Medizinische Fakultät der Martin-Luther-Universität Halle-Wittenberg

The expression of Insulin-like growth factor 2 mRNA-binding protein 1 in ovarian cancer

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Referat

Objective: Insulin-like growth factor 2 binding proteins 1, 2 and 3 (IGF2BP1, 2, 3) belong to a family of oncofetal mRNA-binding proteins that protect oncogenic target transcripts from miRNA-mediated degradation and are thus involved in cellular processes such as cell polarity, migration and proliferation. The protein expression of IGF2BP1 is analysed in ovarian tumours and correlated to clinical and histopathological data at the time of diagnosis and during the course of disease. **Material and methods:** The protein expression of IGF2BP1 was retrospectively analysed by immunohistochemistry (IHC) using FFPE tumour material of a prospective cohort of patients with ovarian cancer (2005-2016, n=71). IHC analyses were performed on primary tumour, peritoneal-, lymph node- and distant metastases with a monoclonal anti-IGF2BP1 antibody. The staining was evaluated by two pathologists, an IRS of 2 or higher was considered a positive reaction. Statistical evaluation was performed with SPSS version 25.0. **Results:** 32 samples (45%) of IGF2BP1-IHC stained cases showed a positive reaction: 38% (n=19) of primary tumours, 36% (n=23) of peritoneal-, 26% (n=10) of lymph node- and 29% (n=4) of distant metastases. The pattern of the IGF2BP1 expression was inter- and intratumourally heterogeneous, up to 100% of stained cells in the tumour tissue. The expression of IGF2BP1 correlated with high FIGO stage, high tumour grade, the presence of lymph node metastases and deviations from standard chemotherapy. Patients with IGF2BP1 expression showed decreased overall survival (OS) and progression-free survival (PFS) compared to patients without IGF2BP1 expression [OS 19 (95% CI 3-36; events n=28) resp. 53 (95% CI 28-78; n=25) months; PFS 14 (95% CI 11-18; n=30) resp. 23 (95% CI 18-29; n=29) months]. IGF2BP1 expression meant that the risk of death doubled (HR 2.028; 95% CI 0.831-2.691). In multivariate analysis, adjusted for age, FIGO stage, grading, histological subtype, macroscopic residual tumour and chemotherapy, the risk of death decreased to the 1.5-fold. **Conclusion:** Protein expression of IGF2BP1 correlates with decreased OS and PFS and is predominantly expressed in more aggressive tumours. For validation as tumour marker prospective studies are necessary.

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List of abbreviations

BRAF	Rapidly accelerated fibrosarcoma B
BRCA	Breast cancer antigen
bTrCP1	Beta-Transducin repeat containing protein 1
CA 125	Cancer antigen 125
CD44	Cluster of differentiation 44
CI	Confidence interval
CRD-BP	c-Myc coding region determinant-binding protein
DAB	3,3'-Diaminobenzidine
EOC	Epithelial ovarian cancer
Ep-CAM	Epithelial cell adhesion molecule
FFPE	Formalin-fixed and paraffin-embedded
FIGO	International Federation of Gynaecology and Obstetrics
HE	Haematoxylin and eosin
HGSC	High-grade serous carcinoma
HMGA2	High-mobility group AT-hook 2
HR	Hazard ratio
HRP	Horseradish peroxidase
IBM	International Business Machines Corporation
IGF2BP	Insulin-like growth factor 2 binding protein
IL8	Interleukin 8
IMP	IGF2 mRNA-binding protein
IRS	Immunoreactivity score
KRAS	Kirsten rat sarcoma
Let-7	Lethal-7
LGSC	Low-grade serous carcinoma
LIN28B	Lin-28 homolog B
MBL	Medical & Biological Laboratories Co.
miR-708	MicroRNA 708
MMMT	Malignant mixed Müllerian tumour
MRD	Macroscopic residual disease
MYC	Myelocytomatosis oncogene
OS	Overall survival
P53	Protein 53

PARP	Poly ADP ribose polymerase
PC	Percentage of positive cells
PFS	Progression free survival
PP	Percentage points
PTEN	Phosphatase and tensin homolog
qRT-PCR	Quantitative real-time polymerase chain reaction
SI	Staining intensity
SPSS	Statistical Package for the Social Sciences
TNM	Tumour, node, metastases
UICC	Union for International Cancer Control
VICKZ	Vg1 RBP/Vera, IMP-1,2,3, CRD-BP, KOC, ZBP-1
WHO	World Health Organisation
ZBP1	Zipcode-binding protein 1

1 Introduction

1.1 Ovarian cancer

1.1.1 Epidemiology, clinic and standard therapy

Ovarian cancer is the second most common cancer of the female genitals. While they account for only a quarter of new gynaecological cancer cases, some half of all gynaecological cancer deaths involve the ovaries. Approximately one in 71 women will develop ovarian cancer during their lifetime (Buttmann-Schweiger and Kraywinkel 2019). The first unspecific symptoms mostly occur at a late stage of disease, and so far, no screening method has been established to show an improvement in disease-related mortality, which is why most ovarian carcinomas are diagnosed in advanced tumour stages in around 80% of cases (Lisio et al. 2019).

In clinical practice, the TNM and FIGO stages are the most relevant classifications for prognosis and therapy. Both describe the extent of a tumour's expansion in ovaries or tubes (T1/FIGO I), small pelvis (T2/FIGO II), or outside the small pelvis (T3), or the presence of lymph node metastasis (N1/FIGO III) or distant metastasis (M1/FIGO IV) with different sub-differences (Prat 2014).

Prognostic factors include age, tumour stage, macroscopic residual tumour, the patient's general condition, histological type, tumour grade, and guidelines-based therapy (Deutsche Krebsgesellschaft, Deutsche Krebshilfe, AWMF (Leitlinienprogramm Onkologie) 2019).

Although many new therapeutic approaches have been tested in recent years, an optimal tumour-free resection result remains the decisive prognostic factor in these cases; even at FIGO stage IV, median overall survival can be extended by 30 months (Du Bois et al. 2009). The gold standard is an extensive debulking surgery involving all visible metastases with en-bloc resection of the uterus, adnexa and often the sigmoid colon, resection of the major omentum, appendix and possibly intestinal parts, lesser omentum and parietal peritoneum, splenectomy and resection of liver capsule metastases with inguinal, paravertebral and paraaortal lymph nodes (Deutsche Krebsgesellschaft, Deutsche Krebshilfe, AWMF (Leitlinienprogramm Onkologie) 2019). Adjuvant first-line chemotherapy with 6 cycles of carboplatin and paclitaxel should follow surgery at FIGO IIB and above. Other therapeutic options in advanced tumour stages or recurrences, including bevacizumab or PARP-inhibitors, have been found to prolong overall survival in recent studies, and were verified and added to the 2018 guidelines (Deutsche Krebsgesellschaft, Deutsche Krebshilfe, AWMF (Leitlinienprogramm Onkologie) 2019).

1.1.2 Subtype classification

In recent decades, researchers have recognised ovarian cancer as a group of diseases instead of one homologous disease. The latest WHO classification of tumours (Kurman 2014) and the TNM classification of UICC (Brierley et al. op. 2017) brought changes in understanding the genesis and histopathology of ovarian epithelial carcinoma. Identical types of epithelial cancer located in either the ovary, the fallopian tube or the peritoneum can therefore be grouped into one disease (Meinhold-Heerlein et al. 2016).

Kurman and Shih (2016) suggest a supplementary classification of the histological subtypes into type I and II tumours. Type I tumours include low-grade serous (LGSC), endometrioid, mucinous, seromucinous, clear cell carcinomas and malignant Brenner tumours (transitional cell carcinomas in the old nomenclature). They account for some 25% of all ovarian carcinomas, are often limited to the organ, and develop via defined preliminary stages. Type II tumours make up 75% of all ovarian cancers, are fast growing, aggressive and responsible for 90% of mortality from ovarian cancer. High-grade serous (HGSC) and undifferentiated carcinomas, as well as carcinosarcomas (alias malignant mixed Müllerian tumours, MMMT), belong into this category. Serous carcinomas show a dual tumour genesis with BRAF or KRAS mutations in LGSCs and p53 or BRCA mutations in HGSCs, and therefore two tumour entities can be assumed here instead of different stages of de-differentiation. The WHO classifies malignant fallopian tube cancer in the same histological subcategories, while peritoneal cancer is only differentiated into high-grade and low-grade serous cancer. Both are rare malignancies but share enough similarities with ovarian cancer to be jointly classified and clinically managed in a similar manner (Deutsche Krebsgesellschaft, Deutsche Krebshilfe, AWMF (Leitlinienprogramm Onkologie) 2019). All histological subtypes can appear as borderline tumours, although these are not addressed in the present work.

1.2 IGF2BP1

Insulin-like growth factor-2 mRNA-binding protein 1 (IGF2BP1) is described as a predominantly pro-survival enhancer for cell growth, differentiation, migration, adhesion, proliferation and metastasis *in vivo* and *in vitro* in embryonic and tumour cells (Bell et al. 2013; Huang et al. 2018; Degrauwe et al. 2016). It belongs to a family of RNA-binding, cytoplasmic proteins (IGF2BPs; alias: VICKZ, CRD-BP, IMPs or ZBPs). By binding and stabilising their targeted mRNA in cytoplasmic protein-RNA complexes, these proteins prevent the premature decay of their target transcripts and control mRNA translation, transport and turnover (Nielsen et al. 2004).

IGF2BP1 and IGF2BP3, as mainly oncofetal proteins, show high expression during embryogenesis and up-regulation, or de novo synthesis, in various tumours (Bell et al. 2013), and are only found in reproductive organs in adult tissue (Hammer et al. 2005). During development, IGF2BP1 is found in various human and mouse organs (Yaniv and Yisraeli 2002). Observations of physiological functions suggest that IGF2BP1 may be involved in the regulation of stem cell functions (Degrauwe et al. 2016). IGF2BPs were identified as regulators of neuronal development, modulating neurite outgrowth and neuronal cell migration (Bell et al. 2013). Knock-out mice for IGF2BP1 have reduced viability, dwarfism and impaired gut development (Hansen et al. 2004), and its over-expression leads to the development of mammary and colorectal cancers in transgenic mice (Hamilton et al. 2013; Tessier et al. 2004). IGF2BP1 seems to be essential for the regulation of CD44 (Vikesaa et al. 2006), MYC (Weidensdorfer et al. 2009), PTEN (Stöhr et al. 2012), bTrCP1 (Elcheva et al. 2009) and other oncogenic mRNAs, leading to the increased protein expression of the encoded proteins. IGF2BP1 has also been reported as antagonising the tumour-suppressive roles of the let-7-family by shielding LIN28B and HMGA2 from attack in ovarian cancer cells (Busch et al. 2016). According to immunohistochemical data, the expression level of IGF2BP1 variates in different neoplasias, with the highest detected expression levels in Hodgkin lymphoma (94%), testicular cancer (90%) and colorectal cancer (81%) (Bell et al. 2013). In the IGF2BP family, a correlation has been demonstrated between the occurrence of metastasis and high expression levels (Vainer et al. 2008), and IGF2BP1 seems to have the highest oncogenic potential, at least *in vitro* (Müller et al. 2018). Few studies, however, have also found that IGF2BP1 has an opposing suppressive association with tumour growth. These mechanisms therefore still need to be further clarified.

1.3 IGF2BP1 in ovarian cancer

Previous studies have found IGF2BP1 expression to be present in ovarian cancer and correlated to poor prognosis and aggressive tumour development at the protein and mRNA level (Köbel et al. 2007; Gu et al. 2004). New anti-IGF2BP1 antibodies have been developed, tested and optimised as part of growing research on the IGF2BP family, and now show improved specificity to the different IGF2BP family members. The available knowledge of IGF2BP1 means that the protein is becoming increasingly interesting as a biomarker and potential therapeutic target in cancer diagnosis and therapy. Translational research can help to

understand the importance of IGF2BP1 in ovarian cancer for potential implementation in clinical routine.

2 Objectives

This work evaluated the protein expression of IGF2BP1 in ovarian cancer. Formalin fixed and paraffin embedded ovarian cancer probes of primary and metastatic tumour were immunophenotyped for IGF2BP1. The study had the following objectives:

- to assess the IGF2BP1 protein expression rate within the cohort,
- to describe the staining pattern within the tumour tissue,
- to analyse the IGF2BP1 protein expression rate in primary tumour tissue and other metastatic tissues of the same patients,
- to describe the association of IGF2BP1 protein expression with clinical and histopathological parameters,
- to analyse overall survival and progression free survival in association with IGF2BP1 expression.

3 Material and methods

3.1 Patients

This study was conducted by a collaboration of the Department of Gynaecology and the Institute of Pathology, Martin Luther University Hospital, Halle-Wittenberg. All patients underwent surgery at the University Hospital Halle-Wittenberg between 2005 and 2016 and their tissue samples and data was collected prospectively at the time of surgery. In five cases, the initial diagnosis and surgery was carried out in another hospital.

All patients gave written informed consent for admission to the tumour bank for research purposes before surgery. The ethics committee approved the tumour bank and an amendment to this IGF2BP1 study was accepted on 14/10/2015.

The inclusion criteria were the diagnosis of primary (n=63) or recurrent (n=3) epithelial cancer originating from the ovary (n=56), the peritoneum (n=1) or the fallopian tube (n=7), or the diagnosis of a malignant mixed Müllerian tumour originating either from the uterus or the ovary (n=7). Patients who met the inclusion criteria, but from whom no FFPE tissue blocks were available, were excluded from the study. The study included 71 patients in total.

