Building a retrospective high-grade serous ovarian carcinoma cohort by combining biobanked tissue samples and longitudinal clinical information

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Tiivistelmä – Referat – Abstract

Ovarian cancer is the fifth most common cause of cancer death among women in Europe; high-grade serous ovarian carcinoma (HGSC) is the most common subtype of ovarian cancer. This report describes the construction and preliminary analysis of a retrospective cohort of HGSC patients. Tissue samples were obtained from Helsinki Biobank, and clinical data was retrieved from several electronic data bases as well as from the paper archives of the HUS Women's hospital.

Over 900 patients were identified for potential inclusion in the cohort. The process of confirming diagnoses is still ongoing. Challenges with the collection and categorization of clinical data are reported and possible solutions discussed. As the confirmation of which patients will be included in the final cohort is not yet complete, analyses in this report are limited to some preliminary descriptions. These initial findings seem to be in concordance with other reports: patients with stage I or II disease and/or complete surgical cytoreduction (R0) have a better 5-year overall survival than those with stage III or IV disease and/or suboptimal surgical outcome. Once the cohort is ready, it will be a unique tool for studying the biology of high-grade ovarian carcinoma.

Avainsanat – Nyckelord – Keywords

ovarian cancer, high grade serous ovarian carcinoma, cohort building, biobank study, retrospective

Säilytyspaikka – Förvaringställe – Where deposited

Muita tietoja – Övriga uppgifter – Additional information

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1 Introduction

Ovarian cancer is the fifth most common cause of cancer death among women in Europe (1), and high-grade serous ovarian carcinoma is the most common subtype of ovarian cancer (2). Over the last decades, research has uncovered considerable new information about the biology of high-grade serous ovarian carcinoma (HGSC). As of today, this has not yet translated into a significantly improved prognosis for patients, though the latest studies on PARP-inhibitors have brought hope that this may soon change (ibid.). High-grade serous ovarian carcinoma is an interesting disease to study as outcomes for patients are quite diverse, i.e. there is considerable variation in the length of overall survival and/or relapse-free survival. That is why it is of great interest to construct a sizable cohort of HGSC patients, that combines patients' tumor samples and clinical data in order to study groups with different disease outcomes and varying responses to treatment and try to find clinical as well as biological factors that can explain these differences.

This report describes how a retrospective cohort of HGSC patients is being built in Olli Carpén's research group at the Department of Pathology within the Faculty of Medicine at the University of Helsinki. Tissue samples were obtained from Helsinki Biobank, and clinical data was retrieved from several electronic data bases as well as paper archives. Biobank data scientist Jani Salmi has analyzed the clinical data to identify subgroups of patients using machine learning technology (Tools for digital phenotyping of disease entities -project). My work within the cohort formation has primarily been to collect clinical data from patients' archived paper files.

The next chapter will introduce the topic of ovarian cancer in general and the subtype of high-grade serous ovarian carcinoma in particular.

2 High-grade serous carcinoma as a subtype of ovarian carcinoma

The research project focuses on high-grade serous ovarian carcinoma as the most common subtype of epithelial ovarian cancer. Past research has shown that ovarian cancer subtypes differ substantially from each other regarding their biology, and today those subtypes are considered different diseases rather than subtypes of the same disease. (3)

2.1 Ovarian cancer classification

There is a great variety of ovarian tumors. The "WHO Classification of Tumours of Female Reproductive Organs" from 2014 provides a detailed classification of the different ovarian tumor types. The classification has changed over time and the newest version reflects advances in molecular biology and pathological research which have led to a better understanding of the disease subtypes. (4) Firstly, one can distinguish *epithelial tumors* from *non-epithelial tumors*. Non-epithelial tumors include categories such as sex cord-stromal tumors or germ cell tumors.

Secondly, within the epithelial tumor category, the WHO classification distinguishes the following subtypes: serous, mucinous, endometrioid, clear cell, Brenner, seromucinous, and undifferentiated. Of the first six epithelial tumor subtypes the classification lists benign, borderline (intermediate) and malignant variants. By definition the undifferentiated category contains only undifferentiated carcinoma. There is one malignant variant of each tumor subtype mentioned, with the exception of serous tumors, which have two malignant forms: low-grade and high-grade serous carcinoma. Serous carcinoma was formerly considered a continuum ranging from grade 1 to grade 3. However, subsequent research found that they are in fact biologically very different and are today called high-grade serous carcinoma (HGSC), which accounts for most of the formerly grade 2 and 3 serous tumors, and low-grade serous carcinoma (LGSC), corresponding to most of the formerly grade 1 tumors. (4) According to accumulated research results, LGSCs evolve through a BRAF- or KRAS-mutation, whereas HGSCs evolve through a p53-mutation (5). Though they are now thought to be two distinct diseases, very rarely a low-grade serous carcinoma can develop into a high-grade serous carcinoma (4).

