly basis, but the list serves to illustrate the diversity of marker molecules associated with this disease. Seven categories have been employed in this thesis: oncofoetal antigens, carcinoplacental antigens, enzyme antigens, proto-oncogene antigens, polyclonal antibody defined antigens, chemically defined antigens, and monoclonal antibody defined antigens, see table 2.2.

Many of the monoclonal antibodies listed are directed against determinants upon the same PEM (polymorphic epithelial mucin) molecule, these will be indicated by an asterisk. Most of these markers have not been fully characterised. A description of the characteristics of the more promising "novel" tumour markers investigated in this thesis will be given after table 2.2.

Table 2.2 ***Putative ovarian tumour markers***

Classification	Reference
Oncofoetal antigens	
Carcinoembryonic antigen (CEA)	van Nagell *et al.* (1978)
α-foetoprotein (AFP)	Stanhope *et al.* (1979)
Tumour-associated trypsin inhibitor (TATI)	Stenman *et al.* (1982)
Carcinoplacental antigens	
Tissue polypeptide antigen (TPA)	Bjorklund (1972)
Human chorionic gonadotrophin (β HCG)	Fishman *et al.* (1975)
Enzyme antigens	
Placental-like alkaline phosphatase (PLAP)	Fishman *et al.* (1968)
Lactate dehydrogenase (LDH)	Awais *et al.* (1973)
Galactosyltransferase (GT)	Bhattacharya *et al.*(1976)
Ribonuclease (RNAase)	Sheid *et al.* (1977)
Cystine aminopeptidase (CAP)	Blum and Sirota (1977)
Leucine aminopeptidase (LAP)	Blum and Sirota (1977)
Cystidine 5'-monophosphate	Chatterjee *et al.* (1978)
N-acetylneuraminic acid(CMP-NANA)	Chatterjee *et al.* (1978)
Sialyl-tranferase	Khoo (1979)
Serous ovarian neoplastic amylase (SONA)	Vankley (1981)
α-L-fucosidase	Barlow *et al.* (1981)
5'-nucleotidase	Chatterjee *et al.* (1981)
β-hexoseaminidase	Chatterjee *et al.* (1982)
Proto-oncogene antigens	
c-myc, c-fos (nuclear proteins)	Slamon *et al.* (1984)
c-fms (receptor tyrosine kinase)	Slamon *et al.* (1984)
c-HA-ras (membrane bound GTPase)	Krontiris *et al.* (1985)
c-Ki-ras (membrane bound GTPase)	Filmus & Buick (1985)
c-neu/c-erb-B2/HER-2 (receptor tyrosine kinase)	Slamon *et al.* (1989)

Table 2.2 *continued.....*

Classification	Reference
Polyclonal antibody defined antigens	
OCAA, OCAA-1	Bhattacharya & Barlow (1973)
OV_1, OV_2	Order *et al.* (1975)
Fucose-rich glycoprotein (FRGP)	Hamazaki & Hotta (1975)
Immunogenic ovarian tumour antigen (IOTA)	Stolbach *et al.* (1979)
Thermostable antigen (TA)	Burton *et al.* (1976)
A1-A4	Burton *et al.* (1977)
M1-M4	Bara *et al.* (1977)
OCA	Knauf and Urbach (1978)
OVC-1, OVC-2	Imamura *et al.* (1978)
OCAA 2-5	Bhattacharya & Chatterjee (1980)
Dawson Antigen	Dawson *et al.* (1980)
Chemically defined antigens	
Fibrin degradation products (FDP)	Svanberg & Astedt (1975)
CSAp	Pant *et al.* (1978)
Pepsinogens	Hirsch-Marie *et al.* (1978)
β-Oncofoetal antigen (BOFA)	Goldenberg *et al.* (1978a)
Circulating immune complexes	Poulton *et al.* (1978)
β2-Microglobulin	Khoo *et al.* (1979)
Prostaglandin F	Sanders *et al.* (1980)
CX.1	Wass *et al.* (1981)
β1-PAM	Stimson & Farqharson (1981)
cGMP	Turner *et al.* (1982)
Ferritin	Yabushita *et al.* (1985)
Acute phase proteins, IAP	Koebl *et al.* (1988)
Sialic acid	Goldhirsch *et al.* (1988)
Prostacyclin, thromboxane	Aitokallio-Tallberg *et al.* (1988)
M-CSF	Kacinski *et al.* (1989)

Table 2.2 ***continued.....***

MAb defined antigens	Antigen	Reference
Ovarian carcinoma		
OC125	High M_r glycoprotein	Bast *et al.* (1981)
NB/70K	70 KD glycoprotein	Knauf & Urbach (1981)
GP48	48 KD glycoprotein	Bhattacharya *et al.* (1982)
ID3	High M_r glycoprotein	Bhattacharya *et al.* (1982)
MOV 2	High M_r glycoprotein	Colnaghi *et al.* (1982)
OC133	80 KD protein	Berkowitz *et al.* (1983)
MF 116	105 KD glycoprotein	Mattes *et al.* (1984)
$4F_4,7A_{10}$	48 KD glycoprotein	Bhattacharya *et al.* (1984)
OVTL3	High M_r glycoprotein	Epenetos *et al.* (1987)
OV632	High M_r glycoprotein	Flueren *et al.* (1987)
MOV 8, MOV 19	Low M_r glycolipids	Mottolese *et al.* (1987)
NB 12123	70 KD protein	Knauf & Bast (1988)
CASA, OSA	High M_r glycoprotein	McGuckin *et al.* (1990)
Breast carcinoma		
*DF3	High M_r glycoprotein	Abe & Kufe (1981)
*HMFG1, *HMFG2	High M_r glycoproteins	Taylor-Papadimitriou *et al.* (1981)
*AUAI	High M_r glycoprotein	Arklie *et al.* (1981)
*B72.3	High M_r glycoprotein	Nuti *et al.* (1982)
*115 D8	High M_r glycoprotein	Hilkens *et al.* (1984)
*NCRC 11	High M_r glycoprotein	Ellis *et al.* (1984)
F36/22	High M_r glycoprotein	Croghan *et al.* (1984)
2G3	High M_r glycoprotein	Frankel *et al.* (1985)
369 F10	High M_r glycoprotein	Frankel *et al.* (1985)
200 F9	High M_r glycoprotein	Frankel *et al.* (1985)
41 B4	230 KD glycoprotein	Frankel *et al.* (1985)
520 C9	200 KD glycoprotein	Frankel *et al.* (1985)
113 F1	200/100/60/40 KD protein	Frankel *et al.* (1985)
454 A12, 454 E4,	96 KD transferrin receptor	Frankel *et al.* (1985)
454 C11	200 KD glycoprotein	Frankel *et al.* (1985)
493 D1	71 KD protein	Frankel *et al.* (1985)
9C6	70 KD protein	Frankel *et al.* (1985)
33 F8	66 KD protein	Frankel *et al.* (1985)
677 B8	65/61 KD protein	Frankel *et al.* (1985)
317 G5	43 KD glycoprotein	Frankel *et al.* (1985)
90K	90 KD glycoprotein	Scambia *et al.* (1988)
2C8,2F7	60 KD glycoprotein	Bhattacharya *et al.* (1985)
*SM3	High M_r glycoprotein	Burchell *et al.* (1987)
CA54/61	High M_r glycoprotein	Nozawa *et al.* (1989)
Colon carcinoma		
NS199	Low M_r glycolipid	Koprowski *et al.* (1979)
Pancreatic carcinoma		
DU-PAN-2	High M_r glycoprotein	Metzgar *et al.* (1982)
Osteogenic sarcoma		
791T/36	72 KD protein	Embleton *et al.* (1981)
Laryngeal carcinoma		
Ca 1	High M_r glycoprotein	Woods *et al.* (1982)

2.4.1 CA125

The OC125 monoclonal antibody was produced by Bast *et al.* (1981) using a modified method of Köhler and Milstein (1975). BALB/c mice were immunized using the cell line OVCA 433 established from a patient with stage III serous cystadenocarcinoma of the ovary. After immunization, spleen cells from the mice were fused with a plasmacytoma cell line, and the supernatants from resultant colonies were screened using indirect immunofluorescence. OC125, the most promising clone isolated, was selected for its reactivity with the OVCA 433 cell line and other EOC cell lines, but lack of reactivity with a B lymphocyte line established from the same patient. OC125 defines an antigenic determinant designated CA125, cancer antigen 125.

CA125 is found elevated in a number of pathological and physiological states, particularly reproductive states. Kabawat *et al.* (1983a), using a variety of immunofluorescence techniques, demonstrated expression of CA125 in first and second trimester foetal tissues derived from coelomic epithelium (Müllerian ducts, cells lining the foetal peritoneum, pleura, and pericardium) and amniotic epithelium. CA125, however, was absent in cells lining the foetal ovary or any other foetal tissues. CA125 also reacted with adult derivatives of coelomic epithelium including cells from the Fallopian tubes, endometrium and endocervix (Kabawat *et al.*, 1983a). No reactivity was observed with normal ovarian epithelium, although CA125 was expressed in cells lining inclusion cysts, papillary excresences and adhesions where surface epithelial cells had undergone metaplasia. Reactivity was also found in mesothelial cells lining adult pleura, pericardium and peritoneum. From these observations, Kabawat *et al.* (1983b) concluded that CA125 is a differentiation antigen associated with coelomic epithelium and its normal and neoplastic derivatives. Subsequent studies found CA125 expression in sections of normal ovarian epithelium in addition to normal epithelium of the pancreas, colon, gall bladder, stomach, lung and kidney (Dietel *et al.*, 1986, Nouwen *et al.*, 1986 and 1987).

Of particular clinical interest were the findings of elevated serum CA125 in patients with endometriosis (for review see Kenemans *et al.*, 1988), during menstruation (Pittaway *et al.*, 1987) and early in pregnancy (Niloff *et al.*, 1984a). Jacobs *et al.* (1988a) investigated the compartmental distribution of CA125 activity in tissue homogenates from the female reproductive tract. The highest tissue levels were detected in first trimester decidual homogenate and were greater than those found in non-pregnant endometrium and term decidua. These findings parallel the rise in serum CA125 during first trimester of pregnancy, and are consistent with the hypothesis that CA125 is a product of normal endometrium/decidua, suggesting that CA125 elevation during pregnancy is of decidual origin (Quirk *et al.*, 1988). CA125 is also elevated in endometrial and cervical mucus (de Briujn *et al.*, 1986) in agreement with the above findings. Indeed, CA125 serial measurement has been advocated for follow-up of patients with endometriosis.

The highest CA125 levels are found in patients with EOC. Elevated serum levels of CA125 were originally reported in greater than 80% of EOC patients, and reflected the course of disease in the majority of these patients (Bast *et al.*, 1983). Consequently, a large research effort has established the role of CA125 in several aspects of EOC management (Canney *et al.*, 1984, Crombach *et al.*, 1985, Bast *et al.*, 1985, Niloff *et al.*, 1986, Lavin *et al.*, 1987, Brioschi *et al.*, 1987, Schilthuis *et al.*, 1987, Lambert *et al.*, 1987). Kabawat *et al.* (1983b) first demonstrated the association of CA125 in tumours of serous, endometrioid and clear cell types but not mucinous tumours. Subsequent studies found increased tissue expression in patients with all histological types of ovarian cancer, and in a high proportion of malignancies arising in the lung, breast, stomach, liver, gall bladder, pancreas, kidney and large bowel (Bast *et al.*, 1983, Haga *et al.*, 1986). It is expressed by epithelial ovarian tumours and other tissues of Müllerian origin, notably endometrial, endocervix, and fallopian tube malignancies (Niloff *et al.*, 1984b).

The determinant CA125 is found on a high molecular weight glycoprotein, the function of which is unknown. Due to its complex nature, information regarding

the physical and immunological nature of this antigen is limited. Studies of the nature of CA125 are also hampered by the inability so far to clone the gene that encodes it. Several investigators have determined the molecular weight of the CA125-bearing molecule. Using Western blotting, CA125 activity in ovarian cancer serum, amniotic fluid, human milk, and ovarian cancer cell line supernatants was associated with a moiety of greater than 1000 KD and a lower molecular weight moiety of 200 - 400 KD (Davis *et al.*, 1986, O'Brien *et al.*, 1986). The nature of the determinant has been investigated using a variety of chemical, physical and enzymatic treatments. CA125 activity was destroyed by treatment with periodate, mild alkali and neuraminidase, suggesting that it is a sialylated saccharide bound to protein by alkali-labile linkage (Hanisch *et al.*, 1985). However, Davis *et al.* (1986) found periodate treatment, at a concentration sufficient to oxidize carbohydrate, to have no effect on CA125, while heating to 100°c not surprisingly destroyed activity. They concluded that the determinant must be proteinaceous in nature. The precise nature of the CA125 determinant however remains unclear. Davis *et al.* (1986) found a carbohydrate content of 26%, lower than that of a typical mucin (see section 2.4.3) In addition, CA125 contains N-linked saccharides, while mucins are typically O-linked.

Whilst co-expression of CA125 with the antigenic determinants CA199, and DF3 and B72.3, have been found on the same molecular complex (Davis *et al.*, 1986), reports of serum levels indicate independent expression.

2.4.2 $HMFG_2$

Fat globules found in milk are surrounded by a membrane which is acquired as they are extruded from the acinar cells of the mammary gland by a process of reverse pinocytosis (Dowben *et al.*, 1967). The membrane should therefore be representative of the apical cell membrane of the secretory mammary cell and contain components appropriate for this stage of differentiation. It is relatively easy to isolate the non-lipid components of the milk fat globule membrane by extracting the fat globules with lipid solvents. Polyclonal antisera

raised to delipidated Human Milk Fat Globule (HMFG) and made specific by adsorption with various human cells (Ceriani *et al.*, 1977) proved to be a useful tool for the identification of breast epithelial cells in culture and tissue sections. It was also useful for localising mammary tumours in mice (Wilbanks *et al.*, 1981). Such delipidated preparations have been used to raise monoclonal antibodies in an attempt to obtain specific markers for the characterisation of breast epithelial cells *in vitro* and *in vivo*, and also for defining differentiation stages in normal and malignant breast epithelial cells (Taylor-Papadimitriou *et al.*, 1981).

Taylor-Papadimitriou *et al.* (1981) produced monoclonal antibodies by injecting female BALB/c mice, first with delipidated HMFG, then with a booster injection three weeks later with either the same delipidated HMFG or with milk epithelial cells grown in culture for two weeks ($HMFG_1$ and $HMFG_2$ respectively). Two antibodies, 1.10.F3 ($HMFG_1$) and 3.14.A3 ($HMFG_2$), reacted strongly with 7/8 breast cancer cell lines but were unreactive with lymphoblastoid cell lines, fibroblast cell lines, or other epithelial cell lines (except HeLa derivatives). Both of these antibodies showed strong binding, by indirect immunoperoxidase staining of paraffin-embedded and frozen tissue sections, to malignant breast cells (Arklie *et al.*, 1981). Both antibodies were strongly expressed in actively secreting mammary epithelial cells, but not on most normal cells found in the non-lactating breast (Arklie *et al.*, 1981), and they exhibited a luminal binding pattern. Primary breast cancer tissue of all cell types, including; ductal, lobular, medullary, mucoid, and Pagets disease of the nipple, stained with both antibodies (Arklie *et al.*, 1981), whilst lymph node metastases stained positive with 3.14.A3 ($HMFG_2$). Well differentiated cells stained more strongly than poorly differentiated cells. Of the small numbers of other tumour types initially examined, only three stained positive; ovary, lung, and uterus (Arklie *et al.*, 1981). $HMFG_2$ was also shown to stain bladder carcinomas positively (Conn *et al.*, 1988) and over 90% of epithelial ovarian tumours (Ward *et al.*, 1987a).

The HMFG component produced by lactating mammary glands was originally purified by Shimizu and Yamauchi (1982), and characterised as a mucin. Later,

it was identified by Burchell *et al.* (1983) and Griffiths *et al.* (1987) as being the antigenic component recognized by a range of monoclonal antibodies raised against HMFG preparations (see table 2.1). It has been variously termed PAS-O (Shimizu and Yamauchi, 1982), NPEP - non-penetrating glycoprotein (Ceriani *et al.*, 1983), EMA - epithelial membrane antigen (Ormerod *et al.*, 1983), MAM-6 (Hilkens *et al.*, 1984), PUM - polmorphic urinary mucin (Swallow *et al.*, 1986), ESM - epithelial sialomucin (Hilkens, 1988), and most recently PEM - polymorphic epithelial mucin (Hilgers *et al.*, 1989).

Mucus glycoproteins, commonly referred to as mucins, have been difficult to analyse because of their complexity. PEM, however, is a "simple" non-gel forming mucin that has been relatively well characterised biochemically. Mucins have the following characteristic properties: a high molecular weight (usually >400 KD); a high serine and threonine content in the protein backbone; mainly O-linked glycans (attached via N-acetylgalactosamine to the hydroxyl oxygen of serine and threonine, in contrast to the N-linkage via the amide nitrogen of asparagine to N-acetylglucosamine in most cell membrane and plasma glycoproteins) which may comprise >50% of the weight of the molecule; an apical localization in normal epithelia, and a density intermediate between those of plasma glycoproteins and proteoglycans (Hilkens, 1988).

Burchell *et al.* (1983) showed several intermediate glycoproteins in Western blots, using the monoclonal antibody $HMFG_2$, of cell lysates of T47D breast cancer cells. These were thought to represent long-lived intermediates in the glycosylation process (Griffiths *et al.*, 1987). The biosynthesis of O-linked sugars has not been elucidated in great detail; there is no consensus on the timing and localization of the initiation of O-linked carbohydrates, for review see Hilkens, 1988. PEM exhibits extensive polymorphism (hence the name) which has been detected by various monoclonal antibodies and lectins (Walker, 1990). This polymorphism, first noted at the glycoprotein level (Swallow *et al.*, 1986 and 1987), is a result of genetic polymorphism. Heterogeneity in the size of the apomucin, shown by Burchell *et al.* (1983), occurs due to a variable number (20-100) of tandem repeat units present in the gene (Gendler *et al.*,

1988). Initial sequencing of partial cDNA clones showed that the tandem repeat sequence consisted of 60 base pairs, which were highly rich in guanine and cytosine and coded for a peptide of 20 amino acids rich in serine, threonine and proline (Gendler *et al.*, 1987 and 1988). The gene has been mapped to band 22q of chromosome 1 (Swallow *et al.*, 1987), and recently Gendler *et al.* (1990) have reported the full sequence for PEM. The core protein was originally estimated to be 68 KD after hydrogen fluoride deglycosylation of the mucin purified by affinity chromatography from mixed samples of human milk (Burchell *et al.*, 1987). Over one quarter of all amino acids in PEM contain potential glycosylation sites.

Some monoclonal antibodies raised against PEM are directed to the carbohydrate moiety or a combined protein-carbohydrate epitope, but most are directed to the protein backbone, including $HMFG_2$ (Price *et al.*, 1991). $HMFG_2$ recognises a synthetic peptide corresponding to the amino acid sequence predicted by the tandem repeat sequence. The epitope consists of three amino acid residues - asparagine - threonine - arginine (Price *et al.*, 1991). The monoclonal antibody SM-3 raised specifically against the core protein of PEM (Burchell *et al.*, 1987) does not react with the fully processed mucin but reacts with the aberrantly processed PEM produced by breast cancer cells. This antibody may therefore form the basis of a more cancer specific assay.

2.4.3 CA153

The tumour marker CA153 is defined by two monoclonal antibodies, DF3 (Kufe *et al.*, 1984) and 115D8 (Hilkens *et al.*, 1985), raised against a membrane-enriched fraction of the human breast cancer cell line MCF-7 and delipidated HMFG (see previous section) respectively. Kufe *et al.* (1984) originally showed differential reactivity of DF3 with malignant and benign breast tumours. Hilkens *et al.* (1984) using a sandwich assay, which employed 115D8 as both catcher and tracer antibodies, found elevated MAM-6 in a high proportion of patients with metastatic breast, ovarian, prostate and cervical carcinomas. Tobias *et al.*

(1985) were the first to develop a sandwich immunoassay using DF3 as catcher and 115D8 as tracer antibodies to define the tumour marker designated CA153.

Using this assay, Tobias *et al.* (1985) found elevated serum CA153 levels in 79% of patients with metastatic breast cancer. Fewer than 10% of patients with benign breast, liver, lung, ovarian or gastrointestinal tumours had elevated serum CA153 (Tobias *et al.*, 1985). Subsequently, Hilkens *et al.* (1985) demonstrated CA153 overexpression by immunohistochemistry in 50% of ovarian malignancies. Scambia *et al.* (1988), in a preliminary longitudinal study of EOC patients, reported elevated CA153 in those with all histological types of EOC, and 20% of those with benign ovarian tumours. The authors also report no incidence of CA153 elevation in fibromyomatosis, endometriosis or endometrial hyperplasia. Serum CA153 is also found raised in carcinomas of the lung, colon, stomach, uterus and pancreas (Tobias *et al.*, 1995). The epitopes recognised by both DF3 and 115D8 are present on PEM described in the previous section (section 2.4.3).

2.4.4 CA724

The monoclonal antibody B72.3 raised by Colcher *et al.* (1981), together with cc49 (Muraro *et al.*, 1988), a second generation MAb with greater affinity and similar specificity to B72.3, form the basis of a double-determinant assay for TAG72 (tumour-associated glycoprotein). Using immunohistochemical techniques, TAG72 was found expressed in all types of EOC (Johnson *et al.*, 1985, Thor *et al.*, 1986). Klug *et al.* (1986) found CA724 elevated in the serum of EOC patients. Scambia *et al.* (1990) found CA724 elevated in the serum of a higher proportion of patients with EOC than cervical or endometrial cancer. CA724 was found not to be raised in breast cancer serum by Scambia *et al.* (1990), in agreement with Klug *et al.* (1986). These findings were surprising considering the monoclonal antibody B72.3 was generated using a membrane enriched extract of metastatic breast tumour tissue, and was originally shown to stain positive with 50% of all breast cancer tissue sections tested (Colcher *et al.*, 1981). Scambia *et al.* (1990) found CA724 serum elevation in fewer than

10% of all benign ovarian tumours and patients with fibromyomatosis. No incidence of CA724 serum elevation was found in patients with endometriosis or endometrial hyperplasia (Scambia *et al.*, 1990). In addition, Thor *et al.* (1986) found CA724 elevation in fewer than 10% of patients with benign ovarian tumours and normal ovaries, suggesting CA724 may have value in the differential diagnosis of an adnexal mass. CA724 has also proved a useful marker of digestive tract malignancies, notably stomach, colon, pancreas and biliary tract tumours. The epitopes recognised by both B72.3 and cc49 are also present on a high molecular weight glycoprotein complex of greater than 1000 KD.

Much interest has been focused on the surface expression of TAG72 *in vitro* and *in vivo*. Preliminary studies have shown the usefulness of B72.3 in radioimmunoscintigraphy (Surwit *et al.*, 1989). The influence of α-interferon on cell surface expression of CA724 has been investigated by several authors. Greiner *et al.* (1986) demonstrated increased expression of CA724 in response to α-interferon treatment, although Scambia *et al.* (1990) found no evidence of increased CA724 in the circulation of patients receiving α-interferon therapy. This type of therapy may facilitate imaging and targeting with B72.3 by enhancing cell surface expression of TAG72.

2.4.5 CA199

The monoclonal antibody NS 199 that recognises the determinant CA199 was originally raised against a human colon adenocarcinoma derived cell line SW1116 (Koprowski *et al.*, 1979). The distribution of CA199 in normal and malignant tissues has been described by Atkinson *et al.* (1982) using immunohistochemistal techniques. Positive staining was observed in the majority of patients with adenocarcinomas of the pancreas, stomach, colon and in fewer patients with gall bladder tumours. The authors also found positive staining in normal pancreas, stomach, liver, gall bladder and lung (Atkinson *et al.*, 1982).

CA199 is found elevated in the serum from patients with a wide range of gastrointestinal malignancies, including gastric, colon, panceatic, and biliary tract tumours (Del Villano and Zurawski, 1983). Elevation of CA199 in ovarian cancer sera was first demonstrated by Ricolleau *et al.* (1983). In an earlier study, Charpin *et al.* (1982) found positive staining of CA199 in a high proportion of patients with mucinous EOC and advocated CA199 as a marker of mucinous EOC. CA199 has however proved a disappointing marker of other histological types of ovarian tumour (Bast *et al.*, 1984, Canney *et al.*, 1985).

Magnani *et al.* (1983) first identified CA199 as a mucin, after demonstrating that the antibody was directed against a sialylated lacto-N-fucopentose II epitope, an oligosaccharide which is biochemically related to Lewis^a blood group determinant (Magnani *et al.*, 1982).

2.4.6 TATI

Tumour-associated trypsin(ogen) inhibitor (TATI) was originally isolated from the urinary peptide fraction of a patient with stage III serous cystadenocarcinoma of the ovary (Stenman *et al.*, 1982). The oncodevelopmental nature of TATI was implied by findings of high concentrations in tissue extracts of ovarian, endometrial, and cervical cancer, and early (14-16 weeks), but not late (32-40 weeks) amniotic fluid (Stenman *et al.*, 1982). The authors also found trace amounts of TATI in normal brain, liver, spleen, lung, and kidney (Stenman *et al.*, 1982). High serum levels were found in patients with gastric, oesophageal, thyroid and breast cancers and hepatomas. High urinary levels were found in patients with ovarian, endometrial and cervical carcinomas, uterine sarcoma, and severe inflammatory diseases such as acute pancreatitis and broncho-pulmonary disease (Huhtala *et al.*, 1983), but especially hepatobiliary disease (Haglund *et al.*, 1986).

Urinary TATI measurement has generally been found to be more sensitive than serum assay in patients with EOC (Huhtala *et al.*, 1983), however, Halila *et al.* (1988) found serum TATI assay to be sufficiently sensitive for monitoring

patients with mucinous EOC. Expression of TATI appears to decrease with increasing degree of malignancy in patients with mucinous EOC (Halila *et al.*, 1988). They also found higher levels in mucinous cyst fluid than serous cyst fluid (Halila *et al.*, 1987). Several authors have suggested that TATI may add to CA125 in monitoring mucinous EOC (Halila *et al.*, 1987 and 1988, Mogensen *et al.*, 1990), however larger studies are needed to verify this.

Biochemical characterisation of TATI has been relatively easy in comparison to the other ovarian tumour markers investigated in this thesis. TATI is a 6 KD peptide, consisting of 56 amino acids, identical in composition, NH_2 terminal sequence and COOH terminal structure to pancreatic secretory trypsin inhibitor (PSTI) (Stenman *et al.*, 1982, Huhtala *et al.*, 1982), although its origin was extrapancreatic in the patient from whom TATI was originally isolated (Stenman *et al.*, 1982).

Halila *et al.* (1988) suggested that the role of TATI may be to protect normal cells from trypsin autolysis, conversely it may serve a protective role in the tumour. Production of proteolytic enzymes by malignant cells is believed to be essential to the ability of the tumour to degrade extracellular matrix and invade surrounding tissue (for review see Liotta *et al.*, 1986). In a search for the target protease for TATI, Koivunen *et al.* (1991) isolated TAT-2 (tumour-associated trypsinogen 2) from mucinous ovarian cyst fluid. TATI was shown to inhibit TAT-2 activity, thought to be involved in a protease cascade stimulating tumour cell invasion and degradation of extracellular matrix (Koivunen *et al.*, 1990). The authors suggested TATI elevation may be in reponse to TAT-2 expression (Koivunen *et al.*, 1990). Co-ordinate expression of TATI and TAT-2 has also been found by Stenman *et al.* (1990), who also suggest a protective role for TATI. This is consistent with the association of TATI with better differentiated, less invasive tumours (Halila *et al.*, 1988).

2.4.7 c-neu p185

Shih *et al.* (1981) first identified increased expression of the neu oncogene after transfection of NIH/3T3 cells with DNA from ethylnitrosourea-induced rat neuroglioblastomas. The gene was independently identified from normal genomic and cDNA libraries respectively, and termed c-erb B2 (Yamamoto *et al.*, 1986) and HER-2 (Coussens *et al.*, 1985).

C-neu expression has been found on the cell surface of normal adult and foetal tissue from the gastrointestinal, respiratory, reproductive and urinary tracts (Press *et al.*, 1990), and overexpression has been commonly observed in adenocarcinomas (Gullick and Venter, 1988). Neu has potent transforming capabilities in NIH/3T3 cells when expressed in comparable amounts to those observed in some human cancers (Di Fiore *et al.*, 1987). The gene encodes a cell surface glycoprotein of molecular weight 185KD, commonly known as p185, which posseses tyrosine kinase activity (Padhy *et al.*, 1982). C-neu p185 bears extensive sequence homology to epidermal growth factor receptor (EGFR) (Schechter *et al.*, 1984), although the c-neu proto-oncogene is found on band q21 of chromosome 17 (Coussens *et al.*, 1985) and the EGFR proto-oncogene is found on band p11-p13 of chromosome 7 (Meera Khan and Smith, 1984).

In the rat neuroblastoma, site directed mutagenesis has shown c-neu to be activated by virtue of a point mutation at position 664, which changes valine to glutamine in the transmembrane domain, resulting in constitutive expression of activated p185 (Bargmann and Weinberg, 1988). Lemoine *et al.* (1990), however, report no evidence of activating point mutations in the c-erb B2 proto-oncogene in human breast cancer. Although c-neu p185 does not bind to epidermal growth factor (Sefton, 1988), tyrosine phosphorylation of c-neu p185 is stimulated by epidermal growth factor (Connelly and Stern, 1990). Lupu *et al.* (1990) have recently identified a 30 KD factor, gp30, which they claim to be the natural ligand for p185.

Increased expression of the neu oncogene is invariably due to gene amplification, although overexpression has been observed in cancer cell lines in the absence of gene amplification (Gullick, 1990). Tyson *et al.* (1988) first reported neu amplification and overexpression in ovarian tumour tissue, and overexpression in the cell line OVCA 429 in the absence of amplification. The expression of neu has been studied most intensively in breast cancer (Naber *et al.*, 1990). Many similarities have been found between breast and ovarian cancer; 25-30% of tumours at each site exhibit neu amplification and overexpression (Slamon *et al.*, 1989, Berchuck *et al.*, 1990), and approximately 10% of each have single gene copies but overexpress neu (Slamon *et al.*, 1989).

Slamon *et al.* (1989) found neu amplification in 31/120 (26%) primary ovarian tumours; 23 of which were amplified 2-5 fold, and eight were amplified greater than 5-fold. They also found a highly significant correlation between gene amplification and overexpression (Slamon *et al.*, 1989). Patients were grouped according to gene copy number and level of expression, and significant inverse correlations were found between gene amplification and survival in 87 patients, and immunohistochemical staining and survival in 72 patients - although this association was far less significant than the inverse association between DNA amplification and survival. The authors observed no significant correlation between RNA and survival, although they suggest that the number of patients in the study may have been insufficient.

A sandwich ELISA, designated the c-neu ELISA, has recently been developed (Carney *et al.*, 1989) using two monoclonal antibodies NB3 and TA1. These antibodies recognize distinct epitopes on the extracellular domain of c-neu p185, and show no cross reactivity with EGFR (McKenzie *et al.*, 1989). Recent studies have shown that the c-neu ELISA detects a 100 KD glycoprotein in tissue culture supernatant of SK-BR-3 cells that overexpress the c-neu proto-oncogene. This p100 is biochemically related to the c-neu product, and is thought to represent the extracellular domain released from the cellular c-neu protein (Zabrecky *et al.*, 1991). Yuzhong and Clinton (1991) have recently

reported release of a 130 KD glycoprotein, also representing the extracellular domain of c-neu protein, in SK-BR-3 cells. Hayes *et al.* (1989), in a pilot study, found elevated serum levels of c-neu p185, using this assay, in 15% and 23% of patients with localised and metastatic breast cancer respectively. To date, this assay has not been evaluated in ovarian cancer.

CHAPTER 3

Clinical Applications of Epithelial Ovarian Tumour Markers

3.1 INTRODUCTION

The overwhelming feature of ovarian tumour markers discovered pre-1975 is that none has found a role in patient management, whilst monoclonal antibody defined markers, notably CA125, have found roles albeit limited ones. Some of the potential clinical applications of monoclonal antibodies directed against ovarian tumour markers have already been mentioned in chapters 1 and 2. They may be used *in vitro* for serological and immunohistological detection, and *in vivo* for imaging and targeting cytotoxic agents to the tumour. All of these techniques may be applied at one or more stages in the course of this disease. It should perhaps be emphasized at this point that therapeutic applications are still in the early research stages, while serological, immunohistological, and immunoradiological applications are routine in many centres. Many problems need to be resolved before general clinical application of each becomes feasible. In addition to requiring more experience with each technique, it will be a long time before the true benefits of any of these "novel" management strategies can be determined.

Bodmer (1987) has outlined three major aims in the control of cancer, in descending order of priority, as prevention, early detection, and development of specific treatment. It is unrealistic however to expect complete success with all or even any of these measures. In view of the difficulties in determining response to treatment in EOC patients, as described in chapter 1, these aims need to be extended to include a fourth; the ability to monitor response to treatment and determine a patient's disease status without having to resort to further and often unnecessary major surgery.

This chapter will examine each of the aforementioned problem areas. Table 3.1 lists potential clinical applications of MAbs in EOC management. Particular attention will be given to screening and monitoring ovarian cancer, mainly because screening has received much media attention recently and this thesis is concerned with monitoring patients with histologically proven EOC. Many of the principles concerning the use of tumour markers to screen for EOC also

apply to monitoring patients with established disease. First, an overview of imaging and therapy with monoclonal antibodies in ovarian cancer will be given.

Table 3.1 ***Clinical applications of monoclonal antibodies in EOC***

Application	Example(s)
1. **Screening**	Early detection of marker in serum, urine or other body fluid prior to clinical presentation.
2. **Diagnosis**	(i) Detection of marker in serum, urine or other body fluid. (ii) Immunohistology (including cytology) using panels of monoclonal antibodies to (a) determine the occult origin of metastases, (b) differentially diagnose anaplastic tumours, (c) classify tumours morphologically, (d) differentiate benign from malignant lesions, (e) determine prognosis, and (f) predict which markers may be useful for follow-up.
3. **Monitoring**	(i) Determining levels of tumour markers in serum, urine or other body fluids. (ii) Determination of tumour size and location, and any changes in these by radioimmunoscintigraphy (see below).
4. **Immunoimaging**	Detection of tumour masses and occult metastases by injection of radiolabelled monoclonal antibody (radioimmunoscintigraphy), including lymphoscintigraphy.
5. **Immunotherapy**	(i) Active immunotherapy (immunisation). (ii) Systemic (including intralymphatic) using monoclonal antibodies alone, as conjugates with cytotoxic drugs, radioisotopes, or interferons, or as immunoliposomes. (iii) bone marrow clearance using monoclonal antibodies alone, with complement or as conjugates to (a) deplete malignant cells prior to autologous bone marrow transplant, and (b) deplete cells to reduce graft versus host disease in allogenic bone marrow transplant.
6. **Modulation**	(i) Antibody directed antibody-dependent cell mediated cytolysis. (ii) "Arming" of other effector cells, e.g. macrophages and monocytes. (iii) Depletion of blocking factors and suppressor cells.

taken from Daar and Lennox (1987).

3.2 EOC TUMOUR MARKERS AS TARGETS FOR IMAGING AND THERAPY WITH MONOCLONAL ANTIBODIES

Conventional imaging and therapy of EOC suffers many limitations (see chapter 1). Imaging using X-rays, computed tomography, ultrasound, and magnetic resonance imaging can detect at best tumours of 0.5 to 1.0 cm in diameter,

while conventional chemotherapy usually fails because of non-selective toxicity and the development of drug resistance. The concept of a selective and highly efficient "magic bullet" was introduced by Ehrlich last century (Ehrlich, 1898), and was revived recently with the introduction of tumour markers. Several clinical trials using monoclonal antibodies directed against ovarian tumour markers are currently underway.

3.2.1 Route of administration

Whether or not a monoclonal antibody reaches a tumour deposit is dependent on a number of factors, including; the accessibility of the antigen; the size of the tumour deposit(s); the site of tumour deposit(s); the permeability of the epithelium lining blood and lymphatic vessels near the tumour. Given the many physical obstacles that have to be overcome, it is not surprising that the route of antibody administration has an effect upon the outcome of targeting. Systemic (i.v.) administration often results in the trapping of antibodies in normal tissues, in particular the reticulo-endothelial system. As ovarian tumours are mostly localised within the peritoneal cavity, intraperitoneal (i.p.) administration is the best route of administration for detection and therapy with monoclonal antibodies.

Haisma *et al.* (1987) compared i.v. with i.p. administration using ^{131}I labelled OC125 $F(ab')_2$ fragments in 10 patients with ovarian cancer. Rowlinson *et al.* (1987) defined the advantage given by i.p. adminstration as the tumour:tissue ratio following i.p. injection divided by the tumour:tissue ratio following i.v. injection. The i.p./i.v. advantage for normal tissue uptake was, however, poor ranging from only 1 to 10 in Haisma's study.

3.2.2 Immune response elicited by monoclonal antibodies

Although murine monoclonal antibodies are not toxic *per se*, they do elicit an immune response in the majority of patients after both diagnostic and therapeutic administration. One of the earliest groups to target ovarian

carcinomas, the Hammersmith Oncology Group, injected 10 patients with various monoclonal antibodies at a dosage of 250 μg and subsequently administered a therapeutic dose of 2 - 15 mg (Courtney-Luck *et al.*, 1986). Five of their patients developed a human anti-mouse antibody (HAMA) response. Later studies suggested that all patients injected with murine monoclonal antibodies would develop such a response after one or more doses.

HAMAs not only interfere with antibody localisation and uptake by the tumour, but may also cause falsely elevated serum marker levels (Janssen *et al.*, 1989). Several methods have been proposed to circumvent this problem to allow effective repeat administration of antibodies. These include; use of human monoclonal antibodies; use of hybrid antibodies that possess a human IgG frame; host immunosuppression, and; plasmapheresis (for review see Verheijen *et al.*, 1988).

3.2.3 Radioimmunoscintigraphy

The first experiments were conducted by Goldenberg *et al.* (1974) using polyclonal antibodies to CEA to localise xenografts of CEA-bearing human colon carcinoma, and later in patients using ^{131}I labelled antibodies (Goldenberg *et al.*, 1978b). The quality of an immunoscintigram depends on several factors, including the energy of emission, and the physical and biological half-life of the isotope. It is important that the isotope does not emit β particles which would be harmful to normal tissue.

3.2.4 Isotopes

In early studies, the most widely used isotope was ^{131}iodine, which has a high γ emission, relatively long half-life of 8 days, but also significant β emission. Several isotopic labels have been tested, including ^{123}iodine, ^{111}indium, ^{67}gallium, and ^{99m}technetium. *In vivo* dehalogenation was a common problem with iodine labelled antibodies resulting in excretion of free iodine and low tracer accumulation in the tumour (Mach *et al.*, 1980). Technetium-99m has

been advocated as the best label (Britton and Granowska, 1991) for imaging, although the half-life is short (6h), therefore images must be produced within 12h of administration.

Background subtraction techniques are often necessary to reduce the amount of background activity caused by accumulation of labelled antibody outside the target tissue. These may be achieved by subtracting early from late images when most of the antibody has been cleared from non-specific sites. The image obtained using a non-specific antibody may be subtracted from the image obtained with a more specific antibody, as demonstrated by Haisma *et al.* (1984) using MAb 115D8 to localise tumour in mice bearing human breast cancer xenografts. Alternatively, the "blood-pool" activity may be reduced by the use of antibody fragments. Fab and $F(ab')_2$ fragments can be prepared by papain and pepsin digestion respectively of the whole IgG molecule.

3.2.4 Clinical radioimmunoimaging studies

Epenetos *et al.* (1982a and b) were the first to successfully image ovarian cancer using ^{123}I labelled monoclonal antibodies $HMFG_1$ and $HMFG_2$ directed against PEM. Since this report, $HMFG_2$ has been extensively used to image ovarian cancer (Granowska *et al.*, 1984 and 1986, Patiesky *et al.*, 1985, Shepherd *et al.*, 1987, Ward *et al.*, 1987d). These early studies mostly employed ^{123}I and whole antibody. Granowska *et al.* (1986), in a prospective study found a correlation of 95% between pre-operative scans using ^{123}I labeled $HMFG_2$ and findings at primary laparotomy. Kalofonos and Epenetos (1987) were the first to use $F(ab')_2$ fragments of $HMFG_2$ to image ovarian cancer in 14 patients with advanced disease.

Numerous studies have investigated the use of OC125 $F(ab')_2$ fragments to image ovarian cancer (Chatal *et al.*, 1986, Hunter *et al.*, 1987, and Haisma *et al.*, 1987). Positive scans were obtained in patients with normal serum CA125 levels and negative CT scans, suggesting a role for OC125 imaging in detection of occult disease in EOC patients (Granowska *et al.*, 1988).

Other antibodies investigated in this thesis have been employed to image EOC patients. Hnatowich *et al.* (1985) were the first to use an indium label; ^{111}In labelled CA199 successfully visualised 67% of documented tumour sites in patients with various tumours. Greiner *et al.* (1986) used B72.3 to image EOC patients. The use of α-interferon therapy to enhance tumour cell surface expression of TAG 72 was mentioned previously in chapter 2, section 2.4.4, p 50.

3.2.5 Immunotherapy

Unmodified antibodies may mediate their effects through two mechanisms; complement activation and antibody-dependent cell mediated cytotoxicity (ADCC) (see table 3.1, p 58). The first clinical therapeutic use of antibodies in EOC patients involved the use of polyclonal human ovarian anti-tumour serum (HOATS) in a randomised trial comparing its addition to abdominal irradiation, melphalan, and i.p. colloidal ^{32}P (Order *et al.*, 1981). This study was not extended because of the rapid introduction of monoclonal antibodies.

3.2.6 Monoclonal antibody conjugates

It is important that conjugation of monoclonal antibodies with either radiolabels or other cytotoxic agents does not interfere with antibody-antigen interaction. Verheijen *et al.* (1988) have identified several preferable features of monoclonal antibodies for *in vivo* targeting. Thus, a candidate antibody must (1) be directed to a cell surface antigen, (2) bind to its antigen with high affinity, (3) not form immune complexes in the circulation, (4) internalize (for immunoconjugates), (5) mediate ADCC (for unmodified antibodies), and (6) not accumulate in the reticulo-endothelial system. Antigen expression must be high on the tumour cell surface and low on normal cells, i.e. it does not have to be "tumour specific".

Radiolabelled antibodies employing a wide variety of isotopes have been tested therapeutically. Isotopes with a short path length and a short half-life are prefered to minimise damage to normal tissue. β emitters, e.g. ^{131}I, ^{90}Y and ^{32}P,

which have a particle range of several cell diameters, are the most popular radionuclides (Britton and Granowska, 1991). Yttrium-90 has several advantages in that it is a pure β emitter, eliminating hazards to normal tissue. In a clinical study of ^{90}Y labeled OC125, administered intraperitoneally in patients with advanced EOC, Griffin *et al.* (1987) found tumour uptake to be 100 times that of normal tissue, and less than 3% of the dose was excreted in the patients' urine. ^{90}Y labeled $HMFG_2$ has also been used to target ovarian cancer (Snook *et al.*, 1987).

The majority of targeting studies in EOC patients have been performed using labelled OC125 or antibodies directed against epitopes found on PEM, mainly $HMFG_1$ and $HMFG_2$ (for review sees Verheijen *et al.*, 1988, and Britton and Granowska, 1991). The Hammersmith Oncology Group reported a response rate of 25% (7/28) in patients with ovarian carcinoma using i.p. ^{131}I labelled $HMFG_1$, $HMFG_2$ or AUA1, selected by positive immunohistology (Stewart *et al.*, 1987). Poels *et al.* (1986) have recently developed a monoclonal antibody, OV-TL3, directed against an antigen which is not found in the circulation. Preliminary results using this antibody are promising; *in vitro* studies have shown that the biodistribution of OV-TL3 antibody is superior to OC125 (Haisma *et al.*, 1987).

Monoclonal antibodies that have been "humanised" by grafting the hypervariable regions of murine monoclonal antibodies, that determine the antibody binding site, onto human IgG frames have been developed by Verhoeyen *et al.* (1991) for $HMFG_1$, and are currently undergoing clinical trials at Hammersmith Hospital, London. Humanised anti-PLAP (MAb H17E2) has also been genetically engineered in a similar manner (Verhoeyen *et al.*, 1991).

Anti-PEM antibodies are directed against cell surface antigens and probably do not internalise into the tumour cells; this is not a problem with radioimmunotherapy but could be a disadvantage of immunoconjugates. ADCC may be an important factor in the anti-tumour reaction, as most of these antibodies do not bind complement. The problem of tumour antigen

heterogeneity has already been alluded to in chapter 2. Several authors have concluded that the most effective approach to targeting immunotherapy using monoclonal antibodies may be to employ a "cocktail" of complementary antibodies, analogous to the use of panels of antibodies in immunohistochemistry and serology (see sections 3.6 and 3.7, pp. 76-82). The most effective combination, however, remains to be determined.

3.3 PRIMARY PREVENTION

Primary prevention deals with the control of factors known to cause disease. Several risk factors for EOC have been identified, although none has been helpful as yet in aiding its prevention, except oral contraceptive use (Vessey *et al.*, 1987). To recapitulate, family history, early menarche, late menopause, nulliparity, and infertility were amongst the most important risk factors. The only preventable cancer at present is cancer of the cervix, by identification of pre-neoplastic lesions through the use of the Papanicolaou (Pap) smear test. Even so, the proportion of individuals with cervical intra-epithelial neoplasia who progress to frank malignancy is small, and prevention is incomplete. It is estimated that 70-80% of cases are preventable given a 100% accurate diagnostic test and 100% compliance rate. Part of the failure of this screening programme is attributable to poor compliance; as many as nine in ten women diagnosed have never had a smear test.

Elimination of tobacco use, estimated to be responsible for 30-40% of all cancers, would bring greater net benefit to health than all our efforts to treat cancer of all types. Most primary preventative health measures require drastic alterations in our lifestyle, unfortunately there is no consensus yet, exemplified by our inability to control the greatest identifiable cause. Cigarette smoking is not an obvious risk factor for EOC. The inherent difficulties of imposition of a "healthy" lifestyle on any population, means that we must turn our efforts to the next best measure, namely the control of cancer through secondary prevention or screening.

3.4 SCREENING

3.4.1 Introduction

Britain has had a cervical screening programme in operation now for over 20 years, and has been embarking on the implementation of a national breast screening programme following the recommendations of the Forrest Report (DHSS, 1986). In contrast, ovarian cancer screening has received very little attention until recently, and yet it kills twice as many women annually as cervical cancer. Ovarian cancer has long been known as the "silent killer" because of its insidious onset, but also because it rarely received publicity. This situation has changed over the last two years as new reports of promising screening tests have been published (Jacobs *et al.*, 1988b, Campbell *et al.*, 1989, and Bourne *et al.*, 1989). Previous attempts at early detection of ovarian cancer using pelvic examination and cytology met with no success. New developments in monoclonal antibody technology and imaging techniques have brought fresh optimism to the hope of being able to diagnose ovarian cancer earlier when chances of cure and remission are increased because treatment of localised disease is more effective. Early diagnosis in general is believed to be a better approach to the reduction in mortality than the development of specific treatment.

Screening may be defined as "*the presumptive identification of unrecognised disease or defect by the application of tests, examinations, or other procedures that can be applied rapidly*" (Miller, 1985). Thus, a screening test is applied to asymptomatic individuals and is not intended to be diagnostic, where necessary this is confirmed by specialized secondary procedures. Conversion of the simple tenet "early diagnosis leads to better prognosis" into an effective large scale screening programme is a very complex organisational undertaking. Design and ultimately implementation of a screening programme for cancer is fraught with difficult and not entirely apolitical issues, ranging from ethical to economical, when large populations are to be screened in which only a small number of cases will be found. Many original problems encountered with

screening programmes arose because the issue was considered to be clinical rather than epidemiological, with attention focused on cases diagnosed at the expense of the population under study. Consequently, there are many lessons to be learned from those programmes already in existence. At the very least, a screening programme should guarantee an overall benefit to the community and minimal risk that it will be detrimental to certain individuals. At present, screening for cervical and breast cancer are the only two sites backed up by hard evidence as to the benefits at the population level in terms of overall achieveable reduction in mortality rates (Mant and Fowler, 1990). Recently, however, breast cancer screening has come under much dispute (Roberts, 1989), in particular the speed with which it has been introduced in relation to establishment of back-up services (Baum, 1990).

3.4.2 Principles of screening

The principles of screening for disease were first formulated by Wilson and Jungner in 1968 for WHO, comprising the following 10 criteria:-

1. The condition to be screened for should pose an important health problem.
2. The natural history of the disease should be well understood.
3. There should be a recognisable early stage.
4. Treatment of the disease at an early stage should be of more benefit than treatment started at a later stage.
5. There should be a suitable test or examination.
6. The test or examination should be acceptible to the population.
7. There should be adequate available facilities for the diagnosis and treatment of detected abnormalities.
8. For diseases of insidious onset, screening should be repeated at intervals deemed by the natural history of the disease.
9. The chance of physical or psychological harm should be less than the chance of benefit.
10. The cost of a screening programme should be balanced against the benefits it provides.

There are many important factors which may influence the outcome of population screening, some of which are considered below, see sections 3.4.3 to 3.4.6. The degree to which EOC fulfils these criteria will be addressed in section 3.5.

3.4.3 Evaluation of screening

Greenwald *et al.* (1985) have defined cancer control as "*the reduction of cancer incidence, morbidity and mortality through an orderly sequence from research on interventions and their impact in defined populations to the broad systematic application of the research results*". Thus, the basis of any screening programme must be quantification of the extent to which the above are reduced. Ultimately, the impact on mortality rates, through the implementation of randomised controlled trials, where the population is divided into a study group offered screening and a control group that receives the currently accepted programme of routine diagnosis and care, will be the only true measure of the efficacy of screening. Three important phenomena, lead time bias, length bias and selection bias, which may improve the apparent survival in the screened population, are examined below.

3.4.4 Lead time bias

Survival is measured from the date of diagnosis to death, rather than from the date of inception to death. The date of diagnosis may therefore vary considerably, depending on the methods of detection used, without altering the true length of survival from the date of inception. Lead time generated by screening, or the period from detection while the woman is still asymptomatic until the appearance of clinical symptoms which would permit conventional diagnosis, may increase the apparent survival without in fact the individual having benefitted from screening. In such circumstances the patient has to live with the knowledge of her disease for longer.

3.4.5 Length bias

A series of cases diagnosed at screening will be atypical of those arising clinically, since it will contain a disproportionate number of women with slowly developing tumours with probably a better prognosis. Women with rapidly progressing tumours are more likely to present with symptoms before the initiation of, or in the interval between, screening tests. This bias is more likely to be manifest at the initiation of screening and is therefore especially important in studies of short duration.

3.4.6 Selection bias

Selection bias results from entry of a cohort into a screening trial who have a different probability of developing and dying from the disease than the population at large. In self selected populations, it is common to find a higher than normal proportion of women presenting for screening because of a positive family history. These women are more motivated to present for screening because they are more educated in this respect and are more likely to benefit from it. This has been well demonstrated in breast and cervical screening programmes.

3.4.7 Value of a diagnostic test

The validity of a screening test is measured by its sensitivity, specificity, accuracy and predictive powers, see below. These may be expressed as a fraction or a percentage.

Sensitivity is the index of the chance that someone with the disease will have a positive test result, given by the formula:-

$$\textbf{Sensitivity} = TP / TP + FN \ (\times 100\%)$$

The **false negative rate** is given by 1 - sensitivity.

Specificity is the index of the chance that someone without the disease will have a negative test result, given by the formula:-

Specificity = TN / TN + FP (x 100%)

The **false positive rate** is given by 1 - specificity in this thesis, although may be defined in three other ways (Galen, 1990).

Accuracy (or efficiency) is the index of the chance that a test result is correct, given by the formula:-

Accuracy = TP + TN / TP + TN + FP + FN (x 100%)

The predictive value of a positive result (PVP), is the index of the chance that a positive test result means disease is present, given by the formula:-

PVP = TP / TP + FP (x 100%)

The predictive value of a negative result (PVN), is the index of the chance that a negative test result means disease is absent, given by the formula;-

PVN = TN / TN + FN (x 100%)

where TP = true positive, TN = true negative, FP = false positive, and FN = false negative results.

Difficulties in quantifying sensitivity and specificity may be due to uncertainty as to what constitutes an abnormality. This is where knowledge of the natural history of the disease becomes important, often this may only be determined after careful study of a screening programme's findings. Sensitivity is difficult to determine initially as false negatives are not readily apparent, long-term follow-up is required to determine the extent of these. Sensitivity was originally thought to be more important than specificity as it was felt to be crucial to avoid

missing individuals with disease. However, maximising sensitivity usually disproportionately increases cost, and some of the lesions detected at screening may never progress or may be picked up later in a curable state.

When deciding on a cut-off point for any test which is to discriminate between a dichotomous outcome, ie. disease positive and disease negative, a trade-off between sensitivity and specificity has to be made. These two are interrelated, hence, if sensitivity is increased specificity decreases and vice versa. It is more important to maximise specificity and thus minimise the number of individuals who are misclassified as disease positive when dealing with the diagnosis of a disease as serious as cancer. High numbers of false positives generates more work for the back up services, not to mention the unnecessary anxiety caused to the women who are falsely suspected of having cancer. It can be mathematically proven that if false negatives and false positives are of equal clinical concern, as prevalence tends to zero, specificity ascends over sensitivity,

FP + FN = prevalence(1-sensitivity) + (1-prevalence)(1-specificity)
Therefore, if prevalence tends to zero, FP + FN = 1-specificity,
therefore increasing specificity reduces false results (Roulston, 1990).