Most of the patients had their follow-up examinations at the University Hospital Halle-Wittenberg. The referring physicians were asked by post in 2016 to update the present status of all patients whose follow-up remained unclear.

Patient age at the time of diagnosis was between 23 and 82 years with a median age of 68 years, which corresponds to the median age of the onset of the disease in Germany (Buttmann-Schweiger and Kraywinkel 2019). Most of the epithelial cancers were high-grade serous at an advanced disease stage at the time of diagnosis, with 41% of the patients showing distant metastasis at the time of diagnosis, in contrast to the average rate of 26% distant metastasis in Germany (Buttmann-Schweiger and Kraywinkel 2019).

Of all patients who received chemotherapy, 74% received the full guidelines-based first-line chemotherapy of six cycles platinum and taxanes (Deutsche Krebsgesellschaft, Deutsche Krebshilfe, AWMF (Leitlinienprogramm Onkologie) 2019), others received varying numbers of cycles (more cycles in three, and fewer cycles in five cases) or a different platinum-based chemotherapy. Of all patients with chemotherapy, 12% received one to six neoadjuvant cycles. During the median observation time of 30 months, 75% of the patients died, most due to disease progression. **Table 1** shows the detailed characteristics of the total cohort.

Table 1. Patient characteristics and histopathological parameters of the tumours at the time of diagnosis, total cohort and subgroups of type 1 and 2 tumours.

		Total	Type 1	Type 2
		n (%)	n (%)	n (%)
Total		71 (100)	20 (28)	51 (72)
Age	< 60 years	17 (24)	6 (30)	11 (22)
	≥ 60 years	54 (76)	14 (70)	40 (79)
	Median (range)	68 (23-82)	64 (23-80)	69 (31-82)
Diagnosis	Ovarian cancer	56 (79)	18 (90)	38 (75)
	Fallopian tube cancer	7 (10)	2 (10)	5 (10)
	Peritoneal cancer	1 (1)	0	1 (2)
	MMMT	7 (10)	0	7 (14)
Manifestation	One side	39 (55)	14 (70)	25 (49)
	Both sides	26 (37)	5 (25)	21 (41)
	Not assessed	6 (9)	1 (5)	5 (10)
Stage	FIGO I-II	7 (10)	6 (30)	1 (2)
	FIGO III	35 (49)	10 (50)	25 (49)
	FIGO IV (Distant metastasis)	29 (41)	4 (20)	25 (49)
Extent of primary tumour	T1	4 (6)	4 (20)	0
	T2	3 (4)	2 (10)	1 (2)
	T3	64 (90)	14 (70)	50 (98)
Lymph node metastasis	Yes	45 (63)	11 (55)	34 (67)
	No	20 (28)	9 (45)	11 (22)
	Not assessed	6 (9)	0	6 (12)
Grade (Silverberg)	Well differentiated	7 (10)	6 (30)	1 (2)
	Moderately differentiated	17 (24)	10 (50)	7 (14)
	Poorly differentiated	47 (66)	4 (20)	43 (84)
Histological Subtype	Serous high-grade	42 (59)	0	42 (82)
	MMMT	7 (14)	0	7 (14)
	Undifferentiated	2 (3)	0	2 (4)
	Serous low-grade	12 (17)	12 (60)	0
	Endometrioid	4 (6)	4 (20)	0
	Clear cell	2 (3)	2 (10)	0
	Mucinous	1 (1)	1 (5)	0
	Transitional cell	1 (1)	1 (5)	0
Macroscopic residual disease	No residual tumour	36 (51)	15 (75)	21 (41)
	Residual tumour 1-10 mm	23 (32)	4 (20)	19 (37)
	Residual tumour > 10 mm	12 (17)	1 (5)	11 (22)
Preoperative ascites	Yes	52 (73)	14 (70)	38 (75)
	No	18 (25)	6 (30)	12 (24)
	Unknown	1 (1)	0	1 (2)
CA 125 before surgery	Normal	5 (7)	2 (10)	3 (6)
	Elevated	63 (89)	17 (85)	46 (90)
	Unknown	3 (4)	1 (5)	2 (4)
CA 125 at the end of chemotherapy	Normal	38 (54)	14 (70)	25 (49)
	Elevated	16 (23)	2 (10)	14 (28)
	Died before end of chemotherapy	7 (10)	1 (5)	6 (12)
	Unknown	8 (11)	2 (10)	5 (10)
	No chemotherapy given	2 (3)	1 (5)	1 (2)

Albumin before surgery	Lowered	53 (75)	16 (80)	38 (75)
	Normal	3 (4)	1 (5)	2 (4)
	Elevated	1 (1)	1 (5)	0
	Unknown	14 (20)	2 (10)	11 (22)
Intestinal infiltration	Yes	55 (78)	12 (60)	43 (84)
	No	16 (23)	8 (40)	8 (16)
Pleural infiltration	Yes	11 (16)	0	11 (22)
	No	60 (85)	20 (100)	40 (78)
First-line chemotherapy	Carboplatin/Taxan: 6-8 cycles	51 (72)	18 (90)	33 (65)
	Carboplatin/Taxan: <6 cycles	6 (9)	0	6 (12)
	Others	8 (11)	0	8 (16)
	No chemotherapy given	6 (9)	2 (10)	4 (8)
Months of observation	Median (range)	30 (0-168)	31 (2-131)	28 (0-168)
Vital status	Alive	18 (25)	5 (25)	13 (26)
	Dead	53 (75)	15 (75)	38 (75)
	Disease progression, known/suspected	38 (72)	12 (80)	26 (69)
	Complications during/after surgery/ chemotherapy	5 (9)	1 (7)	4 (11)
	Other causes	1 (2)	0	1 (3)
	Unknown	9 (17)	2 (13)	7 (18)

Abbreviations: CA125, cancer antigen 125; FIGO, International Federation of Gynaecology and Obstetrics; MMMT, Malignant mixed Müllerian tumour.

3.2 Tumour tissue

Formalin-fixed and paraffin-embedded (FFPE) tissue was used for the study. Four tissue categories were defined according to the origin of the tissue: primary tumour (n=50), peritoneal metastasis (n=64), lymph node metastasis (n=38) and distant metastasis (n=14).
Between one and five different tumour manifestations were analysed for each patient, by screening slides of five to 60 FFPE tissue blocks each tumour manifestation. From these, one to nine tissue blocks were selected per patient, and one to four tissue blocks per tissue category. Not all tissue categories were available for all patients, and thus the number of samples per patient differed widely. Representative sections were selected by a pathologist for immunohistochemical staining according to the criteria: (a) sufficient tumour tissue, (b) lack of necrosis, (c) representative presence of different tumour phenotypes, and (d) presence of adjacent normal tissue. Additionally, twelve samples from ten patients with tumour-free tissue samples available were used as negative controls. Altogether the study included 228 tissue samples.

3.3 Material

3.3.1 Antibodies, chemicals and enzymes

Antibody Anti-IGF2BP1:	MBL, Japan; Mouse IgG2a κ, Clone 6H6, Code No. RN001M
Antibody diluent:	Zytomed, Berlin
3,3'-Diaminobenzidine:	Zytomed, Berlin, DAB Substrate Kit
Epitope retrieval solution:	Zytomed, Berlin, Citrate buffer diluted 1:10
Ethanol 99%:	Walter-CMP, Kiel
Hemalaun:	Dr. K. Hollborn & Söhne, Leipzig, solution acidic acc. to Mayer
HRP-polymer:	Zytomed, Berlin, ZytoChem-Plus HRP Polymer-Kit
Peroxide solution 3%:	Fischar, Saarbrücken
Postblock reagent:	Zytomed, Berlin, ZytoChem-Plus HRP Polymer-Kit
Wash buffer:	Zytomed, Berlin, diluted 1:20
Xylene:	Walter-CMP, Kiel

3.3.2 Devices

Automated slide stainer:	Leica, Wetzlar, Autostainer XL
Drying cabinet:	Heraeus, Hanau, type T6
Microscope:	Olympus Optical, Japan, model BX50F
Microtome:	Leica, Wetzlar, HistoCore Biocut
Refrigerator:	Thermo Fisher Scientific, Schwerte, model FR157SF
Semi-automatic IHC stainer:	Tecan-Genesis-RSP100, Zytomed, Germany
Water bath:	Gesellschaft für Labortechnik, Burgwedel, type 1002

3.3.3 Consumables

Microscopic slides:	Thermo Fisher Scientific, Schwerte, SuperFrost R Plus
Mounting medium:	Orsatec, Bobingen, Eukitt

3.4 Methods

3.4.1 Immunohistochemistry

FFPE tissue blocks were sliced with a section thickness of 3 µm, then dewaxed with xylene and hydrated using alcohol washes of decreasing concentrations until distilled water. For antigen retrieval, the deparaffinised slides were heated in the steam cooker for 25 min with epitope retrieval solution (pH 6.0, 1:10 dilution). The endogenous peroxidase block involved 3% peroxide solution for 7-10 min. All staining steps were followed by treatment with washing

buffer. The stainings were performed applying a semi-automatic platform according to the manufacturer's instructions. In detail, the primary antibody was diluted 1:100 in antibody diluent and incubated for 30 min, then incubated with postblock reagent for 15 min, then with the HRP-polymer for 20 min. In the last step, the slides were incubated with DAB for 10 min. Counterstaining was performed used hemalaun for 30 sec, blued in tap water followed by alcohol washes of increasing concentrations and xylene and mounted.

Positive controls were performed on normal tissue of the seminiferous ducts of testis and tissue of testicular seminomas according to the Human Protein Atlas (Uhlen et al. 2017). Negative controls were performed on tumour free tissue of the ovary, fallopian tube, uterus, colon polyp, and skin.

3.4.2 Evaluation of Staining

The IGF2BP1 staining was evaluated by one pathologist on 107 samples and by two pathologists on 121 samples. Both were blinded to each other and to the patients' clinical and histopathological data. They were further partially blinded to the patients' diagnoses. The evaluation included the rating of staining intensity (SI) and percentage of positive cells (PC), as well as a description of the morphological pattern of staining.

3.4.3 Quantifying of Staining

The quantification of immunohistochemically analysed IGF2BP1 staining has not been standardised so far. Indeed, various scores with different variables, different evaluation tools and different thresholds are described in the literature. In this study, the immunoreactivity score (IRS) was chosen as the definitive primary score. This IRS is well established to quantify the progesterone and oestrogen receptor status of tumour cells in breast cancer (Remmele and Stegner 1987). The score consists of the variables SI and the percentage points (PP). The SI is rated on a point scale from 0 to 3 (0: no reaction, 1: weak reaction, 2: moderate reaction, 3: strong reaction). The PP are rated on a point scale from 0 to 4 (0: no reaction, 1: less than 10% positive cells, 2: 10%-50% positive cells, 3: 51%-80% positive cells, 4: more than 80% positive cells). The product of both factors gives the IRS with a value between 0 and 12. For the IGF2BP1 staining, an IRS of 0 to 1 was considered as IGF2BP1-negative and an IRS of 2 to 12 as IGF2BP1-positive. The threshold for a positive result was based on the distribution of results, the number of events in each group and the effect on overall survival.

Other scores were applied to the results of the IHC staining in parts of the present study for comparison. The scores are listed and described below for clarity.

PC-Score: Only the percentage of positive cells (PC) was considered. Any amount of stained cells was considered as IGF2BP1-positive.

SI-Score: All slides without any staining (0) or with any number of stained cells with a weak SI (1) were considered IGF1BP1-negative. Slides containing any number of stained cells with moderate (2) or strong (3) SI were considered IGF2BP1-positive.

Kessler-Score (Kessler et al. 2017): This score distinguishes between no reaction, weak reaction and strong reaction. The values are almost analogous to the IRS as the product of SI (0: no reaction to 3: strong reaction) and PP (0: 0%, 1: less than 10%, 2: 10-49%, 3: 50-80%, 4: more than 80% positive cells). Slides without any stained cells (value 0) were considered IGF2BP1-negative. Slides with a value of 1 to 4 were considered IGF2BP1-positive with a weak reaction. Slides with a value of 5 to 12 were considered IGF2BP2-positive with a strong reaction.

3.4.4 Statistics

SPSS (IBM, Armonk, NY, USA) version 25 was used for statistical analysis. Fisher's exact test was applied to all variables with two categories for the analysis of correlations between the expression of IGF2BP1 and clinical, prognostic relevant parameters. The Fisher-Freeman-Halton test was used for variables with more than two categories without a natural order . In all cases, the exact p-value was considered. The primary endpoint overall survival (OS) and the secondary endpoint progression free survival (PFS) were evaluated based on the expression of IGF2BP1 as defined and analysed via the Kaplan Meier Method. The time from the first diagnosis to the death of the patient was defined as OS. The time from the first diagnosis to the occurrence of any event (recurrence, new metastasis and death) was defined as PFS. All patients in the cohort were included in the survival analysis. The survival rates were compared and tested for significance via Log-rank test. A multivariate analysis was carried out with the Cox proportional hazard regression model. P-values < 0,05 were considered significant.

4 Results

4.1 Immunohistochemically determined IGF2BP1 expression profile

4.1.1 IGF2BP1 protein expression in normal tissue (negative controls)

The non-malignant tissue specimens of ten patients were selected as negative controls. The non-malignant FFPE tissue of the ovary (n=6), fallopian tube (n=3), uterus (n=1), colon polyp (n=1) and skin (n=1) were analysed. Six patients had strong IGF2BP1 expression in their cancer tissue, and no IGF2BP1 protein expression was seen in the neoplastic tissues of four patients.

