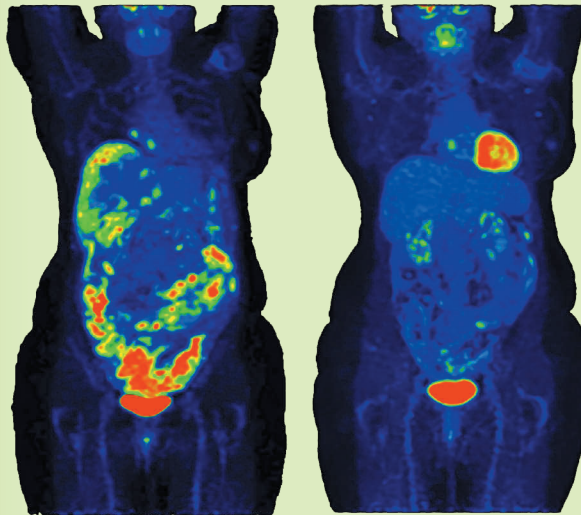




Turun yliopisto
University of Turku

NOVEL PREDICTORS OF RESPONSE AND OUTCOME IN ADVANCED EPITHELIAL OVARIAN CANCER

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To my family

I am among those who think that science has great beauty.
- Marie Curie

ABSTRACT

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Novel predictors of response and outcome in advanced epithelial ovarian cancer

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Neoadjuvant chemotherapy (NACT) followed by interval debulking surgery (IDS) is an alternative treatment approach to primary surgery for primarily inoperable advanced epithelial ovarian cancer (EOC) patients with a poor diagnosis. A clinical challenge has been a lack of reliable noninvasive NACT response assessment methods. Evaluation of the total tumor burden changes with the serum tumor marker HE4 and metabolic ^{18}F -FDG-PET/CT imaging provide novel opportunities for studying NACT response.

A total of 54 advanced EOC patients were recruited in the study; 32 patients treated with NACT followed by IDS and 22 patients referred to primary surgery. Serum HE4 and CA125 were determined at baseline and before each NACT and postoperative chemotherapy cycles. The HE4 and CA125 percentual changes during NACT were determined. In addition to CT imaging, ^{18}F -FDG-PET/CT was performed before and after NACT. The metabolical response to NACT was estimated by using omentum as a reference lesion and by calculating the total metabolic tumor volume (MTV).

The HE4 and CA125 profiles during NACT did not correspond with the anatomical CT response. An HE4 decrease of $>80\%$ during NACT associated with longer survival. The postoperative serum HE4 levels corresponded with the residual tumor in surgery, whereas the same observation was not noted for CA125. In ^{18}F -FDG-PET/CT, the omental SUVmax change during NACT associated with omental histopathological response. The total MTV decrease during NACT corresponded with the primary therapy outcome. The changes in serum HE4 and in ^{18}F -FDG-PET/CT during NACT could be used to identify the patients not responding to NACT.

Keywords: ovarian cancer; neoadjuvant chemotherapy; ^{18}F -FDG-PET/CT; HE4; treatment response

TIIVISTELMÄ

Tuulia Vallius

Uudet ennusteelliset tekijät edenneen munasarjasyövän hoitovasteen ja ennusteen arvioinnissa

Turun yliopisto, Lääketieteellinen tiedekunta, Synnytys- ja naistentautioppi, Turun kliininen tohtoriorjelmä, Valtakunnallinen PET-keskus, Turku, Suomi.

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Leikkausta edeltävää solunsalpaajahoidoa (neoadjuvanttihoito) annetaan huonoennusteisessa edenneessä munasarjasyövässä niille potilaille, jotka diagnoosivaiheessa eivät sovellu leikkaukseen. Kliinisessä työssä haasteena on ollut luotettavien noninvasiivisten menetelmien puuttuminen neoadjuvanttihoitoon vastearvioinnissa. Kokonaistuumorimassan muutoksien arviointi seerumin HE4-merkkiaineella ja ¹⁸F-FDG-PET/TT-kuvantamisella ovat uusia mahdollisuuksia neoadjuvanttihoitovasteen tutkimiseen.

Tutkimukseen rekrytoitiin 54 edennyttä munasarjasyöpää sairastavaa potilasta, joista 32 sai leikkausta edeltävän neoadjuvanttihoitoa ja 22 leikattiin primaaristi. Seerumin HE4- ja CA125-määritykset tehtiin diagnoosivaiheessa sekä ennen josta neoadjuvantti- ja leikkauksen jälkeistä solunsalpaajakuuria. Seerumista määritettiin HE4- ja CA125-pitoisuuksien neoadjuvanttihoitoa aikainen muutos. Varjoainetehosteisen TT-tutkimuksen lisäksi potilaille tehtiin ¹⁸F-FDG-PET/TT-kuvaus ennen ja jälkeen neoadjuvanttihoitoa. ¹⁸F-FDG-PET/TT-kuvista määritettiin vatsapaidan kasvaimen metabolisen aktiivisuuden (maximum standardized uptake value, SUVmax) sekä metabolisesti aktiivisen kokonaistuumoritilavuuden muutos neoadjuvanttihoitoa aikana.

Neoadjuvanttihoitoa aikaiset HE4 ja CA125 profiilit eivät olleet yhteydessä anatomiseen TT-vasteeseen. Yli 80%:n muutos HE4:ssä neoadjuvanttihoitoa aikana ennusti pidempää elossaoloa. Leikkauksen jälkeen määritetty HE4 kuvasti jäännöskasvaimen määrää, mutta samaa havaintoa ei voitu osoittaa CA125:llä. ¹⁸F-FDG-PET/TT:ssä vatsapaidan kasvaimen SUVmax muutos oli yhteydessä kasvaimen histopatologiseen vasteeseen ja kokonaistuumoritilavuuden vähentyminen neoadjuvanttihoitoa aikana ennusti primaarihoitoon vastetta. Seerumin HE4-pitoisuudessa ja ¹⁸F-FDG-PET/TT:ssä nähtävillä neoadjuvanttihoitoa aikaisilla muutoksilla voitiin tunnistaa ne potilaat, jotka eivät hyötäneet neoadjuvanttihoitosta.

Avainsanat: munasarjasyöpä; neoadjuvanttihoito; ¹⁸F-FDG-PET/CT; HE4; hoitovaste

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ABBREVIATIONS

AUC	Area Under the Curve
BMI	Body Mass Index
CA125	Cancer Antigen 125
CI	Confidence Interval
CT	Computed Tomography
DW	Diffusion Weighted
DCE	Dynamic Contrast-Enhanced
EOC	Epithelial Ovarian Cancer
FDG	Fluorodeoxyglucose
FOV	Field Of View
FIGO	International Federation of Gynecology and Obstetrics
GLUT	Glucose Transporter
GCIG	Gynecological Cancer Intergroup
HE4	Human Epididymis protein 4
HR	Hazard Ratio
IDS	Interval Debulking Surgery
LOR	Line Of Response
MRI	Magnetic Resonance Imaging
MTV	Metabolic Tumor Volume
NACT	Neoadjuvant Chemotherapy
OS	Overall Survival
OR	Odds Ratio
PDS	Primary Debulking Surgery
PERCIST	PET Response Criteria in Solid Tumors
PET/CT	Positron Emission Tomography/Computed Tomography
PFI	Platinum Free Interval
PFS	Progression-Free Survival
RF	Radiofrequency
RECIST	Response Evaluation Criteria In Solid Tumors

Abbreviations

RMI	Risk of Malignancy Index
ROC	Receiver Operating Characteristic
ROI	Region Of Interest
ROMA	Risk of Ovarian Malignancy Algorithm
SUL	Standardized Lean Body Mass Uptake Value
SUV	Standardized Uptake Value
TIC	Tubal Intraepithelial Carcinoma
TLG	Total Lesion Glycolysis
TVUS	Transvaginal Ultrasound
VEGF	Vascular Endothelial Growth Factor
VOI	Volume Of Interest
WAP	Whey-Acidic Protein
WFDC2	WAP four-disulfide core domain protein 2

LIST OF ORIGINAL PUBLICATIONS

- I. Vallius T, Hynninen J, Auranen A, Carpén O, Matomäki J, Oksa S, Virtanen J, Grénman S. Serum HE4 and CA125 as predictors of response and outcome during neoadjuvant chemotherapy of advanced high-grade serous ovarian cancer. *Tumor Biology* 2014 Dec;35(12):12389-95.
- II. Vallius T, Peter A, Auranen A, Carpén O, Kemppainen J, Matomäki J, Oksa S, Roering P, Seppänen M, Grénman S, Hynninen J. ¹⁸F-FDG-PET/CT can identify histopathological non-responders to platinum-based neoadjuvant chemotherapy in advanced epithelial ovarian cancer. *Gynecologic Oncology* 2016 Jan;140(1):29-35.
- III. Vallius T, Hynninen J, Auranen A, Matomäki J, Oksa S, Roering P, Grénman S. Postoperative HE4 predicts primary therapy outcome in advanced epithelial ovarian cancer. *Tumor Biology* 2017 Feb;39(2)
- IV. Vallius T, Hynninen J, Kemppainen J, Alves V, Matomäki J, Oksa S, Grénman S, Auranen A, Seppänen M. ¹⁸F-FDG-PET/CT based total metabolic tumor volume change during neoadjuvant chemotherapy predicts outcome in advanced epithelial ovarian cancer. Manuscript.

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1 INTRODUCTION

According to the World Health Organization, epithelial ovarian cancer (EOC) is the seventh most common cancer in women with an estimated 239 000 new diagnoses worldwide in 2012 (Stewart & Wild 2014). In Finland, the five-year relative survival rate in EOC of all stages was 44% between 2012 and 2014 (Finnish Cancer Registry 2016). The most important factors affecting patient's prognosis are age, stage at diagnosis, histopathologic type, the amount of residual tumor after debulking surgery, and platinum sensitivity (Benedet et al. 2000).

EOC is usually diagnosed in an advanced stage. The standard treatment of primary debulking surgery (PDS) followed by adjuvant chemotherapy may not always be feasible in the case of an unresectable advanced-stage disease. Primarily inoperable and unresectable patients can be referred to neoadjuvant chemotherapy (NACT) followed by interval debulking surgery (IDS) without reduction in the patient's outcome (Vergote et al. 2011). However, in some cases the cancer is primarily chemoresistant and does not respond to NACT. The currently used NACT response evaluation methods, serum tumor marker CA125 (Cancer Antigen 125) and computed tomography (CT) imaging (Rustin et al. 2011), have been debated to be insufficient as CA125 is not merely an EOC specific biomarker and the anatomical response in CT requires sufficient time to be visible (Bellati et al. 2012).

Serum tumor marker HE4 (Human epididymis protein 4) and ^{18}F -FDG-PET/CT imaging offer novel approaches to EOC treatment response evaluation. Currently, their role in NACT response evaluation in advanced EOC has not been clarified. Novel non-invasive NACT response assessment methods are needed to identify chemoresistant patients before IDS in order to avoid the potential complications of non-beneficial surgery and a delay in the initiation of second-line chemotherapy.

2 REVIEW OF LITERATURE

2.1 Advanced epithelial ovarian cancer

2.1.1 Diagnostics

2.1.1.1 Clinical diagnosis

Ovarian cancer is usually diagnosed at an advanced stage (Howlader et al. 2015) even though the patients frequently have symptoms also in the earlier stages (Eltabbakh et al. 1999). However, as primary symptoms can be multiple and un-specific for cancer, the diagnosis is easily delayed (Goff et al. 2000). The most common ovarian cancer symptoms include abdominal distension, pain and bloating, loss of appetite, changes in urinary frequency, constipation, back pain and postmenopausal and rectal bleeding (Behtash et al. 2008; Ebell et al. 2016; Goff et al. 2000, 2004; Hamilton et al. 2009). Warning signs predicting ovarian cancer during gynecologic examination are abdominal mass, mass palpable vaginally or rectally and abdominal tenderness (Hamilton et al. 2009). Ascites is frequently present (Feki et al. 2009).

2.1.1.2 Imaging and tumor markers

Transvaginal ultrasound (TVUS) is usually the primary imaging study performed. Multilocular cysts with solid areas are typical TVUS findings associated with ovarian malignancies (Sayasneh et al. 2015). American College of Radiology suggests abdominal and chest contrast-enhanced CT imaging for preoperative staging for patients with adnexal mass suspected of malignancy (Mitchell et al. 2013).

Serum CA125 is an ovarian cancer tumor marker, which is elevated (>35 kU/L) in most serous ovarian cancers (Bast et al. 1983). CA125 is usable in discriminating benign lesions from ovarian malignancies and it is currently used in clinical practice (Duffy et al. 2005; Sölétormos et al. 2016). In patients under age of 40, also serum AFP, HCG and LDH determinations are recommended to rule out germ-cell tumors (Benedet et al. 2000). Jacobs et al. developed the Risk of Malignancy Index (RMI) to aid the evaluation of risk for ovarian cancer in patients with pelvic mass (Jacobs et al. 1990). RMI combines serum CA125 level, TVUS result and menopausal status and it has been shown to discriminate ovarian malignancies from

benign lesions (Jacobs et al. 1990; Tingulstad et al. 1996). Nowadays, the current clinical practice is that if serum CA125 is elevated and the TVUS finding is abnormal, the patient is referred for further evaluation to the gynecologic oncology unit.

2.1.1.3 Histopathologic subtypes

Histopathologic evaluation is performed on tumor tissue samples taken during surgical staging operation. Ovarian tumors have been traditionally divided into six categories: serous, mucinous, endometrioid, clear cell, undifferentiated and unclassified (Benedet et al. 2000). In the histopathologic evaluation, tumor grade is also evaluated and has been traditionally classified either as well (G1), moderately (G2) or poorly (G3) differentiated. However, recent studies on ovarian cancer pathogenesis are leaning towards a division into two groups referring to the cancer development pathways: type I and type II ovarian cancers (Kurman & Shih 2008; Shih & Kurman 2004). Type I tumors, which include low grade serous carcinoma, mucinous, endometrioid and clear cell carcinoma and malignant Brenner tumor, are usually slow growing, genetically stable, low grade tumors. KRAS, BRAF, HER2, PTEN and HFN1 mutations are commonly seen in type I ovarian tumors (Prat 2012). Type II tumors represent a nature of rapid growth and high genetic instability with frequent BRCA and p53 mutations. High grade serous carcinoma, carcinosarcomas, and undifferentiated carcinomas are type II ovarian tumors (Kurman & Shih 2010). The distribution of different histopathological subtypes of ovarian carcinomas is presented in **Figure 1**.

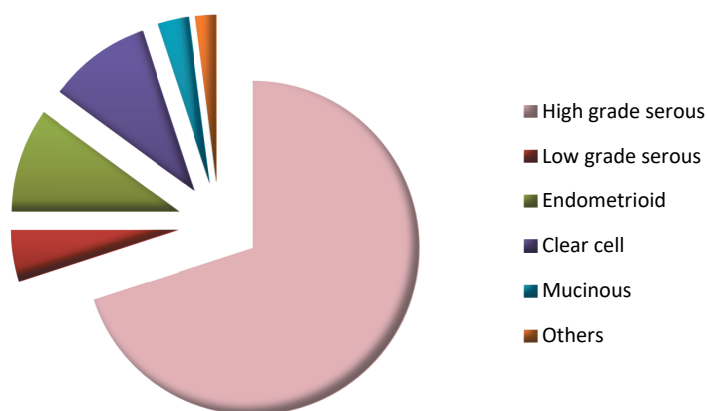


Figure 1 The distribution of different histopathological subtypes of ovarian carcinomas (Prat 2012).

The division of ovarian cancers into types I and II is still artificial as all of these histopathological subtypes have a unique origin, specific genetic mutations, different patterns of spread and sensitivity to chemotherapy. Currently, the precursor lesions of ovarian carcinomas have been suggested as being situated outside the ovaries. The high grade serous carcinomas, which account for 70% of all the ovarian carcinomas, have been proposed to originate from fallopian tube fimbriae (Kurman & Shih 2010; Prat 2012). The development of a high grade serous carcinoma is suggested to occur via either implantation of tubal epithelial cells onto the ovarian surface, which leads to the formation of ovarian inclusion cysts, or shedding and implantation of serous tubal intraepithelial carcinoma (TIC) cells (Kurman & Shih 2010). The low grade serous carcinomas form a completely different entity with an indolent behavior, and at the time of diagnosis, they are usually confined to the ovary. The low grade serous carcinomas are proposed to develop initially from serous cystadenomas to serous borderline tumors. Eventually, the process leads to the development of a low grade serous carcinoma (Kurman & Shih 2010). On the contrary, the high grade serous ovarian carcinomas are commonly wide-spread advanced stage cancers with typically underlying BRCA1, BRCA2 and p53 mutations, which lead to deficiency in DNA double-strand repair system, chromosomal instability and eventually to evolution of carcinoma (Prat 2012). The high grade serous carcinomas frequently have a high proliferation index, Ki-67 (Köbel et al. 2009), and characteristics of sensitivity to platinum-based chemotherapy. The prognosis in high grade serous carcinomas, however, has remained poor due to the high relapse rate (du Bois et al. 2003; Prat 2012).

2.1.2 Staging

EOC staging is surgical, and FIGO (International Federation of Gynecology and Obstetrics) 2014 staging guidelines are currently used (Benedet et al. 2000; Mutch & Prat 2014; Prat 2014). The FIGO staging guidelines are presented in **Table 1**. The evaluation of the tumor stage is done in the primary laparoscopy or laparotomy, where the histopathologic samples of tumor tissue are also taken. Correct primary staging is necessary as the stage at the time of diagnosis is one of the most important prognostic factors in ovarian cancer and affects the choice of primary therapy (Benedet et al. 2000; du Bois et al. 2005).

Ovarian cancer spreads through direct invasion, intraperitoneal seeding, and lymphatic and vascular circulation. Peritoneal metastases are most commonly present, as after penetration of the ovarian capsule the tumor cells are directly in contact with intraperitoneal fluid (Feki et al. 2009; Kyriazi et al. 2010). The peritoneal spread route follows the physiological intraperitoneal fluid flow. The peritoneal

surface of the colon, greater omentum and Fossa Douglas are most often affected. Subdiaphragmatic space is also a frequent metastatic site (Carmignani et al. 2003). Besides peritoneal metastases, para-aortal, pelvic and retroperitoneal lymph node affusions are also frequent (Harter et al. 2007; Tsuruchi et al. 1993). Pleural, liver and lung parenchymal metastases, in addition to supradiaphragmatic lymph node metastases, are also commonly seen in advanced EOC (Bonnefoi et al. 1999; Hynninen et al. 2012).

Table 1 The FIGO 2014 staging guideline used in EOC staging (Prat 2014).