The best measure of the efficacy of a screening test is the PVP, because unlike sensitivity and specificity it is dependent upon the disease prevalence in the population being screened. Two tests, each for different types of cancer, may have equal sensitivity and specificity for their respective diseases, but the test applied to the population in which the disease prevalence is higher will have a higher PVP, making it a more reliable assessment of the disease presence in that particular population.

For example, consider screening two hypothetical populations for EOC with CA125 serum assay, which has a sensitivity of 80% (or false negative rate of 20%) and specificity of 99% (or false positive rate of 1%). Population A consists of postmenopausal women over 50 years old in whom the disease prevalence

is of the order of 40/100,000, and population B consists of postmenopausal women over 50 years old with a positive family history and relative risk of approximately 3, and therefore a disease prevalence of 120/100,000. If 10,000 women from each population are screened, one would expect to find four cases of ovarian cancer in population A and 12 cases in population B. Given a false positive rate of 1%, 100 women from each population would have falsely elevated CA125 levels. The PVP of CA125 assay in population A would be 3.8% [4/(4 + 100)], and 10.7% [12/(12 + 100)] in the high risk population B. Therefore, using the same test in different populations yields a greater PVP in the population with the higher disease prevalence.

3.4.8 Screening outcomes

Apart from attempting to control cancer, the role of screening is to provide individuals with information regarding the likelihood that they may have or may develop cancer. The four possible outcomes of a screening test each has significant implications for the individual concerned, see figure 3.1.

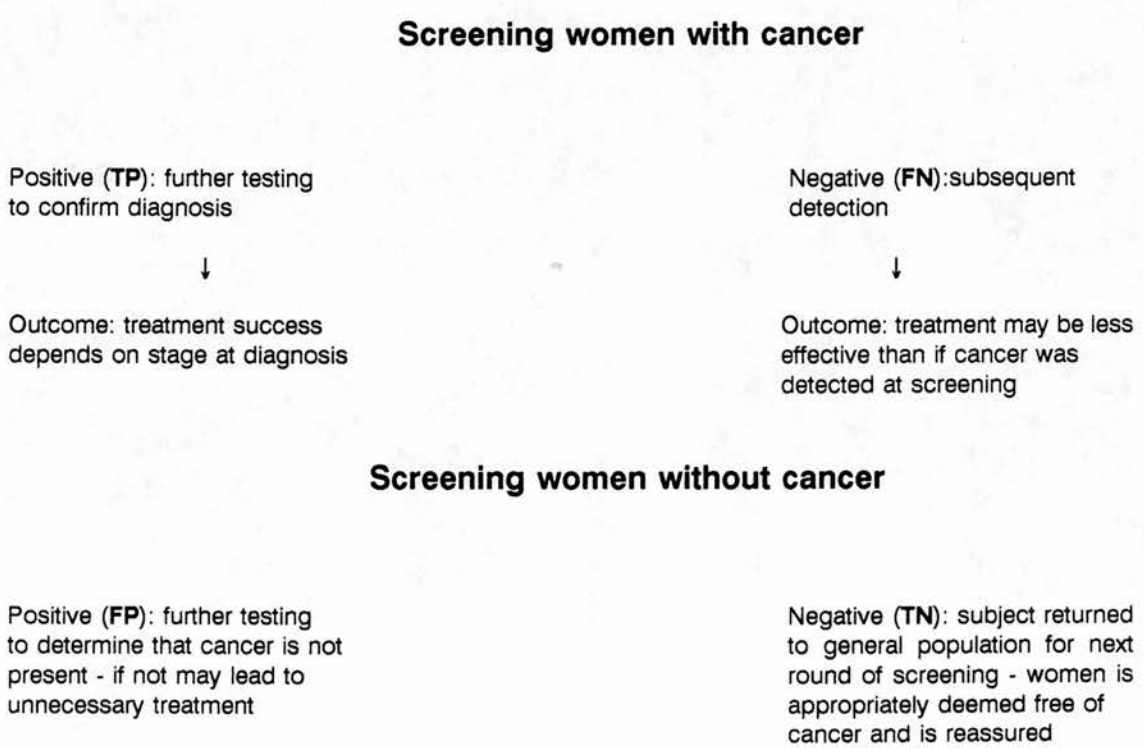

Figure 3.1 ***Screening outcomes*** (modified from Greenwald *et al.*, 1985).

The psychological sequelae of a positive diagnosis is perhaps easier to discern than that resulting from a negative result, or for that matter participation in screening alone. A false result will not only cause undue anxiety but may damage a woman's trust in the programme preventing her from further participation. The success of any screening programme is very dependent upon a high compliance rate from the target population.

3.5 SCREENING STUDIES

Ovarian tissue is not amenable to direct sampling in the way that breast and cervical tissue are. Thus the anatomical site of the ovaries imposes a number of restrictions as to the nature of a screening test. Screening must therefore be performed by techniques which either demonstrate a change in ovarian structure ie. size and morphology, or an alteration of ovarian function ie. reflected by the release of metabolites into the peripheral circulation. Several promising studies involving such techniques have come to light in recent years.

3.5.1 Ultrasound screening for EOC

Campbell and colleagues at King's College Hospital in London, have been performing trials to assess the use of ultrasound for the early detection of ovarian cancer for over 10 years. Initially, they found a good agreement between sector real-time pelvic ultrasound assessment of ovarian size and morphology with that obtained at laparotomy (Campbell *et al.*, 1982). Subsequently, they reported results of a prospective study of 5540 asymptomatic self-referred women over 45 years old, who underwent annual ultrasound for three years, to determine the prevalence of ovarian cancer in this population and assess the predictive value of ultrasound. Eight malignancies were detected of which five were stage 1a and three were metastatic. No false negative results were found, therefore, sensitivity was 100%. The false positive rate was 97.4% and PVP was 2.6%. When tumours of low malignant potential were included among the true positives, PVP rose to 19.3%, indicating a one in five chance of malignancy or potential malignancy with a positive result (Bhan

et al., 1987). This study highlights the difficulties in defining a positive result; as it is unknown what proportion of benign tumours will become frankly malignant (Fox, 1990).

Later, Campbell *et al.* (1989,1990) report detection of an additional metastatic cancer. Screen detected cancers declined in incidence over three successive screens from 1.7% at the first screen to 0.6% and 0.2% at the second and third respectively. Two primary cancers were detected at the first screen and three at the second screen, 16, 18 and 22 months after the first screen. The authors have suggested these results show a need to screen every 12-18 months. A gradual reduction in the false positive rate was noted as more criteria were included in the definition of abnormality. Thus, the false positive rate was lowest when morphology, volume and change in volume were taken into account (women with abnormal scans were rescanned three weeks later to exclude any transient alterations, and if the second scan was abnormal laparotomy was indicated), giving a sensitivity of 100%, specificity of 98.8% and PVP of 2.9% (Campbell *et al.*, 1990).

In addition, Campbell *et al.* (1989) and Bourne *et al.* (1989) have been conducting a comparative study of transabdominal and transvaginal ultrasound screening respectively. Transvaginal ultrasound imaging has two main advantages; the woman does not need a full bladder hence it is less uncomfortable, and the tip of the probe can be placed nearer to the ovary allowing higher frequency ultrasound to give a better resolution of the ovaries. The results of these studies indicate that transvaginal ultrasound is more sensitive than transabdominal ultrasound (Bourne *et al.*, 1989). In addition, the specificity of transvaginal ultrasound can be improved by assessing blood-flow impedance in the ovarian vasculature using colour-flow imaging. Ovarian cancers have a lower impedance to blood flow than benign ovarian tumours as a result of neovascularisation.

Andolf *et al.* (1986) screened 805 women using transabdominal ultrasound, and found one ovarian cancer, two borderline tumours and one caecum cancer.

This study, however, was applied to symptomatic women attending a gynaecology outpatient clinic and is therefore inconclusive with regards to screening in its true sense. Abdominal ultrasound as a secondary procedure to serum CA125 assay and vaginal examination has also been employed (Jacobs *et al.*, 1988b). If effective, utilization of CA125 as a primary screen and ultrasound as a secondary screen, would reduce costs enormously as the cheaper test is used first. The primary test in this incidence needs to have a high specificity to maximise the number of cases detected without referring excessive numbers of women for needless surgery. Inevitably there will be a few followed up unnecessarily.

3.5.2 Screening for EOC with tumour markers

Since the rationale for screening comes from the much improved survival rates in women with FIGO stages I and II disease, it is expedient to look at marker levels in such women. Over a decade ago, Knauf and Urbach (1980) found OCA to be elevated in 70% of women with stages I and II EOC, but also in 10% of normal individuals. The high false positive rate precluded the use of OCA for screening large populations, however, these early observations did suggest that antigens shed from tumour cells could find their way into the peripheral circulation at an early stage of tumour spread. Bast *et al.* (1990) postulated that tumour markers may reach the peripheral circulation in early disease by shedding from cells 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.

Several studies have assessed the sensitivity of pre-operative serum CA125 in women subsequently diagnosed with EOC (for review see Jacobs and Bast, 1989). Jacobs and Bast (1989) in a review of 15 studies report a cumulative elevation of CA125 (>35 Uml^{-1}) in 48/96 (50%) women with stage I EOC at diagnosis. In the first large screening study, Zurawski *et al.* (1987), using a cut-off value of 65 Uml^{-1}, found a false positive CA125 rate of 0.6% in 915 post-menopausal Roman Catholic nuns. Given a disease prevalence of 20-40/100000

in women over 50 years old, 15-30 false positive CA125 results would be encountered with each case detected. Jacobs *et al.* (1990a) have estimated that a screening test with 100% sensitivity would have to have a specificity of 99.6% in order to detect one case of ovarian cancer for every 10 operations performed (see section 3.4.7, pp. 68-71). The authors have assumed that clinicians would be unlikely to employ a test which would result in greater than 10 surgical procedures to detect one case (ie. PVP would have to be at least 10%).

Jacobs *et al.* (1988b) conducted a pilot screening study in 1010 asymptomatic, self-referred postmenopausal women at the Royal London Hospital. If serum CA125 was greater than 30 Uml^{-1}, or vaginal examination abnormal, the woman underwent abdominal ultrasound. If this was abnormal they were referred for surgery. Three women had laparotomies and one was found to have a stage 1b serous EOC, and a CA125 level of 32 Uml^{-1}, within the accepted reference range. Roulston *et al.* (1988) calculated the PVP of Jacobs' results to be 3%. As mentioned in chapter 2 (section 2.3.3, p 38), a well defined high risk population and a high prevalence (10%) are the reasons for the success of β HCG in screening for choriocarcinoma. Both Jacob's and Campbell's screening tests fall far short of an acceptable PVP, because the prevalence of ovarian cancer is low in their test populations. Therefore, a single CA125 assay produces a prohibitively low PVP. No single test will be sufficiently sensitive or specific enough to screen for ovarian cancer. Therefore, most investigators favour a multimodal approach, however, the best is yet to be determined.

Zurawski *et al.* (1990) have recently reported the results of a study conducted in Stockholm from 1985 to 1986, in which 1086 women over 40 years old were screened with CA125. If CA125 levels were >35 Uml^{-1}, the assay was repeated and women were also assessed by transabdominal ultrasound and pelvic examination. The only patient with rising CA125 was found to have a stage II ovarian cancer 20 months after initial CA125 elevation (Zurawski *et al.*, 1990). This study has recently been updated, Einhorn *et al.* (1990) report annual CA125 screening of 5550 women from 1986 to 1988. CA125 was assayed every

three months, and transabdominal ultrasound and pelvic examination performed every six months, in 175 women with CA125 levels >30 Uml^{-1}. Six post-menopausal women had ovarian cancer diagnosed during screening; four of whom had early stage disease. However, three pre-menopausal women, who had normal CA125 levels, also developed ovarian cancers which were missed at screening (Einhorn *et al.*, 1990), illustrating the problem of length bias.

The need for randomised controlled trials to assess screening tests is well recognized. In the meantime pilot studies are continuing to try to identify the most feasible approach. The UKCCCR Subcommittee for coordination of research into Gynaecological Cancers, recognising the growing interest in screening for ovarian cancer, held a workshop in the Royal College of Obstetricians and Gynaecologists in January 1989 to determine whether this would be feasible on a population basis. This workshop has reported its findings and recommendations on screening with reference to the WHO criteria for implementation of a screening programme, see Appendix A.

3.6 DIFFERENTIAL DIAGNOSIS OF BENIGN AND MALIGNANT OVARIAN TUMOURS

As the incidence is greater, more benign tumours will inevitably be detected at screening than malignant. Unfortunately, the true incidence of benign disease is unknown as many cases are recognized incidentally on histological examination of hysterectomy specimens, which may have been performed for other reasons. Initial exploratory laparotomy is most frequently performed by a general gynaecologist. When malignancy is discovered, complete staging and surgical cytoreduction often cannot be achieved. Consequently, the patient must be referred for a second operation.

Several studies have shown that CA125 levels >65 Uml^{-1} in patients with pelvic masses are associated with malignancy in 80-90% of post-menopausal patients (Einhorn *et al.*, 1986, Malkasian *et al.*, 1988, Soper *et al.*, 1990), although Vasilev *et al.* (1988) found no improvement in the discriminatory capacity of

CA125 using this cut-off value compared to 35 Uml^{-1}. Berchuck and Bast (1990) report the PVP of CA125 to increase to 96% when a cut-off value of 95 Uml^{-1} was employed. CA125 is less reliable when used to differentiate benign from malignant ovarian tumours in pre-menopausal women; as CA125 is elevated in a number of benign conditions (see chapter 2, section 2.4.1, p 43). As not all women with EOC express CA125 the PVN is lower than PVP. A negative CA125 result, however, should not discourage a surgeon from performing exploratory laparotomy on a post-menopausal woman with a pelvic mass who is otherwise a candidate for cytoreduction.

Various strategies have been adopted to increase the specificity of detection of malignant ovarian tumours using monoclonal antibodies. Wu *et al.* (1988), in attempt to increase specificity of diagnosis, found the ratio of CA125:CEA useful in discriminating between serous and mucinous ovarian tumours. A ratio of >1000 was associated with serous tumours, while a ratio of <10 was associated with mucinous tumours. CA199 failed to help the discrimination in their study (Wu *et al.*, 1988).

It is more common in studies of differential diagnosis of benign and malignant ovarian tumours to determine coordinate elevation of serum tumour markers. No single tumour marker has 100% sensitivity and specificity for any tumour as a result of tumour antigen heterogeneity. Different combinations of genetic "lesions" resulting in malignant transformation give rise to many different tumour cell phenotypes, each expressing a characteristic set of antigens (see chapter 2, section 2.2, pp. 32-36). Ovarian tumour antigen heterogeneity has been amply demonstrated using immunohistochemical techniques and by serological antigen profiles (Welch *et al.*, 1990).

Other tumour markers, notably CA153 and CA724, not affected by the benign conditions that cause CA125 elevation appear to be the best candidates for panel testing. Finkler *et al.* (1988) found false positive CA125 levels in 50 patients with benign conditions, all of whom had CA125 levels >35 Uml^{-1}, while 42% had CA125 levels >65 Uml^{-1}. CA153 and CA724, however, were only

elevated in 2% and 6% of patients respectively, while NK/70K was raised in 62% of patients. In those with EOC, either CA724 or CA153 was positive in 83% while only 5% of patients with benign adnexal masses had elevated levels of either marker (Finkler *et al.*, 1988). Mogensen *et al.* (1990) have recently advocated the use of TATI assay in addition to CA125 to discriminate benign from malignant mucinous EOC. When multiple markers are used with CA125, diagnostic sensitivity and specificity does not always improve, despite the fact that several markers, including CEA, CA199 and CA724 detect mucinous tumours more frequently than CA125 (Bast *et al.*, 1990).

3.7 MONITORING EOC WITH TUMOUR MARKERS

After cytoreductive surgery, most patients with advanced disease have small volume residual disease that is difficult to detect by physical examination or radiographic procedures (see chapter 1). Although the majority of patients respond to cisplatin based chemotherapy regimes, responses are often short-lived and most of these patients will relapse. Several months may elapse before disease progression becomes clinically evident. CA125 has an established role in monitoring patients with histologically proven EOC, as outlined in sections 3.7.1 to 3.7.5. These areas will be described in more detail in chapters 7, 8 and 9.

3.7.1 Correlation with tumour burden

All studies of serological tumour markers have found significantly higher levels in patients with metastatic disease compared to those with either localised malignant or benign tumours. Correlations with tumour burden are most accurately determined using samples assayed immediately prior to laparotomy, primary or secondary. As mentioned in chapter 1, section 1.9.2, p16, precise measurement of tumour burden is difficult to obtain.

In a review of the literature, Jacobs and Bast (1989), reported serum CA125 elevation prior to second-look in 8/38 (21%) patients with microscopic disease,

14/45 (38%) patients with <1cm disease, 21/46 (46%) patients with <2cm disease, 39/56 (70%) patients with >2cm disease and 39/39 (100%) patients with disease >10cm in diameter. Different disease categories were used in different studies. False negative CA125 results, however, are generally associated with small volume disease, indicating a certain minimal tumour volume necessary to cause elevation of serum CA125. Recall that radiological scanning techniques are also insensitive for tumours of <1cm.

Ward and Cruickshank (1987b) assayed serum $HMFG_2$ in 98 EOC patients two to six weeks after primary surgery. Elevated levels were found in 1/9 (11%) patients with no residual disease, 12/28 (43%) patients with <2cm residual disease, and 37/61 (61%) patients with >2cm residual disease. The statistical significance of these observations was not tested, moreover, the authors had insufficient longitudinal data to determine if serum $HMFG_2$ levels reflected changes in tumour bulk in individual patients with time. Pre-operative $HMFG_2$ levels have not been correlated with tumour burden, and the effect of surgery on post-operative serum levels is unknown.

Scambia *et al.* (1988) correlated serum CA153 levels, measured three weeks after primary surgery, with residual disease. Levels were $>30Uml^{-1}$ in 5/10 (50%) patients with <2cm residual disease, while 10/14 (71%) patients with resdiual disease >2cm had elevated levels. Later, Scambia *et al.* (1990) assayed serum CA724, four to six weeks after primary laparotomy, and found elevated levels in 1/4 (25%) patients with no residual disease, 0/3 with <0.5cm disease, and 5/12 (42%) patients with >0.5cm residual disease. Serum CA199 levels were found elevated in 4/18 (22%) patients with <2cm residual disease, 5/13 (38%) patients with 2-10cm disease, and 7/23 (30%) patients with >10cm residual disease. Halila *et al.* (1988) found elevated serum TATI levels in 2/22 (9%) patients with no evidence of disease, 3/8 (38%) patients with microscopic disease, 1/6 (17%) patients with <1cm disease, 2/12 (17%) patients with >1cm disease, and 3/18 (17%) patients with macroscopic disease at second-look.

The statistical trends of these results are unknown due to the small numbers of patients in each study, although fewer patients with disease <2cm in diameter in general have elevated serum tumour marker levels. In addition, none of these studies distinguishes between patients with different histopathological types of EOC.

3.7.2 Correlation with disease course

There have been numerous studies investigating the correlation between serial serum CA125 levels and the course of disease, and relatively few documenting this information with regard to each of the other ovarian tumour markers mentioned so far. Bast *et al.* (1983) originally found a correlation with serial CA125 and the clinical course in 93% of cases. CA125 levels increased with progressive disease in 17/17 cases and fell with response in 20/21 patients. The authors considered halving or doubling of marker levels to be clinically "significant".

Since this initial report, many studies have confirmed the value of CA125 in determining response to chemotherapy. In a review of the literature, Kenemans *et al.* (1988) reported an overall correlation with course of disease in 87% of patients, ranging from 76% to 95% in different studies. CA125 levels fell with regression in 108/109 (99%) patients, were unchanged in 29/40 (73%) patients with stable disease, and increased in 111/137 (81%) patients with disease progression. As shown in section 3.7.1, the majority of patients with tumour volumes <2cm in diameter have negative CA125 levels. Hence, a negative CA125 result does not preclude the presence of occult disease. Consequently, all studies of CA125 have shown that rising levels are more indicative of progression than falling or negative levels are of disease remission.

In cases where CA125 is elevated in pre-operative serum samples, levels correlate with the clinical disease course in nearly 90% of patients. As mentioned in chapter 2, section 2.4.1, p 44, approximately 85% of all EOC patients express CA125; while fewer patients with mucinous than any other

subtype have elevated serum levels. Complementary markers have therefore been intensely sought which would allow a wider range of patients to be serologically monitored. Studies of other putative ovarian tumour markers to date have shown that although elevated in a high proportion of patients, none is elevated in as many patients as CA125. The correlation between disease course, or response to chemotherapy, and the other markers investigated in this thesis is examined in detail in chapter 8.

3.7.3 Prediction of disease status prior to second-look surgery

A high proportion of patients with no clinically evaluable disease before second-look surgery have false negative CA125 levels. Surgery may be avoided in patients with positive marker results if cytoreduction is not planned. Although several other tumour markers have been found elevated in EOC, none to date has proved more useful than CA125. Even so, approximately 50% of patients with either microscopic or small volume macroscopic residual or recurrent disease at second-look laparotomy have false negative serum CA125 levels.

In one of the earliest reports, Niloff *et al.* (1985) found false negative CA125 levels prior to second-look laparotomy in 22/36 (61%) patients. Kenemans (1990), in a review of the literature, reported a cumulative false negative rate of 48% for CA125 for disease at second-look. Reports of the values for other ovarian tumour markers investigated to date indicate lower sensitivities than CA125 for second-look outcome. These will be examined in detail in chapter 8.

3.7.4 Prediction of recurrence or relapse

Progressively rising CA125 has correlated with recurrence, and provided an average clinical lead time of three months in up to 87% of patients (for review see Kenemans, 1988). However, the value of CA125 lead times has not yet been addressed. The value of other tumour markers in providing clinical lead times is unknown.

3.7.5 Prognosis

There is conflicting evidence regarding the value of pre-operative CA125 assay. Vergote *et al.* (1987) and Van der Burg *et al.* (1988) found an inverse correlation between CA125 levels and second-look outcome and survival respectively, while Sevelda *et al.* (1989) reported no significant association between pre-operative CA125 levels and survival in a larger patient cohort. Cruickshank *et al.* (1987) also found no prognostic value in pre-operative CA125 measurement. Rustin *et al.* (1989) found the pre-treatment level of CA125 of no value in predicting which patients would relapse, but found a seven-fold fall from pre-treatment to after the first cycle of treatment to be of prognostic value. 8/14 (58%) patients who had greater than a seven-fold fall at this time compared to 3/36 (9%) who had less than a seven-fold fall in CA125 were disease free after two years of follow-up. Van der Burg *et al.* (1988) found a half-life of 20 days to give significant discrimination between two prognostic groups. The median time to progression in 16 patients with a CA125 half-life of >20 days was 11 months compared to a median time to progression of 43 months in 21 patients with a CA125 half-life of <20 days. After two cycles of primary chemotherapy, Sevelda *et al.* (1989) and Redman *et al.* (1990) found CA125 to be the most significant predictor of survival. Rosen *et al.* (1989), using the absolute pre-operative CA125 level and CA125 level after surgery, constructed a simple prognostic index. CA125 levels of 0-64 Uml^{-1} were given a score of 1 point, CA125 levels of 65-299 Uml^{-1} were given a score of 2 points and CA125 levels >300 Uml^{-1} were given a score of 3 points. Patients with a combined score of 2 or 3 points had a significantly longer time to recurrence than those with a score of 4, 5, or 6.

Many reports therefore exist concerning the prognostic value of early CA125 assay in predicting response to chemotherapy, second-look outcome, progression free survival and overall survival. There are little data regarding the prognostic value of any of the other marker evaluated in this thesis. Although the prognostic significance of early CA125 assay has been reported by several authors, there is no consensus as to the most useful measurement.

3.8 AIMS OF THIS THESIS

This thesis investigates the use of several monoclonal antibodies in serological monitoring of patients with histologically proven EOC. Its aims are threefold, as follows:-

1. To help clarify the role of serum CA125 assay in the monitoring and prognosis of EOC patients.

2. To evaluate several promising ovarian tumour markers, using monoclonal antibodies directed against epitopes on PEM, in monitoring EOC.

3. To compare these markers to CA125, and determine if any has value in clinical management of EOC in addition to CA125.

PATIENTS AND METHODS

CHAPTER 4

Ovarian Tumour Marker Immunoassays

4.1 INTRODUCTION

The development of the hybridoma technique for the production of monoclonal antibodies (MAbs) has revolutionised cancer diagnosis in vitro (Kupchik, 1988). Countless monoclonal antibodies are available, from both commercial and "in-house" sources. The development of MAbs has been more than adequately covered by numerous texts, see Campbell (1987) for review. Several types of immunoassays exist, each based on the fundamental reaction between antibody and antigen, employing a variety of detection systems, see Tjissen (1987) for review.

Three types of immunoassay were used in this study; immunoradiometric assay (IRMA), radioimmunoassay (RIA), and enzyme-linked immunosorbant assay (ELISA) to determine serum CA125, CA153, CA199, CA724, TATI, $HMFG_2$, and c-neu p185 (described in chapter 2, sections 2.4.1 to 2.4.7, pp. 43-55). Measurement of CA125, CA199, and $HMFG_2$ each employed a single MAb in homologous double-determinant (HoDD) sandwich assays, in which the same antibody was used as both a catcher and tracer molecule. This type of assay is possible where multiple sterically distinct identical epitopes are present on the one antigenic molecule. Measurement of CA153, CA724 and c-neu p185 each employed two MAbs in heterologous double-determinant (HeDD) sandwich assays, in which different antibodies were used as catcher and tracer molecules. This type of assay exploits the ability of different antibodies to recognise sterically distinct epitopes on the same antigenic molecule. TATI was assayed by conventional competitive RIA. CA125, CA153, CA199, CA724, TATI, and c-neu p185 assays were obtained from commercial sources, while $HMFG_2$ was developed "in-house" in collaboration with Unilever Research, Colworth, U.K.. Table 4.1 shows the tumour markers assayed in this study, the MAbs used (the first MAb represents the catcher and the second MAb represents the tracer in double-determinant assays), the types of assay, the source of each assay (full addresses may be found in Appendix B), and the current retail cost of each commercial kit. Each kit provides assay material sufficient for 96-100 tubes, the equivalent cost has been estimated for $HMFG_2$ assay.

Table 4.1 ***Tumour marker assays***

Antigen	Monoclonal Antibodies	Assay Type	Source	Cost/100 tubes
CA125	OC125	HoDD IRMA	CIS, U.K.	£418
CA153	115D8 and DF3	HeDD IRMA	CIS, U.K.	£495
CA199	NS199	HoDD IRMA	CIS, U.K.	£458
CA724	B72.3 and cc49	HeDD IRMA	CIS, U.K.	£433
TATI	Anti-TATI	RIA	Farmos, Finland	*
$HMFG_2$	$HMFG_2$	HoDD ELISA	Unilever, U.K.	£10
p185	NB3 and TA1	HeDD ELISA	Dupont, U.S.A.	*

* assayed free of charge

A novel combined ELISA was developed previously to measure placental-like alkaline phosphatase (PLAP) using the MAb designated H17E2 (I.C.R.F., Lincoln's Inn Fields, London). Microtitre plates were coated with H17E2, and PLAP activity and concentration, or immunoreactivity, measured sequentially on the same plate (Fisken *et al.*, 1989). The sensitivity and specificity of PLAP activity assay were 66% and 53% respectively in patients with advanced disease (stages III and IV), while the sensitivity and specificity of PLAP immunoreactivity were 37% and 73% respectively in stages III and IV.

Therefore, PLAP was not included in the present analysis because this assay gave high false positive and false negative rates and was shown to be of no clinical value in addition to CA125 (Fisken *et al.*, 1989a). This agrees with the study by Haije *et al.* (1987) who also found poor sensitivity using PLAP immunoreactivity assay and poor specificity using PLAP activity measurement in EOC patients.

This chapter describes the principles of each assay, and details the methods used. All commercially obtained assays were performed according to the manufacturer's instructions which are outlined. In addition, the quality control of each assay is described.

4.2 CA125 ASSAY

The first commercial CA125 assay was developed by Klug *et al.* (1984). Since 1984 several commercial assay kits for CA125 have appeared on the market. In an earlier study, the results obtained using three different assay systems available at the time; an IRMA and an ELISA from Abbott Diagnostics, and an IRMA from CIS, were compared (Fisken *et al.*, 1989b). One hundred and thirty two serum samples taken from 42 patients with established EOC were assayed with each method, and the CIS IRMA was found to be the most sensitive and specific assay for CA125. Consequently, this assay was adopted, and for the past three years has been offered routinely in the Dept. Clinical Chemistry, Royal Infirmary, Edinburgh. Currently, eight CA125 assays are available from different commercial sources, each employing the same MAb OC125 but different standard preparations (Milford-Ward,1991). Serial monitoring should therefore be performed using kits from a single source.

4.2.1 CA125 assay principle

The CIS CA125 assay is a simultaneous solid phase IRMA, see figure 4.1 overleaf.

4.2.2 CA125 assay protocol

Each CA125 assay kit provides reagents and materials sufficient for 100 wells, including; 100 OC125 MAb coated beads, one vial of ^{125}I-OC125 tracer MAb, one vial of each CA125 standard (6.5, 30, 80, 200, and 500 Uml^{-1}), one vial of control serum (120 Uml^{-1}), diluent buffer, 6 x 20 well reaction trays, 12 cover seals, and six x 20-place cardboard racks containing plastic counting tubes. All reagents are brought to room temperature (20-25°c) before assaying. Samples and controls are assayed in duplicate and standards are assayed in triplicate.

1. Add 100 µl standards, control, and samples to their respective wells.
2. Add 100 µl ^{125}I-OC125 tracer MAb to each well.
3. Add one OC125 MAb coated bead to each well.
4. Gently tap each tray to release any trapped air bubbles, mix the reagents, and ensure that the beads are completely immersed.
5. Place a cover seal over each tray to prevent evaporation, and incubate overnight (18-22h) at room temperature.
6. Remove cover, aspirate and wash three times with distilled H_2O taking care to avoid overflow from the reaction wells.
7. Transfer the beads from the reaction wells to appropriately labelled counting tubes.
8. Measure the radioactivity of the beads for 60s in a gamma scintillation counter.

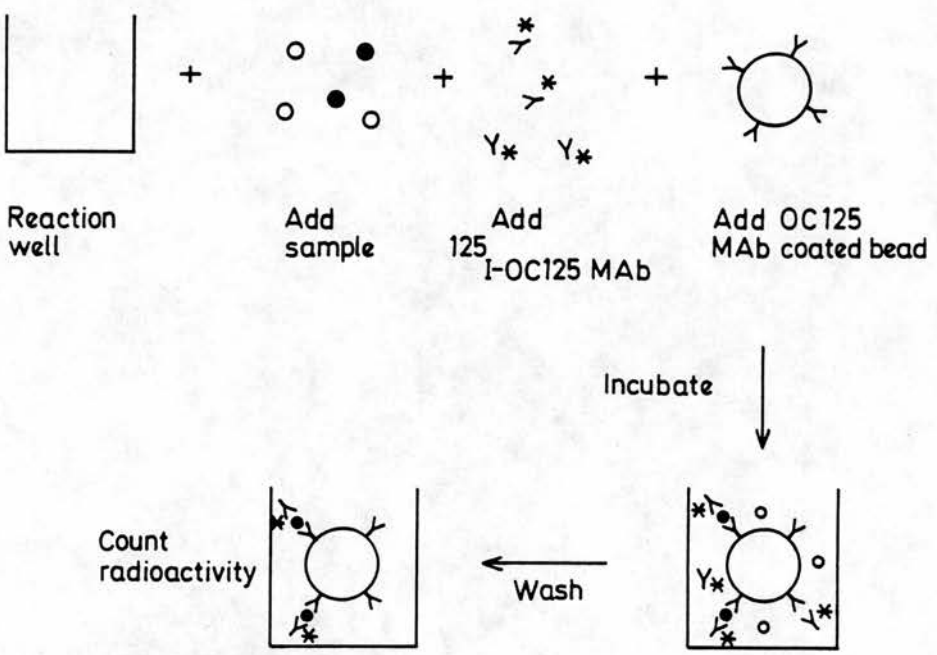

Figure 4.1 ***Simultaneous IRMA***. Polystyrene beads coated with OC125 MAb are incubated simultaneously with the samples and tracer ^{125}I-OC125 MAb. These bind to the immobilised OC125 MAb forming a sandwich complex. Unbound material is removed by washing, and the amount of radioactivity counted is proportional to the concentration of CA125 antigen in the sample.

4.2.3 CA125 standard curve

Sample concentrations were determined by interpolation from a dose-response curve constructed with the standards provided. Samples with CA125 levels >500 Uml^{-1} were diluted one in 10 and re-assayed. Table 4.2 shows the mean counts per minute (cpm), standard deviation (SD), standard error (SE) and coefficients of variation (CV) for each standard value (n=5), see figure 4.2.

Table 4.2 ***CA125 standard values***

CA125 (Uml^{-1})	Mean cpm	SD	SE	CV (%)
6.5	215	32	15.6	14.9
30	483	45	20.1	9.3
80	1182	51	22.8	4.3
200	3119	129	57.7	4.2
500	9049	500	223.6	5.5

4.2.4 CA125 assay precision

The between assay CV of three "in-house" plasma pools, and within assay CV using the diluent and control provided with the CIS kit are shown in table 4.3 (kindly obtained from Dr.C.Sturgeon at the last immunoassay lab review).

Table 4.3 CA125 assay variation

Variation	pool	n	Mean (Uml^{-1})	Target (Uml^{-1})	CV (%)
Inter-assay	1	9	46.3	49	5.1
	2	9	143.2	151	9.7
	3	9	237.4	230	8.0
Intra-assay	1	9	13.9	11	10.3
	2	9	121	120	4.6

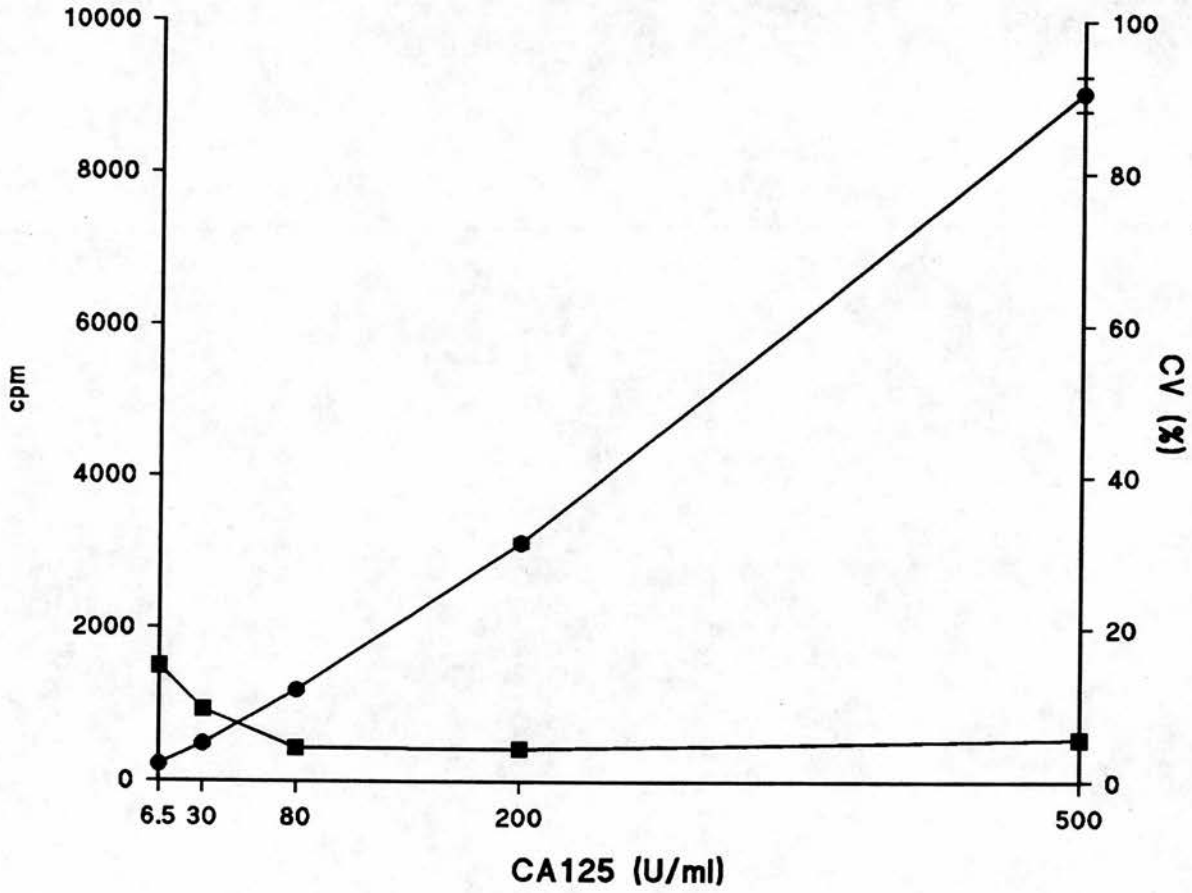

Figure 4.2 ***CA125 standard curve and precision profile***

4.3 CA153 ASSAY

The CA153 assay was originally developed by Tobias *et al.* (1985), and was assayed using the ELSA-CA 153 IRMA in this study.

4.3.1 CA153 assay principle

The ELSA-CA 153 assay is based on a solid phase two-site sequential IRMA, see figure 4.3.

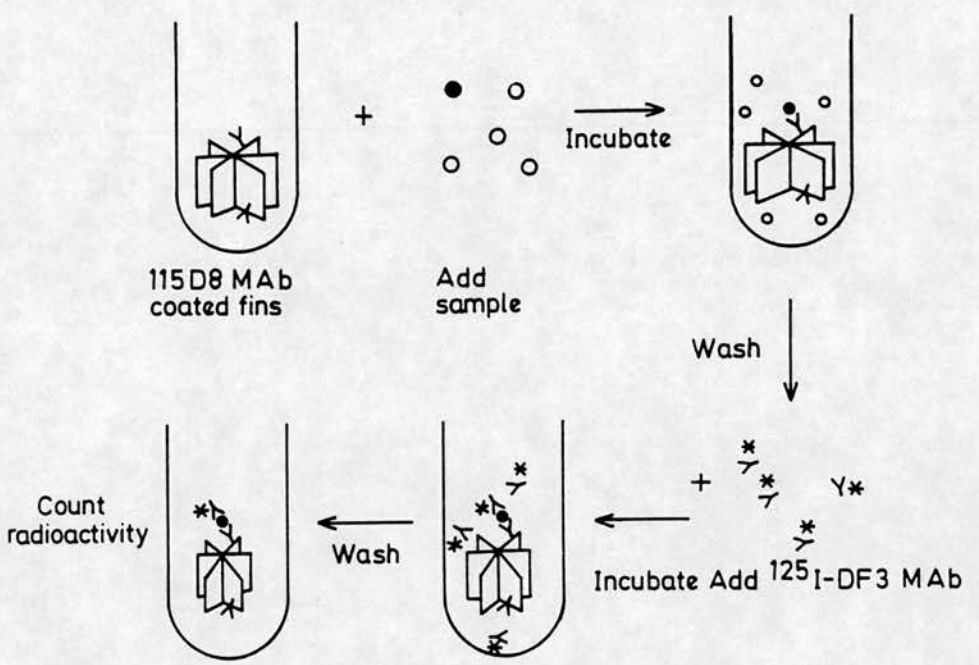

Figure 4.3 ***Sequential two-site IRMA***. Solid phase (plastic fins or ELSAs) coated with 115D8 MAb are first incubated with sample. After washing, ^{125}I-DF3 MAb is added and CA153 Ag present in the sample is sandwiched between the two antibodies. After formation of the bound complex, unbound tracer is removed by washing and the amount of radioactivity counted is proportional to the concentration of CA153 antigen in the sample.

4.3.2 CA153 assay protocol

Each CA153 assay kit contains reagents and materials sufficient for 96 tubes, including; four x 24 tube-packages with 115 D8 MAb bound in excess to the plastic fin (ELSA) jammed into the bottom of the tube, one vial of ^{125}I-DF3 tracer MAb, one vial of each standard (0, 12.5, 25, 50, 100, and 150 Uml^{-1}), one vial of control serum (30 Uml^{-1}), and two vials of diluent buffer. All reagents are brought to room temperature (20-25°c) before assaying. Samples and control serum are assayed in duplicate and standards are assayed in triplicate.

1. Predilute samples and control serum by dispensing 20 µl of each sample or control into appropriately labelled polystyrene tubes. Add 1 ml of diluent buffer into each tube and mix by gently vortexing.
2. Dispense 300 µl of each standard and prediluted sample and control into appropriately labelled 115D8 MAb coated ELSA tubes, and mix by gently vortexing.
3. Incubate for 1h ± 5min at room temperature (18-25°C) while shaking.
4. Aspirate the tubes, and wash three times with 3ml of distilled H_2O.
5. Add 300 µl of ^{125}I-DF3 MAb to each tube and mix by gently vortexing.
6. Incubate for 1h ± 5min at room temperature (18-25°c) while shaking.
7. Aspirate and wash three times as before.
8. Measure radioactivity bound to the ELSA for 60s in a gamma scintillation counter.

4.3.3 CA153 standard curve

The sample concentrations were determined by interpolation using the RiaCalc programme from a dose-response curve constructed with the standards provided. Samples with CA153 levels > 150 Uml^{-1} were diluted one in 10 and re-assayed. Table 4.4 shows the mean cpm, SD, SE, and CV for each standard (n=3).

Table 4.4 ***CA153 standard values***

CA153 (Uml^{-1})	Mean cpm	SD	SE	CV (%)
0	483	369	213	76.3
12.5	13157	1947	1124	14.8
25.0	18805	300	173	1.6
50.0	21238	178	72	0.9
100.0	23526	96	55	0.4
150.0	24138	1712	988	7.1

Figure 4.4 shows the standard curve for CA153 plotted using the values in table 4.4, together with standard errors and precision profile.

4.3.4 CA153 assay precision

One control (30 Uml^{-1}), supplied by the manufacturer, was included in each assay run (n=5). The inter-assay CV was 15.12%, and the intra-assay CV was 4.89% (mean value 30.16 Uml^{-1}).

4.4 CA199 ASSAY

The CA199 assay was originally developed by Del Villano *et al.* (1983), and was measured using the ELSA-CA 19-9 CIS IRMA in this study.

4.4.1 CA199 assay principle

The CIS ELSA-CA 19-9 assay is a solid phase sequential IRMA, similar to CA153 assay, see figure 4.3, except that one MAb is employed as both catcher and tracer MAb. ELSA coated with NS199 MAb are incubated with sample, standard or control. After incubation unbound material is removed by washing and NS199 MAb labelled with ^{125}I is added. After formation of the bound

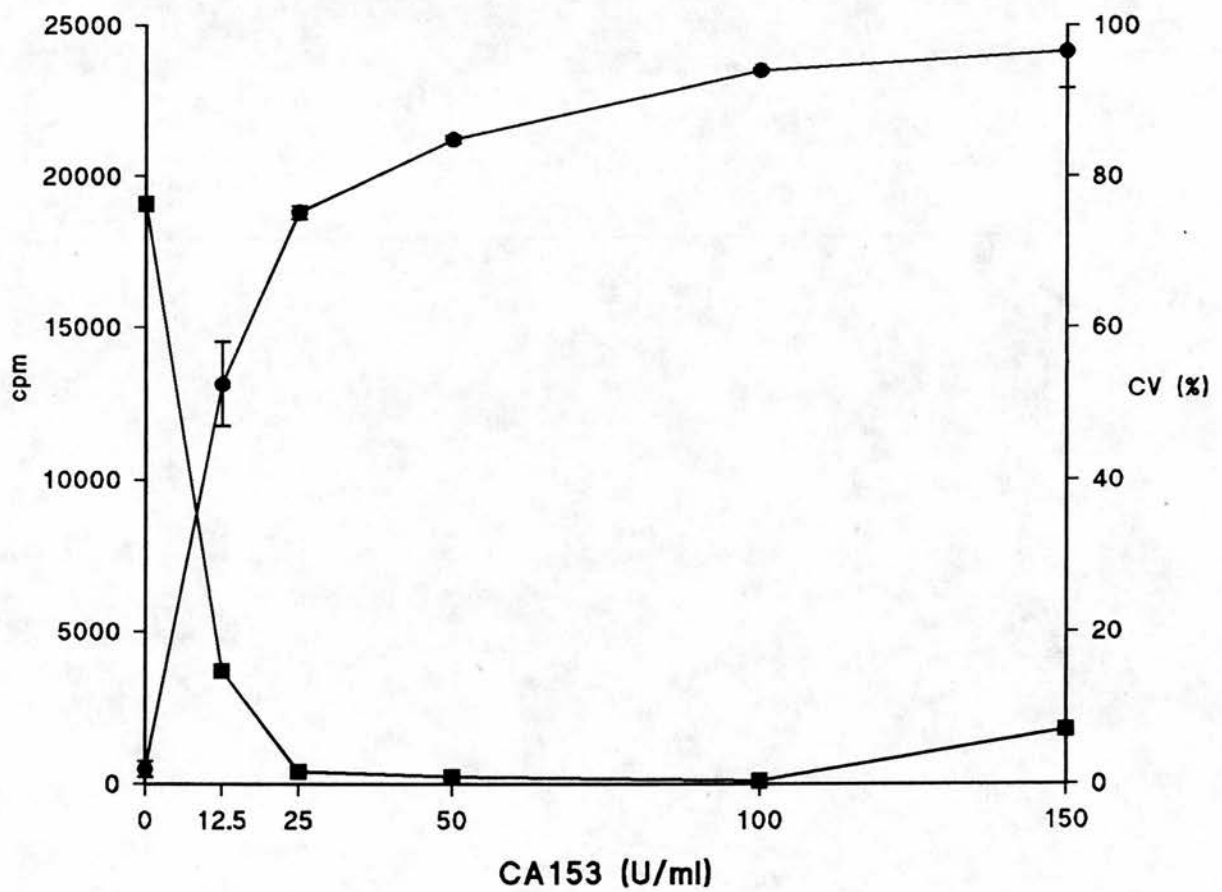

Figure 4.4 ***CA153 standard curve and precision profile***

sandwich complex, unbound tracer is removed by washing. The amount of radioactivity counted is proportional to the concentration of CA199 antigen present in the sample.

4.4.2 CA199 assay protocol

Each CA199 assay kit contains reagents and materials sufficient for 96 tubes, including; 12 x 8 tube-packages with excess NS199 MAb bound to the ELSA jammed into the bottom of the tube, one vial of NS199-^{125}I tracer MAb, one vial of each standard (5, 15, 30, 60, and 120 Uml^{-1}), one vial of control serum (40 Uml^{-1}), and diluent buffer. All reagents are brought to room temperature (20-25°c) before assay. Samples and control serum are assayed in duplicate and standards are assayed in triplicate.

1. Add 100 µl of each standard, sample and control to an appropriately labelled NS199 MAb coated ELSA tube.
2. Add 200 µl of diluent buffer to each tube.
3. Gently mix using a vortex mixer, cover with parafilm to prevent evaporation and incubate for 3h ± 10 min at 37 ± 1°c.
4. Aspirate the tubes and wash three times with 3 ml distilled H_20.
5. Add 300 µl ^{125}I-NS199 tracer MAb to each tube and incubate for 3h 10 min at room temperature (20-25°c).
6. Aspirate, and wash three times as before.
7. Measure radioactivity bound to the ELSA for 60s in a gamma scintillation counter.

4.4.3 CA199 standard curve

The sample concentrations were determined by interpolation using the RiaCalc programme from a dose-response curve constructed with the standards provided. Samples with CA199 levels > 120 Uml^{-1} were diluted one in 10 and re-assayed. Table 4.5 shows the mean cpm, SD, SE, and CV for each standard (n=3).

Table 4.5 ***CA199 standard values***

CA199 (Uml^{-1})	Mean cpm	SD	SE	CV (%)
6.0	859	81	46	9.5
15.0	2041	96	55	4.7
30.0	4306	69	39	1.6
60.0	8271	889	513	10.8
120.0	15387	2193	1266	14.3

Figure 4.5 overleaf shows the standard curve for CA199 plotted using the values in table 4.5, together with standard errors and precision profile.

4.4.4 CA199 assay precision

One control (40 Uml^{-1}), supplied by the manufacturer, was included in each assay run (n=5). Intra-assay CV was 5.7%, and the inter-assay CV was 10.7% (mean value 40.85 Uml^{-1}).

4.5 CA724 ASSAY

The CIS CA724 RIA, an assay originally developed by Colcher *et al.* (1985), was used to measure serum CA724.

4.5.1 CA724 assay principle

The CIS CA724 assay is based on a solid phase two-site sequential IRMA. It is similar to CA153 assay, see figure 4.3, except that beads are used as the solid phase and not plastic fins. Polystyrene beads coated with cc49 MAb are incubated with samples. Unbound material is removed by washing and tracer MAb B72.3 is added resulting in formation of a sandwich complex. Unbound

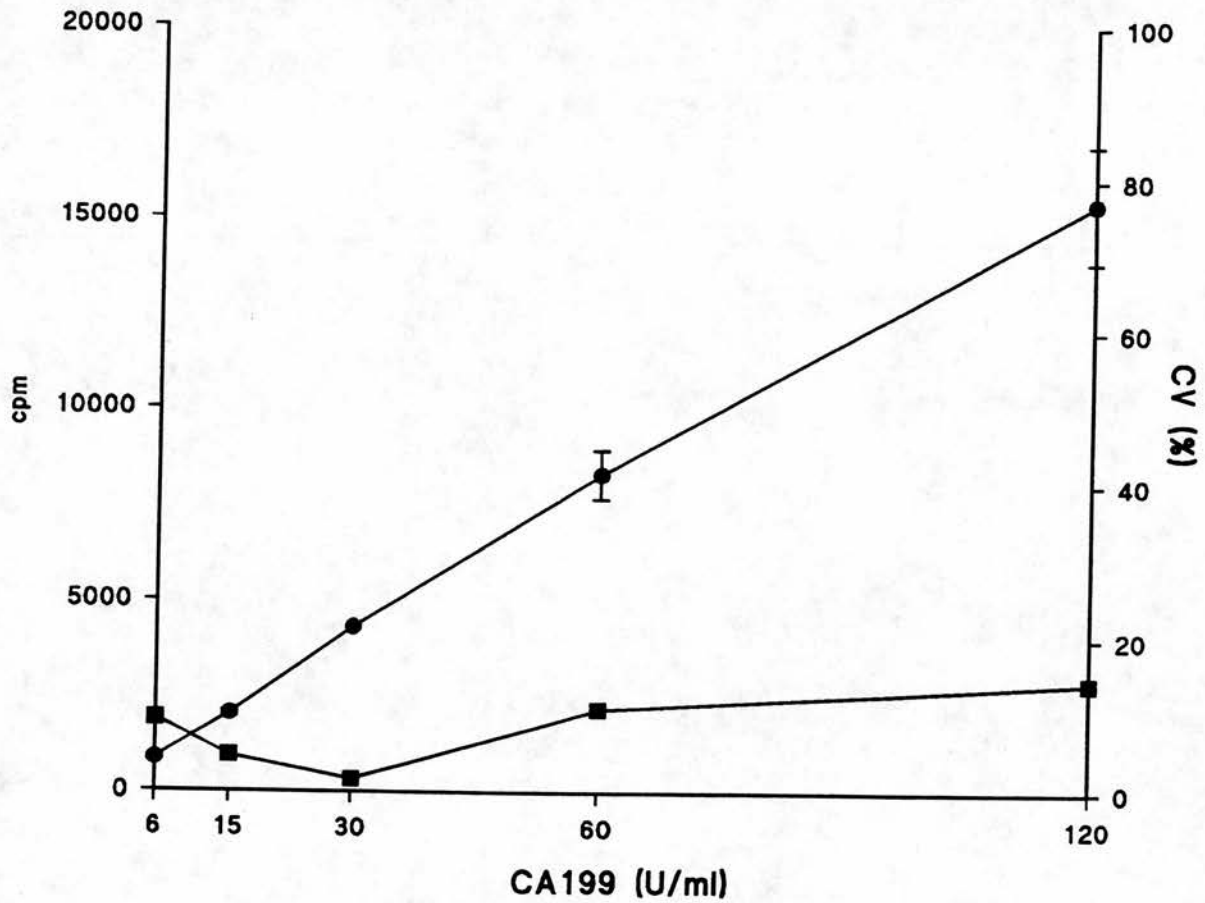

Figure 4.5 ***CA199 standard curve and precision profile***

labelled antibody is removed by washing the beads, and the radioactivity counted is proportional to the concentration of CA724 antigen in the sample.

4.5.2 CA724 assay protocol

Each kit contains reagents and materials sufficient for 100 wells, including; six x 20 well reaction trays, cover seals, cardboard racks containing counting tubes, 100 cc49 MAb coated beads, two vials of ^{125}I-B72.3 MAb, one vial of each standard (3, 10, 20, 35, 50, 75, and 100 Uml^{-1}), one vial of each control serum (9.7 Uml^{-1} and 75 Uml^{-1}) and diluent buffer. All reagents are brought to room temperature (20-25°c) before assaying. Samples and controls are assayed in duplicate and standards are assayed in triplicate.