4.1.2 IGF2BP1 protein expression in spermatogonia cells and testicular seminoma cells (positive controls)

As described in the Human Protein Atlas, normal tissue of the seminiferous ducts of testis shows high expression of IGF2BP1 in spermatogonia cells. Tissues of testicular seminomas also show high or medium expression of IGF2BP1 in 100% of examined cases (Uhlen et al. 2017). These tissues were thus used as positive controls for the IGF2BP1 staining. This data was reproduced and high IGF2BP1 expression was found in spermatogonia cells (**Figure 1**) and also in 100% of testicular seminoma cells (**Figure 2**).

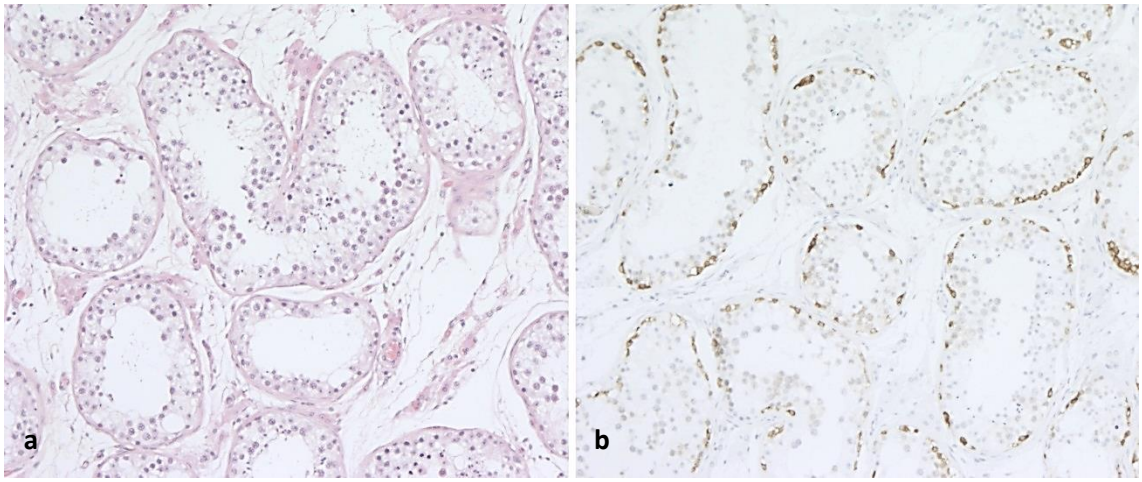

Figure 1. Normal testicular tissue, (a) HE and (b) anti-IGF2BP1 stain, 100x magnification.

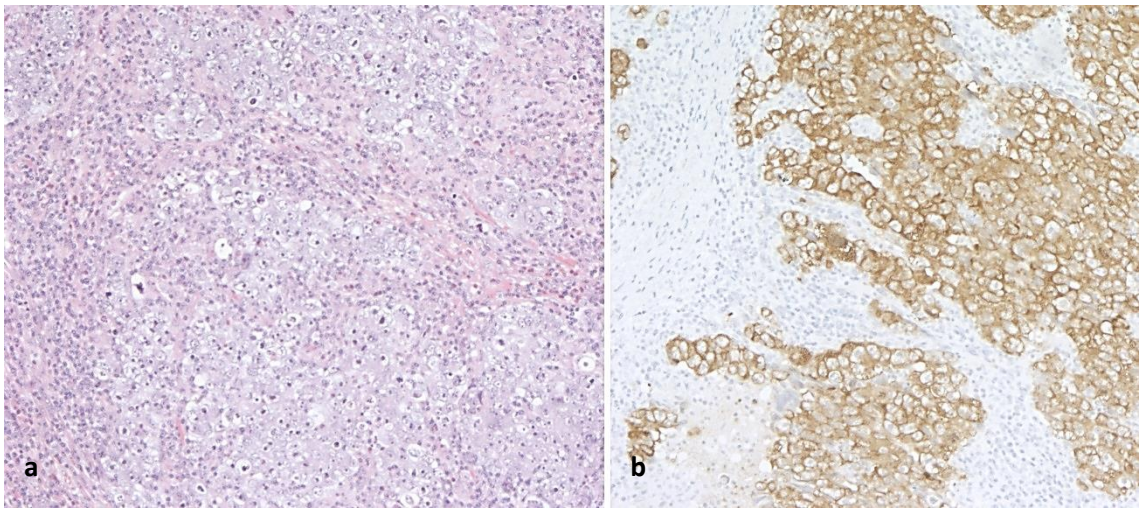

Figure 2. Testicular seminoma tissue, (a) HE and (b) anti-IGF2BP1 stain, 100x magnification.

4.1.3 Proportion and pattern of IGF2BP1 protein expression in ovarian cancer tissue

Of all patients, 45% (n=32) expressed IGF2BP1 as detected via IHC in at least one tissue sample according to the IRS. In 8% (n=6) of the patients, the expression of IGF2BP1 was below the threshold of IRS 2, but above 0. The pattern of the IGF2BP1 expression was inter- and intratumourally heterogeneous. 32% (n=12) of all positive tumours showed a staining reaction in less than 1% of all tumour cells, and in 50% (n=19) of the cases in less than 10% of all tumour cells (**Figure 5, 6, 7**), and seven cases also had a strong IGF2BP1 expression in 95-100% of tumour cells (**Figure 3**). The median PC among IGF2BP1-positive cases was 13%.

In three cases, a borderline tumour was found among non-malignant or malignant areas of ovarian tissues. All borderline tumour tissues were IGF2BP1-negative.

In heterogeneously stained tissue samples, the IGF2BP1-positive cells were found in cell isles inside IGF2BP1-negative tumour areas, sometimes with different isles presenting different staining intensities in the same tissue sample (**Figure 4, 5, 6**).

As expected, all immunohistochemical staining was found to be intracytoplasmic. In most cases, only epithelial tumour cells were stained, however, in some cases with a non-epithelial tumour component, stromal cells were also stained (**Figure 7**).

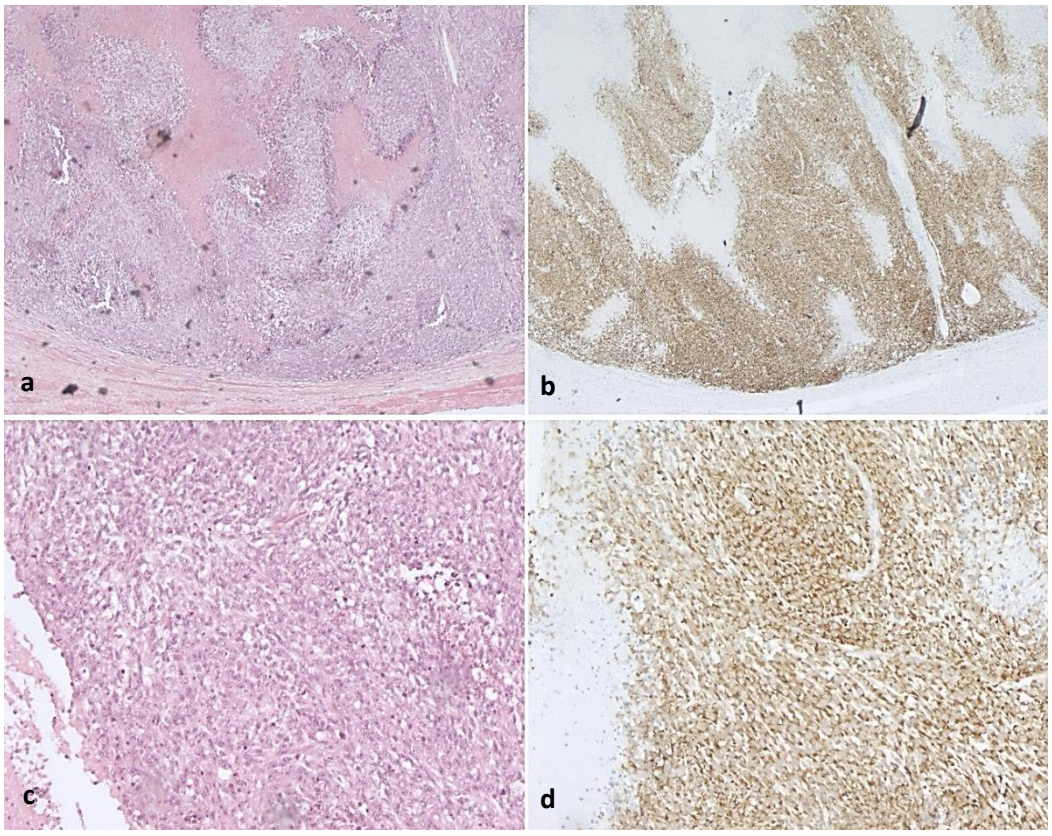

Figure 3. Ovarian tissue with malignant mixed Müllerian tumour, FIGO IIIc, (a) HE and (b) anti-IGF2BP1 stain, 25x magnification, (c) HE and (d) anti-IGF2BP1 stain, 250x magnification. 100% of tumour cells stained, showing strong reaction.

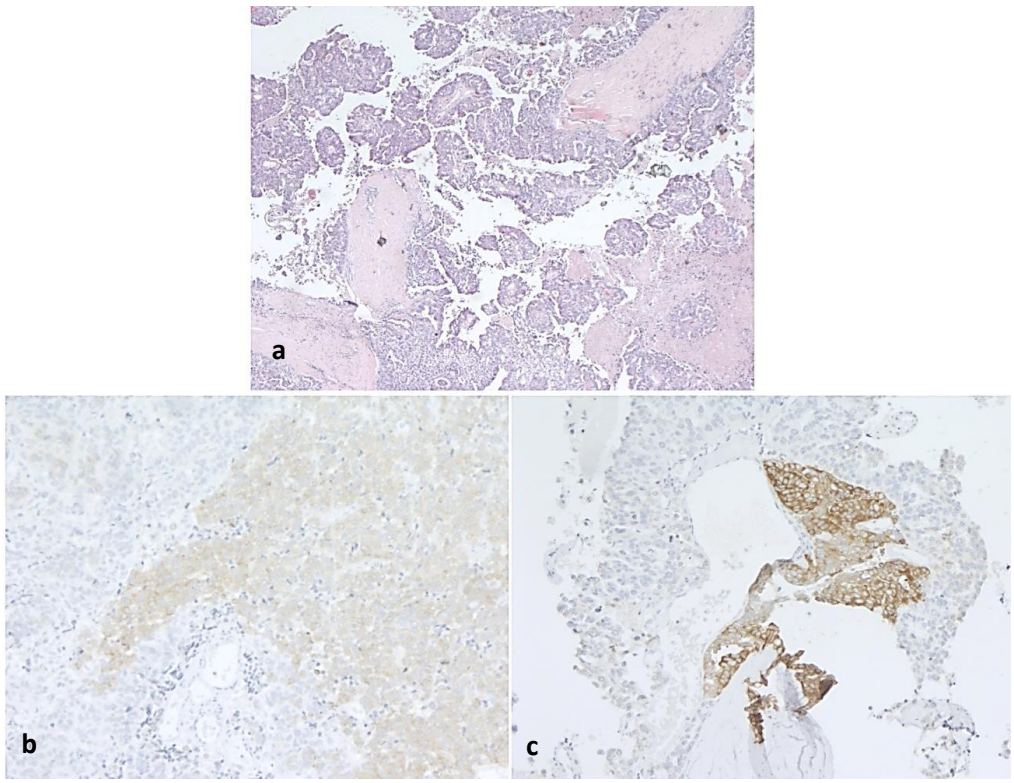

Figure 4. Ovarian tissue with high grade serous ovarian cancer, FIGO IIIc, (a) HE stain, 25x magnification, (b) and (c) anti-IGF2BP1 stain, 100x magnification. 20% of tumour cells stained with weak staining intensity in (b) and 2% of tumour cells stained with strong staining intensity in (c).

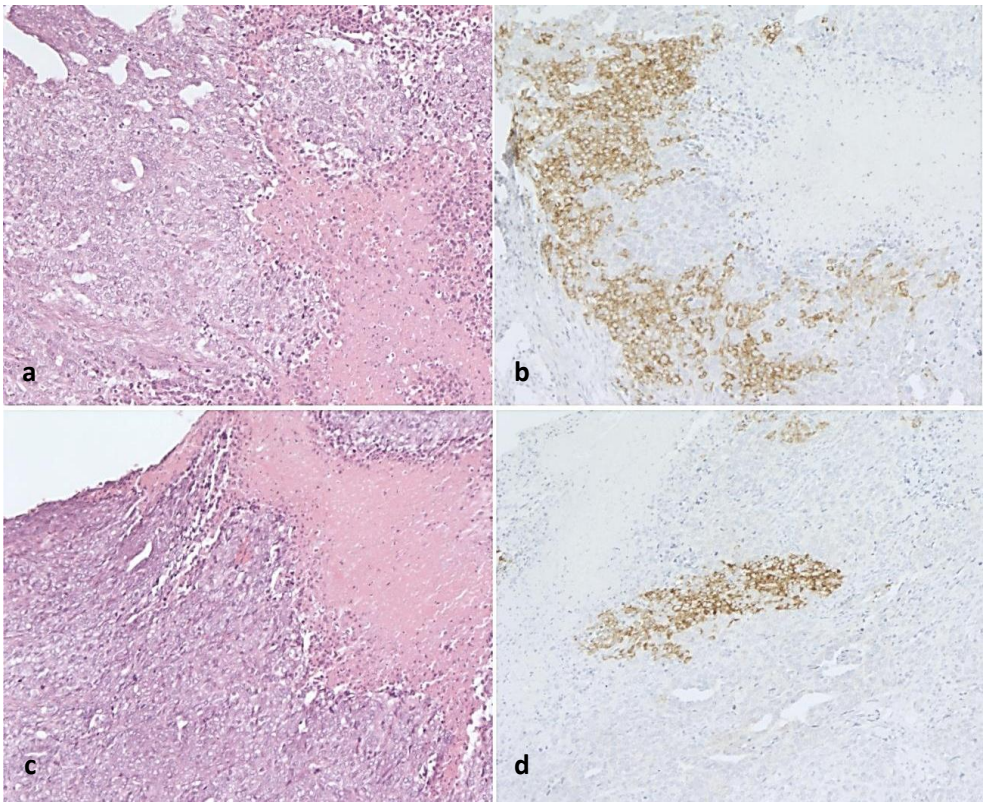

Figure 5. Ovarian tissue with malignant mixed Müllerian tumour of the ovary, FIGO IIIc, (a) and (c) HE and (b) and (d) anti-IGF2BP1 stain, 100x magnification. The tissue shows isles of IGF2BP1-positive tumour cells with 8% of all tumour cells stained with high staining intensity. The staining in (b) is located around a necrotic tumour area, (d) could be an area of intravascular tumour.