About 90% of all malignant ovarian tumors are epithelial tumors, i.e. carcinomas. Of these, over two-thirds are high-grade serous carcinomas (6). Table 1 summarizes the different ovarian carcinoma subtypes and their relative frequencies.

Table 1: Types of ovarian carcinoma, percentages from Prat's review article (6)

Ovarian carcinoma subtype	**% of ovarian carcinomas**
High-grade serous carcinoma (HGSC)	70%
Endometrioid carcinoma (EC)	10%
Clear cell carcinoma (CCC)	10%
Low-grade serous carcinoma (LGSC)	<5%
Mucinous carcinoma (MC)	3%
Others: malignant Brenner tumour, seromucinous carcinoma, undifferentiated carcinoma	<5%

As Mutch points out "primary fallopian tube cancer and primary peritoneal cancer are rare malignancies but share many clinical and morphologic similarities with HGSC" (7). Additionally, the WHO classification states that HGSOC can be seen as an "amalgamation of primary (extra-uterine) `pelvic high-grade serous carcinomas`" as they may arise from the ovary, the fallopian tube or the peritoneum (4). In this sense the word "ovarian" can be somewhat misleading, as HGSC can present without involvement of the ovaries (see below the discussion of the etiology of HGSC). For this reason, the terms high-grade serous ovarian carcinoma (HGSOC) and high-grade serous carcinoma (HGSC) are used synonymously in the following text and we also included such patients in our study, whose disease did not affect the ovaries.

2.2 Epidemiology

Ovarian cancer is the fifth most common cause of cancer death among females in Europe (1). It is estimated that worldwide 225 000 women are diagnosed with ovarian carcinoma every year, most of which are HGSC, and 140 000 die of their disease (4). In Finland, 330 women died of ovarian cancer (of any type) in 2017 (8).

2.3 Etiology

There is some evidence to suggest that lower ovarian cancer risk correlates with later age at menarche, younger age at menopause, using oral contraceptives and parity – all factors leading to a lower number of menstrual cycles over a lifetime. Estrogen hormone therapy after menopause seems to increase ovarian cancer risk. However, many of these studies looked at ovarian cancer in general and did not distinguish between the different histotypes. (4)

Genetically, genomic instability and DNA copy number variations are typical in HGSC, but there are not many consistently mutated genes.(3) The p53 gene is almost always abnormal, and there is a defect in the homologous recombination pathway for DNA repair in approximately 50 percent of HGSCs. (9) Many of these are due to BRCA1 or 2 germline or somatic mutation, or BRCA1 promoter methylation, but other defects in the pathway also lead to HR deficiency, such as high expression of EMSY, which codes for a protein binding to BRCA2 inactivating it (10). Knowledge of HR pathway deficiency is important as these patients may benefit from treatment with PARP-inhibitors (see chapter 2.7).

HGSC was long believed to develop from the surface epithelium of the ovaries, but researchers studying the fallopian tubes of patients with high risk mutations, who had their tubes and ovaries removed in order to prevent ovarian carcinoma, found serous tubal intraepithelial carcinomas (STIC) and tubal carcinomas, suggesting that the fimbriae of the fallopian tube could be the site of origin. (3) Subsequent research also showed that precursor STICs contain the same genetic alterations as later tumors: Labidi-Galy and colleagues suggest that it takes about 7 years from a STIC to the development of HGSC, which then spreads quickly (11). Other studies suggest that there may be some HGSC tumors which arise from precursors in the fallopian tubes, and others which arise without the involvement of the fallopian tubes (9).

2.4 Clinical symptoms, diagnosis, screening, prevention

The average age at HGSC diagnosis is about 63 years (4), e.g. Peres and colleagues report an average age at diagnosis of 61.2 years with a standard deviation of 11.6 years. Their study of patients in the United States also shows the wide age range of HGSC patients; 3% of patients were aged 20-39 years at diagnosis and 5.5% were 80-84 years. (12)

Most patients are diagnosed with advanced disease, when the carcinoma has already spread to the abdominal cavity beyond pelvis, which corresponds to stage III or IV (see chapter 2.6 for staging and chapter 2.8, table 3, for stage at diagnosis). The reason for this is that the clinical symptoms are rather nonspecific, and may include gastrointestinal symptoms such as nausea, early satiety, anorexia, constipation, tenesmus, or bloating, as well as increased urinary frequency, back pain, stomach ache, fatigue, difficulty breathing, or bleeding. (4, 13)

Diagnostic studies include serum tumor marker CA-125 and HE4 levels as well as imaging studies, e.g. ultrasound, CT, MRI or PET-CT. Cancer antigen 125 (CA-125) is usually elevated in HGSC; median levels are 500-1000 U/ml in the setting of advanced disease patients. Imaging reveals complex, hypervascular masses in the pelvis (especially adnexa), ascites, or omental or peritoneal nodules (4). The final diagnosis is usually made based on a histologic sample, e.g. tumor samples from diagnostic or debulking surgery or biopsy. Cytologic samples from ascites or pleural effusions also assist in diagnosis. (14)

