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New insights into the multimodal management of epithelial ovarian cancer

Multimodale prädiktive und prognostische Faktoren zum klinischen Verlauf von Patientinnen mit Ovarialkarzinom

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Abbreviations:

AUC: Area Under the Curve

ARID1A: AT-rich interactive domain 1A gene

BOT: borderline tumors of the ovary

CA125: Cancer Antigen 125

CI: Confidence Interval

EOC: Epithelial Ovarian Cancer

FIGO: International Federation of Obstetricians and Gynecologists

HRD: homologous recombination deficiency

HGSOC: high grade serous ovarian cancer

IDS: interval debulking surgery

AGO Arbeitsgemeinschaft Gynäkologische Onkologie

BRCA1/2 breast cancer gene 1/2

ECOG Performance Status: Eastern Cooperative Oncology Group Performance Status

IMO Intraoperative Mapping of Ovarian cancer

IQR Interquartile Range

IOTA: International Group for Ovarian Tumor Analysis

KRAS: Kirsten rat sarcoma viral oncogene homolog

LGSOC: low grade serous ovarian cancer

MEK: MAPK/Erk kinase

OS: Overall survival

OR: Odds Ratio

PARP: Poly(ADP-Ribose)polymerase

PFS: progression-free survival

PDS: primary debulking surgery

PIK3: phosphatidylinositol-4,5-bisphosphate 3-kinase

pTEN: phosphatase and tensin homolog gene

ROMA: risk of ovarian malignancy algorithm

STIC: serous tubal intraepithelial carcinoma

TOC tumor bank ovarian cancer

TCGA The Cancer Genome Atlas

ROCA: risk of ovarian cancer algorithm

ROMA: risk of ovarian malignancy algorithm

WHO: world health organization

1 Introduction

Ovarian cancer is the fifth most common malignancy in women, but the first cause of death in the developed world due to gynecological cancer (1). The poor prognosis is mostly caused by late diagnosis, most of the patients (75%) being diagnosed with advanced FIGO III and IV stage ovarian cancer when survival rates are poor. The late diagnosis is caused mainly by lack of specific symptoms, of screening and early diagnosis tests. However the 5-years survival rates are around 80 to 90% with FIGO stage I ovarian cancer, compared with 40-50% in advanced stages.

1.1 Etiology and molecular biology

1.1.1 Etiology and molecular biology of epithelial ovarian cancer

The pathogenesis of ovarian cancer is still unclear. Historically ovarian cancer was thought to arise from the ovarian surface epithelial. Although differences in biology, stage, appearance and response to therapy were known, ovarian cancer histological subtypes were regarded as one disease (2). Progresses have been made within the last years. The different histological subtypes of ovarian cancer appear to have different risk factors, precursor lesions, spread patterns, underlying molecular abnormalities and chemotherapy response (2, 3). They are now more correct regarded as different entities. The most common type of ovarian cancer, the high grade serous adenocarcinoma of the ovary (HGSOC) seems to have its origin not in the surface epithelium of the ovary, but from the serous tubal intraepithelial carcinoma (STIC), usually located in the fimbiriated distal portion of the fallopian tube (2, 4, 5). The dysplastic cells will be seeded on the ovarian surface and will develop further into cancer (6). The HGSOC are clinically characterized by extreme aggressive growth, most of the cases being diagnosed in advanced FIGO stages. They respond well to platinum based chemotherapy, but unfortunately they use to relapse, and secondary develop platinum resistance and consequently lead to death (7, 8). From the molecular point of view this subtype is characterized by high genetic instability, ubiquitary p53 loss of function through mutations and in 50% of the patients harbor mutations or epigenetic changes of HRD genes (9).
The low grade serous ovarian cancer seem to develop from cysts (10). These will primary come from the incessant ovulation as postulated by Fathalla et al. (11) many years ago. This precursor lesion will grow slowly, and can develop into borderline tumors due to certain genetic aberration, and finally might transform into low grade ovarian cancer (10). These tumors are characterized by mutation in BRAF, KRAS and ERBB2. Contrary to HGSOC, LGSOC contain very few point mutations (10). From the clinical aspect, they are usually slower growing, usually diagnosed at early stage and therefore associated with better survival rates. Due to low proliferation rates, LGSOC are mostly platinum resistance (2). Due to lack of other standard therapies, most of LGSOC are treated further on with platinum based chemotherapy. A current study analyzing the role of MEK inhibitors as single agent therapy in LGSOC is ongoing (12). There is a huge unmet need to define new therapeutic strategies for LGSOC.

Endometrioid and clear cell carcinoma are the so called endometriosis related ovarian cancer subtypes (2).
Endometriosis is a benign disease but there are hints that suggest that some endometriosis foci might transform into cancer (2). According to current data, the exposure to high concentrations of free iron, obtained through repeated hemorrhages into endometriotic cysts, is a possible cause of carcinogenesis that will lead to endometrioid or clear cell ovarian cancer (13).
Clear cell carcinoma of the ovary has a distinct molecular biology and clinical behavior. There are geographical and racial variations in incidence. In Japan and Asiatic women clear cell ovarian cancer is encountered in more than 11% of the cases, whereas in Caucasian women only in 5%. (14). Clear cell carcinoma is mostly being diagnosed at early stages, when disease is confined only to the ovaries. Between the most encountered genomic alterations there are *ARID1A* and *PIK3CA (2)*. In some cases p53, PTEN, Kras mutations can be encountered.

Mucinous ovarian cancer represents a small and very distinct subset of ovarian carcinomas (15). Most of the tumors are benign or borderline tumors of the ovaries. There are usually diagnosed in FIGO stage I, when disease remained confined to the ovary. Therefore they have a good prognosis following surgery, with the 5-year disease-free survival of 90.8%, compared with serous cancer of the ovary, 75.9% (16). There is a high frequency of intestinal differentiation in BOT (borderline tumors) and in malignant mucinous tumors of the ovary, which are also observed in metastatic gastrointestinal neoplasms. Due to this similarity many scientists suspect that actually mucinous ovarian cancers are metastatic tumors of the gastrointestinal tract. The majority of mucinous malignancies that metastasize to the ovary are having their origin in gastrointestinal tract, pancreas, cervix, breast, and uterus (15). Therefore in case of mucinous ovarian cancer, an appendectomy together with gastroscopy and coloscopy are mandatory for the differential diagnosis. There is overall accepted consensus that true mucinous malignant tumors of the ovary are rare. They seem to come from mucinous borderline tumors and teratomas (17). Few are known about their biomolecular characteristics. KRAS, *RNF4*, ERBB2 and p53 mutations are known so far (17, 18). KRAS mutations are found in approximately 43% to 57% of
mucinous tumors (17, 18). The genetic data suggest that the origin of these tumors is in the benign mucinous cystadenoma that will progress to mucinous borderline tumor of the ovary, before the onset of the invasive disease. This is suggested by the highly incidence of ras mutations in mucinous LMP tumors and adenocarcinoma. Mutations in bRAF and Kras are usually observed in serous borderline but they are not characteristic for adenocarcinomas (19).

1.1.2 Etiology and molecular biology of borderline tumors of the ovary (BOT)

Borderline tumors of the ovary are a distinct entity of ovarian tumors, having a very good prognosis and being usually diagnosed in younger patients (20). They are characterized by complex papillary architecture, multilayed epithelium with tufting, mild nuclear atypia, and slightly increased mitotic activity, without destructive stromal invasion (20).
Borderline tumors have a longer clinical history, although their diagnosis is very difficult, BOT mimicking both malignant and benign sonographic features (21).
In 2014, the new WHO classification differentiated between BOT with and without invasive implants. The last of them being classified as low grade ovarian cancer (22). This new classification took into account the last pathogenesis theory of ovarian cancer.
Serous borderline tumors of the ovary are rarely associated with serous invasive epithelial carcinoma, therefore BOT are probably a different entity. Nevertheless in some cases serous BOT will progress to carcinoma, therefore some relation might exist. In the contrary mucinous BOT are often associated with mucinous EOC. So some BOT are precursors of EOC, some of them not (23).
Sequence mutations in KRAS, BRAF and ERBB2 oncogenes are often seen in BOT (23). Mutations in these genes are encountered in about 2/3 of serous BOT, but are almost not met in HGSOC (23). Mutations in p53 are extraordinary in BOT.
There are hints that BOT will progress into a non-invasive low grade carcinoma and afterwards in an invasive low-grade carcinoma, but there are no relations with HGSOC (23).
The link between mucinous invasive ovarian cancer and mucinous BOT was suggested by clinical and pathological observations. Mucinous carcinomas are associated at time of diagnosis with area of mucinous BOT and of mucinous cystadenoma. Furthermore, point mutations of KRAS gene were observed in mucinous cystadenoma, BOT and EOC lesions (23).

1.2 Hereditary ovarian cancer

BRCA1 and *BRCA2* were identified in 1990s as genes, associated with high risk for breast, epithelial ovarian, fallopian tube, primary peritoneal and other cancers, when mutated (24). These findings strength the idea of hereditary ovarian cancer, that is associated by younger ages, late stages, good response to platinum base chemotherapy and long disease evolution. Further studies documented that the incidence of hereditary ovarian cancer lies between 5% to 15%, depending on the population evaluated. Mutations in BRCA1/2 and mismatch repair genes of Lynch syndrome are mostly responsible for the hereditary ovarian cancers, although most of ovarian cancer cases are sporadic in origin (24).
Primary ovarian cancer treatment consist platinum based chemotherapy. These compounds exert an anti-tumor effect by inducing intra-strand and inter-strand crosslinks in genomic DNA (25). The Cancer Genome Atlas analysis showed that somatic inactivation of BRCA1 and 2 genes through mutations and epigenetic changes (9). The presence of inactivating mutations in BRCA1 and BRCA2 genes, are making the ovarian cancer cells more sensitive to the DNA-damaging effects of platinum compounds. Furthermore data generated by the cancer genome study showed other

genetic mutations of homologous recombination genes, e.g. BR1P1, RAD51C, RAD51D, EMSY, PTEN, ATM, ATR, and Fanconi anemia that combined with BRCA1 and BRCA2 mutations affect approx. 50% of HGSOC patients (9).
The presence of HRD has not only have an impact on genetic counseling, or screening strategies, but also on the development of new targeted therapies. In BRCA-deficient tumors the defect in homologous recombination can be replaced by the PARP pathway, on which base excision repairs relies on (24). If the pathway is blocked via PARP inhibition, the loss of both repair mechanisms leads to accumulation of DNA breaks and, ultimately, cell death (24).
This finding is of major clinical interest, due to the availability of PARPi as therapeutic agent.

