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# ADVANCED EPITHELIAL OVARIAN CANCER – STUDIES ON PREOPERATIVE [<sup>18</sup>F] FDG PET/CT AND HE4 PROFILE DURING PRIMARY CHEMOTHERAPY

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## ABSTRACT

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**Advanced epithelial ovarian cancer – studies on preoperative [<sup>18</sup>F] FDG PET/CT and HE4 profile during primary chemotherapy**

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Epithelial ovarian cancer (EOC) is usually diagnosed in an advanced stage. The prognosis depends highly on the amount of the residual tumor in surgery. In patients with extensive disease, neoadjuvant chemotherapy (NACT) is used to diminish the tumor load before debulking surgery. New non-invasive methods are needed to preoperatively evaluate the disease dissemination and operability. [<sup>18</sup>F] FDG PET/CT (Positron emission tomography/computed tomography) is a promising method for cancer diagnostics and staging. The biomarker profiles during treatment can predict patient's outcome.

This prospective study included 41 EOC patients, 21 treated with primary surgery and 20 with NACT and interval surgery. The performances of preoperative contrast enhanced PET/CT (PET/ceCT) and diagnostic CT (ceCT) were compared. Perioperative visual estimation of tumor spread was studied in primary and interval surgery. The profile of the serum marker HE4 (Human epididymis 4) during primary chemotherapy was evaluated.

In primary surgery, surgical findings were found to form an adequate reference standard for imaging studies. After NACT, the sensitivity for visual estimation of cancer dissemination was significantly worse. Preoperative PET/ceCT was more effective than ceCT alone in detecting extra-abdominal disease spread. The high number of supradiaphragmatic lymph node metastases detected by PET/ceCT at the time of diagnosis brings new insight in EOC spread patterns. The sensitivity of both PET/CT and ceCT remained modest in intra-abdominal areas important to operability. The HE4 profile was in concordance with the CA125 profile during primary chemotherapy. Its role in the evaluation of EOC chemotherapy response will be clarified in further studies.

**Keywords:** Ovarian cancer, positron emission tomography, staging, PET/CT, HE4, neoadjuvant chemotherapy

## TIIVISTELMÄ

**Johanna Hynninen**

**Levinnyt munasarjasyöpä: FDG PET/CT:n käyttö leikkausta edeltävässä arvioinnissa ja seerumin HE4:n muutos ensilinjan solusalpaajahoidon aikana**

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Epiteliaalinen munasarjasyöpä diagnosoidaan yleensä levinneessä vaiheessa. Ennuste riippuu leikkauksessa jäävän jäännöskasvaimen määrästä. Laajalle levinneessä taudissa käytetään neoadjuvantti-solusalpaajahoidoa (NACT) pienentämään kasvainmassaa ennen leikkausta. Uusia ei-kajoavia menetelmiä kaivataan levinneisyyden ja leikatavuuden arviointiin. [<sup>18</sup>F]FDG PET/CT (positroniemissiotomografia/ tietokonetomografia) on lupaava menetelmä syövän diagnostiikkaan ja levinneisyyden arviointiin. Kasvainmerkkiaineiden muutokset hoidon aikana voivat ennustaa hoidon tehoa.

Tähän prospektiiviseen tutkimukseen osallistui 41 munasarjasyöpäpotilasta, joista 21 leikattiin primaaristi ja 20 neoadjuvanttihoidon jälkeen. Ennen leikkausta otettavien varjoainetehosteisen PET/CT:n (PET/ceCT) ja diagnostisen CT:n (ceCT) tuloksia verrattiin toisiinsa. Sekä primaarileikkausten aikana että NACT:n jälkeen tehdyissä leikkauksissa tehtiin visuaalinen arvio taudin levinneisyydestä. Seerumin kasvainmerkkiaine HE4:n (Human epididymis 4) käyttäytymistä ensilinjan sytostaattihoidon aikana seurattiin.

Kirurgin visuaalinen arvio taudin levinneisyydestä primaarileikkauksessa todettiin sopivaksi standardiksi, johon kuvantamistutkimuksia voidaan verrata. NACT:n jälkeen taudin silmämääräisen arvioinnin sensitivisyys löytää syöpäpesäkkeet oli merkittävästi heikompi. Ennen leikkausta otettu FDG PET/ceCT löysi useammin vatsaontelon ulkopuolisia etäpesäkkeitä kuin ceCT. Runsaslukuiset pallean yläpuoliset imusolmukkeet tuovat uutta tietoa taudin leviämistavoista. Sekä PET/ceCT:n että ceCT:n sensitivisyys jäi vaatimattomaksi vatsa-ontelon osissa, jotka ovat kriittisiä arvioitaessa leikkausmahdollisuuksia. Seerumin HE4:n muutos oli samansuuntainen kuin CA 125:n primaarihoidon aikana. HE4:n rooli munasarjasyövän hoitovasteen arvioinnissa tulee selkiytymään jatkotutkimuksissa.

**Avainsanat:** munasarjasyöpä, positroniemissiotomografia, levinneisyys, HE4, neoadjuvanttihoito



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|  |           |
|--|-----------|
| 2.2.4 Treatment response evaluation .....  | 30        |
| 2.3 HE4 IN EOC PRIMARY TREATMENT .....   | 31        |
| 2.3.1 EOC diagnostics .....  | 31        |
| 2.3.2 In predicting operability and survival .....                                       | 32        |
| 2.3.3 In treatment response evaluation .....   | 33        |
| 2.3.4 In follow-up .....   | 34        |
| <b>3 AIMS OF THE STUDY .....</b>   | <b>35</b> |
| <b>4 PATIENTS, MATERIALS AND METHODS.....</b>  | <b>36</b> |
| 4.1 Study design.....  | 37        |
| 4.1.1 Principles of PET/CT .....   | 37        |
| 4.1.1 FDG (fluorodeoxyglucose) .....   | 38        |
| 4.2 FDG PET/CT scanning procedure (Studies I and III).....                               | 39        |
| 4.3 Imaging analysis (Studies I and III) .....   | 39        |
| 4.4 Estimation of disease extent at surgery (Studies I, II and III).....                 | 40        |
| 4.5 Histopathological samples .....  | 40        |
| 4.6 Serum sample collection (Study IV) .....   | 41        |
| 4.7 CA 125 and HE4 analyses (Study IV) .....   | 41        |
| 4.8 Treatment response evaluation (Study IV).....  | 41        |
| 4.9 Statistical analyses .....   | 41        |
| <b>5 RESULTS.....</b>  | <b>43</b> |
| 5.1 The presence of supradiaphragmatic lymph node metastases (Study I).....              | 43        |
| 5.2 The reliability of perioperative visual estimation of tumor extend (Study II) ....   | 44        |
| 5.3 PET/CT vs. CT in EOC staging (Study III) .....                                       | 44        |
| 5.4 PET/CT vs. CT for the evaluation of intra-abdominal disease spread (Study III) ..... | 45        |
| 5.5 HE4 profile during EOC primary chemotherapy (Study IV).....                          | 49        |
| <b>6 DISCUSSION .....</b>  | <b>50</b> |
| 6.1 Methodological considerations and study limitations .....                            | 50        |
| 6.2 Perioperative visual estimation of disease extent.....                               | 51        |
| 6.3 Preoperative PET/ceCT: intra-abdominal disease spread and operability .....          | 52        |
| 6.4 Extra-abdominal disease spread detected with PET/ceCT .....                          | 54        |
| 6.5 Serum HE4 profile during Primary chemotherapy of EOC .....                           | 56        |
| 6.6 Future perspectives.....   | 57        |
| <b>7 CONCLUSIONS.....</b>  | <b>58</b> |
| <b>8 ACKNOWLEDGEMENTS.....</b>   | <b>59</b> |
| <b>APPENDIX 1. ....</b>  | <b>61</b> |
| <b>REFERENCES .....</b>  | <b>62</b> |
| <b>ORIGINAL PUBLICATIONS .....</b>   | <b>73</b> |

**ABBREVIATIONS**

|        |   |
|--------|---|
| EOC    | Epithelial ovarian cancer                             |
| ESUR   | European Society of Urogenital Radiology              |
| CA 125 | Cancer antigen 125                                    |
| CT     | Computed tomography                                   |
| ceCT   | contrast enhanced Computed tomography                 |
| DW-MRI | Diffusion-weighted Magnetic Resonance Imaging         |
| FDG    | Fluorodeoxyglucose                                    |
| FIGO   | International Federation of Gynecology and Obstetrics |
| FNR    | False negative rate                                   |
| FPR    | False positive rate                                   |
| GCIG   | Gynecological Cancer Intergroup                       |
| HE4    | Human epididymis protein 4                            |
| HRT    | Hormone replacement therapy                           |
| IDS    | Interval debulking surgery                            |
| LN     | Lymph node  |
| LNM    | Lymph node metastasis                                 |
| NACT   | Neoadjuvant chemotherapy                              |
| NPV    | Negative predictive value                             |
| PET    | Positron emission tomography                          |
| PET/CT | Positron emission tomography/Computed tomography      |
| PDS    | Primary debulking surgery                             |
| PFS    | Progression-free survival                             |
| PPV    | Positive predictive value                             |
| OS     | Overall survival                                      |
| RECIST | Response Evaluation Criteria in Solid Tumors          |
| SUVmax | Maximum standard uptake value                         |
| TVS    | Transvaginal ultrasound                               |
| VOI    | Volume of interest                                    |

## LIST OF ORIGINAL PUBLICATIONS

This dissertation is based on the following original publications, which are referred to in the text by the Roman numerals I–IV.

- I Hynninen J, Auranen A, Carpén O, Dean K, Seppänen M, Kempainen J, Lavonius M, Lisinen I, Virtanen J, Grénman S: FDG PET/CT in staging of advanced epithelial ovarian cancer: frequency of supradiaphragmatic lymph node metastasis challenges the traditional pattern of disease spread. *Gynecol Oncol.* 2012 Jul; 126(1): 64-8.
- II Hynninen J, Lavonius M, Oksa S, Grénman S, Carpén Olli, Auranen Annika: Is perioperative visual estimation of intra-abdominal tumor spread reliable in ovarian cancer surgery after neoadjuvant chemotherapy? *Gynecol Oncol* 2013 Feb; 128(2): 229-232.
- III Hynninen J, Kempainen J, Lavonius M, Virtanen J, Matomäki J, Oksa S, Carpén O, Grénman S, Seppänen M, Auranen A: A prospective comparison of integrated FDG-PET/contrast-enhanced CT and contrast-enhanced CT for pretreatment imaging of advanced epithelial ovarian cancer. *Gynecol Oncol* 2013 Nov; 131(2): 389-94.
- IV Hynninen J, Auranen A, Dean K, Lavonius M, Carpen O , Perheentupa A, Seppänen M, Grénman S: Serum HE4 profile during primary chemotherapy of Epithelial Ovarian Cancer. *Int J Gynecol Cancer.* 2011 Dec; 21(9): 1573-8.

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## 1 INTRODUCTION

Ovarian cancer is a rare disease with incidence of 8.3/100 000 women in Finland (Finnish Cancer Registry 2014). In 2012, ovarian cancer was diagnosed in 466 women and there were 336 deaths due to ovarian cancer in Finland. It is the commonest cause of death from gynecological malignancy. In the United States and the Western world, ovarian cancer is the 5<sup>th</sup> most frequent cancer to cause death in women (Siegel et al. 2014).

About 90 % of the malignant ovarian tumors are of epithelial origin. Early epithelial ovarian cancer (EOC) is often symptomless and, unfortunately, the disease is usually detected in the advanced stage. The cancer cells spread from the ovary to adjacent pelvic organs and around the abdominal cavity via peritoneal fluid circulation. The floating cancer cells can implant on any peritoneal surface. Another route for cancer spread is via lymphatic drainage to the retroperitoneal lymph nodes.

Upfront debulking surgery and platinum-based chemotherapy form the cornerstone of EOC treatment. The amount of residual tumor in surgery is the most critical prognostic factor. The extent of surgery and the use of neoadjuvant chemotherapy before a debulking operation are burning issues on advanced EOC treatment.

The recent development of new imaging methods like FDG PET/CT (Positron emission tomography/computed tomography) has enabled a more precise non-invasive evaluation of disease spread. The use of preoperative PET/CT has revealed distant metastases, which are traditionally considered infrequent at the time of diagnosis (Nam et al. 2010, Fruccio et al. 2013b). The significance of these findings and the use of PET/CT in treatment planning, like in the evaluation of operability, are not yet established.

EOC response to chemotherapy is followed with the serum marker CA 125 (Cancer antigen 125). The novel serum marker HE4 (Human epididymis protein 4) may bring additional value on the evaluation of treatment response and follow-up.

## 2 REVIEW OF THE LITERATURE

### 2.1 EPITHELIAL OVARIAN CANCER (EOC)

#### 2.1.1 Epidemiology

Ovarian cancer is the most common cause of death from a gynecological malignancy in the Western world. The incidence of ovarian cancer in Finland is 8.3/100 000 women (Finnish Cancer Registry 2014). In 2012 there were 466 new cases of ovarian cancer and it was the 5<sup>th</sup> most common cause of cancer death in women in Finland. Incidence grows with age and is the highest in the age group 60–64.

Approximately 14–24 % of ovarian cancer patients have been reported to carry inherited genetic mutations (Walsh et al. 2011, Alsop et al. 2012). The most common BRCA 1 and 2 mutations are inherited in an autosomal dominant fashion. The cumulative lifetime risk for ovarian cancer by the age 70 has been estimated to be around 40 % in BRCA1 mutation carriers and 11–18 % in BRCA2 carriers (Antoniou et al. 2003, Chen et al. 2007). Prophylactic salpingo-oophorectomy reduces the risk (Finch et al. 2006).

Most of the ovarian cancer cases are sporadic. The incidence is higher in developed countries, but the distribution of different subtypes varies (Sung et al. 2014). Infertility is a known risk factor for ovarian cancer (Whitemore et al. 1992). The use of infertility drugs does not increase the risk of invasive EOC but the risk for borderline tumors may be increased (Rizzuto et al. 2013). Endometriosis is particularly associated with increased risk of clear-cell and endometrioid cancer subtypes (Ogawa et al. 2000). Postmenopausal hormone replacement therapy has been demonstrated to increase EOC risk (Morch et al. 2009). In the Million Women study, the risk increased with the duration of HRT use and women who stopped taking HRT had similar risk to that of women who had never used HRT (Beral 2007). The risk of ovarian cancer reduces with greater number of pregnancies, longer duration of oral contraceptive use and greater length of breastfeeding (Whittemore et al. 1992, Danforth et al. 2007).

#### 2.1.2 Classification and carcinogenesis

Ovarian neoplasms are most commonly classified by their origin in the normal ovary. Early ovarian histological development has four major stages. During the process, premordial germ cells migrate to genital ridges formed by coelomic epithelium first. Later peripheral cortex and central medulla are formed. The histogenetic classification categorizes ovarian neoplasms by their cell type: coelomic epithelium, mesenchymal and germ cells (**Table 1**) (DiSaia and Creasman 2012).

**Table 1.** The Histogenetic Classification of malignant ovarian tumors. Modified from DiSaia and Creasman 2012.

| Origin                  | Tumor type   |
|-------------------------|--|
| Epithelium 90%          | Serous<br>Mucinous<br>Endometrioid<br>Clear-cell<br>Undifferentiated carcinoma<br>Carcinosarcoma       |
| Germ cells 3–5%         | Dysgerminoma<br>Immature teratoma<br>Secondary neoplasm from mature cystic teratoma<br>Choriocarcinoma |
| Gonadal stroma 5–10%    | Granulosa cell tumor   |
| Non-specific mesenchyme | Mixed mesodermal sarcoma<br>Lymphoma   |
| Metastatic              | GI tract<br>Breast<br>Endometrium<br>Lymphoma  |

About 90 % of malignant ovarian tumors have epithelial origin. EOC consists of a heterogeneous group of tumors with different histopathological and clinical features and outcomes. The EOC Staging system was updated by FIGO (International Federation of Gynecology and Obstetrics) in 2014. The FIGO committee agreed that the histological types should be defined at the time of diagnosis (Zeppernick and Meinhold-Heerlein 2014). The histological subtypes agreed on by FIGO committee are shown in **Table 2**.

**Table 2.** The frequencies of the main histological subtypes of EOC (Zeppernick et al. 2014)

| The frequency of histological subtypes of EOC |     |
|---|-----|
| High-grade serous (HGSC)                      | 70% |
| Endometrioid                                  | 10% |
| Clear-cell                                    | 10% |
| Mucinous                                      | 3%  |
| Low-grade serous                              | <5% |

### 2.1.2.1 Cell types

Ovarian carcinomas are traditionally thought to arise from ovarian surface epithelium. Epithelium invaginates into the underlying stroma to form inclusion cysts that may undergo a malignant transformation. The different cell types seen in tumors are explained by metaplastic changes during the process (Kurman and Shih 2010).

