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Glycoconjugates as prognostic markers in ovarian cancer

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Working hypothesis

Ovarian cancer has a high mortality, which is mainly caused by the intraperitoneal spread of the tumour cells. This metastatic spread is caused by the ability of the tumour cells to migrate away from the site of the primary tumour and to attach at a distant site within the peritoneum. During this process, cell to cell and cell to matrix interactions are crucial, both processes being mediated by the cell membrane. As the outer coat of all mammalian cells, including cancer cells, consists of a glycocalix, a carbohydrate rich coat, it is proposed that changes in the glycosylation of this coat are associated with intraperitoneal spread. In order to test the hypothesis, that changes of the glycosylation are associated with metastasis, the tumour cells' glycocalix will be investigated by the use of lectins, carbohydrate binding proteins. Furthermore, the expression of the glycosylated cell adhesion molecules CEACAM1 and EGP-2 in ovarian cancer will be immunohistochemically detected. The results of the lectin and cell adhesion molecule studies will then be correlated with the clinical outcome to analyse the prognostic significance of the glycotope and cell adhesion molecule expression.

1 Introduction

1.1 Ovarian cancer

Ovarian cancer is the seventh commonest cause of cancer deaths in women in Germany. On average, 9670 women per year are diagnosed with an ovarian malignancy [ABKD 2004] and in the year 2000 alone 6 100 women died due to this tumour in Germany [Hamburger Krebsdokumentation 1999-2001].

Ovarian cancer has an overall five-year survival of just 46.4% [Heintz et al. 2003]. Although it represents just 5% of malignancies in women, it causes more deaths than any other malignancy of the female genital reproduction tract [ABKD 2004]. At the beginning of the disease there are hardly any symptoms, so that most patients call on the physician in a late stage of the disease. At this time the tumour has already spread intraperitoneally and has formed a palpable tumour mass, which already represents a stage with a poor prognosis. The late detection and the early intraperitoneal spread contribute primarily to the high death toll of this cancer. The aetiology of ovarian cancer is not completely elucidated but the risk for ovarian cancer raises with the number of ovulation cycle during lifetime [ABKD 2004].

Ovaries are composed of a covering epithelium on its surface and a connective tissue stroma inside. The germ cells in various stage of differentiation are scattered within this connective tissue stroma. Cells derived from all these tissues can undergo benign or malignant transformation and form tumours, which are classified according to their tissue of origin.

Tumours derived from the surface epithelium represent the major tumour group making up to 70% of all ovarian tumours and 90% of the malignant ones. These epithelial ovarian tumours are further subdivided according to the WHO histological classification of tumours of the ovary [Tavassoli und Devilee 2002] (Table 1.1). From these subtypes, the

serous type is the most frequent, accounting for up to 40-53% of all malignant tumours. Other tumour entities like germ cell tumours or tumours derived from the gonadal stroma are uncommon and are therefore not included in the study. The tumours metastatic to the ovaries (most commonly breast or colon cancer) represent only 6-10% of ovarian tumours [Rubin und Faber 1999] and are also not investigated in our study.

Table 1.1: Surface epithelial tumours of the ovary.

The most commonly occurring subtypes of epithelial tumours according to the WHO histological classification of tumours of the ovary are listed. The frequency of each subtype in percentage of all tumours of the ovaries is indicated (modified after Schmidt-Matthiesen et al. 2002).

| Epithelial tumours | Frequency [%] |
|---|---------------|
| Serous tumours | 40-53 |
| Mucinous tumours | 7-15 |
| Endometrioid tumours | 15-22 |
| Clear cell tumours | 4-10 |
| Transitional cell tumours | ~ 1 |
| Other (including undifferentiated carcinomas) | 5-17 |

1.2 Prognostic factors in ovarian cancer

Several factors of essential independent prognostic significance exist according to guidelines of the German Cancer Society and the German Society of Gynaecology and Obstetrics. The tumour staging defined by FIGO (Fédération Internationale de Gynécologie Obstétrique) and by the UICC (Union Internationale Contre le Cancer) together with tumour grading are the most commonly used prognostic factors.

The FIGO staging describes the macroscopic spread of the tumour. Defined criteria rank the FIGO staging from I to IV. In addition, FIGO stages I to III can be further subdivided in a, b and c. This for instance is of particular importance in patients with a tumour in FIGO stage I. A tumour classified as FIGO stage I a is limited to one ovary and a tumour classified as stage I b is limited to both ovaries. But in a tumour classified in FIGO

stage I c the tumour cells have already left the primary tumour tissue and spread into the peritoneal cavity (FIGO stage I c) (Table 1.2).

The UICC subdivide tumour staging in T, N and M. The category T defines the local growth of the primary tumour, the category N defines the involved regional lymph node metastases and M defines distant metastases. Both classifications, FIGO and UICC, have been made comparable [Heintz et al. 2001].

In contrast to staging at the macroscopic level, the grading describes the microscopic differentiation of a malignant tumour. UICC guidelines define grading as follows:

G1 Well differentiated

G2 Moderately differentiated

G3 Poorly or undifferentiated

Gx Grade cannot be assessed.

Poorly differentiated tumours are of higher malignancy than well differentiated ones. This is reflected by a declining 5-year-survival with ascending grading in Figure 1.1. A combination of the factors grade and stage shows that a well differentiated tumour (G1) in FIGO stage III or IV has a reduced 5-year-survival by more than half, instead of a well differentiated tumour (G1) in FIGO stage I or II. This is representative for all degrees of differentiation grades (see Figure 1.1, according to data by [Schmidt-Matthiesen et al. 2002]).

The most frequently used serum marker for patients with ovarian cancer is CA 125. Not all tumour types express this serum marker, so that CA 125 analysis is insufficient as a screening tool. However, if initially present its serum level can be used to predict a relapse after surgical removal of the primary tumour.

The therapy of choice for a particular patient is strongly influenced by the first presentation of the malignancy. In the majority of cases tumour excision including the contralateral adnexa and the major omentum is not avoidable. However, these operations are often only of a palliative character and adjuvant chemotherapy is needed, as residual malignant tissue remains. According to new insights radiotherapy is not a therapeutic option any longer [Heintz et al. 2003]. The remaining residual tumour mass is an independent prognostic factor of epithelial ovarian carcinoma and therefore has important influences on patients survival [Schmidt-Matthiesen et al. 2002]. During initial surgery with an intent to cure it is of pivotal importance to prevent a capsule rupture due to a shortened patients survival effected by dissemination of tumour cells when the capsule has ruptured [Vergote et al. 2001, Sainz de la Cuesta et al. 1994].

Table 1.2: Data for different UICC and FIGO stages.

UICC and FIGO stage describe the macroscopic spread of the tumour of the ovaries. The higher the staging level is the shorter patients 5-year survival. [WHO Classification of Tumours 2002 (Tavassoli et al. 2002), Five-year survival in percentage: patients treated in 1996-1998. FIGO Annual Report 2003 (Heintz et al. 2003)].

| TNM Categories | FIGO Stages | Criteria | 5-year-survival [%] |
|----------------|-------------|---|---------------------|
| TX | | Primary tumour cannot be assessed | |
| T0 | | No evidence of primary tumour | |
| T1 | I | Tumour limited to the ovaries | |
| T1a | IA | Tumour limited to one ovary; capsule intact, no tumour on ovarian surface; no malignant cells in ascites or peritoneal washings | 89.3 |
| T1b | IB | Tumour limited to both ovaries; capsule intact, no tumour on ovarian surface; no malignant cells in ascites or peritoneal washings | 64.8 |
| T1c | IC | Tumour limited to one or both ovaries with any of the following: capsule ruptured, tumour on ovarian surface, malignant cells in ascites or peritoneal washings | 78.2 |
| T2 | II | Tumour involves one or both ovaries with pelvic extension | |
| T2a | IIA | Extension and/or implants on uterus and/or tube(s); no malignant cells in ascites or peritoneal washings | 79.2 |
| T2b | IIB | Extension to other pelvic tissues; no malignant cells in ascites or peritoneal washings | 64.3 |
| T2c | IIC | Pelvic extension (2a or 2b) with malignant cells in ascites or peritoneal washings | 68.2 |
| T3 and/or N1 | III | Tumour involves one or both ovaries with microscopically confirmed peritoneal metastasis outside the pelvis and/or regional lymph nodes metastasis | |
| T3a | IIIA | Microscopic peritoneal metastasis beyond pelvis | 49.2 |
| T3b | IIIB | Macroscopic peritoneal metastasis beyond pelvis 2 cm or less in greatest dimension | 40.8 |
| T3c and/or N1 | IIIC | Peritoneal metastasis beyond the pelvis more than 2 cm in greatest dimension and/or regional lymph nodes metastasis | 28.9 |
| M1 | IV | Distant metastases (excludes peritoneal metastasis) | 13.4 |

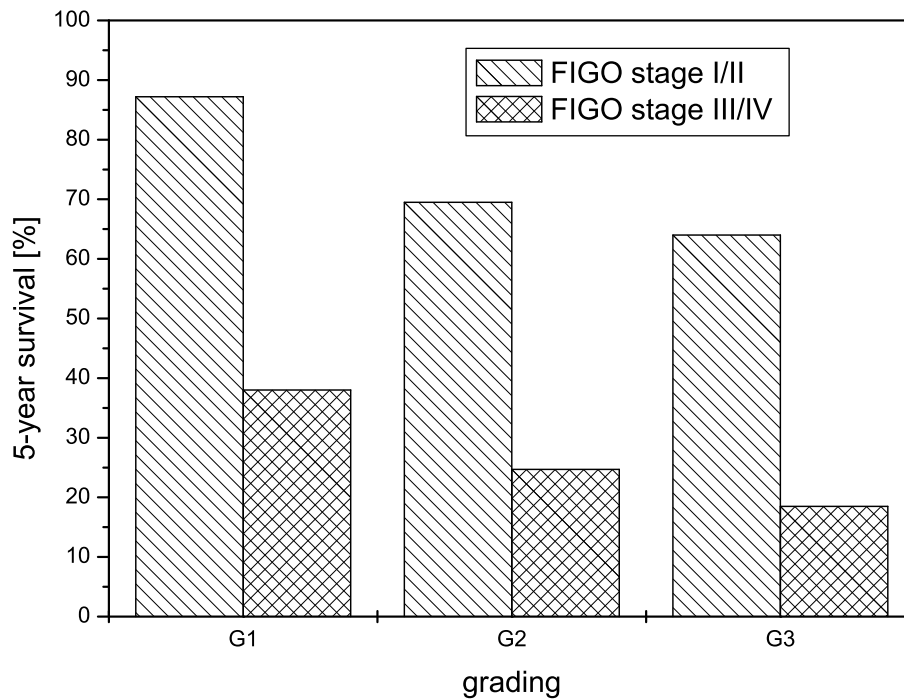


Figure 1.1: Grading and FIGO stage related to 5-year-survival in ovarian tumours.

The 5-year-survival rate declines with ascending grading. A combination of tumour grading and FIGO stage shows the important influence of different FIGO stages. (FIGO Annual Report 1998, modified after Schmidt-Matthiesen et al. 2002)

In addition to the previous factors and also of independent prognostic significance are the age and constitution of the patient and the histological entity of the ovarian tumour. Undifferentiated and clear cell tumours are rather of higher malignancy than transitional cell carcinomas [Dt Krebsgesellschaft 2002]. Compared to endometrial or mucinous type carcinomas the prognosis of serous carcinoma is particularly poor [Schmidt-Matthiesen et al. 2002].

The average age of women with a tumour of the ovaries is 66 years [Hamburger Krebsdokumentation 1999-2001]. As seen in Table 1.3 the incidence rises with advanced age. In general, tumours of younger women are more often of low malignant potential than those in older women and furthermore, younger women with advanced stage invasive epithelial ovarian cancers have in general a better prognosis than older women [Chan et al. 2003].

Table 1.3: Mortality and incidence of ovarian cancer in Germany in the year 2000.

The incidence and the mortality rise with increasing age of the patient [ABKD 2004]. The numbers in the table refer to cases per 100.000.

| Women age in years | Incidence | Mortality |
|--------------------|-----------|-----------|
| under 45 | 4.8 | 0.8 |
| 45 to 60 | 23.7 | 11.7 |
| 60 to 75 | 50.7 | 33.3 |
| 75 and over | 76.0 | 64.4 |
| overall | 23.0 | 14.5 |

1.3 Mechanisms of metastatic spread

The prognosis for women with ovarian cancer is mainly determined by the spread of the disease. If the tumour has spread beyond its anatomical site it is practically incurable.