1.3 Ovarian cancer diagnosis

Ovarian cancer is called "silent killer", and has no specific symptoms in early stages (26). Most of the patients present themselves first with increase in abdominal volume due to ascites and diffuse peritoneal carcinomatosis.
Serological tests, including the classical biomarkers, (eg. Ca125 and HE4) and imagistic analysis are the two pillars of the early diagnosis (27-29). From all imagistic examinations, the most reliable and useful in the clinic is the one of transvaginal ultrasound.
There were several attempts to develop an efficient screening test. The largest study was driven by UK researchers. Hereby over 200.000 postmenopausal women were enrolled. The patients were randomized in three groups: the control group (100.000 women), here no action has been taken; the multimodal screening group (50.000 postmenopausal women) and the ultrasound group (50.000 women). In the multimodal screening group annual CA125 was determined, CA125 velocity was compared and patients were divided into high-, intermediary- or low-risk using the ROCA (risk of ovarian cancer) algorithm (30). CA125 velocity was interpreted using the ROCA algorithm that compares each individuals CA125 profile to the pattern in ovarian cancer and benign diseases. The closer the profile is to known cases of ovarian cancer, the greater the risk for cancer. Based on that the algorithm calculates the percentage risk of having ovarian cancer (31).
High-risk patients, according to ROCA algorithm received a transvaginal ultrasound by an expert ultrasound examiner, as a second stage screening test and then the decision regarding therapy was taken (30). Patients in ultrasound group were examined by transvaginal ultrasound yearly (32). At the annual screen, women with: normal risk of ovarian cancer (ROC), did return to annual screening; intermediate ROC, did repeat the CA-125 in 12 weeks; and with elevated ROC, did repeat CA-125 and transvaginal ultrasound in 6 weeks, or even earlier if results were suggestive of clinical disease (30).

The preliminary results published in 2009 showed that in the ultrasound group 845 surgeries were performed due to suspect adnexal masses, whereas only 22 patients had an ovarian cancer or a borderline tumor. In the multimodal screening group 97 surgeries were performed, 2.9 surgeries per diagnosed ovarian cancer. These results are translated in a better sensitivity (89.5%) and specificity (99.8%) for the multimodal screening group compared to the ultrasound group (75% and 98.2%, respectively). Both arms diagnosed 47.1% and 50% early stage ovarian cancer, respectively (FIGO

stage I and II), significant more than what we know from the literature. Until now there are no data from the control group, and therefore no information about impact of screening on survival rates (32).
The analysis of serial CA125 testing compared with single-threshold (30), ROCA detected 86.4% of the 155 women with invasive epithelial ovarian cancers: in the meanwhile using only the annual serum CA-125 cutoff values of more than 35, more than 30, and more than 22 U/mL would have identified only 41.3%, 48.4%, and 66.5%, respectively (30).
The main aim of the study was to show an advantage in overall survival, therefore there are still no efficient screening tests for ovarian cancer. Nevertheless the study shows a more important clinical value of CA125 velocity as a single-threshold rule for CA-125.
Further attempts have been made in order to increase the number of cases being diagnosed in early stages. The so called gold-standard biomarker for ovarian cancer, the CA125 is a protein being overexpressed in around 80% of ovarian cancer cases, but unfortunately only in 50% of the early cases. Furthermore around 20% of ovarian cancer patients have a normal CA125 (33). Therefore CA125 has a limited value for the early diagnosis. CA125 is elevated in several malignancies but also in benign conditions, such as myoma, pregnancy, benign ovarian cysts, endometriosis. Since many years researchers are trying to discover biomarkers that can compete with CA125. The first serum biomarker that could compete with CA125 was human epididymal protein 4, a protein being first discovered in the human epidiydimal epithelium (34). Similar to CA125, HE4 is overexpressed in serous and endometrioid ovarian cancer patients (34). Elevated HE4 serum levels are present not only in ovarian cancer, but also in endometrium, gastric and non-small cell lung cancer (35, 36, 37). There are data suggesting promotive effects of the HE4 on gastric cancer, endometrial and ovarian cancer (35, 38, 39).
Recent data showed that CA125 is more often elevated within benign ovarian disease, as HE4, especially in endometriosis, making HE4 the preferred biomarker to assess the risk for ovarian cancer in premenopausal pelvic mass patients (40). Both HE4 and CA125 values are elevated in renal and hepatic failure, their value being limited within these cases (41).

1.4 Surgical treatment in ovarian cancer

Although ovarian cancer is diagnosed in advanced stage, maximal tumor debulking can be achieved in around 70% of the patients. Several studies showed the key role of optimal tumor debulking, in terms of no evidence of macroscopically residuals. Optimal primary debulking is associated with a significant benefit in both OS and PFS (42, 43). A metaanalysis of Bristow et al. showed that a decrease of 10% in the residual tumor mass, was associated with an increase of 5.5% of median overall survival (43).
The primary cytoreduction of ovarian cancer requires multiple visceral surgery, that can be performed by trained gynecological oncologists. Reported data showed that patients operated in high volume centers, will have less peri- and postoperative complications and optimal residual mass can be achieved more often (44-46), those increasing the survival rates in ovarian cancer patients.
Despite improvement in surgical techniques, there are still patients in whom optimal tumor debulkingcannot be achieved. These patients will usually relapse and will develop platinum refractory or resistant disease with early recurrence. Furthermore tumor debulking in ovarian cancer patients consists in a multivisceral surgery,

including bowel, diaphragm resection, peritonectomy and splenectomy (47, 48). By now there are no available predictive biomarkers for surgical outcome.
There are still contradictory results if interval debulking (IDS) or primary debulking surgery (PDS) is the treatment of choice in advanced primary ovarian cancer patients. There are two prospective randomized clinical studies analyzing the role of neoadjuvant chemotherapy for ovarian cancer treatment (49-51). Vergote et al. showed in 2010 that patients with advanced epithelial ovarian cancer (EOC) undergoing neoadjuvant chemotherapy, followed by interval debulking have similar overall- (HR for IDS 0.98 95%CI (0.85-1.14)) and progression free- survival rates (HR for IDS 0.99 95%CI (0.87-1.13)) as patients undergoing primary debulking surgery, followed by chemotherapy (49). In the EORTC trial the largest residual tumor was 1cm or smaller in 41.6% of the patients in the PDS arm and 80% in the IDS arm, with a trend of more peri- and postoperative complications in the PDS arm. The 28-days surgical mortality was 2.5% vs. 0.7%, grade 3 or 4 hemorrhage were 7.4% vs. 4.1%, infection rates: 8.1% vs. 1.7% and venous complications 2.6% vs. 0% in PDS vs. IDS arm, respectively (49).
The second randomized clinical study was performed in UK, the first data were presented at ASCO 2013 by Kehoe. The CHORUS trial randomly assigned 552 patients, 550 being eligible for the study (50). The study showed similar results: similar PFS (HR=0.9, 95%CI (0.75, 1,07)) and OS (HR= 0.87, 95% CI (0.71, 1.05)) rates in IDS (median PFS= 11.7 months and median OS=24.5months) and PDS arm (median PFS=10.3 months, median OS=22.8 months). Even in this study, the rate of postoperative complications was significant lower in IDS compared to PDS arm: any grade ¾ complication rates were 2% in PDS vs. 14% in IDS arm. 74% of the patients were discharges within 14 days post-op in PDS vs. 92% of the patients in IDS arm.
The major criticisms to both studies are the low resection rates achieved at primary tumor debulking, short duration of surgery and poor survival rates. Furthermore despite significant increase of total macroscopic tumor clearance from 16% to 40% (50) there were no significant differences in OS and PFS rates.
Therefore further randomized prospective clinical trials are needed in order to elucidate the role of IDS and PDS in advanced ovarian cancer patients. In the next year the randomized international multicentric study, TRUST-Study, analyzing the role of interval debulking vs. primary debulking surgery in advanced ovarian cancer patients will start patients' recruitment.

1.5 Systemic therapy in primary ovarian cancer

The standard chemotherapy in primary ovarian cancer patients consists in a combination of carboplatin and paclitaxel for 6 cycles every three weeks (53).
In early stages ovarian cancer, studies showed patients who undergone complete tumor debulking and adequate staging surgery might not benefit from systemic chemotherapy treatment (53). Nevertheless, the results from ICON1 showed that adjuvant cytotoxic chemotherapy should be considered in high-risk patients, defined as patients with HGSOC or clear cell histology, or presence of stage IC ovarian cancer (53, 54).
Even though about 75% of the patients have clinical complete remission (cCR) after first-line treatment, most of them relapse and eventually die of cancer, indicating the need for further treatment improvements (8). Treatment resistance will eventually emerge in 80-90% of the patients initially diagnosed with widespread of the disease (8). Despite the fact that platinum response is the second most important prognostic

factor for ovarian cancer patients, there are no reliable predictive biomarkers for response to platinum based chemotherapy. The global standard of care remained carboplatin and paclitaxel within the last 20 years (53).

The tumors need blood vessels to be able to grow and invade other spaces. Furthermore studies showed an increased expression in ovarian cancer tissue, blood and ascites samples (55). This observation gave birth to the idea to combine chemotherapy with antiangiogenic drugs in order to improve survival in primary EOC patients.

There were two positive phase III studies in primary settings. The GOG-0218 and ICON 7 studies analyzed the role of adding bevacizumab, a VEGF inhibitor to the standard chemotherapy treatment. The GOG -0218 study showed a benefit in PFS for patients treated with carboplatin/paclitaxel and bevacizumab, 15mg per kilogram of body weight, followed by bevacizumab alone as maintenance therapy (HR=0.717 (95% CI, 0.625 to 0.824; P<0.001) (56). In the ICON 7 study, adding bevacizumab, 7.5mg per kilogram of body weight, to the first line chemotherapy and than continuing the therapy as maintenance regimen resulted in significant improved PFS (HR= 0.81; 95% CI (0.70 to 0.94; P=0.004) (57). None of the studies showed an improved overall survival, mainly due to low number of events, and different chemotherapy regimens in the 2nd and 3rd line including angiogenic drugs.

Both studies showed a benefit in the progression free survival but this wasn't translated in improved overall survival (56, 57).