The cell types of EOC resemble normal cells that line other organs of the female genital tract (Cho and Shih 2009). Serous, endometrioid, mucinous, clear-cell and

transitional-cell tumors exhibit morphological features similar to non-neoplastic epithelial cells in the fallopian tube, endometrium, endocervix, gastrointestinal tract and urinary bladder, respectively. These cell types do not manifest in the normal ovary. This has led to alternative theories of the origin of EOC. It has been suggested that the majority of ovarian high-grade serous carcinomas arise from the high-grade intraepithelial serous carcinomas in the fallopian tube, which then spread to the ovary (Kindelberger et al. 2007). EOC with endometrial and clear-cell types have been associated with endometriosis, which in turn may develop from retrograded menstruation. These tumors may have their origin in the endometrium. Transitional cells are found in nests at the tubal-mesothelial junction and may be the origin of the transitional (Brenner) tumors (Kurman and Shih 2010).

### **2.1.2.2 Histological differentiation**

EOC has traditionally been divided into 3 grades by their histological features. The grade is known to be an independent prognostic factor for patient survival. More recent clinicopathological and molecular studies have shown that division into two categories, type 1 and type 2 tumors reflects better the tumorigenesis and prognosis of different neoplasms (Shih and Kurman 2004). Type 1 low-grade serous tumors may arise stepwise from borderline tumors, they grow slowly and attain a large size while still confined to the ovary. Also endometrioid, clear-cell, and mucinous carcinomas present with the low-grade types. The type 1 tumors are reported to constitute only 25 % of the ovarian cancers and account for approximately 10 % of the ovarian cancer deaths (Kurman and Shih 2010).

High-grade (former grade 2 and 3), serous, malignant mixed mesodermal tumors (carcinosarcomas) and undifferentiated carcinomas form the more aggressive type 2 tumor group: they grow fast and are usually diagnosed in an advanced stage. The low- and high-grade tumor types have distinct molecular changes and mutations. The more common high-grade tumors typically have a very high frequency of TP53 mutations and are genetically unstable (Singer et al. 2005).

### **2.1.3 Symptoms and primary diagnostics**

EOC is a treacherous disease. Early-stage tumors seldom cause any symptoms. The most common reason for seeking medical help is bloating or increased abdominal size caused by ascites, which occurs at a late stage. Other symptoms include fluctuating abdominal pain, difficulty in eating, changes in bowel function and urinary symptoms (Goff 2004). Occasionally, thromboembolic events such as venous thrombosis and pulmonary embolism can be the first signs of EOC. At pelvic examination, the early-stage disease can present as palpable pelvic mass, or often fixed, unilateral or bilateral tumors. In postmenopausal women any palpable ovary is suspicious. At advanced stage, abdominal swelling caused by the ascites is often prominent. Sometimes

“omental cake”, large metastatic growth in the omentum, can be palpable. EOC spread to pelvic peritoneum can be detected by bimanual gynecological examination if there is miliary carcinosis in the fossa Douglas. Dyspnea can be the first symptom of EOC in patients with large pleural effusions.

When a suspicion for malignant ovarian tumor rises, transvaginal ultrasound examination (TVS) is usually the first diagnostic imaging method. The presence of cystic-solid processes, cystic lesions with septae and papillary structures or ascites enhances the suspicion for ovarian malignancy. An elevated serum biomarker CA 125 level supports the possibility. The diagnosis of EOC is, however, always based on an histopathological sample received in surgery or in some cases by an image-guided needle aspiration (Benedet et al. 2000).

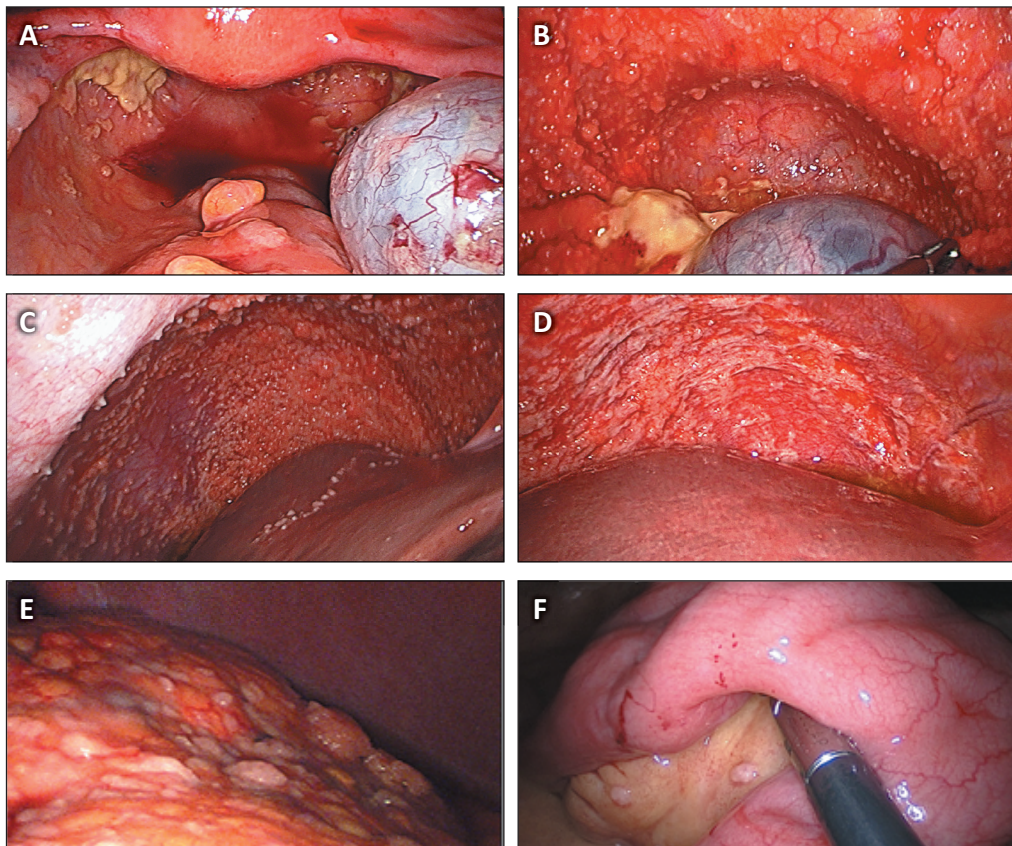
#### **2.1.4 Spread patterns**

EOC spreads from the ovaries via two routes: intra-abdominally along with the peritoneal fluid circulation and to the retroperitoneum via lymphatic drainage. Free-floating tumor cells exfoliating from the primary tumor can implant on any peritoneal surface (Figure 1). Intra-abdominally, miliary carcinosis is often found in the pelvic peritoneum and in the right subdiaphragmatic peritoneal surface. When the disease progresses, the entire peritoneal cavity can be covered with metastatic nodules, including the mesentery of the intestine and the liver surface. The infracolic omentum often forms an “omental cake”, a metastasis that can be larger than the primary tumor itself. EOC can invade the intestinal canal and cause bowel obstruction. However, the intraperitoneal disease often proceeds with mild symptoms and bowel obstruction is more common event with relapsed disease.

The lymphatic drainage from the ovaries leads to retroperitoneal regional lymph nodes (LNs): external and common iliac and para-aortic nodes (Burghardt et al. 1991). In the advanced, stage III-IV disease the nodal involvement is common. Pereira et al. (2007) reported that of 116 stage III-IV patients who underwent systematic lymphadenectomy in Mayo clinic, 84 % had para-aortic and 78 % pelvic LNMs. When an intra-abdominal disease is restricted to the pelvis, LNM is less common. Nomura et al. (2010) reported a 13 % incidence for LNM in 79 patients with apparent stage I and II disease. Lymph node involvement was more common in serous than other histological subtypes. The mean size of metastatic lymph nodes does not differ from that of the benign LNs (Pereira et al. 2007). Intraoperative palpation is not sensitive enough method to find LNM and a systematic lymphadenectomy is needed for adequate staging of the patient (Arango et al. 2000).

EOC is considered to be an intra-abdominal disease. Extra-abdominal metastases are considered infrequent at the time of diagnosis. Cytologically confirmed, malignant, pleural effusion and intraparenchymal liver metastasis are traditionally the most

common lesions that cause upstaging the disease to FIGO stage IV (Bonnetfoi et al. 1999). Less often seen sites for metastasis are lungs and extra-abdominal lymph nodes. Hematogenous spread is uncommon and rare distant metastases to the brain and skin are late manifestations of relapsed disease (Kolomainen et al. 2002, Kim et al. 2012). Bone metastases are also rare and an early onset of bone spread has a strong negative prognostic impact (Sehouli et al. 2013). The improvement of imaging studies in recent years has enabled the finding of small, earlier undetectable, lesions. For example, paracardiac lymph node enlargement is often visible in CT images of advanced EOC patients. Holloway et al. reported in 1997 that the presence of paracardiac lymphadenopathy in CT is a negative prognostic factor. Thereafter, more attention has been paid to supradiaphragmatic lesions. The role of the imaging studies in EOC spread evaluation is presented in chapter 2.2.2.



**Figure 1.** Different spread patterns of ovarian cancer. Photos taken in a laparoscopy. A. Primary tumor on the right ovary. Carcinosis in the pouch of Douglas, below the normal-appearing uterus. B. The anterior pelvic peritoneum is covered with carcinomatous tissue. C, D. The right subdiaphragmatic peritoneal surface is a typical place for EOC spread. E. The infracolic omentum filled with tumor nodules. F. Small metastases in the small bowel mesentery.

### 2.1.5 Staging

Cancer staging defines the extent of disease at presentation and assigns the patients to prognostic subgroups. EOC staging is surgical. The FIGO staging system has been updated recently (**Table 3**) (Mutch and Prat 2014). It is based on the findings in laparotomy with midline incision.

**Table 3.** The FIGO staging system 2014 and TNM classification of EOC. LNM=lymph node metastasis. Modified from Mutch and Prat 2014.

| <b>FIGO stage</b> |   | <b>TNM</b>        |
|-------------------|---|-------------------|
| <b>I</b>          | <b>Tumor limited to ovaries or tubes</b>  |                   |
| IA                | Tumor limited to one ovary, external surface intact   | T1a N0M0          |
| IB                | Tumor limited to both ovaries, external surface intact  | T1b<br>N0M0       |
| IC1               | Tumor confined to ovary/ovaries, capsule rupture during surgery   | T1c N0M0          |
| IC2               | Rupture before surgery or tumor on ovarian/fallopian tube surface   |                   |
| IC3               | Malignant cells in the ascites or peritoneal washing  |                   |
| <b>II</b>         | <b>Tumor limited below pelvic rim</b>   |                   |
| IIA               | Extension and/or implant to uterus and/or tubes and/or ovaries  | T2a N0M0          |
| IIB               | Extension to other intraperitoneal pelvic tissues   | T2b N0M0          |
| <b>III</b>        | <b>Retroperitoneal LNM and/or intraperitoneal spread beyond the pelvis</b>  |                   |
| IIIA1             | Positive retroperitoneal nodes  | T3a N1M0          |
| IIIA1(i)          | Metastasis up to 10 mm in greatest dimension  |                   |
| IIIA1(II)         | Metastasis more than 10 mm in greatest dimension  |                   |
| IIIA2             | Microscopic extrapelvic peritoneal involvement +/- retroperitoneal LNM  |                   |
| IIIB              | Macroscopic peritoneal metastasis beyond the pelvis, 2 cm or less in largest dimension +/- retroperitoneal LNM  | T3b N0-1<br>M0    |
| IIIC              | Abdominal implants beyond the pelvis >2 cm in greatest dimension +/- retroperitoneal LNM (includes extent to liver and spleen capsule but not parenchyma)               | T3c N0-1<br>M0    |
| <b>IV</b>         | <b>Distant metastases</b>   |                   |
| IVA               | Pleural effusion with positive cytology   | Any T Any<br>N M1 |
| IVB               | Distant metastasis beyond the peritoneal cavity. Parenchymal involvement of liver and spleen. Inguinal and extra-abdominal LNM. Transmural bowel invasion into mucosae. |                   |

The adequate staging procedure is described in **Table 4**. Due to the improved techniques and equipment, laparoscopy has become an alternative for laparotomy in staging of the early-stage EOC. It seems to have similar outcomes (Park JY et al. 2008, Park HJ et al. 2013), but has not been validated in randomized trials.

**Table 4.** FIGO guidelines for staging operation (Benedet et al. 2000)

| <b>Guidelines for surgical staging according to FIGO</b>   |
|--|
| Four peritoneal washings (diaphragm, right and left abdomen, pelvis)   |
| Careful inspection and palpation of all peritoneal surfaces  |
| Biopsy of all suspicious lesions   |
| Infracolic omentectomy   |
| Biopsy or resection of any adhesions   |
| In the absence of obvious implants:<br>Random biopsies of normal peritoneum of undersurface of right hemidiaphragm, bladder reflection, cul-de-sac, right and left paracolic recesses, and both pelvic sidewalls |
| Lymphadenectomy of pelvic and para-aortic nodes  |
| Total abdominal hysterectomy, bilateral salpingo-oophorectomy, and excision of masses when prudent   |
| Appendectomy for mucinous tumors   |

The classical FIGO staging approves that imaging with computed tomography (CT) can be valuable in disease evaluation to certain extent, but histological confirmation of the findings is required. Chest X-ray commonly serves as a screen for pleural metastases (Benedet et al. 2000). In order to determine a patient to stage IV due to pleural effusion, the pleural cytology must be examined.

### 2.1.6 Prognostic factors

The most important prognostic factors for patient survival are FIGO stage, histological subtype and complete tumor debulking in surgery. In retrospective review of 1895 stage III patients, age, performance status, tumor histology and residual tumor in surgery were independent predictors of prognosis (Winter et al. 2007).

### 2.1.7 Treatment of primary EOC

#### 2.1.7.1 Staging surgery

Staging surgery is mandatory in the treatment of apparent stage I and II ovarian cancer. The operation technique is described in **Table 4**. The need for adjuvant chemotherapy is based on the surgical staging. It also gives information on the patient's prognosis. Fertility-sparing surgery is an option for young patients who want to have children and

have the disease restricted to the ovaries (Fruscio et al. 2013a). The uterus and the healthy ovary are preserved after a careful exploration of the entire abdominal cavity and a systematic pelvic and para-aortic lymphadenectomy. Long-term survival with fertility-sparing surgery has been reported to be comparable with radically operated patients with 5-year overall survival of 90.8 % (Kajiyama et al. 2011). Grade 3 tumors are associated more frequently with lethal distant recurrences. Instead, disease relapse in the ovaries can usually be managed successfully (Fruscio et al. 2013a).

With the disease restricted to the pelvis, it is recommended to remove tumors without rupturing the neoplasm to avoid cancer cells seeding to the peritoneal cavity.

### **2.1.7.2 Upfront debulking surgery**

#### ***2.1.7.2.1 The importance of residual tumor***

Primary debulking surgery (PDS) is the cornerstone of the advanced stage EOC treatment. Since Griffiths' publication almost 40 years ago, the extent of cytoreductive surgery and the residual tumor after surgery have been recognized as the most important factors determining the survival of patients with advanced disease (Griffiths 1975). In the early 1990s, Hoskins et al. performed an analysis of over 500 patients from GOG 52 and 97 studies and showed that the size of the residual tumor (categorized as less than 1 cm, 1–2 cm or over 2 cm) was an independent predictor of overall survival (Hoskins et al. 1992, 1994). This finding has been confirmed in multiple reports since then.

The strength of the data presented on the prognostic significance of the residual tumor in surgery has, however, been questioned (Covens 2000). The most commonly used categorization of the residual tumor describes the maximal diameter of individual tumor implants, not the total number of remaining tumor nodules. Yet, patients with diffuse carcinomatous seeding <1 cm have significantly worse prognosis than patients with individual minimal residual nodules (Farias-Eisner et al. 1994).

Based on the analysis of a prospective randomized SCOTROC trial, Crawford et al. (2005) have suggested that an increased PFS associated with optimal surgery is achieved in patients with less advanced disease. They conclude that the extent of the disease rather than the aggressive surgical procedures defined the patient's prognosis. This conclusion has been questioned strongly by the advocates of extensive surgery (Chi et al. 2011).