A special anatomical feature of ovarian tumours is that tumour cells spread in the majority of cases into the peritoneal cavity (FIGO stage I c, as described in chapter 1.2). The peritoneal fluid is used as a transport vehicle and thus tumour cells may spread as far as to the liver and diaphragm from where they may penetrate into the pleural cavity. Lymphatic spread is also common in ovarian cancer affecting mostly pelvic and paraaortal lymph nodes. Rather unusual and often in a terminal stage or in a relapse metastatic deposits are formed through haematogenous spread into the lung or bone marrow [Schmidt-Matthiesen et al. 2002].

The metastatic process is composed of a sequel of single steps so far inadequately understood starting with the loosening of individual tumour cells or small clusters from the primary tumour. Once they are enabled to migrate and do so, they interact with the surrounding extracellular matrix and the neighbouring cells. If they have migrated into the peritoneal cavity, they have to survive as single cells or small lumps of cells to adhere to the mesothelial cells that form the peritoneal lining. After they have accomplished adherence, they proliferate locally and form a secondary tumour mass. Cell to cell and cell to matrix interactions of those cells, mediated by the cell membrane surrounding all human cells, are of particular importance in this process.

The cell membrane's basic structure consists of a lipid bilayer, in which membrane proteins are embedded. On the outside of the cell membrane carbohydrate chains are covalently linked to both lipids and proteins, collectively called glycoconjugates. All these glycoconjugates form the glycocalyx as the outermost coat of the cells (Figure 1.2).

As metastasis represents a breakdown of the normal intercellular communication, many studies have analysed the carbohydrate residues of malignant cells in order to detect abnormalities in the sugar residues indicative of metastasis. To analyse these carbohydrate residues lectin histochemistry has been employed.

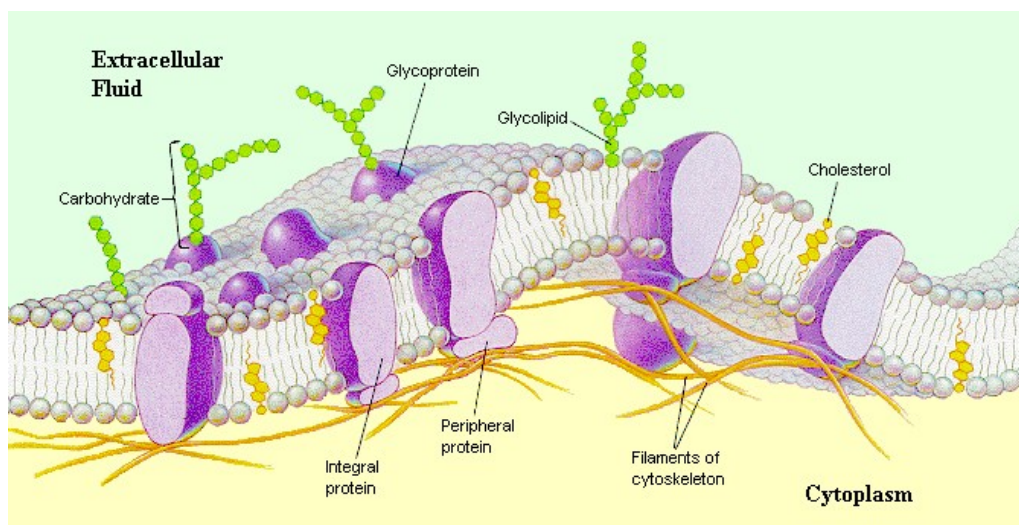


Figure 1.2: Structure of a human cell membrane.

The lipid bilayer contains membrane proteins and lipids linked to carbohydrate residues on the outer surface. These carbohydrate residues are important in cell to cell and cell to matrix interactions. In many tumour entities, changes in these sugars are associated with metastasis formation. [Reference: <http://www.omega.dawsoncollege.qc.ca/ray/cellmemb/membrane.jpg> (30.10.2003)]

1.4 Lectins

Lectins are carbohydrate binding proteins of non-immune origin that agglutinate cells and/or precipitate polysaccharides or glycoconjugates [Goldstein et al. 1980]. The nomenclature of lectins arises from the species of origin and their abbreviation usually

refers to its Latin or common name. The carbohydrate specificity of lectins is generally described by the (mono)saccharide that best inhibits this agglutination (Table 1.4).

Table 1.4: Lectins used in this study.

The species of origin, the nominal carbohydrate specificity and the abbreviation are listed.

Carbohydrate abbreviation: galNAc: N-acetyl galactosamine, gal: galactose, fuc: fucose.

| Common abbreviation | Source of lectin: Latin name | Source of lectin: Common name | Inhibitory carbohydrate |
|---------------------|------------------------------|-------------------------------|-----------------------------------|
| HPA | <i>Helix pomatia</i> | Roman or edible snail | D-galNAc, glcNAc, gal |
| ML-I | <i>Viscum album</i> | European mistle-toe | gal, neuraminic acid |
| PHA-L | <i>Phaseolus vulgaris</i> | Red kidney bean | Carbohydrates of the complex type |
| UEA-I | <i>Ulex europaeus</i> | Gorse or furze | a-L-fuc |

The present study focuses on four lectins, which have already been used to study glycoconjugate changes in malignant tumours and which represent different carbohydrate specificities.

From all these lectins, HPA has been most widely used in metastasis research. HPA binding is associated with a poor prognosis in cancer of the breast [Thomas et al. 1993, Leatham und Brooks 1987], colon [Konno et al. 2002, Schumacher et al. 1994], lung [Laack et al. 2002b], prostate [Shiraishi et al. 1992], stomach [Kakeji et al. 1994] and oesophageal cancer [Yoshida et al. 1993] as well as malignant melanoma [Thies et al. 2001a]. HPA recognises the sugar residue GalNAc, which is part of the blood group A antigen [Matsui et al. 2001] and the Tn epitope [Springer 1989].

The lectin UEA-I binds L-fucose (fuc), which is associated with blood group antigen O(H), so that UEA-I agglutinates most strongly erythrocytes of blood group O(H) [Pereira et al. 1978, Boyd und Shapleigh 1954]. In breast cancer research tumours with a distinctive binding for UEA-I were associated to a shorter patients survival [Fenlon et al. 1987] and in endometrial carcinoma the high affinity of the tumour cells for UEA-I was associated with a poor outcome [Ambros und Kurman 1993]. Furthermore, a comparative study

[Arenas et al. 1999] of non-malignant human prostate and prostatic carcinoma showed an increased expression of fucose residues in prostatic carcinomas. In squamous cell carcinomas of the uterine cervix diffuse UEA-I binding correlated with poor survival of the patients [Davidson et al. 1998].

Beta 1-6 branched oligosaccharides are recognised by PHA-L. These specific carbohydrates are reported to be of prognostic significance in breast cancer [Fernandes et al. 1991], colon cancer [Seelentag et al. 1998], B-cell lymphoma [Suzuki et al. 1999] and oral squamous cell carcinoma [Tanda et al. 1996].

ML-I, a lectin of the European mistletoe, is specific for galactose (gal). In malignant melanoma cells ML-I binding was associated with metastasis formation [Thies et al. 2001b].

1.5 CEACAM1 and EGP-2

As mentioned above adhesion to mesothelial cells is a discerning feature in ovarian cancer, which is mediated by the glycocalix. The carbohydrate residues of the glycocalix recognised by the lectins are mainly glycoproteins. Although most of the membrane proteins are glycosylated, cell adhesion molecules are of particular context if the metastatic process is investigated. These molecules are not only glycosylated but the carbohydrate residues of these molecules have been implicated to be involved in their adhesive function [Kannagi et al. 2004]. This study focuses on the cell adhesion molecules, CEACAM1 and Ep-CAM.

The expression of CEACAM1 occurs in healthy tissues as well as malignant tumours. The healthy tissues expressing CEACAM1 include granulocytes, leucocytes, dendritic cells and epithelia of various organs [Horst und Wagner 2004]. Examples of malignant tumours expressing CEACAM1 are adenocarcinoma of the lung and malignant melanomas. CEACAM1 expression is linked to prognosis in a similar way as is HPA in adenocarcinoma of the lung [Laack et al. 2002a] and malignant melanomas [Thies et al. 2002]. Hence it was included in this study as well. Several studies indicate that the loss or down regulation of CEACAM1 plays an important role in defining the aggressiveness of tumour behaviour. This assumed tumour suppressive activity was investigated in tumours of the mammary gland [Riethdorf et al. 1997], the colorectum [Neumaier et al. 1993], the

prostate [Luo et al. 1999] and the endometrium [Bamberger et al. 1998]. CEACAM1, formerly known as biliary glycoprotein (BGP) [Svenberg 1976], NCA-160, C-CAM (cell-cell adhesion molecule), C-CAM105, H4A or pp120, belongs to the carcinoembryonic antigen (CEA)-related adhesion molecules and is a member of the immunoglobulin superfamily. CEACAM1 has many biological functions such as homophilic and heterophilic adhesion, tumour suppression, invasion promotion and it modulates the immune response and the angiogenesis [Horst und Wagner 2004]. CEACAM1 itself is highly glycosylated and is the major protein carrier of selectin binding carbohydrate groups Lewis X and sialyl Lewis X [Stocks und Kerr 1993], although more precise characterisations of these glycan epitopes are needed [Lucka et al. 2004].

The last cell adhesion molecule investigated was the epithelial cell adhesion molecule (Ep-CAM), epithelial glycoprotein-2 (EGP-2) or 17-1A. Ep-CAM is expressed in non-squamous carcinomas as well as in normal epithelial tissue and its immunoreactivity was investigated for different cell lines and solid tumours [Balzar et al. 1999, Jojovic et al. 1998].

A main feature of epithelial carcinomas is the loss of their epithelial morphology to invade. For that the cells must have mesenchymal characteristics, so that this process is so called epithelial-mesenchymal transition [Birchmeier et al. 1996]. It was shown that EGP-2 is down-regulated during the epithelial-mesenchymal transition of tumour cells in a study by Jojovic et al. [Jojovic et al. 1998]. In this study, human cancer cell lines were transplanted into severe combined immunodeficient mice (SCID) and the EGP-2 expression was analysed in spontaneous lung metastases. They showed that EGP-2 was expressed in the primary tumour tissue of colon cancer cell lines. In contrast, EGP-2 was not expressed in the small lung metastases of this colon cancer cell lines, while it was re-expressed in larger metastases. In conclusion, it seems that EGP-2 was first down regulated and then up-regulated again. A further reason to investigate this molecule is that it is a target structure for so far experimental immunotoxins [Di Paolo et al. 2003] and knowing its expression pattern in ovarian cancer would be a prior requirement to its use as a target in animal models and in clinical immunotoxin trials.

2 Tissue blocks and methods

Initially 71 cases were evaluated in a preliminary investigation (first cohort). Following initial statistical analysis we then proceeded to further investigate these lectins and/or cell adhesion molecule expression that showed promising results in the statistical analysis (for tests see 2.4). Hence, after having investigated the first 71 patients, a second cohort with 93 patients was included resulting in a total number of 164 patients. For comparative reasons six non-malignant ovarian tissue blocks of four patients were investigated (in two cases both ovaries were investigated). The formalin fixed and paraffin wax embedded tissue blocks were retrieved from the files of the University-Hospital Hamburg-Eppendorf (UKE), Institute of Gynaecopathology. The blocks originated from patients treated in the period from 1985 to 2002 at the Clinics of Gynaecology, University-Hospital Hamburg-Eppendorf (UKE).