At ASCO 2014, Gourley et al presented the results of the subanalysis performed in part of ICON7 samples. Using unsupervised hierarchical clustering, three major subgroups of patients: two with angiogenic gene upregulation (the proangiogenic groups) and one with angiogenic gene repression and immune gene upregulation (the immune molecular subgroup) were identified. The survival analysis showed a significant improvement in OS and PFS survival rates (p=0.001 for both) for the immune subgroup of patients. When this signature was evaluated within part of the ICON7 samples, in the control arm better PFS (HR 0.47, 95% CI 0.32, 0.71; p < 0.001) and OS (HR 0.45, 95% CI 0.26, 0.79; p = 0.005) rates were observed in comparison with the pro-angiogenic group. When looking into the bevacizumab arm, those patients in the immune subgroup had significantly worse PFS (p=0.015) when treated with bevacizumab and chemotherapy compared with chemotherapy alone. On the other hand patients in the pro-angiogenic group had a trend to improve survival when adding bevacizumab (PFS of 17.4 months) compared with chemotherapy alone (PFS of 12.3 months) (58). These results are very important and of high clinical interest as they are defining patients subpopulation who might benefit from adding bevacizumab to the first line chemotherapy. Nevertheless, larger studies that confirm these results are needed.

As stated before the high grade serous ovarian cancer is characterized by a high rate of p53 mutations and often deficient in homologous recombination and repair of double-strand DNA breaks (9). This deficiency has led to promising new treatment approaches, both as single agent and in combination with cytotoxic or anti-angiogenic drugs (59).

PARP inhibitors were mainly considered for high grade serous ovarian cancer patients with genomic BRCA1/2 mutations. The data from the TCGA publication, showing increase somatic mutation in homologous recombination genes, has led to reconsideration of that approach (9). The maintenance therapy with Olaparib, a potent PARP inhibitor showed, in platinum sensitive relapsed ovarian cancer patients, that patients with BRCA1 or 2 mutations benefit from such a therapeutically approach (60). These data support the hypothesis that tumors with a homologous recombination

deficiency, will respond to PARP inhibitors. Therefore studies assessing the role of Olaparib in primary ovarian cancer patients are ongoing (SOLO1- Olaparib Maintenance Monotherapy in Patients With BRCA Mutated Ovarian Cancer Following First Line Platinum Based Chemotherapy) (61).
Recent work has showed that homologous recombination (HR) can be suppressed by hypoxia through downregulation of *BRCA1* and *RAD51*; therefore sensitivity to PARP inhibition is increased in hypoxic states (62-65). Hence PARP-inhibitors and anti-angiogenics may have synergistic effects. In a phase 2 clinical study in platinum sensitive ovarian cancer relapses, there was a significant increased median PFS in 44 (17·7 months (95% CI 14·7-not reached)) women who received the combination of olapariband cediranib, vs the PFS in 46 women who received olaparib alone (9·0 months (95% CI 5·7-16·5)); reaching a HR of 0.42 (95% CI 0·23-0·76; p=0·005) (66). New strategies combining Bevacizumab and Olaparib in primary ovarian cancer patients (PAOLA 1study- Platine, Avastin and OLAparib in 1st Line) are on going (67).

1.6 Clinical management of ovarian cancer relapse

The therapeutic scenario for ovarian cancer relapse remains undefined.
The role of cytoreductive surgery in relapse situation remains unclear. Data from prospective trials are missing. So far results from retrospective studies suggest that cancer patients who will be optimal debulked might benefit from such an approach (68, 69). However until now the effective clinical or imagistic criteria to identify the collective of patients who might benefit from this approach are uncertain. The DESKTOP studies, analysed the role of secondary cytoreduction in ovarian cancer patients with first platinum sensitive relapse. The DESKTOP I trial showed in a retrospective study that cytoreduction impact the overall survival rates: 45 months vs. 19 months in completely debulked patients vs. patients with incomplete resection of recurrent disease (68). In the multivariate analysis following factors were associated with maximal tumor reduction: absence of residual mass after primary cytoredcution, ECOG status and ascites volume smaller than 500ml.
All these factors merged into the AGO score. The AGO Score was evaluated prospectively in a multicenter setting, in the DESKTOP II study (70). From 516 patients with first or second platinum sensitive relapse 261 had a positive AGO score and 129 patients received a secondary tumor debulking. The rate of complete resection in AGO score positive first relapsed patients was 76% (95% CI, 69%-83, meaning that in 2 of 3 relapsed ovarian cancer patients the results of the secondary debulking surgery could be predicted correctly (70). The results of the DESKTOP III prospective multicentric clinical trial are expected in order to understand the role of secondary debulking in EOC. DESKTOP III trial is a randomized, prospective, international trials that analyze the role of secondary cytoreduction followed by chemotherapy vs. chemotherapy alone in platinum sensitive relapsed ovarian cancer patients. More than 400 patients with a positive AGO score (Performance status ECOG 0; no residual tumor after primary surgery (if unknown, alternatively primary FIGO stage I/II); absence of ascites (cut off < 500 ml: radiological or ultrasound estimation) have been randomized. The used chemotherapy was a platinum based regimen, but otherwise a physician choice treatment (71). This prospective randomized trial is designed to elucidate the role of surgery or chemotherapy treatment in platinum sensitive ovarian cancer relapse.

The therapeutic strategy is guided by the platinum response. In patients being platinum-sensitive the therapeutic strategy is a re-challenge with carboplatin-based regimen. Usually the second line chemotherapy is a combination of Carboplatin and Pegylated liposomal Doxorubicin (PLD) or Gemcitabine, respectively. In the Phase III clinical trial Calypso, the combination of Carboplatin with PLD showed no inferiority in terms of overall survival rates, but better toxicity profile especially due to less neuropathy (72).

Women with recurrent platinum-sensitive EOC were included in the phase III randomized OCEANS. Patients were randomized in carboplatin plus gemcitabine with or without bevacizumab for 10 cycles maximum, followed by bevacizumab alone until disease progression or toxicity. The results from this study showed an improved PFS in the study group (12 months with bevacizumab vs. 8 months in the placebo group; HR = 0.48; 95% CI, 0.39–0.61) with no improvement in overall survival rates (73).

In a recent study, Ledermann et al investigated the efficacy of a specific PARP inhibitor in high grade serous ovarian cancer patients.131 patients with platinum sensitive EOC relapse were assigned to treatment with Olaparib, whereas 123 patients were included in the placebo group. Around 56% and 50% of the patients, respectively had deleterious or suspected deleterious germline or somatic BRCA mutations. In BRCA mutated patients, median PFS was significantly longer in the olaparib arm than in the placebo group (11.2 months vs. 4.3 months). The same trend was observed in BRCA wild-type patients although the difference was smaller (7.4 months vs. 5.5 months, respectively). There was no impact of PARP inhibition on the overall survival rates in the BRCA positive or negative patients (60).

Current data showed that the combination of antiangiogenic therapy (Cediranib) and PARP inhibitor (Olaparib) have a synergistic effect in recurrent platinum sensitive ovarian cancer patients with a 58% reduced risk of disease progression in the combination arm (66).

Knowing that at least 50% of HGSOC may have homologous recombination deficiency, the response to PARP inhibition is expected to be present even in $BRCA^{wt}$ HRD tumors. ARIEL2, prospectively tested a novel next generation sequencing-based HRD assay and algorithm to predict the response to Rucaparib, a PARP inhibitor. Two hundred six platinum sensitive ovarian cancer patients have been included into the studies. The preliminary results suggested that HRD HGSOC tumors together with BRCA positive tumors are more likely to respond to PARP inhibitors (74).

For platinum resistant ovarian cancer there is no efficient strategy of treatment and systemic treatment is highly dependent on the physician's choice. In platinum resistance setting the prognosis is very poor. In the clinical setting single agent treatment with non-pegylated or pegylated liposomal Doxorubicin (PLD), Topotecan, Gemcitabine and alkylating agents such as Treosulphan or Cyclophosphamide are used, but they have shown a relative modest anti-tumor activity. This is reflected by low response rates less than 20% for each agent and short lasting remissions (75, 76). Paclitaxel weekly at a dose of 80-90 mg/m2/week, seems to be one of the most effective regimen in that situation yielding response rates in the range of 20-60% (77).
Even in platinum resistant setting bevacizumab improved survival in combination with monotherapy, followed by bevacizumab alone as maintenance treatment. The

AURELIA Study analyzed the role of adding Bevacizumab to standard monotherapy in platinum resistant EOC relapsed patients. The PFS hazard ration was 0.48 (95%CI 0.38 to 0.60), in the favor of Bevacizumab treated patients with 3.3 months longer PFS compared with chemotherapy treatment alone. No statistical significant benefit was achieved for the overall survival rates (78).

1.7 Follow-up

According to current guidelines, patients should address to gynecologists for clinical examination and ultrasound every 3 months within the first three years, followed by every 6 months between three and five years, and once yearly after the first five years. Recurrence can be detected in several ways: using the gynecological examination and ultrasound (local relapse or ascites can be detected), clinical symptoms of the patients, using CT, MRI or using the CA125 values. According to GCIG criteria a doubling in CA125-concentration above the upper limit of normal might indicate relapse in ovarian cancer patients (79).
The largest study addressing the value of CA125 for the follow up in ovarian cancer is the study by Gordon et al (80). They analyzed timing of second line chemotherapy according to the CA125 level. Therefore women who developed recurrent disease were randomized to receive chemotherapy on the basis of either recurrence detected by elevated Ca125 (according to GCIG criteria), or based on clinical symptoms or relapse detected by imaging. In the CA125 group 254 patients have been randomized and in the imaging group 233 patients, respectively. Primary aim of this study was the impact of early treatment on overall survival rates, secondary aims were the time to begin 2nd line and 3rd line chemotherapy together with quality of life. The results of the study showed that relapse was diagnosed 4.8 months earlier (HR=0.29 (95% CI 0.24, 0.35) p<0.00001) in the CA125 subgroup, but the early intervention due to rising CA125 concentration did not improve survival (HR=1.00 (95%CI 0.82-1.22) p=0.98). Due to more chemotherapy regimens, patients who started an earlier treatment based on CA125 raising levels had poorer quality of life. The results are still controversial, due to the fact that a possible drawback of the study is that almost all patients received only adjuvant treatment, and the role of secondary or tertiary debulking was not addressed. Nevertheless until now there is no evidence on the role of debulking in ovarian cancer relapse. Because of the palliative setting of relapse disease, further treatment should be initiated on the basis of radiological findings, the clinical features and should take into consideration the preferences of the patient and her oncologists, at least until the results of the DESKTOP III study will be available (53).

2 Aim of the study

Aims of the present research were:

1. To analyze the diagnostic value of different biomarkers (e.g. HE4, CA125, glycanes) in healthy women and patients with pelvic masses, including borderline tumors of the ovary and epithelial ovarian cancer (EOC).
2. To analyzed the role of biomarkers and tumor phenotype in predicting surgical and clinical outcome in patients with advanced EOC.