#### ***2.1.7.2.2 The extent of cytoreductive surgery***

The widely accepted consensus has it that the goal for primary surgery should nowadays be a complete resection of all the visible intra-abdominal disease nodules (du Bois et al. 2009, Chang and Bristow 2012). However, the reasonable extent of surgical procedures is under discussion. The more radical surgery is needed to achieve

complete tumor resection, the more severe complications are possible. High-risk procedures can include diaphragm peritonectomy, liver resection, splenectomy, multiple bowel resections, rectosigmoid colectomy, distal pancreatectomy, gastric resection, extensive lymphadenectomies and intrathoracic surgery. Many series show that these procedures are highly effective for complete cytoreduction with acceptable morbidity and mortality (Chang and Bristow 2012). Single-institution studies from two big centers in OEC surgery, Mayo Clinic and Memorial Sloan Kettering, have underlined the significance of radical cytoreductive surgery in achieving the best treatment results (Chi et al. 2006, Aletti et al. 2006). The study of Chi et al. illustrated that investing in extensive upper abdominal debulking can significantly improve complete debulking rates without compromising the perioperative morbidity. By changing the surgical approach, the median overall survival time in Memorial Sloan Kettering institute increased from 43 to 54 months when time periods 1996–99 and 2001–04 were compared (Chi et al. 2009). The role of extensive debulking surgery can be debated especially in stage IV patients, who have extra-abdominal metastases known to locate in inoperable sites.

The surgical results of advanced EOC vary notably between different hospital types and individual surgeons (Bristow et al. 2002, Kumpulainen et al. 2006). The best quality is achieved in large tertiary centers where operations are performed by gynecologic oncologists who have had formal training in cytoreductive techniques.

### ***2.1.7.2.3 The macroscopic evaluation of disease spread and residual tumor in surgery***

In order to compare the surgical results of different hospitals, complete surgical staging and an exact documentation of the residual tumor are essential. However, no standardized methods or instruments to measure the residual tumor exist. In prevailing practice, neither systematic surgical staging nor the amount of the residual tumor are adequately documented even in the tertiary centers (Gogoi et al. 2012).

The reliability of the visual evaluation of the tumor load in EOC performed by the surgeons has not been studied much. Earlier data on the reliability of perioperative estimation underline the importance of inspecting the whole abdominal cavity in laparotomy (Young et al. 1983, Le et al. 2002). Préfontaine et al. (1994) studied the interobserver variability of intraoperative tumor measurements in a simulated patient at laparotomy and found highly variable results between surgeons. Underestimation of tumor diameter was more common than overestimation.

The interobserver variation on defining the amount of the residual tumor has not been studied. In clinical practice, the individual surgeons report their own results, and this may interfere with the results. The studies comparing the surgical results of different centers are exposed to measurement errors.

### **2.1.7.3 Neoadjuvant chemotherapy**

Despite extensive surgical effort, a certain percent of patients cannot be optimally debulked in PDS. Optimal resection rates range from 20 % at less-experienced centers to over 90 % at centers with extensive expertise in radical surgical procedures (Bristow et al. 2007). In these patients neoadjuvant chemotherapy (NACT) is an alternative treatment modality. The aim of NACT is to reduce the tumor size and extent in order to enable the surgical debulking of the disease. This approach has been suggested especially for patients with stage IV disease or very heavy metastatic tumor load and for patients with poor general condition (Vergote et al. 2011b).

The primary debulking operation has been the cornerstone of EOC treatment for decades. A meta-analysis of 21 non-randomized trials showed that the use of NACT increases the optimal debulking rate (Kang and Nam 2009). The higher optimal debulking rates do not, however, translate into better survival figures (Rosen et al. 2014). EORTC-NCIC study was the first randomized multicenter study that was designed to compare the results of PDS and interval debulking surgery (IDS) and their impact on patient survival (Vergote et al. 2010). The largest residual tumor was 1 cm or less in diameter in 41.6 % of patients in PDS and in 80.6 % of patients in IDS. The survival of the patients treated with NACT and IDS was similar to that of the patients treated with standard PDS. The complete debulking of the macroscopic tumor was the most important predictor of survival in both PDS and IDS.

It is a general consensus that only the patients who respond to NACT benefit from interval debulking attempts. Since surgery is the mainstay of EOC treatment, a decision not to operate should be carefully justified. Currently, there are no clear criteria to identify inoperable patients to avoid unnecessary operations. The role of the imaging studies in evaluating the response for NACT is presented in chapters 2.2.4. Fagotti and co-workers (2010) have suggested that laparoscopy may be a useful additional method for evaluating the operability of the patients who have radiologically and serologically stable disease or a partial response to NACT.

### **2.1.7.4 Patient selection between PDS and NACT+IDS**

PDS is the standard treatment of advanced EOC and most experts prefer it when the disease appears operable. Based on the experience of Leuven and Essen centers, NACT is considered in 15–20 % of all the EOC patients (Vergote et al. 2013). The others can be completely debulked in PDS.

Unfortunately, there are no general criteria for patient selection. The randomized EORCT study found no predictive factors favoring either arm, except for marginally better survival of the patients with metastases less than 5 cm treated with PDS (Vergote et al. 2010). There are two retrospective studies reporting increased survival of stage IV patients treated with NACT (Hou et al. 2007, Rafii et al. 2007).

**Table 5.** Examples of proposed criteria for unresectable disease requiring neoadjuvant chemotherapy. Modified from Bristow et al. 2007.

| Criteria for inoperability   | Reference                            |
|--|--------------------------------------|
| Optimal cytoreduction not achieved with standard operation (TAH, BSO, infracolic omentectomy, rectosigmoid resection, lymphadenectomy) | Ansquer 2001, Morice 2003            |
| "Unresectable" disease in CT scan  | Chan 2003                            |
| >500 ml ascites  |                                      |
| Dense adhesions between bowel and omentum  | Mazzeo 2003                          |
| Carsinosis on small and large bowel  | Mazzeo 2003, Fanfani 2003            |
| Large diaphragmal disease  | Fanfani 2003, Chan 2003, Morice 2003 |
| 2 or more bowel resections needed  | Morice 2003                          |
| Suprarenal adenopathy  | Chan 2003, Morice 2003               |
| Porta hepatis involvement  | Chan 2003, Fanfani 2003, Morice 2003 |
| Liver surface of parenchymal involvement   | Chan 2003                            |

Some reported criteria for unresectable disease requiring NACT are presented in **Table 5**. **Table 6** presents the criteria used for patient selection at the University Hospitals Leuven, Belgium (Vergote et al. 2011b). These criteria are, however, not validated in other centers. A recent prospective multicenter study by Suidan et al. (2014) presented 9 criteria to predict suboptimal debulking and developed a model to predict suboptimal surgery. The predictive score included the patient's age, CA 125, performance status and radiological parameters like suprarenal retroperitoneal lymph nodes >1 cm, diffuse small bowel adhesions/thickening and lesions >1 cm in the small bowel mesentery, the root of the superior mesenteric artery, the perisplenic area and the lesser sac.

**Table 6.** Leuven criteria for using NACT instead of PDS. (Vergote et al. 2011b)

| Leuven criteria for neoadjuvant chemotherapy followed by interval debulking surgery in stage IIIC and IV ovarian carcinoma (about 50 % of the patients with stage IIIC and IV disease) |  |
|--|--|
| 1.   | Tumors larger than 2 cm around the superior mesenteric artery or behind the porta hepatis or   |
| 2.   | Intrahepatic (multiple) metastases or extra-abdominal metastases (excluding resectable inguinal or supraclavicular lymph nodes) larger than 2 cm or                        |
| 3.   | Poor general condition (e.g. >80 years) making a "maximal surgical effort" to no residual tumor impossible, or   |
| 4.   | Extensive serosal invasion (e.g. plaques) of the intestines necessitating bowel resections of >1.5 m.  |
| 5.   | Patients who cannot be (easily) debulked to no residual tumor (e.g. more than 1 bowel resection, expected operative time more than 4 hours, poor general condition, . . .) |

In addition to different imaging methods (chapter 2.2.4.), laparoscopy is also used to evaluate operability in many centers (Angioli et al. 2006, Fagotti et al. 2006). Fagotti et al. (2006) presented a laparoscopy-based model, where the extent of the disease in the liver, the peritoneal surfaces and the bowel infiltration is evaluated and scored. The model was validated later and overall accuracy varied between 77 and 100 % (Fagotti et al. 2008). The recent Cochrane review concluded laparoscopy to be a promising method for the evaluation of operability. However, studies made so far, are small and include variable patient cohorts (Rutten et al. 2014).

The studies focused on predicting operability with different methods face the same problem. Due to a large variety of surgical approaches in different hospitals and countries, the results cannot be generalized. Some committed institutions are able to achieve optimal cytoreduction with extensive upper abdominal procedures in patients who are considered inoperable in many centers. In addition, treatment modality depends on many aspects, not just on the extent of the disease. Optimally, the selection between PDS and NACT should be made by an experienced gynecologic oncologist taking into account the patient's age, co-morbidities, performance status, disease extent and localization of metastases (Vergote et al. 2011b).

The use of NACT is still under lively debate. Centers with high optimal debulking rates in PDS have doubts about the EORTC-NCIC study results due to its low optimal debulking rates in both primary and interval surgery (Chi et al. 2011). NACT defenders point out that the role of PDS as a standard care is based on the non-randomized retrospective studies (Vergote et al. 2011a).

### **2.1.7.5 Chemotherapy on EOC primary treatment**

Platinum-based compounds were introduced to EOC treatment in the 1980s. Former poor response rates in clinical trials improved clearly to 50–80 % (Lambert and Berry 1985, Omura et al. 1986). Later, the combination of cisplatin and paclitaxel was found to be more effective than cisplatin-cyclophosphamide in first-line chemotherapy (McGuire et al. 1996). Carboplatin is as effective as cisplatin and better tolerated (Neijt et al. 2000). Recently, now for over a decade, the paclitaxel-carboplatin combination has been the standard front-line treatment of EOC.

In most of the larger trials, neoadjuvant chemotherapy consists of 3–4 cycles of carboplatin and taxane (Bristow et al. 2007). The chemotherapy continues after IDS with at least three additional cycles (Vergote et al. 2010).

## 2.2 EOC IMAGING

### 2.2.1 Differential diagnostics of pelvic mass

#### 2.2.1.1 TVS

The suspicion of ovarian cancer rises either from the patient's symptoms or from the findings during a gynecological examination. Transvaginal ultrasound is widely available and a relatively inexpensive technique to get more information of a pelvic mass. In ordinary population of ovarian tumors, gray-scale imaging is a reliable tool in differential diagnostics (Valentin 1999). Doppler technology enables the assessment of the distribution of the tumor vessels and the qualitative and quantitative analysis of the tumor blood flow. 2D US combined with Doppler technology is reported to be able to differentiate between malignant and benign ovarian tumors with a 91.0 % sensitivity and a 91.7 % specificity (Dodge et al. 2012).

Some 10 % of the tumors are difficult to classify with US, even for experienced examiners (Jokubkiene et al. 2007). Three-dimensional technique is a promising method for more complicated cases. 3D ultrasound enables a closer evaluation of the structure and vascularity of the tumor. In review of 53 primary studies and 4 meta-analyses on preoperative imaging, Dodge et al. (2012) present that 3D US has both higher sensitivity and specificity when compared to 2D US. In the same analysis, Doppler color flow ultrasound technology was neither as sensitive nor as specific as simple ultrasonography. Quantitative Doppler parameters such as pulsatile index and resistance index had 77–81 % sensitivity and 80–90 % specificity. Jokubkiene et al. (2007) reported that objective quantitative measurement of tumor vascularity with 3D power Doppler is not better than subjective quantification of 2D power Doppler US.

#### 2.2.1.2 MRI

Magnetic Resonance Imaging (MRI) can differentiate various components such as fat, fibrosis, clots and solid parts in a complex adnexal mass by using T1- and T2-weighted sequences. The use of contrast media improves the accuracy. Sensitivity of 91.9 % and specificity of 88.4 % in differential diagnostics have been reported in a meta-analysis of 24 studies (Dodge et al. 2012). MRI is the most useful second-line imaging method for women with indeterminate ovarian mass with gray-scale US (Kinkel et al. 2005).

Dynamic contrast-enhanced MRI (DCE-MRI) can be used to analyze the perfusion of solid tissues. Benign, borderline and malign tumors can be separated by their distinct enhancement patterns (Thomassin-Naggara et al. 2008).

Lately, a functional imaging technique, the diffusion-weighted MRI (DW-MRI) has been introduced to EOC differential diagnostics and staging. DW-MRI provides

information about water mobility, tissue cellularity and integrity of cellular membranes. The apparent diffusion coefficient (ADC) is calculated in order to quantitatively evaluate different tissues (Moyle et al. 2010). ADC can be displayed as a parametric map. Objects with freely moving water such as cysts or bladder appear in a lighter shade of gray (Vargas et al. 2013). For the present, the role of DW-MRI in EOC differential diagnostics is controversial. Nakayama et al. (2005) found no significant differences in ADC values of benign and malignant cystic ovarian lesions. Thomassin-Naggara et al. (2009) made the same conclusion, but in their study DWI signal intensity was an accurate tool for predicting benignity.

### 2.2.1.3 CT

CT is not an ideal tool for evaluating the nature of a pelvic tumor. Some useful morphologic information such as the presence of solid components in the cystic mass can be gained with the use of intravenous contrast agent (Mohaghegh and Rockall 2012). CT is more useful in EOC staging when the extent of the disease is evaluated.

### 2.2.1.4 PET/CT

Benign and malign ovarian tumors can be separated to some extent by their metabolic activity detected with FDG PET/CT. Malignant ovarian tumors are usually much more FDG-avid than the benign or the borderline lesions (Musto et al. 2014). However, an increased FDG uptake has been reported in many benign pelvic processes including uterine fibroids, inflammatory conditions, endometriosis and the normal menstrual cycle (Lerman et al. 2004, Subhas et al. 2005). Incidental ovarian FDG accumulation is seen around the time of ovulation and luteal phase (Kim et al. 2005). SUVmax may vary between histological subtypes of EOC. Serous and endometrioid subtypes are reported to have higher FDG uptake than clear-cell or mucinous subtypes (Tanizaki 2014). The anatomical resolution of PET/CT scanners has improved in current hybrid scanners with advanced technique and by the use of contrast agent in CT.

**Table 7** compiles the main results of PET/CT studies on differential diagnostics of adnexal masses. These studies consist of patients with suspicious ovarian masses and who have already been scheduled for surgery. For example, the patients of Risum et al. (2007) had high suspicion of malignancy based on the Risk of Malignancy Index (RMI) > 150. In addition, many cancer patients in these studies had advanced-stage disease. The histology of the tumors included in different studies is heterogenic. Furthermore, some studies included borderline tumors into the cancer group while others focused on differentiating EOC from borderline and benign tumors. The important question of how to separate benign lesions from local early stage EOC is not properly answered in the current studies.

**Table 7.** Diagnostic performance of PET/CT in characterization of adnexal masses. Results from literature. M=malign, BOT=borderline ovarian tumor, B=benign. ceCT=contrast enhanced CT. The percent of advanced-stage (III/IV) patients is calculated from all primary OC patients. Modified from Fuccio et al. 2011.

| Author           | Number of patients          | Modality             | Sensitivity | Specificity | Accuracy | Amount of stage III/IV patients |
|------------------|-----------------------------|----------------------|-------------|-------------|----------|---------------------------------|
| Risum 2007       | 97<br>M:57/BOT:4/B:40       | PET/ceCT             | 100         | 93          | 97       | 69%<br>(34/49)                  |
| Castellucci 2007 | 50<br>M:32/B:18             | PET/CT               | 87          | 100         | 92       | 56%<br>(18/32)                  |
| Yamamoto 2008    | 30<br>M:10/BOT:4/ B:16      | PET/CT               | 71          | 81          |          | 40%<br>(4/10)                   |
| Nam 2010         | 133<br>M:95/BOT:13<br>/B:25 | PET/ceCT             | 98          | 74          | 92       | 70%<br>(64/91)                  |
| Dauwen 2013      | 69<br>M:45/BOT:11/B:13      | PET/ceCT<br>CT alone | 93<br>96    | 77<br>38    | 90<br>86 | 80%<br>(36/45)                  |
| Tanizaki 2014    | 166<br>M:67/BOT:14/B 79     | PET/CT               | 81          | 95          |          | 22%<br>(15/67)                  |

## 2.2.2 Radiological staging of EOC

EOC staging today is surgical. FIGO and ESUR (European Society of Urogenital Radiology) guidelines recommend CT with coverage of the base of the lungs to the inguinal region as the imaging technique of choice for preoperative staging (Benedet et al. 2000 and Forstner et al. 2010). MRI is reserved for situations where CT is contraindicated, such as in renal insufficiency and pregnancy. Additional benefit of PET/CT to conventional CT is not yet fully established and PET/CT is not included in clinical guidelines.