2.1 Lectin histochemistry

From the paraffin wax embedded tissue blocks 5 μm sections were cut using a microtome (Microm, Techno-med GmbH, Bielefeld, Germany) and applied on Adhesion Micro Slides (HistoBond[®], medite GmbH, Burgdorf, Germany). Slides were then deparaffinized in xylene and rehydrated in a series of graded ethanols to distilled water. Trizma Base (Sigma, Steinheim Germany), sodium chloride (J.T. Baker, Deventer, Netherlands) and hydrochloric acid dissolved in distilled water (Merck, Darmstadt, Germany) compose Tris-buffered saline (TBS). The slides were incubated in 0.1% trypsin (Biochrom KG, Berlin, Germany) dissolved in TBS (pH 7.6) with calcium chloride (1 mM) and magnesium chloride added (1 mM) (Merck, Darmstadt, Germany) (lectin buffer, pH 7.6) and were incubated at 37 °C for 15 minutes. To stop the trypsin digestion, the slides were washed in running tap water for 10 minutes. Afterwards slides were washed three times

in lectin buffer for five minutes. Incubation with biotin-conjugated lectin [(10 $\mu\text{g/ml}$) Sigma, Darmstadt, Germany] in lectin buffer followed in a humid chamber for one hour at room temperature. Slides were then washed three times five minutes in TBS. Alkaline phosphatase-labelled streptavidin was used for visualisation of the biotin-conjugated lectin binding sites. The slides were incubated with the Vectastain[®] ABC KIT solution (Vectastain[®], Vector, ABC KIT, Burlingame, CA) in a humid chamber for 30 minutes. Afterwards slides were washed three times in TBS for five minutes. Substrate solution was composed of Sodium-Nitrite (Sigma, Darmstadt, Germany), distilled water, dimethylformamide (Sigma, Darmstadt, Germany), TBS (pH 8.24) and Tween (Sigma, Darmstadt, Germany). Moreover Naphtol-AS-biphosphate (Sigma, Darmstadt, Germany) together with hexatozized New fuchsin was used as a substrate. Slides were covered by this substrate solution and incubated in dark for 20 minutes. To stop the reaction, the slides were washed in running tap water for five minutes and were then transferred into distilled water. Slides were counterstained in Mayers hemalum solution (Merck, Darmstadt, Germany) 1:1 in distilled water for 10 seconds and then blued in running tap water for 10 minutes. After aqueous mounting media precipitation (crystal/mountTM, Biømeda, Foster City, CA) the slides were covered using a resinous permanent mounting media (Clariøn, Biømeda, Foster City, CA). In each staining procedure lectin incubation was omitted for one slide and thus used as negative control. Sugar specificity for the lectins was tested with galNAc (Sigma, Steinheim, Germany) for HPA, fuc (Sigma, Steinheim, Germany) for UEA-I, thyroglobulin (Sigma, Steinheim, Germany) for PHA-L and gal (Serva, Heidelberg, Germany) for ML-I. Before incubating the slides with the lectin, they were incubated with the appropriate specific sugar (each in 100 mM). As a general stain a section of each paraffin wax block was stained with hematoxylin- and eosin in addition.

2.2 Immunohistochemistry

2.2.1 CEACAM1

Rehydrated slides were microwaved at 500 Watt five times for two minutes each in 10 mmol/l citrate buffer (pH 6.0) and allowed to cool down for 20 minutes. They were washed in TBS twice for five minutes. All antibodies were diluted in DAKO antibody diluent with background reducing components (DAKO, Glostrup, Denmark). To

block non-specific binding sites slides were covered with normal rabbit serum (DAKO, Glostrup, Denmark) in a dilution 1:10 for 20 minutes in a humid chamber, followed by incubation with the monoclonal CEACAM 1 antibody 4D1/C2 (a kind gift of Prof. Dr. Wagener, UKE) at 8 $\mu\text{g/ml}$ over night at 4 °C. The next day the slides were washed in TBS three times for five minutes. Slides were then incubated with biotinylated rabbit anti-mouse immunoglobulins diluted 1:40 in DAKO antibody diluent for 20 minutes and then rinsed in TBS three times for five minutes. Antibody binding was detected by the same alkaline phosphatase-labelled streptavidin method as described for lectin histochemistry, as well as counterstaining and mounting. For each staining procedure in immunohistochemistry the negative control slides were incubated with TBS buffer instead of the primary antibody.

2.2.2 EGP-2

Rehydrated slides were incubated in protease (1 mg/ml, Protease type XXIV: bacterial; Sigma, Deisenhofen, Germany) diluted in prewarmed (37 °C) TBS for 15 minutes. They were then washed in cold TBS three times for five minutes, followed by incubation with 10% normal rabbit serum (DAKO, Glostrup, Denmark) to block non-specific binding sites in a humid chamber for 20 minutes. All antibodies were diluted in DAKO antibody diluent with background reducing components. Incubation with the MOC-31 antibody (a kind gift of PD Dr. U. Zangemeister-Wittke, University of Zürich) at 15 $\mu\text{g/ml}$ was carried out at 4 °C over night in a humid chamber. The next morning the slides were washed in TBS three times for five minutes and then incubated with rabbit anti-mouse immunoglobulins (DAKO, Glostrup, Denmark) as secondary antibody in a dilution 1:40 for 20 minutes. The same antibody detecting, counterstaining and mounting techniques were used as described previously.

2.3 Analysis of staining pattern

The staining of the tumour cells was assessed using a semi-quantitative scale. For the lectins of the first cohort the following five-step scale was used: A negative symbol (-) was assigned if less than 5 % of the tumour cells were stained, a plus (+) was assigned if 5 to 20% of the tumour cells were stained, a double plus (++) if 21 to 50% of the tumour

cells were stained, three plus (+++) if 51-80% of the tumour cells were stained and four plus (++++) if more than 80% of the tumour cells were stained. Statistical analysis for the first cohort revealed that a simplified three-step scale allows a more efficient method for analysing the results. Therefore staining pattern analysis of CEACAM1 (first cohort) and the lectins UEA-I and HPA (second cohort) used the following scale: A negative symbol (-) was assigned if less than 5 % of the tumour cells were stained. A plus (+) was assigned if 5 to 50% of the tumour cells were stained, a double plus (++) if more than 50% of the tumour cells were stained. For the second statistical analysis the first results were reclassified according to the three step scale applied for the second cohort. Two observers analysed the slides independently. Cases in which opinions differed were discussed in a meeting and a consensus was achieved. The slides were examined under a Zeiss Axioplan photomicroscope (Carl Zeiss, Jena GmbH, Germany) and photographed with the Axiocam MRc5 (Zeiss, Munich, Germany).

2.4 Statistical methods

The clinical course of all patients was followed up. The overall survival time was defined as interval from the date of diagnosis to the date of death or to the last date of information for living patients. Relapse free time was defined as interval from the date of diagnosis to the date of the first progression of the disease. All these data together with the clinical stage (according to WHO criteria), tumour grade, histological tumour entity and patients age were subscribed in Excel for Windows version 9. The staining results for UEA-I, HPA, PHAL and ML-I together with the results for CEACAM-1 were added. A chi square test was used to analyse possible correlations between each lectin and each antibody and the clinical and histological data. All variables were analysed by the Cox regression model to evaluate the prognostic significance of the factors. Cox-Regression analysis was performed using SPSS for Windows version 9 (SPSS Inc, Chicago, Illinois, USA). First a univariate analysis for each factor separately was performed. A P-value of less than 0.05 was defined as statistically significant. These statistically significant variables were further included in a multivariate analysis using forward variables selection (likelihood-ratio). In addition survival curves according to the Kaplan-Meier method [Kaplan und Meier 1958] were carried out for statistically significant variables.

3 Results

3.1 Analysis of the first cohort

Initially 71 cases were investigated and used as preliminary test. Following statistical analysis we investigated further 93 cases only with the promising results. Therefore UEA-I and HPA are not included in this chapter, they will be shown in the results of the second cohort only.

3.1.1 Patients characteristics and statistical analysis

Patients (n=71) mean age was 58.10 (standard deviation 12.60) with a range from 24 to 82 years. 5.6% of the patients (n=4) had a tumour classified as stage I, 8.5% of the patients (n=6) had a tumour classified as stage II, the majority, namely 45.1% of the patients (n=32) had a tumour classified in stage III, followed by tumours classified as stage IV with 40.8% of the patients (n=29). According to the grade of differentiation 12.7% cases (n=9) presented with a well differentiated grade (G1), 33.8% cases (n=24) presented with a moderately differentiated grade (G2) and 53.5% cases (n=38) presented with a poorly differentiated (G3) grade. Four different subtypes of epithelial tumours of the ovary were diagnosed. Most of them were classified as serous tumours (71.8%; n=51). Only 11.3% tumours (n=8) were classified as mucinous tumours, 8.5% tumours (n=6) were classified as endometrioid tumours and also 8.5% tumours (n=6) were classified as being of the undifferentiated type.

For all 71 cases the follow-up was available. The mean follow-up time was 26.08 months with a minimum of 1 month and a maximum of 152 months (standard deviation 28.84). After the follow-up time 55 (77.5%) patients died and only 16 (22.5%) patients survived. During follow up time 57 patients (80.3%) had a relapse and the mean relapse free time was 19.49 months (standard deviation 28.82).

Table 3.1: Stage and age are prognostic factors of the first cohort. This table shows the overall survival as well as the relapse free time both for the univariate as well as the multivariate analysis. For the significant multivariate values the Exp (β) (Hazard ratio) is calculated.

| Analysis for overall survival | | | |
|--------------------------------|--------------------|----------------------|-----------------------|
| Factor | Univariate P-value | Multivariate P-value | Hazard ratio (95% CI) |
| stage I+II | 0.041 | ns (0.053) | - |
| stage III (vs I+II) | ns | ns | - |
| stage IV (vs I+II) | 0.041 | ns (0.053) | - |
| age | 0.033 | 0.036 | 1.8 (1.0-3.2) |
| grade | ns | - | - |
| histology | ns | - | - |
| Analysis for relapse free time | | | |
| stage I+II | 0.031 | 0.04 | - |
| stage III (vs I+II) | ns | ns (0.081) | 2.4 (0.9-6.2) |
| stage IV (vs I+II) | 0.036 | 0.014 | 3.3 (1.3-8.7) |
| age | ns | - | - |
| grade | ns | - | - |
| histology | ns | - | - |

Table 3.1 shows the results of the univariate and multivariate analysis. Univariate analysis revealed that stage ($P=0.041$) and age ($P=0.033$) were significantly correlated with patients' overall survival. Those patients whose tumours were classified as stage I or stage II significantly survived longer than patients whose tumours were classified as stage IV ($P=0.041$). According to age the patients were divided into two groups. One group with the patients 65 years and younger and a second one with the patients older than 65 years. Therefore according to age those patients who were 65 years and younger significantly survived longer than patients older than 65 years ($P=0.033$).

If correlated to relapse free time only stage had statistically significant power ($P=0.031$). Patients with tumours in stage I or II had a significant longer relapse free time than patients whose tumours were classified as stage IV ($P=0.036$).

The grade and histological tumour type did not have any significant influence on overall survival [grade ($P=0.722$), histology ($P=0.217$)]. According to the relapse free time age, grade and histological tumour type did not have any significant influence on relapse free time [age ($P=0.147$), grade ($P=0.387$), histology ($P=0.277$)].

A Cox regression model using stepwise forward selection method (Analysis of Likelihood) evaluated for patients older than 65 years a 1.8 times higher risk to die than for patients 65 years or younger ($P=0.036$; 95% CI: 1.0-3.2). The Cox regression model was not evaluable for stage as stage was not included in this analysis due to a non-significant P-value ($P=0.053$).

According to relapse free time, patients with tumours classified as stage IV had a 3.3 times (95% CI: 1.3-8.7) higher risk to relapse than patients with tumours classified as stage I or II. Patients with tumours classified as stage III had a 2.4 times (95% CI: 0.9-6.2) higher risk to relapse than patients with tumours classified as stage I or II.

3.1.2 Lectin histochemistry

All of the 71 tissue sections were stained for PHA-L, ML-I, UEA-I and HPA. Of these, only UEA-I and HPA staining results showed to be statistically significant, thus they were used for the second cohort.

In all control slides without lectin incubation no staining was observed. The sugar specificity controls always resulted in a complete inhibition of the staining.

3.1.2.1 Binding characteristics and statistical analysis of PHA-L

Eleven tumours (15.5%) showed PHA-L binding sites in less than 5% of the tumour cells (-), in 17 (23.9%) tumours 5-20% of the tumour cells were stained by PHA-L (+), in 17 (23.9%) tumours 21-50% of the tumour cells were stained (++) and in 17 (23.9%) tumours 51-80% of the tumour cells were stained (+++), and in only nine (12.7%) tumours more than 80% of the tumour cells were stained (++++). In statistical analysis no significant correlation between PHA-L binding sites and overall survival ($P=0.885$) or relapse free time ($P=0.719$) was revealed. Chi square test revealed no correlation between PHA-L expression and the other variables.