FIGO stage	Tumor status
I	Tumor confined to ovaries
IA	Tumor limited to one ovary/fallopian tube, capsule intact, no tumor on surface, negative cytology washing
IB	Tumor limited to both ovaries or fallopian tubes
IC	Tumor limited to one or both ovaries, with any of following:
IC1	Surgical spill
IC2	Capsule rupture before surgery or tumor on ovarian/fallopian tube surface
IC3	Malignant cells in the ascites or peritoneal washings
II	Tumor involves one or both ovaries/fallopian tubes with pelvic extension below pelvic brim or peritoneal cancer
IIA	Extension/implant on uterus/fallopian tubes
IIB	Extension to other pelvic intraperitoneal tissues
III	Tumor involves one or both ovaries/fallopian tubes or primary peritoneal cancer, with cytologically/histologically confirmed spread to the peritoneum outside the pelvis / metastasis to the retroperitoneal lymph nodes
IIIA	Positive retroperitoneal lymph nodes \pm microscopic peritoneal involvement beyond the pelvis
IIIA1	Positive retroperitoneal lymph nodes only
IIIA1(i)	Metastasis \leq 10 mm
IIIA1(ii)	Metastasis $>$ 10 mm
IIIA2	Microscopic extrapelvic peritoneal involvement \pm positive retroperitoneal lymph nodes
IIIB	Macroscopic, extrapelvic peritoneal metastasis \leq 2 cm \pm positive retroperitoneal lymph nodes, includes extension to capsule of liver/spleen.
IIIC	Macroscopic, extrapelvic peritoneal metastasis $>$ 2 cm \pm positive retroperitoneal lymph nodes, includes extension to capsule of liver/spleen.
IV	Distant metastasis excluding peritoneal metastases
IVA	Pleural effusion with positive cytology
IVB	Hepatic/splenic parenchymal metastasis, metastasis to extra-abdominal organs (including inguinal lymph nodes and lymph nodes outside of the abdominal cavity)

2.1.3 Primary treatment

2.1.3.1 Primary surgery and adjuvant chemotherapy

The primary treatment of advanced EOC consists of complete surgical resection of all tumor tissue with primary debulking surgery (PDS) followed by adjuvant platinum-based chemotherapy. Complete resection is considered to be the goal of surgery as patients with residual tumor have worse prognosis compared to those with no macroscopic disease (Bristow et al. 2002; du Bois et al. 2009; E. L. Eisenhauer et al. 2008). The recommended standard primary surgery consists of radical tumor debulking, bilateral salpingo-oophorectomy, total hysterectomy, omentectomy, peritoneal debulking and when needed, bowel surgery and other debulking in the upper abdomen (Wimberger et al. 2006). A systematic pelvic and para-aortic lymphadenectomy also seems to prolong the overall survival (OS) if complete debulking is achieved (Du Bois et al. 2010). However, in the case of clinically negative lymph nodes in patients with a complete resection, the survival benefit of routine lymphadenectomy was not achieved (Harter et al. 2017).

Adjuvant chemotherapy of platinum combined with taxane is the current primary choice in advanced EOC first-line treatment (du Bois et al. 2003; McGuire et al. 1996; Ozols et al. 2003). At present, six to eight three-week cycles of intravenous carboplatin (AUC 5 or 6, day 1) combined with either weekly (60-80 mg/m², days 1, 8 and 15) or 21-day schedule (175 mg/m², day 1) paclitaxel are recommended (Bookman 2016; Chan et al. 2016; Karam et al. 2017; Morgan et al. 2011). Bevacizumab is a monoclonal antibody targeted against vascular endothelial growth factor (VEGF) and it is a novel addition to the primary therapy of advanced EOC. In a double-blinded phase III trial, patients with newly diagnosed advanced EOC received either standard carboplatin-paclitaxel chemotherapy, chemotherapy with initial bevacizumab or chemotherapy with prolonged bevacizumab. The use of prolonged bevacizumab after chemotherapy was associated with longer PFS (Burger et al. 2012; Perren et al. 2011). In advanced EOC patients with the highest risk of relapse, the use of bevacizumab in addition to standard first-line chemotherapy may improve OS (Oza et al. 2015).

2.1.3.2 Neoadjuvant chemotherapy and interval debulking surgery

As the majority of EOC patients are diagnosed at an advanced stage, there are many patients who have an extensive tumor burden and a reduced general condition at the time of diagnosis. If a patient is primarily considered inoperable and the

possibility of R0 resection is poor, the debulking surgery is postponed and neoadjuvant chemotherapy (NACT) is started to reduce the tumor burden (Wright et al. 2016). Operability can be assessed reliably during diagnostic laparoscopy (Angioli et al. 2006; Fagotti et al. 2005, 2008). The goal of NACT is to reduce the tumor burden so that optimal cytoreduction in interval debulking surgery (IDS) after NACT is achieved. NACT consists of three to four cycles of carboplatin combined with paclitaxel. After IDS, adjuvant chemotherapy is continued until completion of a total of six to eight cycles (Wright et al. 2016).

The role of NACT in EOC primary treatment has remained controversial. A meta-analysis of Kang et al. showed that NACT treated patients are more likely to be completely resected compared to primary debulked patients (Kang & Nam 2009). Two randomized trials presented that if complete resection is achieved in IDS, the patients have a similar prognosis compared to completely resected PDS patients (Kehoe et al. 2015; Vergote et al. 2011). However, Rosen et al. debated that even if R0 resection was more often achieved in the NACT group in a non-randomized patient cohort, the R0 resected patients in the PDS group still had a longer survival (B. Rosen et al. 2014). Therefore, it seems that NACT should be applied to a carefully selected patient group. NACT treated patients are suggested as having fewer surgery-related complications and lower postoperative mortality (Kehoe et al. 2015; Vergote et al. 2010).

2.1.4 Primary treatment response evaluation

2.1.4.1 Current clinical guidelines

In EOC, CT imaging and serum CA125 are currently applied in the treatment response evaluation (E. A. Eisenhauer et al. 2009; Rustin et al. 2011). Response Evaluation Criteria in Solid Tumors (RECIST) (Therasse et al. 2000) was created as an anatomic response evaluation in solid tumors and the updated version RECIST 1.1 was released in 2009 (E. A. Eisenhauer et al. 2009). According to RECIST 1.1 criteria, up to five measurable target lesions in CT imaging are selected for response evaluation. A maximum of two lesions can represent the same organ. A lesion is considered measurable with size ≥ 10 mm. In the case of a pathological lymph node, the minimum size for the short axis must be 15 mm. Non-measurable i.e. non-target lesions relevant to EOC include ascites, pleural effusion, peritoneal thickening and all other lesions with sizes below the defined measurement limits (E. A. Eisenhauer et al. 2009). RECIST 1.1 response criteria are presented in **Table 2** and **Table 3**.

Table 2 The definition of target lesion treatment response according to RECIST 1.1 criteria (E. A. Eisenhauer et al. 2009).

Treatment response	Target lesions
Complete response (CR)	Disappearance of target lesions. Any previous pathological lymph nodes must have short axis < 10mm.
Partial response (PR)	≥ 30% reduction in the sum of diameters of target lesions.
Progressive disease (PD)	≥ 20% increase in the sum of diameters of target lesions AND ≥ 5 mm absolute increase OR Presence of any new lesions.
Stable disease (SD)	Increase or decrease in the sum of target lesion diameters does not fulfill the PR or PD criteria.

Table 3 Evaluation of non-target lesions treatment response according to RECIST 1.1 criteria (E. A. Eisenhauer et al. 2009).

Treatment response	Non-target lesions
Complete response (CR)	Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must have short axis < 10 mm.
Non-CR / Non-PD	≥ 1 non-target lesion persistent OR maintenance of tumor marker level above normal.
Progressive disease (PD)	Unequivocal progression of existing non-target lesions OR Appearance of one or more new lesions

After RECIST 1.1 criteria were published, the Gynaecological Cancer Intergroup (GCIG) released a response evaluation recommendation, which incorporates RECIST 1.1 and serum CA125 response (Rustin et al. 2011). GCIG CA125 response definition is valid for patients who primarily present serum CA125 values at least twice the upper normal reference limit. According to this guideline, CA125 response is defined as at least a 50% reduction in the CA125 level compared to the pretreatment sample. The result must be confirmed and maintained for at least 28 days. Progression is defined as an increase of at least twice the upper reference limit in CA125, in two different samples obtained at least one week apart. If CA125 does not normalize during the given therapy, the progression is defined as an increased CA125 that is greater than twice the nadir value. **Table 4** presents the combined RECIST 1.1 and CA125 response evaluation in detail.

Table 4 Evaluation of the best overall response according to the GCIg definition incorporating RECIST 1.1 and serum CA125. Modified from Rustin et al. 2011.

Target lesion	Non-target lesion	New lesions	CA125	Overall best response
CR	CR	No	Normal	CR
CR	Non-CR / Non-PD	No	Not PD	PR
CR	CR	No	PR but not normal	PR
CR	NE	No	PR	PR
PR	Non-PD / NAE	No	Not PD	PR
NAE	Non-PD	No	PR	PR
PD > 28 days from CA125 PR			PR	PR
SD	Non-PD	No	PR	PR
SD	Non-PD / NAE	No	Not PR and not PD	SD
PD ≤ 28 days from CA125 PR			PR	PD
PD	any	Yes / no	any	PD
any	PD	Yes / no	any	PD
any	any	Yes	any	PD
any	any	Yes / no	PD	PD

2.2 Serum tumor markers expressed in EOC

2.2.1 CA125

2.2.1.1 Structure, physiological expression and function

Serum CA125 is a mucin type glycoprotein encoded by MUC16 gene (Yin & Lloyd 2001). The CA125 molecule has been described to consist of three major domains: the extracellular amino terminal, the multiple repeat domains, and the trans/intramembranous carboxy terminal domain (O'Brien et al. 2001). During human fetal development, the expression of mucins is connected to respiratory tract development. In adults, the physiological significance of MUC16 has been associated with defense against pathogens and production of a protective mucus layer (Hattrup & Gendler 2008).

In adult tissues, CA125 is physiologically expressed on the cell surface of the epithelium of fallopian tubes, ovaries, endometrium and endocervix, and also on the

surface of pleural, pericardial and peritoneal mesothelium cells (Kabawat et al. 1983). It is also expressed on the surface of airway epithelium cells (Davies et al. 2007). In non-pregnant healthy women, the circulating CA125 in serum has been suggested to originate from peritoneum and endometrium (Bischof 1993). In a cohort of 888 healthy individuals, only 1% presented CA125 serum values above 35 U/mL (Bast et al. 1983), which is nowadays still considered as an upper range of normal CA125 variation. The physiological half-life of CA125 is 4.8 days (Canney et al. 1984).

CA125 levels decrease with age and premenopausal women have been presented to have higher CA125 levels compared to postmenopausal women (Haga et al. 1986; Pauler et al. 2001). CA125 levels vary according to the menstrual cycle (Kan et al. 1992), which is seen especially in the patients with endometriosis (Hallamaa et al. 2012). The changes in endometrial tissue during the menstrual cycle explain the CA125 fluctuation in serum (Kafali et al. 2007). During the first trimester of pregnancy, CA125 levels elevate physiologically due to active decidual synthesis (Haga et al. 1986; Jacobs et al. 1988). Pauler et al. evaluated serum CA125 levels of 18 748 postmenopausal women followed over 12 years within an ovarian cancer screening trial (Pauler et al. 2001). In their analysis, healthy Caucasian women had higher CA125 levels than Africans or Asians. Smokers presented lower CA125 levels compared to non-smokers and the same correlation was found with caffeine consumption. Previous hysterectomy and the use of hormone replacement therapy also reduce serum CA125 levels (Grover et al. 1992).

2.2.1.2 Expression in pathological conditions and in malignancies

Benign gynecological diseases, such as endometriosis, Meigs' syndrome, and uncontrolled ovarian hyperstimulation syndrome can increase serum CA125 levels (Gulec et al. 2014; M. Hirsch et al. 2016; Morán-Mendoza et al. 2006). Benign serous epithelial, sex cord stromal, dermoid and fibroid tumors also elevate serum CA125. Increased CA125 levels in sera are also found during inflammatory processes such as abscesses and pelvic inflammatory disease (Moore, Miller, Steinhoff, et al. 2012). Surgical operations are known to increase serum CA125 temporarily (Miralles et al. 2003; Talbot et al. 1989; Yedema et al. 1993).

The effect of renal insufficiency on CA125 interpretation is controversial (Menzin et al. 1995; Odagiri et al. 1991). Liver fibrosis and cirrhosis cause elevated serum CA125 levels (Eerdekens et al. 1985; Haglund et al. 1991; Schöniger-Hekele & Müller 2006). Pericardial effusion (Seo et al. 1993) and cardiac failure (Nägele et al. 1999) also increase serum CA125. Patients with ascites and pleural fluid are known to present elevated serum CA125 values, even if the causes of the effusions

were non-malignant (Mezger et al. 1988). Serum CA125 increase has been presented in tuberculous peritonitis (Simsek et al. 1997). Zeimet et al. studied peritoneal mesothelial and ovarian cancer cells and reported that CA125 shedding and release was greater in mesothelial cells compared to that of ovarian cancer cells. Interleukin-1 beta and tumor necrosis factor alpha increased the secretion of CA125 in the mesothelial cells (Zeimet et al. 1996).

Serum CA125 levels are most typically increased in EOC (Bast et al. 1983) and the majority (80%) of ovarian cancers express CA125 (D. G. Rosen et al. 2005). The density of CA125 expression on the ovarian carcinoma cell surface has been presented to be ten times compared to that of the ovarian surface epithelium (Marth et al. 1996). CA125 may have an important role in ovarian cancer pathogenesis as it can offer adhesion through interaction with mesothelin between the ovarian cancer cell surface and mesothelial cells on the peritoneal surface (Bast & Spriggs 2011). Serum CA125 levels have been presented as varying according to the cancer progression or regression (Canney et al. 1984). Serum CA125 levels are higher in ovarian cancer patients with ascites compared with those with no ascites (Topalak et al. 2002). Serum CA125 is currently the standard tumor marker used in EOC treatment response evaluation and during follow-up (Rustin et al. 2011), even if the routine follow-up of CA125 levels in asymptomatic patients after completion of primary treatment does not offer any survival benefit (Rustin et al. 2010).

CA125 is not an ovarian cancer specific tumor marker. Elevated CA125 levels have also been detected in other gynecological cancers, such as adenocarcinomas of the fallopian tube, endometrium and endocervix (Canney et al. 1984; Niloff et al. 1984). Peritoneal carcinomatosis due to non-ovarian malignancy increases serum CA125 (Topalak et al. 2002). Breast cancer (Leonard et al. 2004) and bladder cancer (Margel et al. 2007) can cause elevated serum CA125 levels. Moreover, patients with colorectal adenocarcinoma in addition to liver, pancreatic, gallbladder and metastatic gastric cancers have been reported to have elevated serum CA125 levels, especially in an advanced stage of the disease (Canney et al. 1984; Chaube et al. 2006; Fujimura et al. 2002; Haglund et al. 1991, 1986). Hepatocellular carcinoma increases CA125 level in ascites (Bergmann et al. 1987). Malignant peritoneal mesothelioma, sarcoma and Non-Hodgkin's lymphoma patients can also have elevated serum CA125 levels (Kato et al. 2004; Lazzarino et al. 1998; Simsek et al. 1996).

2.2.2 HE4

2.2.2.1 Structure, physiological expression and function

Human epididymis protein 4 (HE4) is a secretory whey-acidic (WAP) protein with a four-disulfide core domain (Kirchhoff et al. 1991). The gene WAP four-disulfide core domain protein 2 (WFDC2) that encodes HE4 is situated in chromosome 20q12-13.1 (Clauss et al. 2002). In normal tissues, HE4 is mainly expressed in the epithelia of reproductive tracts and central respiratory airways: it is expressed in the epithelium of trachea, lung, epididymis, endometrium, endocervix, and fallopian tubes (Drapkin et al. 2005; Galgano et al. 2006). The salivary gland, thyroid, pituitary gland, kidney, and Bartholin's gland also express HE4 (Galgano et al. 2006). The biological role of HE4 is largely unknown, but it has been suspected as being associated with immune defense (Bingle et al. 2006).

Serum HE4 levels increase with age, smoking and the degree of renal insufficiency (Bolstad et al. 2012; Nagy et al. 2012). Even though HE4 levels significantly differ between various age groups, the menopausal status may not affect the serum HE4 interpretation (Moore, Miller, Eklund, et al. 2012). Male sex and high body mass index (BMI) are associated with lower serum HE4 levels (Bolstad et al. 2012). Only a mild variation in serum HE4 levels has been observed during different menstrual phases (Anastasi, Granato, et al. 2010; Hallamaa et al. 2012), whereas serum HE4 values have been reported to be decreased during pregnancy compared to non-pregnant females (Moore, Miller, Eklund, et al. 2012). The use of oral contraceptives does not affect serum HE4 levels (Hallamaa et al. 2012). In a Nordic cohort, the serum HE4 levels ≤ 70 pmol/L were considered to be within the normal range (Bolstad et al. 2012). In Turku University Hospital, the upper normal ranges of serum HE4 suggested for pre- and postmenopausal women are 70 pmol/L and 140 pmol/L, respectively. In contrast, the upper normal range limits of < 70 pmol/L (women < 50 yrs) and < 90 pmol/L (women > 50 yrs) are used in the Helsinki University Hospital area.

2.2.2.2 Expression in pathological conditions and in malignancies

HE4 is expressed in mucinous cystadenomas and to a lesser extent, serous cystadenomas (Georgakopoulos et al. 2012). Endometriosis rarely increases serum HE4 (Huhtinen et al. 2009; Moore, Miller, Steinhoff, et al. 2012) and serum HE4 levels have been presented to be similar in patients with endometriosis and healthy controls (Hallamaa et al. 2012). Benign liver and lung diseases increase serum HE4

levels (Escudero et al. 2011; Hertlein et al. 2012). Increased serum HE4 levels have also been reported in heart failure patients, even after the age, sex and renal function were adjusted (De Boer et al. 2013).

HE4 is expressed in most ovarian borderline and malignant lesions (Georgakopoulos et al. 2012). The majority of serous and endometrioid epithelial ovarian cancers express HE4 and secrete it as a N-glycosylated protein (Drapkin et al. 2005; Hellström et al. 2003; Jiang et al. 2015). In contrast, only a half of clear cell carcinomas express HE4 (Drapkin et al. 2005). In studies performed with ovarian cancer cell lines, the HE4 overexpression promotes in vitro cancer cell proliferation, adhesion, invasion and metastasis (Lu et al. 2012; Moore et al. 2014; Zhu et al. 2015). In endometrial cancer, cancer cell proliferation and tumor growth are also promoted by HE4 overexpression (Li et al. 2013). In vitro, HE4 overexpression in ovarian cancer cells is also associated with the development of cisplatin resistance (Ribeiro et al. 2016).

The use of serum HE4 has been studied in differential diagnostics in patients with pelvic mass both alone and in combination with serum CA125 in several studies. In these studies, HE4 has been presented to be able to detect ovarian cancer from benign lesions (Anastasi, Marchei, et al. 2010; Escudero et al. 2011; Hamed et al. 2013; Holcomb et al. 2011; Huhtinen et al. 2009; Jacob et al. 2011; Montagnana et al. 2009; Moore et al. 2008). In addition, a Risk of Ovarian Malignancy Algorithm (ROMA) was developed, a diagnostic score which combines serum HE4 and CA125 (Moore et al. 2009), as the combination of CA125 and HE4 was presented to have a better sensitivity in ovarian cancer detection than either one alone (Escudero et al. 2011; Moore et al. 2008; Park et al. 2011). When compared to RMI, ROMA has better sensitivity in predicting the possibility of EOC (Moore et al. 2010). The usefulness of ROMA has also been validated in an Italian multicentric study (Romagnolo et al. 2016). Besides ovarian cancer, also endometrial carcinoma, salivary gland and pancreaticobiliary cancers, mesothelioma and lung adenocarcinoma express HE4 the most. To a lesser extent, also breast, gastric and prostate cancers express HE4. Hepatocellular, colon and urinary track carcinomas have also been reported to have HE4 expression (Galgano et al. 2006).