Neoadjuvant chemotherapy with interval surgery has become a treatment option in primarily inoperable EOC. Patient selection between primary surgery and NACT is a burning issue. The significance of radiological staging is growing since more accurate information on disease extent influences treatment decisions. The present review of literature focuses especially on the impact of PET/CT on EOC staging.

### 2.2.2.1 MRI

Staging accuracy of MRI is reported to be between 78–88 % (Forstner 2007). Limitations of MRI include problems of covering the entire abdomen with high-resolution images and longer examination and interpretation times. The strength of MRI compared to CT is detecting the local spread of tumor to the pelvis (Forstner et al. 1995). Overall sensitivity of gadolinium-enhanced MRI in depicting peritoneal disease is comparable with CT, 95 % and 92 %, respectively (Tempany et al. 2000).

Diffusion-weighted MRI (DW-MRI) is a new promising method for cancer staging. Compared to PET/CT it does not involve ionic radiation and is widely available. Intra-abdominal lesions of only 5 mm can be revealed (Kyriazi et al. 2010). Some preliminary reports of DW-MRI in EOC staging are available. In a recent prospective study of Michielsen et al. with 32 EOC patients, DW-MRI showed a higher accuracy of 91 % compared to PET/CT (71 %) and CT (75 %) for detecting peritoneal tumor nodules. The performance on retroperitoneal lymph nodes and distance metastases were comparable with PET/CT (Michielsen et al. 2014).

### 2.2.2.2 CT

Staging by cross-sectional imaging requires a complete coverage of the abdominal cavity. The use of oral contrast media is essential in order to differentiate bowel loops from peritoneal implants. Inclusion of lung bases enables the evaluation of pleural fluid and cardiophrenic LNs. If pleural effusion or suprarenal lymphadenopathy is detected, further evaluation of the chest region is recommended (Forstner 2010). Staging accuracy for CT ranges between 53–92 % in the literature (Forstner et al. 1995, Tempany et al. 2000). The study of Coakley et al. (2002) brings out that the sensitivity of the spiral CT on detecting peritoneal metastases reduces when implants are smaller than 1 cm. Their overall sensitivity of 85–93% for peritoneal metastases decreased to 25–50% in lesions less than 1 cm. Patients with only small-size peritoneal metastases cannot be evaluated sufficiently with CT.

### 2.2.2.3 PET/CT

PET imaging is most useful in combination with anatomical imaging. The introduction of hybrid PET/CT (positron emission tomography/computed tomography) scanner in the late 1990s made it possible to combine the information of functional and anatomical imaging in a single device and scanning session (Beyer et al. 2000). The areas with an abnormal tracer uptake could be localized to specific anatomical structures such as lymph nodes. PET/CT also helps to identify false positive PET findings. Sensitivity can improve when an activity interpreted as physiological is shown to localize in a pathological site on the fused CT scan. The challenges of combining PET and CT include issues such as the patient's motion, respiration and bowel movements between PET and CT scanning. These artifacts can be largely corrected with modern devices. The spatial resolution of 5 mm combined with the metabolic information has made PET/CT an interesting tool for cancer staging (Kitajima et al. 2011).

**Table 8** presents the results of studies on radiological EOC staging with PET/CT compared to FIGO surgical staging. According to the studies presented so far, PET/CT seems to outperform CT in accuracy. **Table 9** gathers the studies of FDG PET/CT on primary EOC staging.

**Table 8.** The studies comparing the staging accuracy of PET/CT and CT using surgical FIGO stage as reference standard. Modified from Fuccio et al. 2011.

| Authors          | Patient number | Patients with FIGO stage III-IV | PET/CT accuracy | ceCT accuracy |
|------------------|----------------|---------------------------------|-----------------|---------------|
| Castelluzzi 2007 | 32             | 18                              | 69% (22/32)     | 53% (17/32)   |
| Kitajima 2008    | 40             | 15                              | 75% (30/40)     | 55% (22/40)   |
| Nam 2010         | 91             | 64                              | 78% (71/91)     | -             |
| Dauwen 2013      | 56             | 36                              | 57% (31/56)     | 55% (32/56)   |

The use of PET/CT on EOC preoperative evaluation has also revealed co-existent malignancies. These include thyroid cancers, early stage breast cancers and renal cancer (Castellucci et al. 2007, Nam et al. 2010, Dauwen et al. 2010).

#### 2.2.2.3.1 *Intra-abdominal spread (T staging)*

Nam et al. (2010) compared the performance of PET/CT and the conventional CT in detecting peritoneal metastases and found them equally useful with 94 % of sensitivity. On the contrary, in Dauwens study of 56 EOC patients, the sensitivity of PET/CT was worse but specificity better than that of CT (Dauwen et al. 2013). The results were alike when detecting tumor deposits on the bowel wall and mesentery. De Iaco et al. (2011) made a detailed analysis of intra-abdominal PET/CT staging by comparing the imaging results with the laparoscopy findings in 9 quadrants of the abdominopelvic area, totally in 360 areas of 40 patients. They found sensitivity of 78.9 % and specificity of 68.4 % for PET/CT. False negative findings were common (28.9 %) in areas with tumor lesions <5 mm (DeIaco 2011). Limited spatial resolution and less FDG-avid mucinous tumors are known causes for false negative PET results (Kyriazi et al. 2010). At the same time, focal physiological radiotracer activity in bowel loops and urinary system can lead to false positive interpretations (Subhas et al. 2005).

The overall advantage of PET/CT in tumor staging is generally more due to the lymph node staging and in detecting unexpected distant metastases than in detecting local tumor spread in adjacent organs (Kitajima et al. 2011).

#### 2.2.2.3.2 *Lymph nodes (N staging)*

In anatomical imaging, lymph nodes are evaluated by size and structure. The most accepted criterion for the suspicion of malignancy is the short axis dimension of LN over 10 mm (Torabi et al. 2004). Any defined threshold diameter is, however, a compromise, since LNM is sometimes microscopic and again lymph nodes may be enlarged due to benign inflammatory processes. The strength of PET imaging is the ability to detect the abnormal glucose metabolism of cancer cells regardless of lesion size. PET/CT can detect relatively tiny metastatic LNs from 5–9 mm in size (Kitajima et al. 2011). Kitajima et al. (2008) compared PET/CT and CT in retroperitoneal LN staging of EOC with lymphadenectomy specimen as reference standard. Node-based

sensitivity and specificity for PET/CT were 81.3 % and 96.6 %, respectively, and the corresponding figures for CT were 37.5 % and 100 %. The sensitivity of PET/CT for LNM has also been reported to be good in apparent early-stage tumors (Signorelli et al. 2013). In a meta-analysis of 882 patients, PET/CT was the most accurate imaging method for the detection of LNM in EOC patients (Yuan et al. 2012). There was no significant difference between the diagnostic performance of MRI and CT. Both primary and treated EOC patients were included in the meta-analysis. In a recent prospective study by Michielsen et al. (2014), DW-MRI outperformed CT and was comparable with PET/CT in retroperitoneal nodal staging of EOC patients.

So far, radiological staging cannot replace surgical staging with lymphadenectomies. Histopathological examination of lymph nodes is essential to accurately stage the patient.

#### **2.2.2.3.3 Extra-abdominal spread (M-staging)**

PET/CT detects a greater number of distant metastases than CT alone (Table 8). Risum et al. (2010) reported that in their patient material of 64 FIGO stage III and 2 stage IV patients, PET/CT indicated stage IV for 27 (41 %) of the patients. Supradiaphragmatic lymph nodes were the most common sites for distant metastases. In the analysis by Fruscio et al. (2013) of 95 FIGO stage III-IV patients, 25 were upstaged to stage IV by PET/CT, 16 of them due to supradiaphragmatic LNM. In a retrospective analysis of 53 EOC patients, abnormal mediastinal FDG uptake was common (32 %) in the preoperative FDG PET/CT scan (Bats et al. 2011).

The supradiaphragmatic LNMs detected with PET/CT are reported to locate in the parasternal, supraclavicular, axillary and mediastinal LNs (Risum et al. 2010, Fruscio et al. 2013). Some of the detected superficial LNMs have been confirmed with biopsy. The intrathoracic nodes, however, are not reachable with US-guided biopsy and surgical procedures to obtain histological confirmation are generally considered too invasive. The lack of histological verification is a common limitation on studies concerning the accuracy of PET/CT in supradiaphragmatic lesions.

The prognostic relevance of the distant metastases found with PET/CT but not with conventional CT is not fully established. Risum et al. (2010) reported a small difference in survival in PET/CT stage III and IV patients in their material. However, in the multivariate analysis the residual tumor after surgery was the only statistically significant prognostic factor (Risum et al. 2010, Risum et al. 2011). The study of Bats et al. (2012) with patients with increased mediastinal FDG uptake at the time of diagnosis reached a similar conclusion. In Fruscio et al. (2013) study, PET/CT stage IV patients had lower response to standard chemotherapy than stage III patients. The prognoses of the patient groups did not differ significantly, and the authors concluded that the intra-abdominal disease is more likely to lead patients to death.

**Table 9.** Studies on FDG PET/CT in EOC primary staging. From studies that also concern differential diagnostics of pelvic mass, only the patients with EOC are included. \* Results of FGD PET were combined with information of abdominopelvic CT taken in a different session.

| Study                   | N  | Modality | Comparative imaging methods | T staging  | N staging   | M staging   | Reference standard  |
|-------------------------|----|----------|-----------------------------|--|---|---|---|
| <b>Castellucci 2007</b> | 32 | PET/CT   | CT                          | 9 pt's miss-staged between stages IA-IIIc, overall concordance 69%               |   | 5 stage IV (liver, pleura, mediastinum, supraclavicular LNM) CT found 2       | Surgical, histopathology                                    |
| <b>Kitajima 2008</b>    | 40 | PET/ceCT | CT                          | Lesion-based sens. 62%, spes. 97% inside pelvis                                  | Nodal-based sens. 81%, spes. 98% (sens. better than CT) | Patients with apparent distant metastases were treated with NACT and excluded | Surgical, histopathology                                    |
| <b>Risum 2010</b>       | 66 | PET/ceCT | -                           |  |   | 41% of stage III pt's upstaged to stage IV, 23 due to distant LNM             | Surgery, No histological verification of distant metastases |
| <b>Nam 2010</b>         | 91 | PET/ceCT | CT, MRI                     | Sens. 95%, spes. 83% (equal to CT) overall concordance 78%                       | Sens. 84%, spes. 93% (sens. better than CT)             | 15 unpredicted supra-abdominal LNM, 5 biopsy confirmed                        | Surgical, histopathology                                    |
| <b>De Iaco 2011</b>     | 40 | PET/CT   | -                           | Sens. 79% , spes. 68% for peritoneal disease                                     | -   | -   | Laparoscopy with biopsies                                   |
| <b>Fruscio 2013</b>     | 95 | PET/CT   |                             |  |   | PET/CT upstaged 25 pt's to stage IV, 16 due to supradiaphragmatic LNM         | Surgery, No histologic verification of distant metastases   |
| <b>Dauwen 2013</b>      | 56 | PET/ceCT | CT                          | Generally lower sens., higher spec. than CT in different intra-abdominal regions | Sens. 50% (CT 47%)                                      | 9 pt's upstaged to stage IV (4 biopsies, 5 extensive radiological LNM)        | Surgery, histology, follow-up                               |
| <b>Michielsen 2014</b>  | 32 | PET/ceCT | CT, WB-DW-MRI               | DW-MRI better than PET/CT and CT in peritoneal staging                           | Equal to DW-MRI in accuracy, better than CT             | Equal to DW-MRI, better than CT in thoracic lymphadenopathies                 | Surgical, histopathology, DW-MRI when biopsy not possible   |

### 2.2.3 Evaluation of operability

When the disease is inoperable at the time of diagnosis, the patient can be treated with neoadjuvant chemotherapy and interval debulking surgery. A non-invasive method for operability assessment would save patients from undergoing useless surgical explorations.

Several studies have focused on the identification of specific radiological predictors of suboptimal debulking. The significance of CT features such as peritoneal thickening; tumor implants  $\geq 2$  cm in the peritoneum, bowel mesentery, diaphragm, liver or porta hepatis; suprarenal para-aortic lymph nodes ( $\geq 1$  cm); extension of omental tumor to spleen, stomach, or lesser sac; and pelvic sidewall disease has been assessed (Bristow et al. 2000, Dowdy et al. 2004, Axtell et al. 2007, Ferrendina et al. 2009). Bristow et al. (2000) presented a predictive index score including 13 different radiological parameters for the identification of patients likely to undergo suboptimal debulking. Unfortunately, this scoring system could not be validated in subsequent multicenter studies (Axtell et al. 2007, Gemer et al. 2009). Overall, the wide range of the use of different CT parameters makes these studies hard to compare with each other.

More recently, also PET/CT predictors for operability have been searched. In Risum and co-workers study (2008) the presence of large bowel mesentery implants on PET/CT predicted incomplete debulking. Fruscio et al. reported that FIGO stage III patients who were upstaged to stage IV by PET/CT had lower optimal cytoreduction rates than the patients whose disease was limited to abdomen also by PET/CT (Fruscio et al. 2013b).

### 2.2.4 Treatment response evaluation

The radiological assessment of the change in the tumor burden is important in the clinical evaluation of treatment response. CT is the most commonly used method, since it is widely available, reproducible and cost-effective. MRI is also a useful method. Abdominal and transvaginal sonography is operator-dependent and the techniques and measurement directions can vary between examinations.

RECIST guideline (Response Evaluation Criteria in Solid Tumors) is used in clinical trials to evaluate the radiological response to chemotherapy. The patients are classified into four categories by their response to treatment: complete responders, partial responders or as having stable disease or progression. RECIST was published in 2000 and since then many investigators and authorities have adopted it in the assessment of treatment outcome (Eisenhauer et al. 2009). The new version, RECIST 1.1, was published in 2009. The role of the functional imaging, i.e., FDG PET/CT and DW-MRI was discussed within the expert group. The consensus, however, was to still lean

on anatomical imaging. The only exception was to include FDG PET/CT as an adjacent tool for detecting progressive disease. Larger trials to determine the role of functional imaging in the treatment response evaluation are needed.

Anatomical imaging is insufficient, particularly when evaluating response to newer cancer therapies that aim at stabilizing the disease. PERCIST is a proposed guideline for PET imaging in the treatment response evaluation (Wahl et al. 2009). It includes standards for PET protocol and for assessing normal reference tissue values. In addition, it integrates the PET measurements with RECIST criteria. At least 30 % decline of SUV measurements is proposed to be a cut-off value for a medically relevant therapy response (Wahl et al. 2009).

So far, only a few studies handle PET or PET/CT in the treatment response evaluation of EOC primary treatment. These studies are more focused on predicting the treatment response than on the response assessment. In preliminary reports the percent change of SUVmax during NACT seems to correlate with histopathological response (Nishiyama et al. 2008, Martoni et al. 2011). In a study with 33 patients treated with NACT, over 20 % decline of SUVmax in FDG PET scan made after first chemotherapy cycle was more accurate than CA 125 or histopathological response criteria in predicting the patient outcome (Avril et al. 2005).

## 2.3 HE4 IN EOC PRIMARY TREATMENT

Human epididymis protein 4 (HE4) was first discovered in human epididymis (Kirchhoff et al. 1991). It is a small-sized secreted protein belonging to the “four disulfide” acid protein group. In addition to the male reproductive system HE4 is expressed in various normal tissues such as trachea and salivary gland and to less extent in lung, prostate, thyroid and kidney (Galgano et al. 2006). Normal ovarian epithelial tissue does not express HE4. Drapkin et al. (2005) found that the HE4 gene was overexpressed in EOC, especially in serous (93 %) and endometrial subtypes (100 %). Modest expression is also found in adenocarcinoma of the lung, breast, transitional cell and pancreatic carcinoma (Galgano et al. 2006).

### 2.3.1 EOC diagnostics

Cancer antigen 125 (CA 125) is the most commonly used and most studied serum biomarker in ovarian cancer. Serum CA 125 level is elevated in 80 % of the patients with serous epithelial ovarian cancer, more commonly in the advanced-stage disease. When examining patients with a pelvic mass, CA 125 can be combined with ultrasound to separate benign and malign tumors. Low specificity of CA 125, however, limits its use in differential diagnostics. CA 125 is often elevated also in benign

gynecological conditions like endometriosis and in non-neoplastic diseases such as heart failure and lung disease (Miralles et al. 2003, Bedaiwy and Falcone 2004).