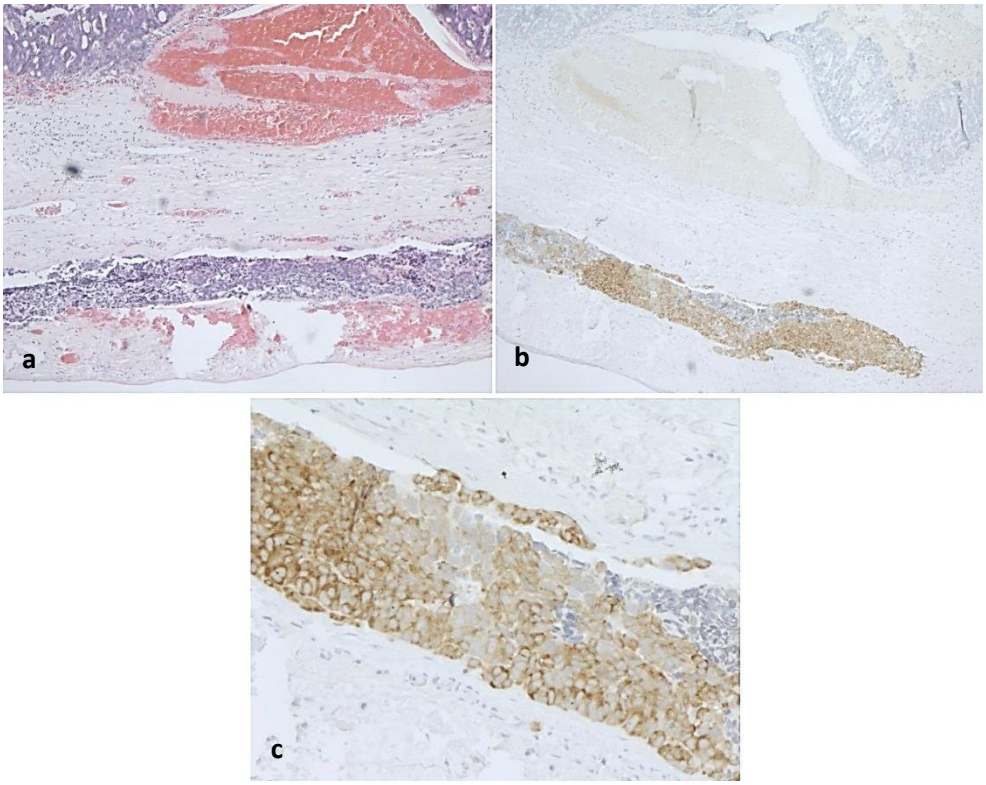

Figure 6. Ovarian tissue with high grade serous ovarian cancer, FIGO IIIc, (a) HE and (b) anti-IGF2BP1 stain, 100x magnification, (c) anti-IGF2BP1 stain, 400x magnification. 1% of all tumour cells stained with high staining intensity. The figure shows a strongly IGF2BP1-positive intravascular tumour portion.

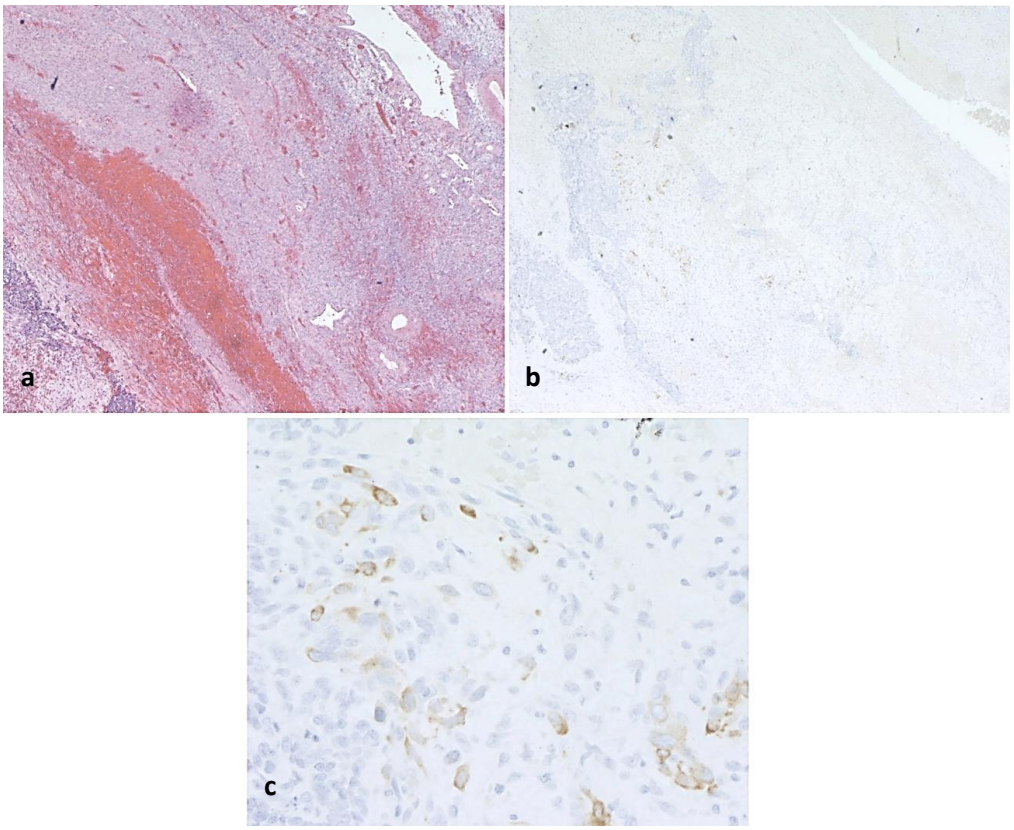

Figure 7. Ovarian tissue with endometrioid ovarian cancer, FIGO IIIc, (a) HE and (b) anti-IGF2BP1 stain, 25x magnification, (c) anti-IGF1BP1 stain, 250x magnification. 5% of tumour cells stained, including stroma cells (c).

4.1.4 IGF2BP1 protein expression in primary tumour tissue and corresponding metastatic tissues

In the different tissue categories, a slightly higher expression of IGF2BP1 could be seen in primary tumour tissue and peritoneal metastasis tissue compared to lymph node and distant metastasis, without a clear trend emerging (**Table 2**).

Table 2. Frequencies of the IGF2BP1 expression in different tissue categories.

		negative		positive	
	n	n	%	n	%
Primary tumour	50	31	62	19	38
Peritoneal metastasis	64	41	64	23	36
Lymph node metastasis	38	28	74	10	26
Distant metastasis	14	10	71	4	29

Of the 50 patients with available primary tumour tissue, corresponding peritoneal metastasis tissue was collected in 45 cases, tissue of lymph node metastasis in 29 cases and tissue of distant metastasis in eleven cases. There was a significant correlation between the IGF2BP1 expression in primary tumours and peritoneal metastasis as well as lymph node metastasis;

due to small case numbers the effect was not significant in distant metastasis (**Table 3**). There was a strong impact in all tissue categories with a Phi rating from 0.436 to 0.624.

Table 3. Comparison of the expression of IGF2BP1 in primary tumour tissue and corresponding metastasis tissue. P-value was analysed via Fisher's exact test.

		Primary tumour					
		Total	negative		positive		p
		n	n	%	n	%	
	Total	50	31	62	19	38	
Peritoneal metastasis	**negative**	29	22	82	7	39	0.005
	positive	16	5	18	11	61	
Lymph node metastasis	**negative**	21	14	93	7	50	0.014
	positive	8	1	7	7	50	
Distant metastasis	**negative**	7	7	78	0	0	0.109
	positive	4	2	22	2	100	

4.1.5 Association of IGF2BP1 protein expression with type 1 and 2 tumours

Type 2 tumours showed more expression of IGF2BP1, at 51%, but only 30% of type 1 tumours were IGF2BP1-positive, although the difference was not significant. There were more IGF2BP1-positive tumours, among type 2 tumours, with a high percentage of positive cells (26% of type 2 compared to 17% of type 1 tumours with >50% stained cells), but there were no relevant differences between the staining patterns. Using other scores, the percentage of IGF2BP1-positive cases was 25% cf. 45% for SI-Score and 30% cf. 63% for PC-Score in type 1 cf. 2 tumours, and a high reaction, according to Kessler-Score, was found in 10% cf. 24%. **Table 4** shows the comparison of the IGF2BP1 expressions corresponding to tumour category. In this and the following comparisons, the different score values of different tissue categories from the same patient were summarised into one value per patient, using the highest value.

Table 4. Frequencies of the IGF2BP1 expression in type 1 and type 2 tumours.

	Total	negative		positive		p
	n	n	%	n	%	
Total	71	39	55	32	45	
Type 1	20	14	70	6	30	0.123
Type 2	51	25	49	26	51	

4.2 Correlation of IGF2BP1 protein expression to histopathological, clinical and prognostic parameters

In the histopathological parameters, IGF2BP1-positive tumours significantly more often demonstrated a higher extent of primary tumour (T-status) and higher tumour stages at first diagnosis (0% positive at FIGO stage I or II cf. 50% positive at FIGO stage III or IV), the presence of lymph node metastasis (53% positive with N1 cf. 15% with N0), and a poor level of differentiation (55% positive with grade 3 cf. 25% with grade 1-2). Interestingly, among the type 2 tumours, all cases of malignant mixed Müllerian tumours (n=7) and undifferentiated tumours (n=2) showed an expression of IGF2BP1, while serous high-grade carcinomas presented IGF2BP1 expression in only 41%. A significant correlation with histological subtype could be seen only for type 2 tumours. The effect on tumour grade was also stronger within the group of type 2 tumours, although not significant in either tumour type. In spite of the small case numbers, expression of IGF2BP1 showed a higher effect on tumour stage and on the presence of lymph node metastasis for type 1 tumours than for type 2 tumours.

In the clinical parameters, the expression of IGF2BP1 correlated with intestinal infiltration at the time of surgery in type 1 tumours (50% positive with intestinal infiltration cf. 0% without) and deviations from the standard chemotherapy in type 2 tumours. There was also a pattern of higher age at the time of first diagnosis with IGF2BP1 expression (p=0.053), with a stronger effect in type 2 tumours (p=0.097 in type 2 cf. p=1 in type 1 tumours).

Correlations in the other IHC scores were comparable to the chosen primary score (IRS, threshold 2). Using a threshold of 3 instead of 2 in the IRS, there was a significant correlation between IGF2BP1 expression and an elevated CA 125 value at the end of chemotherapy (50% positive cf. 18% among patients with a normal CA 125 value), especially in type 2 tumours (p=0.033), suggesting a low response to therapy.

There was no correlation between the expression of IGF2BP1 and mono- or bilateral tumour manifestation, presence of distant metastasis, macroscopic residual disease, presence of preoperative ascites, preoperative value of CA 125 or albumin, pleural infiltration at the time of surgery, or the cause of death. **Table 5** summarises the results of this analysis for the total cohort,

Table 6 for the sub-cohort with type 1, and **Table 7** for the sub-cohort with type 2 tumours. Except for the histological subtypes, variables with n<5 in at least one subgroup are not displayed.

Table 5. Patient characteristics and histopathological parameters with IGF2BP1 expression for the total cohort. The p-value was calculated via Fisher's exact test cf. Mann-Whitney-U test.

	Total in subgroup	Total IGF2BP1-positive in subgroup		p
	n	n	%	

Total		71			
Age	< 60 years	17	4	23	0.053
	≥ 60 years	54	28	52	
Manifestation	One side	39	15	39	0.612
	Both sides	26	12	46	
Stage	FIGO I-II	7	0	0	0.014
	FIGO III-IV	64	32	50	
Lymph node metastasis	Yes	45	24	53	0.006
	No	20	3	15	
Distant metastasis	Yes	29	14	48	0.809
	No	42	18	43	
Grade	1+2	24	6	25	0.023
	3	47	26	55	
Histologic subtype	Serous high-grade	42	17	41	0.005
	MMMT	7	7	100	
	Undifferentiated	2	2	100	
	Serous low-grade	12	4	33	
	Endometrioid	4	1	25	
	Clear cell	2	0	0	
	Mucinous	1	0	0	
	Transitional cell	1	1	100	
Macroscopic residual disease	No residual tumour	36	14	39	0.344
	Residual tumour	35	18	51	
Preoperative ascites	Yes	52	24	46	0.784
	No	18	7	39	
CA 125 before surgery	Normal	5	3	60	0.648
	Elevated	63	27	43	
CA 125 normalised after chemotherapy	Normal	39	16	41	0.377
	Elevated	16	9	56	
Intestinal infiltration	Yes	55	28	51	0.089
	No	16	4	25	
Pleural infiltration	Yes	11	4	36	0.743
	No	60	28	47	
First-line chemotherapy	Carboplatin/Taxan: 6-8 cycles	51	17	33	0.003
	Others/No chemotherapy	20	15	75	

Abbreviations: CA125, cancer antigen 125; FIGO, International Federation of Gynaecology and Obstetrics; MMMT, Malignant mixed Müllerian tumour.