1. Add 100 µl sample, standard, or control to appropriately labelled wells.
2. Add 100 µl diluent buffer to each well.
3. Add one cc49 MAb coated bead to each well, apply cover seal and gently tap the tray to ensure reagents are mixed and that the beads are immersed.
4. Incubate for 4h ± 5min at 37°c.
5. Aspirate, and wash three times with distilled H_2O.
6. Add 200 µl ^{125}I-B72.3 tracer MAb and gently tap trays to mix.
7. Incubate for 18h ± 2h at 2°c - 8°c.
8. Aspirate, and wash three times as before.
9. Transfer beads to appropriately labelled counting tubes.
10. Count radioactivity for 60s in a gamma scintillation counter.

4.5.3 CA724 standard curve

The sample concentrations were determined by interpolation using the RiaCalc programme from a dose-response curve constructed using the standards supplied. Table 4.6 shows the mean cpm, SD, SE, and CV (%) for each standard (n=2).

Table 4.6 ***CA724 standard values***

CA724 (Uml^{-1})	Mean cpm	SD	SE	CV (%)
3.0	358	34	24	9.4
10.0	1277	14	10	1.1
20.0	2098	166	117	7.9
35.0	3578	62	44	1.7
50.0	4957	96	68	1.9
75.0	7630	122	86	1.6
100.0	9585	869	614	9.1

Figure 4.6 shows the standard curve for CA724 plotted using the values in table 4.6, together with standard errors and precision profile.

4.5.4 CA724 assay precision

CVs were calculated using two controls from each of the two assay runs; one low concentration (9.7 Uml^{-1}) and one high concentration (75 Uml^{-1}) supplied by the manufacturer. The intra-assay CVs for low and high concentrations were 7.46% and 6.22% respectively. The inter-assay CVs for low and high concentrations were 17.39% and 2.95% respectively. Mean values for the low and high assay controls were 7.36 Uml^{-1} and 73.94 Uml^{-1} respectively.

4.6 TATI ASSAY

The Farmos Spectria TATI RIA, an assay originally developed by Stenman *et al.* (1982), was used to assay serum TATI.

4.6.1 TATI assay principle

The Farmos TATI RIA is based on the widely used radioimmunoassay technique, see figure 4.7, p 102.

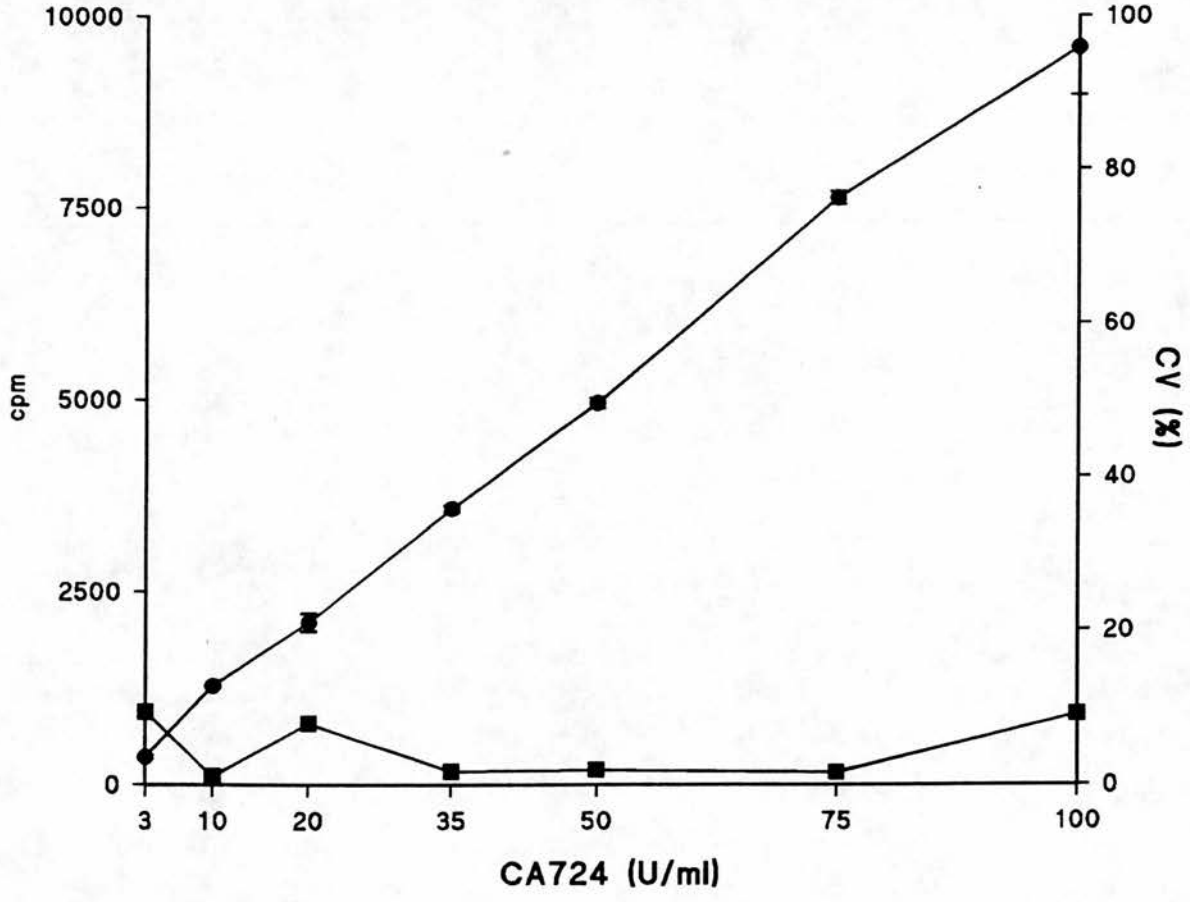

Figure 4.6 ***CA724 standard curve and precision profile***

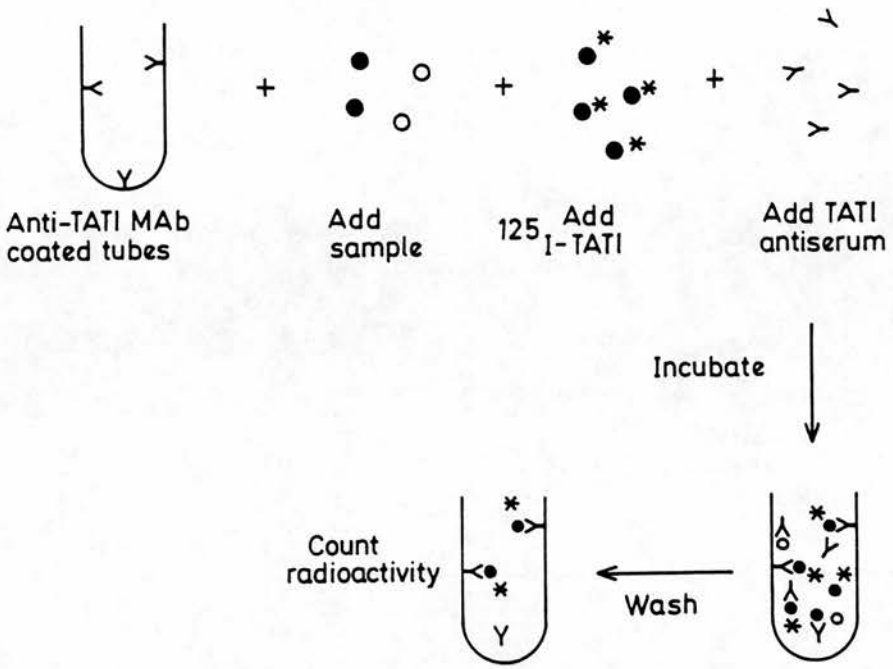

Figure 4.7 ***Competitive RIA***. Sample containing an unknown amount of TATI is incubated with a standard amount of ^{125}I labelled TATI, which competes for binding to a limited number of high affinity binding sites of the solid phase antibody. After washing to remove unbound labelled antigen the radioactivity counted is inversely proportional to the amount of TATI antigen in the sample.

4.6.2 TATI assay protocol

Each TATI kit provides reagents and materials sufficient for 100 tubes, including; two x 50 tubes coated with anti-TATI MAb, two vials of ^{125}I-TATI, one vial of each TATI standard (0.5, 5, 15, 45, 150, and 450μgl^{-1}), one vial of each control serum (12-23μgl^{-1} and 136-206μgl^{-1}), diluent buffer, and washing solution. All reagents are brought to room temperature (20-25°) before assaying. Samples and controls are assayed in duplicate and standards are assayed in triplicate.

1. Add 25 µl of sample, standard or control to appropriately labelled anti-TATI MAb coated tubes. Leave two tubes blank for background.
2. Add 200 µl ^{125}I-TATI to all tubes, including two for determination of non-specific binding and two for determination of total count.
3. Add 200 µl TATI antiserum to all tubes except those for determination of non-specific binding and total count. Add 200 µl H_2O to the non-specific binding tubes.
4. Shake gently and incubate overnight (18h) at room temperature.
5. Decant tubes and tap out any remaining liquid onto absorbent paper.
6. Wash with 1.0 ml wash solution and tap out any remaining liquid.
7. Count radioactivity for 60s in a gamma scintillation counter.

4.6.3 TATI standard curve

The sample concentrations were determined by interpolation using the RiaCalc programme from a dose-response curve constructed with the standards supplied. Samples with TATI levels > 450 μgl^{-1} were diluted one in 10 and re-assayed. Table 4.7 shows the mean cpm, SD, SE, and CV (%) for each standard (n=9).

Table 4.7 ***TATI standard values***

TATI (μgl^{-1})	Mean cpm	SD	SE	CV (%)
5.0	13726	438	146	3.2
15.0	9788	547	182	5.6
45.0	6237	599	200	9.6
150.0	3267	306	102	9.4
450.0	1789	354	118	19.8

Figure 4.8 shows the standard curve for TATI plotted using the values in table 4.7, together with standard errors and precision profile.

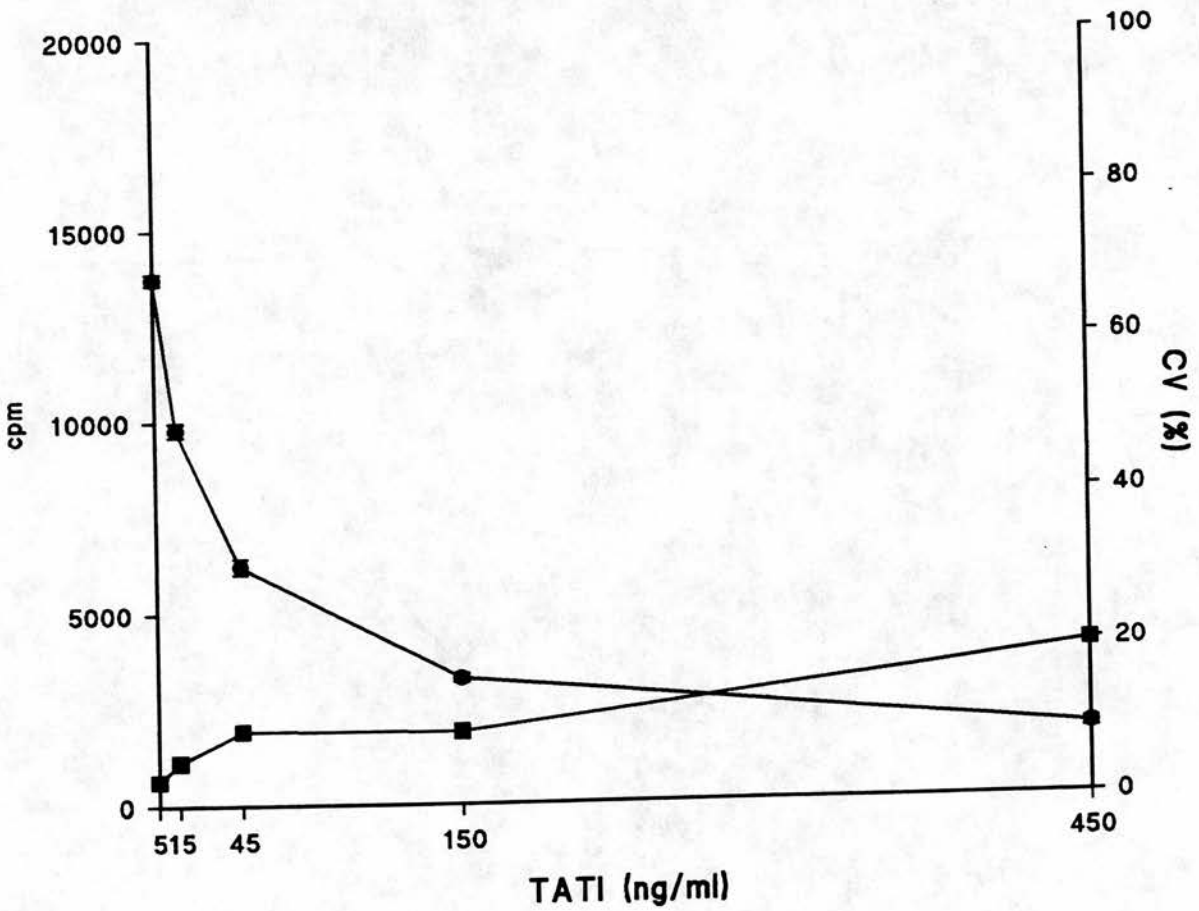

Figure 4.8 ***TATI standard curve and precision profile***

4.6.4 TATI assay precision

CVs were calculated using two controls from each assay run (n=9); one of low concentration (12-23 μgl^{-1}), the other of high concentration (136-206 μgl^{-1}) supplied by the manufacturer. The intra-assay CVs for low and high concentrations were 13.3% and 8.6% respectively, while the inter-assay CVs for low and high concentrations were 6.3% and 14.1% respectively. Mean values for the low and high controls were 14.75 μgl^{-1} and 188.9 μgl^{-1} respectively.

4.7 $HMFG_2$ ASSAY

Several monoclonal antibodies have been developed which recognize different epitopes on the polymorphic epithelial mucin (PEM) found in the human milk fat globule membrane, see section 2.4.3, chapter 2. The antibody used in this study was raised by Taylor-Papadimitriou *et al.* (1981), and is obtainable commercially from Unipath. $HMFG_2$ standards and horseradish peroxidase anti-$HMFG_2$ Ab conjugate were kindly prepared by I.Jönrup at Unilever.

4.7.1 $HMFG_2$ assay principle

Several different types of immunoassay have been developed for $HMFG_2$, including RIAs (Burchell *et al.*, 1984, Ward and Cruickshank, 1987b), ELISAs (Dhokia *et al.*, 1986, Ashorn *et al.*, 1989) and a chemiluminescent ELISA employing a camera to detect and quantitate $HMFG_2$ (Badley,A., personal communication). Poor sensitivity was experienced in this study using a double determinant sandwich RIA.

The poor sensitivity experienced in attempts to develop a RIA in this study was possibly due to a detection system which employed a ^{125}I labelled polyclonal Ab (SAPU, UK). The homologous double-determinant assay principle used in the finally adopted ELISA was applied. ^{125}I-anti-mouse IgG was used as a tracer after formation of the $HMFG_2$ MAb - **HMFG** - $HMFG_2$ MAb complex. The assays

developed by Burchell *et al.* (1984) and Ward and Cruickshank (1987b) employed directly iodinated $HMFG_2$ MAb as tracer.

At this time, an $HMFG_2$ MAb-HRP conjugate became available from Unilever, and this was used to develop a simple, rapid, sensitive ELISA. The ELISA developed by Dhokia *et al.* (1986), where samples were treated with a low pH (pH 2.0) to expose the epitope, in an attempt to improve assay sensitivity, was found in this study to be unreproducible. Optimal conditions were arrived at after systematic variation of reagents and their concentrations, incubation periods, incubation temperatures. Harsh treatments, such as use of strong acidic conditions to disrupt serum complexes that may mask specific epitopes, were unnecessary.

4.7.2 Preparation of $HMFG_2$-horseradish peroxidase conjugate

Horseradish peroxidase (HRP) enzyme was conjugated to $HMFG_2$ IgG in a 1:1 ratio. 5 mg HRP (Sigma Type VI) was dissolved in 1 ml distilled H_20, and oxidised by the addition of 0.4 ml freshly prepared 0.1M sodium metaperiodate for 20 min at room temperature in the dark, while gently stirring occasionally. Oxidised HRP was then dialysed overnight at 4°c with 1L 1 mM acetate buffer pH 4.4, stirring continuously. The pH was brought to pH 9.0 with 0.2 M carbonate buffer pH 9.5. 5 mg $HMFG_2$ MAb in 1 ml carbonate buffer pH 9.5 was added and stirred gently for 2h at room temperature in the dark. 0.1 ml of freshly prepared sodium borohydride (5 $mgml^{-1}$ in distilled H_2O) was added and incubated at 4°c for 2h. The conjugate was finally dialysed with phosphate buffered saline (PBS) pH 7.4 containing 0.01% w/v Thimerosal, and was stored in this buffer at 4°c in the dark.

4.7.3 Purification of the milk mucin

Human breast milk was centrifuged at 10000g for 30min to isolate the skimmed milk fraction. HMFG was prepared from human skimmed milk by affinity chromatography on an $HMFG_1$ sepharose column prepared by purification of

tissue culture supernatant using a Protein A column and coupling of the purified MAb to cyanogen bromide activated sepharose (Pharmacia) as described by the manufacturer's instructions. Human skimmed milk was passed in batches of 100 ml through the column followed by washing with PBS pH 7.4. Bound antigen was eluted using 0.1 M glycine pH 2.5 and the fractions registering absorbance at 280 nm were pooled and dialysed against 0.25M acetic acid and freeze dried. Solutions made from this material were stored at - 20°c.

4.7.4 Deglycosylation of HMFG

Purified HMFG was partially deglycosylated by hydrolysis with anhydrous hydrogen fluoride for 1h at 4°c, as described by Mort and Lamport (1977). Longer incubation results in complete deglycosylation of HMFG which does not react as strongly with $HMFG_2$ antibody.

4.7.5 Preparation of $HMFG_2$ standards

$HMFG_2$ standards were prepared by the method of Burchell *et al.* (1987) using the preparatory steps described in 4.7.3 and 4.7.4. Standards were prepared in PBS pH 7.4, containing 7% bovine serum albumin (BSA) and 0.01% w/v Thimerosal as preservative, to avoid interference from HMFG normally present at varying levels in normal human sera. HMFG concentrations were set by reference to an original preparation isolated by Dr. S. Mather (St. Bartholomew's Hospital, London). 1 mg of the freeze dried powder was arbitrarily equal to 10^6 units. Deglycosylated HMFG was calibrated against a preparation obtained from Dr. J. Taylor-Papadimitriou (I.C.R.F., Lincoln's Inn Fields, London) in a similar way. Aliquoted standards were stable for at least 2 weeks at 4°c, whilst deglycosylated HMFG kept for only 1 week at 4°c.

4.7.6 $HMFG_2$ assay protocol

All samples were assayed in duplicate. Microtitre plates (M129B, Dynatech, Billingshurst, Kent, U.K.) were coated overnight at 4°c with 50 µl 5.0 $\mu g ml^{-1}$

$HMFG_2$ MAb in 0.05M carbonate buffer pH 9.6. The plates were washed three times with 100 µl 0.15M PBS pH 7.4 containing 0.05% polyoxyethylene sorbitan monolaurate (Tween 20) (PBS/Tween 20). 25 µl neat serum, standard, or control were incubated with 25 µl PBS/Tween 20 for 30min at 37°c in a shaking incubator (Dynatech). The plates were washed three times with PBS/Tween 20, and 50 µl $HMFG_2$ MAb-HRP conjugate (see 4.7.3) at 1:1000 in PBS/Tween 20 incubated for 30min at 37°c. After three final washes, 100 µl peroxidase substrate: 0.04% w/v 0-phenylenediamine and 0.02% v/v H_2O_2 in 0.15M citrate phosphate buffer pH 5.0 was added. The reaction was stopped after 30min incubation at 37°c with the addition of 50 µl 2.5M H_2SO_4, and optical density determined at 492nm with a Titertek Multiscan, see figure 4.9.

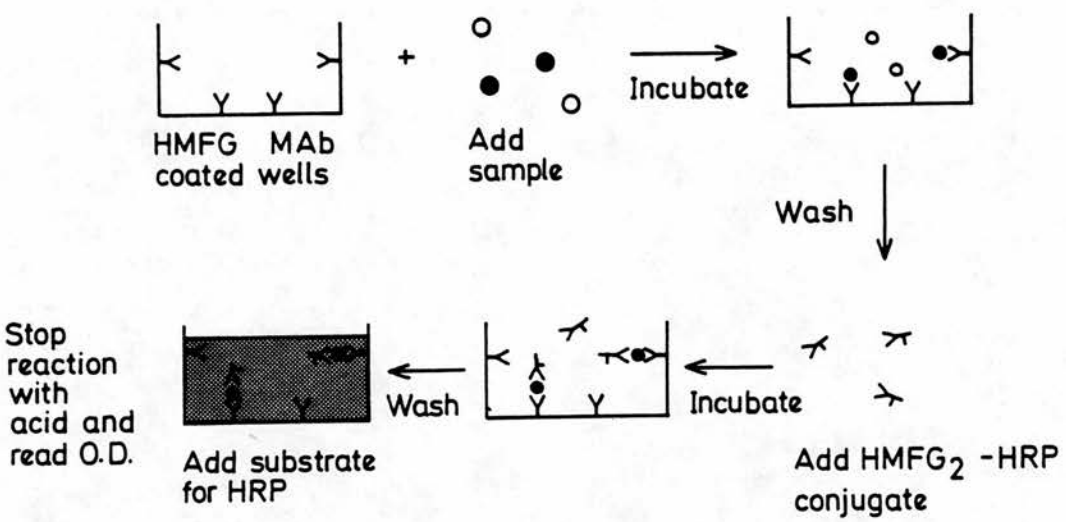

Figure 4.9 ***Sandwich ELISA with HRP detection system***. Microtitre wells are coated by overnight incubation with $HMFG_2$ MAb. After washing to remove unbound MAb, samples are incubated. After a further wash, $HMFG_2$ MAb-HRP conjugate is added and $HMFG_2$ antigen present in the sample is sandwiched between the two antibodies forming a complex bound to the solid phase. A final wash removes unbound conjugate and substrate is added to develop the colour. The reaction is stopped by the addition of acid, and the optical density measured is proportional to the concentration of $HMFG_2$ antigen in the sample.

Control and unknown sample concentrations were interpolated manually from dose-response curves constructed using standard preparations at the following concentrations: 0, 50, 100, 200, 350, and 500 Uml^{-1}. One standard curve was included for every two ELISA plates assayed. Table 4.8 shows the mean optical densities (o.d.), SD, SE, and CV (%) for each standard (n=22).

Table 4.8 ***$HMFG_2$ standard values***

$HMFG_2$ (Uml^{-1})	Mean o.d.	SD	SE	CV (%)
0.0	0.215	0.068	0.014	31.6
50.0	0.394	0.073	0.016	18.5
100.0	0.586	0.102	0.022	17.4
200.0	0.943	0.155	0.033	16.4
350.0	1.337	0.184	0.042	13.8
500.0	1.656	0.317	0.073	19.1

Figure 4.10 shows the standard curve for $HMFG_2$ plotted using the values in table 4.8, together with standard errors and precision profile.

4.7.8 $HMFG_2$ assay precision

CVs were calculated using two controls included in each assay run (n=22); one of low concentration (50 Uml^{-1}) and one of high concentration (150 Uml^{-1}). The intra-assay CVs for low and high concentrations were 6.8% and 4.93% respectively, while the inter-assay CVs for low and high concentrations were 14.9% and 6.56% respectively. Mean values for the low and high controls were 43.5 Uml^{-1} and 149.8 Uml^{-1} respectively.

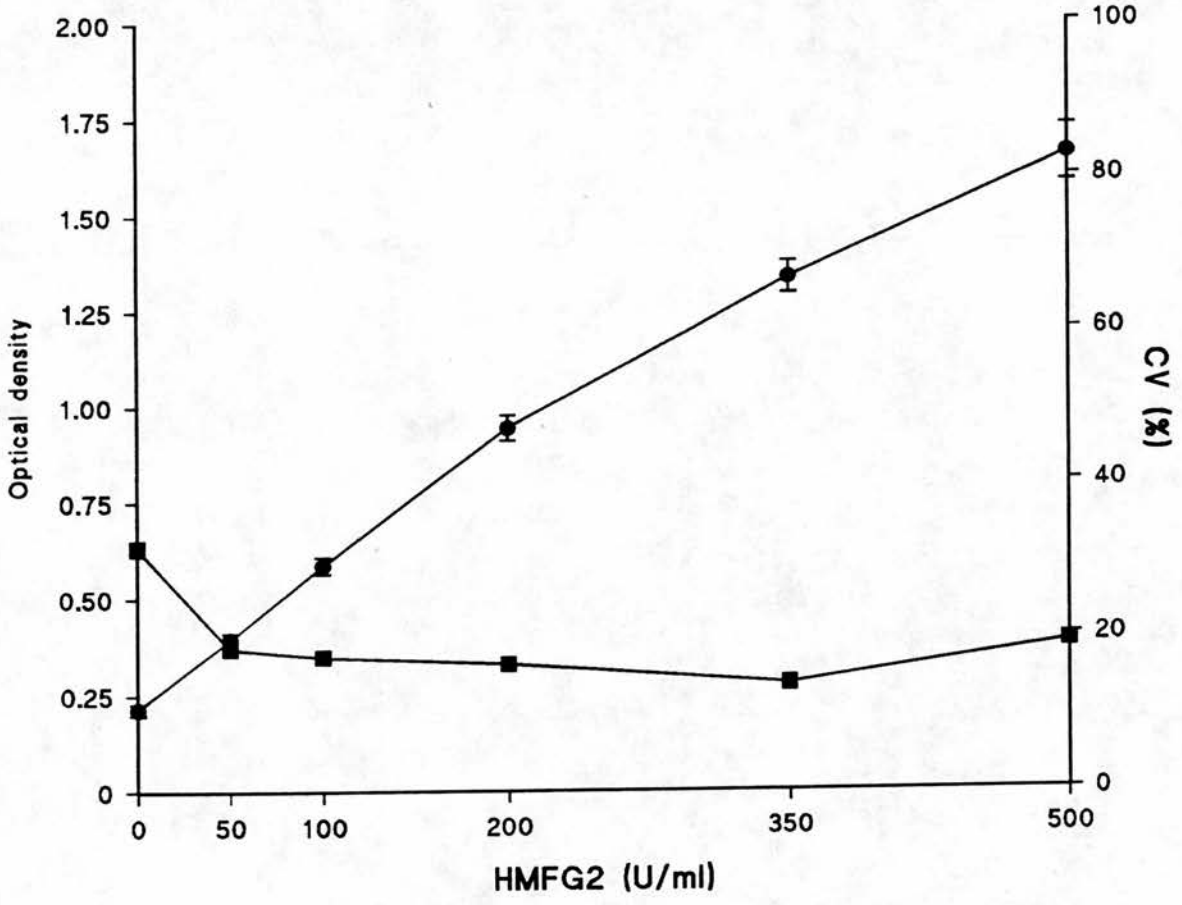

Figure 4.10 ***HMFG$_2$ standard curve and precision profile***

4.8 P185 ASSAY

An assay for the neu (c-erbB 2/HER-2) oncogene product, c-neu p185, has recently been developed, and is available from Dupont/NEN Research Products, U.S.A.. The first study of c-neu p185 in breast cancer serum found 15% and 23% of patients with primary and metastatic breast cancer respectively to have elevated serum c-neu p185 (Hayes *et al.*, 1989).

4.8.1 P185 assay principle

Several monoclonal antibodies have been raised against c-neu p185. Two MAbs, NB3 and TA1, recognise distinct epitopes on the extracellular domain of the human neu oncogene product but do not cross react with the rat neu product (McKenzie *et al.*, 1989). These have been used to develop a heterologous double-determinant sandwich ELISA using biotinylated TA1 tracer MAb and streptavidin-HRP as the detection system, see figure 4.11 overleaf. This assay is similar to $HMFG_2$ assay, except that different Abs are used as catcher and tracer molecules, and the detection system involves the additional biotin/avidin step to improve assay sensitivity.

4.8.2 P185 assay protocol

Samples were kindly assayed for c-neu p185 by Dr.D.F.Hayes and colleagues at the Dana Farber Cancer Institute in Boston, U.S.A. All incubations were performed at room temperature (18-25°c). Samples and control were assayed in duplicate and standards were assayed in triplicate.

1. Serum samples were diluted 1:50 in assay buffer (0.01M PBS pH 7.4 with 1% BSA, 0.1% Tween 20, and 0.1% sodium azide).
2. 100 µl sample, standard, and control were incubated for 18h in microtitre plates coated with NB3 MAb.
3. The plates were washed three times and incubated with 100 µl biotinylated-TA1 MAb in PBS for 30min.

4. The plates were washed three times.
5. 100 µl streptavidin-HRP (diluted in PBS with 1% BSA and 0.01% chloracetamide) was incubated for 15min.
6. Colour was developed by the addition of 100 µl o-phenylenediamine in 0.1M citrate buffer pH 5.0 with 0.01% H_2O_2.
7. The reaction was stopped after 60min with 100 µl 2M H_2SO_4, and absorbance determined at 490nm.

Serum c-neu p185 was quantitated by interpolation from a dose-response curve constructed with serial dilutions of cellular extract from c-neu transfected NIH/3T3 cells. The standard concentrations used were 0, 10, 30, 60, 90, and 120 Uml^{-1}.

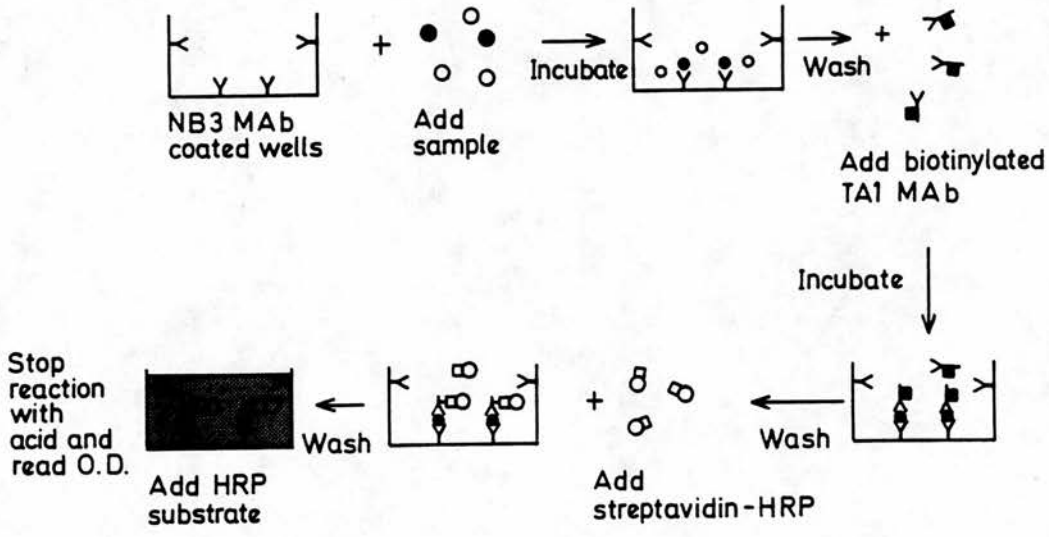

Figure 4.11 ***Sandwich ELISA with avidin/biotin detection system.*** Samples are incubated with NB3 MAb coated microtitre plates. After washing to remove unbound sample, biotinylated-TA1 MAb is added. C-neu p185 present is sandwiched between the two MAbs, and after washing streptavidin-HRP is incubated. After a final wash, the colour is developed with the addition of o-phenylenediamine and the absorbance measured is proportional to the concentration of c-neu p185 in the sample.

4.9 ESTABLISHMENT OF ASSAY CUT-OFF VALUES

Cut-off values may be established in different ways depending on the purpose of the assay. For a review of methods of evaluating and influencing the discriminatory capacity of tumour markers see Makuch and Muenz (1987). It is most common to use the mean value plus two standard deviations of a normal reference population. This is suitable when a differential diagnosis between normal subjects, patients with benign disorders, and patients with malignancy is desirable, i.e. in the context of screening. For the purposes of monitoring patients with established malignancy it is more appropriate to use the disease free patient population as a reference population. This may be done using receiver operating characteristic (ROC) curves which plot the percentage of false positive (FP) results (or 1 - specificity) on the x-axis versus the percentage of true positive (TP) results (or sensitivity) on the y-axis. Choosing a cut-off where the curve begins to plateau gives the best trade-off between sensitivity and specificity. See chapter 3, section 3.4.7, pp. 68-71, for the definition and relative importance of these parameters.

The price of each commercial kit precluded their use to determine our own reference ranges. Therefore, in this study, the cut-off values commonly used were those established by the original investigator and/or recommended by the manufacturer. Table 4.9 shows the cut-off values used for each assay and how each was established. The following values were used throughout all tumour marker analysis.

Table 4.9 ***Assay cut-off values***

Tumour marker	Cut-off value	Method of establishment	Author(s)
CA125	35 Uml^{-1}	↑ in 1% of 888 normal ♀	Bast *et al.*(1981)
CA153	30 Uml^{-1}	↑ in 1% of 1051 normals	Tobias *et al.*(1985)
CA199	33 Uml^{-1}	↑ in 0.6% of 1020 normals	Del Villano *et al.*(1983)
CA724	5 Uml^{-1}	x + 2sd of 66 normals	Scambia *et al.*(1990)
TATI	20 μgl^{-1}	x + 2sd of * normals	Stenman *et al.*(1982)
$HMFG_2$	40 Uml^{-1}	↑ in 5% of 132 normals	Fisken *et al.*(1991)
p185	1900 Uml^{-1}	x + 2sd of 42 normal ♀	Hayes *et al.*(1989)

* unspecified number

In summary, this chapter has described the variety of immunological methods used to measure putative EOC tumour markers, and outlined the reasons for choice of cut-off value - important in determining the clinical applicability of each marker.

CHAPTER 5

EOC Population Characteristics and Management

5.1 INTRODUCTION

Blood samples were collected from patients with established EOC in the post-operative follow-up period for over more than five years, from April 1984 to July 1989. The serum was separated by centrifugation at 1500g for 10 minutes and stored at -20°c until use. The patients all came from Lothian Region, and were eventually treated for their disease at the Western General Hospital and Royal Infirmary in Edinburgh.

After careful and independent retrospective clinical documentation, a total of 1237 samples from 250 patients were included in the final analysis. The length of clinical follow-up in the population was longer than the blood sampling period, in some instances up to a year after the last serum assays were performed. During this time a database combining patients' case histories and serial serum tumour marker levels was being developed to perform statistical analyses. This will be described in chapter 6. Initially, the database was used to collate information on the demographics of the patient population and their management, presented in this chapter.

This chapter is divided into three sections: the first documents the patients' disease characteristics found at diagnosis; the second documents aspects of the patients' management and outcomes of primary therapy; and the third documents the number of tumour marker assays performed. The aim of this chapter is twofold; firstly to show that the population under study is representative of any EOC patient population encountered in a major treatment centre, and secondly to provide a base from which the tumour markers can be evaluated in different clinical settings incurred in this disease.

5.2 PRIMARY DISEASE CHARCTERISTICS OF EOC POPULATION

Table 5.1 shows the findings at primary surgery in all 250 patients. Factors of major prognostic importance were noted, including stage, histological tumour type, tumour grade, presence of adhesions and ascites (and cytology).

*ECOG performance status was also noted prior to initiation of primary chemotherapy.

Table 5.1 ***Findings at primary surgery***

Primary disease characteristics		No. patients
Stage	I	46
	II	27
	III	139
	IV	38
Histopathology	Serous	147
	Endometrioid	41
	Mucinous	22
	Adenocarcinoma	18
	Clear cell	17
	Mixed	3
	Unknown	2
Tumour grade	Well differentiated (WD)	31
	Moderately differentiated (MD)	55
	Poorly differentiated (PD)	126
	Unknown	38
Adhesions	Yes	134
	No	51
	Unknown	65
Ascites	Yes	145
	(Cytology positive	70)
	(Cytology negative	17)
	(Cytology not done	44)
	(Cytology unknown	14)
	No	91
	Unknown	14
*ECOG Performance status	0	115
	1	72
	2	21
	3	4
	4	1
	Unknown	37

*ECOG - European Committee for Obstetrics and Gynaecology

73/250 (29.2%) patients presented with early stage disease, and 177/250 (70.8%) patients presented with advanced disease at initial diagnosis. 17 patients were "upstaged" from early to advanced disease during follow-up. Overall, the mean clinical follow-up was 26.8 months (median 20.3 months and

range 0.5-173.7 months). One patient who had completely resected stage I disease had recurrent stage IV disease 12 years later. Patients who presented with early stage disease had a mean clinical follow-up of 36.7 months (median 31.7 months and range 5.1-173.7 months), while patients who presented with advanced disease had a mean clinical follow-up of 22.7 months (median 17.2 months, and range 0.5-117.6 months). Overall, the mean age at diagnosis was 58.2 years (median 58 years and range 23-81 years). Patients presenting with early disease had a mean age at diagnosis of 55.6 years (median 55 years and range 29-79 years), while patients with advanced disease at diagnosis had a mean age of 59.2 years (median 59 years and range 23-81 years).

Table 5.2 shows the frequency of each tumour type within each stage. The predominance of stage III serous tumours can be clearly seen, accounting for 93/250 (37.2%) of the total.

Table 5.2 ***Frequency of histological type in each stage***

Histological type	FIGO stage I	II	III	IV	Total
Serous	17	15	93	22	147
Endometrioid	12	6	19	4	41
Mucinous	11	3	4	4	22
Adenocarcinoma	-	1	13	4	18
Clear cell	5	2	7	3	17
Mixed	1	-	2	-	3
Unknown	-	-	1	1	2
Total	46	27	139	38	250

Serous papillary cystadenocarcinomas and adenocarcinomas tend to present at a later stage than endometrioid and clear cell tumours, while the majority of mucinous tumours present in the earlier stages. Table 5.3 shows the frequency of each tumour grade within each stage.

The proportions of well and moderately differentiated tumours decrease with progressive disease, while the proportion of poorly differentiated tumours rises with advancing disease. The unknown grade category includes tumours of variable differentiation, in which the composition of different grades is unknown.

Table 5.3 ***Frequency of histological grade in each stage***

Histological grade	FIGO stage I	II	III	IV	Total
WD	9	4	15	3	31
MD	16	8	30	1	55
PD	7	12	79	28	126
Unknown	14	3	15	6	38
Total	46	27	139	38	250

Table 5.4 shows the frequency of each tumour grade within each tumour type. Adenocarcinomas, serous papillary cystadenocarcinomas and endometrioid tumours contain the highest proportion of poorly differentiated cells, while mucinous tumours contain the highest proportion of well differentiated cells.

Table 5.4 ***Frequency of histological grade in each type***

Histological type	Histological grade WD	MD	PD	Unknown	Total
Serous	18	35	81	13	147
Endometrioid	2	8	24	7	41
Mucinous	8	3	2	9	22
Adenocarcinoma	-	3	15	-	18
Clear cell	3	4	3	7	17
Mixed	-	1	1	1	3
Unknown	-	1	-	1	2
Total	31	55	126	38	250

5.3 PATIENT MANAGEMENT AND TREATMENT OUTCOMES

The methods used to treat the patients and determine their responses to primary therapy will be described. Chemotherapy regimes are given in Appendix C. Endpoints include: the percentage of patients responding to first-line chemotherapy; the findings at second-look surgery; progression-free survival; and overall survival.

5.3.1 Primary surgery

A minority of the patients were initially operated on in peripheral hospitals before coming to the Western General and Royal Infirmary in Edinburgh for chemotherapy. The primary operative procedures are shown in table 5.5. TAH, BSO, and omentectomy were performed in 18/46 (39%) of stage I patients, 13/27 (48%) of stage II patients, 38/139 (27.3%) of stage III patients, and 9/38 (23.7%) of stage IV patients.

Table 5.5 ***Primary operative procedures***

Procedure	FIGO stage I	II	III	IV	Total
TAH	42	22	53	14	131
SubTAH	1	1	10	5	17
BSO	41	24	85	24	174
Unilateral SO	2	1	6	2	11
Ovariotomy	3	1	3	2	9
Ovarectomy	-	-	5	2	7
Omentectomy	21	14	63	18	116
Partial omentectomy	1	1	8	5	15
Biopsy only	-	1	40	9	50
Other (palliatve)	-	-	20	2	22

The extent of residual disease after primary surgery was divided into five categories: no residual disease (complete debulk); <2cm residual disease; 2-

5cm residual disease (partial debulk); >5cm residual disease; gross residual disease (no debulk, tumour biopsy only). Optimal debulking was defined as either complete resection or resection to less than 2cm residual disease. The frequency and extent of debulking achieved in each stage is shown in table 5.6.

Table 5.6 ***Frequency and extent of debulking in each stage***

FIGO stage	Residual disease					
	None	<2cm	2-5cm	>5cm	Gross	Total
I	39	7	-	-	-	4
II	4	19	2	1	1	27
III	-	52	34	19	34	139
IV	-	8	13	10	7	38
Total	43	86	49	30	42	250

Optimal debulking was achieved overall in 129/250 (51.6%) of patients. 46/46 (100%) of stage I patients, 23/27 (85.2%) of stage II patients, 52/139 (37.4%) of stage III patients, and 8/38 (21.1%) of stage IV patients were optimally debulked.

Table 5.7 shows the frequency and extent of debulking for each tumour type. Optimal debulking was achieved in 66/147 (44.9%) of patients with serous, 29/41 (70.7%) with endometrioid, 15/22 (68.2%) with mucinous, 7/18 (38.9%) with adenocarcinoma, 11/17 (64.7%) with clear cell, and 1/3 (33.3%) with mixed tumours.

Table 5.7 ***Frequency and extent of debulking in each tumour type***

Tumour type	None	Residual disease <2cm	2-5cm	>5cm	Gross	Total
Serous	17	49	27	22	32	147
Endometrioid	10	19	9	3	-	41
Mucinous	10	5	4	1	2	22
Adenocarcinoma	-	7	3	3	5	18
Clear cell	5	6	5	-	1	17
Mixed	1	-	1	1	-	3
Unknown	-	-	-	-	2	2
Total	43	86	49	30	42	250

Table 5.8 shows the frequency and extent of debulking for each tumour grade. Optimal debulking was achieved in 23/31 (74.2%) of patients with well differentiated tumours, 23/55 (60%) with moderately differentiated tumours, and 49/126 (38.9%) with poorly differentiated tumours.

Table 5.8 ***Frequency and extent of debulking for each tumour grade***

Tumour grade	None	Residual disease <2cm	2-5cm	>5cm	Gross	Total
WD	9	14	5	2	1	31
MD	14	19	7	5	10	55
PD	6	43	27	21	29	126
UNK	14	10	10	2	2	38
Total	43	86	49	30	42	250

Thus, the feasibility of optimal debulking decreased with increasing stage and progressive tumour dedifferentiation. Optimal debulking was more often

achieved in patients with tumours of endometrioid, mucinous and clear cell types, which tend to present earlier (table 5.2) and have a higher proportion, except for endometrioid, of well differentiated cells than serous papillary cyst-adenocarcinomas and adenocarcinomas (table 5.4).

5.3.2 Chemotherapy and responses

The majority of patients in this study were entered into ICRF clinical trials. Responses to first-line chemotherapy, usually started one month after primary surgery, were determined using UICC response criteria (chapter 1, table 1.4, p 27). The methods used to determine responses to therapy in patients with advanced disease are summarized, and the number of second-look operations performed and their outcomes are described for each stage. No account of independent prognostic factors, other than stage, in determining response to therapy has been made. A more detailed breakdown of prognostic groups will be given when the markers are analysed in detail (chapter 9, sections 9.2 and 9.3, pp. 220-233). Treatment of patients is presented according to stage, and outcomes determined at the last case note review (August 1989).

5.3.3 Stage I patients

46 patients presented with stage I disease (3, 24, 10 and 9 had stages I, Ia, Ib and Ic disease respectively). 24 patients received no post-operative therapy, three had whole abdominal radiotherapy, and 19 had chemotherapy (18 adjuvant and one therapeutic). 11 patients have died, and 35 remain alive, two with progressive disease and 33 with no evidence of disease.

Stage I: 2/3 patients had no post-operative therapy and remain disease free, while the other patient had radiotherapy and died from recurrent stage IV disease 13 years later.

Stage Ia: 16/24 patients received no post-operative therapy, none had radio-therapy and six had complete courses of adjuvant chlorambucil. Six patients,

three of whom received chlorambucil, relapsed with stage III (five patients) and stage IV (one patient) disease, have died and 18 remain alive and disease free.

Stage Ib: 4/10 patients received no post-operative therapy, none had radiotherapy, and six had adjuvant chemotherapy. Three patients had complete courses of chlorambucil, one relapsed with stage III disease and died and the other two remain disease free. 1/2 patients completed a course of cisplatin/α-interferon, and one patient had an incomplete course of cisplatin. Both had to stop due to toxicity. All three patients remain disease free. In this group, one patient has died and nine remain alive, one with progressive disease and eight with no evidence of disease.

Stage Ic: 2/9 patients received no post-operative therapy, none had radiotherapy, and seven had chemotherapy. 4/4 completed courses of adjuvant chlorambucil, one relapsed with stage III disease and died, one has progressive disease, and two remain disease free. Two patients had incomplete courses of adjuvant cisplatin and cisplatin/prednimustine due to toxicity and both remain disease free. One patient stopped therapeutic 5-fluorouracil/cisplatin/hexamethylmelamine/prednimustine (5FU/P/H/P) early due to toxicity and had a partial response, but later relapsed with stage IV disease and died. In this group three patients have died and six remain alive, one with progressive disease and five with no evidence of disease.

The second-look procedures performed and their findings are shown in table 5.9. Outcomes were divided into three categories: negative, microscopic disease present or macroscopic disease present. Overall, 21/46 (45.7%) of stage I patients underwent second-look operation, 15/21 had laparotomy and 6/21 had laparoscopy. 10/21 (47.6%) of all second-look procedures were negative, 5/21 (23.8%) patients had microscopic disease and 6/21 (28.6%) patients had macroscopic disease. Four patients were "upstaged" at second-look, three to stage III and one to stage IV, while one patient subsequently relapsed with stage III disease.

Table 5.9 ***Findings at second-look in stage I patients***

Second-look outcome	FIGO Stage I	Ia	Ib	Ic	Total
No. patients	3	24	10	9	46
No. Laparotomies	-	6	7	2	15
Negative	-	1	4	-	5
Microscopic	-	-	3	1	4
Macroscopic	-	4	-	1	5
No. restaged	-	2	-	1	3
No. Laparoscopy	-	-	5	-	16
Negative	-	4	-	-	4
Microscopic	-	-	-	1	1
Macroscopic	-	1	-	-	1
No. restaged	-	1	-	-	1

5.3.4 Stage II patients

27 patients presented with stage II disease (9, 5, 2, and 11 had stage II, IIa, IIb, and IIc respectively). Three received no post-operative therapy, two had whole abdominal radiotherapy, and 22 had chemotherapy (four adjuvant and 18 therapeutic). Eleven patients have died (one death was not due to EOC) and 10 remain alive, two with progressive disease and 13 with no evidence of disease.

Stage II: 1/9 patients had no post-operative therapy, none had radio-therapy, and eight received chemotherapy. Two patients received adjuvant therapy, one had a complete course of chlorambucil, while the other patient refused to finish a course of cisplatin, both remain disease free. Three patients had chlorambucil, two had to stop due to disease progression, the other relapsed later, and all three have died. Two patients had cisplatin/prednimustine, one stopped due to toxicity but had a complete response and remains disease free,

while the other progressed and died. One patient had a complete course of cisplatin but subsequently relapsed and died. In this group, five patients have died and four remain disease free.

Stage IIa: 1/5 patients received no post-operative therapy, two had radiotherapy and two had chemotherapy. One patient had adjuvant chlorambucil and remains disease free, and one had therapeutic cisplatin and remains disease free. Four patients are still alive, one with progressive disease and three with no evidence of disease.

Stage IIb: 1/2 patients had chlorambucil but the response was inevaluable due to unmeasurable disease. One patient had an incomplete course of cisplatin due to toxicity but had a pathological complete response documented at second-look laparotomy. Both patients remain disease free.

Stage IIc: 1/11 patients in this group had radiotherapy, one had adjuvant prednimustine, and nine had therapeutic regimes. Two patients had complete courses of chlorambucil, both achieved a complete response, one subsequently had recurrent stage IV disease and died, while the other remains disease free. Five patients had cisplatin/prednimustine; three completed the regime, one stopped due to toxicity and all four had a complete response, while the other patient stopped due to disease progression. Two have died and three remain disease free. Two patients had cisplatin, one had a complete response and remains disease free, the other stopped due to toxicity, had a subsequent complete response to iproplatin but later had recurrent stage IV disease and died.

The second-look procedures performed and their findings are summarized in table 5.10. Overall, 14/27 (51.8%) of stage II patients underwent second-look operation, 8/14 had laparotomy and 6/14 had laparoscopy.

Table 5.10 ***Findings at second-look in stage II patients***

Second-look outcome	FIGO Stage II	IIa	IIb	IIc	Total
No. patients	9	5	2	11	27
No.Laparotomy	2	2	-	4	8
Negative	-	1	-	3	4
Microscopic	-	-	-	-	-
Macroscopic	2	-	-	1	3
No.upstaged	1	-	-	-	1
No.Laparoscopy	2	-	1	3	6
Negative	2	-	1	3	6
Microscopic	-	-	-	-	-
Macroscopic	-	-	-	-	-
No. upstaged	-	-	-	-	-

10/14 (71.4%) of all second-look procedures were negative, none had microscopic disease, 3/14 had macroscopic disease, and the outcome of one was unknown. One patient was "upstaged" to stage IV at second-look, three patients who had a negative second-look subsequently relapsed with stage IV disease.

5.3.5 Stage III patients

4/139 patients received no post-operative therapy due to frailty, two had whole abdominal radiotherapy, the treatment of one patient is unknown as case notes could not be found at the time of assessment, and the remaining 132 patients had chemotherapy. 97/139 patients have died and 42 are still alive; six with progressive disease, three with suspected progressive disease due to rising CA125 levels, eight with stable disease, 17 in complete remission, five with inevaluable disease, and three whose disease status is unknown.

The responses to first line therapy could not be determined in 15 patients; four who had no therapy, two who had radiotherapy, four who had adjuvant chemotherapy, and five whose case notes could not be found at the time of assessment. A further nine patients were inevaluable. 65/115 (56.5%) of patients with evaluable disease responded to first line therapy. 39/65 (60%) of these were complete responses and 26/65 (40%) were partial responses. 25/39 (64%) of those who achieved a complete response, 7/26 (26.9%) of those who achieved a partial response, 2/21 (9.5%) of those who remained stable, and 0/29 patients who progressed on therapy remain alive.

66/115 (57.4%) of patients completed their chemotherapy regimes, while 49/115 (42.6%) had incomplete regimes. The reasons for stopping are shown in table 5.11.

Table 5.11 ***Reasons for stopping chemotherapy in patients with stage III***

Reason for stopping	Response CR	PR	SD	PD	Total
Regime complete	30	21	11	4	66
Toxicity	9	5	2	1	17
Progressive disease	-	-	8	24	32
Patient refused	-	-	-	-	-
Total	39	26	21	29	115

Responses to individual regimes are shown in table 5.12, with the number of pathologically documented responses shown in brackets. Thus, 29/39 (74%) of complete reponses and 13/26 (50%) partial responses were documented pathologically.

Table 5.12 ***Responses to individual regimes in patients with stage III***

Chemotherapy regime	Response CR	PR	SD	PD	Total
Cisplatin/pred.	19 (17)	8 (3)	2	10	39
5-FU/P/H/P	6 (5)	5 (4)	6	1	18
Chlorambucil	3	3	8	16	30
Cisplatin	4 (2)	6 (6)	3	1	14
Prednimustine	3 (2)	2	2	1	8
Cisplatin/α-interferon	2 (2)	-	-	-	2
Carboplatin	1	-	-	-	1
Cisplatin/adriamycin	-	1	-	-	1
Cisplatin/CMF	1 (1)	-	-	-	1
CHIP	-	1	-	-	1
Total	39 (29)	26 (13)	21	29	115

The methods used to assess response to first line therapy are summarized in table 5.13. 49/115 (42.6%) of all responses were assessed by second-look operation, 39/115 (33.9%) were assessed clinically, 26/115 (22.6%) were assessed radiologically, and 1/115 (0.8%) were determined by CA125 assay. The patient assessed by CA125 had rising levels with progressive disease.