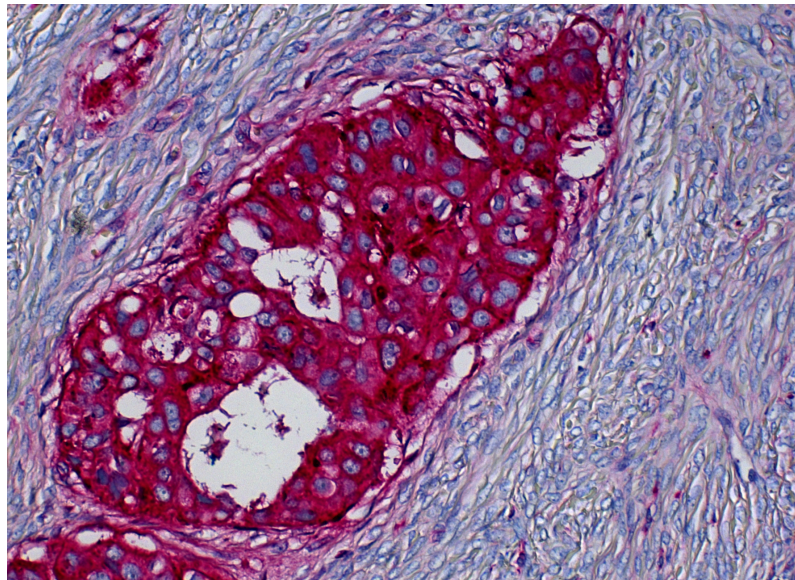


Figure 3.1: PHA-L binding sites in ovarian cancer cells.

Very intense staining of ovarian cancer cells with PHA-L. This sample was derived from a 73 year old cancer patient, who died four months after diagnosis. The classification of this tumour was G3 and stage III.

3.1.2.2 Binding characteristics and statistical analysis of ML-I

In 14 tumours (19.7%) ML-I bound less than 5% (-) of the cancer cells. 5-20% of the tumour cells (+) were stained in 20 of the tumours (28.2%), 21-50% of the tumour cells (++) were stained in 22 tumours (31%) and 51-80% of the tumour cells (+++) were stained

in 13 tumours (18.3%) and in only two tumours (2.8%) more than 80% of the tumours (+++++) were stained.

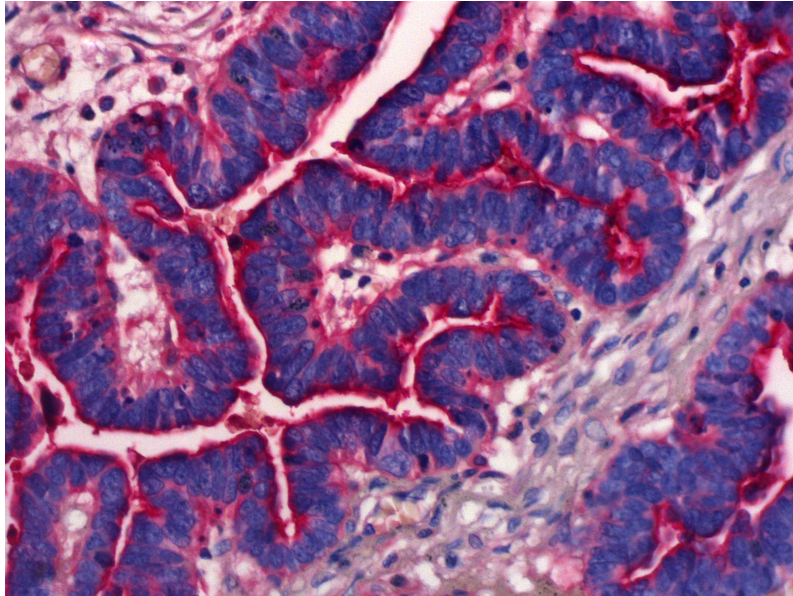


Figure 3.2: ML-I positive ovarian cancer cells.

Of significance here is that the apical ML-I reactivity is more pronounced than the basal ML-I reactivity. This sample was obtained from a 66 year old cancer patient, who died 56 months after diagnosis. The classification of this tumour was G2 and stage IV.

No significant correlation between ML-I binding sites and overall survival ($P=0.992$) or relapse free time ($P=0.887$) was revealed. Chi square test revealed no correlation between ML-I expression and the other variables.

3.1.2.3 Non-malignant ovaries

Of the six non-malignant ovaries the connective tissue stroma was stained by PHA-L in three cases. In one case cysts and their lining epithelium was stained as well. In two cases the corpus albicans was stained and in one case no specific staining for PHA-L was assessed.

In all six cases of non malignant ovarian tissues ML-I binding sites were occasionally demonstrated in the tunica media of the blood vessels and in the corpus albicans. In two cases the cysts lining epithelium was stained.

3.1.3 Immunohistochemistry

Seventy-one patients with a tumour of the ovaries were investigated using anti-CEACAM1 and EGP-2 antibodies.

All the control slides incubated without the primary antibody showed no positive staining.

3.1.3.1 CEACAM1 characteristics and statistical analysis

Thirty-four tumours (47.9%) were classified as CEACAM1 negative, in 32 tumours (45%) CEACAM1 expression was presented in up to 50% of the tumour cells and in 5 tumours (7%) CEACAM1 expression was detected in more than 51% of the tumour cells.

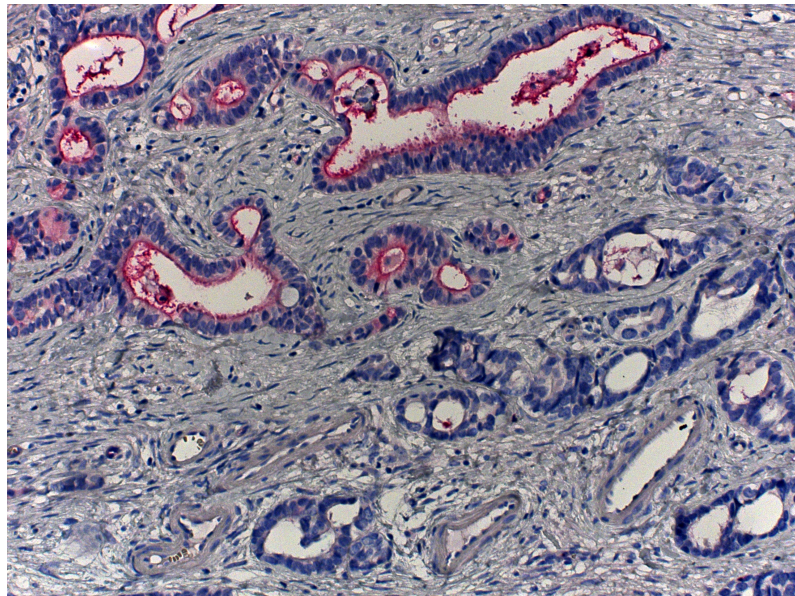


Figure 3.3: The CEACAM1 positive ovarian cancer cells are shown in the upper left hand corner in contrast to the CEACAM1 negative ovarian cancer cells which are demonstrated in the lower right hand corner.

This sample was derived from a 70 year old cancer patient, who died 39 months after diagnosis. The classification of this tumour was G1 and stage III.

No significant correlation between CEACAM1 expression and overall survival ($P=0.938$) or relapse free time ($P=0.792$) was revealed. Chi square test showed no correlation between the other variables and CEACAM1 expression.

3.1.3.2 EGP-2 characteristics

In all investigated 73 tissue blocks the ovarian cancer cells expressed EGP-2 abundantly. Therefore these results were not included in statistical analysis.

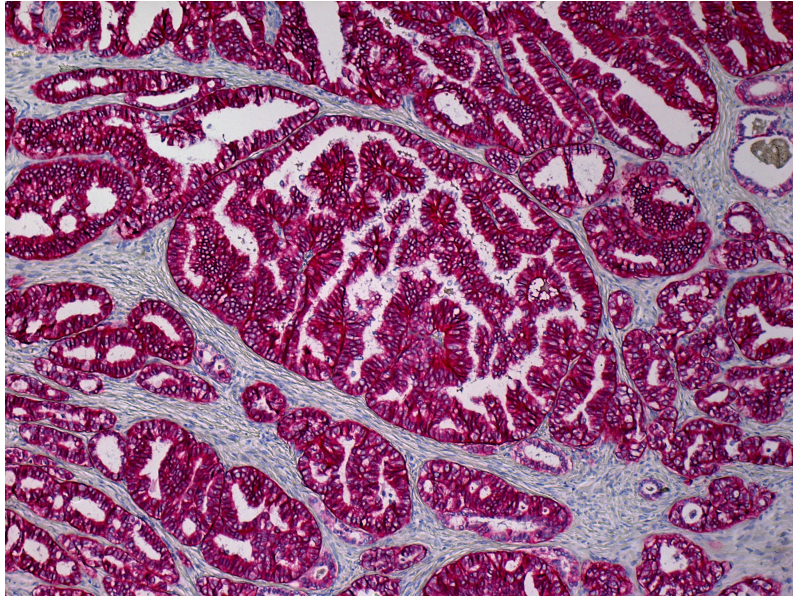


Figure 3.4: All investigated ovarian tumour cells expressed EGP-2. The expression of EGP-2 was combined membranous and cytoplasmatic and was equally impressive in all patients. This sample was obtained from the same patient as in Figure 3.3.

3.1.3.3 Non-malignant ovaries

In the six cases of non-malignant ovarian tissue occasionally limited stretches of small blood vessels expressed CEACAM1. Granulocytes were very intensively labelled.

For the non-malignant ovarian tissue EGP-2 expression was evaluated in the epithelium lining the cyst. The epithelium of the fallopian tube was very intensively labelled and therefore could be used as internal positive control.

3.2 Analysis of the second cohort

After initial statistical analysis of the 71 staining results of the first cohort we proceeded to investigate the additional 93 blocks, but only with these results that showed to be statistically significant, namely UEA-I and HPA. Therefore the second cohort included 164 patient cases.

3.2.1 Patients characteristics and statistical analysis

The patients mean age was 57.50 years (range: 24 to 83). Table 3.2 shows that the older patient group in this study is more frequent (n=121, 74.8%) than the younger patient group (n=43, 26.2%). The Tables 3.2, 3.3, 3.4 and 3.5 each include the number of patients from the FIGO Annual Report 2003 for comparison [Heintz et al. 2003].

Table 3.2: The age distribution in our study and the FIGO Annual Report 2003 [Heintz et al. 2003].

The group of ≥ 50 yrs is the most frequent group in both studies.

| Patients age | Our study | FIGO Annual Report 2003 |
|---------------|---------------|-------------------------|
| <50yrs | n=43 (26.2%) | n=1910 (33.5%) |
| ≥ 50 yrs | n=121 (73.8%) | n=3784 (66.5%) |
| Total | n=164 (100%) | n=5694 (100%) |

Stage I tumours were diagnosed in 11% of our cases (n=18), stage II tumours in 7.9% cases (n=13), stage III tumours in 50.6% cases (n=83) and stage IV tumours in 30.5% cases (n=50) (see Table 3.3).

12.2% of the tumours (n=20) were graded as well differentiated (G1), 29.9% (n=49) as moderately differentiated (G2) and 57.9% (n=95) as poorly or undifferentiated tumours (G3) (see Table 3.4).

All tumours were classified as epithelial tumours and were further subdivided according to the current WHO Classification. The serous carcinoma was the predominant histological tumour entity (n=127, 77.4%), followed by the mucinous type (n=16, 9.8%), endometri-

Table 3.3: Distribution by stage compared between our study and the FIGO Annual Report 2003 [Heintz et al. 2003].

The distribution of stage III between our cohort and the FIGO Annual Report is approximately equal. Stage IV is on second position in our study which is in contrast to the FIGO Annual Report in which stage I is the second frequent group.

| Stage | Our study | FIGO Annual Report 2003 |
|-------|--------------|-------------------------|
| I | n=18 (11%) | n=1085 (27%) |
| II | n=13 (7.9%) | n=384 (9.6%) |
| III | n=83 (50.6%) | n=2024 (50.5%) |
| IV | n=50 (30.5%) | n=511 (12.8%) |
| Total | n=164 (100%) | n=4004 (100%) |

Table 3.4: Distribution by grade of differentiation compared between our study and the FIGO Annual Report 2003 [Heintz et al. 2003].