Table 6. Patient characteristics and histopathological parameters with IGF2BP1 expression for type 1 tumours. The p-value was calculated via Fisher's exact test cf. Mann-Whitney-U test.

		Total in subgroup	**Type 1 IGF2BP1-positive in subgroup**		**p**
		n	**n**	**%**	
Total		20			
Age	< 60 years	6	1	17	0.613
	≥ 60 years	14	5	36	
Manifestation	One side	14	5	36	0.257
	Both sides	5	0	0	
Stage	FIGO I-II	6	0	0	0.115

	FIGO III-IV	14	6	43	
Lymph node metastasis	Yes	11	6	55	0.014
	No	9	0	0	
Macroscopic residual disease	No residual tumour	15	4	27	0.613
	Residual tumour	5	2	40	
Preoperative ascites	Yes	14	4	29	1
	No	6	2	33	
Intestinal infiltration	Yes	12	6	50	0.042
	No	8	0	0	

Abbreviations: FIGO, International Federation of Gynaecology and Obstetrics.

Table 7. Patient characteristics and histopathological parameters with IGF2BP1 expression for type 2 tumours. The p-value was calculated via Fisher's exact test cf. Mann-Whitney-U test.

		Total in subgroup	**Type 2 IGF2BP1-positive in subgroup**		**p**
		n	**n**	**%**	
Total		51			
Age	< 60 years	11	3	27	0.097
	≥ 60 years	40	23	58	
Manifestation	One side	25	10	40	0.375
	Both sides	21	12	57	
Lymph node metastasis	Yes	34	18	53	0.177
	No	11	3	27	
Distant metastasis	Yes	25	13	52	1
	No	26	13	50	
Grade	1+2	8	2	25	0.14
	3	43	24	56	
Macroscopic residual disease	No residual tumour	21	10	48	0.779
	Residual tumour	30	16	53	
Preoperative ascites	Yes	38	20	53	0.742
	No	12	5	42	
CA 125 normalised after chemotherapy	Normal	25	11	44	0.514
	Elevated	14	8	57	
Intestinal infiltration	Yes	43	22	51	1
	No	8	4	50	
Pleural infiltration	Yes	11	4	36	0.324
	No	40	22	55	
First-line chemotherapy	Carboplatin/Taxan: 6-8 cycles	33	11	33	0.001
	Others/No chemotherapy	18	15	83	

Abbreviations: CA125, cancer antigen 125.

4.3 Univariate survival time analysis via Kaplan Meier method

4.3.1 Comparing different scores via log rank test

Different scores, some of which have been used in published studies, were applied to the IGF2BP1 staining of the cohort and analysed via Kaplan Meier method and log rank test. The application of four of the tested scores showed a significantly different probability of survival in the log rank test. The IRS with a threshold of 2 was chosen as the primary score for further analysis. The other marked scores were considered separately in parts of the results and discussion. **Table 8** shows the summary of all tested scores.

Table 8. Composition of different scores with their corresponding log rank tests of the Kaplan Meier analyses that have been applied to the IGF2BP1 staining of the total cohort for the end point OS. The marked values showed a significantly different probability of survival.

Variable	Reference in literature	Negative staining		Intermediate staining		Positive staining		p-value
		Definition	n (%)	Definition	n (%)	Definition	n (%)	
PC		0%	33 (47)			>0%	38 (53)	0.037
PC		≤ 1%	47 (66)			2-100%	24 (34)	0.15
PC	**(Köbel et al. 2007)**	< 5%	49 (69)			5-100%	22 (31)	0.206
PC	**(Cornejo et al. 2012)**	<5%	49 (69)	5-25%	8 (11)	26-100%	14 (20)	0.394
SI		0-1	43 (61)			2-3	28 (39)	0.004
SI x PP	**(Remmele and Stegner 1987)**	0-2	50 (70)			3-12	21 (30)	0.014
SI x PP	**(Remmele and Stegner 1987)**	0-1	39 (55)			2-12	32 (45)	0.009
SI + PP	**(Ohno et al. 2009)**	0-4	60 (85)			5-12	11 (15)	0.125
SI x PP	**(Zhou et al. 2015)**	0-4	60 (85)			5-12	11 (15)	0.125
SI x PP	**(Hsieh et al. 2013)**	1-3	56 (79)	4-6	6 (8)	7-12	9 (13)	0.067
SI x PP	**(Kessler et al. 2017)**	0	33 (46)	1-4	24 (34)	5-12	14 (20)	0.019

Abbreviations: OS, overall survival; PC, percentage of positive cells; PP, percentage points; SI, staining intensity.

The impact of IGF2BP1 on OS and PFS was also evaluated: a subgroup analysis was stratified according to lymph node metastasis, tumour grade, macroscopic residual disease, and type 1 and 2 tumours prognostic factors.

4.3.2 Correlation of IGF2BP1 protein expression and overall survival cf. progression free survival

First, the total cohort was stratified according to the dualistic model of Kurman and Shih (2016), since type 1 and 2 tumours differ strongly in characteristics and OS, but there was no significant difference in overall survival in this cohort (p=0.878 in the log rank test) or in the expression of IGF2BP1 for tumour type 1 and 2. The whole cohort is thus considered together in the following.

Patients with expression of IGF2BP1 showed a significantly reduced OS and PFS in contrast to patients without IGF2BP1 expression (p=0.009 for OS and p=0.014 for PFS in the log rank test). The median OS of patients was 19 months with expression of IGF2BP1 and 53 months without

IGF2BP1 expression (**Figure 9**), and the median OS of the total cohort was 34 months (**Figure 8**). The probability of five year OS was 21% (95%-CI 7-36%) with IGF2BP1 expression cf. 39% (95%-CI 23-55%) without IGF2BP1 expression. The median PFS of patients was 14 months with expression of IGF2BP1 and 23 months without IGF2BP1 expression (**Figure 9**), in contrast to 19 months for the total cohort (**Figure 8**). The probability of 5 year PFS was 7% (95%-CI <1-17%) with IGF2BP1 expression cf. 26% (95%-CI 11-40%) without IGF2BP1 expression.

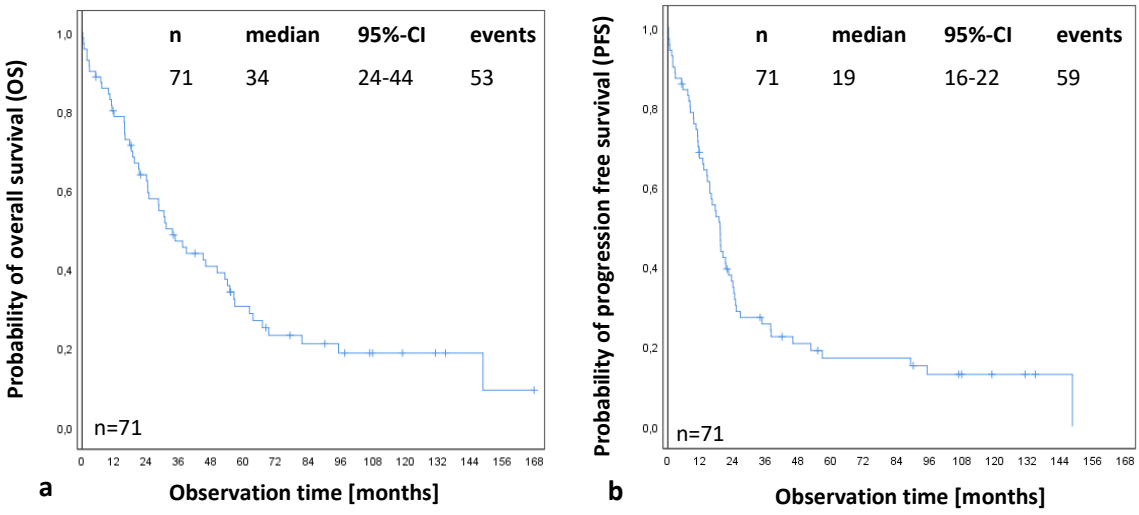

Figure 8. Kaplan Meier Curve with the cumulative (a) overall survival and (b) progression free survival of the total cohort.

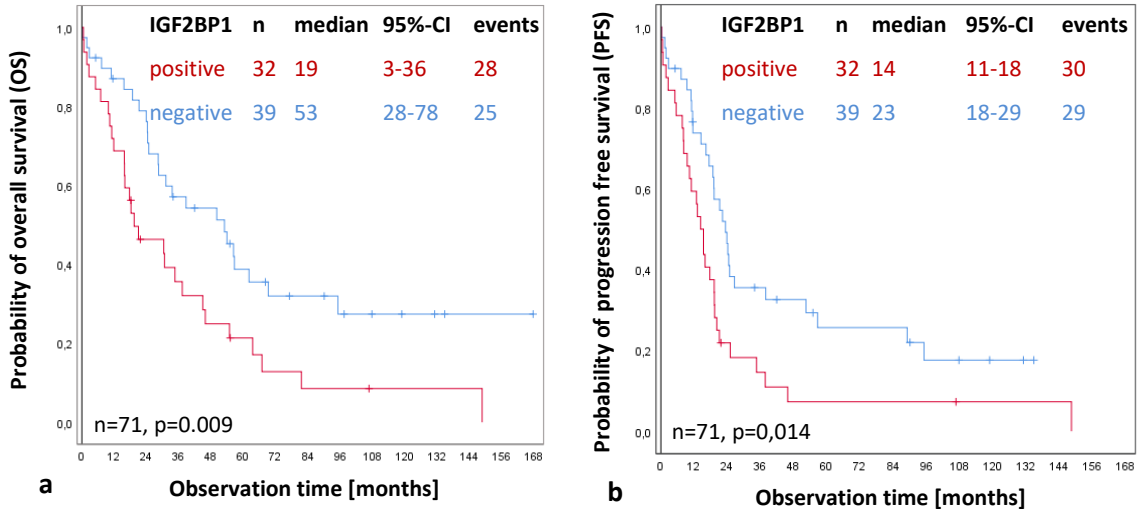

Figure 9. Kaplan Meier Curve with the cumulative (a) OS and (b) PFS of the IGF2BP1-positive and –negative subgroups. Patients with expression of IGF2BP1 show significantly reduced OS and PFS.

4.3.3 Subgroup analysis of overall survival

No effect of IGF2BP1 on the course of disease was detected in the subgroup analysis stratified for lymph node metastasis (p=0.159 for the cohort with presence of lymph node metastasis, p=0.830 for absence of lymph node metastasis).

Stratified for tumour grading, the expression of IGF2BP1 was correlated with reduced OS in poorly differentiated tumours (**Figure 10**).

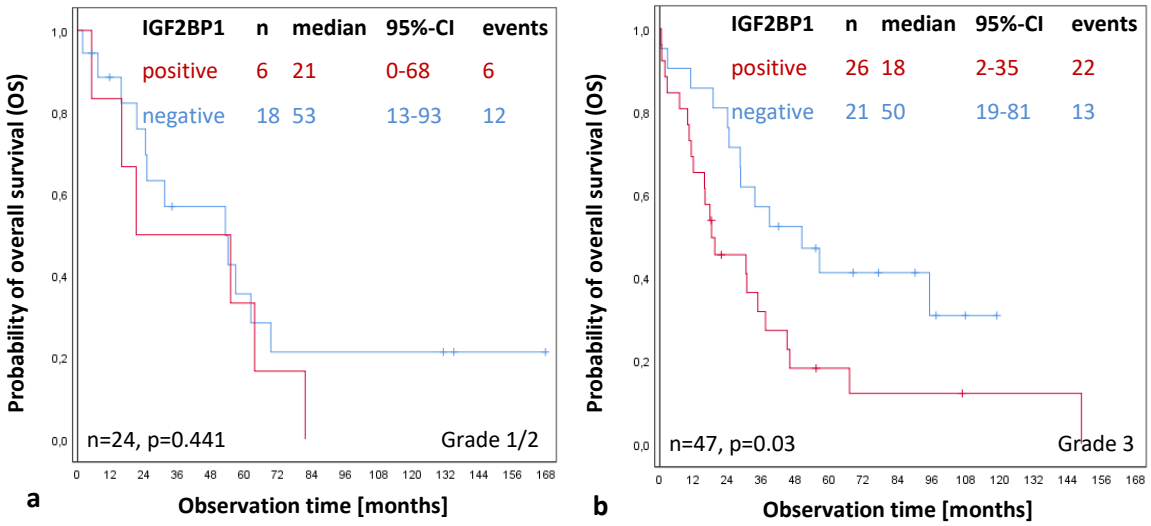

Figure 10. Kaplan Meier Curve with the OS of the IGF2BP1-positive and –negative subgroups stratified for tumour grade (a) 1/2 and (b) 3. Expression of IGF2BP1 correlates with poor OS in poorly differentiated tumours.

Analysis of type 1 and type 2 tumours showed a significantly reduced OS only in type 2 tumours (**Figure 11**).