HE4 is a promising serum marker in differential diagnostic of women with pelvic mass (Moore et al. 2008, Plebani et al. 2012). In endometriosis HE4 increased less often than CA 125 (Huhtinen et al. 2009). A ROMA algorithm, that combines the patient's menopause status, CA 125 and HE4 values, has been reported to outperform the commonly used Risk of Malignancy Index (RMI) (Moore et al. 2010). In an independent validation study of Van Gorp et al. (2011), HE4 and ROMA did not increase the detection of malignant disease compared with CA 125 alone. More recently Sandri et al. (2013) reported that in postmenopausal patients ROMA index had better accuracy in differentiating benign and malignant ovarian masses than CA 125.

Since EOC is often symptomless at the early, curable stage, a lot of attention is paid to the early detection and screening of EOC. Most studied screening algorithms include serial CA125 measurements and ultrasound examinations (Menon et al. 2005). In the future, multimarker assays combining CA 125, HE4 and other markers may be used in the early detection of EOC (Yurkovetsky et al. 2010). A recent study of Karlan et al. (2014) found HE4 useful in the secondary screening of the high-risk patients with a rising CA 125 at primary screening.

While HE4 is on the threshold of becoming a widely used serum marker in EOC clinical treatment, it must be kept in mind that also many non-neoplastic factors influence the serum HE4 level. HE4 concentrations increase with age (Bolstad et al. 2012, Park et al. 2012, Moore et al. 2012, Urban et al. 2012). Age-specific thresholds for HE4 have been suggested (Urban et al. 2012). Elevated HE4 concentrations are seen in chronic kidney disease with different levels of impairment of renal function (Nagy et al. 2012). Bolstad et al. (2012) reported an increasing trend of HE4 levels even when creatine level increased within physiological limits. Smoking elevates the HE4 levels by 20–30 % (Bostad et al. 2012, Urban et al. 2012). In addition, various diagnostic thresholds are applied in different HE4 studies. The comparability of results obtained by different commercially available assays must be confirmed (Ferraro and Panteghini 2014).

### **2.3.2 In predicting operability and survival**

The level of CA 125 at the time of diagnosis has been compared with surgical results and patient outcomes. In a study of Cooper et al. (2002), preoperative CA 125 level was an independent prognostic factor for survival but not for optimal cytoreduction. On the other hand, Latimer et al. (1996) reported that the initial CA 125 level did not correlate with survival in the patients whose levels dropped to normal limit during primary chemotherapy.

Some studies concerning advanced EOC patients treated with NACT have also been published. The usefulness of CA 125 in predicting complete debulking in IDS was studied by Rodriguez et al. (2012) and Furukawa et al. (2013). In these studies Pre-IDS CA125 values predicted surgical outcome in IDS but CA 125 values at the time of diagnosis did not. Pre-IDS values such as CA 125 <100 (Rodriguez et al. 2012) and CA 125 <20 (Furukawa et al. 2013) have been reported to be associated with no residual tumor in IDS. Complete debulking rates in these studies were 47.5 % and 61.3 %, respectively.

The usefulness of HE4 as a predictor for operability has been studied recently. In a multicenter study with 275 primary EOC patients, the preoperative serum HE4 level predicted optimal cytoreduction in PDS (Braicu et al. 2013). Sensitivity and specificity improved when CA 125 was combined with HE4. Angioli et al. (2013) reported that the combination of preoperative HE4 <262 pmol/l and ascites <500 ml predicted complete cytoreduction in PDS with the sensitivity of 100 % and the specificity of 89.5 %. At the same time, Yang and al. (2013) reported that with the cut-off value of 600 pmol/l, HE4 predicted incomplete cytoreduction with 77 % sensitivity and 32 % specificity. One reason for the different results in the studies concerning operability is the differences in surgical approaches between institutions.

### **2.3.3 In treatment response evaluation**

If CA 125 is elevated at the time of EOC diagnosis, it is commonly used to monitor the treatment response. In clinical trials analyzing new therapeutic agents, CA 125 and radiological RECIST criteria are used to define the treatment response. According to GCIG recommendation, both RECIST criteria and the decrease of CA 125 to less than 35 IU/L must be achieved in order to consider the patient as a complete responder (Eisenhauer et al. 2009, Rustin et al. 2011).

The change of the CA 125 level during primary chemotherapy has been studied as a predictive factor for patient outcome. Several multivariate analyses have demonstrated that CA 125 half-life and nadir concentrations are independent prognostic factors for recurrence (van der Burg et al. 1988, Riedinger et al. 2006). For patients who reach complete response to primary therapy, a rapid normalization of CA 125 by the third cycle of chemotherapy predicts longer progression-free survival (PFS) and overall survival (OS) (Rocconi et al. 2009). The fast decline of CA 125 to less than 35 IU/L over initial 2 cycles is an independent predictor of survival also in suboptimally debulked patients (Markman et al. 2006).

Serum HE4 is elevated at the time of diagnosis in most, particularly serous EOC cases. HE4 level seems to decrease during chemotherapy treatment but its behavior has not been evaluated in detail. In a recent study with 76 patients, HE4 level > 70 IU/L at the third chemotherapy cycle seemed to predict platinum resistance (Angioli et al. 2014).

### **2.3.4 In follow-up**

When EOC relapses, CA 125 often rises before the patient has symptoms or detectable clinical or radiological findings appear (van der Burg et al. 1990, Rustin et al. 1996). The use of serial CA 125 measurements during follow-up of symptomless patients is, however, controversial. A randomized trial with 529 patients showed no survival benefit with early treatment of asymptomatic patients (Rustin et al. 2010). If CA 125 is measured during follow-up, the treatment options and philosophy must be discussed thoroughly with the patient. Many clinicians wait for symptoms to appear before starting the treatment of the relapse with cytotoxic agents.

In addition to CA 125, serum HE4 is the only other FDA-approved biomarker for EOC follow-up. Yet, so far there have been few studies focusing on HE4 in EOC monitoring (Piovano et al. 2014). In the first prospective trial with 34 patients with EOC relapse, Plotti et al. (2012) concluded that adding HE4 to CA 125 improves the detection of recurrence. Combining CA 125 and HE4 at the cut-off of 70 pmol/l, the sensitivity to detect recurrent OC was 76 % with a specificity of 100 % (Plotti et al. 2012). In small patient materials with serial serum HE4 measurements, HE4 appears to be comparable to CA 125 and in some patients it begins to rise earlier than CA 125 (Anastasi et al. 2010, Manganaro et al. 2013). The clinical impact of HE4 in EOC follow-up is not established.

### 3 AIMS OF THE STUDY

The objective on this prospective study on primary EOC was to investigate new clinically relevant methods for primary EOC staging and treatment response evaluation. The main focus was on preoperative assessment of advanced EOC with PET/CT imaging.

The specific aims were:

1. To study FDG PET/CT in radiological staging of advanced EOC with special focus on the presence and distribution of extra-abdominal lymph node metastasis.
2. To compare the surgeons' visual estimation of intra-abdominal tumor spread at primary and interval surgery to histopathological samples. The study was done in order to form a surgical reference standard for imaging results.
3. To compare integrated FDG PET/contrast-enhanced CT and contrast-enhanced CT in EOC pre-treatment imaging: staging and assessment of intra-abdominal tumor load.
4. To describe the profile of the novel serum marker HE4 during EOC primary chemotherapy in order to evaluate its potential for further studies of EOC prognosis.

## 4 PATIENTS, MATERIALS AND METHODS

From October 2009, a prospective clinical trial for EOC patients has been conducted at the Department of Obstetrics and Gynecology, Turku University Hospital, Finland. The Ethics committee of the Intermunicipal Hospital District of Southwestern Finland approved the study protocol and it was registered in ClinicalTrials.gov (Identifier: NCT01276574). The data in present studies include patients recruited from October 2009 to March 2012.

All patients with suspected advanced ovarian, fallopian, or peritoneal cancer on the basis of physical examination, ultrasound, and with the presence of ascites or increased CA 125 antigen level were eligible to participate in this prospective clinical trial. Exclusion criteria included age > 80 years, diabetes mellitus and history of previous cancer. The study was presented to eligible patients at the time of the pre-treatment visit. Patients willing to participate allowed their written informed consent. A summary of patient data in studies I-IV is presented in **Table 10**.

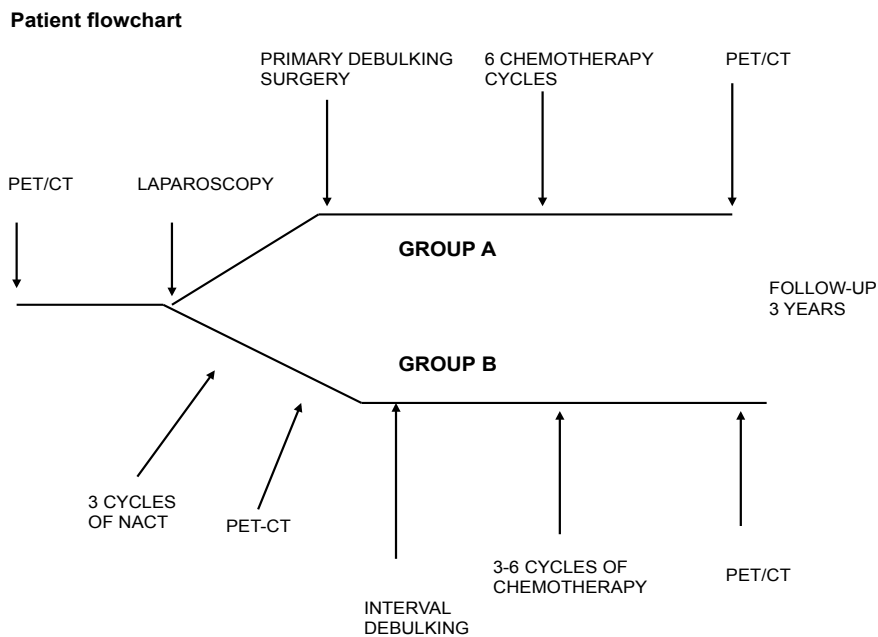
**Table 10.** Patients. The FIGO staging system used in the studies was accepted in 2006.

|                       | Study I            | Study II           | Study III          | Study IV           |
|-----------------------|--------------------|--------------------|--------------------|--------------------|
| Number of subjects    | 30                 | 39                 | 41                 | 11                 |
| Age<br>median (range) | 64 (48-79)         | 65 (48-79)         | 65 (45-79)         | 67 (52-79)         |
| FIGO stage            |                    |                    |                    |                    |
| I                     | 0                  | 0                  | 2                  | 0                  |
| II                    | 1                  | 2                  | 2                  | 0                  |
| III                   | 17                 | 21                 | 21                 | 7                  |
| IV                    | 12                 | 16                 | 16                 | 4                  |
| Histology             |                    |                    |                    |                    |
| Serous                | 25                 | 35                 | 37                 | 9                  |
| Mucinous              | 2                  | 1                  | 1                  | 0                  |
| Clear-cell            | 1                  | 1                  | 1                  | 1                  |
| Endometrioid          | 1                  | 1                  | 1                  | 0                  |
| Undifferentiated      | 1                  | 1                  | 1                  | 1                  |
| Origin                |                    |                    |                    |                    |
| Ovary                 | 27                 | 34                 | 35                 | 10                 |
| Fallopian tube        | 1                  | 2                  | 2                  |                    |
| Peritoneal surface    | 2                  | 3                  | 4                  |                    |
| Unknown               |                    |                    |                    | 1                  |
| Treatment (PDS/NACT)  | 16/14              | 19/20              | 21/20              | 7/4                |
| Recruitment time      | 10/2009-<br>7/2011 | 10/2009-<br>3/2012 | 10/2009-<br>3/2012 | 10/2009-<br>6/2010 |

## 4.1 STUDY DESIGN

The flowchart of the treatment of the patients and the imaging studies are presented in **Figure 2**. The treatment consisted of either primary surgery followed by 6 cycles of platinum- and taxane-based chemotherapy (group A) or 3 cycles of NACT followed by IDS and 3–6 additional chemotherapy cycles (group B). The treatment modality for each patient was determined based on the surgeon's estimation of operability in diagnostic laparoscopy or laparotomy. In laparoscopy, Fagotti scoring system (Fagotti et al. 2006, 2008) was used for decision. The surgeon was also aware of the results of the preoperative imaging studies.

Contrast enhanced FDG PET/CT (PET/ceCT) scans were performed within 2 weeks prior to the primary surgery, after the possible NACT and 4 weeks after the completion of the primary chemotherapy. If disease relapse was suspected during the follow-up period due to clinical symptoms, physical examination, rising pattern of CA 125 level or routine CT examination, the situation was evaluated with PET/ceCT.



**Figure 2.** Patient flowchart.

### 4.1.1 Principles of PET/CT

Positron emission tomography is an imaging method that uses short-lived radioisotopes attached to tracers and produces a three-dimensional image of functional processes in the body. The system is based on the detection of pairs of gamma rays, which are

emitted indirectly by positron-emitting radionuclide. The tracer is chemically incorporated into the biologically active molecule and injected intravenously to the patient. After the IV injection of the tracer, the active molecule concentrates into the tissues of interest. The waiting period varies between different tracers.

The radioisotope emits a positron, which travels a short distance, usually less than one mm in the tissue. The encounter of positron and electron produces annihilation photons that can be detected with the scanning device. The detectors of a PET scanner are arranged in a ring-shaped patterns around the patient. The data is sent further to the processing unit. The image reconstruction is made using various mathematical models, and the attenuation corrections are made.

PET allows for the recording of the data in three dimensions. Thin (3–4 mm) tomograms can be recorded and displayed in coronal, transverse and sagittal planes. Quantitative assessment of the change of the tracer uptake in lesions of interest is particularly useful in the follow-up studies with repeated PET scans. Standardized uptake value (SUV) is a measurement unit in which the concentration of a radiotracer in a defined region divided by an injected dose normalized most often for the patient's body weight.

#### **4.1.1 FDG (fluorodeoxyglucose)**

Radiotracer is a compound where the short-lived radioisotope is incorporated with the matters normally used by body such as glucose. Also molecules, which bind to the receptors, can be labeled in order to study, for example, the sites of drug action. The most commonly used tracer is  $^{18}\text{F}$ -FDG, a glucose analogue labeled with fluorine-18.

The two alternative ways of how glucose is metabolized in cells have been known since the 1930s. Otto Warburg recognized that glucose can be metabolized either by combination of oxygen, oxygenation, or by glycolysis (Miles and Williams 2008). The latter is essential for the metabolism of the cancer cells. A high glycolytic rate, activated glucose transporters and regionally raised blood flow in many tumors make the cancer cells visible in FDG PET scan (Bomanji et al. 2001).

FDG is a non-specific tracer and it also detects inflammatory and infective sites in addition to malignant tumors. Normal physiological FDG-uptake patterns must also be taken into account when interpreting scans. These include, for example, diffuse or focal uptake of thyroid, breast tissue of premenopausal women and bowel activity (Prakash et al. 2010). FDG is excreted via kidneys and a large amount of activity is seen in the renal collecting system and bladder. A drawback of this tracer is that some subtypes of the ovarian cancer can be falsely negative due to the inherent low metabolic rate of these tumors. Further, micrometastases cannot usually be detected with the PET method reliably.

## 4.2 FDG PET/CT SCANNING PROCEDURE (STUDIES I AND III)

Preoperative whole body contrast-enhanced FDG PET/CT (64-row Discovery STE or VCT, General Electric Medical Systems, Milwaukee, WI, USA) was performed within 2 weeks before open laparoscopy or laparotomy. All patients fasted for a minimum of 6 h and their serum glucose was controlled before the intravenous injection of 4 Mbq/kg <sup>18</sup>F-FDG. They were asked to void before the scan started. Low-dose PET/CT (kV 120, Smart mA range 10–80) from skull base to mid-thigh was performed after 50–60 min from injection. This was followed with a whole body diagnostic contrast-enhanced CT (ceCT) scan (kV 120, Smart mA range 100–440) after an automated intravenous injection of contrast agent. Oral contrast agent was not used. Transaxial, coronal and sagittal images were reconstructed with 128x128 matrix size in fully 3D mode using ML-OSEM reconstruction algorithm. The follow-up scans were performed with the same scanner as the preoperative scans. The time interval from injection to scanning was the same within 10 minutes.

## 4.3 IMAGING ANALYSIS (STUDIES I AND III)

Imaging analysis was performed using ADW 4.5 workstation. Integrated FDG PET/ceCT images were interpreted by two experienced nuclear medicine physicians. They were blinded to the surgical findings. The analysis was performed visually and semi-quantitatively. The mean and maximum standard uptake values (SUVs) were calculated from the lesions with focal tracer accumulation and defined as the ratio of activity per milliliter of tissue to the activity in the injected dose corrected by decay and by the patient's body weight in the volume of interest (VOI). VOIs were placed around the regions of increased FDG uptake. Cut-off threshold from the SUVmax was 42 %. Any focal tracer accumulation was interpreted as a pathological finding, although readers took into account and excluded the most typical physiological FDG accumulations from analysis. Lymph nodes with increased glucose uptake were considered positive for metastatic spread regardless of their size. The SUVmax values of the lymph nodes were registered, but no threshold level to differ benign and malignant nodes was used.