The distribution of the grade of differentiation is approximately similar in both studies.

| Grade of differentiation | Our study | FIGO Annual Report 2003 |
|--------------------------|--------------|-------------------------|
| G1 | n=20 (12.2%) | n=650 (19.3%) |
| G2 | n=49 (29.9%) | n=1098 (32.6%) |
| G3 | n=95 (57.9%) | n=1623 (48.1%) |
| Total | n=164 (100%) | n=3371 (100%) |

oid type (n=10, 6.1%) and undifferentiated type (n=10, 6.1%) and one case of clear cell carcinoma (0.6%) (see Table 3.5).

Table 3.5: Distribution of the histological tumour type compared between our study and the FIGO Annual Report 2003 [Heintz et al. 2003].

Both studies mainly include the serous tumour type but in our study much more tumours were classified as serous subtype.

| Histological tumour type | Our study | FIGO Annual Report 2003 |
|--------------------------|---------------|-------------------------|
| serous | n=127 (77.4%) | n=2322 (52.9%) |
| mucinous | n=16 (9.8%) | n=600 (13.7%) |
| endometrioid | n=10 (6.1%) | n=802 (18.3%) |
| undifferentiated | n=10 (6.1%) | n=316 (7.2%) |
| clear cell | n=1 (0.6%) | n=349 (8.0%) |
| Total | n=164 (100%) | n=4389 (100%) |

The clinical course of all 164 patients was followed up for a maximum time of 152 months (mean 26.79 months; standard deviation 24 months). Eighty-two patients (50%) died during follow-up time and 82 patients (50%) were still alive after follow-up time. Seventy-one patients (43.3%) did not relapse during follow-up time and the majority, namely 93 patients (56.7%), did relapse.

Table 3.6 shows the results of the statistical analysis of the patients characteristics (stage, age, grade and histological tumour type) of the second cohort. P-value of univariate and multivariate analysis are listed, additionally for multivariate analysis the hazard ratio [Exp (β)] and the 95% confidence interval (95% CI) are shown.

In univariate analysis stage ($P < 0.0005$) and age ($P = 0.013$) are significantly correlated to overall survival. Furthermore they are also a prognostic indicator if correlated to relapse free time (for stage $P < 0.0005$; for age $P = 0.015$). Tumours classified as stage IV had a significant shorter overall survival ($P < 0.0005$) and relapse free time ($P < 0.0005$) compared to tumours classified as stage I or II. Older patients (older than 65 years) had a significant shorter overall survival ($P = 0.013$) and relapse free time ($P = 0.015$) compared to younger patients (65 years and younger).

Table 3.6: Stage and age are potential prognostic factors for the second cohort. This table shows the overall survival as well as the relapse free time both for the univariate as well as the multivariate analysis. For the significant multivariate values the Exp (β) (Hazard ratio) is calculated.

| Analysis for overall survival | | | |
|--------------------------------|--------------------|----------------------|-----------------------|
| Factor | Univariate P-value | Multivariate P-value | Hazard ratio (95% CI) |
| stage I+II | <0.0005 | <0.0005 | - |
| stage III (vs I+II) | ns | 0.001 | 4.5 (1.7-11.5) |
| stage IV (vs I+II) | <0.0005 | <0.0005 | 7.3 (2.8-18.6) |
| age | 0.013 | 0.042 | 1.6 (1.0-2.6) |
| grade | ns | - | - |
| histology | ns | - | - |
| Analysis for relapse free time | | | |
| stage I+II | <0.0005 | <0.0005 | - |
| stage III (vs I+II) | ns | <0.0005 | 6.2 (2.5-15.7) |
| stage IV (vs I+II) | <0.0005 | <0.0005 | 9.6 (3.7-24.6) |
| age | 0.015 | 0.029 | 1.7 (1.0-2.6) |
| grade | ns | - | - |
| histology | ns (0.069) | - | - |

The grade and histological tumour type did not have any significant influence on overall survival [grade ($P=0.442$), histology ($P=0.131$)] or relapse free time [grade ($P=0.154$), histology ($P=0.069$)].

In multivariate analysis the stepwise forward variables selection (Analysis of Likelihood) was used and revealed stage and age as independent statistically significant if correlated to patients overall survival [stage ($P<0.0005$), age ($P=0.042$)] and relapse free time [stage ($P<0.0005$), age ($P=0.029$)]. The hazard ratio for stage III classified tumours showed a 4.5 times higher risk (95% CI: 1.7-11.5) and for stage IV classified tumours a 7.3 times higher risk (95% CI: 2.8-18.6) of dying than for tumours classified as stage I or II. Older patients (older than 65 years) had a 1.6 times higher risk (95% CI: 1.0-2.6) dying of the tumour than younger patients (65 years and younger). According to relapse free time the hazard ratio for stage III classified tumours showed a 6.2 times higher risk (95% CI: 2.5-15.7) and for stage IV classified tumours a 9.6 times higher risk (95% CI: 3.7-24.6) to relapse than for tumours classified as stage I or II. The risk to relapse is 1.7 times higher (95% CI: 1.0-2.6) for older patients than for younger ones. The results of overall survival compared to the results of the relapse free time show similar P-values and risk estimates for the prognostic factors.

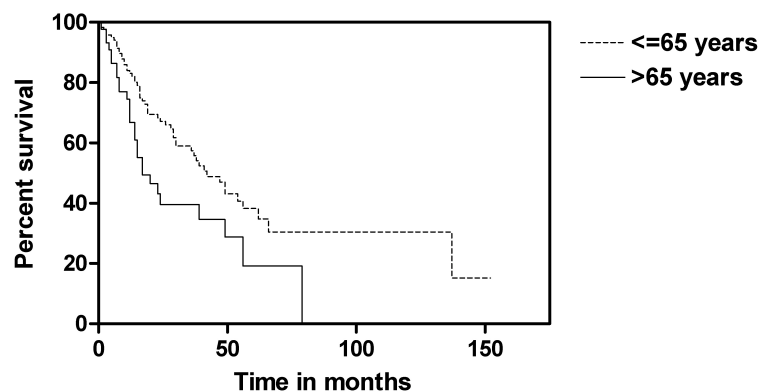


Figure 3.5: Kaplan-Meier plot of overall survival for age. Patients who were older than 65 years ($n=44$) had a significantly poorer survival than patients 65 years and younger ($n=120$).

Figure 3.5 shows the Kaplan-Meier survival plot for age. The vertical scale represents the survival in percentage and the horizontal scale represents the follow-up time in months. The progression of the curve for the older group runs beneath the curve for the younger group. The mean survival time for the group of patients who are 65 years or younger ($n=120$) is 64 months (Standard Error 7, 95% CI: 50-79) and for the group of patients older than 65 years ($n=44$) the mean survival time is only 33 months (Standard Error 5, 95% CI: 23-43). Considering the Kaplan-Meier analysis for age, the overall survival rate is 37% after 56 months in the group of younger patient compared to a survival rate of just 19% after the same time for the older group of patients.

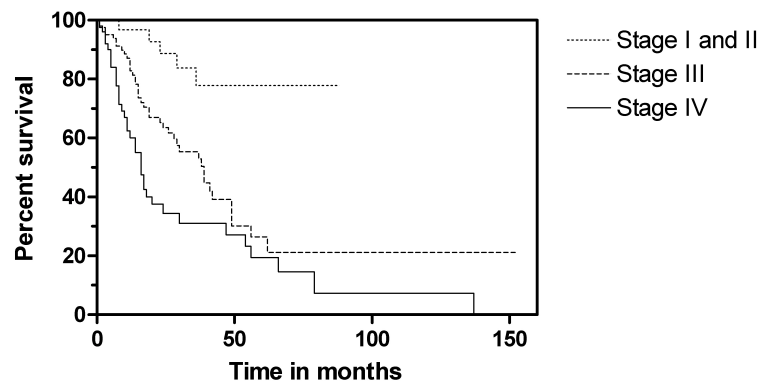


Figure 3.6: Kaplan-Meier plot of overall survival for stage.

Patients with a tumour classified as stage IV ($n=50$) and patients with a tumour classified as stage III ($n=83$) had a significantly poorer survival than patients with a tumour classified as stage I and II ($n=31$).

The Kaplan-Meier survival plot for stage is shown in Figure 3.6. It shows the survival in percentage on the vertical scale and the follow-up time in months on the horizontal scale. The curve for the stage I and II runs above the curve for stage III and this curve again runs above the curve for stage IV. The mean survival rate for the group of patients with a tumour classified as stage I and II ($n=31$) is 74 months (Standard Error 6, 95% CI: 63-85), for the group of patients with a tumour classified as stage III ($n=83$) the mean survival time is 54 months (Standard Error 8, 95% CI: 38-71) and for the group of patients with a tumour classified as stage IV ($n=50$) the mean survival time is only 33 months (Standard

Error 7, 95% CI: 20-46). For instance, after 29 months in the group of tumours classified as stage I and II the overall survival time is 77.8%, compared to an overall survival time of 56.8% at the same time for the group of tumours classified in stage III. And after 30 months in the group of tumours classified in stage IV the overall survival time is only 30.97%.

3.2.2 Lectin histochemistry

All 164 patient cases were stained for UEA-I and HPA.

For all control slides without lectin incubation no staining was observed. The sugar specificity controls always resulted in a complete inhibition of the staining.

3.2.2.1 Binding characteristics and statistical analysis for UEA-I

The tumours of 43 patients (26.2%) showed no binding for UEA-I, 72 tumours (43.9%) showed 5 to 50% UEA-I positive labelled tumour cells and in 49 tumours (29.9%) more than 50% of the tumour cells were labelled by UEA-I.

Table 3.7 (see page 36) shows univariate and multivariate analysis with P-value and Hazard ratio [Exp (β)] and 95% confidence interval (95% CI) for UEA-I and HPA. Univariate analysis revealed that UEA-I has significant influence on overall survival ($P=0.018$). Analysis of UEA-I binding assigned with + versus no binding was significantly correlated to overall survival ($P=0.006$), as well as correlated to relapse free survival ($P=0.033$).

Multivariate Cox regression analysis using the stepwise forward variables selection (Analysis of Likelihood) also demonstrated results for UEA-I binding ($P=0.032$). Patients whose tumours were assigned with one plus had a 2.1 times higher risk of dying of the tumour than patients with a tumour with no UEA-I binding ($P=0.012$; 95% CI: 1.1-3.8). Compare the confidence interval of UEA-I for this result with the results for stage in Table 3.6 and note that the confidence interval for UEA-I is smaller.

The Kaplan-Meier survival curve for UEA-I binding shows the survival in percentage on the vertical scale and the follow-up time in months on the horizontal scale (Figure 3.8). The progression of the survival curves in this figure showed that the curve for the group of tumours with 5 to 50% of the tumour cells labelled (UEA-I +) runs beneath the curve for negative tumours (UEA-I -) and in addition it runs beneath the curve for tumours with more than 50% of the tumour cells labelled (UEA-I ++). The mean survival time for the

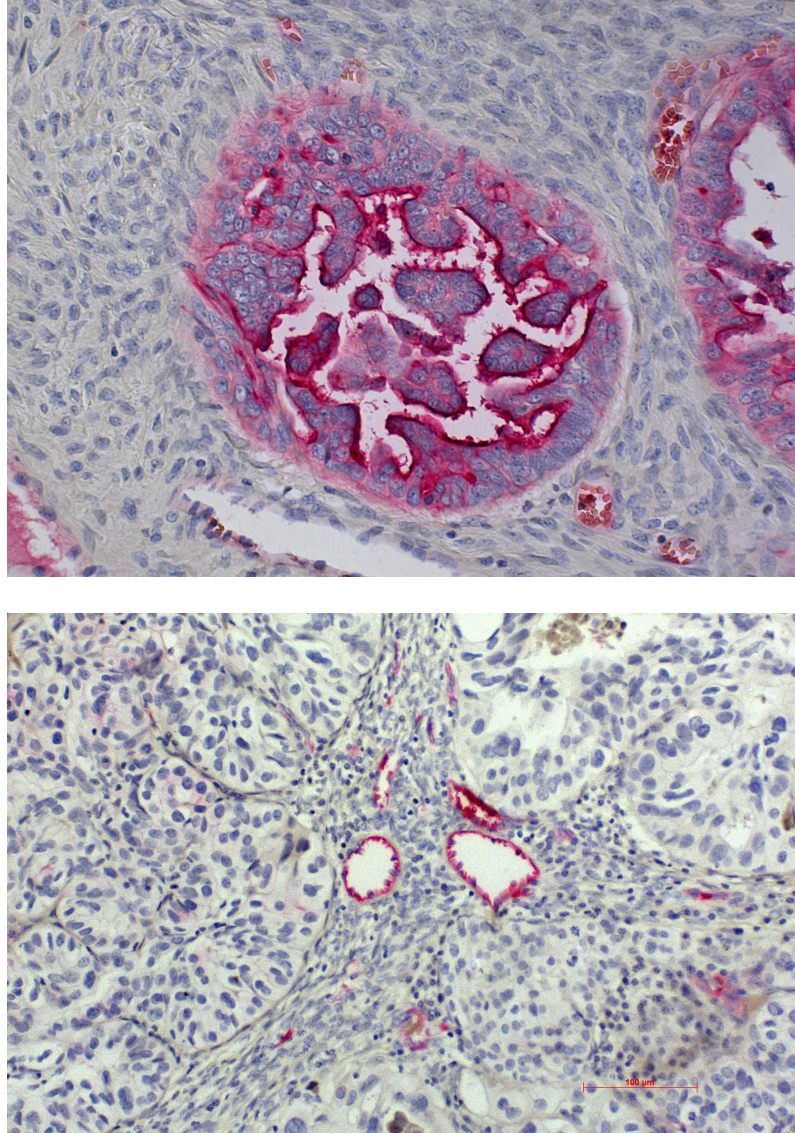


Figure 3.7: The top photomicrograph shows UEA-I positive ovarian cancer cells, whereas the bottom photomicrograph shows UEA-I negative ovarian cancer cells. The bottom photomicrograph demonstrates positive blood vessels inbetween UEA-I negative ovarian cancer cells. The tumour in the top photomicrograph was derived from a 62 year old cancer patient, who died one months after diagnosis. The classification of this tumour was G2 and stage III. The bottom tumour was derived from a 63 year old cancer patient, who was still alive after nine months of follow-up time. The staging of this tumour was G3 and stage II.