At the time of diagnosis, the advanced FIGO stage III/IV EOC patients have greater serum HE4 levels compared to the patients with FIGO stage I/II EOC (Braicu et al. 2013; Kalapotharakos et al. 2012; Paek et al. 2011; Trudel et al. 2012). The association of preoperative HE4 to the debulking surgery outcome has been studied: Angioli et al. evaluated preoperative HE4 values taken before diagnostic laparoscopy (Angioli et al. 2013). In their study, preoperative HE4 ≤ 262 pmol/L together with ascites < 500 mL had a sensitivity of 100% and specificity of 89.5% in predicting the possibility of optimal cytoreduction in primary

debulking surgery. Another study performed by Braicu et al. presented that a preoperative combination of CA125 \leq 500 U/mL and HE4 \leq 500 pmol/L predicted optimal cytoreduction (Braicu et al. 2013). In primary debulked patients, lower preoperative HE4 values are associated with longer PFS (Aarenstrup Karlsen et al. 2016; Bandiera et al. 2011; Kong et al. 2012; Paek et al. 2011; Trudel et al. 2012) and OS (Bandiera et al. 2011; Kalapotharakos et al. 2012).

After surgery, serum HE4 values have been associated with platinum sensitivity. Angioli et al. presented that HE4 values during primary chemotherapy differed in platinum sensitive and resistant patients (Angioli et al. 2014). Serum HE4 seems also usable in the follow-up after EOC primary treatment in detecting ovarian cancer recurrence (Anastasi, Marchei, et al. 2010). Nassir et al. presented that the combination of HE4 and CA125 during follow-up after first-line EOC chemotherapy predicted recurrence better than either one alone (Nassir et al. 2016). In recurrent EOC, HE4 levels are reported to associate to presence of peritoneal carcinomatosis, the amount of ascites, secondary cytoreductive surgery outcome, and OS (Braicu et al. 2014).

2.2.3 Other tumor markers

The use of several other tumor markers has also been studied in the detection and during primary chemotherapy of EOC. In general, other tumor markers may provide additional information, for example, when evaluating the possibility of malignancy in case of CA125-negative ovarian tumor (D. G. Rosen et al. 2005). Primarily elevated serum levels of CA19-9 and CA15-3 are commonly seen in mucinous carcinomas, but less frequently in serous ovarian cancers (Gadducci et al. 1992). Elevated serum CEA (Høgdall et al. 2008) and CA72-4 are also associated with mucinous carcinomas (Fioretti et al. 1992). In the primary chemotherapy response evaluation and in the detection of disease progression, however, none of these tumor markers have provided an advantage compared to serum CA125 in initially CA125-positive patients (Fioretti et al. 1992).

Serum mesothelin (McIntosh et al. 2004) and human kallikrein 6 (Diamandis et al. 2003) in addition to CA125 have been proposed to improve ovarian cancer detection. Elevated serum inhibin levels are seen mainly in patients with granulosa cell tumors, but also mucinous and endometrioid carcinomas. In serous carcinomas, inhibin is less frequently elevated (Robertson et al. 2002). Elevated serum interleukin 6 has been associated with poor prognosis in ovarian cancer patients predominantly with a serous histology, but it has offered no additional benefit to CA125 in the detection of cancer (Scambia et al. 1995). Serum macrophage colony stimulating factor (M-CSF) levels are suggested to increase in EOC compared to benign

ovarian tumors, but in treatment response evaluation M-CSF does not provide any advantage over a CA125 assay (Gadducci et al. 1998). The serum concentrations of vascular endothelial growth factor (VEGF) are also increased in ovarian cancer, however, VEGF has not been found to be a clinically useful tool in differentiating ovarian cancer from benign lesions (Obermair et al. 1998). In addition, novel multiple biomarker panels have been developed to aid EOC detection in patients with a pelvic mass (Coleman et al. 2016).

2.3 Anatomic and functional EOC imaging

2.3.1 CT

CT images are computer-processed images, which are produced with multiple cross-section X-ray images taken from different angles (Herman 2009). CT imaging of abdomen, pelvis and chest is a recommended primary imaging modality for women with a suspected ovarian malignancy for a staging and operability assessment (Wright et al. 2016).

CT imaging has been studied in preoperative staging and operability assessment in EOC patients who have been referred to upfront surgery. Several studies have presented that CT has high specificity in identifying the absence of cancer lesions (Glaser et al. 2013; Nasser et al. 2016). However, while some studies have debated that preoperative CT may not be sensitive enough to predict the possibility of an optimal debulking surgery outcome in the presence of tumor tissue (MacKintosh et al. 2014; Mullany et al. 2004; Nasser et al. 2016), several studies have presented contradictory results (Borley et al. 2014; Ferrandina et al. 2009; Fujwara et al. 2011; Jung et al. 2010). One of the limitations of CT imaging is its poor ability to identify peritoneal lesions, especially when no ascites is present and the diameter of the peritoneal tumor nodule is below 1 cm (Kyriazi et al. 2010). Use of multi-detector CT instead of single-detector CT improves the peritoneal nodule identification (Funicelli et al. 2010), but functional imaging methods also offer improvement in peritoneal carcinomatosis detection.

2.3.2 MRI and DW-MRI

Magnetic resonance imaging (MRI) has a good soft-tissue contrast and ability to produce either two- or three-dimensional images without exposing patient to ionizing radiation (Moser et al. 2009). In brief, the MRI scanner comprises of a main

magnet and gradient and radiofrequency (RF) systems. The main magnet is constantly on and induces external magnetic field, which arranges the protons to align and spin in a hydrogen nuclei at a certain frequency. Gradient coils produce the changing gradient fields needed for signal localization. RF coils transmit RF pulses, which cause spin excitation. RF coils are also used as receiving coils for measuring the emitted signal after spin relaxation. The signals from the tissue protons and their localization are used in the image formation. Longitudinal (T1) and transverse (T2) relaxation times vary between different tissues and therefore provide contrast differences. MRI systems with 1.5 T or 3 T field strength are currently standard in clinical use (Moser et al. 2009).

Dynamic contrast-enhanced (DCE) MRI provides information on tissue perfusion in addition to anatomic imaging by using a paramagnetic contrast agent such as gadolinium. Diffusion-weighted (DW) MRI measures the Brownian motion of water molecules between compartments. Apparent diffusion coefficient (ADC) represents the diffusion within tissue and the change in ADC can be used in the assessment of tissue pathology. DW-MRI can be utilized in cancer therapy response monitoring (Moser et al. 2009).

Gadolinium-enhanced MRI has been presented to have a good ability to accurately detect benign and malignant lesions in patients with complex adnexal mass (Hricak et al. 2000). A meta-analysis by Liu et al. (Liu et al. 2007) concluded that MRI, CT and US behave in a comparable manner in the detection of ovarian cancer. However, MRI and CT studies showed a higher number of complex ovarian masses compared to US studies included in their meta-analysis. US studies showed a higher incidence of simple cysts, which may increase the performance of US. Currently, the American College of Radiology recommends the use of MRI in EOC differential diagnostics for patients with contraindication for CT contrast agents, pregnant women and for those with inconclusive CT findings (Mitchell et al. 2013).

Although MRI has been presented as performing equally well to CT imaging in EOC staging (Tempany et al. 2000), DW-MRI has been described to give an advantage compared to CT (Michielsen et al. 2014). In detection of ovarian cancer, DW-MRI is usable in evaluating peritoneal dissemination (Fujii et al. 2008). MRI might also have an advantage over CT in detecting small peritoneal implants of less than 2 cm in diameter (Tempany et al. 2000). The higher costs and motion artifacts can be considered as limitations of MRI (Mitchell et al. 2013).

2.3.3 PET/CT

PET/CT is an imaging modality combining the advantages of both metabolic and anatomic imaging. It is non-invasive and in addition to oncology, it is also routinely used in neurologic and cardiologic imaging (E. E. Kim et al. 2013). PET imaging is based on the use of tracer molecules labeled with radionuclides (Turkington 2001). ^{18}F -fluorodeoxyglucose (^{18}F -FDG) is a commonly used tracer in cancer imaging. ^{18}F -FDG is an ^{18}F -radioisotope labeled glucose analogue, which is also used in EOC. In general, medical imaging also utilizes other radioisotopes such as ^{68}Ga , ^{15}O , ^{11}C , ^{13}N (E. E. Kim et al. 2013). The half-life of ^{18}F is 110 minutes (Turkington 2001). ^{18}F -FDG is transported into cells via glucose transporters (GLUTs). The increased metabolic rate in tumor cells, due to tumor hypoxia and increased proliferation, leads to an increased need for glucose molecules for energy production and the expression of high levels of GLUTs and hexokinases. Hexokinase phosphorylates ^{18}F -FDG, which is then trapped inside the tumor cell. As the expression of glucose-6-phosphatase is reduced in cancer cells, phosphorylated FDG molecules are collected inside cancer cells. In addition to malignant cancer cells, ^{18}F -FDG accumulates in other cells with an increased glucose consumption, such as inflammatory cells (Basu et al. 2014).

When a radionucleus decays, a proton is converted into a neutron leading to a positron and a neutrino being emitted (Basu et al. 2014). ^{18}F -isotope emits a positron, which travels a distance of 1 to 2.5 mm (positron range) in tissue before losing kinetic energy due to scattering (Mettler & Guiberteau 2006). A positron and an electron annihilate each other upon colliding and as a result two 511-keV photons are emitted in approximately 180° opposite directions (Turkington 2001). Both of these photons are coincidentally detected within 6 to 12 nanoseconds in the detectors situated on the opposite sides of the PET scanner and the source of the emitted positrons, referred to as the line of response (LOR), is then identified.

PET imaging is commonly performed with integrated PET/CT scanners. In a CT image, a correct anatomical counterpart for focal ^{18}F -FDG uptake in the PET image is visualized. CT images are also utilized in the attenuation correction process (Basu et al. 2011). In the beginning of ^{18}F -FDG-PET/CT imaging, the blood glucose level is determined and the patient is weighed. ^{18}F -FDG is administered by intravenous injection and the catheter is flushed with saline. The patient waits at rest for approximately 45 to 60 min after the tracer injection for the onset of the imaging. PET/CT imaging is ordinarily performed in a supine position with arms up (Mettler & Guiberteau 2006). The radiation exposure of the whole-body ^{18}F -FDG-PET/CT imaging comprises of the amount of injected ^{18}F -FDG and the CT imaging. The effective dose of ^{18}F -FDG is 1.9×10^{-2} mSv/MBq and the dose caused by the CT imaging is dependent on the intended use of CT (Boellaard et al. 2014).

The factors lowering PET image quality are scattering, attenuation, random events, noise and spatial resolution. Scattering results from the annihilated photon interacting with tissue and changing its direction before reaching the detector. This effect can be reduced by using shields blocking photons originating outside the field of view (FOV) (Turkington 2001) and a specific energy window, which excludes photons outside the chosen energy range (Mettler & Guiberteau 2006). Random events occur when two photons hit detector within the accepted time frame for true events (within 6 to 12 ns). Both scattering and random events increase image noise (Turkington 2001). Photon absorption in the tissue or a scattering out of the detector causes loss of true coincidences i.e. attenuation. Attenuation can account for as much as 50% to 95% of the true events depending on the patient's size and body composition (Mettler & Guiberteau 2006). Attenuation is greater in deeper body parts and in areas with intense ^{18}F -FDG uptake, lesser in certain tissues such as lungs (Turkington 2001). Attenuation correction is important as it decreases image noise, artifacts, and distortion (Mettler & Guiberteau 2006). Attenuation correction is achieved by calculation and taking a reference scan (Turkington 2001). Spatial resolution refers to the ability to distinguish two adjacent points as separate and its main limitations include detector size, positron range and a slight variety in the angle of the annihilated photons ($180^\circ \pm 2^\circ$). Using smaller sized crystals in detectors can improve spatial resolution (Moses 2011). The time-of-flight PET/CT scanners also improve the reliability of the imaging results. The time interval between the detection of the two emitted photons is used to calculate the precise localization of the emission point (Surti 2015).

^{18}F -FDG accumulates physiologically in the brain and the heart. The cortical tissue and the basal ganglia have relatively high ^{18}F -FDG uptake compared to that of e.g. white matter. In heart muscle, the left ventricle dominates ^{18}F -FDG uptake whereas the right ventricle and atria do not accumulate ^{18}F -FDG (Basu et al. 2014; Cook et al. 2004). Liver, spleen, bone marrow, stomach and small intestine present low physiological ^{18}F -FDG accumulation and also the tonsils, thyroid gland, endometrium and testes uptake ^{18}F -FDG to some extent. Physiological accumulation of ^{18}F -FDG can be intense in the colon (Cook et al. 2004; Shreve et al. 1999). In premenopausal women, the physiological ^{18}F -FDG accumulation in the ovaries (S. K. Kim et al. 2005), endometrium (Lerman et al. 2004) and in fallopian tubes (Yun et al. 2010) is dependent on the phase of menstrual cycle. During lactation, elevated ^{18}F -FDG uptake in the breast may be seen. Shoulder and neck muscle tension and brown fat activation can also cause symmetrical focal ^{18}F -FDG uptake (Cook et al. 2004). ^{18}F -FDG does not reabsorb in renal tubules and is therefore excreted via the kidneys and urinary tract.

Blood glucose and insulin levels influence ^{18}F -FDG uptake and therefore, all the patients are asked to fast for four to six hours before administration of ^{18}F -FDG.

Insulin increases ^{18}F -FDG uptake in muscle and fat, which causes a reduction in tumor-to-background ratio (Basu et al. 2014). Analyzing thoracic lymph nodes is also easier after fasting as the physiological ^{18}F -FDG accumulation in the heart is lower due to fatty acid metabolism (Cook et al. 2004). To decrease the muscle uptake during ^{18}F -FDG-PET/CT imaging, the patient should lie at rest and not talk after tracer injection. Heavy exercise should also be avoided for 24 hours before ^{18}F -FDG-PET/CT imaging. The use of insulin preceding ^{18}F -FDG-PET/CT imaging is not recommended (Mettler & Guiberteau 2006).

Some benign diseases, such as Paget's disease and chronic granulomatous inflammatory diseases (sarcoidosis and tuberculosis), can be falsely mistaken for malignancy due to increased ^{18}F -FDG accumulation (Cook et al. 1996). Other infectious diseases, such as pneumonia and sinusitis, cause ^{18}F -FDG uptake due to leukocyte infiltration. Degenerative and inflammatory changes in joints as well as healing fractures also uptake ^{18}F -FDG (Shreve et al. 1999). After chemotherapy, thymus rebound (Brink et al. 2001) and increase in bone marrow ^{18}F -FDG uptake (Basu et al. 2014) are commonly known phenomena. The use of granulocyte colony stimulating factors also cause elevated ^{18}F -FDG uptake in bone marrow (Basu et al. 2011).

The most commonly determined quantitative parameters to describe tracer uptake in PET imaging include standardized uptake value (SUV), standardized lean body mass uptake (SUL), total lesion glycolysis (TLG) and metabolic tumor volume (MTV). SUV is calculated by dividing the maximum region of interest (ROI) activity by the per kilogram administered tracer activity. The unit for SUV is g/mL (Mettler & Guiberteau 2006). SUV_{max} is the maximum measured activity in a single pixel in the ROI, whereas SUV_{peak} refers to the maximum measured activity in approximately 1-cm³-volume ROI. SUV_{mean} is defined as the mean activity in ROI. SUVs can be corrected for lean body mass to reduce errors caused by differences in body fat composition. MTV is calculated as the sum of volumes of all metabolically active tumor lesions. TLG is the sum of all tumor lesions SUV_{mean} multiplied by MTV. The volume of interest (VOI) in the liver is used as a reference in PET imaging analysis (Wahl et al. 2009). ^{18}F -FDG-PET/CT is reproducible imaging method and the spontaneous fluctuation in short-term glucose metabolism is suggested to be low (Rockall et al. 2014; Weber et al. 1999). The interobserver agreement in ^{18}F -FDG-PET/CT imaging analysis is reported to be substantial and the variability might even be a minor issue in image interpretation compared to that of CT (Jacene et al. 2009; Senft et al. 2011).

^{18}F -FDG-PET/CT has been presented to be superior to US, CT and MRI in discriminating benign and malignant ovarian lesions (Castellucci et al. 2007; Nam et al. 2010; Risum et al. 2007). However, PET/CT is debated to be limited in the

detection of early stage cancers (Grab et al. 2000) and small tumor lesions of diameter less than 5mm, such as seen in peritoneal carcinomatosis (De Iaco et al. 2011). In EOC staging, ^{18}F -FDG-PET/CT is presented to be superior compared to CT (Castellucci et al. 2007; Fruscio et al. 2013; Hynninen et al. 2012; Kitajima et al. 2008) and MRI (Nam et al. 2010). On the contrary, DW-MRI might perform better compared to ^{18}F -FDG-PET/CT in peritoneal metastases detection (Michielsen et al. 2014). ^{18}F -FDG-PET/CT can reliably detect lymph node metastases (Signorelli et al. 2013). A meta-analysis by Yuan et al. (Yuan et al. 2010) concluded that ^{18}F -FDG-PET/CT can detect pelvic and para-aortic lymph node metastases even more accurately than CT or MRI.

^{18}F -FDG-PET/CT has been studied in operability assessment in both primarily debulked patients and in patients treated with NACT. Preoperatively, ^{18}F -FDG-PET/CT is presented as being useful in predicting incomplete cytoreduction (Shim et al. 2015), but, when used with no contrast-enhanced, it seems equal to MRI and CT in the detection of peritoneal carcinomatosis (Schmidt et al. 2015). When selecting patients for neoadjuvant chemotherapy instead of primary debulking surgery, the criteria of PET/CT stage IV, PET positive large mesentery bowel infiltrates and pleural exudates have been suggested for patient selection (Risum et al. 2012). Patients with PET/CT stage III have been suggested to have longer survival compared to FIGO stage III patients (Risum et al. 2010). The survival difference can be explained by the more accurate staging related to PET/CT compared to CT.

Compared to CT, ^{18}F -FDG-PET/CT is also superior in detecting ovarian cancer recurrence (Bhosale et al. 2010; Risum et al. 2009; Sebastian et al. 2008). A positive ^{18}F -FDG-PET/CT scan can identify EOC patients with a high risk of relapse (Caobelli et al. 2015) or recurrent cancer (Bhosale et al. 2010; Bristow et al. 2003; Thrall et al. 2007) if, after EOC primary therapy or during follow-up, the CA125 rises but the CT findings are normal or equivocal. ^{18}F -FDG-PET/CT might even detect ovarian cancer relapse prior to a CA125 rise (Pan et al. 2011). However, one of the limitations of ^{18}F -FDG-PET/CT is its poor ability to detect low-volume disease (Pannu et al. 2004).