Because of the delayed diagnosis of HGSC, and ovarian cancer in general, the possibility of curative treatment becomes very rare (compared to early stage disease). Therefore, considerable effort has been devoted to developing effective screening methods. Serum CA-125 levels alone or combined with transvaginal ultrasound and/or additional markers such as HE4, MMP-7 or CA-72-4 have been tested as potential screening tools. (15) Circulating tumor cells and cell-free DNA could be a means for early diagnosis, monitoring disease progression, and even for identifying clinically actionable alterations in HGSC (16). The use of liquid biopsies for ovarian cancer screening and disease monitoring is being studied (17), however, none of these measures has yet met the standards of sensitivity, specificity, and practicality that would enable them to be used

for mass screening. Research efforts continue, and there will hopefully be an effective screening method in the future.

Preventive surgery is recommended for known high risk groups, i.e. carriers of BRCA1 or BRCA2 mutations. Germline BRCA-mutations are associated with a considerably higher risk of ovarian cancer; there is a 20-65% lifetime risk for patients with a BRCA1 mutation and 11-37% for patients with a BRCA2 mutation. Almost all of the BRCA-associated ovarian cancers are HGSCs. (4) The Finnish Society of Obstetrics and Gynecology recommends the excision of fallopian tubes and ovaries for BRCA1 carriers by the age of 40 and for BRCA2 carriers by the age of 45 (18).

2.5 Morphology and immunohistochemistry

The WHO classification defines HGSC morphology as "a carcinoma composed of epithelial cells displaying papillary, glandular (often slit-like) and solid patterns with high-grade nuclear atypia" (3).

Immunohistochemical staining is often used by pathologists to verify a morphologic diagnosis. HGSC is characterized by positive staining for the marker WT1 and by abnormal TP53 staining. Aberrant TP53 staining can mean either its complete absence, or diffuse overexpression. However, there is no single antibody, or combination of antibodies, that can define this histotype with 100% accuracy. In Köbel et al.'s study, 96.7% of HGSC cases stained positive for WT1 and 93.9% of HGSC cases showed aberrant TP53 staining. 91.7% of HGSC cases showed both WT1 positivity and TP53 aberrancy, while there were only very few cases that showed those two characteristics while not being HGSC (less than 1% of cases with these staining characteristics). (19)

According to a simplified algorithm one could first test for WT1 staining, positive staining meaning that the histotype is probably either low-grade or high-grade serous, and negative staining meaning it is more likely to be clear cell, mucinous or endometrioid. The pattern of TP53 staining then differs between low-grade and high-grade serous carcinomas, being aberrant in HGSCs and normal in LGSCs. (5)

In their recent paper Köbel and colleagues test a third version of their "Calculator of Ovarian carcinoma Subtype/ histotype Probability" (COSPv3). They conclude that the integration of IHC-stains can improve histotyping, however one cannot (yet) rely on it alone, since in 7% of cases COSPv3´s predictions were discordant with the benchmark integrated histotype, which was based on morphological and IHC information. (20)

2.6 Staging

Cancer staging is an important tool that helps to understand the patient's condition, allows for outcome prediction to a certain degree and assists in choosing the appropriate treatment. For ovarian cancer, the staging classification of the International Federation of Gynecology and Obstetrics (FIGO), first published in 1973, is widely used[1]. (7) It was also used in the clinical reports assessed in this study and therefore it will be presented here shortly. The classification has been revised over the years and the latest version is from 2014, which is summarized in table 2. As pointed out later, we used the 2014 classification and re-staged patients accordingly based on medical reports.

HGSC is characterized by spread within the peritoneal cavity, which has no physical barriers to metastasis, which is believed to enable spreading of the disease to distant sites within the cavity (21). FIGO stage I indicates that the tumor is confined to the ovaries; in stage II it has spread within the pelvis, but not above the pelvic brim. Stage III means that the disease has spread to the peritoneal cavity outside the pelvis and/or to the retroperitoneal lymph nodes. Stage IV disease means intraparenchymal hepatic or splenic metastasis, or spread of the disease to distant sites outside the peritoneal cavity, such as lungs or the umbilicus.

Staging in ovarian cancer is based on the staging surgery, radiographic images, and histologic results from washings, ascites, pleural effusions and biopsies. Patients with FIGO stage I and II disease, where cancer is confined to the pelvis, have a much better

[1] Also the TNM staging is used, see table 1 in Mutch's paper for the Figo staging system and the corresponding TNM (7). The German current care guideline for ovarian cancer (S3-Leitlinie Diagnostik, Therapie und Nachsorge maligner Ovarialtumoren, p. 66) states that pathologists should always report the TNM classification (5).

prognosis than patients with spread outside of the pelvis (stage III or IV). Unfortunately, most HGSC are stage III or IV at diagnosis (see chapter 2.8).