Two experienced radiologists, who had no knowledge of either the PET/CT scans or findings at surgery, evaluated ceCT images, which were viewed in coronal, axial, and sagittal sections and appropriate windowings were applied.

Peritoneal implantation was diagnosed when nodular, plaque-like, or infiltrative soft tissue lesions or abnormal enhancement were seen in the peritoneal fat or on the peritoneal surface. Omental invasion was diagnosed when there was an infiltrative, nodular, or cake-like appearance of enhanced soft tissue in the omentum. Invasion of the bowel was diagnosed when irregular thickening or distortion with abnormal

enhancement was registered in the bowel wall. LNs with a short-axis diameter greater than 10 mm were generally defined as malignant (Kitajima et al. 2008). Furthermore, regardless of the node size, the presence of central necrosis and multiple round-shaped nodes in an unusual anatomical area were considered a sign of malignancy, and the presence of fatty hilum was considered a benign sign.

All the imaging data were collected into similar detailed worksheets to enable precise comparison between the two imaging methods and surgical findings. Contrast enhanced CT and PET/ceCT evaluations included the systematic assessment of 22 intra-abdominal anatomical sites and 18 sites outside the peritoneal cavity. In case there were multiple findings in the observed area, the two largest lesions were measured and those SUVmax and SUVavg values were calculated. A translated version of the worksheet used is presented in **Appendix I**. It joins together surgical and radiological parameters.

#### 4.4 ESTIMATION OF DISEASE EXTENT AT SURGERY (STUDIES I, II AND III)

The operation started with open laparoscopy (34 patients) or laparotomy (8 patients). The possible ascites was removed and abdominal cavity was inspected organ by organ. Visual estimation by the surgeon was performed by consensus of two operators and each region was categorized as macroscopically malignant, healthy/benign appearing (e.g. postoperative adhesions, liver cyst) or not evaluable. In case of conflict between visual estimation and the biopsy results, the histopathological results were chosen as the reference standard for imaging studies. The surgeons were aware of the findings in preoperative PET/ceCT. Tumor lesions detected in PET/ceCT were searched in surgery and biopsied, if possible.

The data were collected into detailed worksheets with similar categorization of anatomical areas, which were used in imaging analyses (**Appendix I**). An assisting nurse filled the form during the operation according to the surgeon's instructions.

When laparoscopy was immediately followed by laparotomy, the abdominal cavity was re-evaluated in order to define the visual findings in laparoscopy.

#### 4.5 HISTOPATHOLOGICAL SAMPLES

In both PDS and IDS, the primary tumor, the removed lymph nodes and the samples from all removed tumor specimens were sent to histopathological analysis. In addition, at the beginning of the operation, multiple biopsies were obtained from areas defined visually as carcinomatous or healthy tissue.

#### 4.6 SERUM SAMPLE COLLECTION (STUDY IV)

Serum samples were collected before the primary surgery, a day before every chemotherapy cycle and a day before the interval debulking surgery (7–10 times). During follow-up, serum samples were drawn every 3–6 months until disease relapse or for 3 years.

The CA 125 samples were analyzed for clinical use by TYKSLAB within a day from the sample collection. The serum samples for HE4 analysis were collected into non-heparinized tubes and centrifuged for 15 minutes at 3000 rpm (800g) after a 30-minute incubation at room temperature. They were stored at -80°C and analyzed later.

#### 4.7 CA 125 AND HE4 ANALYSES (STUDY IV)

Cancer antigen 125 concentrations were analyzed using the electrochemiluminescence immunoassay (ECLIA) method (Modular E170 automatic analyzer, Roche Diagnostic GmbH, Mannheim, Germany). A cancer antigen 125 level of less than 35 IU/L was used as the normal cut-off value. HE4 concentrations were analyzed by enzyme-linked immunosorbent (EIA) assay (Fujirebio Diagnostics Inc, Malvern, PA, USA) according to the manufacturer's instructions. An HE4 level of <140 pmol/l for healthy postmenopausal women was considered normal.

#### 4.8 TREATMENT RESPONSE EVALUATION (STUDY IV)

The profiles of the serum markers HE4 and CA 125 were compared against the patient's radiological response to first-line chemotherapy. The chemotherapy response was defined according to Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 criteria (Eisenhauer et al. 2009). The patients were classified as complete responders (CR), partial responders (PR), as having stable disease (SD) or progressive disease (PD). Four patients were treated with NACT and an interval debulking attempt. Their metabolic response to NACT was evaluated with FDG PET/CT. The lesion with the lowest change in the FDG uptake was used for metabolic response evaluation.

#### 4.9 STATISTICAL ANALYSES

Statistical analyses were performed using SAS 9.2 software (SAS Institute Inc., Cary, NC, USA) (I), SAS 9.3 software (SAS Institute Inc., Cary, NC, USA) (III) and IBM SPSS statistics software (version 20) (II).

In study I, the characteristics of the patients with and without LNM above the diaphragm in PET/CT were compared using Fisher's exact test and Wilcoxon two-

sample test for continuous variables. A P-value  $<0.05$  was considered statistically significant.

In study II, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of the surgeons' visual estimation were calculated for primary surgery and in interval surgery. Histopathological results served as reference standards. The findings obtained in primary and interval surgeries after NACT were compared using  $\chi^2$  test and Fisher's exact test when appreciable. A P-value  $<0.05$  was considered significant.

In study III, the sensitivity, specificity, accuracy and positive (PPV) and negative predictive values (NPV) were calculated for hybrid PET/CT and contrast-enhanced CT using operative and histopathological findings as the reference standards. The utility of imaging methods was studied on both per-patient and per-area bases. The results of PET/CT and CT were compared with the McNemar test. The retroperitoneal LNs were assessed on a per-nodal-station basis and compared with the Durkalsky McNemar test. Only the patients with a histological reference standard from LNs were included in the LN analysis. For extra-abdominal areas not detectable during surgery, PET/CT and CT were compared using the kappa coefficient and positive agreement between the imaging studies was determined.

In study IV, only descriptive comparisons of HE4 and CA 125 profiles were made. The small amount of patients did not allow us to make statistical calculations.

## 5 RESULTS

### 5.1 THE PRESENCE OF SUPRADIAPHRAGMATIC LYMPH NODE METASTASES (STUDY I)

The preoperative FDG PET/ceCT suggested supradiaphragmatic LNM in 20 of the 30 examined patients. Seven of these patients were classified to stage IV by FIGO staging system. Contrast-enhanced CT revealed supradiaphragmatic disease in 10/30 patients. The anatomical distribution of the PET-positive lymph nodes is presented in **Table 11**. Histological verification was obtained from four patients, three with PET-positive axillary LNs and one with suspicious subclavian LN. All the biopsied LNs were confirmed to be metastatic. The abnormal tracer accumulation was as common in parasternal LNMs as in the cardiophrenic angle, a generally known location for EOC metastasis (Figure 3).

The patients with supradiaphragmatic LNM detected with PET/ceCT had significantly more ascites ( $p=0.011$ ), higher pre-treatment CA 125 values ( $p=0.013$ ) and more frequently carcinomatosis in the subdiaphragmal peritoneal surface in the operation ( $p=0.033$ ). Instead, the primary tumor's metabolic activity, histological grade or the presence of pleural fluid were not associated with supradiaphragmatic LNM.

Follow-up PET/CT was available in 19/20 patients. Five patients with complete response according to RECIST 1.1 criteria demonstrated 8 metabolically active supradiaphragmatic LNs in the pre-treatment PET/CT, and only one LN remained active after chemotherapy. Similarly in 8 partial responders, 11/20 LNs remained positive during chemotherapy and in 6 patients with disease progression, 12/21 LNs remained positive and one new active LN appeared.

**Table 11.** Anatomical distribution of supradiaphragmatic lymph node metastasis of 20 EOC patients detected with pre-treatment PET/ceCT and contrast-enhanced CT. The Lymph node size is the maximum short diameter. Modified from Hynninen et al. 2012.

| <i>Lymph node site</i>       | <b>Patients with PET-positive LNs</b> | <b>Patients with abnormal LNs in CT</b> | <b>Size of PET- positive LNs (mean) (mm)</b> | <b>SUVmax</b> | <b>Biopsy confirmation</b> |
|------------------------------|---------------------------------------|---|--|---------------|----------------------------|
| Supradiaphragmatic           | 20                                    | 10                                      |  |               |                            |
| cardiophrenic                | 14                                    | 6                                       | 8–28   | 1.1–5.7 (3.7) |                            |
| parasternal                  | 14                                    | 1                                       | 4–11   | 2.7–4.3 (3.2) |                            |
| middle/posterior mediastinal | 8                                     | 4                                       | 5–22   | 2.3–7.5 (4.2) |                            |
| axillary                     | 6                                     | 1                                       | 6–14   | 1.3–6 (3.3)   | 3                          |
| subclavian                   | 1                                     | 1                                       | 20   | 5.2           | 1                          |
| more than 1 site             | 14                                    | 3                                       |  |               |                            |

## 5.2 THE RELIABILITY OF PERIOPERATIVE VISUAL ESTIMATION OF TUMOR EXTEND (STUDY II)

220 samples collected from the primary surgery and 92 samples from the interval surgery after NACT were compared with the histopathological results. The results are presented in **Table 12**. The sensitivity and accuracy to visually distinguish cancer from healthy tissues were better in primary surgery than after NACT, whereas the specificity was similar. Microscopically carcinomatous areas had benign visual appearance more often after NACT than at primary surgery.

**Table 12.** Comparison between the visual assessment by surgeons and the histopathological results. Multiple anatomical regions in the peritoneal cavity were evaluated at the beginning of the operation. Histopathology of simultaneous biopsies served as a reference standard. PPV=positive predictive value, NPV=negative predictive value, FPR=false positive rate, FNR=false negative rate. Adapted from Hynninen et al. 2013a.

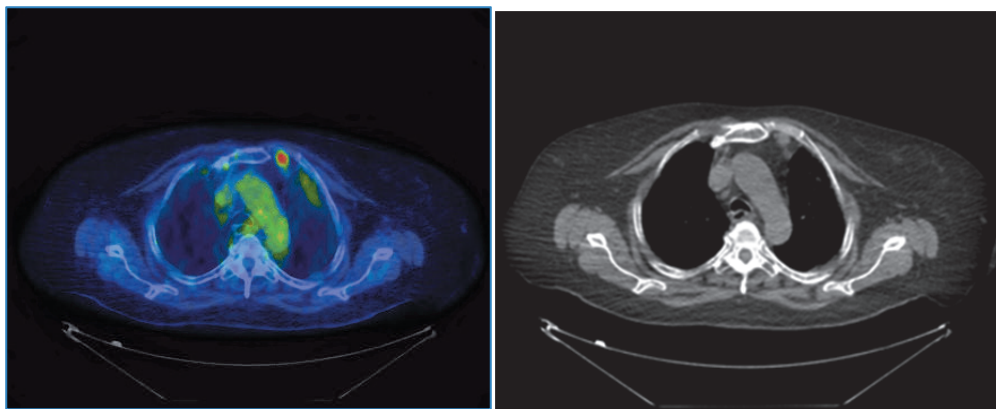
|   | Primary surgery<br>(n=39) | Interval surgery<br>after NACT (n=16) | Primary vs.<br>interval surgery (p) |
|---|---------------------------|---------------------------------------|-------------------------------------|
| Evaluated samples in<br>the peritoneal cavity | 220                       | 92                                    |                                     |
| Sensitivity                                   | 98% (191/195)             | 86% (61/71)                           | 0.0004                              |
| Specificity                                   | 76% (19/25)               | 76% (16/21)                           | 1.000 (NS)                          |
| Accuracy                                      | 95% (210/220)             | 84% (77/92)                           | 0.001                               |
| PPV   | 97% (191/197)             | 92% (61/66)                           | 0.150 (NS)                          |
| NPV   | 83% (19/23)               | 62% (16/26)                           | 0.125 (NS)                          |
| FPR   | 24%                       | 24%                                   |                                     |
| FNR   | 2%                        | 14%                                   |                                     |

## 5.3 PET/CT VS. CT IN EOC STAGING (STUDY III)

Preoperative ceCT suggested extra-abdominal disease spread in 25/41 patients and PET/ceCT in 32/41 patients. The seven patients with findings detected by PET/CT but not by ceCT had metabolically active nodes in the axillar, parasternal, mediastinal and cardiophrenic regions. The most discordant area for suspected metastasis was the parasternal lymph node chain, where metastasis was predicted in 19 patients by PET/CT and in 1 patient by ceCT (**Figure 3**). The parasternal LNM lack histological verification, since they were not reachable with US-guided biopsy. However, all of these patients also had suspected metastasis in other supradiaphragmatic lymph node areas.

Sixteen out of 41 patients had FIGO stage IV disease. The staging system demands a histological confirmation of extra-abdominal lesions. Six patients were upstaged to stage IV due to positive pleural cytology, four due to biopsy-confirmed LNM. In addition, three patients had intraparenchymal liver metastasis and four had tumors in

the umbilicus. In FIGO stage IV patients, abnormal metabolic activity was commonly detected in multiple areas outside the peritoneal cavity.



**Figure 3.** A patient with PET/CT stage IV disease. Abnormal metabolic activity was detected in two parasternal lymph nodes. The larger LN (SUVmax 4.8) was suspicious also in ceCT.

#### 5.4 PET/CT VS. CT FOR THE EVALUATION OF INTRA-ABDOMINAL DISEASE SPREAD (STUDY III)

Data from 21 patients treated with PDS and 20 NACT patients with diagnostic laparoscopy (17) or laparotomy (3) were included in the study. In the overall site-based analysis, the sensitivity for PET/CT and CT was 51 % (237 out of 463) and 41 % (196 out of 484), respectively, whereas the specificity was 89 % (205 out of 230) and 92 % (216 out of 236) and the accuracy was 64 % (442 out of 693) and 57 % (412 out of 720), respectively. PET/CT outperformed CT in sensitivity ( $P=0.017$ ), but in specificity the two imaging methods were equal.

The performance of PET/CT and CT in various intra-abdominal regions was analyzed and combined on per-patient basis. The results are shown in **Table 13**. The sensitivity of PET/CT was superior to CT in subdiaphragmatic peritoneal surface ( $p=0.020$ ), omentum ( $p=0.17$ ), bowel mesentery ( $p=0.001$ ) and large bowel serosae ( $p=0.004$ ). Specificity was generally good with both imaging methods and did not differ significantly.

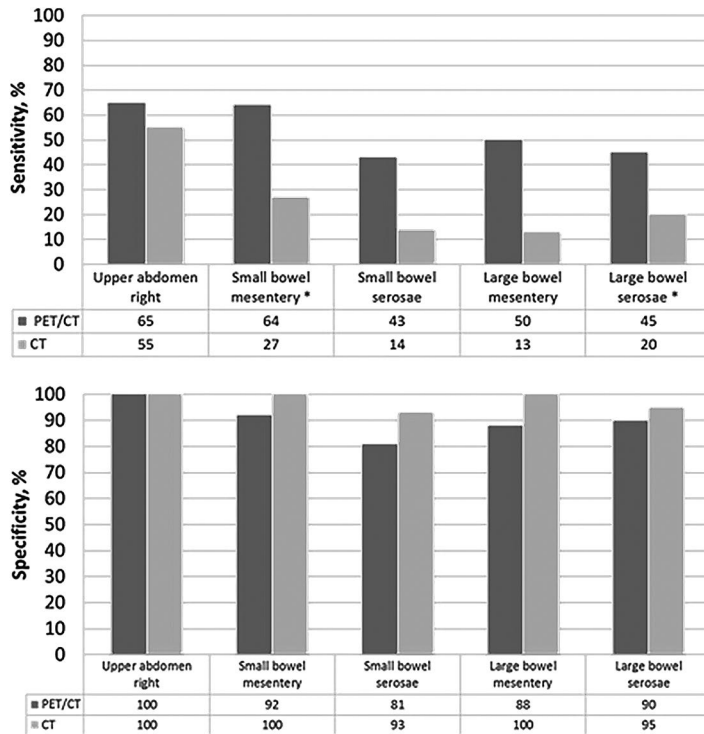
Some critical areas for complete tumor debulking in surgery, the upper abdomen, bowel mesentery and bowel serosae, are presented separately in **Figure 4**. The section “upper right abdomen” consists of measurements of the dorsal diaphragmatic peritoneum and dorsal liver surface. In this area, total tumor resection requires peritonectomy and the great liver veins penetrate the diaphragm nearby. The sensitivity figures in high-risk areas were generally low with both imaging methods and the difference between PET/ceCT and ceCT remained insignificant in the areas demanding extensive upper abdominal surgery. In some cases, the information gained from preoperative PET/CeCT guided the operator to search disease in unusual places (**Figure 5**).