Volumetric ^{18}F -FDG-PET/CT parameters MTV and TLG, which cover a patient's all malignant tumor lesions, have been evaluated as prognostic factors in EOC. To-date, only four studies focusing on the topic in primary treated EOC patients have been published (Chung et al. 2012; Lee et al. 2014; Liao et al. 2013; Yamamoto et al. 2016). Chung et al. examined total MTV and TLG values in pretreatment ^{18}F -FDG-PET/CT of 55 ovarian cancer patients (Chung et al. 2012). They suggested that patients with baseline MTV $>70\text{cm}^3$ and TLG $>563\text{g}$ are likely to have disease progression earlier compared to those with an initially lesser tumor burden. The study by Liao et al. focused on postsurgical total MTV and TLG evaluation (Liao

et al. 2013). 47 ovarian cancer patients underwent ^{18}F -FDG-PET/CT after PDS. The postsurgical TLG was reported to be an independent prognostic factor associated with OS. The median survival times for patient groups with TLG <20, TLG 20-70 and TLG >70 were 72, 26 and 10 months, respectively.

Lee et al. also evaluated the pretreatment MTV and TLG of 166 ovarian cancer patients with various histologies and stages (Lee et al. 2014). They presented that baseline MTV >25cm³ and TLG >100g were both associated with shorter PFS. The patients with no macroscopic tumor after PDS had also significantly lower baseline values of MTV and TLG. Yamamoto et al. had a patient cohort of 37 patients with newly-diagnosed ovarian cancer who underwent ^{18}F -FDG-PET/CT before PDS (Yamamoto et al. 2016). Nineteen of these patients had serous ovarian carcinoma. They reported that, at baseline, both MTV and TLG correlated with serum CA125. Patients with low TLG at diagnosis were also likely to have longer PFS.

In recurrent ovarian cancer patients, MTV and TLG before secondary debulking surgery have been presented as being associate with the surgery outcome and PFS (Vargas et al. 2015). Patients with MTV < 7.52 ml and TLG < 35.94 g had longer PFS compared to those with greater tumor volumes. Kim et al. suggested that, at the time of the first EOC relapse, MTV and TLG are also predictors of OS (C.-Y. Kim et al. 2015). The patients with MTV > 92 cm³ and TLG > 332 g during a relapse had only a mean survival of 11 months compared to 56 months in the group of lesser tumor volume.

2.3.4 PET/MRI

Similar to PET/CT, also PET/MRI imaging also combines the advantages of metabolic, morphological and functional imaging, but with significantly lower exposure to ionizing radiation (F. W. Hirsch et al. 2013). The ability of PET/MRI to detect malignant lesions has been presented to be equal (Beiderwellen et al. 2015; Drzezga et al. 2012; Grueneisen et al. 2015; Queiroz et al. 2015) or even superior (Fiaschetti et al. 2011) compared to that of PET/CT. PET/MRI may be a feasible choice of imaging modality, when a good soft tissue contrast is warranted, for instance in musculoskeletal and pelvic imaging (Antoch & Bockisch 2009; Von Schulthess & Schlemmer 2009).

PET/MRI is suggested to be usable in differentiating benign from malignant lesions in patients with an adnexal lesion suspected of ovarian cancer (Fiaschetti et al. 2011) and recurrent ovarian and cervical cancer (Beiderwellen et al. 2015; Grueneisen et al. 2015). Compared to PET/CT, PET/MRI has been presented to have better sensitivity and accuracy in detecting recurrent gynecological

malignancy (Kitajima et al. 2014). PET/MRI is also presented to perform better than PET/CT in tumor delineation, especially in cervical and endometrial cancer (Queiroz et al. 2015). In patients with various gynecological malignancies, fused PET/T2-weighted MR images performed more accurately in detecting the anatomical site of ^{18}F -FDG accumulation and detected smaller sized pathological lesions than PET/T1-weighted MR or PET/CT images (Nakajo et al. 2010). In contrast, PET/CT might have an advantage over PET/MRI in detecting small-size lung parenchymal metastases (Beiderwellen et al. 2015). The imaging protocol of PET/CT is also more rapid compared to that of PET/MRI (Von Schulthess & Schlemmer 2009).

2.4 EOC treatment response evaluation

2.4.1 Tumor markers

In general, response to cancer therapy can be studied with either direct surgical evaluation or with non-invasive imaging studies performed before and after chemotherapy. Indirect information on treatment response can be obtained with evaluating cancer specific serum tumor markers during chemotherapy. In EOC, serum CA125, in addition to CT imaging, is routinely used in primary therapy response evaluation (E. A. Eisenhauer et al. 2009; Rustin et al. 2011). The primary therapy response consists of both surgical debulking and response to the adjuvant chemotherapy, whereas response to NACT is merely affected by chemosensitivity. However, despite the preceding cancer treatment, the correct evaluation of the treatment response is important in order to avoid unbeneficial and potentially risky treatments.

During primary chemotherapy of EOC, serum CA125 levels have been presented to correspond to the patient's outcome. Early CA125 normalization after the first (Fisken et al. 1993) and second (Gadducci et al. 1994; Markman M, Federico M, Liu PY, Hannigan E 2006; Rocconi RP, Matthews KS, Kemper MK, Hoskins KE, Huh WK, Straughn JM 2009) adjuvant chemotherapy cycle is associated with a longer OS. Serum CA125 normalization and CA125 half-time during primary chemotherapy have been associated with a clinical and a pathological complete response (Gadducci et al. 1994). Moreover, the CA125 nadir values below the normal upper range (<35 U/mL) of <10 U/mL (Crawford & Peace 2005) and <20 U/mL (Riedinger et al. 2006) during or after primary chemotherapy have been suggested to refer to better outcome. Serum CA125 kinetics have also been presented as following the change in tumor size during chemotherapy in recurrent

ovarian cancer (Wilboux et al. 2014). However, in recurrent ovarian cancer, increasing CA125 values during early cycles of second-line chemotherapy have been reported even in chemotherapy-responding patients (Coleman et al. 2007).

The behavior of serum CA125 during NACT has also been evaluated in several studies (Furukawa et al. 2013; Le, Hopkins, et al. 2007; Menczer et al. 2011; Pelissier et al. 2014; Rodriguez et al. 2012; Tate et al. 2005). CA125 less than 75 U/mL after third NACT cycle has been presented to associate with longer relapse-free survival and OS (Pelissier et al. 2014). Tate et al. evaluated CA125 regression during NACT and concluded that CA125 regression coefficient was a prognostic factor for overall survival (Tate et al. 2005). Furukawa et al. presented that CA125 less than 20 U/mL after NACT predicts complete cytoreduction in IDS and a longer OS (Furukawa et al. 2013). In the study by Rodriguez et al., pre-IDS CA125 less than 100 U/mL was associated with complete cytoreduction (Rodriguez et al. 2012). However, few studies considering the use of CA125 in NACT response evaluation have shown contradictory results as to whether CA125 can be reliably used in response evaluation. In the study by Le et al., the CA125 decrease of more than 50% from baseline during NACT was referred to as a positive CA125 response (Le, Hopkins, et al. 2007). In the study, 83% of all the patients (n=91) were responders to NACT according to this definition. However, there was no difference in the PFS in CA125 responders compared to non-responders. Menzer et al. evaluated 37 NACT-treated patients who all had a CA125 reduction of more than 50% during NACT (Menczer et al. 2011). CA125 normalization (<35 U/mL) or a CA125 decrease of more than 90% during NACT did not associate with a better outcome.

The use of serum HE4 in EOC treatment response assessment has only been discussed in three studies (Angioli et al. 2014; Hynninen et al. 2011; Pelissier et al. 2016). Hynninen et al (Hynninen et al. 2011) evaluated serum HE4 samples during EOC primary chemotherapy in a patient cohort including both primary debulked and NACT treated patients. A decrease in serum HE4 below 140 pmol/L after the second adjuvant chemotherapy cycle after PDS was seen in all the patients with a complete response, whereas the CA125 normalization was achieved in a later phase of the chemotherapy. The serum HE4 profiles obtained during NACT were also in concordance with the anatomic and metabolic treatment response seen in CT and ¹⁸F-FDG-PET/CT. Angioli et al (Angioli et al. 2014) obtained serum HE4 samples during EOC first-line chemotherapy from patients with no macroscopic tumor residuals after PDS. The platinum sensitive and resistant patients were reported to have statistically significant difference in serum HE4, but not in the CA125, values before the first, third and sixth adjuvant chemotherapy cycles. The platinum resistant patients had persistently elevated serum HE4 levels >70 pmol/L during the primary chemotherapy. Pelissier et al (Pelissier et al. 2016) measured

serum HE4 levels before and after three cycles of NACT and presented that, after NACT, patients with serum HE4 ≤ 252 pmol/L were likely to be optimally debulked in IDS. Low serum HE4 levels before IDS were also associated with platinum sensitivity.

To conclude, as serum CA125 levels may be increased due to inflammation, peritoneal irritation after abdominal surgery and other non-malignant conditions, the assessment of treatment response with merely CA125 may lead to false interpretation of a negative response in some cases. Moreover, in NACT treated patients, CA125 might have its limitations in response evaluation. Serum HE4 might be more cancer specific and therefore offer an advantage in treatment response evaluation in addition to CA125.

2.4.2 Anatomic imaging

CT imaging is the currently used standard imaging modality in EOC primary treatment response evaluation. Although RECIST 1.1 criteria (E. A. Eisenhauer et al. 2009) are generally accepted in cancer treatment response evaluation, some limitations considering the use of these criteria in EOC have been presented. Biliaci et al. (Bilici et al. 2010) presented that radiological CR after NACT defined according to RECIST 1.1 criteria (**Table 2**) did not predict PFS or OS. Similarly, in the study by Menczer et al (Menczer et al. 2011), median PFS did not differ in patients with marked improvement seen in CT after NACT compared to those with merely some or no improvement. Marked improvement was defined as a reduction of more than 50% in the size or number of the tumor lesions seen in CT. Yildirim et al. (Yildirim et al. 2012) reported that in a patient cohort of 35 primarily inoperable advanced EOC patients, 34% had PR and 66% SD after NACT according to RECIST criteria. Despite the poor response status, 83% and 35% of these patients could still be optimally resected to no macroscopic disease. As CT imaging has been debated to have restrictions in NACT response evaluation (Bellati et al. 2012), other non-invasive imaging modalities are needed to identify the chemosensitive and chemoresistant patients.

2.4.3 Functional imaging

As anatomic imaging has its limitations in EOC treatment response evaluation, functional imaging can offer precise information on the change in tumor metabolism during cancer treatment. ^{18}F -FDG-PET/CT can be used in early response assessment, which is not possible with anatomic imaging (Khiewvan et al. 2017).

However, no specific standard response evaluation criteria for functional imaging in EOC currently exist. PET Response Criteria in Solid Tumors (PERCIST) version 1.0 criteria has been introduced for ^{18}F -FDG-PET/CT based treatment response evaluation (Wahl et al. 2009). PERCIST 1.0 criteria evaluate SULmax of up to five separate tumor lesions (up to two per organ) with the most intense ^{18}F -FDG-uptake. The percentual change in SULpeak from pretreatment to posttreatment imaging is calculated. Complete metabolic response is defined as disappearance of all increased metabolic activity. More than 30% and 0.8 unit decrease in the target lesion SULpeak is referred as partial metabolic response. In contrast, progressive disease is defined as more than 30% increase in the target lesion SULpeak or appearance of any new lesions. It is notable, that a target lesion is the most intense lesion at the time of imaging and therefore not necessarily the same lesion at baseline and at follow-up. The response evaluation is preferably performed with a single lesion with the hottest SULpeak. However, the sum of SULs of up to five most active lesions can also be optionally evaluated.

A few studies considering the use of ^{18}F -FDG-PET/CT in EOC treatment response evaluation have been performed. In a study conducted by Sironi et al. (Sironi et al. 2004), 31 patients with ovarian cancer of various histology underwent ^{18}F -FDG-PET/CT followed by second-look laparotomy at the end of postoperative adjuvant first-line chemotherapy. Histological samples of PET/CT positive lesions from corresponding anatomical sites were obtained during surgery. The positive predictive value for ^{18}F -FDG-PET/CT in detecting persistent tumor lesions was 89%. Similarly, a study comparing ^{18}F -FDG-PET to second-look laparotomy, showed that ^{18}F -FDG-PET imaging was equal to surgery in detecting persistent disease after six adjuvant chemotherapy cycles (S. Kim et al. 2004). PFS of the patients with negative and positive PET at the end of primary therapy were 40.5 ± 11.6 months and 23.7 ± 5.3 months, respectively. Picchio et al. also reported that ^{18}F -FDG-PET combined with CT can accurately detect persistent disease after completion of primary chemotherapy (Picchio et al. 2003).

Avril et al. evaluated SUVmax decrease in a single lesion after one and three NACT cycles (Avril et al. 2005). A threshold of -55% change in the SUVmax after third NACT cycle in a lesion with the lowest metabolic activity decrease identified the metabolic responders, who had a longer OS compared to the metabolic non-responders (38.9 vs. 19.7 months, respectively). Martoni et al. presented that a decrease of 100% in SUVmax of the primary tumor after three NACT cycles predicts complete pathological response and an optimal surgery outcome after a total of six NACT cycles (Martoni et al. 2011). Nishiyama et al. performed ^{18}F -FDG-PET/CT before and after NACT for 21 patients with gynecological malignancy, amongst whom there were eight ovarian cancer patients (Nishiyama et al. 2008). The decrease of 65% in SUVmax of the primary tumor during NACT identified

pathological responders from non-responders (sensitivity 90%, specificity 81.8%, accuracy 85.7%).

The importance of the total tumor burden in EOC treatment response evaluation has not been studied. However, as preoperative MTV and TLG have been reported to predict outcome in EOC, these parameters might also serve as a new approach to EOC treatment response assessment.

DW-MRI and DCE-MRI have been studied in EOC treatment response evaluation. Kyriazi et al. evaluated chemotherapy-induced changes with DW-MRI in 42 patients with either newly diagnosed or relapsed advanced ovarian cancer (Kyriazi et al. 2011). MRI was performed at baseline and after the first and third chemotherapy cycles and up to five target lesions per patient were evaluated. In addition to MRI, response to chemotherapy was evaluated with CA125 decline and CT imaging. Responders had a higher whole tumor volume change compared to non-responders after three chemotherapy cycles and a significant change in ADC histogram parameters. A greater increase in ADC in ovarian tumors after three cycles of NACT in chemotherapy responders was also presented by Sala et al. (Sala et al. 2012).

2.4.4 Histopathology

In EOC patients, tumor tissue samples for histopathology can be obtained during primary staging laparoscopy, PDS and IDS. The histopathology of samples from primary laparoscopy or PDS cannot be directly used for treatment response evaluation, as they simply provide information on the morphological type and cancer dissemination (stage) at the baseline. However, the primary evaluation is indispensable and must be performed carefully in order to obtain the correct diagnosis and determine the prognostic histopathological features. The histopathologic samples obtained after NACT during IDS provide possibilities for reliable chemotherapy response evaluation. The post-NACT tumor tissue samples demonstrate direct chemotherapy-induced changes in tumor cells without interference from the preceding surgery.

The changes seen in EOC after chemotherapy comprise both stromal changes and changes in surviving tumor cells. Fibrosis, inflammatory cells, cholesterol clefts, hemosiderin pigment, calcified psammoma bodies (McCluggage et al. 2002), necrosis and foamy macrophages (Sassen et al. 2007) are common chemotherapy-induced stromal findings. After chemotherapy, residual tumor cells are likely to appear as giant tumor cells with irregular, enlarged nuclei with chromatin

accumulation and prominent nucleoli and either densely eosinophilic or clear cytoplasm (McCluggage et al. 2002).

Currently, no standard criteria for histopathologic response grading in EOC exists. A few studies focusing on histopathologic response evaluation after NACT have presented the histopathologic response as a prognostic factor (Ferron et al. 2009; Le, Williams, et al. 2007; Muraji et al. 2013; Petrillo et al. 2014; Sassen et al. 2007). However, the features evaluated (presented in **Table 5**) and the histopathologic response classifications used in these studies varied. Le et al. evaluated necrosis, fibrosis, macrophage infiltration and tumor-induced inflammation (Le, Williams, et al. 2007). They presented that better histopathologic response was associated with longer survival. Petrillo et al. defined complete pathologic response as no residual malignant cells in IDS specimens (Petrillo et al. 2014). In their study, complete pathologic response predicted longer PFS and OS compared to patients with microscopic or macroscopic tumor cell foci in IDS samples. Even if microscopic or macroscopic pathologic responders were R0 resected in IDS, they still had a poorer prognosis compared to R0 resected complete pathologic responders. In the study by Muraji et al., histopathologic response was assessed with a four-graded scale according to viable cancer cells, necrosis, fibrosis, and tumor-induced inflammation (Muraji et al. 2013). In their study, poor histopathologic responders had inferior OS compared to those with marked response to chemotherapy. When evaluating only R0 resected patients, the patients with better histopathologic response also had a longer PFS and a lower risk of platinum-resistant relapse. Sassen et al. presented that of all the chemotherapy-induced changes, only the pattern and extent of the residual tumor after NACT correlated with the OS (Sassen et al. 2007). Ferron et al. concluded that although the R0 resected NACT patients with persistent viable tumor cells had earlier relapses compared to those with no histological residual disease, there was no difference in overall survival (Ferron et al. 2009).

Table 5 The histopathologic features previously used in EOC treatment response evaluation after NACT.

Authors	Year of publication	n=	The features used in histopathologic response evaluation
Le et al.	2007	61	Fibrosis Necrosis Macrophage infiltration Tumor induced inflammation
Sassen et al.	2007	49 (+ control group n=35)	Fibrosis Necrosis Macrophage infiltration Tumor induced inflammation
Ferron et al.	2009	58	Mitotic index Fibrosis Necrosis
Muraji et al.	2013	124	Fibrosis Necrosis Tumor induced inflammation Residual tumor cells
Petrillo et al.	2014	322	Residual tumor cells

3 AIMS OF THE STUDY

The aim of this study was to investigate the use of serum HE4 and ^{18}F -FDG-PET/CT in the NACT response evaluation and in prediction of outcome in an advanced stage EOC patient cohort. The methods routinely used in NACT response assessment are CT imaging and the serum tumor marker CA125, both of which have been debated to be limited in response evaluation. It is clinically relevant to be able to identify the patients who are chemoresistant to first-line therapy and subsequently would still be unresectable after three NACT cycles in order to avoid unbeneficial and potentially riskful surgical operations. The specified study aims:

- I. To evaluate serum HE4 and CA125 profile changes during NACT and to compare these to radiologic NACT response as evaluated by CT and RECIST 1.1 criteria, IDS outcome, platinum sensitivity, PFS and OS (study I).
- II. To study the postoperative and nadir serum HE4 and CA125 values during postoperative chemotherapy after both PDS and NACT followed by IDS and to compare these to the residual tumor after surgery, primary therapy outcome and PFS (study III).
- III. To evaluate the metabolic response to NACT with ^{18}F -FDG-PET/CT in omentum and to compare the reduction in SUVmax to the histopathological response seen in the corresponding omental tumor tissue samples taken during IDS (study II).
- IV. To evaluate the change in the total MTV in ^{18}F -FDG-PET/CT during NACT and to examine if the total MTV change corresponds to the primary therapy outcome, PFS and OS (study IV).