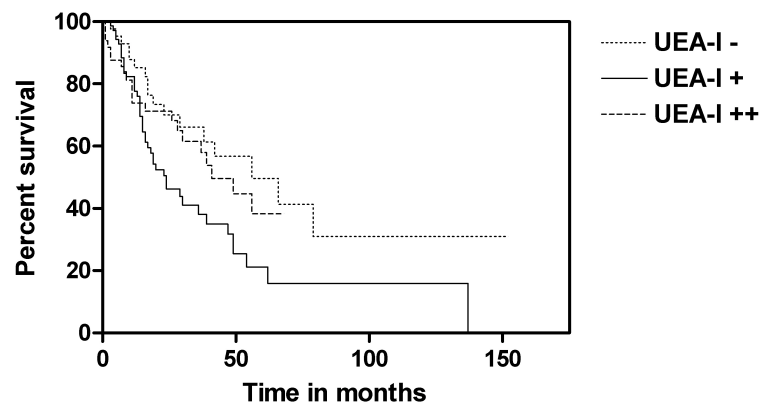


Figure 3.8: Kaplan-Meier plot of overall survival for UEA-I binding. Patients with tumours with 5 to 50% positive labelled tumour cells by UEA-I (n=72) had a significant poorer survival than patients whose tumours were UEA-I negative (n=43).

group of negative tumours for UEA-I (n=43) was 74 months (median Standard Error 13, 95% CI: 49-98), for the group of patients with 5 to 50% of the tumour cells labelled the mean survival time is 42 months (Standard Error 7, 95% CI: 28-56) and for the group of patients with more than 50% of the tumour cells labelled mean survival time is 41 months (Standard Error 4, 95% CI: 33-50). If we compare the mean survival time of the UEA-I negative group with the UEA-I double plus (++) group after 56 months follow-up time the mean survival time of the UEA-I negative group is 49.59% and for the UEA-I double plus (++) group 38.30%. But already after just 54 months the survival time for the UEA-I one plus (+) group is only 20.89%.

3.2.2.2 Binding characteristics and statistical analysis for HPA

The tumours of 39 patients (23.8%) showed no binding for HPA, 65 tumours (39.6%) showed up to 50% HPA positive labelled tumour cells and 60 tumours (36.6%) showed more than 50% of the tumour cells labelled by HPA.

The predictive value of HPA binding was limited to overall survival (Table 3.7). Univariate analysis revealed that the survival of patients whose tumours were assigned with one

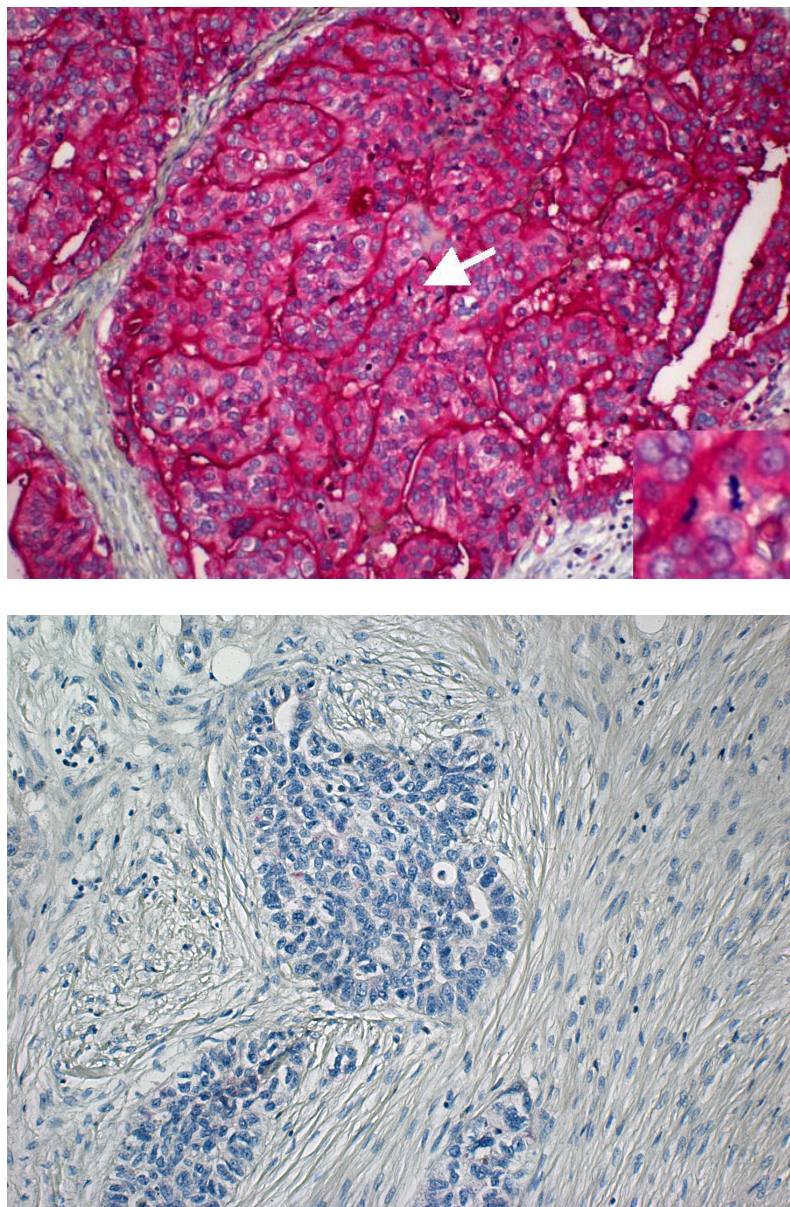


Figure 3.9: The top photomicrograph shows HPA positive ovarian cancer cells, whereas the bottom photomicrograph shows HPA negative ovarian cancer cells. Note that the stromal tissue shows no binding sites for HPA. In the top photomicrograph the arrow indicate mitosis, shown in the inset in detail in the right hand bottom corner.

Table 3.7: UEA-I and HPA are potential prognostic factors.

This table shows the overall survival as well as the relapse free time both for the univariate as well as the multivariate analysis. For the significant multivariate values the Exp (β) (Hazard ratio) is calculated.

| Analysis for overall survival | | | |
|--------------------------------|--------------------|----------------------|-----------------------|
| Factor | Univariate P-value | Multivariate P-value | Hazard ratio (95% CI) |
| UEA-I | 0.018 | 0.032 | - |
| UEA-I + (vs negative) | 0.006 | 0.012 | 2.1 (1.1-3.8) |
| UEA-I ++ (vs negative) | ns | ns | - |
| HPA | ns | - | - |
| HPA + (vs negative) | 0.045 | ns | ns |
| HPA ++ (vs negative) | ns | - | - |
| Analysis for relapse free time | | | |
| UEA-I | ns (0.088) | ns | - |
| UEA-I + (vs negative) | 0.033 | ns | - |
| UEA-I ++ (vs negative) | ns | ns | - |
| HPA | ns | - | - |
| HPA + (vs negative) | ns (0.067) | ns | ns |
| HPA ++ (vs negative) | ns | - | - |

plus, compared with the survival of patients whose tumours showed no HPA binding was significantly shortened ($P=0.045$).

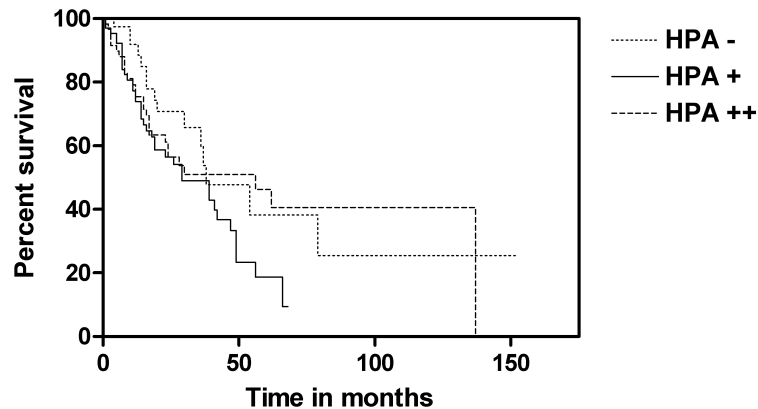


Figure 3.10: Kaplan-Meier plot of overall survival for HPA binding. Patients with tumours with up to 50% positive labelled tumour cells by HPA ($n=65$) had significant poorer survival than patients whose tumours were HPA negative ($n=39$).

Figure 3.10 shows the Kaplan-Meier survival analysis HPA binding. The curve for HPA one plus runs beneath the other two curves. This observation is also reflected if the mean survival time of the three HPA groups (negative, one plus and double plus) is compared. The mean survival time for the group with HPA one plus ($n=65$) is only 34 months (Standard Error 3, 95% CI: 27-40), compared to 67 months (Standard Error 9, 95% CI: 49-86) for the group with HPA double plus ($n=60$) and 66 months (Standard Error 14, 95% CI: 39-93) for the HPA negative group ($n=39$).

3.2.2.3 Non-malignant ovaries

For non malignant ovarian tissue UEA I histochemistry binding sites were demonstrated in different intense pattern in the blood vessels, erythrocytes (five cases), the mucus of the cysts and in the fallopian tube.

HPA histochemistry of non-malignant ovarian tissue showed binding sites in the endothelium of blood vessels. In two cases the erythrocytes were HPA labelled as well. The cyst lining epithelium and their containing mucus showed binding sites for HPA in two cases.

3.2.2.4 Chi square test of UEA-I and HPA

No correlation of UEA-I binding and HPA binding was evaluated in the chi square test (Likelihood ratio=0.392) (see Table 3.8).

Table 3.8: Chi square test of UEA-I and HPA.

No significant correlation between UEA-I binding and HPA binding was revealed.

| | UEA-I - | | UEA-I + | | UEA-I ++ | | Total | |
|--------|---------|---------|---------|---------|----------|---------|-------|---------|
| HPA - | 14 | (32.6%) | 17 | (23.3%) | 8 | (18.4%) | 39 | (24.2%) |
| HPA + | 18 | (41.9%) | 27 | (37.0%) | 20 | (40.8%) | 65 | (39.4%) |
| HPA ++ | 11 | (25.6%) | 29 | (39.7%) | 20 | (40.8%) | 60 | (36.4%) |
| Total | 43 | (100%) | 73 | (100%) | 48 | (100%) | 164 | (100%) |

4 Discussion

4.1 Patient selection criteria

In the present study two patient cohorts were investigated. The first group was composed of 71 patients and was used for a preliminary investigation. The second enlarged group included additional 93 patients and therefore consisted of 164 cases altogether. During the course of the discussion only the second cohort will be referred to when not specified otherwise.