Table 5.13 ***Method of assessing response in patients with stage III***

Method of assessment	Response CR	PR	SD	PD	Total
Laparotomy	18	15	3	-	36
Laparoscopy	11	1	1	-	13
CT scan	2	2	2	7	13
US scan	1	4	2	4	11
X-Ray	-	-	1	1	2
Clinical	7	4	12	16	39
CA125	-	-	-	1	1
Total	39	26	21	29	115

5.3.6 Stage IV patients

All but one patient with stage IV disease, who was moribund after surgery, had post-operative chemotherapy. Eight patients in this group remain alive, two with progressive disease, four with stable disease, and two with inevaluable disease. 18/34 (52.9%) of stage IV patients with evaluable disease responded to first-line therapy. 4/18 (22.2%) of these were complete responses and 14/18 (77.7%) were partial responses. There was a higher proportion of complete responders in stage III than stage IV patients. 1/4 (25%) of those stage IV patients who achieved a complete response, 5/14 (35.7%) of those who achieved a partial response, 1/4 (25%) of those who remained stable, and 1/12 (8.3%) of those who progressed on first line therapy remain alive.

The reasons for stopping chemotherapy are shown in table 5.14. The majority 20/34 (58.8%) completed their regime, while 14/34 (41.2%) had incomplete regimes.

Table 5.14 ***Reasons for stopping therapy in patients with stage IV***

Reason for stopping	Response CR	PR	SD	PD	Total
Regime complete	4	13	3	-	20
Toxicity	-	1	-	1	2
Progressive disease	-	-	1	11	12
Patient refused	-	-	-	-	-
Total	4	14	4	12	34

Responses to individual regimes are shown in table 5.15, with the number of pathologically documented responses shown in brackets. 3/4 (75%) of complete responses and 4/14 (28.6%) of partial responses were documented pathologically.

Table 5.15 ***Responses to different regimes in patients with stage IV***

Chemotherapy regime	Response CR	PR	SD	PD	Total
Cisplatin/prednimustine	4 (3)	9 (2)	3	4	20
Chlorambucil	-	1	1	6	8
Cisplatin	-	1	-	1	2
Prednimustine	-	3 (2)	-	-	3
Carboplatin	-	-	-	1	1
Total	4 (3)	14 (4)	4	12	34

The methods used to assess response to first line therapy are summarized in table 5.16. 7/34 (20.5%) of the total were assessed by second-look surgery, 13/34 (38.2%) by clinical examination, and 14/34 (41.2%) by radiological scanning.

Table 5.16 ***Method of assessing response in patients with stage IV***

Method of assessment	Response CR	PR	SD	PD	Total
Laparotomy	3	2	-	-	5
Laparoscopy	-	2	-	-	2
CT scan	1	6	2	1	10
US scan	-	1	1	2	4
X-ray	-	-	-	-	-
Clinical	-	3	1	9	13
CA125	-	-	-	-	-
Total	4	14	4	12	34

The findings at second-look surgery were summarized in tables 5.9 and 5.10 for patients with stages I and II disease respectively. Table 5.17 summarizes the findings in patients with stages III and IV disease. While second-look surgery

was performed in 21/46 (45.7%) of stage I patients and 14/27 (51.8%) of stage II patients, 65/139 (46.8%) of stage III and 7/38 (18.4%) of stage IV patients had second-look surgery. 31/65 (47.7%) of stage III and 3/7 (42.9%) of stage IV patients had a negative second-look operation. 8/65 (12.3%) of stage III and 2/7 (28.5%) of stage IV patients had microscopic disease at second-look operation. 15/65 (23%) of stage III and 1/7 (14.3%) of stage IV patients had macroscopic disease at second-look operation. 10/65 (15.4%) of stage III and 1/7 (14.3%) of stage IV patients had bulky disease at second-look operation.

Table 5.17 ***Findings at second-look in patients with stages III and IV***

Second-look outcome	Stage III	Stage IV	Total
No.patients	139	38	177
No. Laparotomy	51	5	56
Negative	20	3	23
Microscopic disease	7	1	8
Macroscopic disease	13	-	13
Bulky disease	10	1	11
Unknown	1	-	1
Debulked	5	1	6
No. Laparoscopy	14	2	16
Negative	11	-	11
Microscopic disease	1	1	2
Macroscopic disease	2	1	3
Bulky disease	-	-	-
Unknown	-	-	-

The most common reason for performing a second-look procedure was to determine response to therapy in patients clinically free of disease. Although 42 patients had inoperable disease at primary surgery, only six patients were subsequently rendered operable and had interval debulking at second-look after several courses of chemotherapy.

5.3.7 Progression-free survival

Progression free survival was calculated for those patients where it was possible to give a date of progression. This date inevitably will be later than the "true" date of progression, depending on how it was assessed and how closely the patient was being monitored at the time. Time to progression, shown in table 5.18, was calculated by subtracting the date of primary diagnosis from the date of progression.

Table 5.18 ***Progression-free survival in each stage***

Stage	No. patients alive (%)	Progression-free survival (months) Mean	Median	Range
I	7 / 17 (41)	24.4	16.3	3.2 - 155.6
II	3 / 12 (25)	20.0	14.4	4.3 - 42.6
III	11 / 94 (12)	14.4	9.9	1.6 - 57.1
IV	5 / 31 (16)	10.6	8.4	1.4 - 38.3

5.3.8 Survival

The percentage survival, expressed as the possible number of patients alive (including the number of patients who had died and the number alive followed up for a length of time) over the total number alive is shown in table 5.19. The two year survival for stages I, II, III, and IV was 97%, 80%, 40.8%, and 21.1% respectively, and the five year survival rate was 50%, 16.7%, 8.6%, and 0% respectively. Five year survival rates are lower than often presented in the literature, possibly due to the small numbers followed up for this length of time.

Table 5.19 ***Survival in each stage***

Survival	FIGO Stage I	II	III	IV
1 year	42/43 (97.7%)	24/27 (88.9%)	95/137 (69.3%)	25/38 (65.8%)
2 years	32/33 (97.0%)	20/25 (80.0%)	49/120 (40.8%)	7/32 (21.9%)
3 years	18/26 (69.0%)	15/21 (71.4%)	26/112 (23.2%)	2/31 (6.5%)
4 years	13/21 (62.0%)	7/16 (43.3%)	17/108 (15.7%)	1/30 (3.3%)
5 years	8/16 (50.0%)	2/12 (16.7%)	9/104 (8.6%)	0/30 (0%)

5.4 TUMOUR MARKER "FOLLOW-UP"

The main purpose of this chapter is to lay the foundations of the clinical settings in which the tumour markers in this study were measured. Whether tumour markers have anything useful to add to the total clinical picture of EOC will be addressed in chapters 7, 8 and 9.

The number of assays performed for each marker, the length of "marker" follow-up as opposed to "clinical" follow-up (see section 5.2, pp. 117-118) will be described. CA125 was assayed in all 1237 samples from the total population of 250 patients, while the other markers were assayed in smaller numbers of patients samples. "In-house" assays, HMFG2 and p185, were assayed in greater numbers than the commercial assays, the major restriction being the expense of the commercially available assay kits. The time the assays were performed and the number of serum aliquots remaining were also important factors in the choice of samples assayed.

Table 5.20 shows the numbers of each tumour marker assay performed in the total population. In addition to this, the mean, median and range is shown for the number of samples assayed per patient.

Table 5.20 ***Number of tumour marker assays***

Tumour marker	No. patients	No. samples	No. samples per patient Mean	Median	Range
CA125	250	1237	4.8	4.0	1-17
$HMFG_2$	215	880	3.9	3.0	1-13
p185	173	601	3.4	3.0	1-10
TATI	117	346	2.9	2.0	1-8
CA153	42	192	4.3	4.0	1-13
CA199	42	193	4.3	4.0	1-13
CA724	20	70	3.3	3.0	1-6

Tables 5.21 and 5.22 show the number of assays performed in patients with early and advanced disease respectively. The numbers of samples in tables 5.21 and 5.22 add up to give the total numbers found in table 5.20, however, there is a discrepancy in the numbers of patients. This is because seven patients who were "upstaged" from early to advanced stage during follow-up had samples taken during both phases of their disease, and not all patients had samples assayed for each marker.

Table 5.21 ***Number of tumour marker assays in early stage disease***

Tumour marker	No. patients	No. samples	No. samples per patient Mean	Median	Range
CA125	63	286	4.5	3.0	1-16
$HMFG_2$	62	236	3.8	3.0	1-13
p185	52	158	3.0	3.0	1-9
TATI	39	111	2.8	3.0	1-8
CA153	18	86	4.8	4.5	1-13
CA199	18	86	4.8	4.5	1-13
CA724	8	32	4.0	4.5	1-6

Table 5.22 ***Number of tumour marker assays in advanced stage disease***

Tumour marker	No. patients	No. samples	No. samples per patient Mean	Median	Range
CA125	194	951	4.9	4.0	1-17
$HMFG_2$	159	644	4.1	3.0	1-13
p185	125	443	3.6	3.0	1-10
TATI	80	235	2.9	2.0	1-8
CA153	27	106	3.9	4.0	1-11
CA199	27	107	3.9	4.0	1-11
CA724	13	38	2.9	3.0	1-6

The length of "marker" follow-up was calculated in months by subtracting the first sample date from the last. Overall, the mean CA125 "follow-up" time was 11.5 months (median 7.6 months and range 0-54.4 months). Patients with early stage disease had a mean CA125 "follow-up" of 14.5 months (median 11.8 months and range 0-50.7 months). Patients with advanced disease had a mean CA125 "follow-up" of 10.3 months (median 6.6 months and range 54.4 months).

Each marker result was scored true positive, true negative, false positive or false negative, see Appendix D for scoring system. The clinical settings, outlined in the first two sections in this chapter, in which the markers were evaluated, numbers permitting, will be described in the chapters 7, 8 and 9.

CHAPTER 6

Establishment of an EOC Patient Database

6.1 INTRODUCTION

A database comprising the epithelial ovarian cancer patients' case histories together with their serum tumour marker levels was developed in collaboration with Unilever Research at the Colworth Laboratory, Sharnbrook, U.K.. The database was constructed using a VT 220 IBM terminal on the URL Colworth MARS - VAX/VMS mainframe computer system, and statistical analyses performed using the SAS (Statistical Analysis System) software package. A similar, but less comprehensive system had previously been used for analysis of breast tumour markers (Robertson, 1989), also in collaboration with Unilever Research. Robertson's study set out to determine if there was a correlation between tumour marker levels and UICC response to therapy 2, 4, and 6 months after its initiation, and to construct a clinically useful prognostic index with the appropriate markers.

6.2 NECESSITY OF THE DATABASE FOR CLINICAL ANALYSIS

Epithelial ovarian cancer is a multifarious disease; patients fall into several different prognostic groups, each requiring a different treat-ment strategy. From the outset of this study blood sampling was to be performed on a regular monthly basis, particularly during chemotherapy, and subsequently during regular clinic visits. In practice, however, the timing and number of blood samples obtained from each patient varied greatly. A number of factors had an influence on the range of samples obtained from the patient population. The list below is by no means exhaustive, but illustrates several such factors inherent in this and any clinical study of a similar nature.

Factors influencing the range of samples obtained:-

1. The time between April 1984 and July 1989 that each patient was entered into the study; this determined the length of follow-up each patient had and ultimately affected analyses of progression and survival.

2. The period in the course of disease and treatment during which the patient was entered into the study; this affected the number of samples obtained at specific times. For example, patients already receiving second-line chemotherapy could obviously not be included in a multivariate analysis of markers as early prognostic factors.

3. Several clinical trials were conducted concurrently. Thus, where possible the treatment the patient received had to be taken into account in order to eliminate the effects of different treatments on marker levels.

4. Clinicians remembering to take samples at appropriate times during treatment. For example, the number of samples obtained immediately prior to second-look was poor. If surgery was deferred for some reason in a patient awaiting second-look, then such a patient often did not have a repeat sample sent for marker assay at the appropriate time. This was frequently the case and greatly reduced the possible number of samples with which to correlate marker levels with second-look outcome.

5. Samples reaching the laboratory for separation of serum within an appropriate time. Blood samples were discarded if they reached the laboratory from the wards and clinics greater than two days after being taken if they had been left at room temperature. Unfortunately this was relatively common, up to 10% of samples arrived too late.

6. When and how many of the assays were performed for each marker (as described in chapter 5, section 5.4, pp. 134-136). The commercial assays were not performed on a routine basis, except for CA125, therefore the range of samples assayed depended on which samples were available at the time of receiving the marker assay kits.

The complexities described above contributed to the necessity of developing this database to answer clinical questions retrospectively. Upon it's completion, the availability of sufficient numbers of "appropriate" tumour marker data to

analyse became clear immediately and clinical and statistical analyses were greatly facilitated.

6.3 CONSTRUCTION OF THE DATABASE

With hindsight, construction of two separate databases; one containing the case histories and the other containing the serial tumour marker levels would have been more straightforward. However, one database was developed containing patients case histories and serial tumour marker data. Consequently, information was entered on a sample basis rather than on a patient basis. This meant that for each blood sample taken from a patient, all diagnostic and other clinical details had to be entered, with the sample date as the "fixed" entry, resulting in much unavoidable duplication of clinical information in the database.

For each blood sample, the maximum number of variables or "fields" was 59. The total number of samples included in the final analysis was 1237 resulting in a maximum of 72,983 data entries, which occupied 413696 bytes (1/2 Mbyte) of computer memory. The fields were visualised within 3 computer screens. Screens 1 and 2 displayed all the clinical details pertaining to that sample date, while the remainder displayed the tumour marker levels at that date.

The fields were ordered in a logical sequence to ensure ease of data entry and ease of selection of samples or patient groups of interest to facilitate programming. Some of the entry options are self-explanatory, however the majority consist of several codes. Short codes were constructed for clinical information to reduce the amount of computer memory required. Whilst some of these are generally accepted abbreviations, others were constructed purely for the purpose of this database and are decoded later. The list overleaf shows the fields in order together with their respective entry options.

6.3.1 Order of database fields and entries

Screen 1

	Field	Data entry option
1.	Sample number	0001 - 2000
2.	Patient name	e.g. SMITH-MARY
3.	Date of birth	e.g. 01/01/33
4.	Sample date	e.g. 01/01/88
5.	Patient age	e.g. 55
6.	Cancer stage	FIGO I - IV
7.	Histopathological grade	0 - 4
8.	Cancer subtype	SPC, MUC, etc...
9.	Smoker	Y/N/U
10.	Performance status	0 - 4
11.	Ascites	Y/N/U
12.	Cytology	NEG/POS/UNK/ND
13.	Adhesions	Y/N/U
14.	Date diagnosis/surgery 1	e.g. 01/12/87
15.	Operative procedure 1	0 - 9
16.	Debulk	C/Y/P/N/B
17.	Date second-look surgery	e.g. 01/07/88
18.	Operative procedure 2	0 - 9
19.	Second-look outcome	NEG/MIC/MAC/BUL
20.	Drug therapy	Y/N
21.	Current therapy	CIS/PRED/etc...

Screen 2

	Field	Data entry option
22.	Type of therapy	T/A/P
23.	Date first given	e.g. 01/01/88
24.	Regime number	0 - 6
25.	Number of cycles	0 - 20
26.	% Completion of regime	0 - 100
27.	Reason for stopping regime	PC/TOX/etc...
28.	Clinical disease	Y/N
29.	Evaluable disease	ED/NED
30.	Response to therapy	NA/NE/CR/PR/SD/PD
31.	Date response assessed	e.g. 01/07/88
32.	Method response assessed	LRT/LSC/etc...
33.	Date of progression	e.g. 01/01/89
34.	Method progression assessed	LRT/LSC/etc...
35.	Date last seen (if alive)	e.g. 01/07/89
36.	Current status (if alive)	NE/CR/PR/SD/PD
37.	Date of death	e.g. 01/08/89
38.	Survival (months)	0 - 100
39.	CA125 censored	< or >
40.	CA125 result	0 - 500
41.	CA125 qualifier	TP/TN/FP/FN
42.	HMFG2 censored	< or >

Screen 3

	Field	Data entry option
43.	HMFG2 result	0 - 500
44.	HMFG2 qualifier	TP/TN/FP/FN
45.	CA153 censored	< or >
46.	CA153 result	0 - 500
47.	CA153 qualifier	TP/TN/FP/FN
48.	CA199 censored	< or >
49.	CA199 result	0 - 500
50.	CA199 qualifier	TP/TN/FP/FN
51.	CA724 censored	< or >
52.	CA724 result	0 - 500
53.	CA724 qualifier	TP/TN/FP/FN
54.	TATI censored	< or >
55.	TATI result	0 - 500
56.	TATI qualifier	TP/TN/FP/FN
57.	NEU censored	< or >
58.	NEU result	0 - 10000
59.	NEU qualifier	TP/TN/FP/FN

6.3.2 Information entry

Entry of marker data, unlike clinical information, presented no problems. These data were "censored", for example if a marker was undetectable it was given a value of less than the limit of detection of the assay, i.e. <5 Uml^{-1} for CA125, instead of the value zero. This resulted in data enhancement for SAS package handling.

Some of the initial diagnostic information e.g. operative procedure and histology remained constant throughout the blood sampling period. However, the majority of clinical information e.g. therapy regimes and responses changed with time, as did the marker levels. Therefore the resulting database consisted of longitudinal or continual clinical information and discrete marker data.

Thus, certain "rules" regarding entry of clinical information had to be developed in order to maximise the use of information gained from the case notes relating to the blood sampling time. This will become clearer when the rules are examined. For example, a blood sample was taken from a patient three months after completing a course of cisplatin and a routine CT scan taken two months

previously showed the patient to have a complete response. The "response" recorded with that sample date was CR if it was later confirmed after reviewing retrospectively all available clinical information.

Responses to therapy are conventionally documented upon completion of therapy using UICC response criteria (see chapter 1, table 1.2, p 27). Blood samples were very rarely taken precisely at this time, therefore to maximise the use of the marker data obtained throughout the disease course a special system for documenting "response" or disease status at all times was implemented, as described in the example above. It must be stressed that this was purely for research purposes. It was vital to have a consistent clinical base from which the markers could be evaluated. Field number 28 was also created to aid this purpose. Using all the information available the presence or absence or disease was judged in retrospect to enable a marker scoring system to be used to evaluate their sensitivity, specificity, accuracy and predictive powers.

Data printouts were painstakingly checked and double-checked for mistakes. One or two mistakes in entering marker data became apparent when simple programmes were run. For example, typing in a value of 165 Uml^{-1} for CA724 instead of 16.5 Uml^{-1} would have resulted in a gross over-estimate of mean levels. Clinical information on the other hand was less reliable and less amenable to error testing; many discrepancies in details arose from different sources. Where possible information was obtained from the case notes, which were less contradictory, rather than the sample forms which tended to be less reliable. Great care was taken to ensure consistency in clinical documentation.

6.3.3 Field information entry and applications

The list overleaf shows the decoding of the field options. Data that may change with time are marked with an asterisk. Brief rules for entry of information are given where appropriate. Finally, some general applications are indicated: selection (S), correlation (C), prognosis (P), and various (V) including combinations of the first three analyses.

Field	Entry	Decode	Use	Rules for data entry
* 1.	1000	-	-	-
2.	NAME	-	S	-
3.	DOB	-	P	-
4.	DATE	-	V	-
* 5.	AGE	-	P	Age at sampling
* 6.	I - IV	FIGO stage	P	Stage at sampling
* 7.	0	Unknown	-	-
	1	Well diff.	V	-
	2	Moderate diff.	V	-
	3	Poorly diff.	V	-
	4	Variable diff.	V	Enter if mixture of grades is unknown
8.	SPC	Serous	V	-
	MUC	Mucinous	V	-
	ENDO	Endometrioid	V	-
	CC	Clear cell	V	-
	ADENO	Adenocarcinoma	V	-
	MIX	Mixed	V	-
	UNK	Unknown	-	-
* 9.	Y/N	Yes/No	C	-
10.	0 - 4	ECOG performance status	P	Initial pre-therapy value only
11.	Y/N/U	Yes/No/Unknown	P	-
12.	NEG/POS	Negative/Positive P	-	
	UNK/ND	Unknown/Not done	-	-
13.	Y/N/U	Yes/No/Unknown	P	-
14.	DATE	-	V	-
15.	0	Biopsy only	V	-
	1	TAH	V	-
	2	BSO	V	-
	3	Omentectomy	V	-
	4	Partial oment.	V	-
	5	Infracolic oment. V	-	
	6	Oophorectomy	V	Left or right
	7	Ovariotomy	V	Bilateral or one
	8	Ovarectomy	V	Bilateral or one
	9	OtherVeg.	-	Palliative procedures
16.	C	Complete	V	No residual disease
	Y	Yes	V	< 2cm
	P	Partial	V	2 - 5 cm
	N	No	V	> 5cm
	B	Biopsy only	V	Gross disease left
* 17.	DATE	-	V	-
* 18.	As 16.			
* 19.	NEG	Negative	V	-
	MIC	Microscopic	V	-
	MAC	Macroscopic	V	-
	BUL	Bulky disease	V	-
* 20.	Y/N	Yes/No	S	Y - if patient is on Rx, or has had it in the past, N - if patient has never had Rx
* 21.	NIL	Not on Rx	S	Patient may be pre or post regime
	CIS	Cisplatin	V	-
	CARBO	Carboplatin	V	-
	PRED	Prednimustine	V	-
	CHLOR	Chlorambucil	V	-
	AIFN	a-Interferon	V	-
	MEL	Melphalan	V	-
	MITOX	Mitoxantrone	V	-
	MITOZ	Mitozolamide	V	-
	IFOS	Ifosfamide	V	-

Field	Entry	Decode	Use	Rules for data entry
* 21.	BLEO	Bleomycin	V	-
	ADRIA	Adriamycin	V	-
	MC	Mitomycin C	V	-
	CYCLO	Cyclophosphamide	V	-
	BIANT	Biantrazole	V	-
	5U/P/-H/P	5-Fluorouracil/ Cisplatin/Hexa-methylmelamine Prednimustine	V	-
* 22.	T	Therapeutic	S	-
	A	Adjuvant	S	-
	P	Palliative	S	-
* 23.	DATE	-	V	Date of first cycle of most recent regime
* 24.	0 - 6	-	V	Number of regimes patient has had including the current
* 25.	0 - 20	-	P	Number of cycles of current Rx given by that sample date
* 26.	0 - 100	-	V	-
* 27.	PC	Protocol complete	S	-
	TOX	Toxicity	S	-
	PD	Progression	S	-
	REF	Patient refusal	S	-
* 28.	Y/N	Yes/No	V	Disease presence or absence judged in retrospect using all available information
* 29.	ED/NED	Evaluable/ non-evaluable disease	V	Clinically, radiologically, or surgically
* 30.	NA	Not applicable	V	Patients on adjuvant Rx
	NE	Not evaluable	V	No measurable disease
	CR	Complete response	V	-
	PR	Partial response	V	-
	SD	Stable disease	V	-
	PD	Progression	V	-
* 31.	DATE	-	V	Date of most recent assessment, + or - one month
* 32.	CLIN	Clinical	V	-
	LRT	Laparotomy	V	-
	LSC	Laparoscopy	V	-
	XRAY	X-Ray	V	-
	US	Ultrasound scan	V	-
	CT	Computed tomography	V	-
	BIOC	Biochemical	V	-
	CA125	CA125 assay	V	-
* 33.	DATE	-	V	Date of most recent assessment
* 34.	As 32.	-	P	-
35.	DATE	-	P	-
36.	AS 30.	-	P	-
37.	DATE	-	P	-
38.	0 - 100	Months survival since diagnosis	P	-

6.4 STATISTICAL ANALYSIS

The database was used initially to collate the information on the patient populations demographics, their management, and the results of treatment, presented in chapter 5. Once the database was established, the ability to answer retrospectively certain clinical questions was greatly facilitated. Longitudinal plots of serial marker levels were constructed for all patients to help identify which patients would provide information regarding various clinical correlations. In addition, they helped to define exclusion criteria necessary to develop or modify existing programmes.

Programmes were written using the SAS software package to perform the appropriate statistical analysis. As marker data show a positively skewed and not a Gaussian distribution, non-parametric statistical tests were commonly employed. Tumour marker data were first standardised by logarithmic conversion (log10) before performing many of the following statistical tests.

The F test (one way analysis of variance) was used to determine the correlation between post-operative residual disease and marker levels in samples assayed 1-4 weeks after primary surgery (chapter 8, section 8.2, pp. 181-184). The Kruskal Wallis test was used to determine the correlation with overall response to chemotherapy (chapter 8, section 8.3, pp. 185-197). The difference between marker response in complete and partial responders was determined by the Mann Whitney test (chapter 8, section 8.3, pp. 185-197). The difference between pre-treatment and post-treatment marker levels in patients who received first-line chemotherapy was determined by the Wilcoxon Signed Rank test (chapter 8, section 8.3, pp. 185-197). The sensitivity, specificity, accuracy and predictive values for pre-second-look marker data (chapter 8, section 8.4, pp. 198-206) were calculated using the formulae given in chapter 3, section 3.4.7, pp. 68-71.

The SAS Lifetest procedure (SAS/STAT Users Guide, 1989) using the chi-squared statistic for the Wilcoxon test was used to perform both univariate and multivariate analyses of prognostic factors (chapter 9, section 9.2, pp. 220-225). Cox's proportional hazard model was not available on SAS. Kaplan Meier progression free survival and overall survival curves were constructed (chapter 9, section 9.3, pp. 225-233), and differences between progression free survival and survival curves were determined using the Log Rank test (Peto *et al.*, 1977).

RESULTS AND DISCUSSION

CHAPTER 7

EOC Tumour Marker Elevations

7.1 INTRODUCTION

The ability of serum tumour markers to discriminate between the presence and absence of viable malignant cells, far less determine the tumour volume, depends not only on the marker, the type of malignancy and the assay conditions, but also on the method used to assign a cut-off value above which marker levels are considered "abnormal". The choice of cut-off values for the marker assays in this study were described in chapter 4, table 4.9, p 114.

All studies have found significantly elevated marker levels in patients with advanced disease, irrespective of marker or tumour type. Kabawat *et al.* (1983b) first described the association of CA125 with serous, endometrioid, clear cell, mixed, and undifferentiated epithelial ovarian malignancies, but not mucinous tumours, using immunohistochemistry. Subsequent studies found elevated serum levels in patients with mucinous EOC, although levels were generally lower and were raised in fewer patients than those with non-mucinous tumours (for reviews see Kenemans *et al.*, 1988, and Jacobs and Bast, 1989). Each of the markers described in this thesis has been found raised in serum from patients with all histological types of ovarian cancer, and each has been advocated for a particular histological type(s) - commonly mucinous or non-mucinous (see chapter 2). There are few studies in the literature describing the association between levels of serum CA125, or other markers, and tumour grade. This chapter therefore examines the distribution of elevated levels of CA125, CA153, CA199, CA724, TATI, $HMFG_2$, and p185 in patients with different disease stages, histological types and tumour grades.

No single tumour marker has 100% sensitivity and specificity for any particular stage or histological variety of EOC. This is due to the heterogeneity of antigen expression in epithelial ovarian tumours, demonstrated in numerous immuno-histochemical and serological studies (Bast *et al.*, 1990, Esteban and Battifora, 1990, Welch *et al.*, 1990). The performances of each tumour marker assay, in terms of overall sensitivity and specificity for EOC, are compared using Receiver Operating Characteristic (ROC) curves (Makuch and Muenz, 1987).

ROC curves were also used to compare the general performance of each marker in patients with different tumour types (Hanley and McNeil, 1982).

Tumour antigen heterogeneity presents problems not only in diagnosis and monitoring, but also in imaging and therapy with monoclonal antibodies. The ideal "panel" or combination of markers has not yet been identified for any of the above applications although many have been evaluated (Einhorn *et al.*, 1989, Bast *et al.*, 1990, Lahousen *et al.*, 1990). The value of each marker assayed in this thesis was therefore determined in addition to the universally accepted best single serum marker, CA125.

7.2 EOC TUMOUR MARKER ELEVATIONS

Every sample collected was assayed for CA125, whereas fewer assays were performed for the other markers for reasons given in chapter 5, section 5.4, p134. The numbers and percentages of patients with each disease stage who had at least one positive marker result are shown in table 7.1.

As stated earlier, the cut-off values employed for CA125, CA153, CA199, CA724, TATI, $HMFG_2$ and p185 were 35 Uml^{-1}, 30 Uml^{-1}, 33 Uml^{-1}, 5 Uml^{-1}, 20 μgl^{-1}, 40 Uml^{-1} and 1900 Uml^{-1} respectively. Overall, in descending order; CA125, $HMFG_2$, CA153, TATI, CA724, CA199 and p185 were elevated in 211/261 (80.8%), 134/221 (60.6%), 21/46 (45.6%), 50/117 (42.7%), 9/22 (40.9%), 15/46 (32.6%) and 21/179 (11.7%) patients respectively (table 7.1). There is a discrepancy in the number of patients; several patients were re-staged after disease progression and had markers assayed during both stages of their disease, these patients were counted twice. CA125 was elevated in a greater proportion of patients with all FIGO stages than any of the other markers. Apart from CA125, $HMFG_2$ was elevated in a greater proportion of patients with all disease stages than any of the other markers.

Table 7.1 ***Proportion of patients with elevated marker levels in each stage***

Tumour marker	FIGO Stage I	II	III	IV	Total
CA125	19 / 38 (50.0%)	16 / 25 (64.0%)	126 / 147 (85.7%)	50 / 51 (98.0%)	211 / 261 (80.8%)
CA153	2 / 9 (22.2%)	3 / 9 (33.3%)	11 / 18 (61.1%)	5 / 10 (50.0%)	21 / 46 (45.6%)
CA199	3 / 9 (33.3%)	3 / 9 (33.3%)	5 / 18 (27.8%)	4 / 10 (40.0%)	15 / 46 (32.6%)
CA724	1 / 4 (25.0%)	2 / 4 (50.0%)	2 / 8 (25.0%)	4 / 6 (66.7%)	9 / 22 (40.9%)
TATI	4 / 22 (18.2%)	8 / 17 (47.0%)	25 / 57 (43.8%)	13 / 21 (61.9%)	50 / 117 (42.7%)
$HMFG_2$	18 / 38 (47.0%)	13 / 24 (54.2%)	71 / 115 (61.7%)	32 / 44 (72.7%)	134 / 221 (60.6%)
p185	1 / 33 (3.3%)	1 / 19 (5.3%)	12 / 88 (13.6%)	7 / 39 (17.9%)	21 / 179 (11.7%)

Figures 7.1 to 7.7 show the distribution of marker levels in patients with each disease stage. The numbers of patients with elevated levels correspond to those given in table 7.1. As no pre-operative samples were available, figures 7.1 to 7.7 were constructed using the highest level of each marker from each series of samples assayed per patient. The first, last, middle, highest, lowest or a random value could have been used for this purpose. However, in the absence of pre-operative samples, the highest value was chosen to represent the number of patients with elevated levels (table 7.1); this measurement also gave the closest approximation to marker sensitivity for each disease stage.

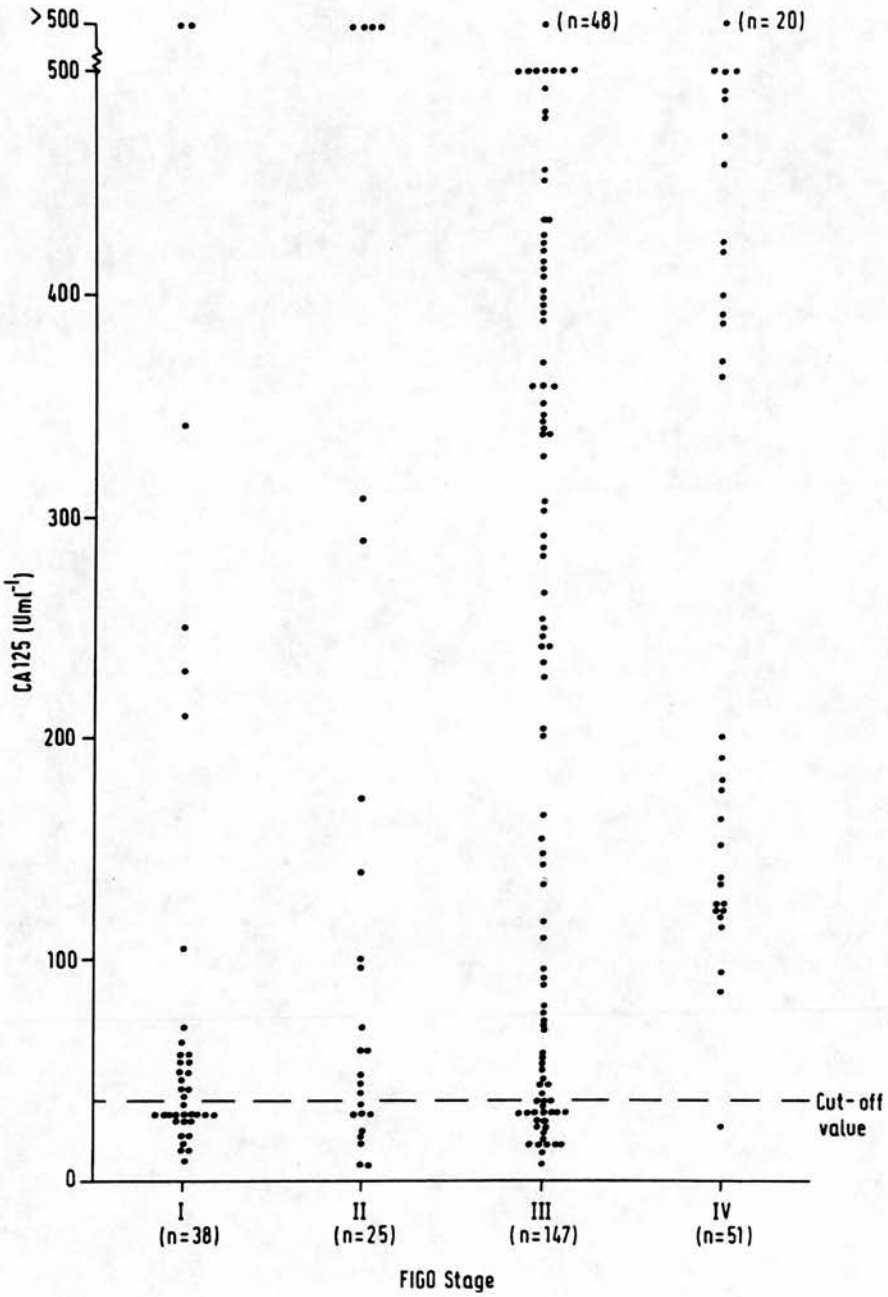

Figure 7.1 ***CA125 levels; distribution according to FIGO stage***

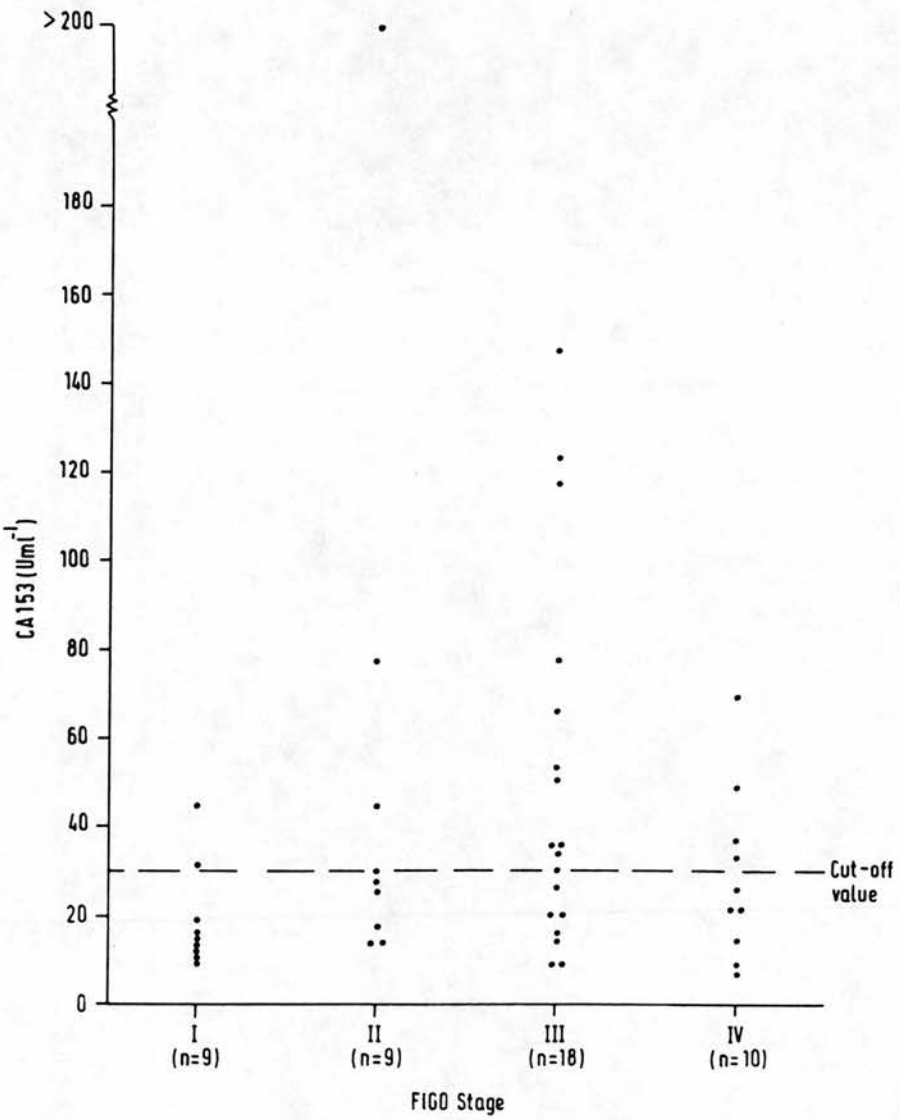

Figure 7.2 ***CA153 levels; distribution according to FIGO stage***

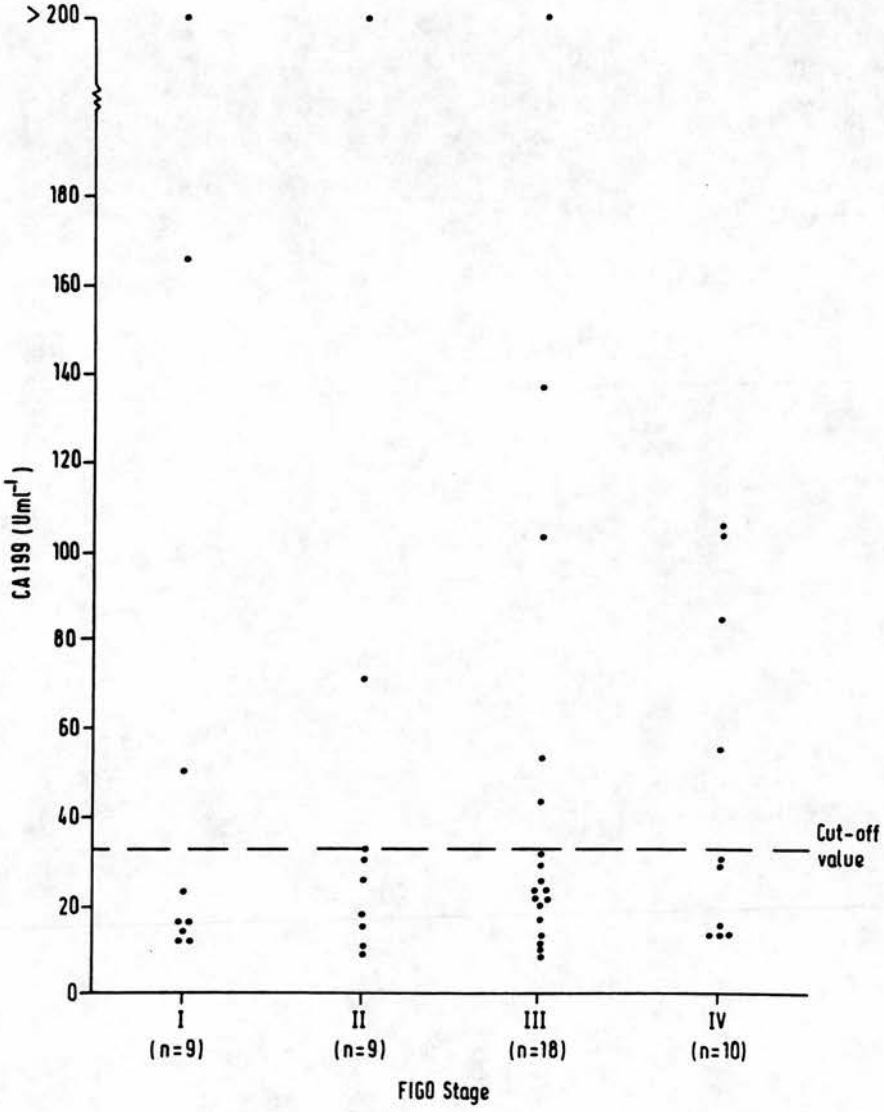

Figure 7.3 ***CA199 levels; distribution according to FIGO stage***

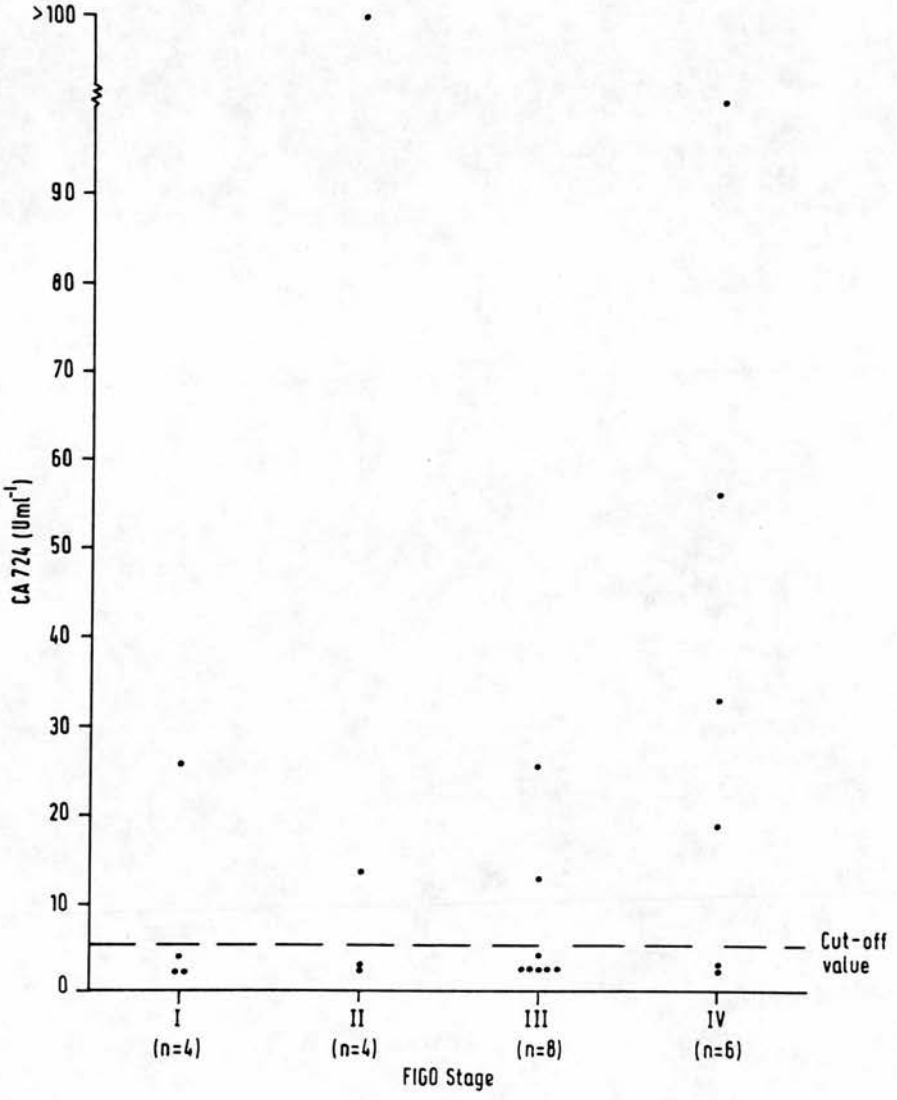

Figure 7.4 ***CA724 levels; distribution according to FIGO stage***

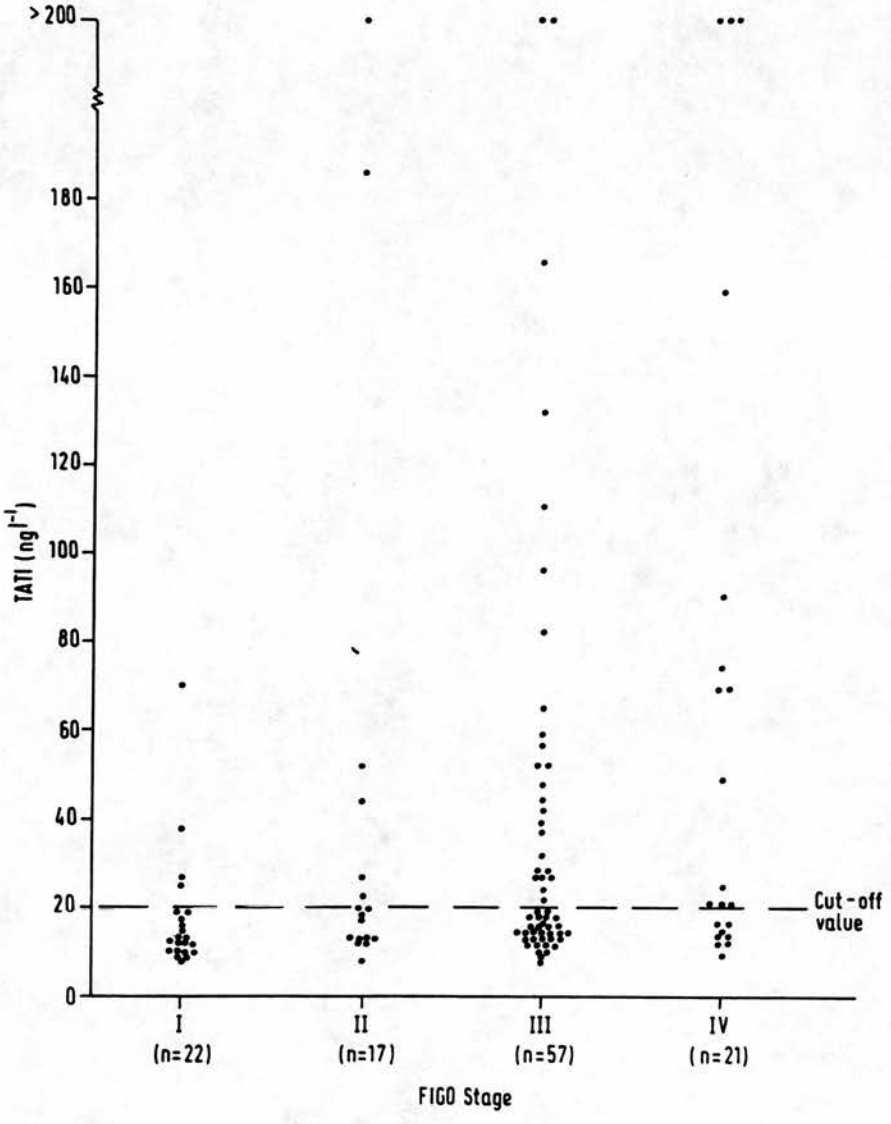

Figure 7.5 ***TATI levels; distribution according to FIGO stage***

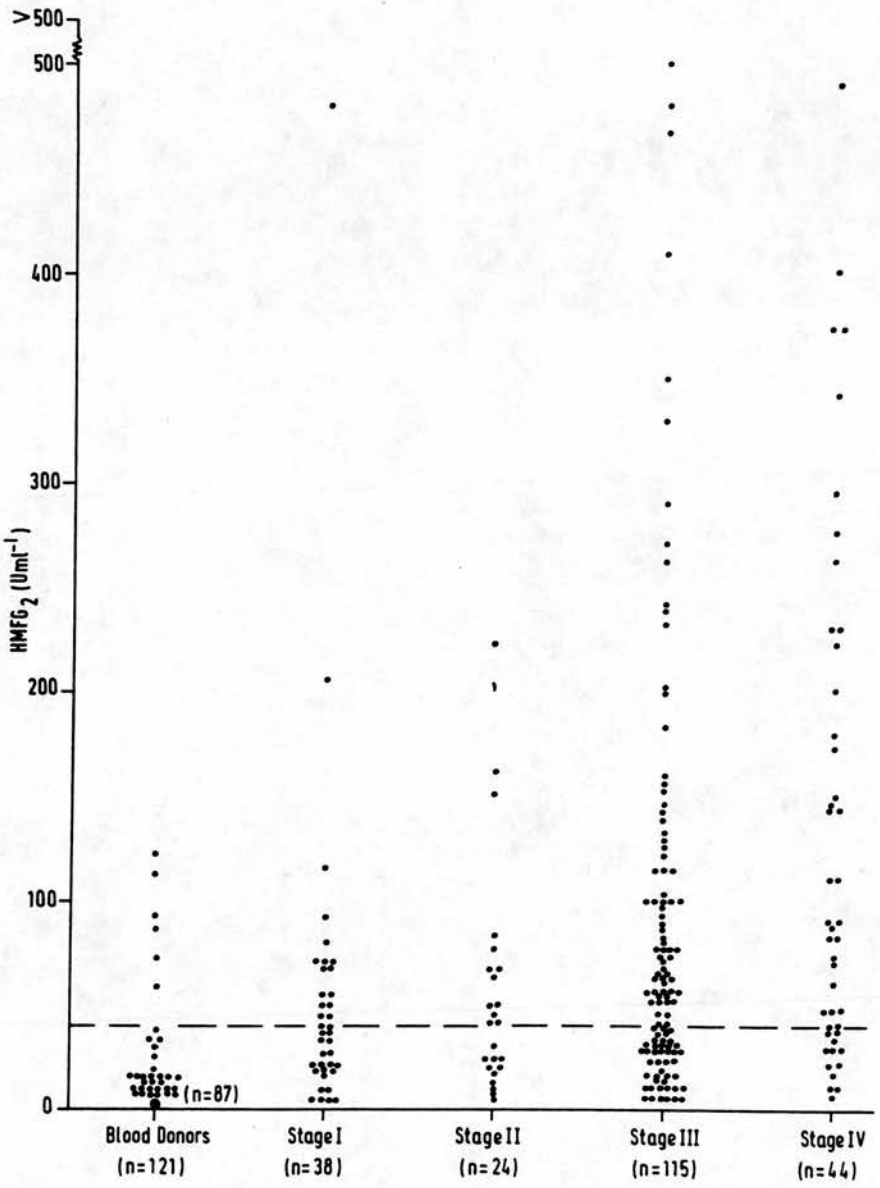

Figure 7.6 ***HMFG$_2$ levels; distribution according to FIGO stage***

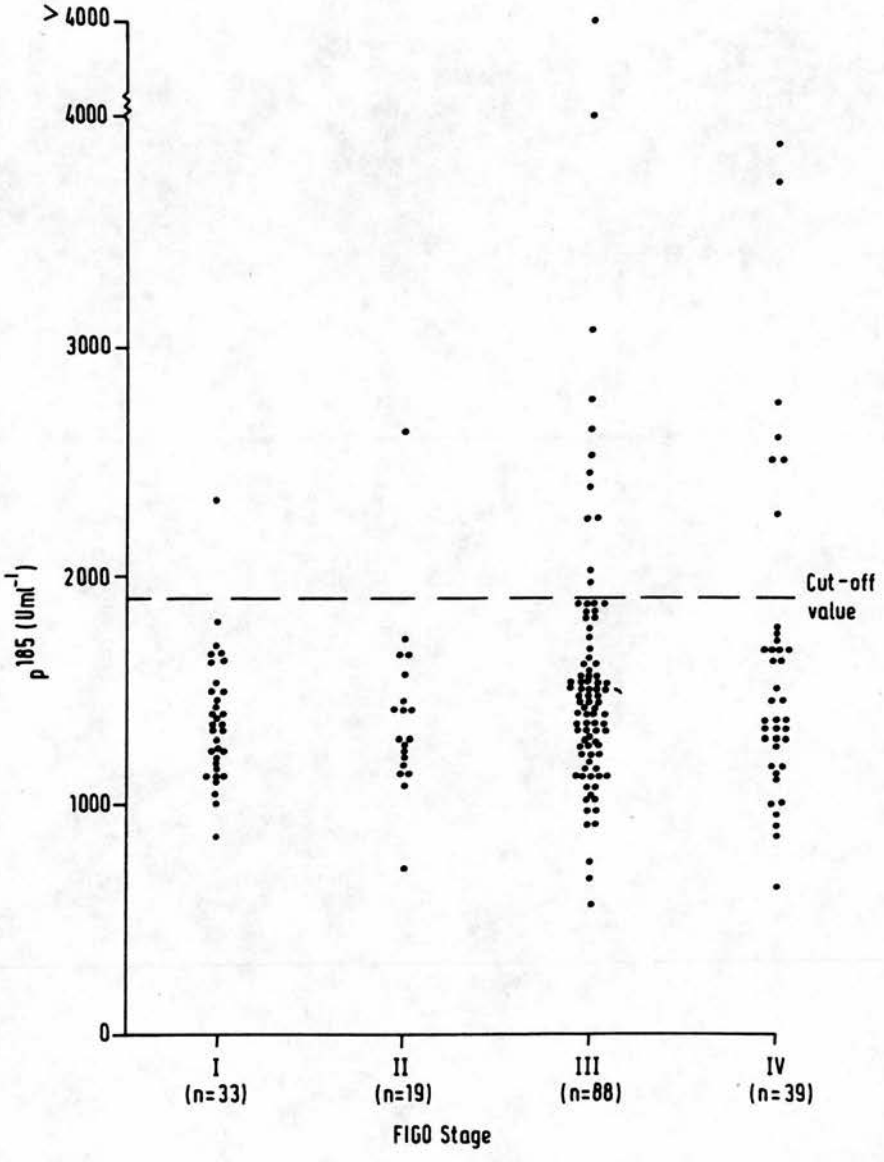

Figure 7.7 ***p185 levels; distribution according to FIGO stage***

The numbers and percentages of patients with each histological type who had at least one elevated level of each marker are shown in table 7.2.