4 MATERIALS AND METHODS

4.1 Study design

This study was a part of a larger prospective observational trial evaluating the role of ^{18}F -FDG-PET/CT imaging and novel serum biomarkers in the primary treatment of advanced EOC (Epithelial Ovarian Cancer- staging and response to chemotherapy evaluated by PET/CT (MUPET)). The study was conducted in the Department of Obstetrics and Gynecology, Turku University Hospital, Finland. The local ethics committee approved the study protocol (ClinicalTrials.gov Identifier: NCT01276574). This dissertation study was designed to focus on the NACT treated patients of the MUPET trial patient cohort. The flowchart in **Figure 2** illustrates the study design and timing of the obtained ^{18}F -FDG-PET/CT images and serum samples in comparison to surgical operations and chemotherapy cycles.

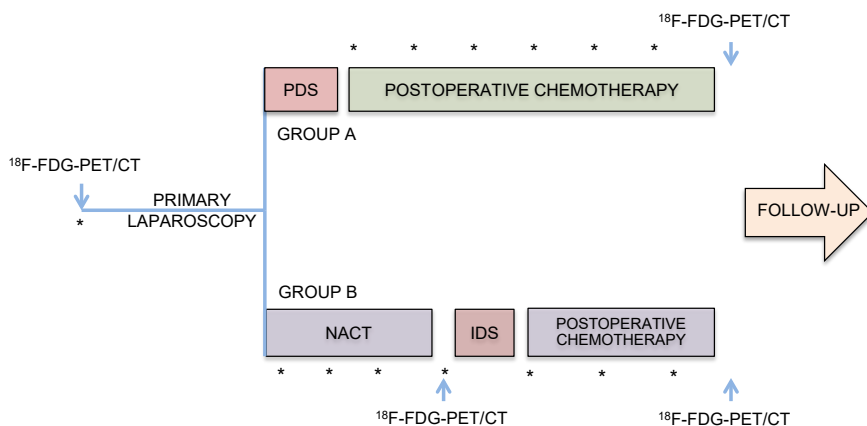


Figure 2 Flowchart of the study design. The obtained serum samples are marked with *. PDS = primary debulking surgery, NACT = neoadjuvant chemotherapy, IDS = interval debulking surgery.

4.2 Study population

The study patients were recruited between December 2009 and March 2014. All the new patients with suspected ovarian malignancy admitted to the Department of Obstetrics and Gynecology were offered a chance to participate in the MUPET study. The inclusion criteria for the MUPET study were new advanced EOC, primary peritoneal or fallopian tube cancer and age of 18 to 80 years. All the patients signed an informed consent. The exclusion criteria were a medical history of diabetes or previous cancer.

The inclusion criteria for studies I-IV:

- I. Primarily inoperable FIGO stage III/IV EOC patients referred to NACT, serum HE4 and CA125 samples obtained before and after NACT and CT imaging performed before IDS.
- II. Primarily inoperable FIGO stage III/IV EOC patients referred to NACT, ^{18}F -FDG-PET/CT performed before and after NACT and omental histopathological samples obtained after NACT available.
- III. FIGO stage III/IV EOC patients, PDS performed or referred to NACT followed by IDS and at least three postoperative serum HE4 and CA125 samples available.
- IV. Primarily inoperable FIGO stage III/IV EOC patients referred to NACT and ^{18}F -FDG-PET/CT performed before and after NACT.

The distribution of the patient cohort according to the inclusion in the original publications is presented in **Table 6**. The characteristics of the patient cohort are presented in **Table 7**. The subpopulations in studies I-IV are described in detail in the original publications I-IV.

Table 6 The distribution of patients included in the original publications.

Patient ID	HE4 profile during NACT 2014	SUVmax change and histopathologi- cal response 2016	HE4 profile during postoperative chemotherapy 2017	Total MTV change during NACT 2017
	n=25	n=26	n=49	n=29
1	x		x	x
2			x	
3			x	
4	x	x	x	x
5			x	
6			x	
7			x	
8			x	
9			x	
12	x	x	x	x
13			x	
14	x	x	x	x
17	x	x	x	x
18	x	x	x	x
19	x			x
22	x			x
23			x	
25			x	
28			x	
30	x	x	x	x
31			x	
32	x	x	x	x
33	x	x		x
34	x	x	x	x
35			x	
36			x	
39	x			x
40	x	x	x	x
42			x	
44	x	x	x	x
45	x	x	x	
47			x	
48	x	x	x	x
49	x	x	x	x
54	x	x	x	
55			x	
56	x	x	x	x
57			x	
58	x	x	x	x
59	x	x	x	x
60	x	x	x	x
62	x	x	x	x
63	x	x	x	x
64			x	
67		x	x	x
68		x	x	x
70			x	
71				x
73		x	x	x
75			x	
78			x	
83			x	
86		x	x	x
87		x	x	x

Table 7 Patient characteristics presented in the whole patient cohort and in the subgroups of PDS and NACT+IDS.

	All n=54 (100%)	NACT n=32 (59%)	PDS n=22 (41%)
Age, median (range)	64 (30-80)	64 (38-80)	62.5 (30-79)
Stage			
IIIB	2 (4%)	2 (4%)	-
IIIC	26 (48%)	13 (24%)	13 (24%)
IVA	8 (15%)	7 (13%)	1 (2%)
IVB	18 (33%)	10 (19%)	8 (15%)
Serum creatinine (umol/L) at diagnosis, median (range)	64 (45-118)	64 (48-118)	65 (45-101)
Histology			
High grade	48 (89%)	31 (57%)	17 (31%)
Low grade	5 (9%)	1 (2%)	4 (7%)
Clear cell	1 (2%)	-	1 (2%)
Number of primary chemotherapy cycles, median (range)	6 (3-12)	6.5 (3-12)	6 (6-10)
NACT cycles			
3		29	
4		3	
Surgery outcome			
No macroscopic tumor	13 (24%)	7 (13%)	6 (11%)
Tumor residual <1cm	28 (52%)	19 (35%)	9 (17%)
Tumor residual >1cm	9 (17%)	2 (4%)	7 (13%)
No IDS attempt	4 (7%)	4 (7%)	
Primary therapy outcome			
CR	29 (54%)	14 (26%)	15 (28%)
PR	13 (24%)	7 (13%)	6 (11%)
PD	7 (13%)	6 (11%)	1 (2%)
PD after NACT	5 (9%)	5 (9%)	

4.3 Surgical treatment (The selection between PDS and NACT+IDS)

All the patients underwent CT and ¹⁸F-FDG-PET/CT imaging at the baseline, prior to primary laparoscopy. The tumor staging and operability were subsequently evaluated in laparoscopy. An experienced gynecologic oncologist performed all the

surgical operations. The Fagotti scoring system (Fagotti et al. 2008) was utilized for evaluating the possibility of complete tumor resection. The preoperative imaging studies were available for the surgeon and also utilized in the clinical decision-making. During laparoscopic evaluation, tumor tissue samples for histopathological evaluation and verifying diagnosis were obtained systematically.

The patients were divided into two groups according to the operability at the time of the diagnosis (**Figure 2**). In Group A, the primary laparoscopy was converted into laparotomy. The patients in Group B received three to four cycles of NACT, after which the operability was again evaluated. If the patient had profited from NACT, IDS was performed. The results of CT and ^{18}F -FDG-PET/CT imaging, the behavior of serum CA125 during NACT and the patient's clinical condition were all available to the clinician when evaluating the possibilities of tumor resection in IDS.

4.4 First-line chemotherapy

All the study patients received the standard platinum-based primary chemotherapy used in EOC. The chemotherapy cycles consisted of 21-day cycles of paclitaxel 175 mg/m² and carboplatin AUC 5. In the group A, the patients received six to ten postoperative chemotherapy cycles. In the group B, the patients received three to four cycles of NACT at first and, in addition, three to six postoperative chemotherapy cycles after IDS. A total of five patients received only carboplatin due to a reduced general condition. In six patients, the adjuvant chemotherapy was combined with bevacizumab, which was continued as maintenance after primary therapy.

4.5 Primary therapy response evaluation

The primary therapy response was evaluated with CT and serum CA125 after the last postoperative chemotherapy cycle. The response definitions in the criteria incorporating RECIST 1.1 and CA125 (presented in chapter 2.1.4.1) were followed (E. A. Eisenhauer et al. 2009; Rustin et al. 2011). The patients were classified as having a complete response (CR), partial response (PR), stable disease (SD) or progressive disease (PD).

4.6 Follow-up

After completion of the primary therapy, the patients with CR or PR proceeded to follow-up. During follow-up, serum CA125 samples were routinely obtained every three months. All the patients were followed until disease progression was noted. The disease progression was defined as serum CA125 rise >70 U/mL or at least two times the CA125 nadir value confirmed twice as suggested by the GCIG incorporated response criteria (Rustin et al. 2011) or a clinical suspicion of relapse. In case of a suspected relapse, ^{18}F -FDG-PET/CT was performed as indicated by the study protocol. If the disease progression was recorded after NACT (n=5) or during postoperative chemotherapy (n=7), the disease was considered platinum refractory and the patient was directly referred to second-line chemotherapy.

4.7 Serum sample collection and analysis

4.7.1 CA125

Venous blood samples were obtained systematically according to the study protocol. The samples were collected at the baseline before surgery and before each postoperative adjuvant chemotherapy cycle. In NACT treated patients, the samples were also obtained before each NACT cycle and before IDS. The blood samples were incubated for 30 minutes at room temperature in non-heparinized tubes and were subsequently centrifuged for 15 minutes at 3,000 rpm (800 g). The serum obtained was used for tumor marker analyses.

Serum CA125 concentrations (U/mL) were determined quantitatively by using automatic electrochemiluminescence immunoassay (ECLIA) analyzer (Modular E170 automatic analyzer, Roche Diagnostic GmbH, Mannheim, Germany).

4.7.2 HE4

Serum HE4 concentrations (pmol/L) were analyzed from frozen serum samples. The serum was stored at -20°C or -80°C in 2 ml cryotubes. Enzyme-linked immunosorbent assay (ELISA) kits by Fujirebio Diagnostics Inc. (Malvern, PA, USA) were used to manually analyze the serum HE4 concentrations. The manufacturer's instructions were followed in the analyses.

4.7.3 Tumor marker evaluation (studies I-III)

Further on, the serum HE4 and CA125 are both being referred to as the tumor markers.

In study I, the tumor markers before and after NACT and their changes during NACT were evaluated. The tumor marker values at the time of diagnosis (pre-NACT) and after NACT (pre-IDS) were used to calculate the percentual change during NACT. The patients were divided into two groups according to the reduction in tumor markers during NACT (reduction of $<$ or $>$ 80%). The selected division was based on the literature (Rodriguez et al. 2012).

In study II, the tumor marker halftimes during NACT were obtained using the serum samples preceding each NACT cycle and IDS. A fitted linear model for the tumor markers logarithm was used and the $\log(1/2)$ was divided by the slope of the regression line to obtain the halftimes.

In study III, the preoperative, postoperative and nadir tumor marker values and the perioperative tumor marker changes were evaluated. The preoperative serum tumor markers were defined as the tumor markers closest to PDS or IDS and postoperative serum tumor markers as the samples closest to the onset of chemotherapy. Median number of 6 (range 3 to 10) postoperative HE4 samples and 5 (range 3 to 9) CA125 samples were analyzed. The perioperative percentual change was calculated using preoperative and postoperative tumor marker values. The tumor marker nadir values were defined as the lowest values during postoperative primary chemotherapy. The preoperative HE4 and CA125 samples were obtained before surgery within median time period of 1 day (range 0 to 41 days) and 1 day (range 0 to 48 days), respectively. From surgery to postoperative HE4 and CA125 sample analysis, a median time interval was 19 days (range 4 to 33 days).

4.8 Imaging and imaging analysis

4.8.1 CT

CT imaging is the standard imaging modality used in EOC diagnostics and response evaluation (E. A. Eisenhauer et al. 2009). All the study patients underwent contrast-enhanced diagnostic whole-body CT imaging at baseline and after completion of postoperative chemotherapy. CT was also performed after NACT. Experienced radiologist evaluated the obtained CT images.

In study I, the NACT response was evaluated according to the RECIST 1.1 criteria (E. A. Eisenhauer et al. 2009) with the selected target and non-target lesions of CT images before and after NACT. Up to five target lesions, with maximum of two per organ, were chosen for response evaluation. The partial responders were further divided into two groups, as the majority of the patients had partial response after NACT. The cutoff value for the division was a 50% reduction in target lesions, which was similar to the study by Menczer et al. (Menczer et al. 2011).

4.8.2 ^{18}F -FDG-PET/CT

In addition to CT imaging, ^{18}F -FDG-PET/CT was performed at baseline and after the last primary chemotherapy cycle. NACT treated patients in the group B underwent ^{18}F -FDG-PET/CT after NACT. The median time interval from the last NACT cycle to ^{18}F -FDG-PET/CT was 22 days (range 4 to 35 days). ^{18}F -FDG-PET/CT was also performed in case of suspected relapse. The ^{18}F -FDG-PET/CT performed at the end of the primary treatment was blinded for the clinician.

^{18}F -FDG-PET/CT was obtained from the base of the skull to mid-thigh. ^{18}F -FDG-PET/CT imaging was performed with either a 64-row Discovery STE or VCT (General Electric Medical Systems, Milwaukee, WI, USA). The patients were required to fast for a minimum of six hours before the administration of ^{18}F -FDG. Serum glucose level measurement preceded the tracer injection. Attenuation correction was done with low-dose CT. In NACT patients, PET imaging begun 55 \pm 6 (mean \pm SD) min after intravenous injection of 302 \pm 53 (mean \pm SD) MBq of ^{18}F -FDG. When comparing the baseline and post-NACT ^{18}F -FDG-PET/CT images, the imaging studies were initiated in a median difference of 3 min (range 0 to 14 min).

ADW 4.5 and 4.6 workstations (General Electric Medical Systems, Milwaukee, WI, USA) were used in PET imaging analysis. The PET images were reconstructed with the ML-OSEM reconstruction algorithm in 3D mode and 128×128 matrix size. The liver reference volume of interest (VOI) was used for background activity. The SUVmax values were corrected for body weight and the injected tracer dose. Two experienced nuclear medicine physicians analyzed all the ^{18}F -FDG-PET/CT images blinded to the patient's clinical outcome.

In study II, the pre- and post-NACT ^{18}F -FDG-PET/CT images were systematically evaluated and all the metabolically active lesions were recorded. The omentum was chosen as a lesion of interest as the omentum was resected in IDS from all the patients, which allowed the histopathological response evaluation of the equivalent

anatomical site. The pre- and post-NACT SUV_{max} values of omentum were used to calculate the SUV_{max} change during NACT.

In study IV, the obtained baseline and post-NACT ¹⁸F-FDG-PET/CT images were used to evaluate the total metabolic tumor volume (MTV) and its change during NACT. PET VCAR (Volume Computer Assisted Reading) program was used to semi-automatically identify the metabolically active lesions of the baseline and post-NACT ¹⁸F-FDG-PET/CT images. The suggested lesions were manually either bookmarked as a pathological lesion or deleted in case of physiological uptake, such as ureters and bladder. MTV was determined with an estimated threshold with weight factor 0.6 used as the segmentation algorithm. The total MTV composed of the sum volume of all lesions. The pre- and post-NACT target SUV_{peak}, target SUV_{max} and target SUL_{max} were also recorded. A lesion with the highest SUV_{max} at the time point was considered as a target lesion and therefore, a patient's target lesion was not necessarily the same before and after NACT. The pre- and post-NACT MTVs were recorded and the percentual change in MTV during NACT was calculated. Target SUV_{peak}, target SUL_{peak}, target SUV_{max} and target SUL_{max} behaved similarly in the statistical analyses and therefore, to simplify reporting, the target SUV_{peak} was chosen. The pre- and post-NACT target SUV_{peak} values were used to calculate the percentual change in target SUV_{peak} during NACT.

4.9 Tissue samples and histopathological analysis

Samples for histopathology were obtained during primary laparoscopy, PDS and IDS. Multiple biopsies were systematically taken from all areas of visual carcinomatosis at the beginning of the surgical operations and all the resected tumor samples were also evaluated histopathologically. The samples were fixed in formalin, processed and embedded paraffin using routine pathology techniques. Tissue sections were stained with hematoxylin and eosin (H&E). For Ki-67 immunostaining, Ventana Benchmark XT device (Roche Tissue Diagnostics/Ventana Medical Systems, Tucson, AZ, USA) was used. To calculate the percentage of Ki-67 immunoreactive tumor cells, at least 300 tumor cells per sample were evaluated from three selected hot spot areas, where the Ki-67 staining was particularly prominent. The evaluations were carried out without knowledge of the imaging studies or clinical response to chemotherapy.

In study II, the histopathological response evaluation after NACT focused on the omental tissue samples obtained during IDS. The chemotherapy-induced changes evaluated from the slides were the amount of necrosis, fibrosis, histiocytes and lymphocytes and viable tumor cells. Analyses were performed independently by

two evaluators. The histopathological response was subsequently classified as a good, moderate or poor response based on the extent of the combined morphological changes. The histopathological responders were defined as the patients with moderate or good histopathological response to NACT. The patients with poor histopathological response were referred to as histopathological non-responders. The Ki-67 immunostaining was performed for evaluating the proliferative activity in the omental and ovarian tumor samples after NACT.

4.10 Statistical analyses

The statistical analyses were performed using SAS for Windows versions 9.3 and 9.4 and R softwares. In order to find cutoff values for continuous variables, the receiver operating characteristic (ROC) analysis was used to calculate the area under the curve (AUC). P-values <0.05 were considered as statistically significant. PFS was the primary end-point and was defined as the time from diagnosis to the disease progression.

In study I, three patients were excluded from the analyses considering tumor marker change due to lack of pre-IDS tumor marker values. The pre-NACT and pre-IDS HE4 and CA125 values were compared to IDS outcome. ROC analysis was used to evaluate the ability of pre-IDS tumor markers to predict surgical outcome. With ROC-analysis, an optimal cutoff value for identifying patients with suboptimal (>1 cm) IDS outcome was selected. The changes in tumor markers during NACT were compared with CT response, IDS outcome, platinum-free interval (PFI), progression-free survival (PFS) and overall survival (OS). PFI was defined as the time from the end of primary chemotherapy to disease progression and was calculated in all the treatment responders after primary therapy. Somers' D was used to calculate the strength of the association between the tumor markers and CT response and outcome. A Kaplan-Meier analysis was performed to evaluate PFS and OS in the patient groups with a tumor marker decrease $>80\%$ and $<80\%$ during NACT. A log rank test was used to compare the differences between the survival curves.

In study II, a median number of 3 (range 1 to 10) omental histopathological samples per patient were evaluated. Ki-67 immunostaining was performed in 17 patients. ROC analysis was used to select an optimal cutoff value for omental SUVmax decrease during NACT to identify the histopathological nonresponders from responders. The associations between omental SUVmax decrease during NACT and histopathological response were evaluated with the cumulative logit model. Spearman's correlation coefficient was used study the association between two continuous variables. The Mann-Whitney U test was used to compare the

continuous variables between two groups. Univariate analysis between predictor variables (omental SUVmax decrease, histopathological response, HE4 halftime) and PFS was performed.