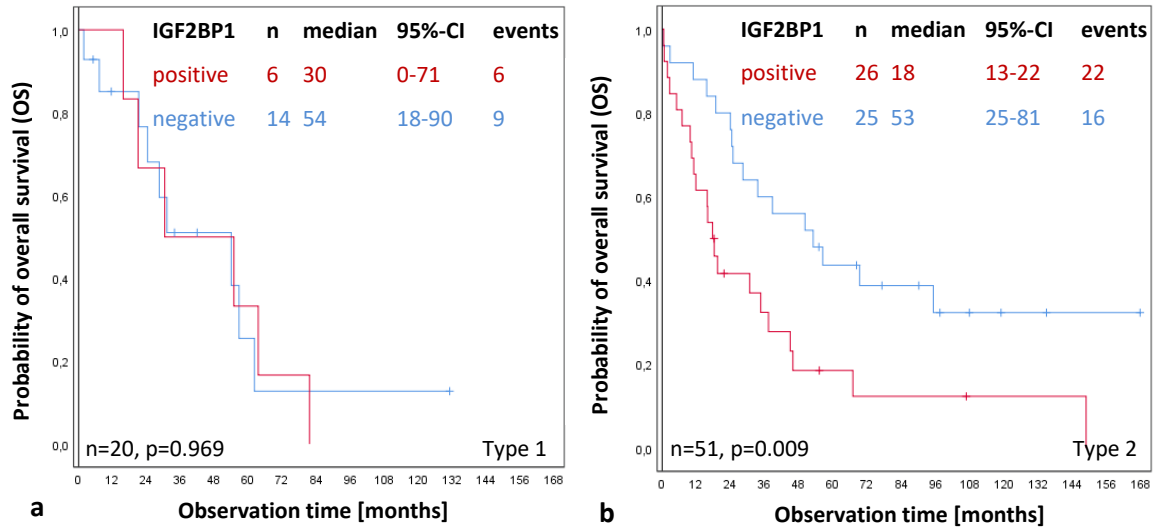

Figure 11. Kaplan Meier Curve with the OS of the IGF2BP1-positive and –negative subgroups stratified for tumour type (a) 1 and (b) 2. Expression of IGF2BP1 correlates with poor OS in type 2 tumours.

Macroscopic residual disease is the most important known prognostic factor for ovarian cancer, yet in this cohort the presence of macroscopic residual tumour showed no effect on OS or PFS (**Figure 12**). Stratifying for macroscopic residual disease after surgery, the expression of

IGF2BP1 correlated significantly with reduced OS in the subgroup with macroscopic residual tumour. This trend was also seen in the subgroup without macroscopic residual disease, without being significant (**Figure 13**). No correlation was found between the presence of macroscopic residual tumour and survival either in tumours with or without IGF2BP1 expression, although there was a trend of reduced OS with residual tumour in the IGF2BP1-positive subgroup. (**Figure 14**)

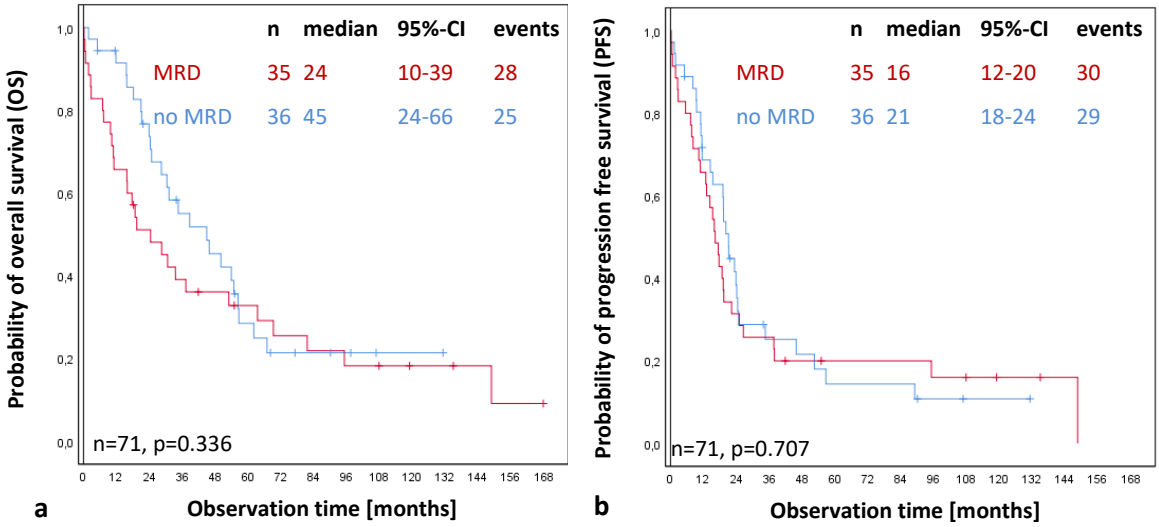

Figure 12. Kaplan Meier Curve with the cumulative (a) OS and (2) PFS of the subgroups with and without macroscopic residual disease (MRD). The results show no significant difference between the subgroups.

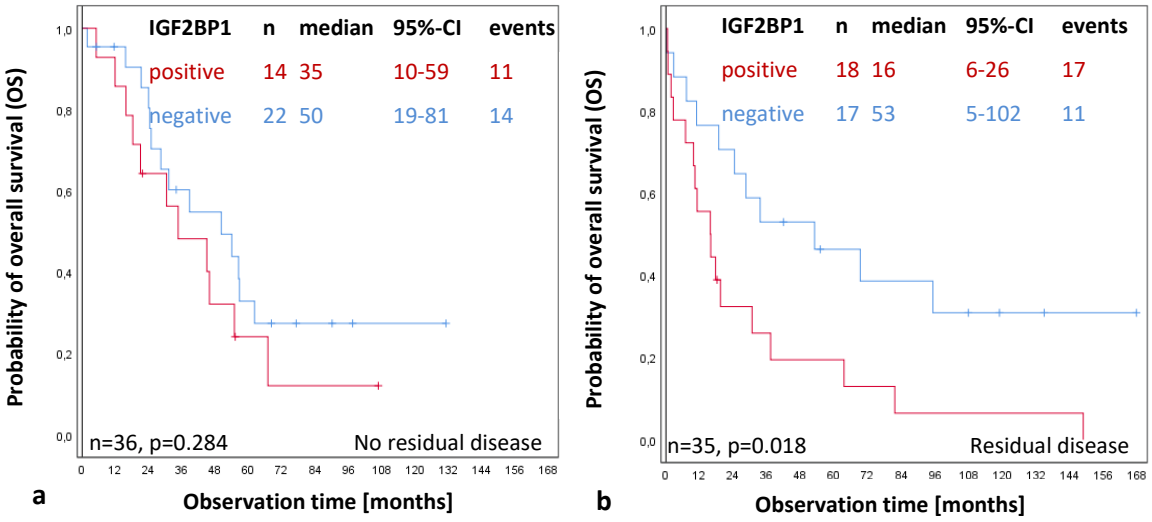

Figure 13. Kaplan Meier Curve with the OS of the IGF2BP1-positive and –negative subgroups stratified for presence of residual disease after surgery. Expression of IGF2BP1 correlates significantly with poor OS in tumours with residual disease (b).

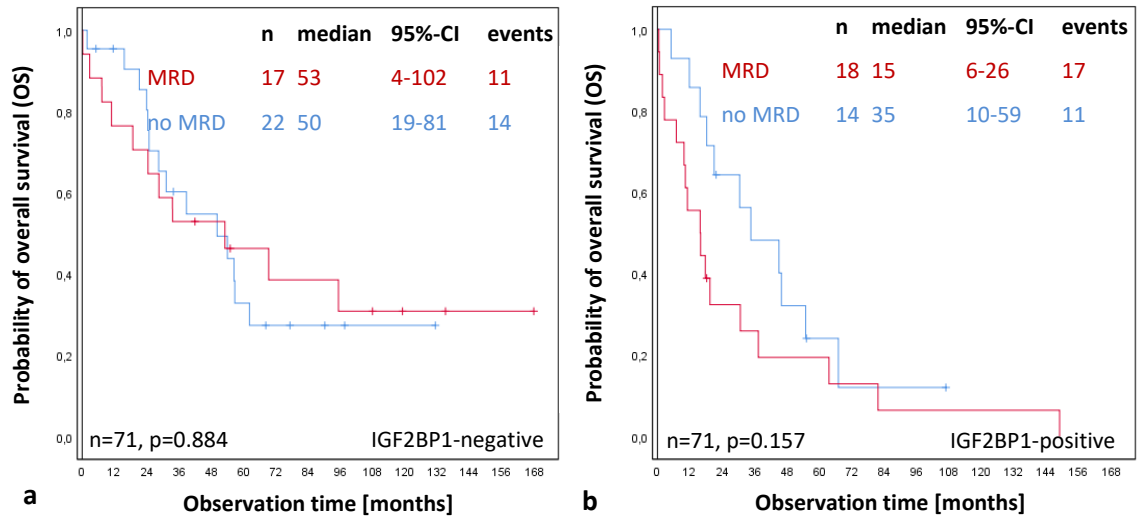

Figure 14. Kaplan Meier Curve with the OS of the subgroups with and without macroscopic residual disease (MRD), stratified for expression of IGF2BP1. Presence of residual disease does not correlate with overall survival either in the IGF2BP1-positive (b) or –negative (a) subgroup.

4.4 Survival time analysis via Cox regression model

4.4.1 Overall survival

We analysed the effect of patient characteristics on their OS via the Cox regression model. In the univariate analysis, the variables histological type, lymph node metastasis, distant metastasis, pleural infiltration and first-line chemotherapy showed a significant impact on OS. Interestingly, there was no significant effect on the known prognostic factors age at diagnosis, tumour stage, tumour grade, tumour type, and macroscopic residual disease. The trend,

however, generally met the expectation. The expression of IGF2BP1 showed one of the highest effects of all characteristics, with a risk of death two times higher than without IGF2BP1 expression. **Table 9** shows the results of the univariate Cox analysis in detail.

Table 9. Univariate analysis of the Cox regression model for the patient characteristics and the expression of IGF2BP1 for the end point OS. The marked subgroups were used as reference.

		n	HR	95%-CI	p
IGF2BP1	negative	39			
	positive	32	**2.028**	(1.176 - 3.497)	0.011
Age	< 60 years	17			
	≥ 60 years	54	**1.258**	(0.63 - 2.511)	0.515
Epithelial or mixed tumour	Epithelial carcinoma	64			
	MMMT	7	**3.159**	(1.169 - 8.537)	0.023
Manifestation	One side	39			
	Both sides	26	**1.359**	(0.76 - 2.431)	0.301
Stage	FIGO I-II	7			
	FIGO III-IV	64	**1.798**	(0.56 - 5.774)	0.324
Lymph node metastasis	Yes	45	**2.095**	(1.066 - 4.114)	0.032
	No	20			
Distant metastasis	Yes	29	**1.787**	(1.039 - 3.073)	0.036
	No	42			
Grade	1+2	24			
	3	47	**1.14**	(0.644 - 2.015)	0.653
Type of tumour	Type 1	20			
	Type 2	51	**0.954**	(0.522 - 1.744)	0.878
Macroscopic residual disease	No residual tumour	36			
	Residual tumour	35	**1.307**	(0.756 - 2.261)	0.337
Preoperative ascites	Yes	52	**1.085**	(0.593 - 1.985)	0.792
	No	18			
CA 125 before surgery	Normal	5			
	Elevated	63	**2.077**	(0.503 - 8.575)	0.313
CA 125 normalised after chemotherapy	Normal	39			
	Elevated	16	**1.148**	(0.571 - 2.305)	0.699
Albumin before surgery	Lowered	54			
	Normal or elevated	4	**2.153**	(0.752 - 6.162)	0.153
Intestinal infiltration	Yes	55	**1.149**	(0.589 - 2.243)	0.684
	No	16			
Pleural infiltration	Yes	11	**2.436**	(1.229 - 4.829)	0.011
	No	60			
First-line chemotherapy	Carboplatin/Taxan: 6-8 cycles	51			
	Others/No chemotherapy	20	**2.074**	(1.132 - 3.802)	0.018

Abbreviations: CA125, cancer antigen 125; FIGO, International Federation of Gynaecology and Obstetrics; HR, hazard ratio; IGF2BP1, insulin-like growth factor 2 mRNA-binding protein 1; MMMT, Malignant mixed Müllerian tumour.

For the multivariate analysis, the expression of IGF2BP1 was adjusted to the factors that show the strongest effect on OS according to the literature: age at diagnosis, tumour stage, grading,

histological subtype cf. type of tumour, macroscopic residual disease, and first-line chemotherapy. In this analysis, IGF2BP1 failed to provide additional prognostic information and showed no effect on the OS (**Table 10**). In this cohort the known prognostic factors did not show the expected effect on OS, and the IGF2BP1 expression was adjusted in a second multivariate analysis to the factors that showed the highest significance in the univariate analysis: lymph node metastasis, distant metastasis, histological subtype cf. epithelial or mixed tumour, pleural infiltration and first-line chemotherapy, as well as macroscopic residual disease as the most important clinical prognostic factor. Again, IGF2BP1 showed no prognostic relevance. In this analysis, pleural infiltration had the highest impact on OS (**Table 11**).

Table 10. Multivariate analysis of the Cox regression model for the end point OS for the expression of IGF2BP1 adjusted to the most significant prognostic factors in clinical practise. The marked subgroups were used as reference.

		n	HR	95%-CI	p
IGF2BP1	negative	39			
	positive	32	**1.473**	(0.761 - 2.851)	0.25
Age	< 60 years	17			
	≥ 60 years	54	**1.217**	(0.589 - 2.515)	0.596
Stage	FIGO I-II	7			
	FIGO III-IV	64	**1.75**	(0.449 - 6.819)	0.42
Grade	1+2	24			
	3	47	**1.247**	(0.563 - 2.761)	0.587
Type of tumour	Type 1	20			
	Type 2	51	**0.585**	(0.229 - 1.493)	0.262
Macroscopic residual disease	No residual tumour	36			
	Residual tumour	35	**1.274**	(0.704 - 2.308)	0.424
First-line chemotherapy	Carboplatin/Taxan: 6-8 cycles	51			
	Others/No chemotherapy	20	**1.868**	(0.903 - 3.866)	0.092

Abbreviations: CI, confidence interval; FIGO, International Federation of Gynaecology and Obstetrics; HR, hazard ratio; IGF2BP1, insulin-like growth factor 2 mRNA-binding protein 1.