**Table 13.** The sensitivity, specificity and accuracy of hybrid PET/CT and CT for the detection of intra-abdominal disease spread in 41 ovarian cancer patients. Analysis was performed on a per-patient basis. TP=true positive, FN=false negative, TN=true negative, FP=false positive. PPV=positive predictive value, NPV=negative predictive value. The category “High-risk upper abdomen” consists of sites demanding extensive upper abdominal surgery to achieve complete tumor resection. Right=dorsal subdiaphragmatic peritoneum and dorsal liver surface. Left=ventricle, bursa omentalis, spleen and the tail of the pancreas. Modified from Hynninen et al.2013b.

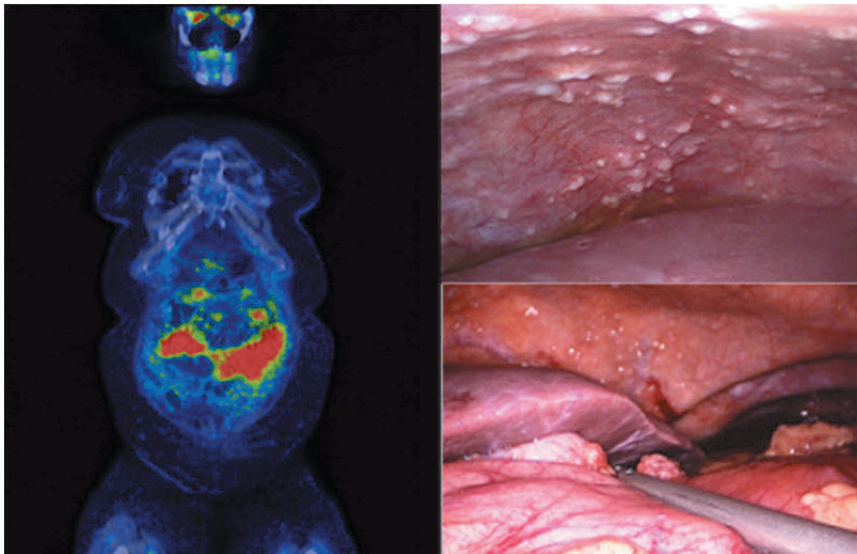
| Site                              | TP              | FN | TN | FP | Sensitivity (%) | Specificity (%) | Accuracy (%) | PPV (%) | NPV (%) | P-value for sensitivity |
|-----------------------------------|-----------------|----|----|----|-----------------|-----------------|--------------|---------|---------|-------------------------|
| <b>Bowel mesentery</b>            | Small intestine |    |    |    |                 |                 |              |         |         |                         |
|                                   | PET/CT          | 16 | 9  | 12 | 1               | 64              | 74           | 94      | 57      | <b>0.020*</b>           |
|                                   | CT              | 7  | 19 | 13 | 0               | 27              | 51           | 100     | 41      |                         |
|                                   | Large intestine |    |    |    |                 |                 |              |         |         |                         |
|                                   | PET/CT          | 15 | 15 | 7  | 1               | 50              | 88           | 94      | 32      | <b>0.005*</b>           |
|                                   | CT              | 4  | 27 | 8  | 0               | 13              | 31           | 100     | 23      |                         |
| <b>Bowel serosae infiltration</b> | Altogether      |    |    |    |                 |                 |              |         |         |                         |
|                                   | PET/CT          | 31 | 24 | 19 | 2               | 56              | 66           | 94      | 44      | <b>0.001*</b>           |
|                                   | CT              | 11 | 46 | 24 | 0               | 19              | 41           | 100     | 31      |                         |
|                                   | Small intestine |    |    |    |                 |                 |              |         |         |                         |
|                                   | PET/CT          | 6  | 8  | 21 | 5               | 43              | 81           | 68      | 72      | <b>0.103</b>            |
|                                   | CT              | 2  | 12 | 25 | 2               | 14              | 93           | 66      | 68      |                         |
| Large intestine together          | PET/CT          | 29 | 35 | 37 | 4               | 45              | 63           | 89      | 51      | <b>0.004*</b>           |
|                                   | CT              | 13 | 53 | 40 | 2               | 20              | 49           | 87      | 37      |                         |
|                                   | Sigmoid colon   |    |    |    |                 |                 |              |         |         |                         |
|                                   | PET/CT          | 19 | 7  | 8  | 2               | 73              | 80           | 91      | 53      | <b>0.001*</b>           |

| Site                             | TP | FN | TN | FP | Sensitivity (%) | Specificity (%) | Accuracy (%) | PPV (%) | NPV (%) | P-value for sensitivity |
|----------------------------------|----|----|----|----|-----------------|-----------------|--------------|---------|---------|-------------------------|
| CT                               | 5  | 20 | 9  | 0  | 20              | 100             | 41           | 100     | 31      |                         |
| Transversum                      |    |    |    |    |                 |                 |              |         |         |                         |
| PET/CT                           | 2  | 18 | 16 | 0  | 10              | 100             | 50           | 100     | 47      | <b>0.564</b>            |
| CT                               | 5  | 18 | 14 | 2  | 22              | 88              | 49           | 71      | 44      |                         |
| <b>Diaphragm</b>                 |    |    |    |    |                 |                 |              |         |         |                         |
| PET/CT                           | 25 | 9  | 6  | 0  | 74              | 100             | 78           | 100     | 40      | <b>0.020*</b>           |
| CT                               | 16 | 18 | 6  | 0  | 47              | 100             | 55           | 100     | 25      |                         |
| <b>Omentum</b>                   |    |    |    |    |                 |                 |              |         |         |                         |
| PET/CT                           | 31 | 3  | 6  | 1  | 91              | 86              | 90           | 97      | 67      | <b>0.017*</b>           |
| CT                               | 27 | 7  | 5  | 2  | 79              | 71              | 78           | 93      | 42      |                         |
| <b>"High-risk upper abdomen"</b> |    |    |    |    |                 |                 |              |         |         |                         |
| Right                            |    |    |    |    |                 |                 |              |         |         |                         |
| PET/CT                           | 20 | 11 | 5  | 0  | 65              | 100             | 70           | 100     | 31      | <b>0.317</b>            |
| CT                               | 17 | 14 | 5  | 0  | 55              | 100             | 61           | 100     | 26      |                         |
| Left                             |    |    |    |    |                 |                 |              |         |         |                         |
| PET/CT                           | 3  | 11 | 6  | 2  | 21              | 75              | 41           | 60      | 35      | <b>0.059</b>            |
| CT                               | 8  | 6  | 8  | 0  | 57              | 100             | 72           | 100     | 57      |                         |

\*= difference in sensitivity is statistically significant (p<0.05)



**Figure 4.** The sensitivity and specificity of PET/ceCT and CT in selected intra-abdominal areas important to operability. \*= difference between PET/ceCT and ceCT is statistically significant.

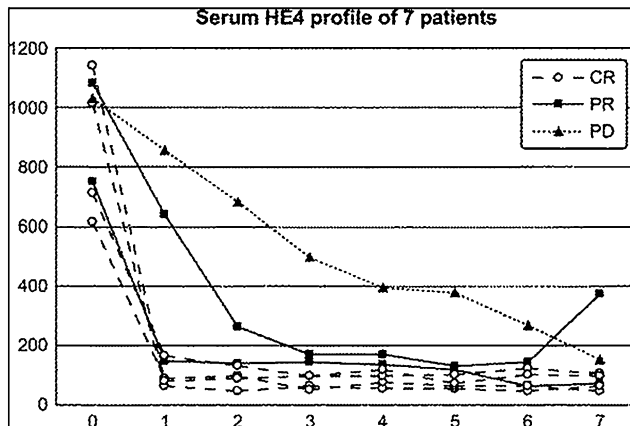


**Figure 5.** The omental metastasis seen in preoperative PET/ceCT reaches to the spleen. A smaller tumor was detected near the gall bladder. In laparoscopy, the patient had extensive disease including subdiaphragmatic carcinosis and a metastatic tumor in the lesser omentum. The tumor is in sight, when the ventricle is pressed down with the instrument. The patient was referred to neoadjuvant chemotherapy.

## 5.5 HE4 PROFILE DURING EOC PRIMARY CHEMOTHERAPY (STUDY IV)

Seven patients treated with PDS and four patients treated with NACT were included in this pilot study. HE4 profiles were pictured with CA 125 for the descriptive comparison of the biomarkers. The two biomarkers generally changed parallel to each other during primary chemotherapy. **Figure 6** presents the HE4 profiles during the primary treatment of the seven patients treated with primary debulking surgery. The patients are divided into 3 groups by their radiological response at the end of chemotherapy; complete responders (4), partial responders (3) and disease progression (1). The HE4 levels seem to decline more rapidly in patients who will achieve complete radiological response at the end of primary treatment.

In four NACT patients, the percent changes of the biomarkers during NACT were compared with anatomical response detected with CT and metabolic change detected with PET/CT. For the two partial radiological responders, HE4 and CA 125 performed similarly. One patient, who had both markers clearly elevated, responded poorly to chemotherapy (stable disease by CT, 23 % decline of SUVmax). HE4 change (-19 %) was better in line with radiological evaluation than CA 124 change (-93 %). The results of this descriptive pilot study could not be statistically evaluated due to small number of patients.



**Figure 6.** HE4 profiles (unit pmol/l) of 7 patients treated with primary debulking surgery and 6 cycles of chemotherapy. 0=pre-treatment value, 1-6=values a day before each chemotherapy cycle, 7= post-treatment value 1–3 months after the 6<sup>th</sup> cycle.

Adapted from Hynninen et al. 2011.

## 6 DISCUSSION

Epithelial ovarian cancer is usually diagnosed in advanced, incurable stage. The development of surgical techniques and chemotherapy regimen has, however, provided the patients prolonged disease-free survival with decent quality of life.

Debulking surgery forms the cornerstone of advanced EOC treatment, but the timing of the surgery is a matter of debate. Accurate information of the disease extent gained from preoperative imaging studies can help treatment planning. A non-invasive method for the operability assessment would save inoperable patients from exposure to useless surgical explorations. Radiological predictors for suboptimal debulking have been searched (Bristow et al. 2000, Dowdy et al. 2004, Axtell et al. 2007, Ferrendina et al. 2009). The wide range of various CT parameters used makes these studies hard to compare with each other and a validated system to predict operability is lacking. Overall, to determine the operability of a patient is a complex issue. In addition to parameters measurable with imaging, the patient's general condition, other illnesses, the surgeon's skills and the treatment policy of the institutions affect the decision when to operate.

Traditionally EOC is thought to have two main spread patterns: intraperitoneal and lymphatic spread to the retroperitoneal LNs. The apparent presence of LNM above the diaphragm is a new finding in recent PET studies and the route of cancer cells to distant LNs is not well established. The posterior route of lymph channels from para-aortic nodes through the diaphragm in aortic hiatus and further to the thoracic duct is well known to gynecologic oncologists who perform staging lymphadenectomies and cytoreductive surgery. Less attention is paid to lymph drainage from the peritoneal cavity. The lymph drains dominantly via small capillaries through the diaphragm reaching collecting lymphatics beneath the pleurae. The lymph nodes at the thoracic aspect of diaphragm were classified by Rouvière in 1932. The lymphatic drainage from the anterior cardiophrenic group continues to the parasternal lymph whereas the lateral cardiophrenic group generally drains to the anterior mediastinal chain.

### 6.1 METHODOLOGICAL CONSIDERATIONS AND STUDY LIMITATIONS

The patient population of the present study consisted mainly of advanced-stage cases. This kind of patient cohort with widely spread disease enabled specially the comparison of sensitivity and the false negative findings of perioperative visual estimation, PET/ceCT and ceCT, but the amount of false positive findings cannot be reliably investigated. For example, omental disease was present in 34/41 patients. Since it was such a common finding, false positive results are unlikely. Positive and negative predicting values are dependent on the incidence of the phenomenon.

We studied the surgeon's visual estimation of intra-abdominal disease in order to evaluate whether it can be used as a reference standard when two imaging methods, PET/ceCT and ceCT were compared. The comparison of the accuracy of the visual evaluation in PDS and IDS was not the original intention. This is a limitation to this prospective study. At the same time, the surgeon's possible views on the ongoing debate concerning the use of NACT in advanced EOC could not interfere with the results in this setting. The visual estimation of tumor spread was formed by consensus of two operators. This increased the reliability of the estimation, but the interobserver variation between the statements of the individual surgeons could not be studied.

The frequency of random biopsies from healthy-appearing tissues was rather low in both PDS (23) and IDS (26). This may have interfered with the results. The low number of random biopsies partly resulted from the fact that the disease was so extensive in many patients. When practically all the peritoneal surfaces were covered with carcinomatous tissue, the number of random biopsies performed was low.

The results on preoperative PET/ceCT and conventional ceCT were compared using detailed worksheet (**Appendix I**). The surgical findings served as reference standard and were gathered in a similar form. The division of abdominal cavity into 22 anatomical sites in the data collection form enabled the study of the performance of the imaging methods on a single tumor lesion level. This is a clear strength of the study: every area was evaluated perioperatively, so all the lesions detected with PET/ceCT or ceCT had their own surgical reference. The downside to this detailed data collection is that in some cases the readers of PET/ceCT and CT may have categorized the same tumor focus into adjacent anatomical areas. The subjective interpretation of the readers may have interfered with the results.

In most patients with primarily inoperable disease, the surgical findings were gathered with a diagnostic laparoscopy. Due to extensive disease and adhesions, some areas were not evaluable in surgery and were left out of the analysis, in which PET/CT and CT were compared. This may have interfered with the results. In addition, inter-observer variability was not studied and this is a limitation to study.

## 6.2 PERIOPERATIVE VISUAL ESTIMATION OF DISEASE EXTENT

In primary surgery, perioperative visual estimation of tumor spread showed 98 % sensitivity, 76 % specificity and 95 % accuracy. These figures convinced us to use the surgical findings as the reference standard when EOC non-invasive staging with imaging was studied. Earlier data on the reliability of perioperative estimation of EOC dissemination underline the importance of inspecting the whole abdominal cavity in laparotomy (Young 1983, Le 2002). More recent retrospective studies have focused on the role of random biopsies in staging of apparent early-stage cancer (Powless et al. 2009, Shroff et al. 2011, Lee et al. 2014). The amount of patients upstaged solely based

on random biopsies of healthy-appearing peritoneum has been reported to be approximately 4 %. Our findings are in line with the conclusion indicating that random biopsies seem to add little to the careful inspection of all peritoneal surfaces in PDS.

Our results indicate that the surgeon's perioperative visual impression of the disease spread is affected by NACT. In cases of primary surgery, the areas positive for microscopic tumor tissue in biopsy samples also had detectable macroscopic tumor (FNR 2 %), leading to removal of the tissue when possible. In contrast, after NACT, 14 % of the focuses with microscopic cancer cells in biopsy specimens had a benign visual appearance in the perioperative assessment, suggesting that based on the visual estimation alone, the surgeons may have left minimal residual tumor in potentially resectable areas.

The goal of debulking surgery is removal of all macroscopic tumor in both PDS and IDS (Chi et al. 2006, Chang et al. 2012, Vergote et al. 2010). Both earlier retrospective studies (Kang and Nam 2009) and randomized EORCT/NCIC trial (Vergote et al. 2010) report superior debulking results in IDS compared to PDS. These better figures do not, however, result in better survival. It can be hypothesized that similar tumor nodules are in fact more completely removed in PDS than in IDS. For example, carcinomatous peritoneal surfaces in paracolic gutters are stripped off in PDS, but the same area may look macroscopically normal after NACT resulting in microscopic residual disease left in IDS. Our study supports this hypothesis on its part. However, since we did not evaluate the residual tumor after surgery but the tumor extent at the beginning of operation, any direct conclusion on the possible differences in microscopic residual tumor in PDS and IDS cannot be made.

There are no earlier studies reporting on the perioperative visual evaluation of the disease spread in IDS. The subject should be studied in a larger prospective trial designed to evaluate the accuracy of the visual estimation. It has been suggested that in IDS, where complete macroscopic resection is achieved with less radical operations, residual cancer stem cells remain in the scar tissue (Lim et al. 2010). If this hypothesis can be confirmed, in order to decrease the microscopic residual disease, all the tracers of the tumor like scar tissue and adhesions should be removed in IDS. However, with more extensive radical operations some benefits now linked to IDS, like lower morbidity, are lost.