In study III, the patients excluded from the analyses considering tumor marker change perioperatively, postoperative biomarkers or HE4 nadir values, were those who did not have either pre- or postoperative HE4 (n=11) or CA125 (n=3) or lacked all the HE4 samples after IDS (n=1). Two debulking surgery outcome groups were identified: patients with no macroscopic tumor (R=0) and patients with a residual tumor >0 (R>0). Four of the R0 resected patients and five of the R>0 resected patients were missing the postoperative serum HE4 sample. Linear models were used to compare the postoperative tumor markers and residual tumor after surgery. Cumulative logit model was used to study the associations between the predictor variables (perioperative tumor marker change, postoperative and nadir tumor marker values) and primary therapy outcome. Cox regression analysis was used to study the associations between these variables and PFS. The results are reported by the patient cohort combined (the PDS and NACT groups conjoined), as interactions between the patient subgroup and tumor markers were not statistically significant. The association of postoperative CA125 to PFS was an exception to this, as an interaction between PDS and NACT groups was observed. Both tumor markers were evaluated as predictor variables simultaneously in a fitted model to study the predictor's ability to predict the response beyond the effect of the other predictor. The postoperative tumor markers, FIGO stage and debulking surgery outcome were variables evaluated as prognostic factors for PFS with log rank test and multivariate analysis.

In study IV, the pre-NACT MTV, post-NACT MTV, MTV change during NACT, pre-NACT target SUVpeak, post-NACT target SUVpeak and target SUVpeak change during NACT were evaluated as predictor variables. The cumulative logit model was used to study the effect of these variables on the primary therapy outcome. IDS outcome groups R=0 and R>0 were identified. Four patients with PD response after NACT were referred directly to second-line chemotherapy and were included in the R>0 group in the statistical analyses. The ability of the total MTV change during NACT to identify the patients with PD and SD response after primary therapy and R0 surgery outcome in IDS was evaluated with ROC-analysis. IDS outcome (0/>0), FIGO stage (III/IV) and total MTV change during NACT were evaluated as predictors for PFS with multivariate analysis. The effect of the total MTV and target SUVpeak change during NACT on OS was estimated with survival analysis. Cox regression was used in PFS and OS analyses.

5 RESULTS

5.1 Serum HE4 in EOC treatment response evaluation

5.1.1 HE4 profile during NACT (study I)

The median serum HE4 before NACT, after NACT and the change during NACT were 3252 pmol/L (range 156 to 3252 pmol/L), 149 pmol/L (range 35 to 874 pmol/L) and -85% (range -20% to -98%), respectively. The median pre-NACT CA125, pre-IDS CA125 and CA125 change during NACT were 885 U/mL (range 55 to 14625 U/mL), 46 U/mL (range 7 to 2144 U/mL) and -94 % (range +80 % to -99 %).

All the patients (n=22) presented substantial reduction in serum HE4 levels during NACT regardless of the anatomical NACT response evaluated by CT. Although the serum CA125 changes during NACT were statistically different in the CT response groups (Somers' D 0.44, CI (Confidence Interval)=0.07-0.81), all the patients showed considerable decrease in the serum CA125 levels. The serum HE4 and CA125 changes during NACT in the various CT response groups are presented in **Table 8**.

Table 8 The change in serum HE4 and CA125 levels during NACT in the patient cohort and in the subgroups as divided according to the anatomical NACT response evaluated by CT. Modified from Vallius et al. 2014.

	n=	HE4 change median (range)	CA125 change median (range)
All	22	-85% (-20% to -98%)	-94% (80% to -99%)
CT response			
Complete	0	-	-
Partial, >50% reduction in target lesions (PR1)	6 (27%)	-92% (-69% to -98%)	-97% (-85% to -99%)
Partial, <50% reduction in target lesions (PR2)	7 (32%)	-82% (-54% to -93%)	-95 % (-77% to -98%)
Stable disease (SD)	4 (18%)	-77% (-68% to -95%)	-92% (-83% to -98%)
Progressive disease (PD)	5 (23%)	-83% (-20% to -92%)	-83% (80% to -92%)

Neither the pre-NACT HE4 values nor the HE4 change during NACT corresponded to the surgery outcome. On the contrary, the pre-IDS HE4 values differed between

the R0, R<1 and R>1 IDS outcome groups (Somers' D 0.83, CI=0.65-1.00). With ROC-analysis, an optimal cutoff value of 645 pmol/L for pre-IDS HE4 was selected to identify the patients with R>1 IDS outcome (sensitivity 67%, specificity 95%, AUC (Area Under the Curve) 0.877, the curve presented in **Figure 3**). **Figure 4** illustrates the pre-IDS HE4 values according to the CT response and IDS outcome.

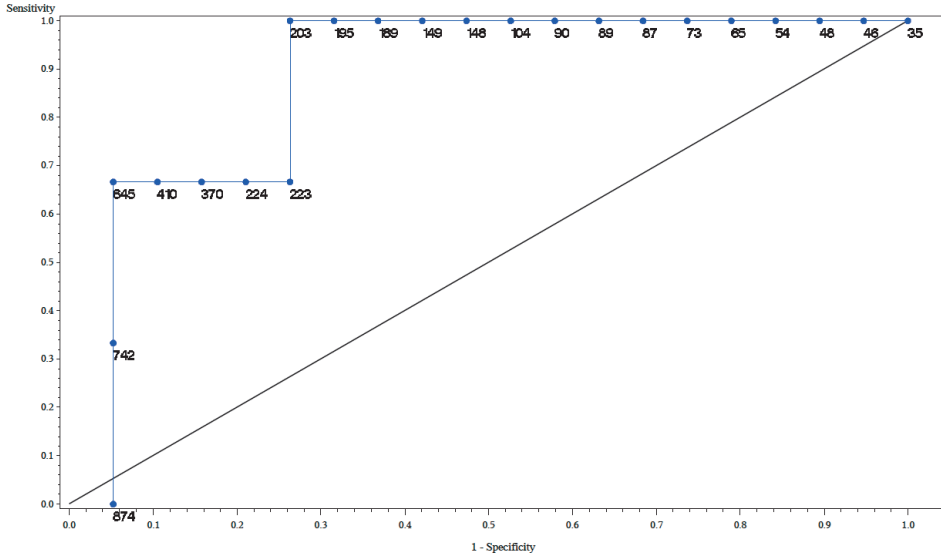
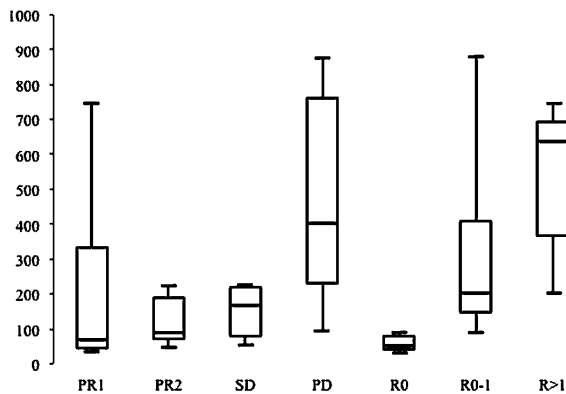


Figure 3 The ROC curve of an analysis performed for differing the patients according to the IDS outcome (R>1 vs. others). A cut-off value of 645 pmol/L (sensitivity 67%, specificity 95%, AUC 0.877) identified the R>1 patients.



PR1: partial response >50% reduction in target lesions, PR2: partial response <50% reduction in target lesions, SD: stable disease, PD: progressive disease.

Figure 4 A box-plot illustration of the serum HE4 (pmol/L) levels after NACT in the various CT response groups (PR1, PR2, SD, PD) and IDS outcome groups (R0, R0-1, R>1).

The pre-IDS CA125 values were lower (Somers' D 0.65, CI=0.37-0.92) and CA125 changes during NACT were more substantial (Somers' D 0.64, CI=0.38-0.89) in patients with no macroscopic disease in IDS compared to those with R<1 and R>1 tumor residuals. When pre-IDS CA125 was evaluated as a predictor of suboptimal (R>1) IDS outcome, a cutoff value of 389 U/mL was considered optimal for identifying patients who would not benefit IDS (sensitivity 67 %, specificity 100 %, AUC 0.947).

When analyzing the survival data, the patients with CA125 decrease >80% during NACT had longer PFS compared to those with CA125 decrease <80% (p=0.0002). The same observation was not seen in HE4. However, the HE4 decrease >80% during NACT provided an OS advantage compared to the patients presenting HE4 decrease <80% during NACT (p=0.01). A similar trend was seen when evaluating the association between the CA125 change and OS, but the results were not statistically significant (p=0.35). The median survival times in the patient cohort are presented in **Table 9**.

Table 9 The median survival times of the patient cohort presented in years. The statistically significant associations are marked with * and **.

	All	HE4 decrease during NACT		CA125 decrease during NACT	
	n=22	>80% (n=14)	<80% (n=8)	>80% (n=19)	<80% (n=3)
PFS (yrs)	1.02	1.32	0.81	1.30**	0.41**
PFI (yrs)	0.75	0.99	0.43	***	***
OS (yrs)	2.81	3.38*	1.60*	3.38	1.13

PFS: progression free survival, PFI: platinum free interval, OS: overall survival. *p=0.01, **p=0.0002, ***All the patients with CR after primary therapy had CA125 decrease >80 % during NACT.

5.1.2 HE4 profile during postoperative adjuvant chemotherapy (study III)

The serum HE4 values obtained during primary therapy are presented in **Table 10**. The postoperative HE4 values corresponded with the amount of residual tumor in surgery (p<0.0001, R²=0.36) in the combined patient cohort (n=40), whereas the same observation was not seen with postoperative CA125. In univariate analyses, the postoperative HE4 predicted primary therapy response (p=0.005, OR (Odds Ratio)=0.01, 95% CI=0.003-0.01) and PFS (p=0.03, HR (Hazard Ratio)=1.00, 95% CI=1.00-1.01). The multivariate analysis performed with FIGO stage (III/IV), surgery outcome (R0/R>0) and postoperative HE4 and CA125 suggested that the

postoperative serum HE4 was the only prognostic variable for PFS ($p=0.04$, HR=1.003, 95% CI 1.00-1.01).

Table 10 The serum HE4 values (pmol/L) during EOC primary therapy. Modified from Vallius et al. 2017.

HE4 (pmol/L)	All	PDS	NACT+IDS
	Median (range)	Median (range)	Median (range)
at diagnosis	732 (59 – 12128)	573 (59 – 1391)	1070 (156 – 12128)
pre-IDS			104 (35 – 477)
postoperative	99 (34 – 856)	96 (34 – 856)	99 (39 – 384)
nadir	52 (25 – 257)	48 (25 – 204)	69 (31 – 257)
at the end of primary therapy	53 (25 – 431)	48 (25 – 431)	61 (31 – 175)

The postoperative HE4 values according to the surgery outcome and primary therapy response are presented in **Table 11**. Of all the R0 resected patients ($n=13$), a total of seven patients initially had an FIGO stage IIIC disease whereas six patients presented an FIGO stage IVA or IVB disease at the time of diagnosis. The median postoperative HE4 in R0 resected FIGO stage III and IV patients were 50 pmol/L (range 34 to 89 pmol/L) and 44 pmol/L (range 36 to 58 pmol/L), respectively.

Table 11 The postoperative HE4 (pmol/L) values presented according to the surgery outcome and primary therapy outcome.

	Postoperative HE4, median (range)		
	All	PDS	NACT+IDS
Surgery outcome			
R0	48 (34 – 89)	58 (34 – 89)	48 (39 – 50)
R>0	142 (47 – 856)	147 (69 – 856)	118 (47 – 384)
Primary therapy response			
CR	80 (34 – 279)	80 (34 – 261)	76 (39 – 279)
PR	164 (61 – 639)	197 (69 – 639)	157 (61 – 266)
PD	205 (47 – 856)	856	197 (47 – 384)

R0: no macroscopic disease, R>0: residual tumor >0, CR: complete response, PR: partial response, PD: progressive disease.

The HE4 levels at the time of diagnosis did not predict PFS ($p=0.24$). The statistically significant associations between the perioperative percentual change in the tumor markers and primary therapy response or PFS were not seen. However, the HE4 nadir values during postoperative chemotherapy were associated with primary therapy response ($p=0.0002$) and PFS ($p=0.009$, HR=1.01, 95% CI=1.01-

1.02). The HE4 nadir values and the HE4 values at the end of primary therapy are presented in **Table 12** and **Table 13**. The median times for HE4 nadir to be reached were 119 days after PDS (range 19 to 238 days) and 62 days after IDS (range 5 to 149 days). The median number of postoperative chemotherapy cycles after which the HE4 nadir was reached was three (range 0 to 8).

Table 12 The serum HE4 nadir values obtained during postoperative chemotherapy. The HE4 nadir values are presented in the whole patient cohort and in the subgroups of primary debulking surgery (PDS) and neoadjuvant chemotherapy followed by interval debulking surgery (NACT+IDS).

Primary therapy response	Nadir HE4 (pmol/L), median (range)		
	All	PDS	NACT+IDS
CR	49 (25 to 160)	45 (25 to 100)	57 (31 to 160)
PR	80 (38 to 153)	56 (38 to 133)	100 (42 to 153)
PD	175 (38 to 257)	204	135 (38 to 257)

CR: complete response, PR: partial response, PD: progressive disease.

Table 13 The serum HE4 values at the end of primary therapy. The HE4 values are presented in the whole patient cohort and in the subgroups of primary debulking surgery (PDS) and neoadjuvant chemotherapy followed by interval debulking surgery (NACT+IDS).

Primary therapy response	HE4 at the end of primary therapy (pmol/L), median (range)		
	All	PDS	NACT+IDS
CR	50 (25 to 122)	45 (25 to 122)	57 (31 to 111)
PR	74 (40 to 431)	63 (40 to 431)	116 (42 to 175)
PD	*		

CR: complete response, PR: partial response, PD: progressive disease. *The HE4 at the end of primary therapy was determined if a complete or partial response was achieved.

The CA125 at the time of diagnosis was associated with PFS ($p=0.01$, $HR=1.45$, $95\% CI=1.09-1.94$). The postoperative CA125 did not correspond to the primary therapy outcome, but it was associated with PFS in the combined patient cohort ($p=0.002$, $HR=1.00$, $95\% CI=1.001-1.004$) and in patients treated with NACT ($p=0.006$, $HR=1.00$, $95\% CI=1.00-1.01$). The patients with lower CA125 nadir values during postoperative chemotherapy were likely to have better response to primary therapy ($p<0.0001$) and longer PFS ($p<0.0001$, $HR=1.01$, $95\% CI=1.01-1.02$). The median nadir CA125 values in the combined patient cohort, in PDS and NACT treated patients were 13 U/mL (range 4 to 447 U/mL), 12 U/mL (range 4 to 162 U/mL) and 15 U/mL (range 4 to 447 U/mL), respectively. CA125 nadir values were reached with median of 118 days (range 19 to 238 days) after PDS and 69 days (range 5 to 172 days) after IDS. When evaluating primary therapy

outcome, the nadir tumor markers combined predicted a primary therapy outcome better than either one alone. The main results of the statistical analyses concerning serum tumor marker profiles during NACT and postoperative chemotherapy are presented in **Table 14**.

Table 14 Serum tumor markers in the prediction of response and outcome in advanced EOC.

Study	n	CT response after NACT		surgery outcome			primary therapy outcome			PFS			OS				
		Somers' D	95% CI	p-value	analyzed groups	Somers' D	R ²	95% CI	p-value	OR	Somers' D	95% CI		p-value	HR	Somers' D	95% CI
HE4																	
before NACT	I 25	-0.07	-0.41-0.26	NS	R<1 vs R>1	0.34		-0.10-0.78	NS	0.18		-0.25-0.62	NS				
after NACT	I 25	0.36	-0.07-0.78	NS	R<1 vs R>1	0.83		0.65-1.00	<0.05	0.46		-0.04-0.96	NS	0.28		NS	
change during NACT	I 25	0.37	-0.03-0.77	NS	R<1 vs R>1	0.24		-0.19-0.67	NS	0.37		-0.15-0.89	NS	0.37		0.02	0.01
post-operative nadir	III 40	NE			R0 vs R>0		0.36		<0.0001	0.01		0.003-0.01	0.005	1.00		1.00-1.01	0.03
	III 48	NE			NE					0.02		0.01-0.04	0.0002	1.01		1.01-1.02	0.009
CA125																	
before NACT	I 25	-0.26	-0.64-0.13	NS	R<1 vs R>1	0.17		-0.35-0.70	NS	0.04		-0.25-0.58	NS				
after NACT	I 25	0.06	-0.44-0.55	NS	R<1 vs R>1	0.65		0.37-0.92	<0.05	0.34		-0.16-0.84	NS	0.23		NS	
change during NACT	I 25	0.44	0.07-0.81	<0.05	R<1 vs R>1	0.64		0.38-0.89	<0.05	0.64		0.26-1.00	<0.05	0.48		0.003	NS
post-operative nadir	III 48	NE			R0 vs R>0				NS			0.006-0.09	NS	1.003		1.001-1.004	0.002
	III 49	NE			NE					0.05		0.09	<0.0001	1.01		1.01-1.02	<0.0001

OR: Odds Ratio, HR: Hazard Ratio, CI: Confidence Interval, p: p-value, NE: not evaluated, NS: non significant.

5.2 The use of ^{18}F -FDG-PET/CT imaging in NACT response evaluation

5.2.1 ^{18}F -FDG-PET/CT compared to histopathological response (study II)

The median omental SUVmax decrease during NACT was 64 % (range 16 to 84 %). The SUVmax decrease in omentum in the three histopathological response groups is presented in **Table 15**. The decrease in SUVmax differed between the poor, moderate and good histopathological responders ($p=0.004$, OR=0.9, CI=0.84-0.97). However, omental SUVmax change was not a predictor of primary therapy response ($p=0.32$, OR=1.02, CI=0.98-1.06) or PFS ($p=0.33$, HR=0.99, CI=0.96-1.01). The median PFS of the whole patient cohort and that of the poor, moderate and good histopathological responders were 1.1, 0.9, 1.2 and 1.4 years, respectively. A statistically significant difference in PFS was seen between the histopathological response groups ($p=0.05$).

Table 15 The percentual change in omental SUVmax during NACT in histopathological response and primary therapy outcome groups.

	n=	SUVmax change during NACT median (range)
Histopathological response		
Good	4	-71% (-67% to -76%)
Moderate	13	-68% (-52% to -84%)
Poor	9	-32% (-16% to -76%)
Primary therapy response		
CR	14	-67% (-31% to 84%)
PR	6	-64% (-28% to -82%)
PD	6	-62% (-16% to 82%)

*statistically significant association between the variables.