Table 7.2 ***Proportion of patients with elevated marker levels in each histological type***

Tumour marker	Histological type Serous	*PDA	Endometrioid	Clear cell	Mucinous
CA125	127 / 151 (84.1%)	17 / 18 (94.4%)	35 / 46 (76.0%)	11 / 17 (64.7%)	15 / 23 (65.2%)
CA153	12 / 20 (60.0%)	1 / 1 (100%)	6 / 16 (37.5%)	1 / 1 (100%)	0 / 7 (0%)
CA199	5 / 20 (25.0%)	1 / 1 (100%)	7 / 16 (43.8%)	0 / 1 (0%)	2 / 7 (28.6%)
CA724	3 / 9 (33.3%)	1 / 1 (100%)	3 / 8 (37.5%)	0 / 1 (0%)	2 / 3 (66.7%)
TATI	31 / 65 (47.7%)	3 / 4 (75.0%)	8 / 22 (36.4%)	4 / 7 (57.1%)	3 / 17 (17.4%)
$HMFG_2$	86 / 126 (68.3%)	9 / 11 (81.8%)	21 / 42 (50.0%)	9 / 15 (60.0%)	7 / 22 (31.8%)
p185	13 / 102 (12.7%)	3 / 7 (42.8%)	3 / 31 (9.7%)	1 / 15 (6.7%)	1 / 19 (5.3%)

* Poorly differentiated adenocarcinoma

Ignoring those tumour types where only one patient had a particular marker assay, CA125 was elevated in a higher proportion of patients with all histological types than all the other markers, except for CA724 which was elevated in a slightly greater percentage of patients with mucinous tumours, although the number of patients was small (table 7.2). Apart from CA125, $HMFG_2$ was elevated in a greater proportion of patients with all histological types than any of the other markers. In general, fewer patients with mucinous

tumours had elevated markers than those with non-mucinous tumours, except for CA724.

The numbers and percentages of patients with well differentiated, moderately differentiated and poorly differentiated tumours who had elevated levels of at least one marker are shown in table 7.3.

Table 7.3 ***Proportion of patients with elevated marker levels with tumour grade***

Tumour marker	Tumour Differentiation		
	Well	Moderate	Poor
CA125	26 / 36 (72.2%)	43 / 60 (71.7%)	119 / 134 (88.8%)
CA153	1 / 7 (14.2%)	6 / 14 (42.8%)	12 / 18 (66.7%)
CA199	2 / 7 (28.6%)	5 / 14 (35.7%)	5 / 18 (27.8%)
CA724	2 / 4 (50.0%)	4 / 9 (44.4%)	3 / 8 (37.5%)
TATI	5 / 18 (27.8%)	13 / 30 (43.3%)	27 / 72 (37.5%)
$HMFG_2$	16 / 32 (50.0%)	31 / 51 (60.8%)	77 / 112 (68.8%)
p185	2 / 26 (7.7%)	2 / 39 (5.1%)	18 / 87 (20.6%)

The percentages of patients with elevated marker levels with well, moderately and poorly differentiated tumours shown in table 7.3 is displayed in figure 7.8. The percentage of patients with serum marker elevations increased with tumour grade for CA125 (although there was no difference between those with well and moderately differentiated tumours), CA153 and $HMFG_2$. The opposite trend was observed for CA724. Serum expression of p185 was highest in patients with poorly differentiated tumours, whereas serum expression of both CA199 and TATI were highest in those with moderately differentiated tumours.

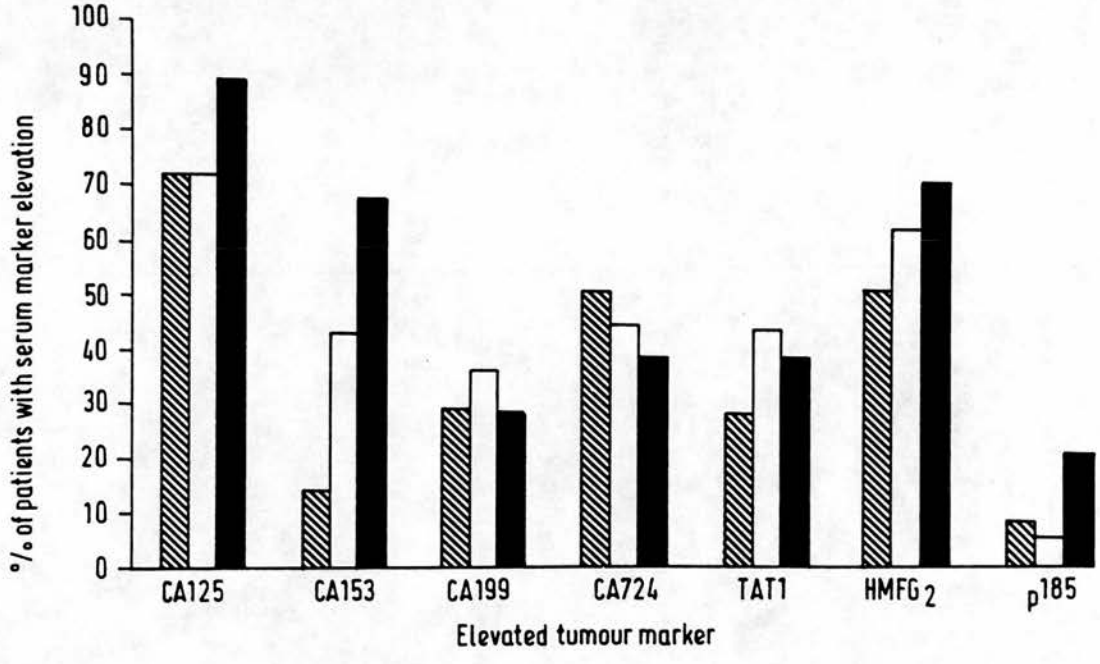

Figure 7.8 ***Association of serum marker elevation with tumour grade.*** The percentage of patients with elevated levels of each serum tumour marker investigated in this thesis with well differentiated (▧), moderately differentiated(□) and poorly differentiated (■) tumours is shown.

7.3 MARKER SENSITIVITY, SPECIFICITY, ACCURACY AND PREDICTIVE VALUES

The formulae given in chapter 3, section 3.4.7, pp. 68-69, can be used to determine the sensitivity, specificity, accuracy, and predictive values of a positive and negative marker result. These parameters are usually calculated pre-operatively to determine if a particular marker can distinguish benign from malignant disease or indicate the stage or extent of disease. A general calculation of these parameters is clinically meaningless. They were however determined using samples assayed for CA125, $HMFG_2$ and TATI prior to second-look operation to ascertain the value of each test in predicting disease presence, see chapter 8, table 8.14, p 205.

7.4 RECEIVER OPERATING CHARACTERISTIC (ROC) CURVES

The relationship between test sensitivity and specificity, as the proportion of false negative and false positive results vary inversely with cut-off value, can be simply shown in a receiver operating characteristic (ROC) curve (Makuch and Muenz, 1987). ROC curves are an excellent way of graphically illustrating the performance of a test; sensitivity or true positive rate (y-axis), is plotted as a function of 1-specificity or false positive rate (x-axis). As the cutoff value increases, the closer the curve lies to the y-axis the better, i.e. the more sensitive and specific, the test. Increasing the cut-off value to maximise specificity reduces sensitivity and vice-versa. A cut-off value is usually chosen to give the best trade off between the two, as described in section 3.4.7, p 70. The choice of cut-off value depends on the stringency of test requirements; for example, in the case of a disease as serious as cancer, where cure is often unobtainable, it is desirable to maximise the specificity of a diagnostic test (Galen and Gambino, 1975).

7.4.1 ROC curve comparison of overall sensitivity and specificity of each tumour marker assay

ROC curves were constructed for each tumour marker using all samples scored retrospectively as true positive, true negative, false positive or false negative (see Appendix D for scoring system). Figure 7.9 compares computer (SAS) generated ROC curves for each marker. Figure 7.9 clearly shows that CA125 is superior to all the other markers in the evaluation of EOC.

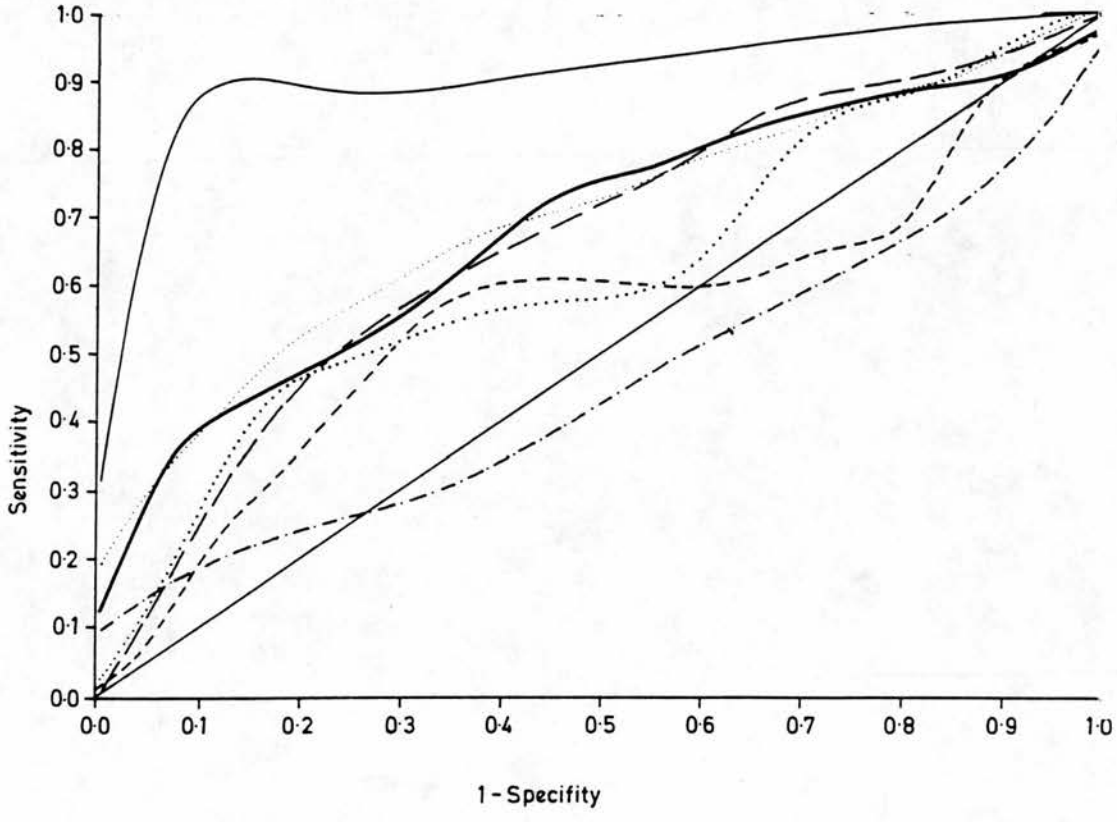

Figure 7.9 ***ROC curve comparison of markers.*** Computer generated ROC curves for CA125 (____), CA153 (____), CA199 (.....), CA724 (------), TATI (.....), $HMFG_2$ (- - -), and p185 (- . - . -) are compared.

7.4.2 ROC curve comparisons of assay performance for different histological tumour types

The most common quantitative index describing the ROC curve is the area under it. Areas may be compared using the Wilcoxon rank test to determine if there are any significant differences in sensitivity and specificity between different tests (Hanley and McNeil, 1982). These comparisons were made between different histological types for each marker. Differences were designated significant only if $p<0.05$. CA125 was a significantly more sensitive and specific marker for poorly differentiated adenocarcinomas (PDA) than either serous or endometrioid tumours. There were no significant differences between any other tumour types. CA153 was a significantly better marker for PDA than serous, endometrioid and mucinous tumours. CA153 was also a significantly better marker for either serous or endometrioid than mucinous tumours. No other differences were observed. CA199 was a significantly better marker for PDA than serous, endometrioid and mucinous tumours. CA199 was, however, a significantly better marker for mucinous than either serous or endometrioid tumours. CA724 was also a significantly better marker for mucinous than both serous and endometrioid tumours. No other differences were observed. TATI was a significantly better marker for PDA than serous, endometrioid and mucinous tumours. TATI was significantly poorer marker for serous than either endometrioid or clear cell tumours, and a significantly poorer marker for mucinous than of clear cell tumours. There were no significant differences between any histological types for $HMFG_2$ or p185, although $HMFG_2$ was least sensitive and specific for mucinous tumours and most sensitive and specific for PDA.

7.5 TUMOUR MARKER COMBINATIONS OR "PANELS"

The value of each marker in addition to CA125 was investigated in two ways. A stepwise discriminant analysis was performed to determine whether any marker could improve the ability of CA125 to discriminate simply between disease activity and inactivity, ignoring pre-determined cut-off values. Secondly,

assuming positive CA125 results to be correct, each of the remaining markers was evaluated in a series of CA125-negative samples. These analyses were not performed for p185 because it was elevated in only 11.7% of patients (table 7.1), the vast majority of whom had markedly raised CA125 levels.

7.5.1 Stepwise discriminant analysis

The number of samples that were assayed for CA125 and each of the remaining markers differed. The number of positive samples, i.e. samples taken from patients who either had evaluable disease or were judged retrospectively to have active disease at the time of assay, out of the total number of samples assayed for each marker were: 108/184 (58.7%) for CA153; 108/185 (58.4%) for CA199; 47/69 (68.1%) for CA724; 199/357 (59.1%) for TATI; and 505/810 (62.3%) for $HMFG_2$. Therefore the active disease prevalences in populations assayed for each marker were similar.

The correlations between CA125 and the other markers in the stepwise discriminant analysis were as follows: CA153 ($n=184, r=0.455, p>0.05$), CA199 ($n=185, r=0.197, p>0.05$), CA724 ($n=69, r=0.403, p>0.05$), TATI ($n=357, r=0.029, p<0.0001$) and $HMFG_2$ ($n=810, r=0.183, p<0.005$). Therefore, CA153, CA199 and CA724 did not add significantly to the discriminatory capacity of CA125, while TATI ($p<0.0001$) and $HMFG_2$ (0.005) did add significantly to CA125.

7.5.2 Series assay of markers in addition to CA125

Marker assays may be performed in a parallel or series manner (Galen and Gambino, 1975). Samples assayed in parallel are simultaneously assayed for each marker. To reduce the overall cost and labour intensity, samples may be assayed in series. The most specific marker is assayed first and subsequent assays are performed on samples which give negative results with this test. In this instance, CA125 is also the most sensitive test compared to any of the other markers, though this may not always be the case. The extent to which

any of the markers in this study improved sensitivity, specificity, and predictive values when assayed in CA125 negative samples compared to CA125 assay alone is shown in table 7.5.

Table 7.5 ***Series analysis of each marker in addition to CA125***

	% change with additional assay of:				
	CA153	CA199	CA724	TATI	$HMFG_2$
No.samples	94	95	30	173	329
Sensitivity	↑ 0.5	↑ 0.6	↑ 0.1	↑ 3.0	↑ 2.9
Specificity	↓ 3.4	↓ 6.7	0	↓ 11.8	↓ 17.3
Accuracy	↓ 0.1	↓ 0.3	↓ 0.7	↓ 0.3	↓ 3.0
PVP	↓ 0.4	↓ 0.8	0	↓ 2.3	↓ 6.5
PVN	↑ 1.1	↑ 0.7	↑ 4.8	↑ 7.8	↑ 3.5

Different populations were assayed for each marker, hence direct comparisons cannot be drawn between markers. Sensitivity, and concomitantly PVN, are increased with the additional measurement of all markers compared to CA125 alone. CA153, CA199, and CA724 each improve sensitivity by < 1.0%, while TATI and $HMFG_2$ improve sensitivity by 3.0% and 2.9% respectively. While sensitivity increases marginally, specificity is more markedly reduced resulting an overall decrease in accuracy. TATI and $HMFG_2$ reduced specificity overall by 11.8% and 17.3% respectively.

To ascertain which patients might benefit from additional marker assay to CA125, the changes in sensitivity and specificity resulting from TATI and $HMFG_2$ assay compared to CA125 assay alone were examined in more detail. Patients were given a negative score if CA125 and either TATI or $HMFG_2$ were both negative and a positive score if one or both markers were elevated. A true negative score with CA125 alone became a false positive score in patients with either false positive TATI or $HMFG_2$ levels. A false negative score with CA125 alone became a true positive score in patients with either true positive TATI or

$HMFG_2$ levels. The number of patients whose scores were converted from true negative to false positive and false negative to true positive after series TATI and $HMFG_2$ assay are shown in table 7.6.

Table 7.6 ***Series addition of TATI and $HMFG_2$ to CA125***

Disease characteristics		No. patients with change in marker score: TATI FN → TP	TATI TN → FP	$HMFG_2$ FN → TP	$HMFG_2$ TN → FP
Stage	I	1	2	2	7
	II	1	5	2	5
	III	5	3	6	11
	IV	5	0	7	1
Type	Serous	10	8	14	16
	Adenocarcinoma	0	0	0	2
	Endometrioid	1	1	3	4
	Clear cell	0	0	1	1
	Mucinous	1	0	0	1
	Mixed	0	1	-	-
Grade	WD	1	1	1	4
	MD	2	6	1	8
	PD	8	3	12	8
Total		12	10	17	24

The number of patients whose marker scores were converted from false negative to true positive outweigh the number of patients whose marker scores changed from true negative to false positive when TATI and $HMFG_2$ assays were performed in series with CA125 only in those with advanced poorly differentiated serous tumours.

In addition, 5/12 (41.6%) patients with true positive results using a combination of CA125 and TATI had clinically evaluable disease at the time of marker assay.

7/17 (41.2%) patients with true positive results using a combination of CA125 and $HMFG_2$ had clinically evaluable disease at the time of marker assay.

7.6 DISCUSSION

CA125 was elevated overall in 81% of patients in agreement with values found in the literature (Jacobs and Bast, 1989). CA125 was elevated in a higher proportion of patients with all disease stages (table 7.1) and histological tumour types (table 7.2) than all the other markers assayed in this thesis, except for CA724 which was elevated in a slightly higher percentage of patients with mucinous tumours (although the number of patients was small). Apart from CA125, $HMFG_2$ was elevated in a greater proportion of patients with all stages (table 7.1) and tumour types (table 7.2) than all other markers.

Ideally, the sensitivity of each marker for a particular stage or type of EOC should be determined by assaying pre-operative samples. As these were unavailable, the highest level obtained in serial samples from each patient was chosen to approximate the number of patients who expressed each marker. Using this approach, the "sensitivity" of CA125 for stages I, II, III, and IV disease was 50%, 64%, 86%, and 98% respectively (table 7.1). Kenemans (1991), in a review of the CA125 literature dating from 1983 to 1990, reported the cumulative sensitivity of CA125 from 16 studies; 81/187 (43%) patients with stage I, 58/68 (85%) patients with stage II, 430/463 (93%) patients with stage III, and 141/145 (97%) patients with stage IV disease had elevated pre-operative CA125 levels. These figures agree with the results obtained in patients with stages I and IV disease in this thesis. However, the "sensitivities" of CA125 for stages II and III were underestimated, probably due to serological monitoring of a minority of patients during remission, highlighting the limitations of this approach to approximating sensitivity.

$HMFG_2$ was elevated in a similar proportion of patients with stage I disease to CA125, but was elevated in approximately 20% fewer patients with stages II-IV disease (table 7.1). The cut-off values used in this thesis were established by the original investigators (chapter 4, table 4.9, p 114), except for $HMFG_2$ which was determined empirically. 5% of normal individuals had elevated $HMFG_2$ levels using the 40 Uml^{-1} cut-off value (figure 7.6). Using their own cut-off value,

Ward and Cruickshank (1987b) reported a false positive rate of 8% in samples from normal individuals, while Burchell *et al.* (1984) originally found a false positive rate of 17%. Ward and Cruickshank (1987b) found elevated pre-operative levels of $HMFG_2$ in 3/9 (33%) patients with stage I disease and 18/29 (62%) patients with stages II to IV disease (they did not distinguish between different stages in patients with advanced disease). Different cut-off values were not employed in this thesis, as the value chosen appeared to be optimal.

CA153, CA199, CA724, TATI and p185 were elevated overall in 46%, 33%, 41%, 43%, and 12% of patients in this study (table 7.1). All of these markers had very poor "sensitivity" for early stage disease. Scambia *et al.* (1988) found elevated pre-operative CA153 levels in 3/11 (27%) patients with stage I disease, similar to the findings in this study (table 7.1). However, the sensitivity for advanced disease was higher; 27/34 (79%) stage III and 11/13 (85%) stage IV patients had elevated pre-operative CA153 (Scambia *et al.*, 1988). CA199 has poorer sensitivity than CA153, Canney *et al.* (1985) found pre-operative CA199 elevated in 29% of patients while Bast *et al.* (1984) found elevated CA199 in only 17% of patients. Recently, Scambia *et al.* (1990) found elevated pre-operative levels of CA724 in 10/15 (67%) patients with stages I and II disease, 11/17 (65%) stage III and 8/12 (67%) stage IV patients. The sensitivity for stage I disease was not reported in this study (Scambia *et al.*, 1990). Kobayashi *et al.* (1989) however reported pre-operative elevation of CA724 in 8.3% of patients with stage I, 25% with stage II, 48% with stage III and 85.7% with stage IV disease. Whilst Scambia *et al.* (1990) used a cut-off value of 7 Uml^{-1}, higher than the value employed in this study, Kobayashi *et al.* (1989) employed a more generous cut-off value of 3.9 Uml^{-1}. Both of these studies reported a higher overall frequency of CA724 elevation than found in this thesis. This is most likely to have resulted from a bias towards assay of patients with negative CA125 levels to determine if the sensitivity could be increased with use of a panel of markers including CA724. This holds true for all of the commercially available markers assayed in small numbers in this thesis. Halila *et al.* (1988) found pre-operative TATI elevated overall in 12/45 (27%) patients, fewer than in this study. The sensitivity of serum p185 for EOC has not been reported to

date, but less than 5% of patients with stages I and II had elevated levels in this study.

In general, tumour markers are elevated in fewer patients with early stage disease, seriously limiting their value in screening for ovarian cancer alone (Roulston, 1990). No tumour marker to date has a sensitivity of greater than 50% for stage I EOC; furthermore, the sensitivity for "true" early disease - stage Ia - is unknown.

CA125 was elevated in the serum of patients with all histological tumour types; fewer patients with mucinous and clear cell tumours had elevated levels than those with serous, endometrioid and poorly differentiated adenocarcinoma (table 7.2). Kenemans (1991), collated data from 21 papers and reported elevated pre-treatment CA125 levels in; 560/597 (94%) patients with serous tumours, 91/97 (94%) patients with undifferentiated tumours, 74/85 (87%) patients with endometrioid tumours, 20/26 (77%) patients with clear cell tumours, 70/119 (59%) patients with mucinous tumours, 10/10 (100%) patients with mixed tumours, and 20/21 (95%) patients with unclassified tumours. The cumulative literature values are slightly higher than the results obtained in this study for reasons given previously. However, there is close agreement regarding the subtypes of tumours that most frequently overexpress CA125.

$HMFG_2$ was most frequently elevated in patients with in descending order poorly differentiated adenocarcinomas, serous, clear cell, endometrioid, and mucinous tumours (table 7.2). There are no data in the literature to compare serum elevation of $HMFG_2$ in patients with different histological types. P185 was also most frequently elevated in patients with poorly differentiated adenocarcinoma, and to a lesser degree in patients with the other subtypes. In descending order, TATI was most frequently elevated in patients with PDA, clear cell, serous, endometrioid and mucinous tumours. TATI has been advocated as a marker of mucinous EOC (Halila *et al.*, 1988, Mogensen *et al.*, 1990), but fewer patients in this study with mucinous tumours had elevated levels than those with non-mucinous tumours. CA153, CA199 and CA724 were

all elevated in the same patient with poorly differentiated adenocarcinoma. There were little data to draw conclusions about the proportions of patients with other tumour types, however CA724 was elevated in a higher proportion of patients with mucinous tumours. Scambia *et al.* (1988) found elevated pre-operative CA153 in 23/30 (77%) patients with serous, 5/10 (50%) with mucinous, 11/14 (78%) with endometrioid, and 2/3 (67%) with undifferentiated tumour types. The range of values was lowest in patients with mucinous tumours. Kobayashi *et al.* (1989) found elevated levels of TAG 72 (CA724) in 39% patients with serous, 52% with mucinous, 44% with endometrioid and 33% with clear cell tumours. The authors advocated CA724 as a marker of mucinous EOC on this basis.

The association between tumour grade and serum marker elevation is not well documented for CA125, or for the other markers. A few studies have reported increasing pre-treatment CA125 levels with decreasing degree of differentiation (Brioschi *et al.*, 1987, Schwartz *et al.*, 1987, Vergote *et al.*, 1987, Zanaboni *et al.*, 1987a), although the correlation was not always statistically significant (Brioschi *et al.*, 1987, Zanaboni *et al.*, 1987a). In this thesis, CA125 levels were elevated in the same proportion of patients with well and moderately differentiated tumours, and in a greater proportion of patients with poorly differentiated tumours (table 7.3 and figure 7.8).

The proportion of patients with elevated serum CA153 and $HMFG_2$ increased with decreasing degree of differentiation (table 7.3 and figure 7.8). P185 was also elevated in serum in substantially more patients with poorly differentiated tumours than well and moderately differentiated tumours (table 7.3 and figure 7.8). CA724 however showed the inverse correlation; degree of expression fell with increasing tumour grade. Both TATI and CA199 were most frequently elevated in patients with moderately differentiated tumours, and in a similar proportion of patients with well and poorly differentiated tumours (table 7.3 and figure 7.8).

The statistical significance of these observations (tables 7.1, 7.2 and 7.3) was not tested because of the difficulties in comparing marker elevations in patients assayed at different times during their illness. The results in this chapter, discussed so far, are only intended to give an approximation of the distribution of each of the above markers in patient populations with different stages, types and grades of tumour. The problem of comparing marker data obtained from patients at different phases in treatment is recurrent throughout this chapter.

Receiver operating characteristic (ROC) curves were plotted for each tumour marker assay using all data available (figure 7.9). These curves may be useful in determining the cut-off value of a diagnostic test depending on the purpose of the test. As all samples were included in the construction of these curves, all that can be concluded from them is that CA125 is, in general, a far better marker of EOC than any of the other markers in this thesis. This is not surprising, as CA125 was elevated in a greater proportion of EOC patients than all of the other markers (table 7.1). There is little difference overall between the other markers, except for p185 which has an accuracy that differs little from chance observation.

ROC curves were also plotted for each histological type (not shown), and the area below compared to determine if there were any significant differences between the performance of each test for different EOC subtypes (Hanley and McNeil, 1982). CA125, CA153, CA199, and TATI were all significantly more sensitive and specific markers of poorly differentiated adenocarcinoma than other types that were compared (see section 7.3.2). These results were to be expected given that each marker was elevated most frequently in patients with poorly differentiated adenocarcinomas (table 7.2), the majority of whom have advanced stage disease. CA724 was most sensitive and specific for mucinous tumours, although only three patients with mucinous tumours had serial CA724 assay. There were no significant differences in overall sensitivity and specificity between $HMFG_2$ or p185 for different histological types, neither of which has been advocated for a particular histological type.

ROC curves however tell nothing about the predictive value of a test; PVP and PVN are heavily dependent on the disease prevalence in a population, unlike sensitivity and specificity (chapter 3, section 3.4.7, pp. 68-71). Consequently, the predictive value of a positive result for each marker assay would be expected to be higher for disease in patients with poorly differentiated adenocarcinomas, and indeed other histological types, than mucinous cystadenocarcinomas, the disease prevalence of the latter being considerably lower. Thus, the sensitivity and specificity of a test may be higher for mucinous compared to non-mucinous tumours, but this does not imply a higher PVP (chapter 3, section 3.4.7, pp. 70-71).

Given that no single test can accurately determine disease status in all patients, "panels" or combinations of markers have been intensely sought for the past eight years since the first clinical report of CA125 (Bast *et al.*, 1983). The value of each marker in addition to CA125 was investigated in two ways in this thesis. Firstly, a stepwise discriminant analysis was performed to determine if any marker could add significantly to the ability of CA125 to discriminate between "disease positive" and "disease negative" samples. If the correlation between two markers is high there is no point assaying both markers; cost may be reduced by assaying the best marker. The correlations between CA125 and CA153 ($r=0.455$) and CA125 and CA724 ($r=0.403$) were relatively high, and only TATI and $HMFG_2$ were able to add significantly to CA125 in this analysis (see section 7.4.1).

Cost was a major limiting factor in the evaluation of the commercially available markers in this thesis, and is likely to pose problems should any panel of markers be adopted routinely. One way of circumventing this problem without reducing diagnostic capability is to assay the most specific marker first, and perform subsequent assays on samples that prove negative with the first test. As CA125 is far superior to any marker discovered to date, it would seem not unreasonable to assay a panel of markers in this "series" manner (Galen and Gambino, 1975) employing CA125 as the first test. Indeed, the majority of studies of marker panels to date have included CA125 as the "gold standard"

to which other markers are compared. This method of panel testing has been adopted in screening studies involving pelvic examination, ultrasound and CA125 assay (Jacobs *et al.*, 1990a). For these purposes a marker may be considered as any test or investigative procedure capable of producing a simple dichotomous outcome in relation to disease presence and not necessarily a quantitative serological assay.

Assay of each additional marker in series with CA125 increased overall sensitivity by less than 1% with combinations of CA125 and CA153, CA125 and CA199, and CA125 and CA724, and by 3% and 2.9% respectively with CA125 and TATI, and CA125 and $HMFG_2$ compared to CA125 assay alone (table 7.5). Specificity was more markedly reduced by each panel, except for CA125 and CA724 as no false positive CA724 results were noted. Thus, CA153 reduced specificity by 3.4%, CA199 by 6.7%, TATI by 11.8%, and $HMFG_2$ by 17.3% (table 7.5). There was a concomitant increase in PVN with a greater fall in PVP, resulting in an overall decrease in accuracy using each panel compared to CA125 assay alone.

Both TATI and $HMFG_2$ increased sensitivity by a marginally greater percentage than CA153, CA199 and CA724, and there were more patients with both CA125 and TATI, and CA125 and $HMFG_2$ data to evaluate. When these marker combinations were investigated in more detail, sensitivity increased, while the specificity of CA125 assay alone was retained, only in patients with advanced poorly differentiated serous tumours, over 40% of whom had clinically evaluable disease at the time of marker assay. It follows that the majority of patients had no clinical evidence of disease at the time TATI or $HMFG_2$ were elevated in CA125 negative samples. There is considerable disagreement in the literature regarding the use of marker panels to improve marker sensitivity. Investigators tend either to advocate the use of various combinations of markers including CA125, or recommend CA125 alone as the optimal test.

Although assay of TATI and $HMFG_2$ in addition to CA125 each improved the detection rate in patients with advanced poorly differentiated serous tumours,

they also generated an equal number of false positive results in patients with other disease stages, types and grades of tumour. The specificity of both of these markers is therefore not sufficiently high to warrant their inclusion in a panel of markers for monitoring all EOC patients. Moreover, these marker panels identified residual or recurrent disease in patients with either clinical evidence of disease and/or limited therapeutic options.

Bast and colleagues concluded that CA125 assay alone was better than a panel of CA125, CA199 and CEA for monitoring EOC a year after the first clinical report of CA125 (Bast *et al.*, 1984). Scambia *et al.* (1990) concluded that CA724 gave no useful additional information to CA125, while Halila *et al.* (1988) concluded that TATI gave no additional information to CA125 in patients with non-mucinous tumours. The number of patients in their study is too small to conclude that TATI is a clinically useful addition to CA125 in patients with mucinous tumours, although recently Mogensen *et al.* (1990) found pre-operative TATI and CA125 assay was able to improve the discrimination between benign and malignant mucinous tumours. Earlier in this study, assay of placental alkaline phosphatase was found to be no use in addition to CA125 due to the high false negative and false positive rate of PLAP assay (Fisken *et al.*, 1989), this was corroborated at the time by Haije *et al.* (1988). Ward *et al.* (1987c) however, reported pre-treatment assay of PLAP and $HMFG_2$ in addition to CA125 to increase sensitivity with CA125 alone from 96% to 100% in 26 patients with advanced disease, and from 18% to 64% in 11 patients with early stage disease. As the disease prevalence in the population with advanced EOC is 100%, their study is worthless. A positive result was defined as elevation of at least one marker in the panel (Ward *et al.*, 1987c), also used in this thesis. The PVP of both PLAP and $HMFG_2$ are, however, not sufficiently high to assist CA125 in screening for early stage disease.

One approach to improving the specificity for screening or diagnosis, and limit the number of false positives that result in unnecessary investigation, is to stipulate that all markers in all panel should be elevated instead of at least one to constitute a positive result (i.e. parallel testing). This approach has been

adopted in screening studies (Bast *et al.*, 1990) where the consequences of false positive results have more immediate widespread implications (chapter 3, section 3.4.8, pp. 71-72). Achieving adequate sensitivity, and not specificity, is more of a problem in monitoring, while achieving adequate specificity is more of a problem in screening. It is unlikely therefore that this approach would improve the ability of markers to detect occult disease in patients with established EOC. Unless markers with similar or superior sensitivity and specificity to CA125 are discovered it is unlikely that marker panels will influence clinical decision making more than CA125 measurement alone.

CHAPTER 8

The Clinical Correlates of Epithelial Ovarian Tumour Markers

8.1 INTRODUCTION

The purpose of this study was to evaluate the role of tumour markers in patients with established epithelial ovarian cancer. Pre-operative tumour marker assay may aid the differential diagnosis of an adnexal mass, which could lead to a more appropriate operation in a substantial proportion of patients with ovarian malignancy (Gadducci *et al.*, 1988, Yedema *et al.*, 1988, Jacobs *et al.*, 1990, Kenemans, 1991, also see chapter 3, section 3.6, p 76). Unfortunately, samples taken prior to primary diagnostic laparotomy were unavailable, therefore the markers' usefulness in this respect could not be assessed.

Ideally, absolute tumour marker levels should reflect viable tumour burden and changes in levels should reflect the response to therapy and course of disease. The former was assessed (i) after primary laparotomy by determining the correlation between post-operative tumour marker levels and residual disease and (ii) before second-look by determining the correlation between pre-second-look tumour marker levels and findings at second-look. The latter was assessed by determining the correlation between the change from pre-treatment to post-treatment levels and response to first-line chemotherapy.

The above correlations are well documented for CA125, but are less well documented for the other tumour markers (for reviews see Jacobs and Bast, 1989, and Kenemans *et al.*, 1991). This chapter investigates the clinical correlates of each tumour marker. More data were available for CA125, TATI, $HMFG_2$, and c-neu p185, hence these were studied more in more detail than CA153 and CA199. CA724 was excluded due to lack of data.

8.2 CORRELATION OF MARKERS WITH POST-OPERATIVE RESIDUAL DISEASE

The primary operative procedures and extent of debulking, in the 250 patients included in this study, were shown in chapter 5 (tables 5.5-5.8, pp. 120-122). Overall, optimal debulking (i.e. residual disease <2cm) was achieved in 129/250 (51.6%) patients; 46/46 (100%) with stage I, 23/27 (85.2%) with stage II, 52/139 (37.4%) with stage III, and 8/38 (21.1%) with stage IV disease had <2cm residual disease. The rate of successful debulking decreased with increasing tumour grade; 23/31 (74.2%) well, 33/55 (60%) moderately and 49/126 (38.9%) poorly differentiated tumours were optimally debulked. Tumour histology also influenced the success rate; 66/147 (44.9%) serous, 29/41 (70.7%) endometrioid, 15/22 (68.2%) mucinous, 7/18 (38.9%) adenocarcinoma, 11/17 (64.7%) clear cell, 1/3 (33.3%) mixed, and 0/2 unknown tumour types were optimally debulked.

It is well known that surgical resection may cause a transient rise in marker levels (Van der Zee *et al.*, 1990). Correlations with tumour burden were therefore assessed in patients who had samples taken 1-4 weeks after primary laparotomy, before the initiation of further therapy. The first blood sample taken for serial monitoring was usually obtained the day before chemotherapy started; approximately four weeks after primary laparotomy.

Seventy seven patients had blood had samples taken during this period. Sixteen patients had stage I disease, nine had stage II, 37 had stage III and 15 had stage IV disease. In this group there were 44 serous, 15 endometrioid, seven mucinous, seven clear cell, two adenocarcinoma, one mixed and one unknown histological type. Twelve tumours were well differentiated, 13 were moderately differentiated, 38 were poorly differentiated, four were variably differentiated (exact compositions were unknown), and 10 were of unknown grade. Complete debulking was achieved in 16 patients, 24 had residual

disease <2cm, 14 had 2-5cm residual disease, nine had >5cm residual disease, and 14 had gross residual disease after biopsy only procedures.

Not all of the patients who had samples taken during this period had every tumour marker assayed. Table 8.1 shows CA125 levels in 76 patients who had samples assayed 1-4 weeks (mean 17.8 days, median 18 days, and range 3-28 days) after primary laparotomy. A wide range of CA125 levels were found in patients in all residual disease categories.

Elevated CA125 was seen in 73.3% of patients who were completely debulked, 87.5% who had <2cm disease, and all patients with >2cm disease. Mean and median post-operative levels increased with residual tumour volume, and this correlation was highly significant ($p<0.0001$). Patients with >2cm disease had significantly higher CA125 levels than those with <2cm disease ($p<0.0001$).

Table 8.1 ***Post-operative CA125 levels***

Residual disease	No. patients with elevated CA125 (%)	CA125 (Uml^{-1}) Mean	SD	Median	Range
None	11 / 15 (73.3)	58.0	46.3	55.5	14-210
< 2cm	21 / 24 (87.5)	238.1	224.0	129.3	17.8-798
2 - 5cm	14 / 14 (100)	472.8	456.6	383.5	50.2-1933
> 5cm	9 / 9 (100)	548.7	440.4	458.0	70-1553
Gross	14 / 14 (100)	480.2	473.4	403.2	88-2000

Table 8.2 shows CA153 levels in 13 patients who had samples assayed 1-4 weeks (mean 17.5 days, median 17 days, and range 7-28 days) after primary laparotomy. CA153 levels increased with tumour burden, but the trend was not significant. Fewer optimally debulked patients had elevated post-operative CA153 levels (33.3%) than those with residual disease >2cm (50%).

Table 8.2 ***Post-operative CA153 levels***

Residual disease	No. patients with elevated CA153 (%)	CA153 (Uml^{-1}) Mean	SD	Median	Range
None	0 / 1 (0)	*11.8	-	-	-
< 2cm	3 / 8 (37.5)	29.8	22.6	23.3	7.3-77.2
2 - 5cm	1 / 3 (33.3)	36.5	23.5	24.6	21.3-63.3
> 5cm	1 / 1 (100)	*65.9	-	-	-

* n=1

Table 8.3 shows CA199 levels in the same 13 patients who had CA153 assay 1-4 weeks after primary surgery. One patient with no residual disease had markedly elevated post-operative CA199. Post-operative levels of CA199 showed no trend with tumour burden.

Table 8.3 ***Post-operative CA199 levels***

Residual disease	No. patients with elevated CA199 (%)	CA199 (Uml^{-1}) Mean	SD	Median	Range
None	1 / 1 (100)	*341	-	-	-
< 2cm	3 / 8 (37.5)	39.2	52.9	15.6	9-166.5
2 - 5cm	0 / 3 (0)	16.3	4.3	14.5	13.3-21.2
> 5cm	1 / 1 (100)	*42.9	-	-	-

* n=1

Table 8.4 shows TATI levels in 28 patients who had samples assayed 1-4 weeks (mean 18 days, median 18 days, and range 3-28 days) after primary laparotomy. A wide range of TATI levels were found in patients in all residual disease categories; 9/22 (40.9%) optimally debulked patients and 3/6 (50%) with residual disease >2cm had elevated levels. Post-operative TATI levels did

not correlate significantly with residual tumour burden and no trends were observed.

Table 8.4 ***Post-operative TATI levels***

Residual disease	No. patients with elevated TATI (%)	TATI (μgl^{-1}) Mean	SD	Median	Range
None	1 / 8 (12.5)	14.5	10.1	10.9	9.1-39.3
< 2cm	8 / 14 (57.1)	29.7	19.2	25.8	7.3-63.4
2 - 5cm	2 / 5 (40)	25.7	19.4	16.2	9.2-56.6
> 5cm	1 / 1 (100)	*29.5	-	-	-

* n=1

Table 8.5 shows $HMFG_2$ levels in 62 patients who had samples assayed 1-4 weeks (mean 17.3 days, median 18 days, and range 3-28 days) after primary laparotomy. There was a wide range of $HMFG_2$ levels in patients in all residual disease categories; 11/34 (32.3%) optimally debulked patients, 2/11 (18.2%) with 2-5cm disease, and 10/17 (58.8%) with >5cm disease had elevated levels.

Table 8.5 ***Post-operative $HMFG_2$ levels***

Residual disease	No. patients with elevated $HMFG_2$ (%)	$HMFG_2$ (Uml^{-1}) Mean	SD	Median	Range
None	3 / 16 (18.8)	26.1	22.5	22.3	5-83.3
< 2cm	8 / 18 (44.4)	38.7	42.4	36.5	5-146.5
2 - 5cm	2 / 11 (18.2)	22.1	21.2	18.3	5-64.6
> 5cm	3 / 6 (50.0)	65.1	52.1	48.3	26.6-166.6
Gross	7 / 11 (63.6)	142.9	151.8	103.2	5-500

With the exception of the partially debulked group, mean and median $HMFG_2$ levels increased with residual tumour volume. This correlation was highly

significant ($p<0.005$). Although $HMFG_2$ levels were not significantly different in patients with <5cm disease, patients with >5cm residual disease had significantly higher levels than those with <5cm residual disease ($p<0.005$).

Table 8.6 shows p185 levels in 38 patients who had samples assayed 1-4 weeks (mean 16.5 days, median 17 days, and range 7-28 days) after primary laparotomy. No patient had elevated p185 after surgery, and there was no correlation with tumour burden, even within the normal range.

Table 8.6 ***Post-operative p185 levels***

Residual disease	No. patients with elevated p185 (%)	p185 (Uml^{-1}) Mean	SD	Median	Range
None	0 / 10 (0)	1121	179	1033	925-1440
< 2cm	0 / 11 (0)	966	191	915	685-1350
2 - 5cm	0 / 5 (0)	1271	367	1260	785-1820
> 5cm	0 / 4 (0)	866	248	833	610-1190
Gross	0 / 8 (0)	1087	434	1013	605-1760

8.3 CORRELATION OF MARKERS WITH RESPONSE TO FIRST-LINE CHEMOTHERAPY

Over the period of this study, up to 10 different chemotherapy regimes were in use, see chapter 5 and Appendix C. Therefore, to ensure adequate sample sizes, patients who received different regimes were grouped according to UICC response; complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD) (see chapter 1, table 1.4, p 27). The correlations between each marker and response were assessed in patients with samples taken immediately prior to first-line chemotherapy, who also had a sample assayed within one month of completion of therapy.

Blood samples were not always taken precisely when a drug regime was completed or when UICC response was determined. Fewer patients who had pre-treatment samples had both pre-treatment and post-treatment samples, however, the majority of patients described in section 8.2 were included. In addition, a few patients who had pre-treatment samples taken outside the 1-4 week post-operative period, as well as post-treatment samples, were included. The treatment of each patient group will be described in more detail in sections 8.3.1 to 8.3.5.

8.3.1 Change in CA125 levels from pre-treatment to post-treatment

Pre-treatment and post-treatment CA125 levels in patients who received first-line chemotherapy are shown in table 8.7.

Table 8.7 ***CA125 levels before and after first-line chemotherapy***

Response	No. patients	Sample	CA125 (Uml^{-1}) Mean	SD	Median	Range
CR	23	pre-Rx	445.2	629.6	227.3	10-2499
		post-Rx	48.6	79.4	30.0	5-330
PR	13	pre-Rx	317.3	245.8	244.0	58.3-865
		post-Rx	31.3	20.6	30.0	7.1-84.2
SD	10	pre-Rx	422.6	257.3	388.7	100-996
		post-Rx	322.6	316.5	219.6	34-1006
PD	14	pre-Rx	496.2	606.1	356.5	68-2410
		post-Rx	1059	1445.8	367.0	27-4920

Figure 8.1 shows the individual changes in CA125 from pre-treatment to post-treatment in the patients in table 8.1, described below in each response category. Overall, change in CA125 levels showed a highly significant correlation with response to first-line chemotherapy ($p<0.005$).

Twenty three patients (stages I-1, II-2, III-18, IV-2) who achieved CR had pre-treatment and post-treatment samples assayed for CA125. Seventeen patients were debulked to <2cm, five were partially debulked, and one had >5cm residual disease. Sixteen patients had cisplatin/prednimustine, three had cisplatin alone, two had cisplatin/α-interferon, and one had 5-fluorouracil/ cisplatin/hexamethylmelamine/prednimustine (5-FU/P/H/P). In this group, CA125 levels fell 10-fold on average; individually they fell in 21/23 (91.3%) and increased in 2/23 (8.7%) patients (figure 8.1). Pre-treatment CA125 levels were significantly higher than post-treatment CA125 levels ($p<0.0001$). Upon completion of therapy, 4/23 (17.4%) patients had false positive CA125 results.

Thirteen patients (stages II-1, III-5, and IV-7) who achieved PR had pre-treatment and post-treatment CA125 assay. Six patients were debulked to <2cm, while seven had >5cm residual disease. Six patients had cisplatin/prednimustine, three had chlorambucil, two had cisplatin alone, one had prednimustine alone, and one had 5-FU/P/H/P. In this group, CA125 levels also fell 10-fold on average; individually they fell in 12/13 (92.3%) and increased in 1/13 (7.7%) patients (figure 8.1). Pre-treatment CA125 levels were significantly higher than post-treatment CA125 levels ($p<0.002$). However, there was no significant difference in CA125 response between complete and partial responders. Upon completion of therapy, 9/13 (69.2%) patients who achieved PR had false negative CA125 results.

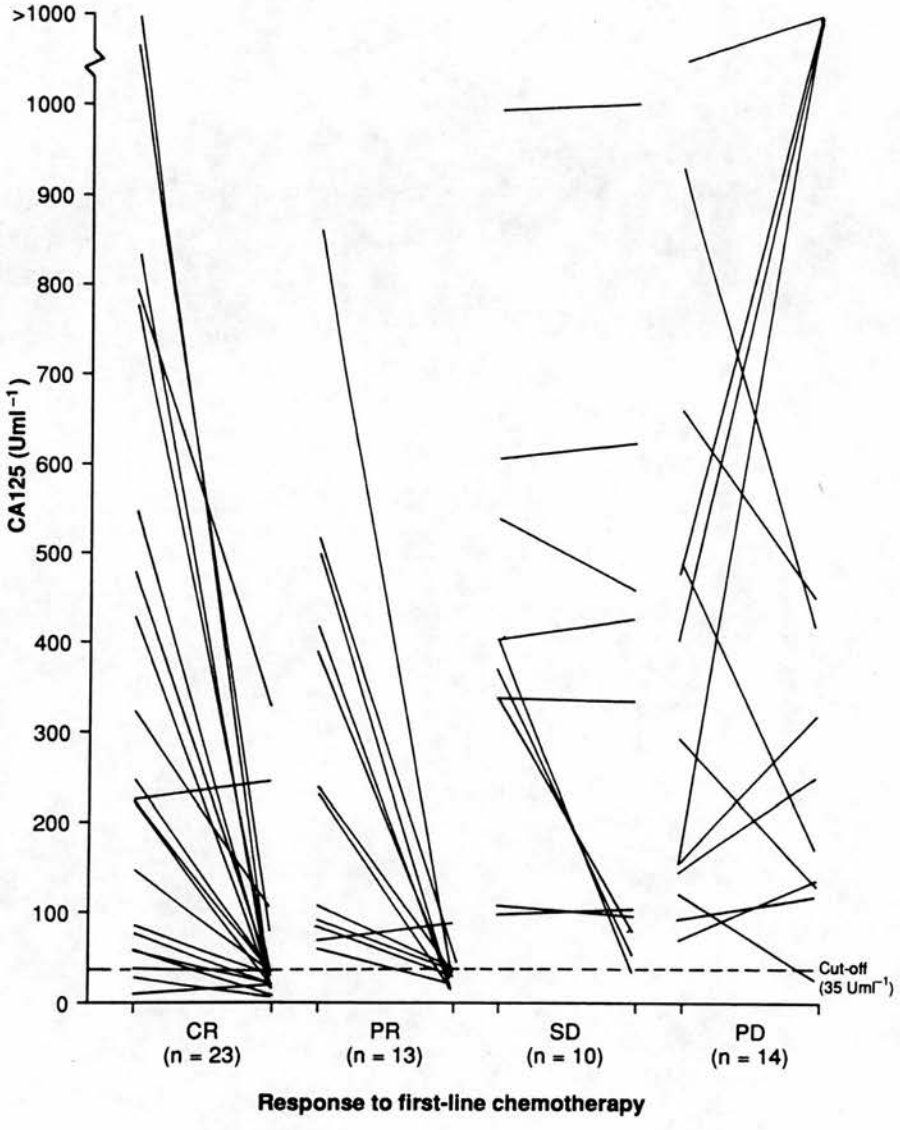

Figure 8.1 ***Change in CA125 levels from pre-treatment to post-treatment values in individual patients in each response category.***

Ten patients (stages II-2, III-7, and IV-1) who remained stable had pre-treatment and post-treatment CA125 assay. One patient was debulked to <2cm, five were partially debulked, and four had residual disease >5cm. Five patients had cisplatin/prednimustine, one had cisplatin, and four had chlorambucil. In this group, CA125 levels remained unchanged in 7/10 (70%) and fell in 3/10 (30%) patients (figure 8.1). There was no significant difference between pre-treatment and post-treatment CA125 levels. Only one patient (10%) had a false negative post-treatment CA125 result.

Fourteen patients (stages III-10, and IV-4) who progressed had pre-treatment and post-treatment CA125 assay. Three patients were debulked to <2cm, five were partially debulked, and eight had residual disease >5cm. Six patients had cisplatin/prednimustine, and eight had chlorambucil. In this group, CA125 levels increased in 9/14 (64.2%) and fell in 5/14 (35.8%) patients (figure 8.1). The mean post-treatment CA125 level was greater than double the mean pre-treatment level (table 8.1), although this difference was not significant. Only one patient (7.1%) had a false negative post-treatment CA125 result.

8.3.2 Change in CA153 and CA199 levels from pre-treatment to post-treatment

CA153 and CA199 were assayed in the same six patients (stages II-1, III-5), all of whom had a complete response to chemotherapy. Five patients were debulked to <2cm, and one was partially debulked. All six patients had cisplatin/prednimustine therapy. There were insufficient data to test for statistical significance.

Pre-treatment CA153 levels were; mean 29.9 Uml^{-1}, sd 23.6 Uml^{-1}, median 24.6 Uml^{-1}, and range 7.3-74.2 Uml^{-1}. Post-treatment CA153 levels were; mean 21.5 Uml^{-1}, sd 11.7 Uml^{-1}, median 23.5 Uml^{-1}, and range 9-36.9 Uml^{-1}. CA153 levels fell in 4/6 (66.7%) patients and were unchanged in 2/6 (33.3%) patients in this group.

Pre-treatment CA199 levels were; mean 22.7 Uml^{-1}, sd 14.2 Uml^{-1}, median 15.9 Uml^{-1}, and range 9-41.5 Uml^{-1}. Post-treatment CA199 levels were; mean 56.5 Uml^{-1}, sd 62.1 Uml^{-1}, median 20.8 Uml^{-1}, and range 8.4-137.7 Uml^{-1}. CA199 levels increased in 4/6 (66.7%) patients and were unchanged in 2/6 (33.3%) patients. There were two (33.3%) false positive CA199 results and one (16.7%) false positive CA153 result post-treatment.

8.3.3 Change in TATI levels from pre-treatment to post-treatment

Pre-treatment and post-treatment TATI levels were measured only in patients who achieved CR and PR, shown in table 8.8. There were insufficient data to test for statistical significance.

Table 8.8 ***TATI levels before and after first-line chemotherapy***

Response	No. patients	Sample	TATI (μgl^{-1}) Mean	SD	Median	Range
CR	6	pre-Rx	30.8	18.4	27.8	9.3-56.6
		post-Rx	13.9	3.7	12.9	11-19.2
PR	3	pre-Rx	68.5	44.5	59	29.5-117
		post-Rx	72.9	98.6	16.3	15.7-186

Six patients (stages I-1, II-1, and III-4) who achieved CR had pre-treatment and post-treatment TATI assay. Five patients were debulked to <2cm, and one was partially debulked. Five patients had cisplatin/prednimustine, and one had cisplatin alone. In this group, TATI levels fell in 5/6 (83.3%) and increased in 1/6 (16.7%) patients. There were no false positive TATI results upon completion of therapy.