***Table 2*:** The 2014 FIGO Ovarian Cancer Staging as outlined by the Society of Gynecologic Oncology (22)

STAGE I: Tumor confined to ovaries	
IA	Tumor limited to 1 ovary, capsule intact, no tumor on surface, negative washings.
IB	Tumor involves both ovaries otherwise like IA.
IC	Tumor limited to 1 or both ovaries
	IC1 Surgical spill
	IC2 Capsule rupture before surgery or tumor on ovarian surface
	IC3 Malignant cells in the ascites or peritoneal washings
STAGE II: Tumor involves 1 or both ovaries with pelvic extension (below the pelvic brim) or primary peritoneal cancer	
IIA	Extension and/or implant on uterus and/or Fallopian tubes
IIB	Extension to other pelvic intraperitoneal tissues
STAGE III: Tumor involves 1 or both ovaries with cytologically or histologically confirmed spread to the peritoneum outside the pelvis and/or metastasis to the retroperitoneal lymph nodes	
IIIA	Positive retroperitoneal lymph nodes and /or microscopic metastasis beyond the pelvis
	IIIA1 Positive retroperitoneal lymph nodes only IIIA1(i) Metastasis ≤ 10 mm IIIA1(ii) Metastasis > 10 mm
	IIIA2 Microscopic, extrapelvic (above the brim) peritoneal involvement ± positive retroperitoneal lymph nodes
IIIB	Macroscopic, extrapelvic, peritoneal metastasis ≤ 2 cm ± positive retroperitoneal lymph nodes. Includes extension to capsule of liver/spleen.
IIIC	Macroscopic, extrapelvic, peritoneal metastasis > 2 cm ± positive retroperitoneal lymph nodes. Includes extension to capsule of liver/spleen.
STAGE IV: Distant metastasis excluding peritoneal metastasis	
IVA	Pleural effusion with positive cytology
IVB	Hepatic and/or splenic parenchymal metastasis, metastasis to extraabdominal organs (including inguinal lymph nodes and lymph nodes outside of the abdominal cavity)

As pointed out earlier (see chapter 2.1) HGSC can also originate from the fallopian tubes or the peritoneum. To account for this, the FIGO staging protocol also exists as a version which includes tubal origin as an alternative in the early stages, i.e. every time the ovary is mentioned in the description of the stage, the text includes “or fallopian tube” (see

Mutch's review article for that version of the FIGO classification (7)). Primary peritoneal cancer is staged starting from stage II in both versions.

2.7 Treatment

After diagnosis one has to decide whether it is beneficial for the patient to undergo primary debulking surgery[2]. Primary means that the surgery takes place before any possible chemotherapy and debulking refers to a surgery that tries to resect all tumor tissue. Debulking surgery usually removes the uterus, adnexa, omentum, ascites and when indicated, the appendix, segments of the intestine, peritoneum, and the parenchyma of any other affected organs. Biopsies of the abdominal cavity as well as cytological samples of ascites and washings are also components of the so-called staging-surgery protocol. Resection of the pelvic and para-aortic lymph nodes is done depending on the circumstances, e.g. it is recommended for stage I and II disease in general, but for stage III and IV only in cases of bulky lymph node metastasis (for more details see for example the KELPO care guidelines of the Finnish Society of Obstetrics and Gynaecology). (18)[3]

One alternative to primary debulking surgery is neoadjuvant chemotherapy (NACT), with later interval debulking surgery if reasonable. The question of optimal timing for the debulking surgery is controversial and has been studied intensively. The Current Guidelines of the German Guideline Program in Oncology conclude that there is no advantage to NACT and interval surgery as compared to post-surgery chemotherapy, and therefore recommend primary debulking surgery followed by chemotherapy. (5) The Finnish guidelines recommend 3 to 4 cycles of NACT followed by interval surgery when, according to examinations such CT scan, it is predicted that primary surgery would not lead to a major cytoreduction. (18)

[2] This chapter relies mainly on information taken from the Finnish and German Care Guidelines for the treatment of ovarian carcinoma . Most of the treatment is identical for the different histotypes of ovarian carcinoma, e.g. surgery treatment and standard chemotherapy.

[3] The Finnish Medical Society Duodecim has withdrawn its Current Care Guideline (i.e. "Käypä Hoito") for Ovarian Cancer from 14th June 2019, the society announced that they will not continue to update the guideline, the last update dates back to 2012. (16) However, the Finnish Society of Obstetrics and Gynaecology publishes so-called KELPO care guidelines for the treatment of epithelial ovarian cancer on its website (18).