Using ROC-analysis, the omental SUVmax change -57% during NACT was selected for optimal cutoff value to identify the histopathological nonresponders to NACT (sensitivity 89%, specificity 88%, AUC 0.91). The patients presenting a SUVmax decrease less than 57% during NACT were more likely to have a poor response to NACT in the histopathological evaluation. Serum HE4 halftime during NACT was also longer in patients with omental SUVmax change less than -57 % compared to those with more substantial SUVmax decrease during NACT ($p=0.02$). The median HE4 halftimes during NACT in all patients and in patients with omental SUVmax decrease <57% and >57% were 27 days (range 16 to 331 days), 45 days (range 21 to 331 days) and 25 days (range 15 to 64 days),

respectively. An example of the omental metabolic response to NACT is presented in **Figure 5**.

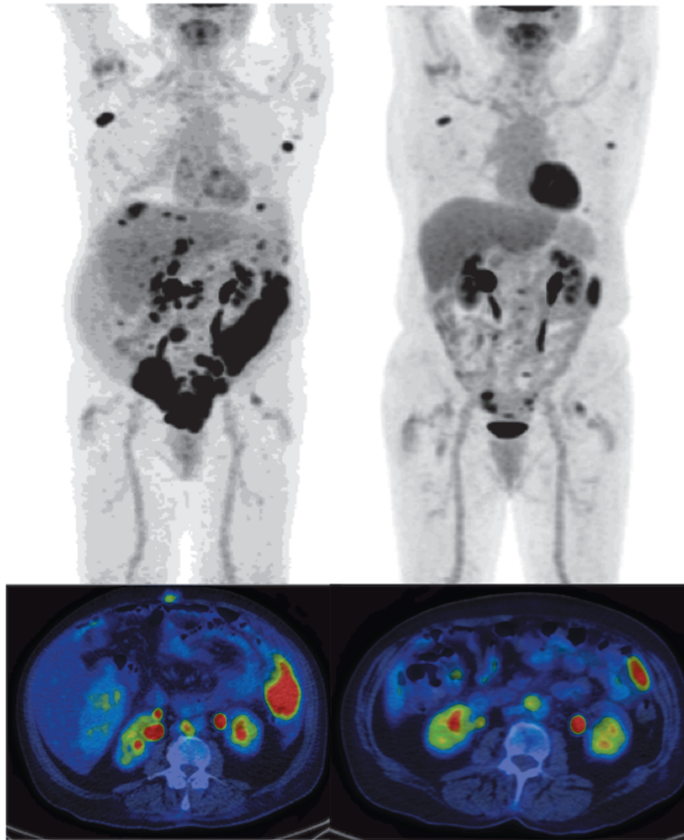


Figure 5 A representative patient of the cohort. A 66-year old female with primarily unresectable FIGO stage IVA EOC. When comparing the ^{18}F -FDG-PET/CT images at baseline (left) and after three cycles of NACT (right), the omental SUVmax change was only -31% during NACT and the histopathological response was later classified as poor.

The median change in ovarian SUVmax during NACT was -63% (range -3 to -90%). The median omental and ovarian Ki-67 were 32% (range 19 to 65%) and 35% (range 6 to 70%), respectively. The decrease in omental SUVmax corresponded with the ovarian SUVmax decrease during NACT ($p=0.05$) and the omental and ovarian Ki-67 were also associated with each other ($p=0.02$).

5.2.2 MTV and MTV change during NACT in ^{18}F -FDG-PET/CT (study IV)

The total MTV values of all analyzed patients and in various primary therapy response groups are presented in **Table 16**.

Table 16 The total MTV values (cm^3) before and after NACT and the percentual total MTV change during NACT. Modified from Vallius et al 2017.

	MTV before NACT median (range), cm^3	MTV after NACT median (range), cm^3	MTV change during NACT median (range), %
All (n=29)	352 (150 to 1322)	51 (0 to 417)	-89% (-24 to -100%)
Primary therapy response			
CR (n=13)	338 (150 to 1322)	24 (0 to 134)	-93% (-77 to -100%)
PR (n=6)	502 (294 to 681)	71 (8 to 156)	-89% (-60 to -98%)
PD/SD (n=10)	383 (160 to 787)	71 (24 to 417)	-75% (-24 to -95%)

The higher total MTV values after NACT predicted a worse primary therapy outcome ($p=0.007$, OR=1.18, 95% CI=1.02-1.37) and a shorter PFS ($p=0.005$, HR=1.09, 95% CI=1.03-1.17). Likewise, the patients with only minor decrease in total MTV during NACT were likely to have a worse primary therapy outcome ($p=0.001$, OR=2.10, 95% CI=1.15-3.81) and a shorter PFS ($p=0.005$, HR=1.41, 95% CI=1.11-1.78). With ROC-analysis, an optimal cutoff value for the total MTV change during NACT was selected to identify the patients who had not responded to NACT. The total MTV change of less than -85% identified the patients with PD response at the end of primary therapy (sensitivity 70%, specificity 78%, AUC 0.79). The total MTV before NACT did not have prognostic significance in the statistical analyses. **Figure 6** illustrates two examples of the total MTV decrease during NACT.

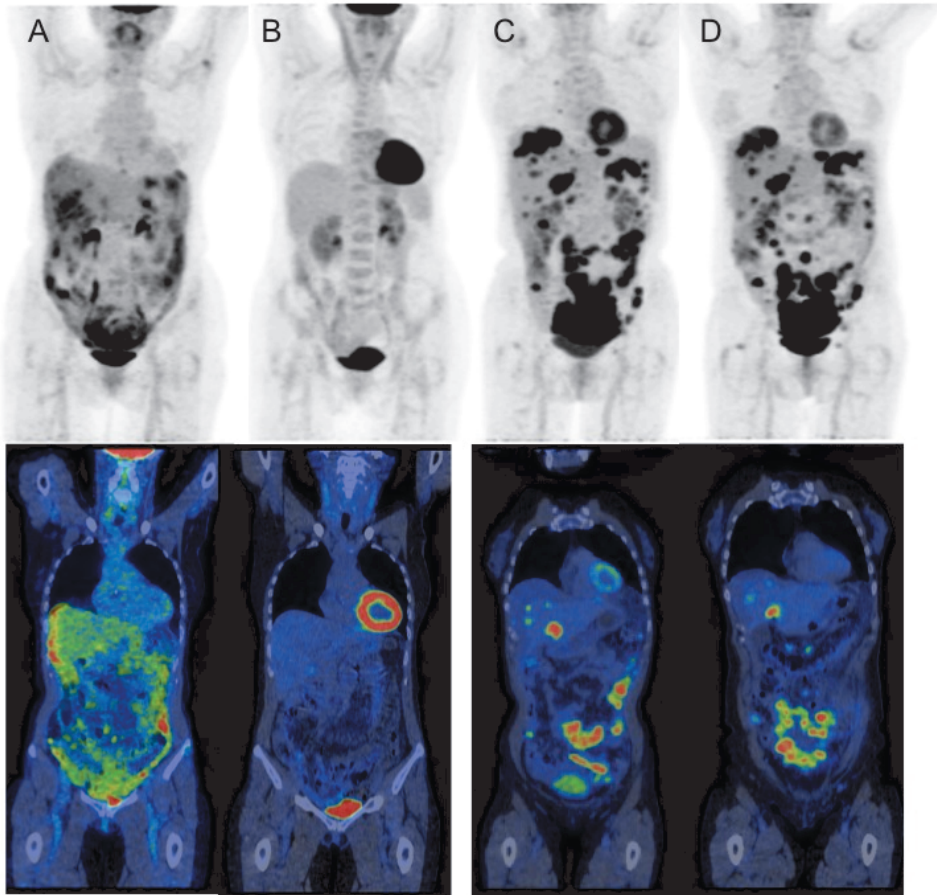


Figure 6 Illustration of the total metabolic tumor volume (MTV) before (A,C) and after (B,D) NACT in ^{18}F -FDG-PET/CT. A 66-year-old female with primarily unresectable FIGO stage IIIC EOC (A,B) and a 63-year-old female with primarily unresectable FIGO stage IVB EOC (C,D). The patients received three cycles of NACT. In the first patient, the MTV decrease during NACT was 95%, complete tumor resection in IDS was achieved and the primary therapy outcome was classified as a complete response. The second patient presented only -24% change in the total MTV during NACT and disease progression after NACT due to new lesions was observed.

A ROC-analysis was performed to evaluate the ability of total MTV decrease during NACT to identify the patients who would be completely debulked in IDS. The total MTV decrease greater than 93% during NACT was considered as optimal (sensitivity 75%, specificity 80%, AUC 0.76) to ensure that all the R0 resected patients would have been correctly referred to IDS initially. Despite the differences in total MTV changes during NACT, they were not statistically significant between the surgery outcome groups R0 and R>0 ($p=0.17$). The surgery outcome was not associated with PFS ($p=0.3$).

The target SUVpeak before NACT (median 10.1 g/mL, range 5.4 to 25.6 g/mL) did not correspond with the primary therapy outcome ($p=0.9$) or PFS ($p=0.8$). A marginally significant difference was observed in the target SUVpeak values after NACT (median 4.6 g/mL, range 2.3 to 14.7 g/mL) between the three primary therapy response groups ($p=0.04$, OR=1.30, 95% CI=0.98-1.73). Furthermore, a minor decrease in the target SUVpeak change during NACT (median -59%, range +15% to -77%) increased the risk of poor primary therapy outcome ($p=0.002$, OR=1.64, 95% CI=1.13-2.37) and shorter PFS ($p=0.01$, HR=1.27, 95% CI=1.06-1.52). **Table 17** summarizes the results of the statistical analyses concerning the observed changes in the tumor metabolism during NACT in the Studies II and IV.

In a multivariate analysis with residual tumor ($0/>0$), FIGO stage (III/IV) and MTV change, the statistically significant variables associated with PFS were MTV change ($p=0.002$, HR=65.1, 95% CI=4.7-907.8) and FIGO stage ($p=0.03$, HR=0.38, 95% CI=0.16-0.90). MTV change ($p=0.04$, HR=6.4, 95% CI=1.0-39.1) and the target SUVpeak change ($p=0.03$, HR=5.3, 95% CI=1.2-22.9) during NACT predicted OS in survival analysis.

Table 17 ^{18}F -FDG-PET/CT parameters in prediction of response and outcome in a NACT treated ovarian cancer patient cohort. Synthesis of the main results of Studies II and IV.

variable	n	Histopathological response			Primary therapy outcome			PFS			OS		
		OR	95% CI	p-value	OR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	p-value
omental SUVmax change during NACT	26	0.90	0.84-0.97	0.004	1.02	0.98-1.06	NS	0.99	0.96-1.01	NS	NE		
target SUV peak after NACT	29	NE			1.30	0.98-1.73	0.04	1.11	0.96-1.27	NS	NE		
change during NACT		NE			1.64	1.13-2.37	0.002	1.27	1.06-1.52	0.01	5.3	1.2-22.9	0.03
total MTV after NACT	29	NE			1.18	1.02-1.37	0.007	1.09	1.03-1.17	0.005	NE		
change during NACT		NE			2.10	1.15-3.81	0.001	1.41	1.11-1.78	0.005	6.4	1.0-39.1	0.04

OR: Odds Ratio, HR: Hazard Ratio, CI: Confidence Interval, NE: not evaluated, NS: non significant.

6 DISCUSSION

6.1 Serum HE4 profile during NACT (study I)

In study I, we presented the data showing that in our patient cohort the anatomical response to NACT evaluated with CT imaging did not correspond to the tumor marker changes during NACT or IDS outcome. According to our results, the lower serum HE4 values after NACT were associated with a better IDS outcome, and the marked serum HE4 decrease during NACT predicted a prolonged OS.

One of the most important prognostic factors in EOC is the amount of residual tumor after debulking surgery (du Bois et al. 2009). The initiation of EOC primary treatment with NACT instead of PDS is a valid option for those patients with advanced inoperable disease at the time of diagnosis, for whom a complete tumor resection in PDS would not be possible. Therefore, the main goal of NACT is to reduce the tumor burden of a primarily unresectable patient. If the tumor burden has not diminished after three to four NACT cycles i.e. the patient has not responded to NACT, the possibilities of complete resection in IDS do not exist. A part of the primarily unresectable patients are chemoresistant to first-line chemotherapy and therefore do not respond to NACT. Noninvasive estimation of treatment response after NACT is important as unbeneficial surgery can delay the onset of second-line chemotherapy and expose the patient to possible complications.

One of the potential limitations of CT imaging in NACT response evaluation is the chemotherapy-induced formation of fibrosis. The anatomical response to chemotherapy seen in CT requires sufficient time to evolve and, therefore, NACT response evaluation with only CT imaging may lead to an underestimation of the chemotherapy response. Our observations of pre-IDS HE4 and CA125 not corresponding with CT response after NACT, but associating with IDS outcome, might support this hypothesis, even though evaluation of the underlying specific mechanisms was out of the main focus of this study. CT imaging may be sufficient in EOC primary therapy response evaluation after PDS and the following adjuvant chemotherapy as the response achieved in that case is induced by both surgery and chemotherapy. In contrast, the evaluation of NACT response is solely based on chemosensitivity assessment, in which case the tumor markers secreted by viable cancer cells and metabolic imaging provide additional valuable information for operability assessment before IDS.

The serum HE4 profile during NACT seems to reflect the patient's chemosensitivity. In our patient cohort, both the pre-IDS HE4 and HE4 decrease during NACT had prognostic importance. The pre-IDS HE4 and CA125 values taken after NACT

differed between the IDS outcome groups. Based on ROC-curves, the optimal cut-off values of pre-IDS HE4 > 645 pmol/L and pre-IDS CA125 > 389 U/mL identified the patients who would still be unresectable after NACT. In the literature, pre-IDS HE4 \leq 226 pmol/L (Plotti et al. 2017) and \leq 252 pmol/L (Pelissier et al. 2016) have been associated with optimal cytoreduction in IDS. Similarly, pre-IDS CA125 values of \leq 20-100 U/mL are described to correspond with the result of no macroscopic disease in IDS. Our suggested cutoff values for pre-IDS tumor markers are relatively high as high specificity was preferred in the cutoff value selection. Above these defined cutoff values the likelihood of any benefit from IDS is particularly low and, on the other hand, all the operable patients would be correctly referred to IDS. A substantial percentual decrease in the serum HE4 and CA125 levels was also required for achieving benefits from NACT. The patients with an HE4 decrease of > 80% during NACT had an OS advantage compared to the patients presenting minor HE4 decreases (OS 3.4 years vs. 1.6 years, $p=0.01$). Even though a similar trend was seen when evaluating CA125 change during NACT, the OS difference was not statistically significant, possibly due to the unbalanced division of the patient cohort.

The division of the patient cohort according to the \geq 80% decrease in the tumor markers during NACT was based on the literature (Rodriguez et al. 2012). The selected cutoff value of 80% used by Rodriguez et al. was the observed median value of serum CA125 decrease during NACT in their patient cohort. The CA125 response definition by GCIg underlines that, for partial response, a CA125 decrease of at least 50% from the baseline during cancer therapy is required (Rustin et al. 2011). However, the direct use of the CA125 response criteria in first-line trials is not recommended, as the definition was originally designed for relapsed ovarian cancer trials. In our patient cohort, a substantial, percentual decrease in serum CA125 during NACT was observed even in the patients with PD at the end of primary therapy. Therefore, we chose a higher cutoff value instead of the division with $<$ and $>$ 50% decrease in the tumor markers during NACT. The median values of the decrease in the tumor markers were not used as cutoff levels. If the HE4 median had been used as a cutoff, there would have been only five patients with a CA125 decrease $<$ 85%. In contrast, with the CA125 median decrease as a cutoff, there would have been only four patients with an HE4 decrease $>$ 94%. Subsequently, when evaluating both tumor markers, the most balanced division of patients might have been achieved with the decrease of \geq 90% during NACT. This unbalanced division might have interfered with the analyses considering the CA125 change during NACT.

It is notable, that the tumor marker values at baseline before NACT did not correspond to the IDS outcome, which may be explained by the advanced stage of the disease at the time of diagnosis in all the study patients. This also indicates, that

chemosensitivity of the cancer cells seems to be more important for a patient's prognosis than the actual amount of the tumor burden detected at the time of diagnosis. This is relevant in particular in patients with a severely advanced disease, such as in the patients of this patient cohort. The previous studies evaluating the behavior of serum CA125 during NACT also support this hypothesis. In studies focusing on advanced inoperable NACT treated EOC patients, the CA125 levels at diagnosis did not associate with the IDS outcome (Furukawa et al. 2013; Rodriguez et al. 2012; Tate et al. 2005) or the platinum-free interval (Pelissier et al. 2016). On the contrary, the pre-IDS serum CA125 levels have been suggested to predict operability and survival (Furukawa et al. 2013; Rodriguez et al. 2012). In addition to serum CA125, monitoring serum HE4 with several serum samples obtained during NACT might also assist to identify the patients with refractory disease after NACT and therefore a poor prognosis.

6.2 Serum HE4 profile during postoperative chemotherapy (study III)

The main results of study III were that the postoperative serum HE4 was in line with the IDS outcome and that the lower postoperative HE4 and HE4 nadir values during EOC primary chemotherapy associated with a better primary therapy outcome and a longer PFS.

Surgery is known to temporarily elevate serum CA125 levels (Mogensen et al. 1993; Talbot et al. 1989), and therefore, in postoperative patients, serum CA125 is not a reliable indicator of residual tumor lesions. CT imaging has also been debated to have limitations in detecting postoperative residual disease (Menczer et al. 2006). In our patient cohort, the postoperative serum CA125 did not correspond with the debulking surgery outcome. However, the patients with a complete resection were observed to have lower postoperative serum HE4 levels compared to patients with macroscopic tumor residuals after surgery. According to our results, the postoperative serum HE4 obtained two to three weeks after debulking surgery seems to reflect the amount of residual tumor better than serum CA125. In general, when evaluating treatment response, it is necessary to know the baseline situation for later comparison. For clinician, it would be useful to have a reliable noninvasive method to evaluate patient's tumor burden before the onset of adjuvant chemotherapy cycles. Postoperative chemotherapy can only be initiated after a recovery period and, at that point, it is important to be certain that the disease has not progressed before the onset of chemotherapy. This is not unambiguous with postoperative CA125 determination. The observations of that the patients with lower postoperative HE4 levels were likely to have a better primary therapy outcome and

longer PFS also suggest that the postoperative serum HE4 could be used to objectively evaluate the amount of residual disease after surgery.

The platinum-sensitive EOC patients have been reported to present lower HE4 values before IDS (Pelissier et al. 2016) and during postoperative chemotherapy compared to the platinum-resistant patients (Angioli et al. 2014). A pilot study including seven patients who underwent PDS and four patients treated with NACT, suggested that HE4 declines rapidly after surgery (Hynninen et al. 2011). However, previous studies considering the significance of serum HE4 nadir values during EOC primary chemotherapy do not exist. In our patient cohort, the HE4 nadir values had prognostic significance. The elevated serum HE4 and CA125 nadir values were associated with a poor primary therapy outcome and a shortened PFS. Both of these tumor markers increased the predictive potential of each other. Serum HE4 nadir in addition to CA125 nadir might be usable in identifying the patients with poor prognosis and high risk of relapse.