Three patients (stages II-1 and IV-2) who achieved PR had pre-treatment and post-treatment TATI assay. Two patients were debulked to <2cm, and one had >5cm residual disease. One patient had cisplatin, and two had chlorambucil. TATI levels fell in 2/3 (66.7%) and increased in 1/3 (33.3%) patients in this group. Two patients (66.7%) had false negative TATI results after therapy.

8.3.4 Change in $HMFG_2$ levels from pre-treatment to post-treatment

Table 8.9 shows $HMFG_2$ levels before and after first-line chemotherapy. Figure 8.2 shows the individual changes in $HMFG_2$ from pre-treatment to post-treatment levels in the patients in table 8.9 described below in each response category. Overall, changes in $HMFG_2$ levels did not show a significant association with response to chemotherapy.

Table 8.9 ***$HMFG_2$ levels before and after first-line chemotherapy***

Response	No. patients	Sample	$HMFG_2$ (Uml^{-1}) Mean	SD	Median	Range
CR	15	pre-Rx	53.6	59.9	38.3	5-202
		post-Rx	24.9	42.6	5.0	5-129.8
PR	9	pre-Rx	37.3	27.6	46.6	5-73.3
		post-Rx	26.8	30.3	11.3	5-86.6
SD	9	pre-Rx	46.3	54.2	18.3	5-162.5
		post-Rx	40.1	27.4	20.0	16.6-78.3
PD	11	pre-Rx	90.3	115.5	53.3	5-350
		post-Rx	74.7	64.5	58.3	5-210

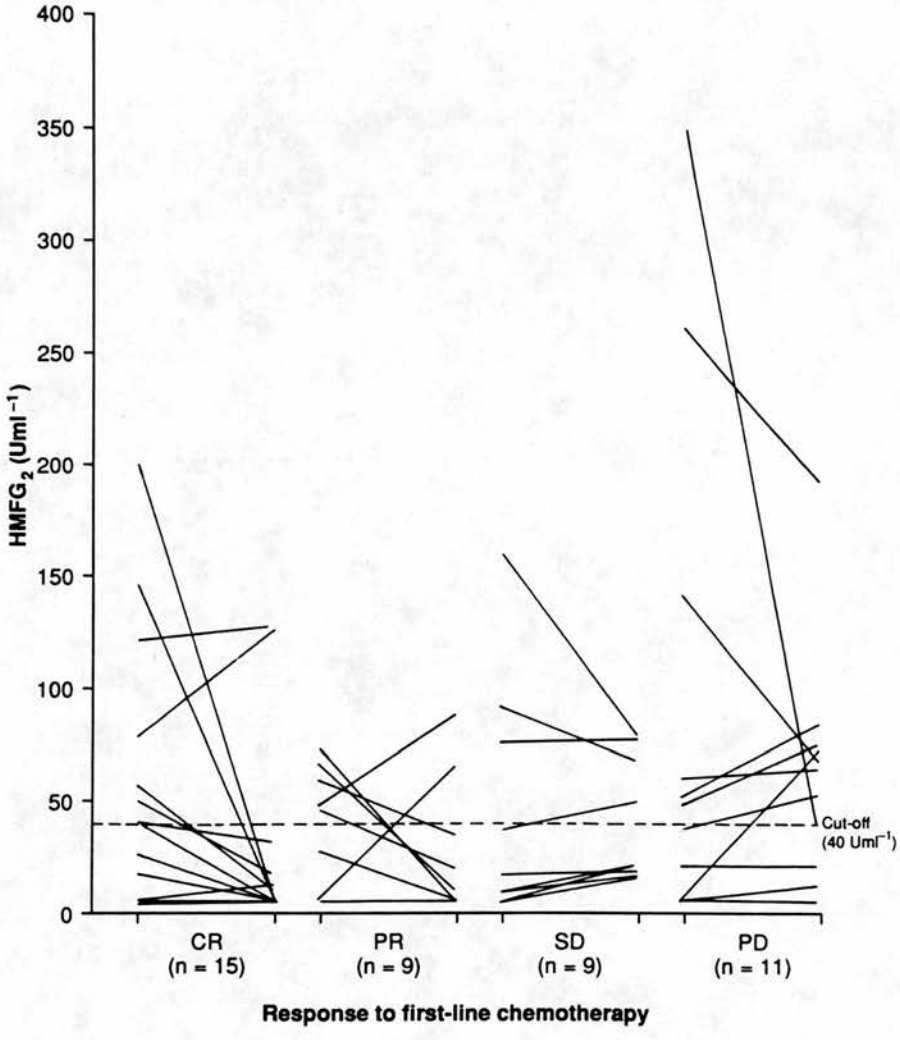

Figure 8.2 ***Change in $HMFG_2$ levels from pre-treatment to post-treatment values in individual patients in each response category.***

Fifteen patients (stages I-1, III-13, and IV-1) who achieved CR had pre-treatment and post-treatment $HMFG_2$ assay. Eleven patients were debulked to <2cm, three were partially debulked, and one had residual disease >5cm. Eleven patients had cisplatin/prednimustine, two had cisplatin alone, and two had cisplatin/α-interferon. In this group, $HMFG_2$ levels fell in 8/15 (53.3%), increased in 3/15 (20%) and were unchanged in 4/15 (26.7%) patients (figure 8.2). The mean pre-treatment $HMFG_2$ level was greater than double the mean post-treatment level (table 8.9), this difference almost achieved significance ($p<0.07$). Two patients (13.3%) had false positive $HMFG_2$ results upon completion of therapy.

Nine patients (stages II-1, III-3, and IV-5) who achieved PR had pre-treatment and post-treatment $HMFG_2$ assay. Four patients were debulked to <2cm, and five had residual disease >5cm. Four patients had cisplatin/prednimustine, two had cisplatin alone, and three had chlorambucil. In this group, $HMFG_2$ levels fell in 5/9 (55.6%), increased in 2/9 (22.2%) and were unchanged in 2/9 (22.2%) patients (figure 8.2). The mean post-treatment $HMFG_2$ level was almost two-thirds that of the mean pre-treatment level (table 8.9), although this difference was not significant. Seven patients (77.8%) had false negative $HMFG_2$ results after therapy.

Nine patients (stages II-2, III-6, and IV-1) who remained stable had pre-treatment and post-treatment $HMFG_2$ assay. One patient was debulked to <2cm, four were partially debulked and four had residual disease >5cm. Five patients had cisplatin/prednimustine, one had cisplatin alone, and three had chlorambucil. In this group, $HMFG_2$ levels increased in 5/9 (55.6%), fell in 2/9 (22.2%) and were unchanged in 2/9 (22.2%) patients (figure 8.2). There was no difference between pre-treatment and post-treatment $HMFG_2$ levels (table 8.9). Five patients (55.6%) had false negative $HMFG_2$ results after therapy.

Eleven patients (stages III-8 and IV-3) who progressed had both pre-treatment and post-treatment $HMFG_2$ assay. Two patients were debulked to <2cm, two

were partially debulked, and seven had residual disease >5cm. Five patients had cisplatin/prednimustine and six had chlorambucil. In this group, $HMFG_2$ levels increased in 4/11 (36.4%), fell in 3/11 (27.2%) and were unchanged in 4/11 (36.4%) patients (figure 8.2). The mean pre-treatment $HMFG_2$ level was slightly higher than the mean post-treatment level (table 8.9), although the difference was not significant. Four (33.3%) patients had false negative $HMFG_2$ results after therapy.

8.3.5 Change in p185 levels from pre-treatment to post-treatment

Table 8.10 shows p185 levels before and after first-line chemotherapy.
Figure 8.3 shows the individual changes in p185 from pre-treatment to post-treatment levels in the patients in table 8.10 described below in each response category. There were insufficient data to test for significance.

Table 8.10 ***p185 levels before and after first-line chemotherapy***

Response	No. patients	Sample	p185 (Uml^{-1}) Mean	SD	Median	Range
CR	5	pre-Rx	929	152	885	795-1190
		post-Rx	1275	198	1290	975-1500
PR	4	pre-Rx	1759	1309	1210	910-3705
		post-Rx	1361	160	1443	1150-1535
SD	5	pre-Rx	1126	345	1000	820-1680
		post-Rx	1361	185	1355	1135-1620
PD	7	pre-Rx	1336	636	940	915-2445
		post-Rx	1230	445	1330	785-2020

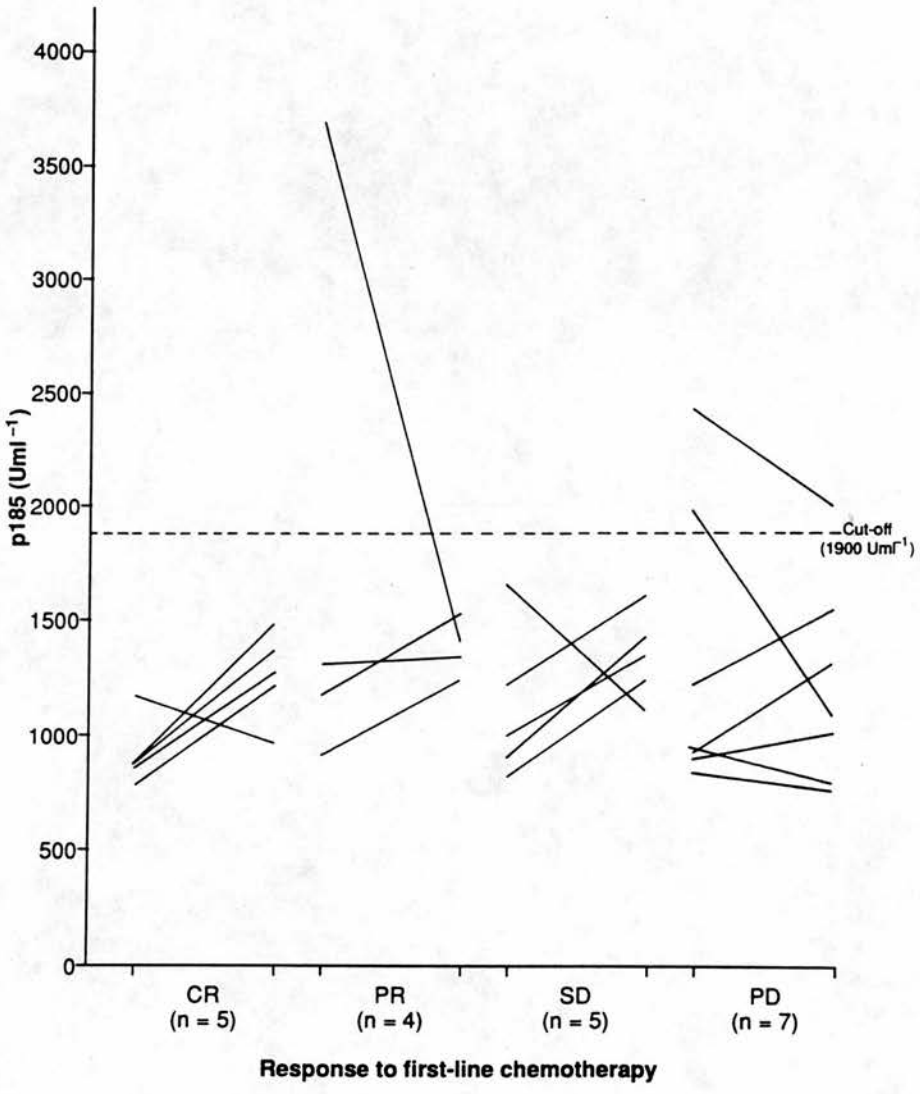

Figure 8.3 ***Changes in c-neu p185 levels from pre-treatment to post-treatment in individual patients in each response category.***

Five patients (stages III-4 and IV-1) who achieved CR had pre-treatment and post-treatment p185 assay. Two were debulked to <2cm, two were partially debulked, and one had >5cm residual disease. Two patients had cisplatin/ prednimustine, two had cisplatin/α-interferon, and one had cisplatin alone. Levels of p185 fell in 1/5 (20%) and increased in 4/5 (80%) patients in this group, however, all values were within the normal range (figure 8.3).

Four patients (stages III-1 and IV-3) who achieved PR had pre-treatment and post-treatment p185 assay. One was debulked to <2cm, two had >5cm residual disease, and one had gross residual disease. One patient had cisplatin/prednimustine, two had cisplatin alone, and one had chlorambucil. Levels of p185 fell in 1/4 (25%), increased in 2/4 (50%), and were unchanged in 1/4 (25%) patients in this group. All patients had false negative p185 levels after therapy (figure 8.3).

Five patients (stages III-4 and IV-1) who remained stable had pre-treatment and post-treatment p185 assay. Three patients were partially debulked and two had gross residual disease. Three patients received cisplatin/prednimustine, one had cisplatin, and one had chlorambucil. Levels of p185 fell in 1/5 (20%) and increased in 4/5 (80%), however, all levels were within the normal range (figure 8.3).

Seven patients (stages III-6 and IV-2) with progressive disease had pre-treatment and post-treatment p185 assay. Three were debulked to <2cm, one was partially debulked and three had gross residual disease. Three patients received cisplatin/prednimustine and four had chlorambucil. Levels of p185 increased in 3/7 (42.8%) and fell in 4/7 (57.2%) patients (figure 8.3), all but one patient had false negative p185 levels after therapy.

8.3.6 Correlation of markers with UICC response to chemotherapy

Various criteria have been used to assess the correlation between the change in tumour marker levels and response to chemotherapy. Generally, doubling or halving of marker levels are considered "clinically significant" changes. The most commonly used criteria for a positive correlation are as follows: CR - 50% or greater decrease in levels; PR - 50% or greater decrease in levels; SD - less than a 50% increase or decrease in levels; PD - 100% or greater increase in levels (Beastall *et al.*, 1991). Using these criteria, the correlations between change in marker level and UICC response in the patients described in sections 8.3.1 to 8.3.5 were determined, see table 8.11.

Table 8.11 ***Correlation of change in marker levels with UICC response***

Tumour marker	No. patients in whom markers correlate with UICC response: CR	PR	SD	PD	Total
CA125	19 / 23	12 / 13	7 / 10	8 / 14	46 / 60 (76.7%)
CA153	1 / 6	-	-	-	1 / 6 (16.7%)
CA199	0 / 6	-	-	-	0 / 6 (0%)
TATI	2 / 6	1 / 3	-	-	3 / 9 (33.3%)
$HMFG_2$	7 / 15	4 / 9	3 / 9	4 / 12	18 / 45 (40.0%)
p185	0 / 5	1 / 4	0 / 5	0 / 7	1 / 21 (4.7%)

Overall, CA125 correlated with response to first-line chemotherapy in 46/60 (76.7%) patients (table 8.11). Changes in CA125 levels agreed with response in 19/23 (82.6%) patients who achieved CR, 12/13 (92.3%) who achieved PR, 7/10 (70%) with SD, and 8/14 (57.1%) with PD. Although CA125 response correlated with CR in 19/23 (82.6%) patients, levels fell in 21/23 (91.3%) patients (figure 8.1); fewer than 21 patients had a CA125 fall of >50%. This applied to changes in all markers in patients in all response categories.

CA153 correlated with CR in 1/6 (16.7%) patients (table 8.11) although levels fell in 4/6 (66.7%) patients. There was no correlation between the change in CA199 levels in six patients who responded completely. Changes in TATI levels correlated with response in 3/9 (33.3%) patients (table 8.11), although marker levels fell in 7/9 (77.8%) patients. Overall, changes in $HMFG_2$ levels correlated with response in 18/45 (40%) patients; 7/15 (46.7%) who achieved CR, 4/9 (44.4%) who achieved PR, 3/9 (33.3%) with SD, and 4/12 (33.3%) with PD. C-neu p185 only correlated with response in one patient who achieved a partial response.

8.4 CORRELATION OF MARKERS WITH SECOND-LOOK OUTCOME

The findings at second-look laparotomy and laparoscopy were described in chapter 5, tables 5.9, 5.10, and 5.17, pp. 125, 127, and 132 respectively. Of the 250 patients in this study, 107 underwent second-look surgery; 21/46 (45.7%) had stage I, 14/27 (51.8%) had stage II, 65/139 (46.8%) had stage III, and 7/38 (18.4%) had stage IV disease at diagnosis. Second-look operation (laparotomy and laparoscopy) revealed no disease in 10/21 (47.6%), microscopic disease in 5/21 (23.8%), and macroscopic disease in 6/21 (28.6%) patients with stage I disease. Second-look operation revealed no disease in 10/14 (71.4%) stage II patients, microscopic disease in 3/14 (21.4%), and the findings were unknown in one case. Second-look operation revealed no disease in 31/65 (47.6%) stage III patients, microscopic disease in 8/65 (12.3%), macroscopic disease in 15/65 (23%), bulky disease in 10/65 (15.3%), and findings were unknown in one case. Three (42.8%) stage IV patients had negative second-look, 2/7 (28.5%) had microscopic disease, 1/7 (14.2%) had macroscopic disease, and 1/7 (14.2%) had bulky disease.

Therefore, the numbers of patients in each outcome group were small. In total, 54 patients had a negative second-look, 18 had microscopic disease, 22 had macroscopic disease, 11 had bulky disease at second-look, and the findings were unknown in two cases. The number of samples taken immediately prior

to second-look was poor; for example, 16/107 (14.9%) patients had blood samples taken for CA125 assay within one month of second-look, 42/107 (39.3%) and 69/107 (64.5%) had CA125 assay within two and three months of second-look respectively, see chapter 6, p 139. Therefore, marker levels in samples taken after primary chemotherapy, up to three months prior to second-look surgery, were assessed.

8.4.1 Marker levels prior to second-look laparoscopy

There were insufficient data to assess CA153 and CA199, and since p185 showed no correlation with either tumour burden or response to therapy, it was not evaluated. Table 8.12 shows the pre-second-look laparoscopy levels of CA125, TATI, and $HMFG_2$ and outcomes in 13 patients (stages I-4, II-1, III-7, and IV-1) who had blood samples taken within three months prior to this procedure (mean 29.4 days, median 21 days, and range 0-56 days). In this group, second-look laparoscopy was performed on average 8.9 months after primary laparotomy (median 8.5 months, and range 5.6-14.7 months). All patients were optimally debulked at primary laparotomy, except for three (patients 8 and 12 were partially debulked and patient 13 had >5cm residual disease). Three patients had no post-operative chemotherapy (all had stage Ia), one had adjuvant chlorambucil and one had adjuvant cisplatin/α-interferon, one had prednimustine, while the remaining seven patients had cisplatin/prednimustine. Eleven patients had a complete clinical response and two had a partial clinical response to first-line chemotherapy. No patient had clinically evaluable disease prior to laparoscopy, except for patient 13 who achieved PR and had a true positive CA125 level prior to operation. Patient 11, who also achieved PR, had false negative CA125 and $HMFG_2$ levels eight days prior to demonstration of microscopic disease. Patients 5 and 6 had false positive CA125 levels before laparoscopy (table 8.12).

Table 8.12 ***CA125, TATI and HMFG$_2$ levels prior to second-look laparoscopy***

No	Stage	Grade	Type	Residual disease	Months after 1° surgery	Days before second -look	Primary Rx	Response	Evaluable disease	Second-look outcome	CA125 (Uml^{-1})	TATI (μgl^{-1})	HMFG$_2$ (Uml^{-1})
1.	Ia	WD	Mucinous	None	5.9	5	None	CR	NED	-ve	32	-	5
2.	Ia	?	Clear cell	None	8.2	50	Chloramb	CR	NED	-ve	30	-	5
3.	Ia	?	Mucinous	None	14.9	21	None	CR	NED	-ve	30	-	10
4.	Ia	MD	Serous	None	14.1	54	None	CR	NED	-ve	30	-	5
5.	II	?	Endometrioid	<2cm	8.6	54	Cisp/Pred	CR	NED	-ve	330	17.7	-
6.	III	WD	Serous	<2cm	5.6	0	Cisp/αIF	CR	NED	-ve	51	-	18.7
7.	III	MD	Clear cell	<2cm	9.5	47	Cisp/Pred	CR	NED	-ve	30	-	5
8.	III	PD	Serous	2-5cm	8.8	0	Cisplatin	CR	NED	-ve	6	17.7	26.6
9.	III	?	Serous	<2cm	9.3	49	Cisp/Pred	CR	NED	-ve	20	-	5
10.	III	?	Endometrioid	<2cm	6.3	18	Cisp/Pred	CR	NED	-ve	25	-	5
11.	III	WD	Serous	<2cm	7.5	8	Cisp/Pred	PR	NED	μ	30	-	34.3
12.	III	PD	Serous	2-5cm	8.5	56	Cisp/Pred	CR	NED	-ve	17	14.1	-
13.	IV	PD	Serous	>5cm	8.3	20	Pred	PR	ED	Macro	470	-	-

Thus, CA125 assay was false negative in 1/13 (7.7%) and false positive in 2/13 (15.4%) patients prior to laparoscopy. TATI assay was true negative in three patients with CR at laparoscopy. $HMFG_2$ assay was false negative in 1/10 (10%) patients and false positive in none prior to laparoscopy.

8.4.2 Marker levels prior to second-look laparotomy

Tables 8.13a and 8.13b show the pre-second-look marker levels in 32 patients (stages I-5, II-2, III-20, and IV-5) who had blood samples taken within three months of second-look laparotomy. This was performed on average 8.8 months after primary laparotomy (median 8.4 months, and range 2.0-37.5 months). Fifteen patients were optimally debulked, seven were partially debulked, nine had >5cm residual disease, and one patient had unknown residual disease. Of the seven patients who had biopsy only at primary laparotomy, secondary debulking was performed in three (patients 11, 12 and 15). Patient 2 had no post-operative chemotherapy (after completely resected stage Ia), three patients had adjuvant chlorambucil, one (patient 6) had whole abdominal radiotherapy, two had prednimustine, four had cisplatin alone, seven had 5-FU/P/H/P, and 13 had cisplatin/prednimustine.

Thirteen patients achieved pathological CR, 12 achieved pathological PR, one had SD, two had PD after first-line chemotherapy. Three patients were inevaluable (patients 4, 15 and 16); patient 4 had early stage disease which was completely debulked and had adjuvant post-operative chlorambucil, although positive washings were found at second-look there was no cytological assessment at primary laparotomy for comparison. The other two inevaluable patients only had biopsy at primary laparotomy for diagnostic purposes; patient 15 was debulked at second-look and patient 16 had unresectable disease. Before operation, 23/32 (71.8%) patients had no evaluable disease and 9/32 (28.1%) patients had evaluable disease.

Table 8.13a ***CA125, TATI, and HMFG$_2$ levels prior to second-look laparotomy***

No	Stage	Grade	Type	Residual disease	Months after 1° surgery	Days before second -look	Primary Rx	Response	Evaluable disease	Second-look outcome	CA125 (Uml^{-1})	TATI (μgl^{-1})	$HMFG_2$ (Uml^{-1})
1.	Ia	MD	Endometrioid	<2cm	6.0	58	Chloramb	CR	NED	-ve	139	-	5
2.	Ia	?	Clear cell	None	6.4	54	None	CR	NED	-ve	30	-	23.3
3.	Ia	MD	Serous	None	7.2	43	Chloramb	PD	NED	Macro	58	15.4	5
4.	Ib	PD	Endometrioid	None	8.6	42	Chloramb	NE	NED	μ	30	-	45
5.	Ib	?	?	None	8.2	63	Cisplatin	CR	NED	-ve	23	8.3	5
6.	IIa	WD	Serous	<2cm	37.5	50	XRT	PD	ED	Macro	95	-	50
7.	IIc	?	Serous	<2cm	9.1	18	Cisp/Pred	CR	NED	-ve	18	32	-
8.	III	MD	Serous	>5cm	9.0	6	Cisp/Pred	PR	ED	Bulky	74	-	-
9.	III	WD	Serous	<2cm	8.4	62	Cisp/Pred	CR	NED	-ve	6	-	-
10.	III	PD	Serous	<2cm	8.9	58	5FUPHP	PR	NED	μ	12	-	-
11.	III	PD	Serous	>5cm	8.4	1	5FUPHP	PR	ED	*Bulky	14	-	-
12.	III	PD	Serous	>5cm	6.7	57	Cisplatin	PR	ED	*Bulky	50	-	46
13.	III	WD	Serous	2-5cm	7.5	48	Cisplatin	PR	NED	Macro	70	-	5
14.	III	MD	Serous	<2cm	9.3	55	5FUPHP	PR	NED	μ	30	10	5
15.	III	PD	Serous	>5cm	3.3	70	Cisplatin	NE	ED	*Bulky	506	-	-
16.	III	MD	Serous	>5cm	2.0	54	Cisp/Pred	NE	ED	Bulky	500	96.7	61.6

Table 8.13b *CA125, TATI, and $HMFG_2$ levels prior to second-look laparotomy (continued)*

No	Stage	Grade	Type	Residual disease	Months after 1° surgery	Days before second -look	Primary Rx	Response	Evaluable disease	Second-look outcome	CA125 (Uml^{-1})	TATI (μgl^{-1})	$HMFG_2$ (Uml^{-1})
17.	III	PD	Serous	<2cm	9.6	70	Cisp/Pred	PR	SUS	Macro	10.7	17.5	5
18.	III	MD	Serous	<2cm	9.4	5	Cisp/Pred	CR	NED	-ve	6	12.8	16.7
19.	III	MD	Serous	<2cm	7.8	19	Cisp/Pred	CR	NED	-ve	6	-	10
20.	III	PD	Serous	2-5cm	8.4	62	5FUPHP	SD	NED	Macro	7	-	-
21.	III	PD	Serous	?	9.3	54	5FUPHP	CR	NED	-ve	17	-	-
22.	III	MD	Serous	>5cm	8.6	45	5FUPHP	SD	ED	Macro	95	-	283
23.	III	MD	Serous	<2cm	7.7	59	Pred	CR	NED	-ve	13	-	-
24.	III	MD	Serous	2-5cm	9.4	58	5FUPHP	PR	NED	μ	340	-	-
25.	III	PD	Serous	>5cm	9.8	55	Cisplatin	PR	ED	Macro	56	15.3	28.3
26.	III	PD	Serous	>5cm	7.6	63	Cisplatin	PR	NED	Bulky	30	-	5
27.	III	PD	Endomet	2-5cm	8.7	49	Cisp/Pred	CR	NED	-ve	20	-	126.5
28.	IV	PD	Serous	2-5cm	7.9	47	Cisp/Pred	CR	NED	-ve	6	-	-
29.	IV	PD	Clear cell	2-5cm	7.8	57	Cisp/Pred	CR	NED	-ve	6	6.6	-
30.	IV	PD	Endomet	2-5cm	7.7	85	Cisp/Pred	CR	NED	-ve	21.2	-	-
31.	IV	PD	Endomet	>5cm	8.9	0	Cisp/Pred	PR	ED	Bulky	13.7	56	60
32.	IV	MD	Serous	<2cm	7.0	27	Pred	PR	NED	μ	10	-	-

CA125 was assayed within three months (mean 46.7 days, median 54 days, and range 0-85 days) of second-look laparotomy in all 32 patients shown in table 8.13. CA125 was elevated in 11 patients; one had no disease, one had microscopic disease, five had macroscopic disease, and four had bulky disease. CA125 was normal in 21 patients; 12 had no disease, four had microscopic disease, two had macroscopic disease, and three had bulky disease. Thus, CA125 was false negative in 9/32 (28.1%) patients and false positive in 1/32 (3.1%) patients prior to second-look laparotomy.

TATI was assayed within three months (mean 42 days, median 54 days, and range 0-70 days) of second-look laparotomy in 11/32 patients shown in table 8.12. TATI was elevated in three patients; one had no disease, and two had bulky disease. TATI was normal in seven patients; three had no disease, one had microscopic disease, and three had macroscopic disease. Thus, TATI was false negative in 4/10 (40%) patients and false positive in 1/10 (10%) patients prior to second-look laparotomy.

$HMFG_2$ was assayed within three months (mean 46 days, median 52 days, and range 0-70 days) of second-look laparotomy in 18/32 patients shown in table 8.13. $HMFG_2$ was elevated in seven patients; one had no disease, one had microscopic disease, two had macroscopic disease, and three had bulky disease. $HMFG_2$ was normal in 11 patients; five had no disease, one had microscopic disease, four had macroscopic disease, and one had bulky disease. Thus, $HMFG_2$ was false negative in 6/18 (33.3%) patients and false positive in 1/18 (5.5%) patients before second-look laparotomy.

8.4.3 Sensitivity, specificity, accuracy, and predictive values of CA125, TATI, and $HMFG_2$ for disease at second-look operation

The sensitivity, specificity, accuracy, and predictive values of CA125, TATI, and $HMFG_2$ in determining outcomes of second-look laparoscopy and laparotomy were calculated in the patients shown in tables 8.12 and 8.13 and described in

the previous sections (8.4.1 and 8.4.2). The results are shown in table 8.14 (the formulae for calculating these parameters were given in section 3.4.7, pp. 68-69). There were insufficient data to calculate these parameters for TATI results prior to laparoscopy.

Table 8.14 ***Value of markers in determining second-look outcome***

Tumour marker	Second-look procedure	No. patients disease positive (%)	% Sensitivity	Specificity	Accuracy	PVP	PVN
CA125	Laparoscopy	2/13 (15.4)	50	82	77	33	90
	Laparotomy	19/32 (59.4)	53	92	69	91	57
TATI	Laparotomy	6/10 (60.0)	33	75	50	67	43
$HMFG_2$	Laparoscopy	1/10 (10.0)	0	100	90	100	90
	Laparotomy	12/18 (66.7)	50	83	61	86	45

The sensitivity of CA125 for detecting disease prior to laparoscopy and laparotomy were poor, 50% and 53% respectively. CA125 was negative prior to laparoscopy in the one patient with microscopic disease. Prior to laparotomy CA125 was negative in 5/6 (83.3%) patients with microscopic disease, 2/8 (25%) with macroscopic disease, and 3/7 (42.8%) with gross disease (these three patients had consistently false negative CA125 values). Specificity was slightly higher for disease at laparotomy than laparoscopy, 92% and 82% respectively. Although the sensitivity and specificity of CA125 for disease at second-look laparoscopy and laparotomy were similar, the predictive value of a positive result was higher for the outcome of laparotomy and predictive value of a negative result was higher for the outcome of laparoscopy. This was simply a result of the higher disease prevalence in the patient group who underwent second-look laparotomy.

TATI had a sensitivity of 33% and specificity of 75% for disease at laparotomy. TATI was false negative in 1/1 and 3/3 patients with microscopic and macroscopic disease respectively. There were insufficient data to calculate

these parameters for laparoscopy, only three patients with CR had TATI assay and all had normal levels (table 8.12).

The sensitivity of $HMFG_2$ before laparoscopy was zero because the $HMFG_2$ level was normal in the only patient with disease (patient 11 had microscopic disease) had a normal $HMFG_2$ level. PVP was 100% for laparoscopy outcome simply because there were no false positive results and the disease prevalence was low (10%). $HMFG_2$ assay had a similar though slightly lower sensitivity and specificity for disease at laparotomy (50% and 83% respectively) compared to CA125 assay, reflecting a similar proportion of false negative and false positive results. $HMFG_2$ was false negative in 1/2 (50%) patients with microscopic disease, 4/6 (66.7%) with macroscopic disease, and 1/4 (25%) with gross disease.

8.5 DISCUSSION

Post-operative levels of CA125 (table 8.1) and $HMFG_2$ (table 8.5), but none of the other markers, showed a significant correlation with residual tumour burden. There was no correlation between p185 levels and tumour volume; levels were within the normal range in all patients assayed post-operatively (table 8.6). There were insufficient data to evaluate the correlation with tumour volume for the other markers in this thesis.

Scambia *et al.* (1988), in a pilot study of CA153 in EOC patients using a cut-off value of 30 Uml^{-1}, found CA153 elevated in 0/5 patients with completely resected tumour, while 5/10 (50%) and 10/14 (71%) patients with <2cm and >2cm residual disease had elevated CA153 levels three weeks after laparotomy. CA153 levels correlated with both pre-operative and post-operative tumour volume in their study; patients with advanced disease had significantly higher CA153 levels than those with stage I disease (Scambia *et al.*, 1988). Scambia *et al.* (1990) later evaluated CA724 in EOC patients and found levels elevated in 1/4 (25%) patients with no residual disease, 0/3 with <0.5cm residual disease, and 5/12 (42%) patients with >0.5cm residual disease using a cut-off value of 7 Uml^{-1}. Halila *et al.* (1988) did not assay TATI in samples taken in the first month after surgery in their study, as Matsuda *et al.* (1985) had previously shown transiently elevated levels of pancreatic secretory trypsin inhibitor (PSTI), which is homologous to TATI, after surgery. There are no data in the literature regarding post-operative serum TATI levels in EOC patients, although in this thesis a higher proportion of patients with residual disease >2cm had elevated post-operative levels than patients with <2cm residual disease (table 8.4). Canney *et al.* (1985) found elevated CA199 after surgery (exact length of time after surgery is unclear) in 4/18 (22%) patients with <2cm residual disease, 5/13 (28%) patients with 2-10cm residual disease, and 7/24 (23%) patients with >10cm residual disease. CA199 levels showed no correlation with residual tumour burden in their study (Canney *et al.*, 1985).

Thus, all tumour markers assayed in this study have been found elevated to some degree after surgery.

CA125 was elevated in a high percentage of patients in all residual disease categories, including all patients with >2cm residual disease (table 8.1). Paradoxically, 73.3% of patients regarded as completely debulked had elevated CA125 1-4 weeks after surgery. In the absence of other disease processes which may cause marker elevation (for review see Kenemans, 1991, also see sections 2.4.1 to 2.4.7, pp. 43-55), abnormal post-operative marker levels in completely resected patients may reflect one or a combination of the following; inaccurate measurement or recording of post-operative residual disease, a slow plasma clearance or release caused by peritoneal trauma. Interestingly, levels of both TATI and $HMFG_2$ were lower in patients documented with 2-5 cm residual disease (after partial debulking) than patients with <2cm disease. This may reflect inaccurate assessment of residual disease, although this was not the case with CA125. However, more patients in this category had CA125 assay than TATI or $HMFG_2$ assay, and there may be unintentional selection of patients with inaccurate recording of residual disease for TATI and $HMFG_2$ assay.

To date, several studies have investigated serum CA125 in the immediate post-operative phase. Redman *et al.* (1988) found elevated CA125 in peritoneal lavage fluid but not in serum, however, they only studied six patients with a post-operative follow-up of five days. Talbot *et al.* (1989) found rising CA125 levels following abdominal surgery in patients with a variety of benign and malignant diseases. Declining post-operative levels were also found in patients with elevated pre-operative CA125, suggesting the effect of laparotomy was masked by removal of the CA125 shedding tumour in these patients (Talbot *et al.*, 1989). More recently, Van der Zee *et al.* (1990) studied post-operative CA125 in three groups of patients, all with normal pre-operative levels, who underwent abdominal surgery for either EOC, cervical cancer or aortic disease. Post-operative CA125 was elevated in 82% of patients irrespective of primary diagnosis. CA125 was measured daily for two weeks after surgery and then

weekly for five weeks in EOC patients. The highest levels were found nine days after laparotomy, gradually returning to normal 3-4 weeks after surgery (Van der Zee *et al.*, 1990). This agrees with the earlier findings of Crombach *et al.* (1985) who reported a return to normal CA125 levels 3-6 weeks after laparotomy in their patients. Thus, CA125, and possibly other markers elevated after surgery, may have limited value in this period (see discussion of prognostic value of early CA125 and $HMFG_2$ assay in chapter 9, p 238).

Chemotherapy may also cause a transient elevation in serum markers. Canney *et al.* (1984) found an acute rise in CA125 levels within the first week of administration of chemotherapy. Such early serum "spikes" after treatment are thought to reflect successful tumour cell lysis and antigen release, and consequently predict a good response (Canney *et al.*, 1984). As tumour markers may take several weeks to return to "baseline" values in the intervening time between surgery and chemotherapy and between cycles of chemotherapy, the most appropriate time (and logistically the easiest) to take samples for monitoring response to chemotherapy, is immediately prior to administration of each cycle. In this thesis, patients had their first blood sample taken for marker assay a median 18 days after laparotomy and within days of initiation of primary chemotherapy. Subsequent samples were usually obtained the day before each cycle of chemotherapy, or at each clinic or ward visit in patients off treatment or in hospital for investigation or other treatment.

Patients were grouped according to UICC response to determine the correlation between change in marker levels with response to first-line chemotherapy. Each group consisted of patients with different stages, grades, tumour types etc., and therefore represented heterogeneous populations. In general, tumour markers were elevated in a greater proportion of patients with advanced disease, poorly differentiated tumours and tumours of serous, adenocarcinoma and clear cell types (see chapter 7, tables 7.1, 7.2, and 7.3, pp. 152, 160 and 161 respectively). Comparisons between different markers must therefore be drawn with caution.

Only CA125 showed a statistically significant correlation with overall response to first-line chemotherapy. Levels fell on average ten-fold in patients who achieved CR and PR, were unchanged in patients with stable disease, and increased two-fold in patients with progressive disease. Levels of CA153, TATI and $HMFG_2$ fell in the majority of responders. There were insufficient data to fully evaluate the correlation with each response for all the other markers, except for $HMFG_2$ and p185. CA153, CA199, CA724 and TATI have been evaluated in more detail by other investigators due to the commercial availability of these tumour markers.

Scambia *et al.* (1988) found rising CA153 levels in 5/5 (100%) patients with PD, and falling levels in 10/12 and 6/8 patients who achieved CR and PR respectively. Levels were unchanged in the other four responders (Scambia *et al.*, 1988). These authors also monitored EOC with CA724 and found falling levels in 10/11 responders and increasing levels in 2/7 non-responders. No distinction was made between CR and PR or between SD and PD (Scambia *et al.*, 1990). Halila *et al.* (1988) found increasing TATI levels in 10/19 (53%) EOC patients with PD, decreasing levels in 4/35 (11%) responders (CR and PR) and unchanged levels in 5/7 (71%) patients with stable disease. In a series of 55 EOC patients monitored with CA199, change in levels correlated with response in 3/3 patients who responded to therapy and 9/9 non-responders in those patients that initially overexpressed CA199 (Canney *et al.*, 1985). In an earlier report, Bast *et al.* (1984) found a correlation between CA199 and response to therapy in only 2/6 (33%) patients with SD who had initially elevated levels. Change in CA199 levels did not correlate with CR, PR or PD in any of their patients (Bast *et al.*, 1984).

In this thesis, $HMFG_2$ levels fell two-fold on average in patients who achieved CR, fell on average by one-third in patients who achieved PR, and were unchanged in those with stable and progressive disease, although levels were highest in patients with PD (table 8.9). To date there have been two studies of HMFG in EOC patients, both involving small numbers of patients with poor

follow-up. Ward and Cruickshank (1987), using the same $HMFG_2$ antibody, found $HMFG_2$ elevated in 2/12 (15%) patients in complete remission and 16/20 (80%) patients at relapse, but they had insufficient samples and follow-up to evaluate the effects response to therapy or course of disease on $HMFG_2$ levels. Patients with progressive disease however had significantly higher $HMFG_2$ levels than those in remission (Ward and Cruickshank, 1987), as also observed in this study. Ashorn *et al.* (1988) reported increasing levels of HMFG, using their antibody designated HMFG III C12, in 6/7 patients with PD, stable levels in 4/5 patients with stable disease, and decreasing levels in 1/5 responders (no distinction was made between CR and PR). Their results were not significant owing to the small number of patients (Ashorn *et al.*, 1988). The authors of both of these studies suggested that overexpression of HMFG may be indicative of a poor prognosis, but did not quantitate their observations further.

P185 levels were elevated in serum from 17/21 (85%) patients with clinically obvious progressive disease (mean CA125 levels in patients with elevated p185 were 1276 Uml^{-1}, median 445 Uml^{-1}, range 14-7157 Uml^{-1}). Not surprisingly, p185 showed no correlation with response to therapy. Strong evidence exists for roles for the oncogenes c-myc, K-ras, neu and their proteins in ovarian cancer pathogenesis (Gullick, 1990), but p185 elevation in serum was of no use in monitoring EOC patients.

In this thesis, 82.6% and 69.7% patients who achieved CR and PR respectively had post-treatment CA125 levels <35 Uml^{-1} (figure 8.1). $HMFG_2$ showed a similar false negative rate in partial responders; 86.7% and 77.8% of patients who achieved CR and PR respectively had post-treatment $HMFG_2$ levels below the cut-off value (figure 8.2). There were no significant differences between pre-treatment and post-treatment CA125 or $HMFG_2$ levels in those who achieved CR and PR. Thus, it was not possible to distinguish CR and PR. The first longitudinal study by Bast *et al.* (1983) concluded that CA125 levels <35 Uml^{-1} may or may not be associated with CR. Since the original report, several authors have corroborated Bast's findings (Canney *et al.*, 1984, Goldhirsch *et*

al., 1988, and Onetto *et al.*, 1989). Two studies have shown, in addition, that upon completion of primary chemotherapy, patients who were disease free always had CA125 levels <35 Uml^{-1} within three months of initiation of therapy (Brioschi *et al.*, 1985, and Lavin *et al.*, 1987).

Change in CA125 levels correlated with overall response in 76.7% of patients, while change in $HMFG_2$ levels correlated with overall response in 40% of patients (table 8.11). The correlation between CA125 and course of disease in earlier studies was consistently higher; CA125 correlated overall with response in 94% (Bast *et al.*,1984), 91.3% (Canney *et al.*, 1984), 89% (Crombach *et al.*, 1985), and 95.7% (Brioschi *et al.*, 1987) of cases, although in a later review of the literature Kenemans *et al.* (1988) report a range of 76% to 95% with an overall correlation of 87%. These studies all used the same criteria for correlation, i.e. a doubling or halving of levels were considered "significant" since the magnitude of these changes are unlikely to be due to chance assay variability. It is however possible that small changes in serum marker levels, even within the normal range, may be an early indication of a changing tumour volume. There were insufficient data to assess the overall correlation with CA153, CA199, and TATI.

Scambia *et al.* (1988) reported a good overall correlation between CA153 and response to therapy in 21/25 (84%) patients. Later they reported an overall correlation between CA724 and response to therapy in 12/18 (66%) patients (Scambia *et al.*, 1990). The authors however use more lenient criteria to obtain these correlations; defining a "significant" change in serum marker level as greater than a 50% decrease or increase in levels (Scambia *et al.*, 1988 and 1990). Halila *et al.* (1988) report an overall correlation between TATI and response to therapy in 19/61 (31%) patients using the generally accepted criteria applied in this thesis. Overall, the correlation between CA199 and response is very poor as less than 30% of EOC patients express this marker in their serum (Bast *et al.*, 1984, Canney *et al.*, 1985).

The percentages of patients in whom CA125 and $HMFG_2$ correlated with response were higher in responders than non-responders (table 8.11). Chemotherapy is thought to permit selective multiplication of cells which have lost the ability to express markers in patients who develop drug resistance. This would explain why levels are not often as high as may be expected in patients with disease progression, and why there was a poorer correlation between non-responders than responders in this study. Furthermore, the size and site of persistent or recurrent lesions may have an influence on serum marker levels.

It is generally accepted that rising CA125 levels are more predictive of progressive disease than falling levels are of response (for reviews see Lambert, 1987, Finkler *et al.*, 1988, and Kenemans, 1991). At first sight this would appear to contradict the previous paragraph. For a "true" correlation, or accurate reflection of post treatment disease status, post-treatment marker levels should be below the cut-off value in patients with CR, and should either remain elevated in patients with PR, SD, or PD, or become elevated with PD. The majority of studies reported in the literature do not apply such strict criteria. Markers returned to levels below the cut-off values upon completion of chemotherapy in most patients who had either complete or partial response in this thesis. It must be concluded that the definitions of response are at present inadequate, as they make no attempt to distinguish between CR and PR. "Marker response", like clinical response, does not equate with pathological response. This is directly attributable to the lack of sensitivity of tumour markers for small volume disease. This problem of definition of "marker" response is currently being addressed by Rustin (personal communication).

At present, monthly CT scanning is the most reliable method of determining disease status (Brioschi *et al.*, 1985) but is insensitive for disease <1cm. The only accurate method of determining response to chemotherapy is by second-look surgery. In this study, 107/250 (42.8%) patients underwent second-look operations. The majority of patients had no clinically evaluable disease prior to

operation, although in total, 1/12 (8.3%) and 9/22 (40.9%) of those had residual or recurrent disease at second-look laparoscopy (table 8.12) and laparotomy (tables 8.13a and 8.13b) respectively.

The sensitivity and specificity of CA125 were similar for the outcome of both second-look laparoscopy and laparotomy (table 8.14). PVP was higher for laparotomy outcome (91%) than laparoscopy outcome (33%) due to the higher disease prevalence in the patient group that underwent laparotomy. Conversely, PVN was higher for the outcome of laparoscopy (90%) than laparotomy (57%) due to the lower disease prevalence in the patient group that underwent laparoscopy. TATI was less sensitive than CA125 for outcome at second-look laparotomy (table 8.14). This was to be expected given the findings of a poorer correlation between TATI levels and response to therapy. The results of $HMFG_2$ prior to laparoscopy are inconclusive because a small number of patients were assayed and only one was disease positive. Prior to laparotomy, $HMFG_2$ was slightly less sensitive and specific than CA125, consequently the PVP and PVN were also lower (table 8.14).

The literature to date shows similar sensitivity and specificity for CA125 prior to second-look operation to those found in this thesis. Niloff *et al.* (1985) first found negative CA125 prior to second-look laparotomy in 22/36 (61%) patients with residual or recurrent disease. Other authors have reported negative CA125 levels in 10/20 (50%) (Alvarez *et al.*, 1987), 11/15 (73%) (Patsner *et al.*,1987), 9/20 (45%) (Zanaboni *et al.*, 1987) patients with disease. Schilthius *et al.*(1987) found microscopic disease in 15/17 (88.2%) and macroscopic disease <2cm in 11/13 (84.6%) patients with negative CA125 prior to second-look laparotomy. Kenemans (1991), in a review of the CA125 literature to date, report a cumulative false negative CA125 rate of 48% using cut-off values of 35 Uml^{-1} and 65 Uml^{-1}. Increasing the cut-off value from 35 Uml^{-1} to 65 Uml^{-1} lowered the false positive rate from 5% to 0% (Kenemans, 1991).

CA153 had a sensitivity of 33% and specificity of 100% prior to second-look laparotomy; 2/2 patients with microscopic disease and 4/7 patients with macroscopic disease had normal CA153 results (Scambia *et al.*, 1988). CA724 had a sensitivity of 38% and specificity of 100% prior to sceond-look laparotomy; 3/3 patients with microscopic disease and 5/10 with macroscopic disease had normal CA724 results (Scambia *et al.*, 1990). In addition, CA724 did not add to CA125 in Scambia's study. Halila *et al.* (1988) found serum TATI elevated in 2/22 patients with no disease, 3/8 with microscopic disease and 3/18 with macroscopic disease pre-sceond-look laparotomy; TATI therefore had a sensitivity of only 19% and specificity of 91% for second-look outcome. No data exists regarding the sensitivity and specificity of CA199 for disease at second-look. Given that CA199 is expressed in fewer than 30% of EOC patients and has shown no correlation with post-operative tumour burden (Canney *et al.*, 1985), the correlation with disease at second-look is expected to be very poor. Hence, none of the markers investigated to date is as sensitive as CA125 for occult disease.

Several alternative methods of determining disease status prior to second-look laparotomy have been evaluated. Mogensen *et al.* (1988) found that pelvic examination under anasthesia gave no additional information to CA125 assay. Recently, Moskovic *et al.* (1991) have shown prospectively that CT scanning is more sensitive than CA125 for detecting disease prior to second-look laparotomy. Perkins *et al.* (1990), in a pilot study comparing novel scanning methods to CA125 serum assay, found radioimmunoscintigraphy using ^{131}I labelled OC125 and magnetic resonance imaging both to be more sensitive than CA125 assay.

Some investigators have advocated different tumour marker panels to increase the sensitivity for disease prior to laparotomy. Lahousen *et al.* (1989) have found a combination of CA125, ferritin, tissue polypeptide antigen (TPA) and CEA useful for deciding whether or not to perform second-look laparotomy. McGuckin *et al.* (1990) have recently developed two novel assays for the

ovarian cancer-associated antigens OSA (ovarian serum antigen) and CASA (cancer associated serum antigen) using the same catcher antibody BC-2 and different tracer antibodies OM-1 and BC-3 for OSA and CASA respectively. They report elevation of OSA and CASA in 82% and 76% of 80 samples compared to 82% elevation with CA125 assay (McGuckin *et al.*, 1990). In addition, the authors claim these assays are more sensitive for occult disease at second-look than CA125, meriting further investigation.

Tumour markers however cannot replace second-look laparotomy as the definitive technique for determining response to chemotherapy and disease status. In the only prospective randomised trial of it's kind, Luesley *et al.* (1988) showed conclusively that second-look has no impact on overall survival. Most authors would agree with the conclusions of Chambers *et al.* (1988) that second-look laparotomy should be reserved for trial situations only, where accurate documen-tation of response (pathological as opposed to clinical) is necessary. However, Lippman *et al.* (1988) argued that resection to <2cm resulted in a significant improvement in survival compared to those patients resected to >2cm at second-look laparotomy. The decreasing trend in recent years in the performance of second-look laparotomy owes more to the demonstration that it lacks survival benefit, rather than the growing use of markers to determine response to first-line therapy. Undoubtedly certain patients do benefit from this procedure. Unless cytoreductive surgery is contemplated, second-look surgery may be avoided in patients with persistently elevated serum tumour markers.

The inability to distinguish CR from PR in patients with no clinically evaluable disease has important implications. It is often possible to convert a partial to a complete response by continuation of therapy. This may improve disease free survival in these patients and ultimately overall survival. Also, patients who respond completely, but who are at a high risk of early relapse, may benefit from consolidation therapy. Up to 30% of patients with negative second-look relapse within five years of operation (Gershenson *et al.*, 1985, Podratz *et al.*,

1988). Prognostic factors that would help identify such patients would be useful in the absence of second-look surgery to determine which patients may benefit from maintenance therapy.

CHAPTER 9

The Prognostic Value of CA125 and $HMFG_2$

9.1 INTRODUCTION

Prognostic factors are sought not only to help understand the natural history of a disease, but also to assist in the guidance and information given to patients, and ultimately to help select treatment. Numerous factors of prognostic importance have been identified in epithelial ovarian cancer patients. These include: **age** (Björkholm *et al.*, 1982, Dembo and Bush, 1982, Schray *et al.*, 1983, Sevelda *et al.*, 1990), **performance status** (Swenerton *et al.*, 1985, Heintz *et al.*, 1988 and 1990), **ascites** (Heintz *et al.*, 1988 and 1990), **stage** (Björkholm *et al.*, 1982, Redman *et al.*, 1986, Slotman *et al.*, 1990), **diameter of primary tumour** (Heintz *et al.*, 1990), **diameter of largest metastasis** (Heintz *et al.*, 1990), **site of metastases** (Heintz *et al.*, 1990), **residual disease** (for reviews see Webb, 1989 and Voest *et al.*, 1989), **histological tumour type** (Björkholm *et al.*, 1982), **tumour grade** (Dembo and Bush, 1982, Schray *et al.*, 1983, Swenerton *et al.*, 1985, Slotman *et al.*, 1990, Sevelda *et al.*, 1990), **ploidy** (Friedlander *et al.*, 1988, Kallioniemi *et al.*, 1988, Klemi *et al.*, 1988), **psammomabody content** (Kuhn *et al.*, 1988), **oestrogen receptor content** (Leake and Owens, 1990), **progesterone receptor content** (Leake and Owens, 1990, Slotman *et al.*, 1990, Sevelda *et al.*, 1990), **transforming growth factor** α (Artega *et al.*, 1988), and **epidermal growth factor receptor** (Bauknecht *et al.*, 1988). These studies have shown that response to treatment and survival are vastly influenced by a variety of inter-connected disease and patient related factors.

Earlier studies of prognostic factors were generally based on univariate analysis (Richardson *et al.*, 1985) which failed to account for the interactions of related variables. Griffiths (1975) was the first to use multivariate analysis to decipher the effects of several factors. Over the past 15 years, there has been growing use of multivariate analysis to identify unrelated factors with the greatest impact on survival. Such independent prognostic factors can be modelled to predict the risk category of an individual patient and are essential for comparison, or stratification, of clinical studies.

Many authors have reported the prognostic significance of early CA125 assay (chapter 3, section 3.7.5, p82). Thus, pre-operative measurement (Lavin *et al.*, 1987, Vergote *et al.*, 1987), post-operative measurement (Redman *et al.*, 1990, Rosen *et al.*, 1990), absolute levels after one (Rosen *et al.*, 1990), two (Sevelda *et al.*, 1989, Redman *et al.*, 1990), and three (Lavin *et al.*, 1987) cycles of primary chemotherapy, the half-life (Van der Burg *et al.*, 1988, Hawkins *et al.*, 1989), and rate of fall (Rustin *et al.*, 1989) after the first cycle of chemotherapy have all been advocated as useful prognostic indicators. However, there is no consensus yet as to which is the most useful indicator. Clarification of the prognostic value of CA125 is essential if this information is to help early treatment decisions. To date, the prognostic value of serum $HMFG_2$ assay has not been determined in ovarian cancer, although Ward and Cruickshank (1987b) and Ashorn *et al.* (1988) suggested overexpression may be a poor prognostic factor.