First of all it will be analysed if our patient cohort is representative by comparing it with the latest FIGO Annual report [Heintz et al. 2003] because this report collects data about carcinoma of the ovary from centers from all over the world.

By comparison of both studies it should be noted that the data of the FIGO Annual Report 2003 [Heintz et al. 2003] were based on patients treated from 1996 until 1998. Our study included patients treated from 1985 until 2002 and therefore it could be speculate that therapy improvement may have contributed to an altered survival of the patients in the two groups of patients. However, an approximately constant rate of the 5-year survival of epithelial carcinoma of low malignant potential (borderline) is reported in the volumes 21-25 of the last five FIGO Annual Reports [Heintz et al. 2003] (see Table 4.1). Therefore no influence of the different period of time in which the patients were recruited from is expected.

The age distribution in our patients' cohort is representative. Both in this study and the FIGO Annual Report most of the patients are 50 years or older (≥ 50 yrs) (see Table 3.2). This is in accordance with the ascending incidence of ovarian cancer with advanced age (see Table 1.3, page 7).

According to the FIGO classification, our study showed a trend towards higher stages. Almost half of the investigated tumours were classified as stage III at time of diagnosis

Table 4.1: Review of the 5-year survival rates reported in volume 21-25 of the FIGO Annual Report [Heintz et al. 2003].

Note the slightly fluctuations but otherwise approximately constant 5-year survival rate of the reports from 1982-98.

| Vol. | Year | Patients (n) | Overall 5-year survival in %, Stage Ia-IV |
|------|---------|--------------|---|
| 21 | 1982-86 | 725 | 89.1 |
| 22 | 1987-89 | 487 | 93.0 |
| 23 | 1990-92 | 302 | 86.2 |
| 24 | 1993-95 | 549 | 87.6 |
| 25 | 1996-98 | 763 | 90.4 |

in our study similar to the FIGO Annual Report 2003 [Heintz et al. 2003]. But stage IV was the second frequent diagnosed stage in our study. In contrast to the other study with stage I as second frequent stage (see Table 3.3). Obviously, stage IV is more represented in our study. This may be contributed by the fact that patients with a more complicated development of the disease call on special trained gynecologist or gynecologic oncologist and they therefore appear at the University Hospital.

Approximately three-quarter of the cases in our cohort were classified of the serous subtype. In the FIGO Annual Report 2003 [Heintz et al. 2003] the serous subtype represents approximately half of the diagnosed histological tumour types. Correspondingly there is a difference in the distribution of the other subtypes (see Table 3.5). However, in both studies the serous subtype was the dominant histological tumour entity.

According to the grade of differentiation both in our study and the FIGO Annual Report 2003 [Heintz et al. 2003] the most frequent diagnosed grade of differentiation was poorly or undifferentiated tumours (G3), followed by moderate differentiated tumours (G2) and then well differentiated tumours (G1) (see Table 3.4). It is noteworthy that more than half of the tumours were diagnosed as G3 and were therefore expected to have a shorter overall survival compared to well or moderately differentiated tumours [Vergote et al. 2001, Heintz et al. 2003].

In conclusion, according to age and grade our collective is comparable with the large collective of the FIGO Annual Report 2003. In contrast, the stage distribution in our patient

cohort was dominated by higher malignant stages. And also in contrast the serous subtype was the predominant classified tumour entity compared to the FIGO Annual Report 2003 [Heintz et al. 2003].

Following statistical analysis the factors stage and age were of prognostic significance in our patients' cohort. This confirmed the defined prognostic factors of the oncology guidelines of ovarian cancer [Dt Krebsgesellschaft 2002]. In contrast to these guidelines the grade of differentiation and the histological tumour entity was not statistically significantly when correlated with overall survival or relapse free time in our study. Nevertheless a trend for the histological tumour entity to be associated with the relapse free time was shown in our investigation ($P=0.069$, see Table 3.6) but this factor did not achieve statistical significance. Note that the P-value for stage, age and histological tumour entity of the first cohort considerably improved by comparison with the P-values of the second cohort. For instance the P-value for stage in the second cohort ($P<0.0005$, see Table 3.6) had a higher prognostic impact than the P-value for stage in the first cohort ($P=0.041$, see Table 3.1). Thus an increased influence of the P-value is expected in a further enlarged patient cohort.

4.2 Lectins as new prognostic marker in ovarian cancer

The present study was undertaken to investigate the lectin binding of UEA-I, HPA, PHA-L and ML-I in ovarian cancer and to correlate its expression to overall survival and relapse free time.

4.2.1 UEA-I as a new prognostic marker in ovarian cancer

Our study revealed that UEA-I is a new prognostic marker in ovarian cancer. Following statistical analysis for UEA-I binding sites the factor UEA-I was associated both to overall survival ($P=0.018$) and relapse free time [$P=0.033$ for UEA-I + (vs UEA-I -)] in univariate analysis. In multivariate analysis UEA-I binding remained as a significant independent prognostic marker for overall survival ($P=0.032$), see Table 3.7. The hazard ratio showed that patients with tumours classified as UEA-I + had a 2.1 times higher risk to die of that

tumour compared to patients with tumours classified as UEA-I -. Both results for uni- and multivariate analysis are limited for tumours assigned with +, not for tumours assigned with ++.

The factors stage and age were also of prognostic significance, and the factor stage has the highest prognostic impact for overall survival ($P < 0.005$) and relapse free time ($P < 0.0005$) so that this factor is deservedly the most common used factor. Although the hazard ratio [$\text{Exp}(\beta)$] for UEA-I + [$\text{Exp}(\beta) = 2.1$, Table 3.7] is lower than for stage III [$\text{Exp}(\beta) = 4.5$, Table 3.6] the narrower confidence interval for UEA-I + shows that this result is more reliable than the result for stage (95% CI for UEA-I +: 1.1-3.8; 95% CI for stage III (vs I+II): 1.7-11.5) .

All these results demonstrated that an increased expression of fucose residues, represented through UEA-I binding sites, in ovarian cancer cells was associated with a higher malignant potential, however, this higher malignant potential was limited to a moderate expression of binding sites only.

4.2.1.1 Comparison with other UEA-I histochemistry studies

In studies of breast cancer [Fenlon et al. 1987], endometrial carcinoma [Ambros und Kurman 1993, Ookuma et al. 1994], squamous cell carcinoma of the uterine cervix [Davidson et al. 1998] and prostatic carcinoma [Arenas et al. 1999] an increased expression of UEA-I binding sites was associated with a higher malignant potential of the cancer cells. However, this does not apply to all types of cancers. In oral squamous cell carcinoma it was shown that tumours with UEA-I binding sites showed a better prognosis and lower metastasis rate than tumours without UEA-I binding sites [Meier et al. 2001].

By comparison of the design of our study to the above mentioned studies, our design best fits to the study of Ambros and Kurman [Ambros und Kurman 1993]. They analysed the extent of UEA-I binding using a semi-quantitative scale divided also into three groups of <10% of cells stained, 11-50% of cells stained and 51-100% of cells stained. Thus only the first group is different compared to our study as our tumours were assigned as negative if <5% of the tumour cells were stained. They showed that UEA-I binding significantly correlated with the depth of invasion and the presence of vascular invasion in endometrial carcinoma. However, in our study the moderate extent of UEA-I binding in ovarian cancer was related to overall survival and relapse free time.

The study by Fenlon et al. [Fenlon et al. 1987] is also similar to our study. They analysed the staining proportion using a semi-quantitative scale divided into two groups of 0-50% and 51-100%. However, they revealed that the group of 51-100% was related to survival and disease free interval of patients with breast carcinoma. Again this is in contrast to our study in which moderate expression was related to overall survival and relapse free time of patients with ovarian cancer.

All of the other above mentioned studies used different scale systems for staining analysis. Most of the studies differentiated only between negative and positive tumour cell staining. Therefore our scaling allows a more precise stratification of the tumour.

Only in oral squamous cell carcinoma tumour cells with no binding sites for UEA-I were of higher malignancy than the tumour cells with binding sites for UEA-I [Meier et al. 2001]. This is in contrast to our study and the above mentioned studies of the different other primary malignancy. Obviously, the influence of fucose residues in the malignant potential is very complex. The influence of different tumour types and their different biological behaviour must be taken into consideration when interpreting the lectin histochemical results.

4.2.1.2 Functional role of fucose residues in metastasis

Fucose residues, specific for UEA-I are over expressed in ovarian tumours that were associated to a shorter overall and relapse free survival. UEA-I + tumours were of higher malignancy than UEA-I - tumours, so that we conclude that these carbohydrate residues were associated with a high malignant potential in the ovarian cancer cells. Again, UEA-I ++ tumours were not associated to overall survival or relapse free time. This observation can not be explained yet. Nevertheless, the association of fucose residues of the UEA-I + tumours and their high malignancy is evidence of a functional role in metastases for these carbohydrate chains, according to findings in many other studies.

Stubbs et al. [1996] could show that core fucosylation has a dramatic effect on the conformation of N-linked oligosaccharides, so that α -1,6-fucosylation of N-glycans alters the functions of different glycoproteins (as growth factor receptors, adhesion molecules and extracellular matrices) [Stubbs et al. 1996]. The α -1,6-fucose is transferred to the innermost GlcNAc residue of complex N-glycans by the enzyme α -1,6-Fucosyltransferase (α -1,6FucT). By comparison of serous adenocarcinoma of the ovaries with non-malignant

ovarian tissue, the serous adenocarcinoma showed a higher α -1,6-Fucosyltransferase (α -1,6FucT) activity. Using lectin blot analysis with LCA (Lens culinaris agglutinin) the serous adenocarcinoma tissues contained more α -1,6-fucose residues than normal ovaries and immunohistochemical studies of serous adenocarcinoma tissues suggest that the expression of 1,6FucT is increased in tumour cells [Takahashi et al. 2000]. All those results are evidence for a change of conformation of N-glycans by α -1,6-fucose residues in ovarian cancer cells and this altered cell surface oligosaccharides can contribute to the malignant potential of the cells.

Another study investigated a rat mammary adenocarcinoma cell line on the basis of nuclear magnetic resonance (NMR) spectroscopy. The approach was to treat the rat mammary adenocarcinoma cell line with fucosidase first before the cells were subcutaneously injected in rats. The study revealed that the injected fucosidase-treated cells metastasised in only 20%, whereas the untreated cells metastasised in 80%. Apparently the removal of the surface fucose by enzyme treatment decreased the metastatic characteristics of the cells [Wright et al. 1988].

Fucose residues also play a major role in cell adhesion, in particular in the selectin mediated adhesion. In a study by Xia et al. [2004] the importance of fucose for this function was demonstrated [Xia et al. 2004]. Human umbilical cord blood cells with a defect in binding to P-selectin resulted in their inability to roll on endothelial selectins in bone marrow vessels of nonobese diabetic/severe combined immune deficiency (NOD/SCID) mice. When treated with GDP-fucose and a 1-3-fucosyltransferase IV these cells improved binding to P- and E-selectin, so that they improved cell rolling under flow. The P- and E-selectin mediated cell rolling is a common feature in leucocytes recruitment during inflammation and possibly metastasis. In the selectin mediated cell rolling the selectin binds to sialylated, fucosylated or sulfated glycans [McEver 1997]. As fucose seems to be mainly involved in this mechanism it is possible that fucose positive ovarian cancer cells may more easily attach to the peritoneum. This phenomenon would support the spread of the tumour cells which could contribute to a higher malignant potential of these cells.

4.2.2 HPA as a possible new prognostic marker in ovarian cancer

Apart from UEA-I, HPA is a potential new prognostic marker in ovarian cancer. In univariate analysis, HPA was associated to overall survival [$P=0.045$ for HPA + (vs HPA -), see Table 3.7]. A trend was also found for HPA being related to relapse free time [$P=0.067$ for HPA + (vs HPA -)]. However, the multivariate analysis revealed no significant results for HPA so that it is not of independent prognostic significance.

Nevertheless, an increased expression of binding sites for HPA, specific for GalNac, in ovarian cancer cells is associated with a higher malignant potential. Again, the higher malignant potential is limited to a moderate (HPA +) expression of HPA binding sites. HPA ++ tumours are not associated to overall survival or relapse free time. This phenomenon can not be explained yet.

4.2.2.1 Comparison with other HPA histochemistry studies

There are other studies of HPA binding, where an association between HPA binding and poor prognosis was found. In lung [Laack et al. 2002b], colon [Schumacher et al. 1994, Konno et al. 2002], breast [Leathem und Brooks 1987, Thomas et al. 1993], oesophagus [Yoshida et al. 1993] and gastric cancer [Takeji et al. 1994] as well as malignant melanoma [Thies et al. 2001a] HPA binding to the tumour cells of the primary malignancy were an unfavourable prognostic indicator.