6.3 ¹⁸F-FDG-PET/CT in NACT response evaluation (study II)

Study II focused on the evaluation of SUVmax decrease during NACT in omentum and its correspondence with NACT-induced histopathological changes seen in the omental tumor tissue samples. The main result was that the patients with poor histopathological response to NACT can be recognized with functional imaging. Functional ¹⁸F-FDG-PET/CT imaging offers a modern, non-invasive way to evaluate chemotherapy response. This is in contrast to the histopathological evaluation, which requires tumor tissue samples obtained during invasive surgery. With ¹⁸F-FDG-PET/CT, changes in the tumor tissue metabolism can be measured. Compared to CT, ¹⁸F-FDG-PET/CT imaging seems to be more accurate in distinguishing viable tumor lesions and scar tissue after chemotherapy. However, no generally accepted definitions for EOC NACT response evaluation with ¹⁸F-FDG-PET/CT exist.

The PERCIST criteria has been suggested for metabolic tumor response assessment in solid tumors (Wahl et al. 2009). Partial metabolic response is defined as at least a 30% reduction in a target lesion's metabolic activity during chemotherapy. A study by Avril et al. suggested that when evaluating a lesion with the lowest change in metabolic activity during NACT, at least a reduction of 55% in SUVmax was needed for a favorable prognosis (Avril et al. 2005). Nishiyama et al. studied histopathological response to NACT and its correlation to the changes seen in ¹⁸F-FDG-PET/CT in patients with various gynecological malignancies (Nishiyama et al. 2008). According to their results, at least a decrease of two-thirds in the SUVmax of a primary tumor was warranted for a histopathological response. Both of

these studies indicate that the decrease in metabolic activity during NACT should be more substantial than presented for partial metabolic response in the PERCIST criteria in order to achieve any benefit from NACT. In this study, the patients with less than a 57% decrease in omental SUVmax during NACT were poor histopathological responders. It seems that the tumor lesion's SUVmax must be reduced at least by half during chemotherapy to achieve a moderate or good histopathological response.

The variance in SUV determination can have a minor effect on the results. In a test-retest setting, the repeated SUV measurements have been suggested to vary by broadly 10% (Minn et al. 1995; Weber et al. 1999). This variance in SUV determination may influence especially the evaluation of tumor lesions with low metabolic activity (Langen et al. 2017). A recent review article focusing on SUV repeatability concluded that SUV reductions of more than 25% and increases of more than 33% are unlikely to be caused by a variance in SUV determination (Lodge 2017). The majority of our patient cohort presented omental SUVmax decrease of more than 25% during NACT referring to chemotherapy-induced decrease in metabolic activity. Nevertheless, the presented cutoff value for SUVmax decrease during NACT should be considered as indicative. The effect of bias caused by possible variance should be excluded with a larger sized patient cohort.

In addition to functional imaging, the serum HE4, secreted mainly by viable ovarian cancer cells, is another indirect way to measure the amount of active tumor tissue during EOC primary treatment. When the tumor burden diminishes, the HE4 level in the serum decreases correspondingly. In Study II, the serum HE4 halftime was longer in patients with an omental SUVmax decrease of less than 57% during NACT. These were the patients with poor response to NACT seen in the direct histopathological evaluation. The previous published data combining PET/CT imaging and serum tumor markers is still limited. After completion of ovarian cancer primary therapy, the predictive potential of posttherapy ^{18}F -FDG-PET/CT and serum CA125 in the recurrence detection has been evaluated (Antunovic et al. 2012; Chu et al. 2016). The combination of metabolic imaging and CA125 increased the diagnostic potential in detecting recurrence (Chu et al. 2016), possibly due to the tumor markers sensitivity to detecting more accurately the low volume disease compared to ^{18}F -FDG-PET/CT.

In Study II, the change in the metabolic activity in ^{18}F -FDG-PET/CT was evaluated in a single specific tumor lesion which had corresponding histopathological samples obtained from the exact anatomical site during surgery. Omentum was selected as a lesion of interest as all the patients had representative omental tissue samples obtained during IDS. This approach offers a reliable basis for direct evaluation of chemotherapy response. However, the histopathological response to

chemotherapy has no standard grading system in EOC. The previously used grading scales presented in Chapter 2.4.4 are variable. The response grading system used in Study II was similar to that of Le et al. (Le, Williams, et al. 2007). The patients were divided into poor, moderate and good histopathological responders according to the amount of necrosis, fibrosis, histiocytes, lymphocytes and viable cancer cells. A statistically significant PFS difference was observed between all the response groups. The previous studies focusing on evaluating the NACT induced histopathological changes have also shown that the histopathological response to NACT predicts survival (Le, Williams, et al. 2007; Muraji et al. 2013; Petrillo et al. 2014). In future, all of these different grading systems might assist in forming a consensus for a clinically useful histopathological response scale.

6.4 The total MTV change during NACT (study IV)

The aim of Study IV was to evaluate the total MTV and its change during NACT in NACT treatment response assessment. According to our results, the patients with high post-NACT MTV values and only a minor decrease in total MTV during NACT were likely to have a poor primary therapy outcome and a short PFS. A substantial decrease in total MTV decrease during NACT also predicted a longer OS.

Ovarian cancer is a model example of a cancer with heterogeneous tumor lesions and a disseminated growth pattern. In advanced stage EOC, the typical findings at the time of diagnostic laparoscopy include peritoneal carcinomatosis and multiple tumor implants in the abdominal cavity. Therefore, following the decrease of the diameter of a single or a few target lesions during NACT may not always reflect the whole treatment response status. According to the PERCIST criteria (Wahl et al. 2009), the preferred lesion of interest is a single lesion with the hottest SULpeak. However, up to five target lesions can be selected for treatment response evaluation. These target lesions after chemotherapy represent the most chemoresistant refractory cancer lesions with the highest metabolic activity. However, evaluation of the total MTV comprises all the pathological tumor lesions and therefore, it might offer a more modern, reliable approach for response evaluation.

When using a specific selected threshold in the MTV determination, MTV seems to be a reproducible parameter (Kitao et al. 2016; Kruse et al. 2015). Although the manual determination of total MTV is time-consuming and prone to errors, a semi-automated program offers reproducible information on the total tumor burden metabolism (Fox et al. 2017). The tumor volume reproducibility has been suggested to be dependent on the evaluated tumor volume, as smaller volumes are bounded by larger variance in the volume measurements. The use of adaptive thresholds,

such as 42% from SUV_{max}, has been described to decrease the variability in repeated volume measurements (Hatt et al. 2017). In this study, the effect of intra- and interobserver variability on MTV determination was not studied. However, the observer-related bias considering the tumor volume determination should be minimized by the semi-automated analyzing method used in the imaging analysis. The difference between the median values of the total MTV decrease during NACT in the CR, PR and PD response groups in this study was not extensive (-93% vs -89% vs -75%, respectively). However, after considering the potential variance in MTV determination, the results are still indicative of a trend towards total MTV being a predictive marker usable in ovarian cancer response evaluation.

No definition for MTV response in EOC has been described previously. For TLG, the PERCIST criteria recommend a routine determination of the five selected target lesions to aid decisions about which response evaluation methods should be used. A decline of at least 40% in TLG during cancer therapy is suggested for a partial response (Wahl et al. 2009). However, the results of Study IV suggest that the MTV decrease during NACT in patients with advanced EOC should be more substantial in order to sufficiently reduce the total tumor burden. The patients with a total MTV decrease of less than 85% during NACT were more likely to present a persistent unresectable disease after NACT and to have disease progression at the end of primary therapy. It is notable that the PERCIST criteria are not directly intended for clinical use but, instead, are originally designed for medical trials investigating drug response in cancer patients. Therefore, more studies focusing strictly on response evaluation in EOC patients are warranted in order to develop clinically applicable response criteria.

In our patient cohort, the total MTV before NACT did not correspond with the patient's outcome in the statistical analyses. Prior to the NACT, all the study patients were considered unresectable and had an extensive tumor burden, which might explain this finding. In contrast, the total MTV after NACT and MTV decrease during NACT were predictors of outcome. These parameters seem to reflect the patient's chemosensitivity and, therefore, could assist in NACT response evaluation in advanced EOC patients.

After chemotherapy, a minimum of a ten-day waiting period is recommended before performing ¹⁸F-FDG-PET/CT imaging for treatment response evaluation. This time interval ensures that, for instance, the possible flare phenomenon seen early after chemotherapy has been bypassed (Wahl et al. 2009). In Study IV, the post-NACT ¹⁸F-FDG-PET/CT imaging was performed at least 13 days after chemotherapy in all the patients, with the exception of one patient who underwent ¹⁸F-FDG-PET/CT four days after the last NACT cycle. After the injection of ¹⁸F-FDG, the baseline and follow-up scans are recommended to be commenced within a 15-

minute time frame to ensure a reliable comparison of these scans (Wahl et al. 2009). In Study IV, this recommendation was followed, which diminishes the physiological variation over time in ^{18}F -FDG metabolism.

6.5 Study limitations

The major limitation of the study is the small number of patients recruited in the study. This is due to the relative low incidence of EOC in addition to the relatively small sizes of Turku University Hospital and Satakunta Central Hospital districts where the patients were recruited. A larger sized patient cohort would increase the reliability of the obtained study results. Nevertheless, the patient cohort consisted of carefully selected newly diagnosed advanced stage III-IV EOC patients, which allowed a reliable basis for treatment response evaluation. The majority of these patients referred to NACT were initially diagnosed with extensive FIGO stage IIIC/IV disease (94%) with high grade serous histological subtype (97 %). The evaluation of inoperability was based on direct visualization of abdominal tumor dissemination and a validated scoring system. Therefore, this patient material represents a very homogeneous patient group. Moreover, all the study patients had serum creatinine levels within the normal range ($< 90 \text{ umol/L}$), with the exception of six patients presenting values from 90 to 118 umol/L at time of diagnosis. The influence of these six patients with marginally elevated creatinine levels on the obtained HE4 results was not evaluated. However, as the patients with severe renal insufficiency were excluded, the analyses regarding serum HE4 can be considered reliable.

According to the latest guidelines of the European Society of Gynaecological Oncology with regard to the quality of debulking surgery in advanced stage III-IV EOC, the goal is that at least half of the patients undergoing debulking surgery should be completely resected. This ensures equality in EOC treatment despite the gynecologic oncology unit and the nationality of the patient. In Study I, only a fourth of the patient cohort achieved R0 resection in IDS, which is a poor rate when compared to international standards (Vergote et al. 2010). One should bear in mind, however, that the ESGO standards refer to an unselected group of FIGO stage III-IV patients, whereas these study patients were chosen for NACT due to signs of a particularly advanced disease, and therefore might present a group of patients, in which a cytoreductive surgery leading to no residual tumors is more difficult to perform. The proportions of FIGO stage IV patients in our patient cohort and in that of the EORTC trial were 54% and 24%, respectively (Vergote et al. 2010). It is also notable, that the patients in EORTC trial were also randomized to the PDS and NACT+IDS treatment arms without an assessment of operability.

As the surgery outcome is highly dependent on the skills of the surgeon, the question remains as to whether the R0 resection rate would be higher in another unit. However, all the operations were performed in a unit specialized in gynaecologic oncology and by experienced gynaecologic oncologists. Moreover, an experienced specialist in gastrointestinal surgery was a part of the operating team when needed. Even though the availability of preoperative ^{18}F FDG-PET/CT images might also cause a potential verification bias, the operability was always carefully assessed using the Fagotti scoring system (Fagotti et al. 2008) and none of the patients was directly referred to NACT due to the PET findings at the time of diagnosis. Therefore, we are quite certain that these patients had truly poor chances of a complete resection at the time of diagnosis. Of the whole patient cohort, the surgery outcome of $R < 1$ was achieved in 81% of patients in IDS and 68% of patients in PDS, which is comparable to the results of the EORTC trial (Vergote et al. 2010). Instead of trying to identify the R0 resected patients with serum tumor markers, the identification of the $R > 1$ patient group was selected as a target in Study I. This is a patient group consisting of the most chemoresistant patients who would still be unresectable after NACT despite the operating unit. These patients, who do not respond to the first-line platinum-taxane combination, could benefit more from second-line treatment, possibly with new immunological treatments offered in clinical trials instead of an IDS attempt.

Another limitation of Study I is that the NACT response was evaluated by using CT imaging. CT imaging has limited ability in the operability assessment (Axtell et al. 2007) and therefore, at the time of diagnosis, the radiological findings and laparoscopic evaluation of the abdominal cavity may not directly correspond. The same clinical challenge, considering the inadequacy of CT in response assessment, is met again after NACT when the operability is re-evaluated (Bilici et al. 2010; Menczer et al. 2011). This can also limit the reliability of the CT imaging as an end-point.

In Study II, the histopathological NACT response was evaluated from the most representative site in the tumor tissue specimens according to the pathologist's visual estimation. The metabolic activity in the omentum in PET was measured by placing the ROI on the area with the most intense ^{18}F -FDG accumulation. Even if the response was assessed using the same tumor lesion in both functional imaging and in histopathological evaluation, there is a possibility that the evaluated sites are not exactly corresponding sites. However, by both methods, the areas of the most visually representative and active omental tumor tissue were selected for response evaluation. Therefore, a patient's selected omental lesions using direct and indirect response assessment methods can still be considered sufficiently corresponding.

In Study III, the associations of residual tumor and the postoperative tumor markers were evaluated. For reference, the patients did not undergo any imaging study after surgery; only the surgeon's estimation of the amount of residual tumor was used. It is true that the surgery outcome is not an objective and unambiguous endpoint. However, the groups of R0 and R>0 were compared in the statistical analyses, which increases the reliability of the analyses. Visual estimation of either no macroscopic tumor or any amount of visible tumor is quite unambiguous and diminishes the inter-observer variability. It is notable, that the compared surgery outcome groups varied between studies I-IV and the consistent use of the same combination, e.g. comparison of merely R0 and R>0 groups throughout the study, might have been a more preferable approach. Furthermore, the inclusion of the five patients with low-grade serous histology is another limitation of Study III as the low-grade serous EOC patients tend to be more chemoresistant compared to the high-grade serous EOC subtypes. Exclusion of these patients would have increased the homogeneity of the patient cohort.

In Study IV, the primary therapy response evaluation was performed by using the results of post-therapy CT scans and serum CA125 at the end of primary therapy. At the end of primary therapy, the treatment response comprises of NACT, IDS and postoperative adjuvant chemotherapy. Even if the total MTV comprises of all the metabolically active tumor lesions, it does not identify the location of the tumor lesions. This information could be important when debating the role of surgery in the primary therapy response evaluation. To be precise, the metabolically active suprarenic lesions, for instance, are not removed in IDS. Therefore, the comparison of the profiles of the serum tumor markers after NACT with the post-NACT total MTV would have increased the reliability these results. Another limitation of Study IV is that two different nuclear physicians evaluated the ¹⁸F-FDG-PET/CT images. However, the semiautomatic analyzing method used decreases the possible inter-observer variability. In addition, the consistent use of a single parameter, either SUVmax or SUVpeak, in both Studies II and IV might have offered a more continuous aspect to the study.

The significance tests used in the study should be interpreted with caution as many statistical tests were performed, thus increasing the chance of making a type I error. Few statistical tests were performed after the significance of certain parameters had been observed. No correction for multiple comparisons was used, which might also increase the chance of false positive findings.

6.6 The clinical importance of response evaluation in the first – line treatment of advanced EOC

Currently, the ^{18}F -FDG-PET/CT imaging and the determination of serum HE4 do not have established roles in EOC treatment response evaluation guidelines. As presented in the Chapter 2.1.4, CT imaging and serum CA125 are clinically routinely used. The evaluation of treatment response is important in order to focus on the correct treatment for each patient. For patients undergoing NACT, the evaluation of the operability after NACT is crucial as unbeneficial surgery only delays the onset of the second-line chemotherapy and exposes the patient to possible surgical complications. As debated earlier, the use of CT-based response evaluation methods might be sufficient in primary debulked patients. However, the RECIST 1.1 criteria seem to be inadequate when evaluating the effects of NACT, and its role in NACT response evaluation and in IDS operability assessment should be reconsidered. As novel and often truly expensive targeted therapies and immunotherapies are being developed, the response evaluation methods used will also have to accept the challenge of evaluating changes in the total tumor burden both more rapidly and more reliably. The metabolic ^{18}F -FDG-PET/CT imaging and the serum tumor marker HE4, in addition to the serum CA125, have potential as future response evaluation methods, as they reflect the early changes in the tumor metabolism.

In Finland, ^{18}F -FDG-PET/CT imaging is available in all the five university hospital gynecologic oncology units. However, the major limitations of its use in EOC treatment response evaluation are the financial aspect and the lack of sufficient evidence in the chemotherapy response assessment. On the other hand, the use of metabolic imaging could profit clinicians as the metabolic response to chemotherapy is achieved in an earlier phase compared to the anatomical response, allowing faster decision-making considering the following treatment options. The radiation exposure related to ^{18}F -FDG-PET/CT is most certainly relevant, when it is performed in trials, but also in clinical use. However, when ^{18}F -FDG-PET/CT is used in treatment response evaluation in advanced EOC patients with high risk of relapse and death caused by EOC, the risk of a secondary malignancy related to the radiation exposure is quite low.

From a patient's perspective, determination of a reliable serum tumor marker in treatment response assessment could be more convenient compared to long, time-consuming imaging protocols. Serum HE4 measurements do not require patient preparation. In addition, the financial costs related to the tumor marker measurements and analyses are naturally fewer compared with those of metabolic imaging. Probably the most benefit in future clinical use would be obtained if both the

metabolical ^{18}F -FDG-PET/CT imaging and serum HE4 could be used in the treatment response evaluation.

When debating the future role of the serum HE4 and ^{18}F -FDG-PET/CT imaging in EOC treatment response assessment, studies with larger patient cohorts are naturally warranted to verify the reported results. In addition, it would be interesting to evaluate the ^{18}F -FDG-PET/CT images obtained at the end of the primary therapy and to study the predictive role of the residual metabolic activity after NACT, IDS, and postoperative adjuvant chemotherapy. The evaluation of the total MTV in addition to the total TLG at the end of first-line therapy and combining the data with that of serum HE4 at the end of primary therapy might help to identify those patients with the highest risk of relapse. In addition to being used and studied as a tumor marker, HE4 could also be considered an interesting target in future drug development for EOC.

7 CONCLUSIONS

1. The change in serum HE4 or CA125 during NACT does not correspond with the anatomic response evaluated by CT. CT imaging may be insufficient for NACT response evaluation.
2. The change in serum tumor markers during NACT needs to be substantial for a favorable prognosis. The patients presenting less than 80% decrease in HE4 and CA125 during NACT were likely to have a shorter overall survival. For HE4 this observation was statistically significant.
3. The postoperative serum HE4 obtained two to three weeks after both primary and interval debulking surgery seems to reflect the amount of residual tumor better than serum CA125.
4. Metabolical ^{18}F -FDG-PET/CT imaging can be used to identify patients with poor histopathological response to NACT. A SUVmax decrease greater than 57% during NACT was required in order to achieve NACT induced histopathological changes.
5. The evaluation of the total MTV decrease during NACT in ^{18}F -FDG-PET/CT is helpful in NACT response evaluation. The patients with a total MTV decrease of less than 85% during NACT might be candidates for second-line chemotherapy and clinical trials, instead of interval debulking surgery.

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Turku, December 2017

A handwritten signature in black ink, appearing to read 'Tuulia Vallius', written in a cursive style.

Tuulia Vallius

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