Based on research results, it is also recommended that the surgery is performed by an experienced gynecologic oncologist, and in some ultra-radical debulking surgeries, in co-operation with surgeons from other specialties, such as gastrointestinal surgeons. (5, 18)

One alternative to the conventional surgical technique is the application of a hyperthermic chemotherapeutic drug directly into the abdominal cavity in connection with the surgery, the so called hyperthermic intraperitoneal chemotherapy (HIPEC). Thus far, there is no strong evidence that justifies the use of HIPEC in the treatment of ovarian carcinoma (5); the Finnish guidelines recommend its use only in the context of clinical trials (18)[4].

Chemotherapeutic treatment is always recommended for HGSC patients, independent of stage[5]. The standard recommendation is 6 cycles of intravenous carboplatin[6] (dose AUC 5) and paclitaxel[7] (dose 175 mg/m^2), infused over 3 hours, with 3 weeks between cycles (5). Paclitaxel can also be given weekly and carboplatin every three weeks. For patients with a poor functional status, considerable comorbidity, or early stage disease, monotherapy with carboplatin can also be considered.

Bevacizumab, a humanized monoclonal antibody that inhibits angiogenesis by targeting and blocking vascular endothelial growth factor (VEGF) (25), can be added to first line chemotherapy in advanced disease. The Finnish guidelines recommend it for stage IIIb and IIIc disease with suboptimal surgery outcome, meaning residual tumor greater than 1 cm, or for stage IV disease (18). This drug has been shown to prolong progression free survival, but an improvement in overall survival could be shown only for subgroups, e.g. high tumor load or stage IV disease (5).

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[4] The same recommendation is given for intraperitoneal chemotherapy, meaning that the cytostatic drug is applied to the peritoneal cavity and not intravenously.

[5] Only patients with FIGO stage 1A well differentiated ovarian carcinoma, who had complete staging surgery, should not receive adjuvant chemotherapy. But for poorly differentiated carcinoma, e.g. HGSC, it is always recommended. (5, 18)

[6] Carboplatin is a platinum-based drug, the parent compound is cisplatin. Its mechanism of action as an anti-cancer drug is described as following: “Carboplatin undergoes activation inside cells and forms reactive platinum complexes that cause the intra- and inter-strand cross-linkage of DNA molecules within the cell. This modifies the DNA structure and inhibits DNA synthesis.”(23)

[7] Paklitaxel is an anti-mitotic drug, it hyper-stabilizes the structure of microtubules and therefore prevents the cell in using its cytoskeleton in a dynamic way. It might also induce apoptosis in cancer cells by binding to Bcl-2-protein. (24)

Patients with HGSC often respond to platinum-based therapy (9). However, most patients relapse at some point, and the relapsed disease is not considered curable in most cases. However, patients can live with the disease for a variable length of time. Treatment of relapsed disease is different for platinum-sensitive and platinum-resistant tumors. In addition to paclitaxel and carboplatin, drugs such as topotecan, gemcitabine, PEGylated liposomal doxorubicin, epirubicin, and etoposide can be used in combination or as single agents, depending on the circumstances. For platinum-sensitive disease, the treatment goal is prolongation of progression-free survival and overall survival; in a platinum-resistant disease relapse the aim is preservation of quality-of-life and symptom management. (5)

For many years there was no major progress in the treatment of HGSC, but in recent years the addition of PARP-inhibitors to the treatment of ovarian carcinoma has been studied and has brought new hope for at least some patients. Poly-ADP-ribose-polymerases (PARP) are proteins which are activated by DNA damage and enable the repair of single-strand DNA breaks. In the presence of a PARP-inhibitors, the single-strand break repair pathway is blocked, which results in double-strand breaks. The double-strand breaks would be repaired by the homologous recombination (HR) pathway, but this pathway is not functioning in, for example, BRCA-mutated or otherwise HR-deficient cells, which eventually leads to cell death.(26) Moore and colleagues reported the first results of the SOLO1 trial, a phase 3 trial studying the efficacy of PARP-inhibitor olaparib used as maintenance therapy after surgery and platinum-based chemotherapy in patients with newly diagnosed FIGO-stage III or IV disease (high-grade serous or endometrioid ovarian cancer, fallopian-tube cancer or primary peritoneal cancer), who had a BRCA1 and/or BRCA2 mutation and who had complete or partial response to the platinum-based chemotherapy. They found a substantial benefit of olaparib maintenance therapy, e.g. 4 years after first-line chemotherapy 53% of the patients of the olaparib group were progression-free, compared to 11% of the placebo group. These findings were the first sign of a major improvement in the therapy of high-grade serous ovarian cancer in a long time. (27) While most of the trial patients had a BRCA1 and/or 2 germline mutation, which is

estimated to be found in up to 25% of HGSC patients[8] (29), the potential utility for patients with a deficiency in homologous recombination (HRD carcinomas) due to another reason such as BRCA1/2 somatic mutation, BRCA1 promotor methylation or EMSY amplification, is compelling (10, 30). Recent research findings suggest, that PARP-inhibitors work well as maintenance therapy following first line platinum-based chemotherapy for patients, who respond to platinum-based chemotherapy, which in turn can be seen as a clinical correlate for HRD (28, 31, 32).