Table 11. Multivariate analysis of the Cox regression model for the end point OS for the expression of IGF2BP1 adjusted to the most significant prognostic factors in the univariate analysis and macroscopic residual disease. The marked subgroups were used as reference.

		n	HR	95%-CI	p
IGF2BP1	negative	39			
	positive	32	**1.315**	(0.67 - 2.581)	0.425
Epithelial or mixed tumour	Epithelial carcinoma	64			
	MMMT	7	**3.109**	(0.945 - 10.229)	0.062
Lymph node metastasis	Yes	45	**1.695**	(0.787 - 3.651)	0.178
	No	20			
Distant metastasis	Yes	29	**1.056**	(0.507 - 2.202)	0.883
	No	42			
Macroscopic residual disease	No residual tumour	36			
	Residual tumour	35	**0.994**	(0.523 - 1.887)	0.984
Pleural	Yes	11	**3.319**	(1.384 - 7.96)	0.007

infiltration	No	60			
First-line chemotherapy	Carboplatin/Taxan: 6-8 cycles	51			
	Others/No chemotherapy	20	**1.647**	(0.803 - 3.381)	0.174

Abbreviations: CI, confidence interval; HR, hazard ratio; IGF2BP1, insulin-like growth factor 2 mRNA-binding protein 1; MMMT, Malignant mixed Müllerian tumour.

4.4.2 Progression free survival

Analogous to the OS, the impact of patient characteristics on the PFS was analysed. In the univariate analysis, the histological type, localisation, lymph node metastasis, pleural infiltration and first-line chemotherapy variables showed a significant effect on the PFS. The tumour stage had a bigger effect on the PFS than on the OS in this analysis, with a risk of death three times higher in higher tumour stages. The death risk for patients with expression of IGF2BP1 was nearly two times higher than for patients without IGF2BP1 expression. **Table 12** shows the results of the univariate Cox analysis for PFS in detail.

Table 12. Univariate analysis of the Cox regression model for the patient characteristics and the expression of IGF2BP1 for the end point PFS. The marked subgroups were used as reference.

		n	HR	95%-CI	p
IGF2BP1	negative	39			
	positive	32	**1.905**	(1.129 - 3.214)	0.016
Age	< 60 years	17			
	≥ 60 years	54	**1.223**	(0.647 - 2.311)	0.536
Epithelial or mixed tumour	Epithelial carcinoma	64			
	MMMT	7	**2.924**	(1.217 - 7.022)	0.016
Manifestation	One side	39			
	Both sides	26	**1.744**	(1.002 - 3.035)	0.049
Stage	FIGO I-II	7			
	FIGO III-IV	64	**3.004**	(0.937 - 9.632)	0.064
Lymph node metastasis	Yes	45	**2.355**	(1.251 - 4.43)	0.008
	No	20			
Distant metastasis	Yes	29	**1.587**	(0.943 - 2.67)	0.082
	No	42			
Grade	1+2	24			
	3	47	**1.149**	(0.662 - 1.994)	0.621
Type of tumour	Type 1	20			
	Type 2	51	**1.139**	(0.631 - 2.056)	0.666
Macroscopic residual disease	No residual tumour	36			
	Residual tumour	35	**1.104**	(0.658 - 1.852)	0.707
Preoperative ascites	Yes	52	**1.227**	(0.688 - 2.19)	0.488
	No	18			
CA 125 before surgery	Normal	5			
	Elevated	63	**1.113**	(0.401 - 3.091)	0.837
CA 125 normalised after chemotherapy	Normal	39			
	Elevated	16	**1.423**	(0.743 - 2.723)	0.287

Albumin before surgery	Lowered	54			
	Normal or elevated	4	**1.587**	(0.566 - 4.454)	0.38
Intestinal infiltration	Yes	55	**1.371**	(0.725 - 2.595)	0.332
	No	16			
Pleural infiltration	Yes	11	**2.091**	(1.065 - 4.102)	0.032
	No	60			
First-line chemotherapy	Carboplatin/Taxan: 6-8 cycles	51			
	Others/No chemotherapy	20	**2.327**	(1.326 - 4.084)	0.003

Abbreviations: CA125, cancer antigen 125; FIGO, International Federation of Gynaecology and Obstetrics; HR, hazard ratio; IGF2BP1, insulin-like growth factor 2 mRNA-binding protein 1; MMMT, Malignant mixed Müllerian tumour.

The most important prognostic factors are relevant for OS and PFS. With adjustment of the IGF2BP1 expression to those parameters in the multivariate analysis, the protein showed no effect on PFS. Tumour stage and first-line chemotherapy had the highest impact on PFS (**Table 13**). In a second multivariate analysis, the expression of IGF2BP1 was adjusted to the factors that were most relevant in the univariate analysis: tumour stage, lymph node metastasis, histological subtype cf. epithelial or mixed tumour, pleural infiltration, first-line chemotherapy, and additionally macroscopic residual disease. Again, the expression of IGF2BP1 did not have any effect on PFS, and pleural infiltration, first-line chemotherapy, and lymph node metastasis had an independent effect on the outcome (**Table 14**).

Table 13. Multivariate analysis of the Cox regression model for the end point PFS for the expression of IGF2BP1 adjusted to the most significant prognostic factors in clinical practise. The marked subgroups were used as reference.

		n	HR	95%-CI	p
IGF2BP1	negative	39			
	positive	32	**1.212**	(0.647 - 2.272)	0.548
Age	< 60 years	17			
	≥ 60 years	54	**1.048**	(0.533 - 2.062)	0.891
Stage	FIGO I-II	7			
	FIGO III-IV	64	**4.004**	(1.043 - 15.373)	0.043
Grade	1+2	24			
	3	47	**1.053**	(0.477 - 2.323)	0.899
Type of tumour	Type 1	20			
	Type 2	51	**0.605**	(0.239 - 1.532)	0.289
Macroscopic residual disease	No residual tumour	36			
	Residual tumour	35	**0.93**	(0.532 - 1.627)	0.799
First-line chemotherapy	Carboplatin/Taxan: 6-8 cycles	51			
	Others/No chemotherapy	20	**2.454**	(1.245 - 4.837)	0.01

Abbreviations: CI, confidence interval; FIGO, International Federation of Gynaecology and Obstetrics; HR, hazard ratio; IGF2BP1, insulin-like growth factor 2 mRNA-binding protein 1.

Table 14. Multivariate analysis of the Cox regression model for the end point PFS for the expression of IGF2BP1 adjusted to the most relevant prognostic factors in the univariate analysis and macroscopic residual disease. The marked subgroups were used as reference.

		n	HR	95%-CI	p
IGF2BP1	negative	39			
	positive	32	**0.938**	(0.482 - 1.823)	0.85
Epithelial or mixed tumour	Epithelial carcinoma	64			
	MMMT	7	**2.526**	(0.813 - 7.854)	0.109
Stage	FIGO I-II	7			
	FIGO III-IV	64	**1.771**	(0.466 - 6.721)	0.401
Lymph node metastasis	Yes	45	**2.101**	(0.991 - 4.456)	0.053
	No	20			
Macroscopic residual disease	No residual tumour	36			
	Residual tumour	35	**0.644**	(0.344 - 1.206)	0.169
Pleural infiltration	Yes	11	**2.818**	(1.349 - 5.888)	0.006
	No	60			
First-line chemotherapy	Carboplatin/Taxan: 6-8 cycles	51			
	Others/No chemotherapy	20	**2.336**	(1.156 - 4.722)	0.018

Abbreviations: CI, confidence interval; FIGO, International Federation of Gynaecology and Obstetrics; HR, hazard ratio; IGF2BP1, insulin-like growth factor 2 mRNA-binding protein 1, MMMT, Malignant mixed Müllerian tumour.

5 Discussion

5.1 Summary of the findings

This study assessed the proportion and pattern of IGF2BP1 protein expression by IHC, and described the association between IGF2BP1 protein expression and the histopathological and clinical parameters of the tumours and patients, including the course of disease.

IGF2BP1 was expressed in 45% (n=32) of this cohort (n=71), and was demonstrated to have prognostic relevance, as patients with IGF2BP1 expression showed decreased OS and PFS and twice the risk of death compared to patients without IGF2BP1 expression. However, in the multivariate analysis, IGF2BP1 expression revealed no independent prognostic significance.

The staining pattern of the IGF2BP1 protein expression was inter- and intratumourally heterogeneous. In the median, the tumours expressed IGF2BP1 in 13% of tumour cells, with maximum variants showing <1% up to 100% stained tumour cells, while in most cases the staining pattern was presented as IGF2BP1-positive cell islands within IGF2BP1-negative tumour areas. The highest expression with 100% of IGF2BP1-positive tissues was found in MMMT. The staining was detected in epithelial tumour cells and was solely intracytoplasmic, where it is known to be primarily located, although it may be involved in the nuclear export of target RNAs and thus found intranuclearly as well (Oleynikov and Singer 2003). In some cases, IGF2BP1 expression was sporadically found in tumour stroma cells. There was no significant difference in IGF2BP1 protein expression between the primary tumour tissue and metastatic tumour tissues of the same patient. Among the histopathological and clinical parameters, IGF2BP1 protein expression was associated with higher tumour stages at first diagnosis, presence of lymph node metastasis, and a poor level of differentiation, confirming the protein's role in tumour aggression. It was also associated with intestinal infiltration at the time of surgery in type 1 tumours and with deviations from the standard chemotherapy in type 2 tumours. Nevertheless, there was no difference between IGF2BP1 expression in type 1 and type 2 tumours.

5.2 IGF2BP1 expression correlates with reduced prognosis and aggressive tumour properties

In agreement with published data, as previously shown on protein (Köbel et al. 2007) and mRNA level (Gu et al. 2004), IGF2BP1 expression is associated with decreased survival, but gives no additional prognostic information when adjusted to other prognostic factors.

A re-evaluation of 1232 serous ovarian carcinomas combining available datasets also demonstrated a significant association of up-regulated mRNA expression with reduced OS (HR=1.34; 95%-CI 1.07-1.67; p=0.011) and PFS (HR=1.57; 95%-CI 1.28-1.92; p<0.001) (Müller et al. 2018). If the results of our cohort are restricted to the cases of HGSC (n=42) for better comparability with previous findings, then our data is generally in line with the dataset results (HR 2.41; 95%-CI 1.18-4.90; p=0.016 in the univariate and HR 1.71; 95%-CI 0.62-4.77; p=0.302 in the multivariate analysis for OS). The results for the sub-cohort of HGSC are nearly the same as for the total cohort.

Our clinical results concerning the association of IGF2BP1 expression and aggressive tumour progression are, firstly, in line with data for cancer-derived cell lines, where an association between the deletion of IGF2BP1 and impairment of growth and metastasis, and, on the other hand, between the up-regulation of IGF2BP1 and an aggressive tumour cell phenotype, was previously demonstrated (Müller et al. 2018). Secondly, our data also corresponds to published clinical data, where IGF2BP1 expression was correlated with higher tumour stages and tumour grading on protein (Köbel et al. 2007) and mRNA level (Gu et al. 2004). The results also correspond to the data from a sub-cohort of the present study (n=29), published by Busch et al. from our group, which showed a correlation between IGF2BP1 expression and FIGO stage at the RNA level via qRT-PCR (Busch et al. 2016), and thus directly validates the results of this study at RNA level.

There was also an association between IGF2BP1 up-regulation cf. over-expression and poor survival for lung adenocarcinoma, oesophageal adenocarcinoma, hepatocellular carcinoma, and neuroblastoma (Huang et al. 2018). An association of IGF2BP1 with advanced stages and poor grading was also shown for hepatocellular carcinoma and neuroblastoma (Huang et al. 2018).

Paradoxically, the expression of IGF2BP1 correlated inversely with grading, lymph node metastasis, distant metastasis, and (lympho-)vascular invasion in gall bladder cancer, and in these cases IGF2BP1 was associated with longer survival time (Kessler et al. 2017). Applying the score used by Kessler and colleagues to the present cohort, the patients in our cohort showed significantly reduced overall survival with high staining intensity (p=0,019 in log rank test). It is not yet known why IGF2BP1 is associated with improved outcomes in some neoplasias. It has been suggested that stromal IGF2BP1 has a tumour-suppressive role in colon carcinomas (Hamilton et al. 2015).

It was homogeneously shown, however, that the data of the present study correlates in vitro and in vivo with published data and thus confirms that IGF2BP1 has a tumour-promoting role in ovarian cancer.

5.3 Evaluation of the expression rates of IGF2BP1 employing immunohistochemistry

There is no standard evaluation of staining signals for IHC with anti-IGF2BP1 antibodies. The IRS was chosen for evaluation in the present study as it includes both SI and PC variables, which separately showed a significant effect on overall survival (p=0.037 for PC-score and p=0.004 for SI-score in log rank test). Moreover, it has been well established for a long time in clinical routine.