3 Personal contribution

3.1 Prognostic biomarkers for risk assessment in patients with pelvic mass

3.1.1 Role of HE4 in the diagnosis of borderline tumors of the ovary

Preoperative HE4 and risk of ovarian malignancy algorithm (ROMA) values do not improve the CA125 diagnostic value for borderline tumors of the ovary (BOT) – a study of the TOC Consortium
Elena Ioana Braicu, Toon Van Gorp, Mani Nassir, Rolf Richter, Radoslav Chekerov, Khayal Gasimli, Dirk Timmerman, Ignace Vergote, Jalid Sehouli. Journal of Ovarian Research 2014, 7:49.

Borderline tumors (BOT) of the ovary are different from malignant or benign epithelial ovarian tumors. They are characterized usually by diagnosis in younger patients, very long clinical history and have usually a very good prognosis (81, 82). From the histological point of view, BOT are characterized by the presence of nuclear atypia without any destructive stromal invasion (81, 82). They can still present microinvasion, lymph nodes involvement as also peritoneal implants. The peritoneal implants might be non-invasive or invasive, in the last case they are classified, according to the new WHO classification as low grade serous ovarian cancer (22).
BOT are usually diagnosed in early stages and chemotherapy is not indicated (85). The standard of care for BOT consists in bilateral oophorectomy, subtotal omentectomy together with comprehensive staging, including peritoneal biopsies, removal of all macroscopic peritoneal implants and peritoneal washings (83, 86, 87). Despite good prognosis, 10 to 30% of the patients will recur and 30% of them will develop an ovarian cancer (20).
BOT is difficult to assess preoperatively. The ultrasound features are overlapping with both invasive and benign ovarian masses. Before surgery only 29-69% of the BOT are classified correctly (21). Until now predicting BOT using CA125 values in pelvic mass patients is controversial discussed. The aim of this study was to analyze the role of HE4 in predicting presence of BOT in pelvic mass patients.

Therefore preoperative serum values were analyzed for both CA125 and HE4 in 167 women with BOT or benign diseases. Overall 63 patients were diagnosed with BOT, whereas 15 patients were presenting invasive implants at the time of diagnosis.

Due to the fact that BOT and benign diseases have different incidence within pre- and postmenopausal patients, the role of HE4 and CA125 was analyzed separately for pre- and postmenopausal patients. In the premenopausal collective of patients both HE4 ($p=0.984$) and CA125 ($p=0.141$) showed no statistical significant differences between benign disease, BOT with and without invasive implants.
Within the postmenopausal patients both biomarkers, HE4 ($p=0.007$) and CA125 ($p=0.003$) differed significantly between benign diseases, BOT independent from the presence of invasive implants. HE4 and CA125 reached an area under the curve (AUC) of 0.732 and 0.778, respectively. The ROMA algorithm, consisting in the combination

of both biomarkers together with menopausal status reached a slightly improved AUC of 0.782, compared with biomarkers alone.
Due to differences in median age within the three subgroups we performed the analysis in matched paired samples. Even in this sub-analysis the performance of HE4, CA125 and ROMA remained poor for the diagnosis of BOT or detection of invasive implants (AUC=0.660, AUC=0.788 and AUC=0.744, respectively).
Our study showed that HE4 alone did not perform better than CA125, alone in predicting the presence of BOT. Furthermore the combination of both biomarkers within ROMA algorithm cannot outperform the prognosis of CA125.

These results underline once more the fact that BOT are a different entity compared to ovarian cancer and new biomarkers or early diagnosis algorithms should be developed and validated in prospective and multicentric setting.

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3.1.2 The serum glycome to discriminate between early stage epithelial ovarian cancer and benign ovarian diseases

Karina Biskup, Elena Ioana Braicu, Jalid Sehouli, Rudolf Tauber, Véronique Blanchard. Dis Markers. 2014;2014:238197.

In a further study we try to identify a new strategy for early detection of ovarian cancer. As mentioned above, until now there are no reliable biomarkers or clinical parameters to screen or diagnose ovarian cancer in early stages.

Glycosylation is post-translational modification of the proteins, having an important impact in their function. Furthermore, glycome modulations were described in different diseases, including inflammation and cancer (88-90). Therefore glycane profile in serum was studied for diagnostic, monitoring and prognostic purposes. Increase in serum fucosylation, antennarity, and sialysation was reported in inflammation and in malignomas (91-95). Recent data showed that changing in N-glycanes are cancer specific (91).

In a previous study we analyzed 63 patients with mostly advanced EOC. Based on the analysis of gylcosilation profile, we defined a new diagnostic tool, GLYCOV, composed of the relative areas of the 11 N-glycan biomarkers, more specific four high-mannose and seven complex-type fucosylated N-glycans (92). The sensitivity of GLYCOV score and CA125 were both 97%, the specificity was higher though for GLYCOV compared with CA125 (98.4% vs. 88.9%, respectively) (92).
Due to the fact that the limitation of CA125 in the diagnosis of ovarian cancer is represented by its overexpression in only 50% of the early cases, we designed a new study, where we compared the diagnosis value of CA125 and GLYCOV score, within patients with early EOC (FIGO stage I 10 patients, FIGO stage II 10 patients). 20 patients being diagnosed with different benign diseases and 33 age-matched healthy patients were included in the control group. N-Glycans were cleaved from serum glycoproteins, permethylated and analyzed using MALDI-TOF mass spectrometry.
The predictive value of GLYCOV within patients with early EOC and healthy patients was analyzed using ROC. The AUC for GLYCOV was with 0.992 superior to that of CA125 (0.884), when analyzing cancer vs. control group. When comparing the early EOC and benign ovarian diseases the GLYCOV score (AUC=0.970) performed better than CA125 (AUC=0.680). This last comparison is of particular clinical relevance, as pelvic mass patients are usually the ones where differential diagnosis should be done.
Our study showed a better sensitivity (95% vs. 60%) and specificity (80% vs. 65%) for the GLYCOV score than for CA125, respectively.
The combination of both GLYCOV score and CA125 didn't increase significantly the sensitivity and specificity of GLYCOV alone. Further prospective and multicentric studies are needed to validate these results. Furthermore the comparison between GLYCOV, HE4, ROMA and also with ultrasound features should be taken into consideration in future studies.

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3.2 Predictive and prognostic role of somatic BRCA1 methylation status in ovarian cancer patients

Ilary Ruscito, Desislava Dimitrova, Ines Vasconcelos, K. Gellhaus, T. Schwachula, F. Bellati, Robert Zeillinger, Pierluigi Benedetti-Panici, Ignace Vergote, Sven Mahner, Dan Cacsire-Tong, Nicole Concin, Silvia Darb-Esfahani, Sandrina Lambrechts, Jalid Sehouli, Sven Olek, Elena Ioana Braicu. Eur J Cancer. 2014 Aug;50(12):2090-8.

High grade serous ovarian cancer is the most common and aggressive form of EOC. Although only 5 to 10% of ovarian cancer is hereditary and mostly linked to inactivation of BRCA1 and BRCA2 genes, the Cancer Genome Atlas publication showed that in fresh frozen tissue samples from HGSOC patients, functional loss of proteins involved in the homologous recombination pathway of DNA repair might be present in up to 50% (9).

In order to improve survival rates in HGSOC patients, new therapeutic targets should be exploited. Therefor there is an increased effort in studying new therapeutic drugs and molecular mechanisms that interfere with deficient homologous recombination pathway. Hypermethylation of CpG island regions of specific oncosupressor gene promoters was identified as a new epigenetic phenomenon involved in the inhibition of tumor suppression and promotion of carcinogenesis (96, 97).

The aim of this study was to analyze the clinical impact of BRCA1 promoter gene methylation status in HGSOC.
Therefore 257 patients with primary HGSOC were enrolled consecutively. Tissue was provided by the tumor bank ovarian cancer (www.TOC-network.de) (207 patients) and from the European OVCAD consortium (50 patients).
Written informed consent was obtained from all participant patients, before tissue samples have been collected. Approval from local ethic committees was provided (EK207/2003, ML2524, HEK190504, EK366 and EK260).
Tissue samples were frozen immediately after removal during surgery in the liquid nitrogen. Before methylation analysis, samples underwent a central histological review, whereas the quality of the tumoral tissue was assessed. Only specimens presenting at least 50% of tumor area were included in the BRCA1 promoter methylation status analysis. Using the bisulfite modification of DNA and methylation-specific PCR we assessed the methylation status of BRCA1 promoter gene.

Median age at diagnosis was 58 years, over 94% of the patients had an advanced HGSOC (FIGO stage III and IV) and optimal debulking, in terms of no macroscopically tumor residuals, was obtained in 63% of the cases. All patients received primary tumor debulking followed by platinum based chemotherapy.

In our study we included a heterogeneous population with a wide range of BRCA1 promoter gene methylation rate. Around 14.8% (34 patients) presented at least 5% of methylation in BRCA1 promoter gene, and were therefore called hypermethylated. Samples were obtained from different anatomic sites. Although no paired samples were available from same patients, no differences in methylation rates in samples originating from different anatomic sites were observed ($p=0.83$). No significant correlation between methylation status and residual tumor mass after surgery ($p=0.585$) or response to platinum based chemotherapy ($p=0.14$) was detected.

In the hypermethylated subgroup, patients were significantly younger (median age 54 years) compared with patients in the hypomethylated group (median age 60 years) ($p=0.008$).

There was no statistically significant impact of BRCA1 methylation status on survival rates ($p=0.566$ for PFS and $p=0.109$ for OS, respectively).

In conclusion only age at first diagnosis was associated with BRCA1 promoter methylation status in our study. Therefore our results suggest that ovarian cancers associated with BRCA1 promoter hypermethylation are more likely to be diagnosed in younger patients.

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3.3 Predictive biomarkers for surgical outcome

The crucial role of primary debulking surgery resulting in no macroscopic residual disease is already accepted worldwide. The role of neoadjuvant chemotherapy followed by interval debulking is not clear yet. Although there might be a subpopulation who will benefit from this approach:

- In patients with a combination of risk factors such as: increased age, low performance status; comorbidities; cachexia/low albumin, enable to undergo extensive cytoreduction with large fluid shifts.
- Emergency surgery in suboptimal setting
- Fresh thromboembolic or cardiovascular event
- Presence of diffuse unresectable intraparechymatous liver / lung metastases
- Presence of diffuse miliary peritoneal carcinosis
- Brain/Bone metastases
- Suboptimal health ressources, such as intensive care units, blood bank availability etc.

In relapse situation due to the lack of prospective data, there are still controversial opinions regarding the role of debulking surgery. Nevertheless data from retrospective studies are showing a benefit for patients undergoing optimal secondary, tertiary or quaternary surgery (68, 69, 98-101).
Nevertheless, until now there are no predictive biomarkers or no reliable clinical parameters to preselect patients who would benefit from debulking surgery in primary or relapse setting.