On the other hand, the response rate of patients with BRCA1/2 mutations to PARP-inhibitors is far less than 100 percent, which may be due to a restored HR pathway or another resistance mechanism (2); hopefully future research will clarify how to identify specific patients who would benefit from PARP-inhibitors. (32, 33)

Immunotherapy is currently utilized with success in some cancers, such as melanoma, but has not yet been successful in the treatment of ovarian carcinoma, despite the fact that tumor infiltration by T-cells has long been a recognized predictor of outcome. One reason could be the heterogeneity of immunological microenvironments within different metastases, as Jiménez-Sáchez and colleagues show in their interesting case study. (34) However, the potential of immunological therapies, also in combination with other therapies, is a subject of on-going research.

2.8 Prognostic factors

The most important prognostic factor for disease outcome is, as in many other cancers, *stage at diagnosis*. (4) Table 3 shows that while 84% of patients diagnosed with stage Ia or Ib disease are alive 5 years after diagnosis, only 32% of patients diagnosed with stage III or IV disease live longer than 5 years after diagnosis.

For patients diagnosed with advanced disease (stage III or IV) *surgery outcome* is the most important prognostic factor: a completely resected tumor means a considerably

[8] The percentages of how much HGSC patients have a BRCA1/2 germline or somatic mutation or another HRD differ somewhat in different sources, e.g. Coleman et al. write of approximately 15% germline and 5% somatic BRCA1 or 2 mutations and in addition up to 30% with HRD because of other genomic alterations (28).

better prognosis. However, the WHO report from 2014 states: “It is not clear at this time whether resectability reflects an intrinsically more favorable disease type, or whether increased surgical effort leads to better outcomes independent of intrinsic tumor characteristics.” This leads to the highly debated question about whether an aggressive surgical approach is justified. Horowitz et al. studied the relationship between disease burden before surgery, the complexity of surgery, and residual tumor size. They conclude that “more aggressive surgery may be warranted if R0 can be achieved”, but also point out that morbidity and mortality resulting from surgery should be considered. They also found that disease burden prior to surgery was a significant prognostic factor. (35)[9]

***Table 3**:* Overall survival of HGSC patients according to stage, data from Peres et al.’ study with data from 17,837 HGSC patients, representative for the US population, NOS = not otherwise specified (12)

	Overall survival		
Stage	**1 year**	**5 years**	**10 years**
Localized = 4,9% of patients *(corresponds to FIGO IA, IB, I-NOS)*	96.8	84.0	67.5
Regional = 17,1% of patients *(FIGO IC, IIA, IIB, IIC, II-NOS)*	93.7	67.7	48.8
Distant = 77,9% of patients *(FIGO IIIA, IIIB, IIIC, III-NOS, IV)*	84.3	32.1	15.0

Rose and colleagues studied prognostic factors for survival in patients with relapsed advanced-stage (stage III or IV) high-grade ovarian carcinoma[10]. *Time to relapse* after primary surgery and chemotherapy (paclitaxel and platinum) accounted for the major part of the prognostic information in their model predicting overall survival after relapse. Also *performance status* and *age* were significant factors in their model, but accounting for much less of the predictive information. (36) Lower performance status and higher age have been found previously to be independent predictors of outcome (progression free survival and overall survival), e.g. in a study with patients with FIGO

[9] This study included cases of advanced epithelial ovarian cancer as well as primary peritoneal cancer, so it was not specific for HGSC.

[10] Their study was not specific for high-grade serous ovarian carcinoma, it included also patients with endometrioid, clear cell, mucinous or mixed subtype, but all patients had high-grade disease. 81.2% of the patients in the study had high-grade serous ovarian carcinoma.

stage III epithelial ovarian cancer (37). The *progression free survival after initial platinum-therapy* as the important prognostic factor for response to second-line treatment was identified about 30 years ago (36).

The *response to neoadjuvant chemotherapy* was also studied and its use as a prognostic factor in HGSC evaluated. Böhm et al. developed a system to quantify response to neoadjuvant chemotherapy in histological samples of the omentum and adnexa from interval debulking surgery (38). In this so-called CRS (Chemotherapy Response Score) system, a CRS score of 1 means "no or minimal tumor response"; 2 means "appreciable tumor response with residual tumor"; and 3 stands for "complete or near-complete response" (for a more detailed description of the three categories and training possibilities see (39)). Ditzel and colleagues found that the CRS score could be useful as a prognostic tool for HGSC. In their study, most notable was the difference between scores 1 and 2 as compared to score 3 with regard to outcome (progression free survival as well as overall survival); patients scored as CRS 3 had a better outcome. (40)

Feigenberg and colleagues present findings to suggest that HGSC patients with *low-volume ascites* at the time of up-front cytoreductive surgery differ from those with high-volume ascites, in that they have better clinical outcomes, their immune related genes are upregulated, and they have more tumor infiltrating cells (41).