Davidson et al. (2014) found that 36% of primary tumours (n=25) and 28% of metastases (n=36) were IGF2BP1-positive in IHC, considering IGF2BP1-positivity as PC of 1% or more. Applying this score in the present study, 38% of primary tumours and 23% of peritoneal metastases were IGF2BP1-positive, and thus correspond to the proportions found by Davidson et al. The similar IGF2BP1 expression in primary tumour tissue and metastatic tissue suggests that the conditions in metastatic tissue differ little from those in the primary tumour and have little effect on the expression of IGF2BP1. Our analysis also showed that the expression of IGF2BP1 in primary tumour tissue is related to the expression in metastatic tissue. Both results suggest that the expression of IGF2BP1 is tumour-immanent and does not manifest itself in the course of tumour progression.

Conversely, Köbel et al. (2007) found IGF2BP1 protein expression in 69% of their cohort of ovarian carcinomas (n=107), and thus significantly more than we detected in the present study. When applying their detection score, that is, considering IGF2BP1-positivity as PC of 5% or more, to this study, a reduction to one third positive IGF2BP1 cases was detected without any correlation with clinical outcome.

One of the main reasons for the discordant IHC results concerning IGF2BP1 expression may be different detection antibodies and staining procedures, for example concerning the selection and dilution of the antibody. Even if the antibody is tested for specificity using western blot, the results can only be transferred to IHC analysis to a limited extent, due to denatured conditions for embedding the tissue (FFPE). Köbel and colleagues noted the cross reactivity of the used anti-IGF2BP1 antibody to other members of the IGF2BP family by western blot, which might also explain the higher detection rate in IHC.

Caution is required regarding differences in the evaluation methods, as not only the expression rates, but also the correlation to survival rates, depend on the score used in IHC. Zhou et al. (2015) chose a score based on the IRS, with a slightly different grouping of PP and a higher threshold. In their study, high IGF2BP1 expression in 61.2% of the cohort correlated with reduced OS and PFS in hepatocellular carcinoma tissues value. Using this score in the present cohort, only 15% of ovarian cancer patients showed high expression without any correlation with prognosis. Other published scores for IGF2BP1 also did not show any association with clinical outcome (Köbel et al. 2007; Zhou et al. 2015) or showed an opposite correlation to the present study (Kessler et al. 2017).

In summary, data in the literature is not very comparable, either to our findings or to other studies, due to different evaluation methods. Not only is a uniform evaluation score required, but also a standard IHC staining protocol and a standard detection antibody, so as to obtain comparable results for IGF2BP1 expression.

5.4 IGF2BP1 in malignant mixed Müllerian tumours

Although a rare entity, there was homogeneous expression of IGF2BP1 in our sub-cohort of MMMT (n=7). MMMT is an aggressive histological subtype of ovarian cancer with epithelial and mesenchymal elements. Those tumours are staged and commonly treated according to epithelial ovarian cancer (EOC), share similar prognostic factors and show worse outcomes compared to EOC (del Carmen et al. 2012). Given the rarity of MMMT, it lacks studies of suitable targets for effective treatment and no improvement in survival rate has been attained in the past few decades, as there are no established guidelines for therapeutic management (Berton-Rigaud et al. 2014). In addition to the strikingly high expression rate, the expression pattern in four out of seven (57%) patients also showed high expression, with PC of 80% or above (cf. 15% in IGF2BP1-positive EOC patients) and a medium or high SI in 100% of cases (cf. 68% in IGF2BP1-positive EOC patients). The patients of this sub-cohort showed the worst outcome of all histological subgroups (median OS 16 months, 95%-CI 6-26 months for MMMT, cf. 37 months, 95%-CI 21-54 months for EOC, p=0.017 in log rank test).

These findings particularly correspond to the association of IGF1BP1 with a mesenchymal-proliferative gene signature. Our group recently demonstrated that IGF2BP1 plays a role in a specific aggressive, mesenchymal-like molecular subtype of HGSC (C5 cluster), which is characterised by high epithelial-to-mesenchymal transition (Tothill et al. 2008; Bley et al. 2020). IGF2BP1 was found to be up-regulated in C5 tumours of EOC patients and cell lines,

promoting SRC activation and increasing ERK2 expression, and suggesting IGF2BP1 as a novel marker for this molecular subtype and associated EOC therapy (Bley et al. 2020).
Given these results for a rare cohort, IGF2BP1 has the potential to become a marker or therapeutic target, especially for MMMT, in future studies.

5.5 Further outlook for IGF2BP1 in ovarian cancer

A further research approach is to examine IGF2BP1 not only in tumour tissue but in serum, and to test its diagnostic ability. So far, few antigens in serum or plasma have been identified that enable an early diagnosis of ovarian cancer. Antigens and autoantibodies, such as anti-IL8 (Lokshin et al. 2006) or epithelial cell adhesion molecule (Ep-CAM) (Kim et al. 2003) could increase the diagnostic power of plasmatic early cancer detection in addition to CA-125. Anti-IGF2BP1 autoantibodies were found in 26.5% of the serum of ovarian cancer patients (Liu et al. 2014). Further studies are necessary to evaluate the diagnostic capability of IGF2BP1.
IGF2BP1 could also play a role in the prognosis and modulation of therapeutic strategies. Cancer patients frequently develop resistance to chemotherapy, and IGF2BP1 seems to play a role for both of the most important therapeutic drugs. The up-regulation of IGF2BP1 in tumour tissue evaluated by IHC was detectable after chemotherapy, and in line with this, cell lines over-expressing IGF2BP1 were more resistant to Paclitaxel-induced cell death (Boyerinas et al. 2012). IGF2BP1 was also found to be up-regulated in platinum-resistant cell lines, and its over-expression reduced chemo sensitivity by reversing the miR-708-mediated susceptibility of ovarian cancer cells to cisplatin. (Qin et al. 2017). Future research might consider that the expression of IGF2BP1 might rather affect the selection of treatment for ovarian cancer patients or itself become a therapeutic target to increase drug sensitivity in ovarian cancer.
The IGF2BP1-dependent cell signalling pathways may also be potential therapeutic targets. Our group used *in vitro* experiments to demonstrate that combined incubation with saracatinib as the SRC-inhibitor and selumetinib as the MEK-inhibitor reduced IGF2BP1-promoted invasive growth in 3D cultures and mouse models (Bley et al. 2020). Both inhibitors had already been tested separately in clinical trials in ovarian cancer patients, but show no clear improvement in therapy response or PFS so far (McNeish et al. 2014; Farley et al. 2013).
With its oncofetal, growth- and survival-promoting properties, IGF2BP1 itself is also an attractive anticancer drug target. Small molecule inhibitors for IGF2BP1 are hard to find, but strategies of identification are being explored for selective inhibitors (Mahapatra et al. 2014). So far, the small molecule BTYNB has been identified as an elective inhibitor of IGF2BP1

binding to c-Myc mRNA, down-regulating mRNA transcripts regulated by IGF2BP1 and leading to inhibited proliferation of IGF2BP1-containing ovarian cancer and melanoma cells *in vitro* (Mahapatra et al. 2017).

Altogether, the effect of IGF2BP1 and IGF2BP1-mediated cell signalling as drug targets or usable inhibitors in cancer therapy seems to be promising and requires further research.

5.6 Notable strengths and limitations of the present work

The strengths of the study lie in the standardised cohort recruitment and quality-assured determination of the results. The study cohort was recruited and samples were collected prospectively. The pre-analytical conditions, that is, the material collection and preparation, were standardised without exception. The commercially purchased detection antibody and staining protocol were validated by testing different antibodies and dilution series directly on FFPE tissue and were selected by experienced pathologists.

The limitations of the study lie in its study design, case numbers and the high-risk cohort. Despite the prospective cohort, the present work is a retrospective study. The sample size of 71 patients was limited, and so was the availability of different tissue category samples, and thus complex statistical investigations for important subgroups were only possible to a limited extent. Larger cohorts with a representative number of each subtype are necessary to determine the importance of IGF2BP1 for each subtype, especially since the different histological subtypes of ovarian cancer are associated with characteristic molecular alterations and increasingly identified as different diseases (Matias-Guiu and Davidson 2014). Generally, the small number of cases limits the validity of the statistical analyses.

The cohort itself was highly heterogeneous with more advanced tumour stages at the time of diagnosis and a worse outcome than would be expected from a representative cohort of patients with ovarian cancer. In order to explain the large deviation of the present cohort from the nationwide average, the sub-cohort in the present study was compared to the total cohort of all cases of ovarian cancer patients who underwent surgery at the University Hospital Halle during the same time period. Of the 171 documented cases, only 29% were remotely metastasised at the time of diagnosis, which is comparable to the average rate of 26% (Buttmann-Schweiger and Kraywinkel 2019). The five-year-probability of survival for the total cohort was 44% (95%-CI 36-52%), which corresponds more closely to the nationwide average of 41% (95%-CI 39-42%) (Buttmann-Schweiger and Kraywinkel 2019) than the five-year-probability of survival of 31% (95%-CI 19-42%) for the present sub-cohort. There was also less

postoperative residual disease in the total cohort (60% without residual tumour) than in the present sub-cohort (51% without residual tumour). The discrepancy between the total cohort and the sub-cohort can be explained by the admission criteria for the tumour bank, as patients with a large tumour mass were more likely to be selected for reservation of fresh frozen material. When interpreting the results, it must therefore be taken into account that they were collected from a high-risk cohort, which does not represent the national average.

5.7 Conclusions and review

In conclusion, analysis of primary and metastatic tumour tissues of ovarian cancer showed IGF2BP1 protein expression that correlates with decreased OS and PFS and parameters for increased tumour aggression. Although the results seem to be auspicious, prospective studies with larger cohorts that enable more detailed differentiation into subgroups of ovarian cancer are necessary to evaluate the qualification of IGF2BP1 as a biomarker and therapeutic target.

5.8 Author’s contribution to the work

The author's own contribution to the work consisted of the creation of the cohort and logistic supervision of the block selection, the IHC staining by a medical-technical assistant and its evaluation by two pathologists. The author also completed the available database with clinical and histopathological variables and survival data from research in pathology reports and medical letters and contact with patients’ primary physicians. The author performed the literature research, statistical analyses by SPSS and the interpretation and presentation of these results for the present paper.

6 Summary

IGF2BP1 is becoming increasingly interesting as a biomarker and potential therapeutic target in cancer diagnosis and therapy. With the help of this work, we were able to add to the existing knowledge on the expression of IGF2BP1 in ovarian carcinomas by describing the association between IGF2BP1 protein expression and detailed clinical and histopathological parameters of the patients and tumours.

IGF2BP1 expression was demonstrated to have a tumour-promotiong role and prognostic relevance in ovarian cancer. In agreement with published data in vitro and in vivo, IGF2BP1 expression is associated with decreased OS and PFS, but gives no additional prognostic information when adjusted to other prognostic factors. Also in agreement with published data, IGF2BP1 expression is associated with higher tumour stages at first diagnosis, presence of lymph node metastasis, and a poor level of differentiation.

As there is no standard evaluation method for IHC with anti-IGF2BP1 antibodies, the findings are hardly comparable to data in the literature. However, the proportions of the staining with 45% of IGF2BP1-positive tumours and, in detail, with around 40% of IGF2BP1-positive primary tumours and around 30% of IGF2BP1-positive metastases, were in line with previous findings. The separate analysis and comparison of metastatic and primary tumour tissues allowed the suggestion that the different conditions in both tissue types have little effect on IGF2BP1 expression, and that the expression of IGF2BP1 is tumour-immanent and does not manifest itself in the course of tumour progression.

Moreover, we recognised a special role of IGF2BP1 in MMMT. It showed an extraordinarily high expression rate in this small, but rare sub-cohort with a poor outcome.

The results suggest that IGF2BP1 has the potential to become a biomarker or therapeutic target for ovarian cancer, and current clinical trials are already testing inhibitors of the IGF2BP1-dependent cell signalling pathways on ovarian cancer patients. However, to definitely evaluate this potential, further, prospective studies are necessary.

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8 Theses

1. IGF2BP1 protein expression was detected by immunohistochemistry in 45% (n=32) of the cohort.
2. The inter- and intratumoural staining pattern of the IGF2BP1 protein expression was heterogeneous with <1% up to 100% stained tumour cells.
3. IGF2BP1 protein expression was always found to be intracytoplasmic in epithelial tumour cells and in single cases in stromal tumour cells.
4. No difference was observed in IGF2BP1 protein expression rate between primary tumour tissue and metastatic tumour tissues of the same patient, suggesting that IGF2BP1 expression is tumour-immanent and does not manifest itself in the course of tumour progression.
5. IGF2BP1 protein expression was associated with higher tumour stages at first diagnosis, the presence of lymph node metastasis, a low level of differentiation, intestinal infiltration and deviations from the standard chemotherapy.
6. Patients with IGF2BP1 protein expression showed decreased overall survival (OS) and progression-free survival (PFS) compared to patients without IGF2BP1 protein expression [median OS 19 (95% CI 3-36) cf. 53 (95% CI 28-78) months; median PFS 14 (95% CI 11-18) cf. 23 (95% CI 18-29) months].
7. IGF2BP1 protein expression meant that the risk of death doubled (HR 2.028; 95% CI 0.831-2.691).
8. IGF2BP1 expression revealed no independent prognostic information in multivariate analysis, adjusted for age, FIGO stage, grading, histological subtype, macroscopic residual tumour and chemotherapy.
9. In summary, the findings confirm the tumour-promoting role of IGF2BP1 in ovarian cancer.

Selbständigkeitserklärung

Hiermit versichere ich, Olga Ungurs, die vorliegende Arbeit „The expression of Insulin-like growth factor 2 mRNA-binding protein 1 in ovarian cancer" selbstständig angefertigt zu haben. Von mir genutzte Hilfsmittel und Literatur sind vollständig angegeben.

Halle (Saale),

Olga Ungurs

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Halle (Saale),

Olga Ungurs

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