3.3.1 Role of histological type on surgical outcome and survival following radical primary tumor debulking of epithelial ovarian, fallopian tube and peritoneal cancers

Elena Ioana Braicu, Jalid Sehouli, Rolf Richter, Klaus Pietzner, Carsten Denkert, Christina Fotopoulou. British Journal of Cancer (2011) 105, 1818-1824

The aim of this study was to analyze in a large cohort of EOC patients, the clinical impact, in terms of influence on surgical outcome and survival rates, of the 2-tier system proposed by Kurman and Shih in 2004 (101). The so called type I tumors, having as prototype the low grade serous EOC, generally behave in an indolent manner, are genetically stable and tend to be diagnosed in early stages. The type II tumors of the ovary, having as prototype the HGSOC, are very aggressive, p53 mutations being present in more than 95% of the cases and are characterized by impaired homologous recombination. They are usually diagnosed in advanced stages and prognosis is very poor (10, 101).

We enrolled retrospectively 632 patients, whereas 100 patients (15.8%) were classified as type I and 532 patients (84.1%) as type II tumors. Forty-four type I tumors (44%) were diagnosed in early stages. Type I patients were significantly younger, were diagnosed at earlier FIGO stages and had lower rates of preoperative ascites, positive lymph nodes and CA125 values.

Estimated 5-year and 2-year OS rates were 56.3% and 59.8% for type I patients vs. 39.3% and 44.9% for type II patients (p=0.021), respectively. Nevertheless, when considering only advanced FIGO IIIC/IV patients, both OS (p=0.779) and PFS (p=0.714) were similar between the histological sub-types. Platinum response also didn't significantly differ within the two subgroups of patients (p=0.314).

Regarding the surgical procedures, in type I patients dissection of para-aortic lymph nodes, extensive peritonectomy, diaphragm stripping and large bowel resection have been performed less frequent as in type II. A lower overall complication rate and a significantly shorter operative time was observed in type I patients. When only advanced stages were analyzed, there were no statistical significant differences regarding surgical procedures or postoperative complications. Complete tumor resection was significantly more often achieved in type I (85%) compared to type II tumors (65.6%) (p=0.001).

The multivariate analysis identified postoperative tumor residuals, mucinous histology and presence of positive lymph nodes as independent predictors of survival. When the analysis was performed only in optimally debulked patients, presence of HGSOC, type II histology and multifocal tumor disseminations were negatively affecting the survival in the multivariate analysis.

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3.3.2 Primary versus secondary cytoreduction for epithelial ovarian cancer: A paired analysis of tumour pattern and surgical outcome.

Elena Ioana Braicu, Jalid Sehouli, Rolf Richter, Klaus Pietzner, Werner Lichtenegger, Christina Fotopoulou. European Journal of Cancer 48 (2012) 687-694.

Although lately a more radical surgical approach is often usedleading to an increased number of optimal resected EOC patients (42, 43), most of patients will relapse, resulting in platinum resistance and consequently leading to death. Personalized therapy attempts failed in improving overall survival rates in EOC (8). Clinical observations regarding treatment failure may understand better temporal heterogeneity and progression of the disease leading to more effective strategies against it (103).

The role of surgery in primary setting is with no doubt crucial for the prolongation of progression free and overall survival. The meaning of surgery in the relapse situation is still unclear. In the last decades, extensive data about the role of primary surgery, platinum based chemotherapy, as also data regarding mutations, epigenetic changes, copy number variations, gene expression of primary EOC have been generated. Nevertheless there are few patients receiving secondary or tertiary cytoreductive surgery, and therefore also few samples collected at disease relapse (8). Even more difficult is to find patients with well-documented, primary and secondary debulking and also patients where paired samples are available.

Understanding the dissemination pattern and temporal tumor heterogeneity will bring more information about the evolution of ovarian cancer and resistant tumoral clones. Therefore we analyzed the role of tumor dissemination pattern in patients with both primary and recurrent disease with the aim of better understanding of tumor behavior and operative outcome in EOC patients. Therefore we included only patients being operated at both primary and relapse situation.

We analyzed the clinical data from 79 patients operated in our center. All data were documented prospectively within our tumor bank for ovarian cancer (www.toc-network.de). The tumor spread, maximal tumor load and residual tumor mass were documented during and at the end of the surgery through an interview with the surgeon and using a validated documentation tool, the intraoperative mapping of ovarian cancer (IMO) (104). Median overall survival and progression free survival to first relapse were 56 months and 16 months, respectively.

Patients developed less ascites in the relapse situation as prior to primary surgery (40.5% vs. 65.3%, p=0.002). In relapse situation patients had higher rates of tumor involvement of the gastric serosa (p=0.003), serosa of small intestine (p=0.001) and mesentery (p=0.012). Regarding surgical procedures: upper abdomen surgery was more frequent used during secondary debulking.

Surgical effort resulted in significant higher maximal tumor debulking rates in primary situation compared to relapse (77% vs. 50%, p<0.001), at similar operative morbidity

(25% vs. 29%; p=0.424). The secondary cytoreduction was associated with significantly higher rates of tumor residuals in the abdominal levels 1 (p=0.008) and level 2 (p=0.001). Maximal tumor load affected mostly level 1 in primary setting (p=0.002) and level 3 at relapse (p=0.045). The residual tumor mass at primary and secondary cytoreduction correlated significantly (p=0.003). The relative risk of any residual tumor mass after secondary debulking was 1.91 in patients with sub-optimal primary debulking surgery versus patients who underwent complete macroscopically tumor resection at PDS.

There was no correlation of tumor dissemination, in terms of peritoneal carcinomatosis, presence of positive lymph nodes and intestinal tumor involvement or at primary vs. secondary tumor debulking.

Despite heavily pretreated patients, surgical morbidity at relapse doesn't seem to increase significantly compared to primary setting when experienced surgeons perform surgery. Nevertheless maybe the temporal tumor heterogeneity leads to reappearance in a more aggressive and therapy resistant profile, which involves higher therapeutic challenges, including more aggressive surgical techniques.

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3.3.3 Role of HE4 in predicting surgical outcome

3.3.3.1 Preoperative HE4 expression in plasma predicts surgical outcome in primary ovarian cancer patients. Results form the OVCAD study.

Elena Ioana Braicu, Christina Fotopoulou, Toon Van Gorp, Rolf Richter, Radoslav Chekerov, Christina Hall, Hermann Butz, Dan Cacsire Castillo-Tong, Sven Mahner, Robert Zeillinger, Nicole Concin, Ignace Vergote, Jalid Sehouli. Gynecologic Oncology 128 (2013) 245-251.

Human epididymal protein 4 was first identified in the epithelium of human epididymis. Further studies showed that it is overexpressed in epithelium of the respiratory and genitourinary tract (105). In 1999, Schummer et al, described for the first time HE4 being expressed in EOC (106).
In 2008 Moore at al, analyzed the role of different biomarkers and their combination in predicting ovarian cancer in pelvic mass patients (38). In this study, the combination of CA125 and HE4 had the best AUC compared to any other biomarker alone, or any other biomarker combination (including triplets and quadruplets). Starting from this results and from the observation that most EOC occur in postmenopausal patients, as also that biomarkers serum concentrations are influenced by age, Moore proposed in a following study a new score for malignancy: the risk of ovarian malignancy algorithm (ROMA) (107). ROMA combines HE4 and CA125 together with menopausal status.
Within the European OVCAD study we included 275 patients with primary epithelial ovarian cancer, who received debulking surgery and platinum based chemotherapy. Blood samples were available prior to surgery and in chemotherapy naïve patients (108).
Aim of our study was to analyze the predictive value of HE4, for surgical outcome in primary EOC.
Most of the patients (94.7%) were diagnosed in advanced FIGO stage (FIGO III and IV) and maximal tumor debulking was achieved in 68.4% of the cases. Over 86% of the patients had HGSOC.
Median HE4 concentrations in plasma and in ascites were 339pM (interquartile range (IQR) 124pM-699pM) and 4339pM (IQR 2117pM-7552pM), respectively. Median value of circulatory CA125 was 456 IU/ml (IQR 123.7-1139.4).
HE4 concentrations increased with advanced FIGO stage ($p=0.004$), increased ascites volume ($p<0.001$), presence of serous histological subtype ($p=0.013$) and high grade ($p=0.034$). Furthermore HE4 significantly correlated with CA125 levels ($p<0.001$).
Both HE4 (AUC=0.635) and CA125 (AUC=0.643) correlated with residual tumor mass in the multivariate analysis. We identified following cut-off values 235pM and 500pM, and 500IU/ml for HE4 and CA125, respectively as having the best sensitivity and specificity. Combining both biomarkers the sensitivity and specificity of predicting surgical outcome increased to 64.8% and 73.5%, respectively.
In the multivariate analysis only CA125 (OR=3.8, 95% CI= 1.78-8.1) together with age (OR=3.33, 95%CI=1.2-9.14) were predictive for impaired surgical outcome. When adjusting after center, FIGO stage, ascites volume and histological subtype, HE4 retained its statistical significance. This analysis lost its significance when adjusted after age.

We created a risk index, composed of HE4 (cut off value of 500pM) and CA125 (cut-off value 500IU/ml). In the multivariate setting, this index was an independent predictive factor for surgical outcome ($p<0.001$).
Furthermore both biomarkers correlated with platinum response ($p=0.009$ for HE4 and $p=0.004$ for CA125). None of the biomarkers were independent prognostic biomarkers for progression free or overall survival.

The results of this study showed that CA125 and HE4, although associated with relative poor sensitivity and specificity might be used in predicting surgical outcome and in identifying patients who will benefit from primary debulking surgery. Nevertheless clinical parameters should be also taken into consideration in order to increase the sensitivity and specificity, eg. presence of ascites, ECOG status, age. Further more complex models, combining clinical parameters with biomarkers are needed to be developed.

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3.3.3.2 HE4 Expression in plasma correlates with surgical outcome and overall survival in patients with first ovarian cancer relapse

Elena Ioana Braicu, Radoslav Chekerov, Rolf Richter, Carmen Pop, Mani Nassir, Hanna Loefgren, Florin Stamatian, Mustafa Zelal Muallem, Christina Hall, Christina Fotopoulou, Jalid Sehouli, Klaus Pietzner. Ann Surg Oncol (2014) 21: 955-962

If the role of surgical debulking is worldwide accepted for the primary situation, there is no enough evidence for the impact of debulking and of residual mass in the recurrent situation. There are retrospective data showing that patients might benefit from this strategy, if macroscopically tumor clearance can be achieved.
Until now there are no validated biomarkers to preselect patients who will benefit from a secondary tumor debulking.
In this study we analyzed the role of HE4 in predicting surgical and clinical outcome in relapsed ovarian cancer patients.
We included 73 consecutive EOC patients with first relapse after primary debulking and platinum based chemotherapy. Optimal tumor debulking could be achieved in 66.7% of all these patients and 86.3% were platinum sensitive.
HE4 correlated with residual mass after secondary tumor debulking (AUC=0.731, p=0.001), and it retained its statistical significance even in the multivariate setting. CA125 did not reach the statistical significance (p=0.075).
Combining HE4 and CA125 the AUC was slightly increased as when using HE4 alone (0.791 vs. 0.731). Furthermore, when performing the multivariate analysis for OS, HE4 together with response to first platinum based chemotherapy were the only independent prognostic factors.
Nevertheless the AUC reveal a poor sensitivity and specificity for both HE4 and CA125, indicating that there is a need to combine clinical features or characteristics with circulating biomarkers.