There are no prognostic tissue markers that can predict response to chemotherapy or disease course yet in clinical use, and one major aim for creating this cohort is to validate potential markers.

3 Aims of research

The aims of the research project were to:

1) **create a retrospective cohort of HGSC patients** treated at the Helsinki University Hospital (HUS) with tissue samples and clinical follow-up data, which can be used for the study of different research questions, e.g. response and resistance in platinum-based chemotherapy. This cohort can also be provided to collaborating research groups, e.g. for the testing of potential biomarkers

2) **divide the cohort into different outcome groups and study disease trajectories**
3) search for **clinical information that can predict the disease course and outcome**

Progress regarding the first research aim, the process of cohort building, will be covered in Chapter 4 on materials and methods. Chapter 5 considers challenges in the collection of clinical data and contains a preliminary description of the cohort, and Chapter 6 gives an outlook on the next steps in the research projects.

4 Materials and methods

4.1 Cohort formation based on available tissue samples

Time frame. Helsinki Biobank holds tissue samples from ovarian cancer (and naturally many other) operations performed at the Helsinki University Hospital dating back several decades. However, only samples from 1982 on are registered in HUS's Pathology Department electronic database, QPATI; only those samples´ pathology reports are accessible and searchable electronically. The Finnish Biobank Act entered into force from 1.9.2013. Before that time it was possible for scientists, with permission from the hospital ethical board and Valvira, to use biobank tissue samples for research without asking the patients for consent or informing them. This changed with the new law and now researchers can use tissue samples taken after 1.9.2013 only if patients have at some point given their consent for use of their samples for research. However, a general consent for inclusion in potential research is rarely requested from patients at the time of tissue collection or when undergoing a clinical procedure. To obtain these declarations of consent later is a very time-consuming and expensive process. For old samples taken before 1.9.2013 an "opt-out" procedure is valid, i.e. individuals have the option to actively object to the use of their samples for research, which only a tiny fraction of patients do. Because of these practical considerations, and also to study overall survival, the period 1.1.1982 – 31.8.2013 was chosen for the creation of the cohort, meaning that the patients' tissue samples were taken during that time. The cohort was created by data scientist Otto Manninen in cooperation with other research

group members. Helsinki Biobank´s scientific-ethical board has given permission to use tissue material and associated clinical data from the biobank (approved 28.4.2017).

Identifying HGSC samples and patients. To identify potential cases for the study cohort, the QPATI database was first searched for diagnostic SNOMED 2/3 codes corresponding to serous carcinoma (M8461*), unspecified carcinoma (M8010*) independent of growth disposition and unspecified poorly differentiated carcinomas (M80203) in the time span 1.1.1982-31.8.2013, which led to 38,000 hits. Exclusion of patients with only cytology samples reduced the number to 34,000 samples, and after filtering for the relevant anatomic sites (ovaries, adnexa, uterus, fallopian tubes, omentum and peritoneum), 5,700 samples were left. These cases were scanned using a search tool that scored freeform diagnosis and diagnosis suffix text for inclusion. As pointed out previously, the definition of and diagnostic criteria for high-grade serous ovarian carcinoma have changed over time, so it was not sufficient to search for the HGSC diagnosis alone. Inclusion criteria were, for example, serous carcinoma, carcinoma serosum, HGSC, high-grade, grade 2, grade 3, gradus II-III, G2, G3, male differentiale, and so on. Exclusion criteria were, for example, low grade, gradus 1, and G1.[11]