The sensitivity of CA125 for occult disease is poor. Nevertheless, CA125 provides a lead time to clinical relapse or recurrence in the majority of patients (for review see Kenemans, 1988). The ability of $HMFG_2$ to provide clinical lead times is unknown. The impact on survival of therapeutic intervention at pre-clinical "serological" diagnosis of relapse however remains to be addressed.

This chapter investigates the prognostic value of early CA125 and $HMFG_2$ assay and the ability of both markers to provide lead times in patients who have responded to their first course of chemotherapy. In addition, the relative importance of routine CA125 assay compared to conventional methods of assessing disease progression is evaluated. The implications for clinical decision making are discussed.

9.2 PROGNOSTIC FACTORS FOR EOC

The initial disease characteristics of the 250 patients included in this study were shown in chapter 5, table 5.1, p 117. During case note review, available prognostic factors of major importance were recorded. These included; residual

disease, stage, tumour grade, histological type, age at diagnosis, performance status (before initiation of chemotherapy), and whether ascites and adhesions were present at primary laparotomy. The prognostic significance of CA125 and $HMFG_2$ together with the above factors were investigated by univariate analysis in patients with advanced disease (FIGO stages III and IV), and the relative importance of each determined using multivariate analysis (Univariate and multivariate analyses were performed using the SAS Lifetest procedure which determines the χ^2 statistic for the Wilcoxon test. Cox's proportional hazard model was not available on SAS.) All histological types were combined because of the small numbers of patients with non-serous tumours. There were insufficient data to analyse prognostic factors in patients with stages I and II disease.

The prognostic significance of each factor was determined in the immediate post-operative period and after each cycle of first-line chemotherapy (irrespective of regime) for its duration. Samples taken from patients after completion of six cycles of therapy were excluded due to insufficient numbers. Samples were usually assayed for markers the day before, and therefore three weeks after, each cycle was administered. One hundred and twenty seven patients had blood samples taken during this period. Not all patients however had samples taken after surgery and after each cycle of chemotherapy. Therefore, different patient groups were assessed at each time point, although there was an overlap of approximately 50% between each group. Consequently, the significance levels quoted at different times are not directly comparable. Section 9.3 will examine one of these patient groups in more detail.

9.2.1 Factors influencing progression free survival in patients with advanced disease

The progression free survival (PFS) of patients with stages III and IV were shown in chapter 5, table 5.18, p 113. True dates of progression depend on the methods and frequency of patient monitoring, and are inevitably earlier than

recorded progression dates. The results of univariate analysis are shown in table 9.1. The number of patients in each group are shown in brackets.

Table 9.1 ***Factors influencing progression free survival during first-line chemotherapy in patients with advanced disease***

	p value (χ^2) after drug cycle number:					
Prognostic factor	Pre-Rx (n=53)	1 (n=58)	2 (n=52)	3 (n=49)	4 (n=43)	5 (n=33)
CA125	ns	0.0005	0.01	0.0005	0.0005	0.05
$HMFG_2$	ns	0.05	0.005	0.01	ns	ns
*RD	0.0005	0.05	0.005	ns	0.002	0.05
Grade	ns	ns	ns	ns	ns	ns
Ascites	ns	ns	ns	ns	ns	ns
Adhesions	ns	ns	ns	ns	ns	ns
*PS	0.005	0.005	ns	0.05	ns	ns
Age	0.0005	0.01	ns	ns	ns	ns

* RD - Residual disease, PS - Performance status

After primary surgery, before the start of chemotherapy, the extent of residual disease ($p<0.0005$), age at diagnosis ($p<0.0005$), and performance status ($p<0.005$) were the most significant predictors of PFS. No other factors were significant at this time. Tumour grade, and presence of ascites or adhesions were not significant predictors of PFS at any time. After one cycle of therapy (approximately two months after surgery), CA125 ($p<0.0005$) was by far the most significant predictor of PFS; performance status ($p<0.005$), residual disease ($p<0.05$), $HMFG_2$ ($p<0.05$), and age at diagnosis ($p<0.01$) were also significantly associated with PFS. After two cycles, residual disease ($p<0.005$), $HMFG_2$ ($p<0.005$), and CA125 ($p<0.01$) were significant. After three cycles, CA125 ($p<0.0005$), $HMFG_2$ ($p<0.01$), and performance status ($p<0.05$) were significant. After four cycles, CA125 ($p<0.0005$) and residual disease ($p<0.002$) were significant. After five cycles, CA125 ($p<0.05$) and residual disease ($p<0.05$) were significant. Thus, CA125 and $HMFG_2$ were both significant predictors of PFS after one cycle of primary chemotherapy, and remained

significant for a further four and two cycles respectively. Table 9.2 shows the results of multivariate analysis of these factors.

Table 9.2 ***Multivariate analysis of prognostic factors influencing progression free survival***

Post cycle number	Most significant prognostic factor	Additional independent prognostic factor(s)
Pre-Rx	Age at diagnosis ($p<0.0005$)	Performance status ($p<0.05$)
1	CA125 ($p<0.0005$)	none
2	$HMFG_2$ ($p<0.005$)	Performance status ($p<0.05$)
3	CA125 ($p<0.0005$)	none
4	CA125 ($p<0.0005$)	none
5	Residual disease ($p<0.05$)	none

After surgery, age at diagnosis was the most significant predictor of PFS ($p<0.0005$), independent of performance status ($p<0.05$). No other factor achieved significance. After one cycle of chemotherapy, CA125 was the most significant predictor of PFS ($p<0.0005$). No other factor achieved significance independently of CA125. After two cycles, $HMFG_2$ was the most significant predictor of PFS ($p<0.005$), independent of performance status ($p<0.05$). After three and four cycles, CA125 was the most significant predictor of PFS ($p<0.0005$). No other factor achieved significance independently of CA125. After five cycles of chemotherapy, residual disease was the most significant predictor of PFS ($p<0.05$), and no other factor achieved significance.

9.2.2 Factors influencing survival in patients with advanced disease

The survival of the total population was described in chapter 5, table 5.19, p 134. The results of univariate analyses of the above factors are shown in table 9.3. The number of patients in each group are shown in brackets.

Table 9.3 ***Factors influencing survival during first-line chemotherapy in patients with advanced disease***

	p value (χ^2) after drug cycle number:					
Prognostic factor	Pre-Rx (n=53)	1 (n=58)	2 (n=52)	3 (n=49)	4 (n=43)	5 (n=33)
CA125	ns	0.005	0.005	0.0005	0.0002	0.05
$HMFG_2$	ns	0.05	0.005	0.002	ns	ns
*RD	0.01	0.02	0.05	ns	0.02	0.05
Grade	ns	ns	ns	ns	ns	ns
Ascites	ns	0.05	ns	ns	ns	ns
Adhesions	ns	0.02	ns	ns	ns	ns
*PS	0.005	0.001	0.05	0.02	0.02	0.05
Age	0.002	0.05	ns	ns	ns	ns

* RD - Residual disease, PS - Performance status

After primary surgery, before the start of chemotherapy, age at diagnosis ($p<0.002$), performance status ($p<0.005$) and the extent of residual disease ($p<0.01$) were significant predictors of survival. Tumour grade was not significant at any time. After one cycle of chemotherapy, performance status ($p<0.001$), CA125 ($p<0.005$), residual disease ($p<0.02$), adhesions ($p<0.02$), age at diagnosis ($p<0.05$), ascites ($p<0.05$), and $HMFG_2$ ($P<0.05$) were all significant. After two cycles, CA125 ($p<0.0005$), $HMFG_2$ ($p<0.0005$), residual disease ($p<0.05$), and performance status ($p<0.05$) were significant. After three cycles, CA125 ($p<0.0005$), $HMFG_2$ ($p<0.002$), and performance status ($p<0.02$) were significant. After four cycles, CA125 ($p<0.0002$), performance status ($p<0.02$), and residual disease ($p<0.02$) were significant. After five cycles, CA125 ($p<0.05$), performance status ($p<0.05$) and residual disease ($p<0.05$) were significant. Thus, CA125 and $HMFG_2$ were significant predictors of overall survival, like progression free survival, after the first cycle of primary chemotherapy and for a further four and two cycles respectively. Table 9.4 shows the results of multivariate analysis of these factors.

Table 9.4 ***Multivariate analysis of prognostic factors influencing survival***

Post cycle number	Most significant prognostic factor	Additional independent prognostic factor(s)
Pre-Rx	Age at diagnosis ($p<0.002$)	Performance status ($p<0.05$)
1	Performance status ($p<0.0001$)	none
2	$HMFG_2$ ($p<0.005$)	Performance status($p<0.005$)
3	CA125 ($p<0.0005$)	none
4	CA125 ($p<0.0002$)	none
5	CA125 ($p<0.005$)	none

After surgery, age at diagnosis was the most significant predictor of survival ($p<0.002$) independent of performance status ($p<0.05$). No other factor achieved significance. After one cycle of chemotherapy, performance status was the most significant predictor of survival ($p<0.0001$), and no other factor achieved significance. After two cycles, $HMFG_2$ was the most significant predictor of survival ($p<0.005$), independent of performance status ($p<0.005$). After three, four, and five cycles, CA125 was the most significant predictor of survival ($p<0.0005$, $p<0.0002$, and $p<0.005$ respectively). No other factors achieved statistical significance at these times.

9.3 VALUE OF CA125 AND $HMFG_2$ MEASUREMENT AFTER ONE CYCLE OF PRIMARY CHEMOTHERAPY

Prognostic information that may assist treatment decisions early during chemotherapy could be extremely useful in two respects. If those patients who are unlikely to respond could be identified early in treatment, unnecessary toxicity could be avoided and valuable resources re-allocated. Alternatively, patients with a good outlook may benefit from dose intensification, thereby shortening the time spent on therapy - possibly improving response rates.

Both CA125 and $HMFG_2$ were significant predictors of total and progression free survival after the first cycle of primary chemotherapy. Fifty seven patients had samples taken at this time; 46 had stage III and 13 had stage IV disease. Forty two patients had serous tumours, nine had endometrioid tumours, three had poorly differentiated adenocarcinomas, one had a clear cell tumour, one had a mucinous tumour, and one had mixed tumour histology. Forty one tumours were poorly differentiated, eight were moderately differentiated and eight were well differentiated. None of these patients had macroscopic tumour clearance at primary laparotomy; 13 had inoperable disease, six had residual disease >5cm, 17 had 2-5 cm residual disease, and 21 had <2cm residual disease. Thirty seven patients had ascites at diagnosis and 49 had adhesions. Twenty six patients had a performance status (PS) of 0, 22 had a PS of 1, seven had a PS of 2, and two patients had a PS of 3 prior to chemotherapy.

Patients who survived for less than six months had markedly high levels of both CA125 and $HMFG_2$ two months after surgery. These patients represented approximately the upper quartile of this group. Consequently, the total group was divided into quartiles on the basis of marker levels to determine if further prognostic groups could be identified.

9.3.1 Identification of patient groups with distinct periods of progression free survival

Dates of progression were obtained for 38 patients who had samples taken after the first cycle of therapy. These patients were divided into quartiles on the basis of CA125 levels, see table 9.5, and PFS curves plotted for each quartile, see figure 9.1.

Table 9.5 ***CA125 levels in patients with poor, intermediate and good progression free survival***

Group	n	Median PFS (months)	No. Patients progression free at 1 year (%)	CA125 (Uml^{-1}) mean	median	range
A	10	4.5	2 / 10 (20.0%)	1308	532	480-6183
B	10	8.5	3 / 10 (30.0%)	390	418.9	252-472
C	9	12.0	4 / 10 (40.0%)	149.9	117.9	66.4-250.9
D	9	19.0	5 / 10 (50.0%)	39.2	35	6-62.9

Patients in group A had a median PFS of 4.5 months, and only two (20%) remained progression free (PF) after one year of follow-up. Patients in group B had a median PFS of 8.5 months, and three (30%) remained progression free at one year. Patients in group C had a median PFS of 12 months, and four (40%) remained progression free at one year. Patients in group D had a median PFS of 19 months, and five (50%) were progression free at one year.

Figure 9.1 shows three distinct prognostic groups; groups A, B and C, and D with poor, intermediate and good PFS respectively (these definitions are arbitrary). The difference between the PFS curves was significant using the Log Rank test ($\chi^2=9.48$, df=3, $p<0.02$). Patients in group A had significantly poorer PFS than those in groups B ($\chi^2=4.27$, df=1, $p<0.05$), C ($\chi^2=4.72$, df=1, $p<0.05$), and D ($\chi^2=8.33$, df=1, $p<0.005$). There was no difference in PFS between groups B and C. Patients in group D had significantly better PFS than those in groups A ($\chi^2=8.33$, df=1, $p<0.005$), B ($\chi^2=4.76$, df=1, $p<0.05$), and C ($\chi^2=5.21$, df=1, $p<0.02$).

Patients in the poor prognostic group had CA125 levels >480 Uml^{-1} (median 532 Uml^{-1}), those in the intermediate prognostic group had CA125 levels in the range 66.4-472 Uml^{-1} (median 252 Uml^{-1}), and those in the good prognostic group had CA125 levels <62.9 Uml^{-1} (median 35 Uml^{-1}).

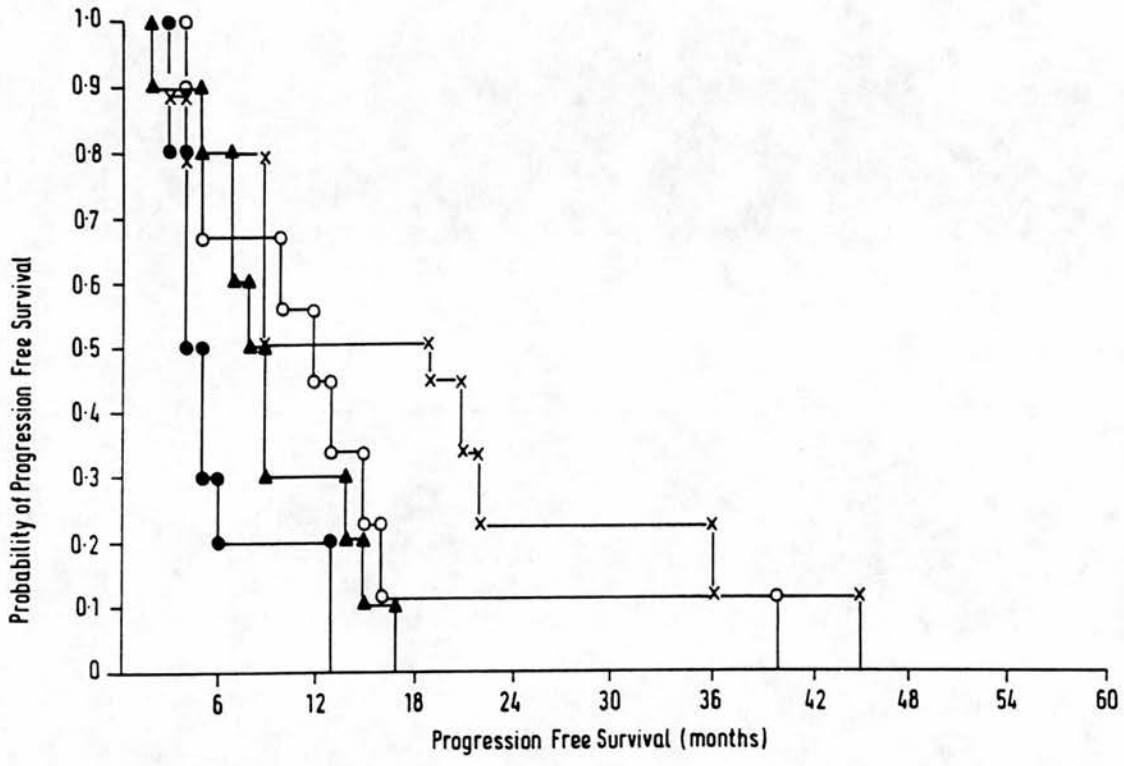

Figure 9.1 ***Progression free survival of patients according to CA125 levels after one cycle of primary chemotherapy.*** Patients in group A (●) had a mean CA125 level of 1308 Uml^{-1}, patients in group B (▲) had a mean CA125 level of 390 Uml^{-1}, patients in group C (○) had a mean CA125 level of 150 Uml^{-1}, and those in group D (×) had a mean CA125 level of 39 Uml^{-1}, see table 9.5. The difference between the four survival curves was significant ($\chi^2=9.48$, df=3, $p<0.02$).

Twenty eight patients with progression dates available had $HMFG_2$ assayed after the first cycle of chemotherapy. This group was divided into quartiles on the basis of $HMFG_2$ results, see table 9.6, and progression free survival curves plotted for each quartile, see figure 9.2.

Patients in all groups had poor median survivals. Thus, groups A, B, C and D had median survival times of four months, six months, nine months and nine months respectively. $HMFG_2$ levels ranged from 5-433 Uml^{-1} in these groups, see table 9.6. There were no significant differences in PFS using the Log Rank test between any two groups with different $HMFG_2$ values.

Table 9.6 ***$HMFG_2$ levels in patients after one cycle of chemotherapy according to progression free survival***

Group	n	Median PFS (months)	No. patients progression free at 1 year (%)	$HMFG_2$ (Uml^{-1}) mean	median	range
A	7	4.0	1 / 7 (14.3%)	178.2	83.3	73-433
B	7	6.0	3 / 7 (42.9%)	55.2	54.9	45-65
C	7	9.0	3 / 7 (42.9%)	32.5	37.6	10-45
D	7	9.0	1 / 7 (14.3%)	6.1	5	5-10

9.3.2 Identification of patient groups with distinct survival periods

The total group of 57 patients were divided into quartiles on the basis of CA125 levels, table 9.7, and survival curves plotted for each quartile, see figure 9.3.

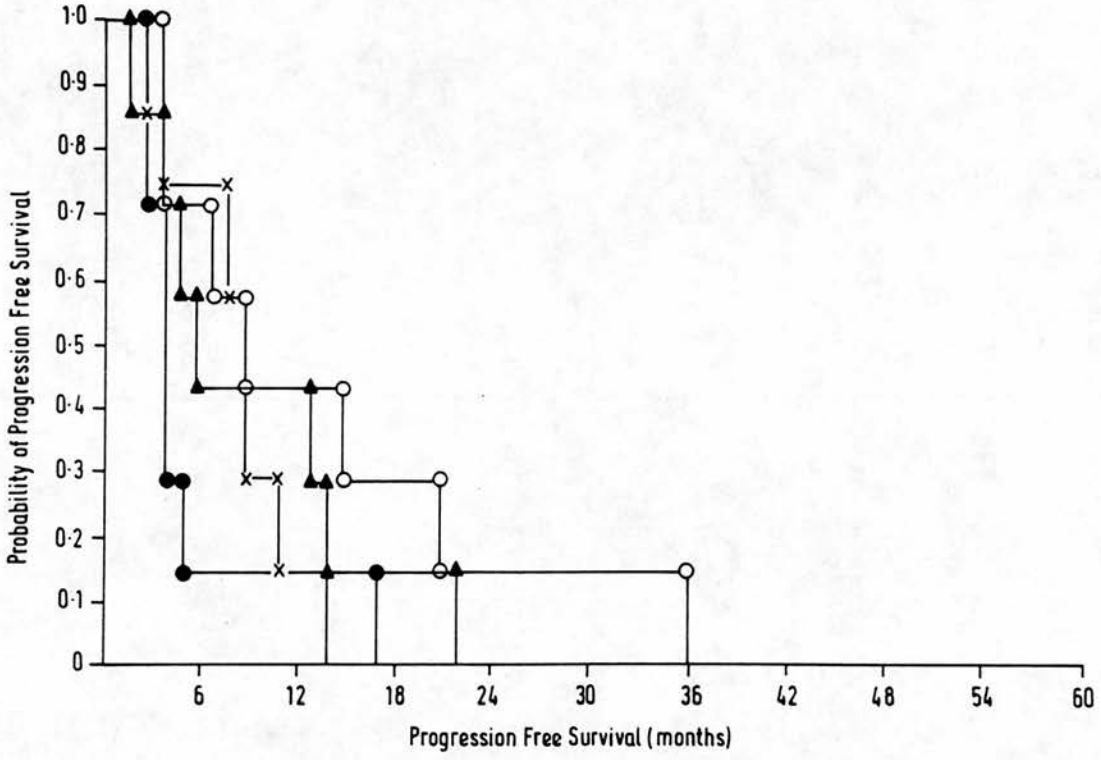

Figure 9.2 ***Progression free survival of patients according to $HMFG_2$ levels after one cycle of primary chemotherapy.*** Patients in group A (●) had a mean $HMFG_2$ level of 83,3 Uml^{-1}, patients in group B (▲) had a mean $HMFG_2$ level of 55.2 Uml^{-1}, patients in group C (○) had a mean $HMFG_2$ level of 32.5 Uml^{-1}, and those in group D (×) had a mean $HMFG_2$ level of 6.1 Uml^{-1}, see table 9.6. The difference between the four survival curves was not significant.

Table 9.7 ***CA125 levels in poor, intermediate and good prognostic groups***

Group	n	Median survival (months)	No. patients alive at 1 year (%)	CA125 (Uml^{-1}) mean	median	range
A	15	7	3 / 15 (20.0%)	1109	500	450-6183
B	14	15	10 / 14 (71.4%)	340	364	228-434
C	14	16	11 / 14 (78.6%)	103	102	58-221
D	14	23	13 / 14 (92.9%)	29	31	6-55

Patients in group A had a very poor median survival of 7 months, only 3 (20%) patients were still alive after one year of follow-up. Patients in groups B and C had similar median survivals of 15 and 16 months respectively. Ten (71.4%) and 11 (78.6%) patients in groups B and C were alive at one year. Patients in group D had a median survival of 23 months, 13 (92.9%) were alive at one year.

Figure 9.3 shows three distinct prognostic groups based on CA125 values at this time. The difference between the survival curves was significant using the Log Rank test ($\chi^2=14.70$, df=3, $p<0.005$). Patients in group A had a significantly poorer survival than those in groups B ($\chi^2=8.12$, df=1, $p<0.005$), C ($\chi^2=8.00$, df=1, $p<0.005$), and D ($\chi^2=13.91$, df=1, $p<0.001$). There was no difference in survival between patients in groups B and C. Patients in group D had significantly better survival than those in groups A ($\chi^2=13.91$, df=1, $p<0.001$), B ($\chi^2=6.67$, df=1, p0.01), and C ($\chi^2=6.58$, df=1, $p<0.02$).

Patients in the poor prognostic group had CA125 levels >450 Uml^{-1} (median 500 Uml^{-1}), those in the intermediate prognostic group had CA125 levels in the range 58-434 Uml^{-1} (median 224 Uml^{-1}), and those in the good prognostic group had CA125 levels <55 Uml^{-1} (median 31 Uml^{-1}, all but one patient had normal levels).

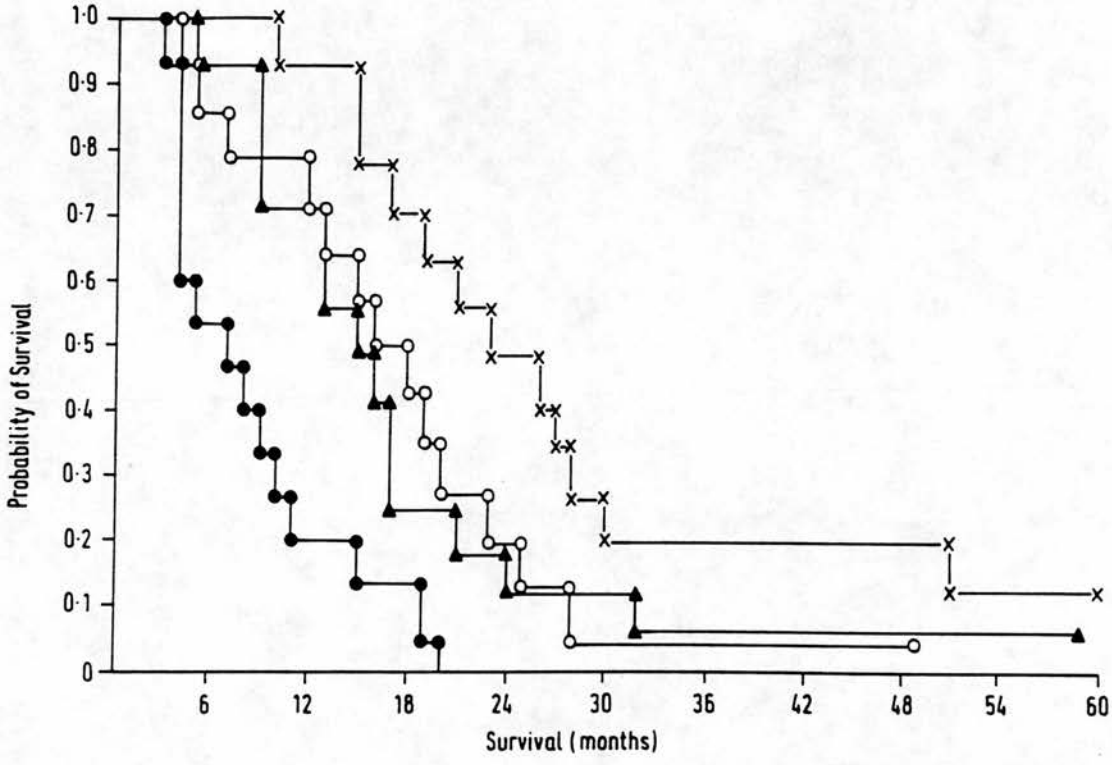

Figure 9.3 ***Survival of patients according to CA125 levels after one cycle of primary chemotherapy.*** Patients in group A (●) had a mean CA125 level of 1109 Uml^{-1}, patients in group B (▲) had a mean CA125 level of 340 Uml^{-1}, patients in group C (○) had a mean CA125 level of 103 Uml^{-1}, and those in group D (×) had a mean CA125 level of 29 Uml^{-1}, see table 9.7. The difference between the four survival curves was significant ($\chi^2=14.7$, df=3, $p<0.005$).

Forty four patients with $HMFG_2$ results after one cycle of therapy were divided into quartiles on the basis of $HMFG_2$ levels, see table 9.8. Survival curves were plotted for each quartile, see figure 9.4.

Table 9.8 ***$HMFG_2$ levels in patients after one cycle of chemotherapy***

Group	n	Median survival (months)	No. patients alive at 1 year (%)	$HMFG_2$ (Uml^{-1}) mean	median	range
A	11	5	4 / 11 (36.6%)	154	90	65-433
B	11	16	8 / 11 (72.7%)	49	48.3	40-64
C	11	13	8 / 11 (72.7%)	24	23.3	10-39
D	11	15	8 / 11 (72.7%)	7	5	5-10

Patients in group A had a poor median survival of five months, while those in groups B, C and D had similar median survivals of 16, 13, and 15 months respectively. There were no significant differences in survival using the Log Rank test between any two patient groups.

9.4 CLINICAL LEAD TIMES TO RELAPSE

Twenty patients were followed serologically from complete (n=10) or partial (n=10) response until clinical relapse. CA125 was assayed in samples from all 20 patients, while $HMFG_2$ was assayed in samples from 15 patients; the stages and histological types of the two groups are shown in table 9.9. The lead time to relapse was calculated in months as the number of days from the first rise in marker levels until the date of appearance of new symptoms divided by 30. Table 9.9 shows the patients with CA125 and $HMFG_2$ lead times. CA125 gave a mean lead time of 8.6 months (median 9.6 months, range 2.0 - 14.8 months) in 14/20 (70%) patients, while $HMFG_2$ gave a mean lead time of 8.6 months (median 9.2 months, range 1.2 - 14.8 months) in 7/15 (47%) patients. Thus, $HMFG_2$ gave similar lead times as CA125 but in fewer patients.

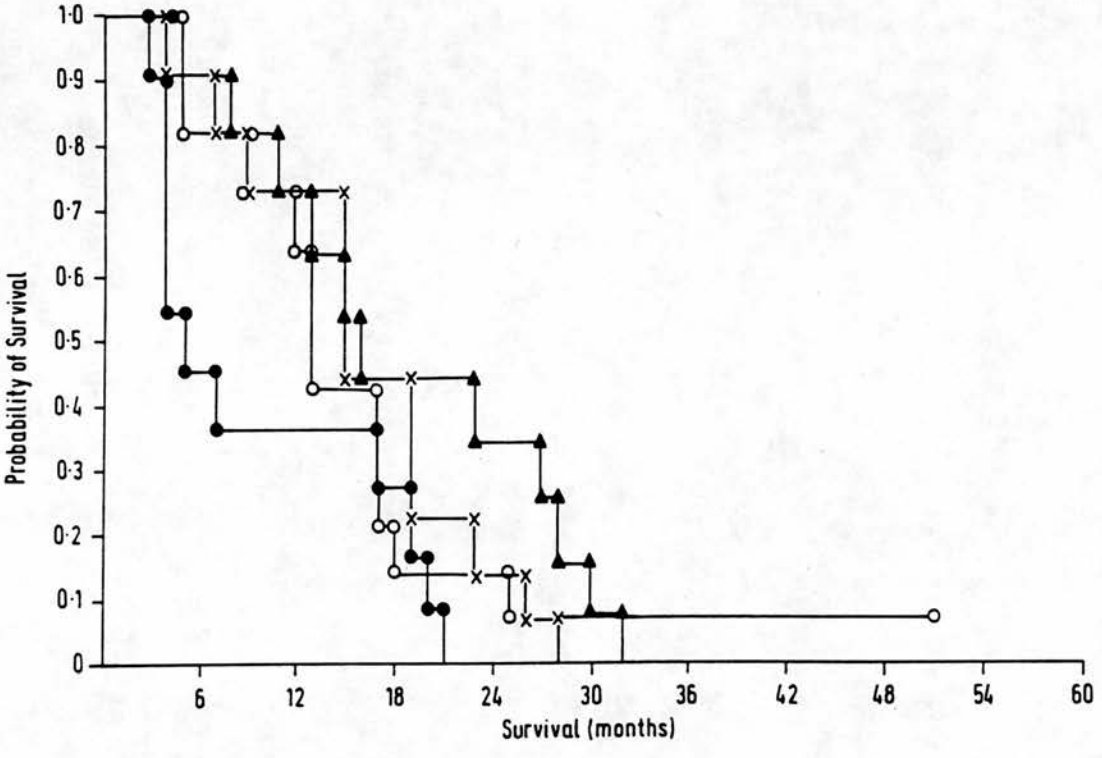

Figure 9.4 ***Survival of patients according to $HMFG_2$ levels after one cycle of primary chemotherapy.*** Patients in group A (●) had a mean $HMFG_2$ level of 154 Uml^{-1}, patients in group B (▲) had a mean $HMFG_2$ level of 49 Uml^{-1}, patients in group C (○) had a mean $HMFG_2$ level of 24 Uml^{-1}, and those in group D (×) had a mean $HMFG_2$ level of 7 Uml^{-1}, see table 9.8. The differences between the four survival curves were not significant.

Table 9.9 ***CA125 and HMFG$_2$ lead times to clinical relapse***

Disease characteristic		Patients with marker lead time CA125	HMFG$_2$
Stage	I	1 / 1 (100%)	0 / 1 (0%)
	II	3 / 3 (100%)	2 / 3 (66.7%)
	III	7 / 14 (50.0%)	5 / 10 (50.0%)
	IV	3 / 4 (75.0%)	0 / 1 (0%)
Histology	Serous	10 / 14 (71.4%)	6 / 10 (60.0%)
	Endometrioid	2 / 2 (100%)	1 / 1 (100%)
	*PDA	2 / 4 (50.0%)	0 / 4 (0%)
Total		14 / 20 (70.0%)	7 / 15 (46.7%)

* Poorly differentiated adenocarcinoma

CA125 gave clinical lead times in patients with disease stages I - IV at diagnosis, while HMFG$_2$ only gave lead times in those with stages II and III, although the numbers in each group were small. CA125 gave a lead time in patients with serous tumours, poorly differentiated adenocarcinoma and endometrioid tumours, while HMFG$_2$ gave a lead time in those with serous and endometrioid tumours. Both markers gave lead times in the same patients, except for one patient with stage III poorly differentiated serous disease who had false negative CA125 and an HMFG$_2$ lead time of 4.6 months. Sampling and follow-up were insufficient to demonstrate lead times in patients with clear cell or mucinous tumours.

9.5 ASSESSMENT OF DISEASE PROGRESSION

One or more methods of assessment, clinical, radiological, surgical or biochemical, may alert the oncologist to the possibility of disease progression. The predominant methods of assessment recorded in the case notes of 154 patients with progressive disease until July 1989 are shown in table 9.10.

Table 9.10 ***Methods of assessing disease progression***

Method of assessment of progression	No. cases (%)
Clinical examination	83 / 154 (53.9)
Computed tomographic scan	27 / 154 (17.5)
Ultrasound scan	23 / 154 (14.9)
Second-look laparotomy	9 / 154 (5.8)
X-ray	8 / 154 (5.2)
CA125 assay	2 / 154 (1.3)
Second-look laparoscopy	2 / 154 (1.3)

Disease progression was most frequently diagnosed during clinical examination; 83 (53.9%) patients were assessed in this manner. Similar numbers, 27 (17.5%) and 23 (14.9%), were diagnosed on CT scan and US scan respectively, while eight (5.2%) patients had disease progression diagnosed by X-ray. Second-look laparotomy and laparoscopy detected nine (5.8%) cases and two (1.3%) cases respectively. CA125 levels led to suspicion of disease progression in only two (1.3%) patients; one had no clinically evaluable disease and no evidence of disease on a recent CT scan, while the second patient had no clinical evidence of disease until one month after CA125 was elevated.

9.6 DISCUSSION

After surgery, residual disease, age and performance status were all significant predictors of progression free survival and overall survival. All published studies agree upon the importance of residual disease (Griffiths, 1975, Swenerton *et al.*, 1985, Webb, 1989, Marsoni *et al.*, 1990), while other prognostic factors vary in significance. These variations may be accounted for by the small sample sizes, generally less than 200 patients, and the different "cocktails" of factors considered in different studies. In addition, the majority of studies, including this thesis, have analysed prognostic factors in patients with advanced disease owing to the greater number of patients who present late.

Several authors, using Cox's proportional hazard model for multivariate analysis, have reported a maximal combination of three or four independent prognostic factors. Thus, Björkholm *et al.* (1982) found stage, age, and histological type, Dembo and Bush (1982) found residual disease, stage, age, and tumour grade, and Schray *et al.* (1983) found residual disease, age and tumour grade to be independent predictors of survival in their studies. None of these studies took performance status into consideration. A later, larger study by Swenerton *et al.* (1985), that retrospectively assessed 16 characteristics in 556 patients, found residual disease, tumour grade and performance status to be independent prognostic factors. Swenerton *et al.* (1985) also reported prognostic factor variation with disease stage. Tumour grade was most important in stages I and II, residual disease was most important in stage III and no other factor was independent of stage IV in predicting survival. Dembo *et al.* (1990) also found tumour grade to be the most powerful predictor of relapse in patients with early stage disease. Although residual disease is *the* most important prognostic factor in ovarian cancer, it is not surprising that it is of no importance in patients with early stage disease, the majority of whom are optimally debulked.

The prognostic significance of tumour grade has been consistently reported to date. However, it was not significant in this thesis, owing to the bias towards

inclusion of patients with poorly differentiated tumours; less than 30% of patients had well and moderately differentiated tumours at any given time. Recently, McGuire (1991) has published a series of guidelines for evaluating prognostic factors. Patient selection bias is a common problem that may mask important factors. Small sample size, a notorious cause of statistical insignificance in randomised trials of chemotherapy regimes, is also a potential problem. Although the sample populations in this thesis are small, the prognostic significance of residual disease, performance status, age, and CA125 are fairly consistent throughout treatment - emphasizing the importance of these factors.

In a large study, Marsoni *et al.* (1990) identified residual disease, stage, age, and tumour type as independent predictors of survival in 514 patients. They also found performance status to be a strong independent factor nullifying the effect of stage and age. Performance status, although a subjective assessment, may therefore represent a "comprehensive" marker of the relationship between a patient's general well-being and the extent of disease expressed by a combination of factors such as stage and age.

Neither CA125 nor $HMFG_2$ were significant predictors of total or progression free survival immediately after surgery. It is well known that surgical intervention causes a transient rise in CA125 (see discussion, chapter 8, p 208). In a recent study, Van der Zee *et al.* (1990) found elevated post-operative levels of CA125 in 82% of patients who underwent abdominal surgery, with normal pre-operative levels, regardless of primary diagnosis. CA125 levels took 3-4 weeks to return to normal in their patients. In this thesis, 82% and 100% of optimally debulked and sub-optimally debulked patients had elevated post-operative CA125 levels (see table 8.1). Although post-operative CA125 levels correlated significantly with residual tumour burden in our study, elevation in serum levels as a result of surgical intervention may partially explain the lack of prognostic significance of CA125 at this time. The same might apply to $HMFG_2$, and indeed other markers, although the prognostic significance of post-operative CA125 has

been found in other studies (Redman *et al.*, 1990, Rosen *et al.*, 1990, Rustin, personal communication).

CA125 was a highly significant predictor of total and progression free survival after one cycle and remained significant throughout primary chemotherapy (tables 9.1 and 9.3). $HMFG_2$ was also significant in this respect after one, two and three cycles of primary chemotherapy. As the length of progression free survival is predictive of overall survival, it not surprising that similar results were obtained in prediction of both, either with the markers or other factors previously discussed. It would be useful to know how the prognostic value of each factor changes with time and disease course, and hence when the most useful information can be obtained.

Information relating to prognosis is desirable as early as possible especially if it can influence treatment for the benefit of the patient. Patients with "advanced" ovarian cancer represent a heterogeneous group, with five year survival varying from as much as 7% to 62% (Marsoni *et al.*, 1990). The knowledge that advanced ovarian cancer patients can be assigned to groups with distinct prognostic characteristics may serve oncologists as a guideline for a more accurate estimate of the trade-off between toxicity and survival offered by chemotherapy. Treating patients with aggressive chemotherapy regimes would not be justified if a poor outcome could be predicted. Although prognostic factors are essential for stratification in clinical studies and to help guide treatment, they cannot however predict how a patient will respond to a particular therapy.

The survival benefits of chemotherapy, in particular platinum combination regimes, are a contentious issue. Population sample sizes are often too small to reveal statistically significant differences in survival between treatment arms. Recently, the MRC Gynaecological Cancer Working Party have initiated an overview of ovarian cancer chemotherapy; by January 1990 they had identified 53 relevant randomised controlled trials with a total of nearly 10,000 patients, to address this issue (MRC Gynaecological Cancer Working Party, 1991).

Many authors have reported the prognostic significance of early CA125 assay, however, there is no consensus yet as to the most useful measurement. There is conflicting evidence regarding the significance of pre-operative CA125 assay; Vergote *et al.* (1987) and Van der Burg *et al.* (1988) found an inverse correlation with survival, while Cruickshank *et al.* (1987) found no correlation, and Sevelda *et al.* (1989) report that CA125 gave no additional information to residual disease and tumour grade. Extensive debulking is likely to override the association between pre-operative CA125 and survival, giving added credence to Sevelda's conclusion.

It was possible to distinguish significantly between three prognostic groups, with relatively poor, intermediate and good PFS and survival (figures 9.1 and 9.3), based on absolute CA125 levels after one cycle of chemotherapy, but not $HMFG_2$. Thus, in approximation, patients with normal CA125 levels had a good prognosis, those with elevated levels but <500 Uml^{-1} had an intermediate prognosis, and those with CA125 levels >500 Uml^{-1} had a poor prognosis (see tables 9.7 and 9.9 for exact values). Fewer patients had $HMFG_2$ assayed, and the total group was slightly biased towards those with a poorer prognosis, again illustrating the problem of selection bias outlined in McGuire's guidelines. Indeed, $HMFG_2$ only just achieved significance at this time ($p<0.05$). Hence, more data are required to assess the prognostic value of $HMFG_2$ in patients with a wider range of prognoses.

Rustin *et al.* (1989) were able to discriminate between different prognostic groups using a seven-fold fall in CA125 levels from pre-treatment to post-treatment, using progression free survival as the endpoint in 54 patients with advanced disease. 58% of patients with greater than a seven-fold fall and 9% of patients with less than a seven-fold fall were disease free after two years of follow-up. Van der Burg *et al.* (1988) found a serum CA125 half-life of greater than 20 days more predictive of a favourable prognosis than a half-life of less than 20 days. There are as yet no data to substantiate these findings.

After two cycles of chemotherapy, Sevelda *et al.* (1989) and Redman *et al.* (1990) found CA125 to be the most significant predictor of survival in 163 and 50 patients respectively. The results of this thesis agree with their findings, in addition $HMFG_2$ and performance status were highly significant at this time. Redman *et al.* (1990) claim to have been able retrospectively to predict outcome with an overall accuracy of 93%; 96% of patients alive at one year were correctly predicted, while 85% dead at one year were correctly predicted.

Although CA125 levels may be used to discriminate significantly between different prognostic groups, they tell us nothing about the accuracy of predicting outcome in the individual patient. Longer follow-up in a larger patient series is necessary to refine the predictive value of CA125. No single biological parameter will give an accurate prediction in all patients due to tumour heterogeneity (Leake and Owens, 1990). Although CA125 was the most significant predictor of PFS in this thesis, it was not independent of performance status in predicting overall survival (table 9.6). Sevelda and Redman did not consider performance status in their evaluations. Of the four patient groups, those in group A with the highest CA125 levels also had at least three other poor prognostic factors. The majority of patients in group A had inoperable bulky disease, poorly differentiated tumours, and a performance status of 2 or 3. At the other end of the spectrum, all patients in group D were optimally debulked and the majority had a performance status of 0. Therefore, although CA125 strongly correlates with outcome, it does not necessarily provide prognostic information that is not either clinically obvious or determinable by several other factors.

Given an accurate prognosis, treatment decisions are still more likely to be influenced by patient desire for active therapy (Cody and Slevin, 1989), by limitations of current drug regimes, and increasingly by financial considerations (Rees, 1991). When deciding whether to stop treatment in patients with no change in markedly elevated CA125 levels after one cycle of primary chemotherapy, Rustin *et al.* (1989) urge caution. Up to 10% of such patients may respond eventually to therapy, and it would be wrong, they argue, to deny

patients this chance however slight. Unfortunately response to chemotherapy rarely results in a significant improvement in long-term survival.

CA125 gave a lead time to clinical recurrence in 70% of responders in this study, while the literature reports indicate up to 87% of responders have a CA125 lead time to relapse (for review see Kenemans, 1988). Both CA125 and $HMFG_2$ gave similar clinical lead times to relapse, $HMFG_2$ in fewer patients. This does not necessarily imply that $HMFG_2$ has similar sensitivity for occult disease to CA125. Indeed, after primary laparotomy, CA125 levels were significantly higher in patients with >2cm compared to those with <2cm residual disease, while $HMFG_2$ levels were significantly higher in patients with >5cm compared to <5cm residual disease (chapter 8, tables 8.1, p 182 and 8.5, p 184). Sampling was performed relatively infrequently, i.e. every few months, in the majority of patients who had marker lead times in this study. It is possible that more frequent sampling would have resulted in longer marker lead times.

Marker elevation during chemotherapy may prompt further investigation leading to discontinuation of treatment, change of treatment, or no action. Marker elevation after chemotherapy may lead to re-initiation of treatment or no action, while elevation in a previously untreated patient may result in initiation of treatment or no action. The course of action given a lead time to relapse or recurrence in a patient with EOC depends ultimately on the available remaining therapeutic options for that patient. No study has yet addressed the question of whether earlier detection of recurrent disease by CA125 monitoring translates into an improved outlook for the patient. As the results of treatment of recurrent disease are so poor, it is doubtful that earlier initiation of therapy would have any impact in the majority of patients. There is understandable reluctance to initiate therapy on the basis of rising marker levels in patients who are otherwise well. It is current practice to wait until other evidence of recurrent disease presents before beginning new therapy.

This caution is underlined by the fact that the predominant method of determining disease progression was clinical examination (table 9.2). This

situation prevailed until two years ago when there was less experience with CA125. Patients diagnosed with early stage disease, completely resected at laparotomy, previously untreated with cisplatin based regimes however may benefit from early treatment of recurrent disease. The impact on survival of therapeutic intervention at pre-clinical "serological" diagnosis of relapse can only be determined by prospective randomised controlled trials.

Similarly, the influence of CA125 upon clinical decision making, whether during first-line or subsequent treatments or during follow-up, can only be addressed in a prospective manner. In what claims to be the first prospective study, the authors at the Royal Marsden Hospital, London, have addressed the influence of scanning techniques on patient management (Gore *et al.*, 1989). In their study, US and CT scans each differed from clinical assessment in 45% of cases, although clinical decision making was influenced in only 10% and 18% of cases respectively. Patient management was more frequently altered when scans were performed for suspicion of relapse rather than for measurement of response or at routine follow-up.

Knowledge of "what is going on" is claimed to be valuable, giving clinicians confidence in treating their patients. This concept has been challenged by Gore *et al.* (1989), begging the question of how important routine CA125 assay is in patient management. This has yet to be addressed and, together with the previous considerations, may have important implications for timing of CA125 assay and allocation of precious Health Board resources. At present there is sufficient information and experience with CA125 to proceed to address these issues.

All the marker evaluations in this thesis have been performed retrospectively. The principal reservation about conclusions drawn from retrospective analyses are that they usually result from "a look to see what the data show" rather than the formal testing of a previous hypothesis. Statistically significant observations may be peculiar to the data set that has been explored retrospectively and may not be open to generalisation. Proof of generalisation of inferences requires that

they be validated on a separate set of patients. Large prospective studies are needed to generate common criteria for defining and reporting risk groups to facilitate comparisons between investigators. Justification of wider routine adoption of the CA125 assay in EOC patient monitoring will be dependent upon the results of such trials.

CONCLUSIONS

CHAPTER 10

Conclusions and Future Prospects

10.1 CONCLUSIONS

CA125, on its own, is not sensitive or specific enough to screen women for ovarian cancer (Fisken *et al.*, 1989b, Rouslton, 1990). A large research effort, however, has established a role for CA125 in monitoring epithelial ovarian cancer patients. Its value lies mainly in determining response to chemotherapy and providing lead times to clinical relapse or recurrence. Many serum assays using monoclonal antibodies directed to PEM detect elevated marker levels in a high proportion of EOC patients, in particular $HMFG_2$. Over 60% of EOC patients in this thesis had elevated $HMFG_2$ serum levels. However, none discovered to date is as sensitive and specific as CA125. Moreover, no marker has proved a clinically useful addition to CA125 in patient monitoring (Fisken *et al.*, 1991). In this study, although both $HMFG_2$ and TATI added significantly to the discrimination of CA125, neither marker was able to provide accurate information in patients with early stage disease with no clinical or radiological evidence of disease.

Although several of the markers assayed in this thesis have been advocated as markers of mucinous tumours, these studies have been based on small numbers of patients. As the incidence of mucinous EOC is far lower than serous EOC it is difficult to obtain sufficient samples for meaningful analysis in any one centre. This also applies to any other non-serous histological types of EOC. In addition, patients with mucinous tumours tend to present at an earlier stage, have better differentiated tumours and a better prognosis in general. Consequently, long-term multicentre studies are necessary to fully evaluate markers such as CA724, TATI, and CA199 in patients with mucinous ovarian carcinomas.

Although CA125 is useful in monitoring response to chemotherapy, the results of many studies, including this thesis (see chapter 8, section 8.3.1, pp. 186-189), have demonstrated that CA125 response cannot discriminate between complete and partial response. CA125 had a sensitivity of 53% in this study for disease at second-look laparotomy, similar to values previously reported in the literature. In addition, neither $HMFG_2$ nor TATI were more sensitive for disease at second-look compared to CA125. Second-look laparotomy remains the most accurate way of

determining true pathological response as opposed to clinical response. This is important, as prognosis may differ substantially between patients with clinical complete response and those with pathological complete response.

CA125 gives a lead time to relapse in the majority of patients. However, clinical examination was the most frequently documented means of determining disease progression in this thesis (see chapter 9, table 9.10, p 236). Prospective randomised trials are needed to determine whether pre-clinical "serological" diagnosis of relapse can be translated into an improved outlook for the patient. At present, tumour markers are limited by currently available drug regimes. Although responses to second-line treatments are poor, patients diagnosed with early stage disease and treated with a first-line platinum regime prior to clinical relapse may benefit in terms of prolonged survival. Until, either more sensitive, widely applicable diagnostic techniques or better treatments become available significant improvements in survival do not appear likely.

Radioimmunoscintigraphy is routinely performed in some oncology units, and has been shown to detect recurrence before serum marker elevation (Granowska *et al.*, 1988). This technique may also be a useful inclusion in a prospective intervention trial. Should such a trial be initiated, the frequency of monitoring would also need to be determined. The demand for CA125 assay in the Immunoassay section of Edinburgh Royal Infirmary has more than doubled over the past two years at considerable cost to the Health Board. Cost may be reduced if an "in-house" assay were available. Wilson and Kalirai (1991) have recently reported the development of an "in-house" ELISA for CA125 with this objective in mind. The correlation coefficient between this assay and a commercial IRMA is 0.7. Further studies using this assay are needed before routine adoption is warranted. Alternatively, new techniques in monoclonal antibody production using plasmid vectors (Winter and Milstein, 1991) may substantially reduce cost, as the most expensive part of any immunoassay is usually the production cost of the antibodies.

Problems regarding standardisation are likely to arise unless one assay method is adopted for either screening or monitoring (Fisken *et al.*, 1989). The MRC Working Party on CA125, coordinated by Dr.G.Rustin, have experienced major difficulties pooling CA125 results from the eight centres involved (including Edinburgh), partly because of discrepancies resulting from the use of different assay kits. In addition, standardisation of clinical information has proved a major problem. The last report of the Working Party agree with the findings in this thesis regarding the prognostic significance of CA125. Prognostic factors are sought to help guide treatment and the information that is given to patients. Both early CA125 and $HMFG_2$ assay were prognostically significant. Each marker was a statistically significant predictor of progression free survival and total survival after the first cycle of primary chemotherapy and throughout first-line treatment (see chapter 9, sections 9.2.1 and 9.2.2, p 221 and p 225 respectively). Statistical significance however, does not necessarily equate with clinical significance, as evidenced in this thesis by the lack of clinical value of series assay of $HMFG_2$ and TATI in addition to CA125 for example. Examination of one of these patient populations revealed that CA125 did not give any additional information to several other clinical parameters, including performance status, age and residual disease.

CA125 may therefore confirm what the clinician already knows or suspects. The value of such confirmatory information is perhaps impossible to determine, although Gore *et al.* (1989) at the Royal Marsden Hospital, London, have challenged a similar concept concerning the value of ultrasound and CT scanning in EOC management. At present, patient management is likely to continue along conventional lines, although routine adoption of novel techniques is becoming more widespread. In this climate of NHS reform, clinicians will increasingly be forced to rationalise their treatments of patients with far advanced cancer (Rees, 1991). Threats to treatment of advanced cancer patients have recently been highlighted in the media in the wake of cuts at Guy's Hospital, London, after opting for trust status.

The best chance an ovarian cancer patient has at present is optimal surgical management. Approximately half of the patients in this thesis were optimally

debulked, although Hacker *et al.* (1989) estimated 85% of all patients may feasibly be optimally resected. This problem has also been recently highlighted in the media. A Working Party was established by the Department of Health to investigate regional treatment variation and define acceptable surgical practices for a number of malignant conditions. Significant differences in five and ten year survival rates of patients with early stage ovarian cancer treated at local and specialist centres in the West of Scotland ("The Cancer Lottery" shown on Panorama, April 1991). Sophisticated management strategies should be available to all patients; unfortunately centres differ widely in the expertise they have to offer and consequently varaitions in outcome are to be expected.

10.2 FUTURE PROSPECTS

A better understanding of the pathogenesis of epithelial ovarian cancer will allow new treatment strategies to be developed. The most interesting in this respect is the work being carried out at the molecular level. Study of the molecular genetics of ovarian cancer is still in its infancy compared to breast, lung and colon cancer. This is not surprising given the large numbers of tumour specimens that need to be collected and the enormous effort required to isolate genetic alterations. A number of genetic abnormalities have been found in ovarian cancer, many in common with those found in breast cancer (for review see Steel, 1991). The tumour suppressor gene, p53, discovered by Lane in 1979, has been intensively studied in breast cancer, and is now receiving much attention in ovarian cancer. Eccles *et al.* (1990) recently reported a high incidence of p53 loss in patients with advanced serous EOC. Russell *et al.* (1990) also report a high incidence of deletion of chromosome 17 in EOC patients. In addition to needing a greater understanding of ovarian cancer pathogenesis, prospective randomised controlled trials addressing the value of CA125 in pre-clinical "serological" and radiological diagnosis of relapse are warranted. Intervention based on rising CA125 levels in the absence of other signs of progression in patients either previously untreated or who have responded to prior therapy may result in an improved prognosis for such patients.

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APPENDICES

APPENDIX A

UKCCCR RECOMMENDATIONS FOR SCREENING FOR EOC

1. Screening for ovarian cancer is of unproven benefit, and should not in the light of current knowledge, be offered as a routine test.

2. Further studies would be necessary before it could be stated whether ovarian cancer screening is effective in reducing deaths from this disease or what is the optimum combination and frequency of tests.

3. Further studies are required to identify high-risk groups. Famlilies identified as being "high-risk" should be referred to centres with a specific interest in the genetic aspects of the disease.

4. Screening of a high-risk group could provide results more quickly and less expensively than screening of a large population of average risk.

5. Any proposed study of a large population would have to be properly structured from the outset, piloted for patient acceptability and carefully monitored and would require extensive funding.