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4 Discussion

4.1 Prognostic biomarkers for risk assessment in patients with pelvic mass

Despite increased aggressiveness in the surgical debulking and attempts of personalized medicine, ovarian cancer remains a deadly disease, with poor increase in survival rates in the last decades (8). This fact is mostly caused by diagnosis in advanced FIGO stages when survival rates are drastically decreased.
For now on there are no diagnostic tools, no gene signature or biomarkers that can predict the risk of malignancy in the general or high-risk population or in the pelvic mass patients.
Therefore there is an unmet need in identifying new diagnostic tools for early detection of ovarian cancer. Ideally these methods should be easy to reproduce and coast effective. Until now there are two different approaches: trying to triage the patients using biomarkers, usually in serum or using imagistic methods, here the transvaginal scan being widely used.
Regarding the transvaginal scan, studies failed in showing a benefit mostly due to lack of standardized diagnostic procedure and due to method subjectivity. At the beginning of this century, the international study group for ovarian tumor analysis (IOTA) was founded. The IOTA group analyzed the role of transvaginal ultrasonography for the differential diagnosis of ovarian tumors. The first limitation of the transvaginal sonography was and still remains the lack of standardized terms and procedures to derive categorical and continuous variables in gynecological ultrasound (109). The first aim of the study group was to standardize the ultrasound examination (109).

Discriminating between benign or malignant adnexal masses is the key point for an optimal clinical management. In most of the pelvic mass patients a benign tumor will be diagnosed (110). Different tools have been used in order to distinguish between benign or malignant adnexal masses, such as biomarkers and/or various prediction models (110). Scores based on morphological appearance of a mass during ultrasound; the risk of malignancy index (RMI): based on CA125, menopausal status and ultrasound characteristics, logistic regressions, neural networks and other computational approaches have been used in order to improve diagnosis (110, 111). The RMI remains the most widely used prediction model for pelvic mass patients, although is based on several ultrasound markers, without being standardized and CA125 (111). In the US, FDA approved CA125, HE4 and OVA1 for diagnostic purposes in pelvic mass patients (112). OVA1 is a tool that combines five biomarkers (CA 125, TTR, ApoA1, β-2 microglobulin, TF), which are identified through serum proteomics using SELDI-TOF-MS. Unfortunately the OVA1 has a high false positive results rate (112, 113), therefore its role in the clinical use is limited.

Afterwards within the IOTA I study several logistic regression models have been analyzed. The LR1 included 12, and LR2 included six, demographic and ultrasound variables. These models were validated in an external cohort of patients. They compared the performance of the logarithmic regression models with the performance of RMI and CA125 using ROC analysis. The AUC of LR1was 0.945, LR2 0.922, RMI 0.865 and of CA125 0.741, showing a better performance of IOTA logistic regressions. In the postmenopausal patients LR1 performed better than all other tests (LR2, RMI, and CA 125) (110).

Despite increase sensitivity and specificity of logarithmic regression models of IOTA group, the major limitation of ultrasound-based algorithm is the subjectivity of the analysis and the technical requirements. IOTA group developed the so called simple rules. These are a simplified version of ultrasound criteria and can predict the presence of malignancy in pelvic mass patients. They defined 5 malignant (M) criteria: presence of ascites, presence of more than 4 papillary projections, presence of moderate or strong blood flow, irregular solid tumors and irregular multilocular-solid lesions larger than 10cm; and 5 rules for benign (B) tumors: unilocular tumor or solid component smaller than 7mm, acoustic shadow, smooth multilocular lesions less than 10cm. Following clinical judgement applies: if only B rules apply, than the tumor mass is more likely benign; if only M rules apply than is malignant, if both B and M rules apply than the patient should be sent to an expert in pelvic ultrasound. Using these simple rules, the IOTA could correctly identify 81% of the benign diseases, 74% of the malignant tumors, but only 50% of the borderline tumors of the ovary (114). Another article published by Fisherova et al, showed that borderline tumors of the ovary are together with other benign tumors, the most difficult masses to correctly diagnose preoperatively, because their macroscopic features overlap with invasive and benign ovarian tumors (21).

Despite worldwide use of ultrasound diagnostic, there are still difficulties in correctly identifying borderline tumors or early EOC. On the other hand the biomarkers are easily to be reproduced, are standardized and are usually cheaper than ultrasound or other imagistic techniques. Nevertheless the lack of effective biomarkers is currently a major obstacle for blood-based early detection in ovarian cancer (115). Hori et al, developed a mathematical model, to quantify the growing time required for a malignant tumor cell population to reach the minimal size so that it will be able to shed enough biomarker into the blood, so that biomarker levels could be detected using available clinical blood biomarker assays. The results showed only after 10.1 years the tumor will reach a volume corresponding to a spherical diameter of about 25.36 mm, and will consequently become detectable by current clinical blood assays and by transvaginal ultrasound (115). Therefore there is an urgent need to discover new diagnostic biomarkers for ovarian cancer. The mathematical models suggest a constant but slowly increase in biomarkers serum levels, suggesting that a biomarker single-threshold might not be that useful as its velocity. This hypothesis is also one of the main findings of the UKCTOCS screening trial (30). Therefore new strategies analyzing the role of biomarker velocity in pelvic mass patients should be developed.

The HE4 is a new biomarker overexpressed in several epithelial tumors, but mostly in ovarian cancer. Since 2008 when the first study by Moore was published, there were many publications focusing on role of CA125 and HE4, or in combination (ROMA algorithm) to predict ovarian cancer in pelvic mass patients. The results obtained are rather contradictory. Moore et al showed that HE4 and ROMA performed better than CA125 alone or in any other biomarker combination (116). In a retrospective study by Van Gorp HE4 and ROMA did not increase the sensitivity and specificity of CA125 alone, neither in pre- nor in postmenopausal patients (117). In a total of 360 pelvic mass patients, a retrospective analysis of different algorithms using ultrasound criteria or biomarkers showed that IOTA LR2 has a better diagnostic performance that ROMA for the characterization of pelvic masses (118).

BOTs are correctly classified before surgery in few cases, only in 29%-69% (21),

therefore the aim of our study was to analyze the diagnostic role of HE4 alone or in combination with CA125. HE4 or ROMA did not perform better than CA125 in the diagnosis of BOT. Both CA125 and HE4 had low sensitivity and specificity for BOT, underlying once more that BOT are a different entity of ovarian tumors, and further biomarker discovery is needed. Using CA125 velocity doesn't seem to improve the percentage of BOTs diagnosed correctly (30). No data are available regarding HE4 velocity. The limitation of our study was the low number of BOT patients included and the lack of information regarding ultrasound features.

Glycans are important for cell-cell adhesion, protein folding, host-pathogen interactions and cell signaling. Distinct differences between glycan profiles are seen between normal and cancer cells. Glycanes are suspected to be involved in cancer transformation and progression (119). A study carried out by our group on 96 subjects showed a modification of N-glycome of total serum glycoproteins in primary early-stage and late-stage patients (92) using MALDI-TOF mass spectrometry. An increase of tri-, tetraantennary fucosylated and sialylated glycans was observed as well as a decrease of high-mannose N-glycans. The glycan structures that were significantly up- and downregulated were combined as a score named GLYCOV. GLYCOV was validated in this second cohort of patients including healthy donors, benign tumors of the ovary and early (FIGO I and II) EOC.
Our findings were in concordance with previous published data that showed a downregulation of high-mannose structure in EOC patients (120, 121). Changes in N-glycanes were present in 9 from 10 FIGO stage I and 10/10 FIGO stage II EOC, suggesting this is a process occurring early in the clinical history of EOC. Furthermore impairments in glycosylation haven't been observed in almost none of benign cases.

There are data suggesting that the regulation of high mannose content is probably linked to the C3 complement, which is the only acute phase protein that carries high mannose N glycans (122). The increase in monofucosylated triantennary trisialylated N-glycan was observed in several solid malignomas and in inflammatory conditions. The mechanism explaining aberrant glycan modulation in EOC patients might correlate with the production of acute phase proteins in the liver that are stimulated by different cytokines (e.g. TNF; IL 1 and IL6) (123-125). Here further studies are needed.

Our study suggests that glycans, especially GLYCOV algorithm might be a potential early diagnostic score for EOC, having a better sensitivity and specificity than CA125. The limitation of the study is of course the relative low number of patients, the monocentric and retrospective character, lack of HE4 values.

4.2 Predictive and prognostic role of somatic BRCA1 methylation status in ovarian cancer patients

Ovarian cancer is mostly sporadic but in 5 to 15% of the cases it is associated with positive family history. The so-called familial EOC is usually associated with mutations in homologous recombination genes, and here primary inactivation of BRCA1 and BRCA2 genes.

High-grade serous ovarian cancer is the most common, but the most aggressive type of EOC. The analysis of 26 observational studies on ovarian cancer patients' survival, on 1213 EOC patients, showed that BRCA1/2 positive patients were having a survival benefit, especially in the BRCA2 positive subgroup (126). The improved overall survival rates in BRCA1/2 positive tumors is partly due to increased response to platinum based chemotherapy.

Recently, the cancer genome study on more than 400 primary high-grade serous EOC patients, showed in up to 50% of the patients a functional loss of proteins involved in the homologous recombination pathway of DNA repair. Hence these tumors phenotypically behave similar to BRCA1/2 mutant cancers, even in the absence of a BRCA1/2 mutation. This higher percentage was achieved by analyzing not only the genomic DNA, but also the somatic mutations and epigenetic changes (9). This phenomenon is called 'BRCAness' and defines DNA homologous recombination deficiency (127, 24).

There are few data regarding the impact of epigenetic changes of BRCA1/2 genes in a homogenous HGSOC cohort. BRCA1 promoter methylation was reported in 5% to 40% of the EOC, depending on analyzed cohort (128-133). Within the TCGA project BRCA1 gene promoter methylation was identified in 11.5% of the HGSOC. BRCA1 and BRCA2 inactivation was the second most frequent alteration following the p53 inactivation. Even in this study, the BRCA1/2 mutations was associated with a better overall survival due to higher sensitivity to DNA-damaging agents, especially platinum-compounds (9).