Again, in contrast to our study the lectin studies with HPA of tumours of the lung [Laack et al. 2002b], colon [Schumacher et al. 1994], breast [Fenlon et al. 1987] and the malignant melanoma [Thies et al. 2001a] differentiated only between positive labelled tumours versus negative tumours. To adapt the scale system of the study by Laack et al. [2002a] and Thies et al. [2001a] to our scale system our staining results were combined. The results of the tumours assigned with + were combined with the staining results of the tumours assigned with ++ (carried out for UEA-I and HPA) and then statistical analysis was performed as before. But those results (data not shown) did not reveal any statistical significance. Consequently the prognostic significance for UEA-I and HPA in this study is only limited for the tumours with 5-50% of the tumour cells labelled so that we have revealed a more differentiated scale system for HPA histochemistry for ovarian cancer.

In comparison with all the other investigated tumour types the mechanism of metastatic spread in ovarian cancer is special. Ovarian cancer spread into the peritoneal cavity by using the peritoneal fluid as transport vehicle. Neither tumours of the lung, colon, breast nor malignant melanoma show a similar mechanism for metastases.

4.2.2.2 Functional role of GalNac residues in metastasis

GalNac residues, as detected by HPA, are associated to a shorter overall in our investigation. HPA + tumours are of higher malignancy than HPA - tumours. Again, HPA ++ tumours are not associated to overall survival or relapse free time. This phenomenon can not be explained yet.

HPA recognises the sugar GalNac, which is part of the Tn epitope [Springer 1989]. The Tn epitope develops if the monosaccharide GalNac binds to the polypeptide backbone in O-linked glycan synthesis. If the Tn epitope is sialylated, the sialyl-Tn (STn) occurs. The expression of the Tn as well as the STn epitope is reported to be synthesized by various cancer types [Brooks et al. 2002]. In a study by Davidson et al. [2000] the expression of Tn and STn in ovarian carcinoma cells in effusions was compared to the expression of Tn and STn in primary ovarian tumours and metastatic lesions [Davidson et al. 2000]. They showed a significant up regulation of Tn and STn in the carcinoma cells of effusions in contrast to both primary tumours and metastatic lesions. Therefore it seems that an up-regulation of Tn and STn represents a transient phenotypic alteration facilitating metastasis. Although our study investigated primary tumour tissue only this transient up-regulation could be an explanation for the fact that in our study the tumours with a moderate expression for HPA were of high malignancy. Thus this up-regulation starts in the primary tumour tissue and enables the cancer cells to spread intraperitoneally. In addition the role of the GalNac residues was investigated in a severe combined immunodeficient (SCID) mouse model [Schumacher und Adam 1997]. Both HPA positive and negative human colon cancer cells and human breast cancer cells were transplanted into SCID mice. The HPA positive cell lines did metastasize in most of the cases whereas most of the HPA negative ones did not metastasize. This study accompanies with the idea of a functional role in metastasis of these carbohydrate chains recognised by HPA.

UEA-I as well as HPA recognise terminal sugar residues and both seem to have prognostic significance in ovarian cancer. The expression of binding sites for HPA in a certain tumour

section does not exclude an expression of binding sites for UEA-I in the parallel tumour section (Chi square test of UEA-I and HPA, see Table 3.8). Hence these terminal sugar chains are actually expressed in combination on the ovarian cancer cells.

4.2.3 PHA-L

The lectin PHA-L was investigated for the initial patient group only (n=71). PHA-L was not associated to overall survival or relapse free time. Although different other studies showed that PHA-L is of prognostic significance in tumours of breast [Fernandes et al. 1991], colon [Seelentag et al. 1998], in B-cell lymphoma [Suzuki et al. 1999] and oral squamous cell carcinoma [Tanda et al. 1996] our study could not reveal PHA-L as prognostic factor for ovarian cancer. PHA-L recognises carbohydrate residues of the complex type. These complex-type oligosaccharides belong to the class of N-linked oligosaccharides and are characterized by the disaccharide $\text{glcNac}(\beta 1-4)\text{Gal}$. The N-acetylglucosamintransferase V is associated to the change in oligosaccharides, detected by PHA-L, in tumours with a high malignant potential [Brooks et al. 2002]. Takahashi et al. [2000] investigated the activity of two different N-acetylglucosamintransferases in normal ovaries, benign ovarian tumours and ovarian epithelial carcinomas. They revealed that the enzyme activity was consistently low in all examined tissues. This is in accordance with our study that showed that PHA-L seems not to appear to be involved in ovarian cancer associated alteration in oligosaccharides.

4.2.4 ML-I

The lectin ML-I was investigated for the initial patient group only (n=71). In addition to other studies of tumours of the colon [Dixon et al. 1994] and lung [Fritz et al. 1999] our study did not demonstrate an association of ML-I binding sites and overall survival or relapse free time.

However, this is in contrast to the study of Thies et al. [2001b], who showed ML-I binding associated with metastasis formation in malignant melanoma cells. One obvious explanation for the discrepancy with the present study could rest on differences in the tumour cell origin. Melanoma cells derive from the neural crest while epithelial carcinomas derive from the endoderm so that differences in cell and tissue specific expression

of glycosyltransferases, which determine the carbohydrate residues on the glycocalix do appear [Fukuda und Hindsgaul 1994].

Another explanation might be found in the differences in the mechanism of metastatic spread of ovarian carcinoma and malignant melanoma. Ovarian cancer mostly spread the intraperitoneal and/or lymphatic way, whereas malignant melanoma cells mostly spread the lymphatic or haematogenous way. In addition, analysis of the staining results of this study differed to the staining analysis study by Thies et. al [2001b]. They evaluated the staining intensity of the labelled tumour cells as weak, intense or very intense in addition to the semiquantitative binding analysis. Only the very intense labelled tumour cells were revealed as prognostic significant in her study.

4.3 Prognostic significance of CEACAM1 and EGP-2

The adhesion molecules CEACAM1 and EGP-2 were investigated for the initial patient group only (n=71). The expression of CEACAM1 was not associated to overall or relapse free survival, so that for ovarian cancer its expression does not seem to be of functional relevance.

However, in adenocarcinoma of the lung [Laack et al. 2002a] and in malignant melanoma [Thies et al. 2002] a prognostic significance for CEACAM1 was revealed. A distinctive feature of ovarian cancer is the early intraperitoneal spread, whereas the haematogenous spread appears in the latest stage of the disease. Thus, ovarian cancer cannot be compared to the other investigated tumour entities mentioned above.

Other studies showed CEACAM1 being dysregulated in different human tumours. For instance when compared to healthy tissue CEACAM1 is down regulated in human colon [Thompson et al. 1994], prostate [Busch et al. 2002] and hepatocellular carcinoma [Hino-da et al. 1990]. In our study 48% of the tumours did not express CEACAM1, 45% of the tumours expressed in 5-50% of the tumour CEACAM1 and in only 7% of the tumours CEACAM1 was expressed in more than 51% of the tumour. In the six investigated non-malignant ovarian tissue no expression of CEACAM1 was present, except of granulocytes and limited stretches of small blood vessels. Consequently, no general statement about the dysregulation of CEACAM1 was revealed for ovarian cancer in this study.

EGP-2, also known as Ep-Cam or 17 1A, is expressed in 100% (n=71) of our analysed ovarian cancer tissues and confirms former studies of ovarian carcinomas [Balzar et al. 1999, Kim et al. 2003]. EGP-2 is also expressed on various other tumours of epithelial origin like breast, pancreas, gonads, the gastrointestinal, respiratory and urinary tract. It is also expressed in some normal epithelial tissue of the gastrointestinal, respiratory and urinary tract, and pancreas, gonads, uterus and cervix [Balzar et al. 1999]. The regularly expression of EGP-2 in ovarian cancer could open perspectives for new imaging options, for early detection of ovarian cancer respectively [Heintzelmann-Schwarz et al. 2004]. Because of its broad distribution in malignant cells, it is a suitable target for immune therapy as well.

Initial treatment in ovarian cancer is the surgical tumour debulking followed by adjuvant chemotherapy for the vast majority of patients. Nevertheless it seems that ovarian cancer treatment stagnates [Heintz et al. 2003]. For future prospects it is important to find new therapy options for patients with ovarian cancer. The monoclonal antibody based strategies are such an option having the advantage of higher specificity and less systemic toxicity in contrast to conventional chemotherapy. As mentioned in the before all investigated ovarian tumours expressed EGP-2. As this transmembrane antigen is expressed by most carcinomas it is commonly used as a target for immunotherapy. In this context the Ep-Cam-specific human antibody, called MT 201 was developed and tested for treatment of an ovarian cancer in an ex vivo model system. MT 201 eliminated malignant cells in samples prepared from tumour tissue of patients with ovarian cancer so that it is indicated that MT 201 can offer an effective therapy for ovarian cancer [Xiang et al. 2003].

In conclusion, of the four investigated lectins UEA-I, HPA, PHA-L and ML was shown that UEA-I and HPA are new prognostic marker in ovarian cancer. In addition UEA-I binding is of independent prognostic significance. The association of overall survival and relapse free time for UEA-I binding and the association of overall survival for HPA binding is limited to the moderate expression of binding sites what has not been described in other lectin histochemistry studies of UEA-I and HPA before. This phenomenon can not be explained yet and must be investigated in future studies. The cell adhesion molecules CEACAM1 and EGP-2 were not found to be of prognostic significance in this study.

5 Abstract

Ovarian cancer represents the seventh common cause of cancer death in Germany. It has a poor survival rate of only 46% and represents the malignant tumour of the female genital tract with the worst prognosis. The poor survival rate is both the consequence of early intraperitoneal spread and of its late detection. Cell to cell and cell to matrix interactions play a functionally important role in this spread. Both are mediated by the cell membrane and in particular its embedded proteins and lipids linked to carbohydrate chains, the glycocalyx. Changes in the glycosylation of the cell membrane have been observed in many different types as a result of malignant transformation. These changes in glycosylation can be detected using lectin histochemistry. In a first approach ovarian cancers from 71 patients were investigated for the binding to the lectins UEA-I, HPA, PHA-L and ML-I. In addition the glycosylated cell adhesion molecules CEACAM1 and EGP-2 were investigated. As statistical analysis revealed a prognostic significance for UEA-I and HPA, additional 93 patients were included in the study (n=164). The expression of the extent of the lectin binding to tumour cells was analysed using a semi quantitative scale, whereas the labelled tumour cells were specified in percentage of the tumour cells stained. The staining results were then correlated with patients' survival time, relapse free time and the clinical data of stage, grade, age and histological entity of the tumour. The univariate statistical analysis revealed a prognostic significance for the lectins UEA-I and HPA, whereas only UEA-I remained of independent prognostic significance in the multivariate analysis. Next to UEA-I and HPA the clinical factors, age and stage were associated to patients overall survival and relapse free time. As UEA-I is specific for fucose and HPA for N-acetyl-galactosamine this study emphasise that those sugar residues may are functionally important for the metastatic spread of ovarian cancer within the peritoneal cavity.

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A Abbreviations

| | |
|---------|--|
| ABC | Avidin Biotin Complex |
| ABKD | Arbeitsgemeinschaft Bevölkerungsbezogener Krebsregister in Deutschland |
| CA 125 | Cancer antigen 125 |
| CI | Confidence interval |
| EGP-2 | Epithelial glycoprotein-2 |
| Exp (β) | Hazard ratio |
| FIGO | Fédération International de Gynécologie Obstétrique |
| fuc | Fucose |
| G1 | Well differentiated tumour |
| G2 | Moderately differentiated tumour |
| G3 | Poorly or undifferentiated tumour |
| gal | Galactose |
| GalNac | N-acetyl galactosamine |
| glc | Glucose |
| glcNac | N-acetyl glucosamine |
| Gx | Grade cannot be assessed |
| HPA | Helix pomatia agglutinin |
| man | Mannose |
| ML-I | Mistletoe lectin-I |
| n | Number |
| ns | Not significant |
| PHA-L | Phytohaemagglutinin-leucoagglutinin |
| TBS | Tris-buffered saline |
| UEA-I | Ulex europeus agglutinin-I |
| UICC | Union Internationale Contre le Cancer |
| WHO | World Health Organization |

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