6. There is need for basic research into the malignant potential of benign and borderline ovarian tumours.

7. Every effort should be made to stage ovarian cancer accurately at diagnosis.

8. Patients suspected of having the disease should have a laparotomy performed by a gynaecologist, preferably one with a special interest in the condition.

APPENDIX B

ADDRESSES OF IMMUNOASSAY KIT SUPPLIERS

CA125, CA153, CA199, and CA724 Immunoradiometric assays

CIS U.K. Ltd.
Unit 5, Lincoln Park,
Business Centre, Lincoln Rd.,
High Wycombe, Buckinghamshire HP12 3RD.

TATI Radioimmunoassay

Farmos Diagnostica,
Farmos Group Ltd.,
SF-90460 Oulunsalo,
Finland.

C-neu p185 Enzyme linked immunoassay

Du Pont Company,
Billerica, MA 01862,
U.S.A.

$HMFG_2$ Enzyme linked immunoassay

Immunology Section,
Unilever Research,
Colworth Laboratories,
Colworth House,
Sharnbrook,
Bedfordshire MK44 ILQ.

ADDRESSES OF $HMFG_2$ ASSAY REAGENT SUPPLIERS

Sigma Chemincal Co. Ltd., Poole, Dorset, U.K.

Horseradish peroxidase (HRP), Tween 20 (polyoxyethylene sorbitan monolaurate), o-phenylenediamine, sulphuric acid (H_2SO_4), sodium borohydride ($NaBH_4$), sodium m-periodate ($NaIO_4$), hydrogen fluoride, thimerosal, bovine serum albumin (BSA), cyanogen bromide activated-sepharose.

BDH Chemicals Ltd., Glasgow, U.K.

Sodium acetate, acetic acid, citric acid, sodium carbonate (Na_2CO_3), sodium hydrogen carbonate ($NaHCO_3$), sodium chloride (NaCl), potassium chloride (KCl), di-sodium hydrogen phosphate (Na_2HPO_4), potassium di-hydrogen phosphate (KH_2PO_4), di-sodium hydrogen phosphate (Na_2HPO_4).

Pharmacia (Laboratory Separation Division), Milton Keynes, Bucks.

Protein A (affinity chromatography).

APPENDIX C

CHEMOTHERAPY REGIMES GIVEN TO EOC PATIENTS

(with typical doses)

Cisplatinum - 160mg/m^2 i.v. every 21 days.

Cisplatinum/Prednimustine - 160mg/m^2 i.v. cisplatinum every 28 days, prednimustine orally days 1-5.

Cispaltinum/α-interferon (i.p.) - 160mg/m^2 i.v. cisplatinum with 25 mu i.p. α-interferon every 21 days.

Prednimustine - 160 mg/m^2 orally days 1-5 every 21 days.

Chlorambucil - 10mg orally for 5-7 days every 21 days.

Carboplatin - 400 mg/m^2 i.v. every 21 days.

5-Fluorouracil/Prednimustine/Hexamethylmelamine/Cisplatin - 600 mg/^{2} i.v. 5-fluorouracil days 1 and 8, 15mg/m^2 oral prednimustine days 2-14, 150 mg/m^2 oral hexamethylmelamine days 2-14, and 30mg/m^2 i.v. cisplatinum days 1 and 8. Cycles repeated every 28 days.

APPENDIX D

SCORING OF TUMOUR MARKER RESULTS

Tumour marker results were retrospectively scored true positive (**TP**), true negative (**TN**), false positive (**FP**) or false negative (**FN**). A marker result was positive if the level obtained was higher than the assigned cut-off value and negative if the level obtained was lower than the assigned cut-off value.

Results were scored at least six months in retrospect using all clinical, radiological and surgical information available at the time of marker assay and proceeding marker assay. If a patient had no clinical, radiological or surgical evidence of disease at the time of marker assay and remained in remission for the following six months, marker levels within the normal range were scored true negative and elevated marker levels were scored as false positive. If a patient had clinical, radiological or surgical evidence of disease at the time of marker assay or relapsed in the proceeding six months, elevated marker levels were scored true positive, and marker levels within the normal range during this time were scored as false negative.

It must be stressed that six months was an arbitrary cut-off point in time. After complete surgical resection, the majority of patients, who had elevated pre-operative marker levels, also have elevated post-operative marker levels. Surgical intervention often causes transient rises in serum marker levels which may take weeks to return to the normal range (see chapter 8, section 8.2, p 181). In addition, marker levels often become elevated in patients longer than six months before clinical or other evidence of relapse presents (see chapter 9, section 9.4, p 233). Long-term follow-up of patients is necessary to determine true marker scores. Consequently, a change in marker levels is more informative than a single value.

PUBLICATIONS

Publications arising from this project:-

Roulston,J.E.,Fisken,J.,Leonard,R.C.F.(1988). Screening for ovarian cancer using CA125 assays. Lancet;**ii**:171-172 (letter to the editor).

Fisken,J.,Leonard,R.C.F.,Shaw,G.,Bowman,A.,Roulston,J.E.(1989). Serum placental-like alkaline phosphatase (PLAP): a novel combined enzyme linked immunoassay for monitoring ovarian cancer. J.Clin.Pathol.;**42**:40-45.

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Fisken,J.,Leonard,R.C.F.,Badley,A.,Jonrup,I.,Aspinall,L.,Sturgeon,C., Roulston,J.E.(1991). Serological monitoring of epithelial ovarian cancer. Dis.Markers; **in press.**

arrhythmia or torsade de pointes. On the tenth day, replacement therapy with thyroxine, 25 µg progressively increased to 100 µg daily, and hydrocortisone, 30 mg daily, was started. One month later, the Q-T interval had shortened to 420 ms (Q-T_c 392 ms, 52/min) and peripheral thyroid hormone levels were normal. She had no palpitation, dizziness, or syncope during nine months of follow-up and the Q-T interval remained normal (400 ms) on the same treatment.

Torsade de pointes related to Q-T prolongation may be a cause of ventricular arrhythmias during hypothyroidism, as demonstrated in our patient and in the case reported by Kumar et al.[2] However, these two patients had secondary hypothyroidism due to panhypopituitarism. By contrast, the case reported by Guthrie et al[1] had peripheral hypothyroidism. Whether the association with other pituitary deficiencies increases the risk of torsade de pointes in Q-T interval prolongation due to hypothyroidism deserves further study.

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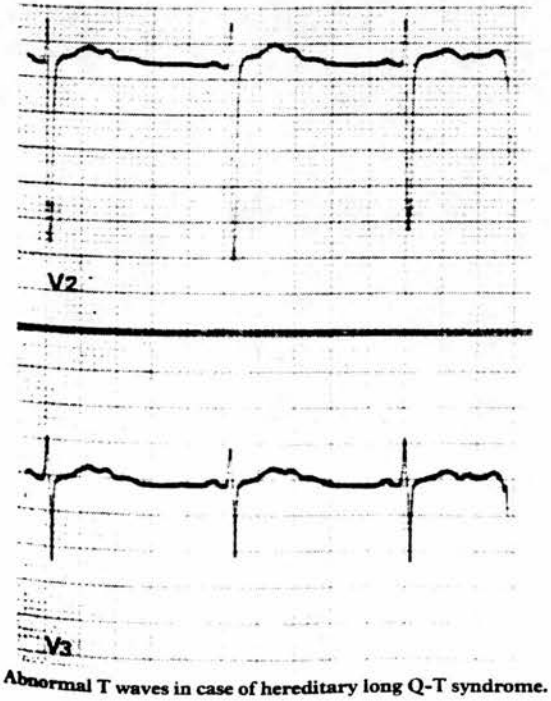
T WAVES IN LONG Q-T SYNDROMES

SIR,—Dr Attwell and Dr Lee (May 21, p 1136) emphasise the measurement of the corrected Q-T interval and its variation with heart rate in the diagnosis of a predisposition to arrhythmias. No mention was made, however, of the fact that in the hereditary prolonged Q-T syndromes, T waves with abnormal morphology are commonly found on the standard electrocardiogram (ECG),[1] and these can be a helpful pointer to the diagnosis.

The figure illustrates the ECG of a 12-year-old girl with Romano-Ward syndrome. These waves have been described as biphasic, bifid, or notched and may also be variably inverted. This abnormality is often much more striking at first glance than the prolongation of the Q-T interval, which may only become apparent after careful measurement and correction with Bazett's formula.



Abnormal T waves in case of hereditary long Q-T syndrome.

The mechanism is said to be differing repolarisation times in different areas of the ventricle. A cellular defect would therefore have to be patchy to produce this effect. In ischaemic heart disease, when similar changes are found,[2] sympathetic imbalance is at least as likely a cause as the myocardial damage. Similarly in alcoholic heart disease, an autonomic neuropathy could be the explanation for Q-T prolongation.

In the hereditary long Q-T syndromes, necropsy studies have failed to demonstrate a consistent abnormality, although James et al have described inflammation or degeneration of the intracardiac nerves and sinoatrial node.[3] There are several reasons for supposing, therefore, that the underlying abnormality may be an imbalance of sympathetic innervation rather than a myocardial cellular defect, at least on current evidence.

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1. Schwartz PJ. Idiopathic long QT syndrome: Progress and questions. *Am Heart J* 1985; 109: 399–415.
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SCREENING FOR EARLY OVARIAN CANCER

SIR,—Professor Campbell and colleagues (Mar 26, p 710) report their preliminary analysis of ultrasound screening for ovarian cancer. They acknowledge that the specificity of the screen was low, but suggest that the high detection rate and predictive value of a negative result "constitute the most important criteria for the evaluation of any screen for a lethal disease". As their screening programme detected all 5 cases of ovarian cancer which occurred in the study populaton during up to 2-years' follow-up, they conclude that ultrasound is "acceptable and effective".

Although high sensitivity is one essential attribute of a screen for cancer, other equally important criteria must be satisfied before a test becomes acceptable. Campbell et al report a specificity of 94·6% and that there was no evidence of morbidity or mortality among the 305 patients (300 false positives) who underwent laparoscopy or laparotomy. They did not, however, consider the psychological morbidity associated with recall for investigation of a positive screening test or the morbidity of laparoscopy or laparotomy even when uncomplicated.

In addition the complication rate of diagnostic laparoscopy is 29·9/1000 (major complication rate 3·9/1000).[1] The annual incidence of ovarian cancer among women over 45 years in the UK is 40/100 000.[2] If these figures are accurate a test with 96·4 specificity used to screen 100 000 women over 45 years of age would detect 40 cases (assuming 100% sensitivity) and produce 5398 false positive results requiring diagnostic surgery. Apart from the cost implications and the difficulty of persuading clinicians to act on a test with such a low positive predictive value, this would result in over 150 complications (21 major), if all patients with positive results were subjected to laparoscopy. Unless the specificity of ultrasound can be improved or benign ovarian tumours are shown to have malignant potential our contention (Feb 6, p 268) that ultrasound alone is unacceptable as a screen for ovarian cancer remains valid.

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2. OPCS cancer statistics, registrations. Cases of diagnosed cancer registered in England and Wales. London: HM Stationery Office, 1983.

SIR,—Jacobs et al[1,2] reported a sensitivity and specificity of 100% for identifying early ovarian cancer with a combination of serum CA-125 over 30 U/ml, positive vaginal examination, and abnormal pelvic ultrasound. However, because of the insensitivity of vaginal examination all subjects with CA-125 over 30 U/ml (31/1010) were examined by ultrasound. 3 were abnormal and were operated on, but only 1 had ovarian cancer—a positive predictive value (PPV)

(true positives/all positives) of 33% in those needing surgery to confirm the diagnosis. In prospective studies the PPV may be lower still; defining a cut-off value on the basis of a population group and then applying that value back to the same group is always likely to increase apparent discrimination.[3] Furthermore, with the interassay coefficient of variation of 9·1%, the 95% confidence interval about their one true positive (in whom CA-125 was 32 U/ml) would be 26 to 38—an equivocal result to say the least. The more usual cut-off value established[4] is 35 U/ml; in that case their sensitivity of 100% (1/1) would fall to 0% (0/1).

Ovarian cancer has a prevalence of 15/100 000.[5] Therefore a test with 100% sensitivity and 99·9% specificity will still yield a PPV of only 13%. Cruickshank[6] suggested a marker panel of CA-125, $HMFG_2$, and placental alkaline phosphatase (PLAP) as more appropriate for screening, and has found an increase in detection rate in patients with localised disease to 64%.[7] He also makes the vital point that such an approach "may increase the false positive rate". It is not difficult to increase apparent sensitivity by sequential assays; tossing a coin four times will yield a pick-up of 93% in a disease-positive group like those studied by Cruickshank and his colleagues. The problem is that specificity will fall with equal sharpness. In our hands PLAP assay did not increase the PPV obtained with CA-125 alone, whether analysed in parallel or in series.[8] Furthermore we find $HMFG_2$ assay in serum to be disturbingly non-reproducible.

A comparative study between the principal CA-125 assays, as suggested by Cruickshank,[6] has been undertaken,[9] and the results indicated a discrepancy in standardisation between the CIS and Abbott assays. This difference does not help to establish true cut-offs. Screening for early ovarian cancer, in the absence of a perfect system, will be hindered by the low disease prevalence and will thereby lead to unacceptably high numbers of false-positives, some of whom, as we have seen, end up in the operating theatre.

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5. Piver SM. Ovarian malignancies: the clinical care of adults and adolescents. Current reviews in obstetrics and gynaecology 4. Singer A, Jordan J, eds. Edinburgh: Churchill Livingstone, 1983: 1.
6. Cruickshank DJ. Screening for ovarian cancer by CA-125 measurement. *Lancet* 1988; i: 540–41.
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MECHANISM OF NITRATE VASODILATORS

SIR,—Dr Yusuf and colleagues (May 14, p 1088) present evidence from an overview of clinical trials that intravenous nitrates reduce mortality in acute myocardial infarction, "the reduction being non-significantly greater with nitroglycerin than with nitroprusside." The lesser effect of nitroprusside has implications about the mechanism of action of "nitrovasodilators" in myocardial infarction. The nitrovasodilators relax vascular smooth muscle by the action of their active moiety, nitric oxide, on soluble guanylate cyclase.[1] Platelet aggregation is also inhibited by elevation of cyclic GMP levels.[2,3] Nitroprusside and nitroglycerin induce similar vasodilator effects at therapeutic concentrations, but platelet aggregation (measured ex vivo and in vitro) is inhibited only by nitroprusside (ref 4 and our unpublished observations), probably because platelets generate nitric oxide from nitroglycerin only at concentrations far in excess of those achieved therapeutically. This suggests that the beneficial effect of nitrovasodilators in myocardial infarction is not attributable to a direct action on platelet function.

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1. Ignarro LJ, Lippton H, Edwards JC, et al. Mechanism of vascular smooth muscle relaxation by organic nitrates, nitrites, nitroprusside and nitric oxide: Evidence for the involvement of S-nitrosothiols as active intermediates. *J Pharmacol Exp Ther* 1981; 218: 739–49.
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EXTREME LABILE BLOOD PRESSURE IN GUILLAIN-BARRÉ SYNDROME

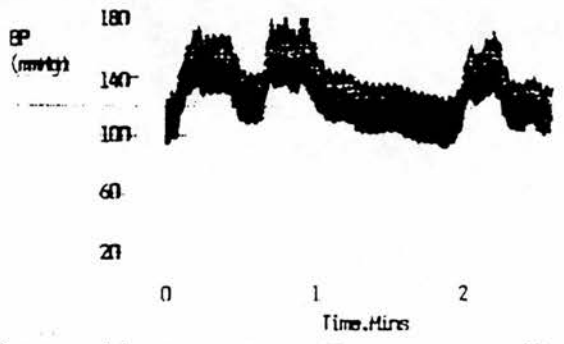
SIR,—Hypertension is a common manifestation of the autonomic disturbances sometimes associated with Guillain-Barré syndrome. Lichtenfeld[1] noted impressive lability of blood pressure with periodicity measured in hours or days. We report here a patient whose blood pressure fluctuated over seconds.

A 14 kg, 4-year-old boy presented with diplopia, ataxia, and developing paralysis, and areflexia. He was ventilated and sedated with diazepam ('Diazemuls'). CSF protein subsequently rose to 1·2 g/l. He eventually recovered fully.

Autonomic disturbances included profuse salivation, gastrointestinal motility, various arrhythmias, and hypertension. On the ninth day of his illness he had a seizure, manifest as slight twitching of one hand, an obtunded level of consciousness and fine, rapid eye movement on fundoscopy. His serum sodium was 131 mmol/l; glucose, calcium, magnesium, and phosphate all normal, as was a computerised tomographic scan. Papilloedema was absent. Direct arterial monitoring revealed the spectacular lability of his blood pressure. The accompanying figure illustrates blood-pressure changes over 3 min; these changes were spontaneous and occurred despite infusions of morphine 1·5 mg/h, midazolam 2 mg/h, hydralazine 2 mg/h, and propranolol 2 mg/h. 24 h urinary hydroxymethylmandelic acid (HMMA) was 22 μmol, three times the upper limit for a normal 14 kg child. Further seizures were noted on EEG, though these were not clinically apparent because of the paralysis.

Control of the seizures was achieved by control of blood pressure and anticonvulsants (phenobarbitone and later phenytoin). Autonomic dysfunction improved in parallel with neurological recovery. At follow-up there was no neurological deficit and normal blood pressure and urinary HMMA levels were normal.

Despite the absence of papilloedema we concluded that the seizures were secondary to hypertensive encephalopathy. This episode has changed our management of patients with Guillain-



Direct arterial pressure print-out illustrating extreme lability of blood pressure over 3 min period, without stimulation and with sedation and antihypertensive medication.

J Clin Pathol 1989;**42**:40–45

Serum placental-like alkaline phosphatase (PLAP): a novel combined enzyme linked immunoassay for monitoring ovarian cancer

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SUMMARY A new combined enzyme linked immunoassay (ELISA) was developed to measure both serum placental-like alkaline phosphatase (PLAP) activity (PLAP A) and concentration (PLAP C) in the same microtitre plate using an Imperial Cancer Research Fund monoclonal antibody, designated H17E2. PLAP A and PLAP C were determined together with an existing marker, CA125, in 397 serial samples from 87 patients with epithelial ovarian cancer. Retrospective assessment showed the sensitivity to increase from 73% with CA125 alone, to 88% using CA125 and PLAP A, and to 93% with all three markers in 261 samples from the patients with known active disease at the time of sampling. When the results for all 397 samples were included in the analysis, however, the specificity, sensitivity, accuracy and predictive powers of this monoclonal antibody were not sufficiently high to assist in the prospective follow up of patients with ovarian cancer. This was due to a significant number of false positive and false negative results.

Our data indicate that PLAP A or PLAP C estimation with H17E2 may, therefore, only be of value in the management of those patients with known active disease who are already known to be "marker positive" for this antigen.

Ovarian cancer produces few local symptoms and presents late in most cases; consequently the mortality is high. Attempts to improve the detection of early stage disease have foundered on the lack of a reliable clinical or radiological screening test. In the past five years screening work has focused on the identification of serological tumour markers which could facilitate earlier diagnosis and disease monitoring, a vital step towards improving the survival of these patients. Although numerous tumour markers have been identified to date,[1] none is specific enough to warrant its use as a primary diagnostic tool, but several have proved useful for monitoring the course of disease.[2] Since the publication of the initial report by Bast *et al*,[3] CA125 has become the accepted test with which other markers are compared.

There has been considerable interest shown recently in the application of placental-like alkaline phosphatase (PLAP) as a marker of epithelial ovarian cancer (EOC). Ectopic expression of PLAP was first discovered in a patient with squamous cell carcinoma of the lung[4] and has subsequently been found in various malignancies[5] including ovarian cancer. Raised serum concentrations of this oncofetal antigen have been found in 44%,[5] 35%,[6] and 40%[7] of patients with ovarian cancer.

PLAP is normally produced by the syncytiotrophoblast of the placenta and has been detected in sera as early as 9 weeks' gestation, increasing considerably during the second half of pregnancy.[8] It is normally undetectable in the sera of healthy subjects and it is this difference between normal adults and patients with cancer which affords it marker potential. Smoking, however, is an established cause of false positive results.[9]

Serum PLAP activity (PLAP A) and concentration (PLAP C) were determined in 387 healthy volunteers and 397 serial samples from 87 patients using a novel combined enzyme linked immunoassay (ELISA), developed by modification of two existing separate assays for PLAP A[10] and PLAP C.[11] Serum CA125 was also determined in all samples and evaluated with PLAP A and PLAP C in the patients with ovarian cancer. Both PLAP A and PLAP C were measured to investigate a recent report[11] that PLAP A decreased and PLAP C increased simultaneously with progression of disease.

Accepted for publication 4 August 1988

Subjects and methods

The Imperial Cancer Research Fund (ICRF) murine monoclonal antibody H17E2 used in this study was produced by immunisation with term placental membranes.[12] It reacts with a heat stable alkaline phosphatase that is more resistant to inhibition by L-Leu than Phe-Ala-Gly-Gly, confirming its recognition of term PLAP as opposed to other isoenzymes of the same family.[12]

Three hundred and ninety seven serial blood samples were collected from 87 patients with EOC over three years. The samples were separated by centrifugation at 1500 *g* for 10 min at 20°C and each serum sample stored in 0·5 ml portions at −20°C. Each sample was thawed once and assayed for PLAP A and PLAP C. A separate portion was assayed for CA125 using CIS ELSA-CA kits (CIS (UK) Ltd, High Wycombe, Buckinghamshire).

Upper limits of normal, defined as the 95th centile for PLAP A and PLAP C were established by assaying samples from 387 healthy blood donors after obtaining informed consent. Smoking habits were noted. All samples were tested in duplicate using a near term pregnancy serum pool as quality control material.

COMBINED PLAP A AND C ELISA

MicroELISA plates (M129B, Dynatech, Billingshurst, Kent) were coated overnight at 4°C with 100 μl/well 1·0 μg ml^{-1} H17E2 monoclonal antibody (supplied by courtesy of the ICRF, Lincoln's Inn Fields, London) in 50 mM carbonate buffer, pH 9·6. The plates were washed three times in 0·15 M phosphate buffered saline (PBS), pH 7·4, containing 0·05% v/v Tween 20 (PBS/Tween 20) to remove unbound antibody; 100 μl serum were then added and incubated for two hours at room temperature. After washing four times in PBS/Tween 20 100 μl phosphatase substrate: 5 mmol l^{-1} disodium p-nitrophenylphosphate (Sigma, Poole, Dorset) in 0·2 mmol l^{-1} diethanolamine buffer (BDH, Glasgow, Scotland) containing 0·5 mmol l^{-1} $MgCl_2$ (pH 9·8) were added and incubated for two hours at 37°C. Optical density was measured at 405 nm using a Titertek Multiskan MCC/340 spectrophotometer (Flow Laboratories, Irvine, Scotland) to determine PLAP A. The plates were then washed four times and 100 μl rabbit anti-human PLAP (Dakopatts, Denmark) at 1/250 dilution in PBS containing 0·5% w/v bovine serum albumin (BSA) were added and incubated for 30 minutes at room temperature. After washing three times in PBS/Tween 20 100 μl peroxidase-conjugated goat anti-rabbit IgG (Sigma, Poole, Dorset) at 1/1000 dilution in PBS/0·5% BSA were added and incubated for 30 minutes at room temperature. The plates were finally washed three times and incubated at room temperature for 45 minutes with 100 μl peroxidase substrate: 0·04% w/v o-phenylenediamine and 0·012% v/v H_2O_2 in 0·15 M citrate-phosphate buffer (pH 5·0) H_2SO_4 (50 μl 2·5 M) were added to stop the reaction and PLAP concentration determined by measuring optical density at 492 nm using a Titertek Multiskan MCC/340.

All patient samples were scored true or false by correlating the clinical state at the time of sampling with the antigen titre. Presence of disease was defined on clinical, radiological, or surgical grounds (laparotomy). Clinical disease activity was defined temporally as declared disease progression or reactivated disease within six months of assay. From this the sensitivity, specificity, accuracy and predictive values of PLAP were determined, alone and in combination with CA125.

DISCRETE PLAP C ASSAY

PLAP C was determined separately to show that preincubation with phosphate substrate for the activity assay did not have any deleterious effects on subsequent PLAP C assay performance in the combined assay.

The plates were coated in the same manner as in the combined assay. After the serum incubation step the plates were washed three times and rabbit anti-human PLAP added. The remainder of the assay was identical with that of the combined concentration assay.

Results

ASSAY PERFORMANCE

The correlation between the combined and discrete PLAP C assay was high ($n = 34$, $r = 0·97$, $y = 1·4\,x$, $p < 0·001$) (fig 1), supporting the use of a combined assay.

Based on results from the near term pregnancy serum pool, the between ($n = 160$) and within ($n = 46$) assay coefficients of variation were: 16% and 7·5% for PLAP A assay, 8·2% and 3·8% for combined PLAP C assay, and 13% and 4·4% for discrete PLAP C assay, respectively.

CORRELATION BETWEEN PLAP A AND C IN BLOOD DONORS

Using the results from 397 blood donors, PLAP A and C were poorly associated ($n = 387$, $r = 0·56$, $y = 0·16\,x$, $p < 0·001$), although still significantly correlated.

PLAP A did not seem to be influenced by smoking in either male or female blood donors ($p > 0·05$). PLAP C, however, did seem to be increased by smoking in both male and female donors ($p < 0·001$).

REFERENCE RANGE FOR PLAP A AND C

PLAP A and PLAP C were not normally distributed in

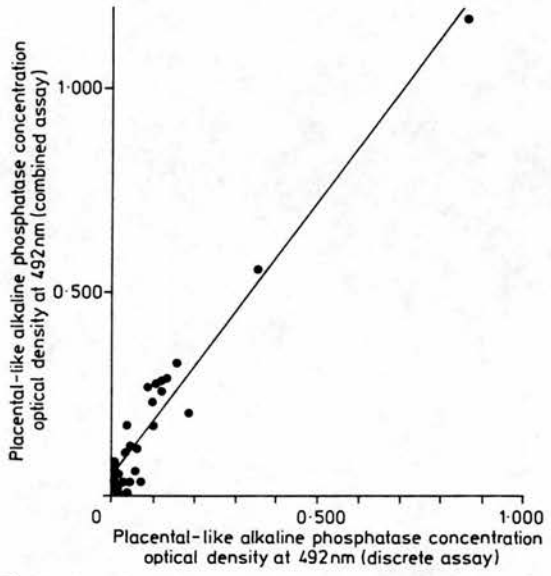

Fig 1 *Correlation between PLAP concentration measured alone and after activity in combined technique.*

the control population, neither did they normalise following logarithmic transformation as tested by Kolmogorov-Smirnov Goodness of Fit Test ($p < 0{\cdot}05$). Therefore, the 95th centiles of the control population, 0·400 and 0·085 for PLAP A and PLAP C, respectively, were used as cut off values. In smokers, however, the 95th centile of the PLAP C reference interval was 0·185; for PLAP A it was unchanged from the non-smokers' value. Therefore, PLAP C abnormality was defined as a value greater than halfway between the 95th centile for non-smokers and smokers because the smoking habits were known in only 10% of the patient population. Assigned cut off values were therefore 0·400 and 0·135 optical density units for PLAP A and PLAP C, respectively.

CORRELATION BETWEEN PLAP A AND C IN PATIENTS WITH CANCER

The correlation between PLAP A and C in patients with ovarian cancer ($n = 397$, $r = 0{\cdot}18$, $p < 0{\cdot}001$) was poorer than that for the blood donors, although still significant.

PLAP A, PLAP C AND CA125 CONCENTRATIONS

The proportion of samples with raised values of PLAP A, PLAP C, and CA125 are shown in table 1. Patients were divided according to the Fédération International de Gynecologie et Obstetrique (FIGO) stage and histopathology. Abnormal values were seen in all stages, but were more numerous in advanced (FIGO stages III and IV) disease.

The sensitivity, specificity, and accuracy of PLAP A, PLAP C, and CA125 for each stage and histopathological type are shown in table 2. Using these criteria PLAP A was more sensitive but less specific than PLAP C for stages III and IV disease, although both performed less well than CA125.

The sensitivity, specificity, accuracy and predictive values of a positive result (PVP) and of a negative result (PVN) for each marker are shown in table 3: all three markers were tested in all 397 samples. This would be the case in a prospective analysis of all sample results.

Table 4 shows the above indices when the combination of all three markers are considered together; A

Table 1 *Proportion of samples with raised marker values*

			No of sample results evaluated (TP and FP)		
	No of subjects	*No of samples*	*PLAP A (OD > 0·4)*	*PLAP C (OD > 0·15)*	*CA125 (35 U/ml)*
Blood donors	387	387	18 (5%)	5 (1%)	
Patients:					
Stage I:					
Serous	5	20	11	9	1
Mucinous	1	5	2	0	0
PDA	1	3	1	2	0
Stage II:					
Serous	4	19	6	2	4
Mucinous	1	3	2	0	0
Stage III					
Serous	40	204	122	81	108
Mucinous	3	12	9	6	3
PDA	12	47	21	5	22
Stage IV:					
Serous	15	64	44	24	44
PDA	5	20	15	4	14
Total	87	397	233 (59%)	133 (33·5%)	196 (49%)

PDA—Poorly differentiated adenocarcinoma.

Table 2 *Marker performance as assessed by histopathology and FIGO staging*

Stage (FIGO)	*Tumour marker*	*Sensitivity (%)*	*Specificity (%)*	*Accuracy (%)*
I	PLAP A	20	43	39
	PLAP C	60	65	64
	CA125	20	100	86
II	PLAP A	0	60	55
	PLAP C	0	90	91
	CA125	100	90	91
III	PLAP A	61	51	58
	PLAP C	37	69	46
	CA125	70	96	78
IV	PLAP A	78	67	76
	PLAP C	38	93	48
	CA125	83	93	85
I + II	PLAP A	14	51	46
	PLAP C	43	77	72
	CA125	43	95	88
III + IV	PLAP A	66	53	63
	PLAP C	37	73	46
	CA125	74	96	80
Histological type (stages I–IV):				
Serous	PLAP A	65	51	60
	PLAP C	42	71	51
	CA125	74	95	81
Mucinous	PLAP A	71	38	50
	PLAP C	14	62	45
	CA125	43	100	80
Poorly differentiated adenocarcinoma:				
	PLAP A	63	65	64
	PLAP C	21	95	44
	CA125	73	95	80

Table 3 *Individual CA125, PLAP A, and PLAP C results*

	CA125	*PLAP A*	*PLAP C*
Sensitivity	190/262 (73%)	169/262 (65%)	98/262 (37%)
Specificity	130/135 (96%)	71/135 (53%)	99/135 (73%)
Accuracy	320/397 (81%)	240/397 (60%)	197/397 (50%)
PVP	190/196 (97%)	169/233 (73%)	98/133 (74%)
PVN	130/201 (65%)	71/164 (43%)	99/264 (38%)

shows positive marker state where one or more than one marker in the panel was increased, and B shows the same indices when PLAP was tested only in the samples which were CA125 negative (so called "series testing").

SERIAL ANTIGEN TITRES IN TWO PATIENTS

Two patients were chosen after analysis of their serial antigen titres, (fig 2) to illustrate retrospectively if PLAP A and PLAP C had been useful for predicting relapse. In case 1 CA125 had been negative until well after clinically evident relapse, and in case 2 an increase in CA125, although preceding relapse, was a late event. An increase in PLAP A in case 1 and an increase in PLAP C in case 2 would have been earlier predictors of relapse. Both patients were followed up after a positive second look laparotomy. Case 1 was receiving chlorambucil when each sample was taken and case 2 received three cycles of Cis-platinum throughout the blood sampling period.

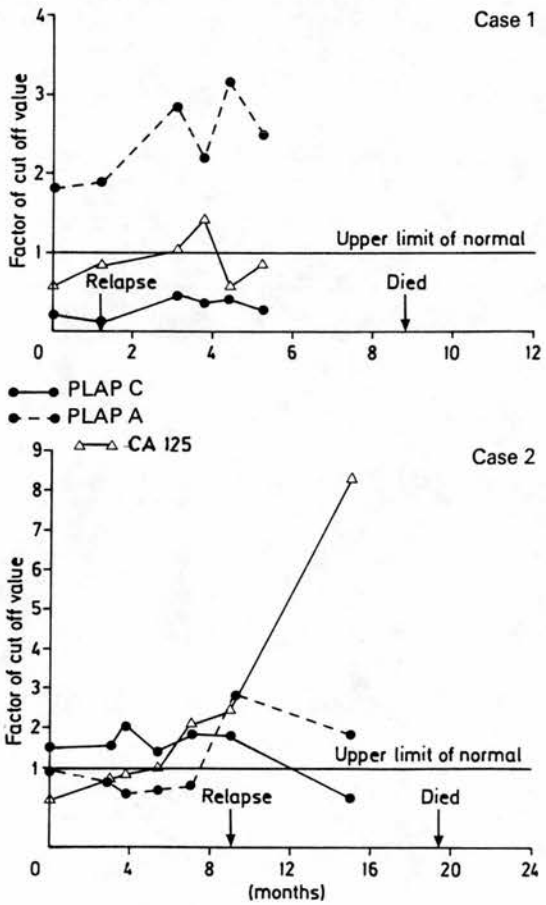

Fig 2 *Case 1: stage III adenocarcinoma. Case 2: stage III serous papillary adenocarcinoma.*

Table 4 *CA125, PLAP A, and PLAP C panel results*

	Sensitivity	*Specificity*	*Accuracy*	*PVP*	*PVN*
A: CA125, PLAP A and PLAP C where ⩾ 1 marker is positive	43/261 (93%)	57/136 (42%)	300/397 (76%)	243/322 (75%)	57/75 (76%)
B: PLAP testing in series on a negative CA125 result	53/71 (75%)	57/130 (44%)	110/201 (55%)	53/126 (42%)	57/75 (76%)

Discussion

Determination of both PLAP A and C in a combined assay has several advantages over separate assays. These include reduced expense (in particular halved monoclonal antibody costs) and reduced operator time and error due to sampling variation. The correlation between the combined and discrete PLAP C assay was high, supporting the use of the combined assay. The gradient of the slope was greater than 1·0, however, for which the reasons are unclear. The greater absorbance in the combined assay was not due to residual p-nitrophenyl phosphate substrate, which showed zero absorbance at wavelengths greater than 470 nm. In the combined assay the catalytic reaction may have induced a conformational change in PLAP which results in enhanced recognition and binding by the rabbit anti-human PLAP.

The data show that PLAP A and PLAP C assays, individually and in combination, are insufficiently sensitive and specific (tables 2 and 3) for the management of women with epithelial ovarian cancer. Several reasons for the failure of PLAP to fulfil expectations may be postulated. Changes in antigen expression during disease progression and increasing tumour dedifferentiation are complex, substantiated by the lack of close correlation we have found between PLAP A and PLAP C in cancer patients when compared with normal controls. These findings contrast with reports from another group who used a different monoclonal antibody raised against PLAP.[14]

Numerous factors influence the expression of PLAP, including smoking, which induces PLAP-like alkaline phosphatase synthesis and secretion by lung alveoli.[15] H17E2 recognises this isoenzyme,[16] which may in part account for a high proportion of false positive results in our series of patients. Whether a "smoking effect" was a source of error in this series is uncertain. The control sera showed a significant increase in the reference interval only in the PLAP C assay; in the patients' sera many more false positive results were seen with PLAP A than with the PLAP C assay. For PLAP C the use of a correction factor based on observations in the large control group might reasonably be expected to have reduced the false positive results associated with smoking. Unfortunately, information on the smoking habits in most patients was unobtainable, hence the effect of smoking on the patients' PLAP values could not be assessed properly.

PLAP has not previously been evaluated in terms of sensitivity, specificity, accuracy and predictive power,[16] although numerous reports advocate its use as a tumour marker in ovarian cancer.[5-8 11 14] When results for PLAP A and PLAP C were combined with CA125 results the overall sensitivity increased from 73% with CA125 alone to 93% with all three markers, where at least one gave a positive result (table 4a). Combining results in this way, however, resulted in a considerable loss of specificity, from 96% for CA125 alone to 42% for the combined results. This loss of specificity is better seen in terms of the relative predictive powers of the test. The PVP of CA125 alone was 97% with a PVN of 65% (table 3), whereas the combined results showed a fall of PVP to 75% and only a relatively small rise in PVN to 76% (tables 4a and b).

PLAP may be assayed in series[17] with CA125. This reduces the total number of PLAP assays required as only CA125-negative samples would require retesting, allowing the PVP of CA125 alone to be retained. Serial analysis of the patients' data (table 4b) showed that the use of PLAP A and PLAP C on samples negative for CA125 (n = 201) gave a PVP of both assays together of only 42%, with PVN remaining unchanged at 76%. It is clear, therefore, that assay of PLAP A and PLAP C did not add significantly to the predictive value of CA125 in these negative samples in which the active disease prevalence, as assessed clinically, was 71/201 (35%).

These data indicate that PLAP, as measured with this monoclonal antibody, confused the interpretation of CA125 results in this cohort of patients. CA125 assay used on its own would seem to be more helpful in clinical decision making. The results of this study agree with those of a recent report by Haije *et al*,[18] who also assayed PLAP activity and concentration by immunoreactivity, but found neither to be useful for general patient follow up and management.

Tucker *et al* have found determination of PLAP activity using H17E2 useful in the follow up of testicular germ cell tumours, particularly seminomas.[10] The applicability of the simple combined assay deserves to be tested in this and possibly other cancers.

The combined assay, using a more specific monoclonal antibody, one which does not react with PLAP induced by smoking,[6] may prove useful in the follow up of patients with ovarian cancer. Increased specificity would then be obtained, possibly at the expense of sensitivity—an acceptable modification in the context of a panel of markers where specificity of each marker is the most important criterion.

Although measurement of PLAP was not found to be generally helpful, a few patients may have benefited from additional PLAP assay (fig 2). It is impossible at this stage to judge which patients will benefit most from prospective serial measurement of PLAP. Appropriate patient selection is essential if PLAP is to be of use in future as an adjunct in monitoring ovarian cancer.

Unfortunately, insufficient preoperative samples were available in this study to assess the value of PLAP

assays in the untreated patient. Preoperative measurement of PLAP may provide a helpful indicator of patients who will benefit from further serial measurement in a manner analogous to CA125 where assay at the time the disease presents helps in the selection of "secretors".[19]

Despite initial promising investigations of PLAP as a tumour marker in ovarian cancer this study and others[18 20] have failed to corroborate them. Work is currently under way using various monoclonal antibodies to investigate further the PLAP molecules and epitopes in conjunction with assay development for other promising markers such as mucin antigens.[13]

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DISEASE MARKERS. VOL. 7, 61–67 (1989)

IMMUNOASSAY OF CA125 IN OVARIAN CANCER: A COMPARISON OF THREE ASSAYS FOR USE IN DIAGNOSIS AND MONITORING

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SUMMARY

There is increasing evidence to support the use of CA125 in the follow-up and management of patients with ovarian cancer and several commercial kits are now available for its measurement. This study investigated and compared the performance of three of them: an enzyme immunoassay (EIA) and an immunoradiometric assay (IRMA) from Abbott Diagnostics, and an IRMA from CIS, U.K.

One hundred and thirty-two serum samples from 42 patients with advanced epithelial ovarian cancer were thawed once and assayed for CA125 using each kit. Both IRMAs performed better than the EIA in terms of CV, sensitivity, specificity, and accuracy. The results confirm the usefulness of CA125 as a marker for ovarian cancer. However, discrepancies between results using different kits suggest the need for improved standardization.

KEY WORDS Ovarian cancer ELISA Immunoradiometric assay Sensitivity Specificity CA125

INTRODUCTION

Since the development of the CA125 immunoradiometric assay (IRMA) using the monoclonal antibody (MoAb) OC125 by Bast *et al.* (1983), many investigators have corroborated their initial findings, establishing CA125 as a useful tumour marker for ovarian epithelial cancer.

CA125 has been found in various tissues, both malignant and non-malignant (Bast *et al.*, 1983). Although not specific for ovarian cancer, CA125 is extremely useful in the follow-up of ovarian cancer patients. Serum antigen levels tend to correlate well with tumour burden and disease status (Lambert, 1987); rising levels are associated with disease progression, whilst falling levels are associated with disease regression. However, a negative result does not preclude the presence of disease (Lambert, 1987); up to 25–30 per cent may be false negative. This is particularly true with mucinous ovarian epithelial carcinomas, where greater than 50 per cent may be false negative (Bast *et al.*, 1983). An increasing level of CA125 was

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found to precede clinical recurrence by a median lead-time of 3 months in one study (Niloff *et al.*, 1986). Recently, the significance of pre-operative CA125 has been reported. It has been suggested that a low pre-operative serum CA125 level is indicative of a favourable prognosis, while a high pre-operative CA125 carries a poorer prognosis (Cruickshank *et al.*, 1987). Pre-operative assay also aids in the selection of 'secretors' whose management may be aided by further serial measurement.

As a result of the relatively low false positive rate in comparison to other markers, CA125 assay is being investigated as a screening test for earlier diagnosis in the 'at risk' population, namely post-menopausal women. Preliminary results from the first U.K. study where an abnormal CA125 and vaginal examination were followed up by pelvic ultrasound have led the authors to suggest the need for further randomized trials (Jacobs *et al.*, 1987, 1988).

Our study investigates and evaluates the performance of three of the commercial assays currently available—an enzyme immunoassay (EIA) and an IRMA from Abbott Diagnostics, and an IRMA from CIS (U.K.)—when used in the assay of samples from patients with ovarian cancer. The results are evaluated in the context of monitoring patients with advanced disease and consideration is also given to the implications of screening for early diagnosis using the CA125 assay.

PATIENTS AND METHODS

Patients

One hundred and thirty-two serial serum samples from 42 patients with FIGO† stage I–IV epithelial ovarian cancer were assayed for CA125 using the kits described. The majority of patients had FIGO stage III and IV disease. All samples were obtained in the post-operative follow-up period.

Methods

The assays were carried out according to manufacturers' instructions supplied with each kit. All kits operate on the same principle: a monoclonal sandwich is formed between fixed OC125 MoAb, CA125 present in the sample, and a radiolabelled (IRMA) or enzyme-labelled (EIA) OC125 tracer MoAb. The antigen concentration is therefore proportional to the amount of tracer bound. All results are interpolated from a standard dose–response curve. The IRMA data were subjected to computer curve fit and analysis by the RIA-Calc program (LKB). The EIA data were analysed and evaluated manually.

The antigen results were scored retrospectively (true or false and positive or negative) according to disease status at the time of sampling. A positive CA125 by any assay was defined as >35 U ml^{-1}, following the initial recommendations of

†Federation International de Gynecologie et Obstetrique.

Table 1. Summary of assay performance

%	Abbott IRMA	CIS IRMA	Abbott EIA
Sensitivity	90·7	80	83·3
Specificity	81·3	92·5	78·8
Accuracy	85·3	87·4	80·6
C.V.	6–10	6–10	10–15
PVP	77·8	88	74·5
PVN	92·4	88	88

Bast *et al.* (1983). Disease status was assessed clinically or surgically (second-look laparotomy).

The sensitivity, specificity, and accuracy of each kit were defined by aggregating the frequencies of true positive (TP), true negative (TN), false positive (FP), and false negative (FN) results. Thus,

$$\text{Sensitivity} = \frac{TP}{TP+FN} \times 100\%$$

$$\text{Specificity} = \frac{TN}{TN+FP} \times 100\%$$

$$\text{Accuracy} = \frac{TP+TN}{TP+TN+FP+FN} \times 100\%$$

For prospective assay and subsequent clinical usage, it is necessary to know the predictive value of a test, i.e. the chance that a test result is correct. The predictive value of a positive result (PVP) and negative result (PVN) were calculated using the following formulae:

$$\text{PVP} = \frac{TP}{TP+FP} \times 100\% \qquad \text{PVN} = \frac{TN}{TN+FN} \times 100\%$$

The sensitivity, specificity, accuracy, coefficient of variation, and predictive values of each kit are shown in Table 1.

RESULTS

Assay Performance

The CVs for each kit (Table 1) show that the IRMAs operate with satisfactory reproducibility within the working range. In terms of precision, both IRMAs were significantly better than the EIA.

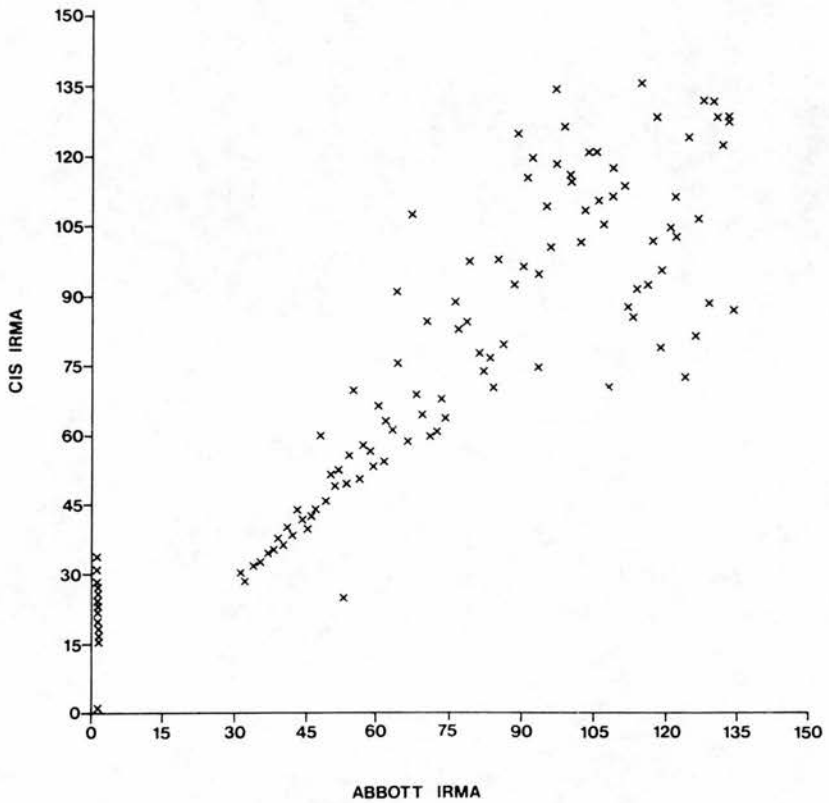

Figure 1. Correlation between Abbott IRMA (x axis) and CIS IRMA (y axis)

The results were correlated using Spearman's Rank Correlation coefficient. The correlation between the IRMAs was good ($n=132$, $r=0{\cdot}93$, $p<0{\cdot}001$, $y=0{\cdot}86x+10{\cdot}7$) but the slope was significantly less than 1·0 (Figure 1). The EIA correlated less well with the IRMAs ($r=0{\cdot}85$, Figure 2, and $r=0{\cdot}86$, Figure 3).

With a slope of less than 1·0 for the correlation between the Abbott IRMA and CIS IRMA, the CIS IRMA consistently gave lower antigen levels than the Abbott IRMA. As the upper limit of normal is the same for both IRMAs, this gave rise to discrepancies in 11/132 (8 per cent) of sample results. Thus, of 11/132 samples judged negative by CIS IRMA but positive with Abbott, five were TP, three were FP, and three (recent samples) need further clinical follow-up.

DISCUSSION

Both IRMA perform better than the EIA in terms of CV, sensitivity, specificity, accuracy, and predictive values (Table 1). In addition, the IRMAs are less time-consuming and cumbersome to carry out than the EIA. Therefore, the IRMA is our method of choice for the CA125 assay. However, although the correlation between the Abbott and CIS IRMAs was good (Figure 1) the slope of the correlation line was

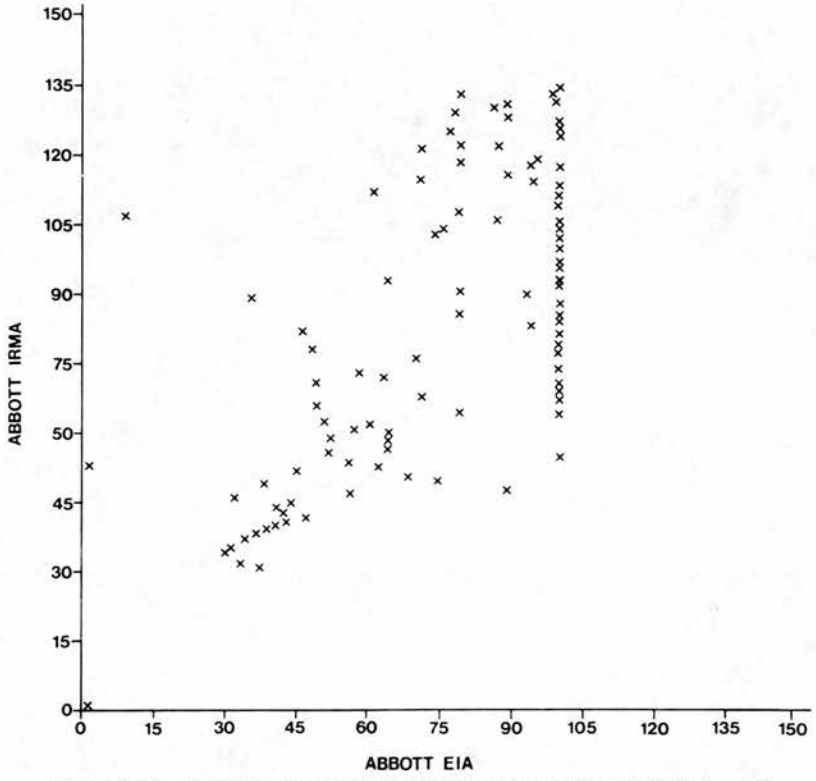

Figure 2. Correlation between Abbott EIA (x axis) and Abbott IRMA (y axis)

significantly less than 1·0, which led to some samples being erroneously classified. Both IRMA manufacturers recommend 35 U ml^{-1} as the upper limit of normal, suggesting a discrepancy in standardization and a need for re-definition of the cut-off value.

The major problem with CA125 assay is the relatively high proportion of false negative results; these reduce overall assay sensitivity (Schilthius *et al.*, 1987), and were particularly noticeable in the CIS IRMA. Although CA125 is not specific to ovarian cancer, false positives are rarely seen in this population and do not constitute a major problem since this marker's main use is in monitoring the progress of established ovarian cancer patients during follow-up therapy. The highest predictive value of a positive test (PVP) was 88 per cent in this study using the CIS IRMA. This would have to be greatly improved to avoid unnecessary trauma to individuals with false positive results, if use of the test were to be extended for profiling lower-risk groups, i.e. if it were to be used for purposes other than the management of known cancer patients. Recent reports (Jacobs *et al.*, 1987, 1988) have, however, suggested such a role for CA125 as part of a multimodal screening system for early diagnosis, but these conclusions have been called into question (Cruickshank, 1988; Roulston *et al.*, 1988).

Ovarian cancer has a prevalence of 15/100 000 of the population (Piver, 1983), therefore a test with 100 per cent sensitivity and 99.9 per cent specificity will produce an unacceptably low PVP of 13 per cent.

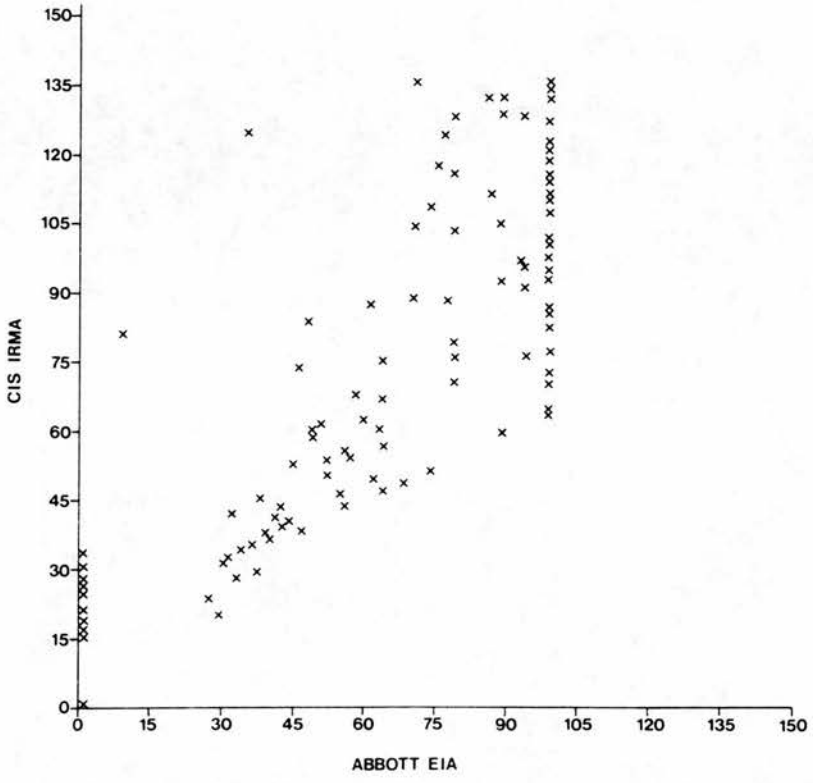

Figure 3. Correlation between Abbott EIA (*x* axis) and CIS IRMA (*y* axis)

In the light of our investigation, improved standardization of the CA125 assay may be advantageous in that it may lead to increased sensitivity when used alone or with a panel of markers as in the current trend (Bast *et al.*, 1984; Dhokia *et al.*, 1986; Knauf *et al.*, 1985; Ward *et al.*, 1987). This may facilitate earlier detection of recurrence and consequently improve management of established ovarian cancer patients.

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