Our study analyzed the role of methylation status in somatic BRCA1 promoter gene, in a homogenous cohort of HGSOC patients. This study was performed in a well clinical characterized cohort of patients. The largest limitation of the study was the lack of information regarding genomic and somatic mutations of BRCA1/2 genes. In 118 patients we had available data with respect to mutation status in genomic BRCA1 exon 11. Exon 11 represents 60% of the BRCA1 gene. In our study, 11 patients presented mutations by gene sequencing. None of the BRCA1 positive patients presented a second inactivation of the gene through methylation. These results were in concordance with those of the TCGA consortium. It seems that the inactivation of BRCA genes follows through mutations or through epigenetic changes, but the both mechanism are unusual to be seen together.

In our study the incidence of BRCA1 promoter hypermethylation was of 14.8% , but there were no association with better PFS or OS rates, or with higher platinum response rates. Only age at first diagnosis was significant lower in hyper-methylated than in BRCA1 non-methylated patients. So our study strengthens once more that BRCA1 positive tumors are diagnosed more often in younger patients.

These results were in concordance with previous published data. Cunnignham et al. showed that epigenetic alterations (hypermethylation) of BRCA1, and Rad51C genes were detected in 10.8% of the cases. The authors showed no differences in PFS and OS with respect to the BRCA1 promoter methylation status (134). In our study we described a slightly higher incidence of BRCA1 promoter methylation, which could be caused by inclusion only of HGSOC patients. Similar results were suggested by TCGA consortium, showing that epigenetically silenced BRCA1 had similar survival data with BRCA1 wild type HGSOC. No impact has been seen on the platinum sensitivity (9). Another study published by Yang et al, showed a positive prognostic effect on survival rates only in BRCA2 mutated tumors but not in BRCA1 mutated or hypermethylated tumors (135). In HGSOC patients, BRCA2 mutations but not BRCA1 mutations or epigenetic changes are associated with improved platinum based chemotherapy response, improved survival and increased genome instability compared with BRCA wild-type tumors (135).

Another recent study showed that the presence of BRCA1/2 mutations are translated in better short-term survival, but this advantage decreases over time, especially after 4.8 years (the HR will become greater than 1, although 0.58 at time 0) and, in BRCA1 carriers can be reversed. The combined 10-year overall survival for BRCA1/2 non-carriers, BRCA1 and BRCA2 carriers was: 30% (95% CI, 28%-31%), 25% (95% CI, 22%-28%), and 35% (95% CI, 30%-41%), respectively. These finding might have important implications in the therapy of both primary and relapsed EOC. These results are also of clinical importance especially when considering the analysis of long-term survival in clinical trials with new agents, especially PARP inhibitors (136). This could be also explained by reversing frame-shift mutations in BRCA1 gene through secondary mutations, therefore developing platinum resistance (137-139). This secondary mutations that might appear in the first, second or successive relapse are of major clinical interest, especially regarding the need of current biopsies before beginning of PARP inhibitors treatment.

Our data suggest that younger ovarian cancer patients might present an epigenetic silencing of BRCA1 gene. Further studies identifying different mechanisms leading to HRD are warranted to tailor the oncologic treatment in HGSOC patients.

In our working group we are planning to analyze the incidence of BRCAness in long term survivors, -patients with no relapse within the first 5 years after platinum based chemotherapy compared to patients who relapsed within the first 2 to 3 years. Moreover we would like to study the tumor heterogeneity, by analyzing the BRCAness in paired primary tumor and metastasis, as also in paired primary and recurrent HGSOC tissue samples.

4.3 Predictive biomarkers for surgical outcome

The corner stone of ovarian cancer treatment is surgery (45). Explorative laparotomy and histology are crucial for the staging of EOC. Especially in advanced cases, when the disease has spread throughout the peritoneal cavity, cytoreductive surgery is critical, and removing all macroscopic tumor masses results in better progression free and overall survival (42-46). Most of the patients are diagnosed in advanced stages. Therefore extensive multivisceral surgery is needed in the majority of the cases. In order to achieve high quality surgery, removal of the tumor should be performed without complications, or harm done to the patients (45). The absence of macroscopically tumor residuals is associated with optimal survival rates (45). Studies showed that surgical outcome in EOC is improved in high volume centers with large expertise in gynecological oncology. During tumor debulking often upper abdominal surgery is needed. Even in the hands of the best surgeons restrictions to the extent of surgery, and therefore suboptimal surgical outcome, might appear. This is mainly caused by tumor or patients related factors, such as tumor spread, invasion of vital organs or co-morbidities (45). There are no predictive biomarkers, clinical parameters or algorithms yet to be able to predict patients who will not benefit from primary cytoreductive surgery.

Another unclear area is the role of tumor debulking for relapse situation. Prospective results from DESKTOP III clinical trial are expected. Until now data from retrospective studies, shows a benefit for optimal debulked patients undergoing secondary, tertiary and quaternary debulking (68, 69, 99, 101).

The molecular biology of the tumor might play an important role even for surgical outcome. Ovarian cancer is not one disease, but many diseases under the same name. Different histological subtypes of ovarian cancer seem to originate in the fallopian tube epithelial or precursor lesions in the ovaries. Therefore a 2-pier system was proposed, with type I tumors being diagnosed usually in younger patients and at earler stages, and having a longer course and type II tumors with HGSOC as prototype, being usually diagnosed in advanced stages and having an aggressive course (23).

We evaluated the impact of histological type on surgical and clinical outcome after primary tumor debulking EOC patients, in a retrospective cohort of patients. All patients have been operated by four experienced gynecological oncologists, in a comprehensive center for ovarian cancer with special training in abdominal surgery. Therefore the bias due to insufficient surgical skills can be excluded.

Our study was performed retrospectively, but used a validated and systematic documentation tool, such as IMO. Hereby tumor pattern, surgical procedures and tumor residuals are described and are documented prospectively through an interview with the surgeon immediately during and after surgery.

The results demonstrated that type I patients were significantly younger at time of first diagnoses compared with type II patients, and were diagnosed at earlier FIGO stages. Ascites, lymph node involvement had a lower incidence in type I patients, therefore even higher rates of optimal tumor debulking, less complications and shorter surgical times could be achieved. When the subanalysis was performed only in advanced stages (FIGO III and IV) the histological type didn’t retain any statistical significance, and had no prognostic value on survival rates or surgical outcome. When focusing on

advanced stages only the presence of a mucinous histology had a negative impact on survival when compared with low-grade serous tumors. Extrapelvic dissemination was more often encountered in the presence of high-grade serous histology.

According to the current guidelines primary EOC patients will be treated with Paclitaxel/Carboplatin with or without bevacizumab. Although there is enough evidence that low-grade tumors, clear cells and mucinous ovarian cancer do not respond well to platinum based chemotherapy, histological subtyping is still not taken into consideration in decision making process in primary setting (140). Wimberger et al presented the results of a retrospective large cohort analysis, where the multivariate analysis of OS identified following independent prognostic factors: mucinous histological subtype together with surgical outcome, multiple sites of metastases and ECOG status (141).

According to the ovarian tumorigenesis theory, mucinous and clear cell subtypes are associated with better survival rates. This was the case in our study, but this was mainly due by being diagnosed in early stages. When the analysis was performed only for advanced stages the histological subtype did not retain its significance any more.

When performing a sub analysis of only in optimal debulked patients, in terms of no macroscopically residuals, type II histology was associated with significantly poor survival. This might be explained by a potential 'higher aggressiveness' of type II cancers that might be associated with mutant p53. Nevertheless the exact underlying mechanisms have to be investigated in future trials. In the study by Eltabbakha et al it was found that p53 expression by immunhistochemical staining was a highly significant predictor for cytoreductibility. Complete cytoreduction was 5.6 times more likely to be achievied in women with tumors expressing p53 in a mild or moderate setting compared with the ones with stronger p53 expression (142).

In a further study we analyzed the tumor pattern in paired, primary and relapse debulking surgery, in order to better understand the role and the limitations of secondary cytoreduction, and also to maybe detect clinical patterns that might predict resection. Our study revealed that cytoreduction was associated with larger tumor residuals after secondary cytoreduction, due to relapses in patterns less accessible to complete resection, such as mesentery, upper abdomen and gastrointestinal serosa. No other predictors for surgical outcome have been identified. Residual masses after primary and secondary cytoreduction correlated significantly.

Our results showed that there is a different tumor spread in primary vs. relapse setting, with poor surgical outcome in relapse situation. This suggesting a more aggressive way of re-appearance of the disease, maybe due to clones diversity. Furthermore we detected no clinical parameters, or tumor spread in the primary setting that could predict the type of relapse onset.

There are hints that platinum resistance may be attributed to originally preexisting clones that are present already by the time of first diagnosis (143). These clones will become more present with every relapse and will be responsible for the acquired platinum resistance. This theory might also explain the different tumor pattern in primary and relapse situation on same patients, making surgery more difficult and more extensive in order to be able to achieve no macroscopically residual disease.

In the relapse situation lower ascites volumes have been observed but also lower maximal tumor debulking rates. Retrospective data have showed the role of cytoreduction surgery at relapse, when patients seem to benefit if macroscopically tumor clearance is reached (68, 69, 99, 101). Although in relapse situation, patients were pretreated, the morbidity rates were not increased significantly, those data being in concordance with DESKTOP results published by Harter et al (68). We observed a trend of increase morbidity rates associated with secondary debulking, although no statistical significance has been reached. Therefore the indication for secondary cytoreduction must be well balanced and performed in optimal setting in order not to harm the patients. Until now, unfortunately, we have not yet reliable clinical, imagistic or serologic markers to predict surgical outcome.

Furthermore we analyzed the role of HE4 alone or in combination with CA125 in the prognoses of surgical outcome after primary or secondary debulking. Most of the studies focused until now on the diagnostic role of HE4. There are few data about its role in predicting surgery outcome (144).

If ovarian cancer cells or the tumor environment mostly overexpress HE4 and CA125, the removal of the tumor should be translated to postoperative reduction of the circulatory levels of HE4 and CA125. Also, higher serum HE4 and CA125 in EOC patients should reflect the extensive tumor pattern and persisting higher circulatory tumor marker values could be a sign of suboptimal tumor debulking.

In our studies, in primary setting CA125 was performing slightly better than HE4 in predicting surgical outcome, but the combination of these both biomarkers slightly increased the sensitivity and specificity. For primary EOC HE4 and CA125 did not impact the OS and PFS. In the multivariate analysis only CA125 and FIGO stage II were independent predictive biomarkers for surgical outcome.