### 6.3 PREOPERATIVE PET/CECT: INTRA-ABDOMINAL DISEASE SPREAD AND OPERABILITY

Preoperative imaging should provide accurate information on tumor location and have good sensitivity to detect all the cancer lesions. In this context, low sensitivity means underestimation of disease spread. A portion of cancerous areas is interpreted as healthy. In turn, with low specificity, too high portion of healthy areas is falsely

considered carcinomatous. In case of low sensitivity of preoperative imaging, the full extent of EOC is not revealed until in the operation. If the inoperable patients were found with the preoperative imaging study, unnecessary operations could be avoided.

In our study, PET/ceCT outperformed ceCT in the overall site-based analysis in sensitivity (51 % vs. 41 %) ( $P=0.017$ ), but in specificity the two imaging methods were equal (89 % vs. 92 %). The overall sensitivity of PET/ceCT was lower than in the earlier studies; Kitajima et al. (2008) reported a lesion-based sensitivity of 62 % for intrapelvic tumor lesions and Nam et al. (2010) a sensitivity of 95 % for extra-ovarian intra-abdominal lesions. The differences between the results can be explained by differences in the reference standards. When peritoneal cavity is evaluated carefully region by region in surgery and the data are collected in a systematic manner, the number of findings is higher and a true lesion-based analysis is possible. The study setting of Kitajima et al. was more similar to ours. Their perioperative evaluation consisted of 17 specific intra-abdominal sites against 22 in our study. The somewhat low sensitivity in our study can be partly explained by the microscopic disease; when there was a discrepancy between the results of visual estimation and biopsy, the latter was chosen as the reference standard.

Intestinal FDG uptake is known to cause false positive findings in the GI track (Cook et al. 2004). Bowel movements, intestinal mucosae or intraluminal contents are possible reasons. In the present study, the false positive findings were surprisingly uncommon in both the bowel mesentery and the serosae. This might reflect our patient material: many patients had tumor lesions in those areas. Some of the PET findings may have been physiological FDG accumulation, but there was also true tumor in the same area and they were interpreted as true positives. As discussed earlier, the patient material is not ideal for studying false negative findings.

The performance of ceCT in our study was very similar to a recent study by Glaser et al. (2013). CT is known to have low sensitivity rates (7–28%) when small peritoneal lesions less than 1 cm are concerned (Kyriazi et al. 2010).

In the present study, we did not analyze whether the preoperative imaging results could predict the surgical outcome. This can be seen as a limitation. However, the surgical techniques and the procedures used vary between institutions. The extent of surgery depends also on the patient's general condition and other illnesses. Since the operability results from a single-institution study cannot be generalized, we chose instead to make a detailed analysis of the performance of PET/ceCT on different intra-abdominal regions.

The sensitivity of PET/CT was superior to CT in the regions important to operability. However, the sensitivity figures remained low in both PET/ceCT and ceCT. The performance of PET/ceCT was quite similar to earlier studies which report their lesion-specific figures organ by organ. As an example, the sensitivity to detect carcinosis on

bowel serosae, which was 56 % in our patient material, has previously reported to vary from 57 % to 75 % (Kitajima et al. 2008, Nam et al. 2010). A recent study of Michielsen et al. (2014) focused on DW-MRI on EOC staging. In their study, DW-MRI outperformed PET/CT and CT in peritoneal surfaces and bowel mesentery. More studies are needed to determine the potential of DW-MRI in operability evaluation.

Earlier, De Iaco et al. (2011) have presented a detailed comparison of PET/CT and laparoscopy in different intra-abdominal quadrants. In line with our results, they reported that false negative PET findings were common (29 %) in places with disseminated tumor nodules smaller than 0.5 cm. De Iaco et al. (2011) suggest that PET/CT could be used in combination with laparoscopy. In our experience, it could bring additive value to laparoscopy when the tumor is disseminated in unusual places or the visibility in laparoscopy is limited.

Even though we did not directly study operability, it can be concluded, that due to only moderate sensitivity figures, PET/ceCT is insufficient as a single non-invasive method for the evaluation of operability in EOC patients. Patients with suspected EOC should be referred to centers with the capacity of radical surgery. This should be done regardless of the results of the preoperative imaging studies, since the disease can be much more widely spread than predicted.

#### 6.4 EXTRA-ABDOMINAL DISEASE SPREAD DETECTED WITH PET/CECT

The exact knowledge of the disease extent and distribution is important in cancer treatment planning, especially when the role of the personalized, targeted therapies is evolving. In this prospective study, the preoperative PET/ceCT suggested stage IV disease in a notable number of patients. Sixteen out of 41 patients had FIGO stage IV disease, but 32/41 patients had distant metastases according to PET/CT. The most common cause for FIGO stage IV was positive pleural cytology (6 patients). Four patients were upstaged to FIGO stage IV due to biopsy confirmed LNM suspicious in PET/CT. The remaining patients with suspected stage IV disease on the basis of PET/CT had metabolically active or enlarged mediastinal, cardiophrenic or parasternal LNs, typically in multiple locations.

Our findings are in line with earlier PET studies, in which greater number of distant metastases were detected with PET/CT than CT alone (Castelluzzi et al. 2007, Nam et al. 2010, Risum et al. 2010). The Danish study (Risum et al. 2010) had the most similar population to ours. Of their 66 advanced-stage EOC patients, 64 had FIGO stage III and 2 had FIGO stage IV disease, while PET/CT indicated stage IV in 27/66 (41 %) patients.

Histological verification is the gold standard for imaging studies. We were able to confirm metastatic disease in one patient with PET positive subclavian and in three patients with PET positive axillary LNs. Unfortunately, it was technically impossible to obtain a histological verification from deep intrathoracic LNs. This is a limitation to the study.

In the present study, lymph nodes with focally increased glucose uptake higher than background activity were considered positive for metastatic spread. The SUVmax values of the lymph nodes were registered, but we did not use any threshold level to differ benign and malign nodes. The small size of many LNs impairs usefulness of SUV values due to lack of partial volume correction effect. We consider that the PET/CT positive supradiaphragmatic LNs in our study are likely to represent metastatic lesions. Earlier, Kitajima et al. (2008) showed that PET/CT is an accurate method for evaluation of EOC retroperitoneal LNM when compared to the lymphadenectomy specimen. False positive findings were uncommon. In our study, false positive findings due to FDG uptake caused by infection are unlikely. Our patients had no history of lung disease and the parasternal lymph node chain and the cardiophrenic angle are atypical places for infections.

When we analyzed the distribution of PET positive supradiaphragmatic LNs, the parasternal lymph node chain and the cardiophrenic angle were the most common places for abnormal metabolic activity. Particularly, the high number of LNM in parasternal lymph node chain was a new finding. While many PET/CT studies report supradiaphragmatic LNM on EOC patients, their distribution has not been the focus of previous studies. In the study of Risum et al. (2008) five out of 25 distant LNMs were in the parasternal area. When we compared the performance of PET/ceCT and CT, the most discordant area was the parasternal lymph node chain, where metastatic disease was proposed in 19 patients by PET/ceCT and in 1 patient by ceCT. This may explain why EOC spread to this area has not previously been registered.

The cardiophrenic LNs are a formerly known anatomical area for EOC spread. The lymph node enlargement in CT has been reported to be a significant adverse prognostic factor (Holloway et al. 1997). Lately, some reports on cytoreductive surgery extending the cardiophrenic angle have been presented. Both video-assisted thoracic surgery (Lim et al. 2009) and transabdominal approach (Yoo et al. 2013) have been used. The impact of these procedures on patient survival is not verified. In our analysis of PET/CT stage IV patients, eleven out of 14 patients with cardiophrenic LNM also exhibited abnormal activity in the parasternal area. Our findings question the role of extensive cytoreduction on cardiophrenic LNs. Is it justified if most of the patients have disease also in the upper afferent lymph nodes?

The distribution of PET positive supradiaphragmatic LNs in our study go nicely together with the old anatomical studies illustrating the lymphatic circulation from abdominal cavity through the diaphragm. This is an example of how the development of

functional imaging can provide new information on cancer spread patterns and help to better understand the biological behavior of the disease. We suggest that EOC spreads to the parasternal LNs from the peritoneal cavity via transdiaphragmal invasion of cancer cells to the lymphatic system. In our analysis of 30 patients, the patients with supradiaphragmatic LNM detected with PET/CT had significantly more ascites and more frequently carcinomatosis in subdiaphragmal peritoneal surface than the patients whose disease was restricted to the abdominal cavity. This finding supports the hypothesis of transdiaphragmal lymphatic spread in advanced EOC. However, causality of these findings and the presence of the supradiaphragmatic LNM cannot be demonstrated with this study.

The clinical significance of the PET/CT positive supradiaphragmatic LNs needs further studies. In study of Risum et al. (2010), the survival of PET/CT stage III patients was significantly longer than that of PET/CT IV patients. In multivariate analysis the residual tumor after surgery was, however, the only statistically significant prognostic factor. Later, Frucsio et al. (2013) reported similar prognosis on PET/CT stage III and IV patients, yet the response to chemotherapy was worse in PET/CT stage IV EOC. The follow-up of our patient cohort will on its part cast light on this matter. Today, the presence of PET/CT stage IV disease should not affect the extent of intra-abdominal debulking surgery. The surgical goal also for these patients should be complete tumor debulking. In the future, when the prognostic value of PET/CT stage IV disease is clarified, the presence of PET/CT IV disease may direct the clinical decisions like patient selection to targeted therapies.

## 6.5 SERUM HE4 PROFILE DURING PRIMARY CHEMOTHERAPY OF EOC

The intention of our pilot study was to illustrate the behavior of HE4 during EOC primary chemotherapy. In currently available literature, there are no earlier data on this matter. Our patient number was small and they represented different outcomes (progression, partial and complete response). The data did not enable statistical comparison of the biomarkers. This is an obvious limitation to the study.

In general, HE4 profiles were similar to CA125 profiles during primary chemotherapy. When patients with different treatment outcomes were compared, HE4 levels seemed to decline more rapidly in patients who later achieve complete radiological response at the end of the primary treatment. Any direct conclusions on the clinical usefulness of HE4 cannot be made based on this small patient group. Instead, the pilot study encouraged us to plan further studies with larger patient cohort on HE4 as a predictive factor in EOC treatment (Vallius et al. 2014). The strength of our patient material is the well-documented radiological response evaluation with ceCT and PET/ceCT.

## 6.6 FUTURE PERSPECTIVES

The value of PET/ceCT in EOC preoperative imaging seems to be more in the staging and in detecting distant metastases than in the operability assessment. In order to find the best preoperative imaging method for EOC, studies designed to compare FDG PET/CT and DW-MRI are needed. Our study setting enables us to focus also on chemotherapy response evaluation with PET/ceCT in the future. Neoadjuvant chemotherapy provides a reliable setting to study the early chemotherapy response, since the metabolic changes measured with PET/CT can be compared with the findings in IDS.

The behavior of the supradiaphragmatic LNMs during the treatment as well as at the time of clinical progression is one of our study areas in the future. Its prognostic value is another important matter.

HE4 is a potentially useful serum biomarker for EOC clinical treatment. Our study material enables us to study its prognostic value and use in follow-up. With the current cytotoxic therapies, early treatment of symptomless disease relapse is not recommended (Rustin et al. 2010). The development of new, targeted therapies may, however, increase the significance of detecting early, symptomless recurrence. Serum marker HE4 could be a potential, cost-effective tool for this matter.

## 7 CONCLUSIONS

1. In primary surgery, perioperative visual estimation of tumor spread showed 98 % sensitivity, 76 % specificity and 95 % accuracy. These figures justify the use of surgical findings as a reference standard when EOC is evaluated with imaging techniques.
2. The surgeon's perioperative visual impression of the disease spread is affected by NACT. In interval surgery, areas with benign visual appearance had more often microscopic disease in histopathological evaluation. Macroradical operation may mean less complete tumor debulking at IDS than that at PDS.
3. Based on the present study, the evaluation of operability cannot solely be based on preoperative PET/ceCT or ceCT. A non-invasive method of operability assessment is currently lacking.
4. PET/ceCT is more effective than ceCT in detecting extra-abdominal disease spread in advanced EOC patients.
5. The large number of supradiaphragmatic lymph node metastases detected with pretreatment PET/ceCT challenges the traditional view on EOC spread patterns. Their prognostic significance is, however, unclear.
6. Serum HE4 is a valid candidate for larger trials focusing on the evaluation of EOC treatment response and prognosis.

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The MOT dictionary for obvious reasons.

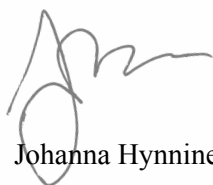
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Turku, December 2014



Johanna Hynninen

## APPENDIX 1.

| Patient number, date       |        |      |                  |                             |             |        |        |
|----------------------------|--------|------|------------------|-----------------------------|-------------|--------|--------|
| Anatomical region          | PET/CT | ceCT | Surgical finding | Histopathologic al findings | Lesion size | SUVmax | SUVavg |
| <b>Thoracic region</b>     |        |      |                  |                             |             |        |        |
| Parasternal LNs            |        |      |                  |                             |             |        |        |
| Subclavian LNs             |        |      |                  |                             |             |        |        |
| Axillary LNs               |        |      |                  |                             |             |        |        |
| Cardiophrenic angle        |        |      |                  |                             |             |        |        |
| Mediastinal LNs            |        |      |                  |                             |             |        |        |
| Pleural fluid dx/sin       |        |      |                  |                             |             |        |        |
| Lung dx/sin                |        |      |                  |                             |             |        |        |
| <b>Liver parenchyma</b>    |        |      |                  |                             |             |        |        |
| Right/ Left,               |        |      |                  |                             |             |        |        |
| Ventral/ dorsal            |        |      |                  |                             |             |        |        |
| Liver surface              |        |      |                  |                             |             |        |        |
| Right/left,                |        |      |                  |                             |             |        |        |
| Ventral/dorsal             |        |      |                  |                             |             |        |        |
| Ascites                    |        |      |                  |                             |             |        |        |
| <b>Peritoneal surfaces</b> |        |      |                  |                             |             |        |        |
| Diaphragm Right            |        |      |                  |                             |             |        |        |
| Ventral/dorsal             |        |      |                  |                             |             |        |        |
| Diaphragm Left             |        |      |                  |                             |             |        |        |
| Ventral/dorsal             |        |      |                  |                             |             |        |        |
| Middle abdomen             |        |      |                  |                             |             |        |        |
| Pelvis                     |        |      |                  |                             |             |        |        |
| Fossa Douglas              |        |      |                  |                             |             |        |        |
| Bladder surface            |        |      |                  |                             |             |        |        |
| Porta hepatis              |        |      |                  |                             |             |        |        |
| Ventricle                  |        |      |                  |                             |             |        |        |
| Bursa omentalis            |        |      |                  |                             |             |        |        |
| Spleen, tail of            |        |      |                  |                             |             |        |        |
| pancreas                   |        |      |                  |                             |             |        |        |
| The omentum                |        |      |                  |                             |             |        |        |
| <b>Bowel</b>               |        |      |                  |                             |             |        |        |
| Small intestine            |        |      |                  |                             |             |        |        |
| serosae                    |        |      |                  |                             |             |        |        |
| Small intestine            |        |      |                  |                             |             |        |        |
| mesentery                  |        |      |                  |                             |             |        |        |
| Appendix and               |        |      |                  |                             |             |        |        |
| caecum                     |        |      |                  |                             |             |        |        |
| Colon transversum          |        |      |                  |                             |             |        |        |
| Rectosigmae                |        |      |                  |                             |             |        |        |
| Large intestine            |        |      |                  |                             |             |        |        |
| mesentery                  |        |      |                  |                             |             |        |        |
| Kidneys                    |        |      |                  |                             |             |        |        |
| Adrenal glands             |        |      |                  |                             |             |        |        |
| <b>Para-aortal LNs</b>     |        |      |                  |                             |             |        |        |
| Cranial/caudal to          |        |      |                  |                             |             |        |        |
| renal vein                 |        |      |                  |                             |             |        |        |
| <b>Pelvic LNs</b>          |        |      |                  |                             |             |        |        |
| Right/left                 |        |      |                  |                             |             |        |        |
| <b>Inguinal LNs</b>        |        |      |                  |                             |             |        |        |
| Right/left                 |        |      |                  |                             |             |        |        |
| <b>Uterus</b>              |        |      |                  |                             |             |        |        |
| <b>Ovary</b>               |        |      |                  |                             |             |        |        |
| Right/left                 |        |      |                  |                             |             |        |        |
| <b>Fallopian tubes</b>     |        |      |                  |                             |             |        |        |
| Right/left                 |        |      |                  |                             |             |        |        |

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