Previous studies analyzed the role of CA125 in predicting surgical outcome in primary ovarian cancer patients. However, there is no established CA125 cut-off value to predict surgical outcome. Chi et al analyzed the CA125 cut-off value of 500 U/ml, showing a sensitivity and specificity of 78% and of 73%, respectively in predicting preoperative residual disease (145). Other studies have demonstrated that a decrease of more than 75 % in CA-125 levels from primary debulking to the start of adjuvant chemotherapy was associated with better PFS rates in EOC patients (146).

In a recent publication by Angioli et al. combing preoperative HE4 levels (cut off value of 262pM), together with presence of ascites (less or more than 500ml) resulted in a sensitivity of 100% and a specificity of 89.5%, with a positive predicting value of 94% and negative predicting value of 100% (144). These results are much different from what we generated within our study, and this can be explained by the fact that Angioli, strongly preselected the patients. All patients underwent prior to study inclusion through a diagnostic laparoscopy. The laparoscopy consisted a triage to identify patients more likely to be optimal debulked or in being candidates for neoadjuvant treatment. The predictive role of HE4 for surgical outcome was evaluated only in the first group of patients. Angioli analyzed a significant lower number of preselected patients.

In the patients having first relapse of EOC and undergoing secondary cytoreduction, the results showed that HE4 is an independent predictive factor for maximal tumor

debulking, and together with platinum response they are independent predictive factors for OS.

The DESKTOP III Study recruited more than 140 patients. The DESKTOP III trial is evaluating prospectively the role of tumor debulking followed by platinum based chemotherapy vs. platinum based chemotherapy alone in platinum sensitive patients with first relapse and positive AGO score (71). According to DESKTOP I and II clinical trials, the AGO score could predict patients who are most likely to be optimal secondary tumor debulked. AGO score consists only in clinical parameters: residual mass after first cytoreduction, volume of ascites and ECOG status. For further prospective trials, the combination of biomarkers (eg. HE4 and CA125) with clinical parameters (eg. AGO score) could improve the sensitivity and specificity of both tests.

Our data showed that higher CA125 circulatory levels were more often detected in patients who experience relapse in the middle abdomen vs. patients with no relapse in this region. HE4 showed higher circulatory levels in the presence of upper abdomen relapse. These differences could be explained with regard to tumor heterogeneity (147) suggesting that CA125 and HE4 might predict tumor pattern at relapse. These data are insufficient for drawing conclusions and larger studies are needed.

A study by Kong et al included 80 patients with primary EOC. Results showed that increased HE4 levels were significantly associated with poor PFS in multivariate setting ($p = 0.0179$) (148).

Other data suggest that increased circulatory levels of HE4 are an independent prognostic factor for PFS and OS in primary EOC patients. (149)

The two studies showed that HE4 and CA125 are predictive markers for surgical outcome, although sensitivity and specificity were relative low.

There are preclinical data showing that HE4 might be responsible for tumor progression (38, 39). This might explain the correlation with OS, PFS but also with residual mass after surgery.

5 Future directions

The presented articles showed a possible role of Glycanes, HE4 and CA125 in early diagnosis of EOC. Furthermore HE4 and CA125 seem to predict surgical outcome in primary and relapse ovarian cancer patients. Nevertheless the here presented data need to be validated in a prospective multicenter study. Therefore we designed the prospective, multicenter BERLINER study, which is on going. Within this study, pelvic mass patients in whom surgical treatment is recommended are included. All patients will undergo a standardize ultrasound according to IOTA criteria. Clinical data, including menstrual status, family history, symptoms are documented. Within this study the diagnostic role of HE4 and Ca125 alone or in combination together with ultrasound criteria will be analyzed. Furthermore blood samples, serum and plasma, will be collected for validation of the GLYCOV algorithm but also for the discovery of further possible biomarkers. This study prospectively included more than 1300 pelvic mass patients. CA125 and HE4 will be analyzed centralized in Labor Berlin. Together with gynecological departments at Charité, 5 other gynecological clinics from Vivantes are participating into this trial. In order to address the importance of biomarker velocity in around 25% of the pacients a second serum sample was collected. New biomarker discovery using *in silico* analysis of publicly available data together with gene expression, proteomics and immunohistochemistry are planned for the near future.

In order to understand better the impact of histological subtypes on surgical outcome, omics should be applied in different tumors and correlated with surgical outcome in future studies. Furthermore, surgical outcome should be assessed according to P53 status as well as BRCAness in the primary tumors.

Tumor heterogeneity should be taken in consideration; therefore it is mandatory to analyze temporary and spatial heterogeneity. Hence paired samples from primary sites and metastatic lesions will be analyzed in same patients. Furthermore, genetic changes using a predefined gene panel set will be used in order to identify differences in paired primary and relapsed ovarian cancer samples, but also in serial relapses obtained from similar patients. For the paired primary and relapsed ovarian cancer patients, we identified within the OCTIPS consortium, a FP7 European project, more than 100 patients, where both primary and relapse samples are available. BRCAness in paired primary and relapse samples is of major clinical interest due to importance of companion diagnosis for therapy with PARP inhibitors.

Furthermore we will analyze the dissemination pattern in OCTIPS patients, this time also with regard to patients receiving interval debulking surgery vs. primary debulking surgery. In this way we hope to get a better understanding of the evolution of resistant clones.

6 Abstract

Although a plethora of biomarkers have been analyzes in the last decades and ultrasound criteria have been developed, ovarian cancer is mostly diagnosed in advanced stages.

Despite more extensive surgical procedures and improvement in surgical outcome, ovarian cancer remains a deadly disease with most of the patients developing relapse and platinum resistance.

Although progresses in understanding the molecular biology of EOC have been done in the last years, targeted therapies failed to improve overall survival rates in ovarian cancer patients.

The here presented data focuses on three major bottle necks of multimodal management of ovarian cancer: predictive biomarkers for early diagnosis of EOC, role and limitations of BRCA1 epigenetic changes and predictive biomarkers and clinical parameters for clinical outcome.

Borderline tumors of the ovary (BOT) are a special entity of epithelial tumors, usually having a benign behavior. Nevertheless, optimal surgical staging is mandatory, as BOT could be a precursor of low grade serous ovarian cancers. Using ultrasound, BOT together with some benign tumors, are the most difficult pelvic tumors to be assessed. They have both malignant as also benign characteristics. In our study, presented here, we analyzed the role of HE4 and CA125 alone and in combination within ROMA algorithm to predict the presence of BOT in pelvic mass patients. Our results showed that HE4 and ROMA (an algorithm combining CA125, Both biomarkers together with ROMA had poor sensitivity and specificity. Therefore there is still an urgent need of new diagnostic strategies with regards to BOT.

Furthermore we analyzed the role of GLYCOV for the diagnosis of early ovarian cancer. GLYCOV is a newly discovered biomarker panel, formed by 11 N-glycan biomarkers, more specific four high-mannose and seven complex-type fucosylated N-glycans. Regarding to available data CA125 is increased in up to 80% of ovarian cancer patients, but only in 50% of early stages. Therefore CA125 remains a poor biomarker for early detection. In our study, GLYCOV had a better sensitivity and specificity than CA125 alone, with an AUC of 0.992 in detecting early stages EOC (FIGO stage I and II). This study included 20 FIGO I and II ovarian cancer patients, therefore further validation studies are needed.

Homologous recombination deficiency (HRD) has been reported in up to 50% of high grade serous ovarian cancer patients. Mutations in BRCA1 and 2 genes are usually responsible for HRD. Epigenetic changes of BRCA1/2 promoter as also functional loss of proteins involved in HDR showed similar behavior as BRCA1/2 mutant tumors. Current trials showed that PARP inhibition increased progression free survival in BRCA mutant tumors. Therefore understanding inactivation mechanisms of the BRCA1 gene is of high clinical importance. In our study, we showed that methylation of BRCA1 promoter gene in HGSOC was not associated with platinum response, or with better overall or progression free survival rates. Hyper-methylation was associated with younger age at first diagnosis. No significant correlation between methylation

status and surgical outcome have been detected.

Residual mass after primary tumor debulking is an important prognostic factor. Optimal surgery can be achieved in ca. 60-70% of the patients, if primary debulking is performed in high volume centers. Nevertheless in a sub-group of patients optimal surgical outcome cannot be achieved. Until now there are no predictive biomarkers or clinical parameters for residual tumor mass. We analyzed the role of CA125 and HE4 in predicting surgical outcome in primary EOC. CA125 performed better than HE4 alone, but the combination of both biomarkers increased the sensitivity and specificity. When looking at different histological subtypes, we found out that in early stages, type I tumors have better survival rates, less extensive surgery, less extensive tumor spread. This was mostly due to diagnosis in early stages. Nevertheless when analyzing only the advanced FIGO stages, there was no differences in survival rates between type I and type II EOC. In advanced stages, tumor residuals together with positive lymph nodes and extrapelvic dissemination were independent prognostic factors for PFS and OS.

If surgery is well accepted for primary situation, controversial discussions are surrounding the indication of secondary debulking surgery for ovarian cancer relapse. In our presented data, we analyzed the differences in surgical outcome and dissemination pattern in paired primary and relapse ovarian cancer patients. Complete tumor resection was significantly more often achieved in primary debulked patients as in first relapse. Residual mass at first surgery correlated with surgical outcome at relapse. No clinical parameters could predict surgical outcome in relapse situation. Dissemination pattern differed between primary and relapsed situation: with higher incidence of ascites at first diagnosis, and significant more involvement of upper abdomen in recurrent setting. In relapsed ovarian cancer diffuse mesenterial and peritoneal spread have been reported, leading to sub-optimal surgical outcome. There were no significant differences within postoperative complications.

Looking for the classical biomarkers, HE4 and CA125, and their ability to predict surgical outcome, our study showed that both HE4 and CA125 are correlating with tumor residuals in patients with first platinum sensitive relapses. Nevertheless the sensitivity and specificity was poor, so further prospective multicentric studies evaluating biomarkers in combination with clinical parameters (eg. ECOG status, ascites, residual mass after primary debulking surgery) are needed. Furthermore, HE4 together with response to first platinum based chemotherapy were the only independent prognostic factors for OS.

These data are the fundament for further validation and discovery studies towards personalized management of ovarian cancer patients.

7 Literature

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9 Erklärung

§ 4 Abs. 3 (k) der HabOMed der Charité

Hiermit erkläre ich, dass

- weder früher noch gleichzeitig ein Habilitationsverfahren durchgeführt oder angemeldet wurde,
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- mir die geltende Habilitationsordnung bekannt ist.

Ich erkläre ferner, dass mir die Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis bekannt ist und ich mich zur Einhaltung dieser Satzung verpflichte.

Berlin, den 18.12.2015