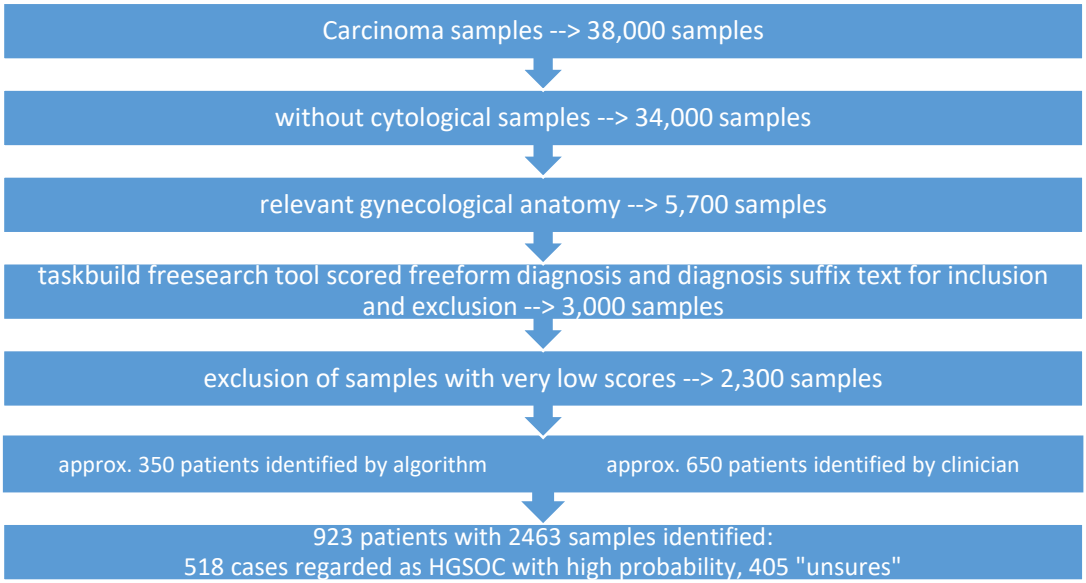

Figure 1: Process of identifying patients for the cohort

[11] These were exclusion and inclusion criteria at the level of samples. The identification of a “high-grade” sample led to the inclusion of the correspondent patient into the cohort, whereas the identification of a “low grade” sample did not lead to the exclusion of the patient from the cohort, but to the exclusion of the sample from the samples to screen, because it would be possible that another sample of the same patient would be “high-grade”.

After this step 3,000 samples were left. Those 3,000 samples were scored with the aim of identifying HGSC samples. Samples with very low scores, e.g. those which included only the phrase “male differentiatum”, were excluded. Further, all samples from men were excluded. Of the remaining 2,300 samples, about 350 patients with a high-probability true HGSC diagnosis were detected by keywords, such as “high-grade” and “serous carcinoma”. The rest of the samples were evaluated by MD Anniina Färkkilä, via identifying key words or sentences from pathology reports, both via excluding e.g. tumors of other origin (gastric, pancreas), and by positive key-words (e.g. “high”) and reading through the individual pathology reports. This step identified about 650 more candidate samples. In total, samples from 923 patients were identified; 518 were regarded as having a high probability of being HGSC, and 405 were “uncertain” cases. Each patient had one to four tissue samples from different time points and/or different tumor sites available in the biobank, so in total, 2,463 samples were identified.

Confirmation of diagnoses. As the cohort still contains many uncertain cases, and also because of changes in the classification and diagnosis of ovarian cancer, the next step is to verify diagnoses and include only patients with a confirmed histologic diagnosis of HGSC. In a first confirmation step, pathologists Anni Virtanen and Anna Laury re-evaluate the morphology of the tumor samples and exclude cases which are not HGCS. This review process is ongoing on at the time of writing this report[12]: of the 405 “uncertain” cases, thus far 51 have been included and 61 excluded[13]; of the 518 “relatively certain” cases 262 cases were checked, of which 215 were included and 47 excluded. As a second confirmation step, IHC staining for p53 and WT1 will be done on all samples that are eventually included as part of a TMA (see TMA formation below).

4.2 Addition of clinical data to the cohort using electronic data bases and paper archives

The next step was collection of clinical data for the patients included in the cohort. Data scientist Jani Salmi was responsible for collecting the necessary information from electronic databases of the Helsinki University hospital (DigPhen-project). Clinical data,

[12] The following numbers are from October 2019.
[13] Cases were excluded based on morphology and in some rare cases, due to insufficient tumor sample size.

such as comorbidities (as determined by the patients' ICD10 diagnosis codes six months before as well as one year after the diagnosis of HGSC), measurements of CA-125 tumor marker levels, other blood analyses, and medications received, were obtained from electronic health records of the Helsinki University hospital.

Survival information, i.e. *date of death* and *cause of death* were obtained from Statistics Finland for deceased patients. At the time of writing this report, information on the death of patients is available only through the year 2015. The information about deaths occurring from 2016-2018 has been requested and will be included once delivered. This means that for the moment, we can study the 5-year-overall survival only for cases diagnosed in 2010 or earlier.

As some of the necessary data, particularly information concerning surgery and chemotherapy treatment, were not easily available electronically, these had to be collected from the paper archives. One challenge of clinical data collection in general, but especially when it is collected "by hand" from archived paper files, is to decide which kind of information to include in the study cohort data pool and in how much detail. The goal is to collect as much detailed information as is necessary, while keeping the data set as compact as possible in order to not make it a too time-consuming process. The decision regarding which data to obtain from the paper files was made in cooperation with gynecologists Taru Tuomi and Mikko Loukovaara, data scientist Jani Salmi, and pathologist Olli Carpén.

The following data was obtained from or calculated based on the information from the paper archive of the Helsinki Women's Hospital (see also data collection sheet in appendix):

- patient baseline age, weight, height
- date of diagnosis
- ASA performance grade at first surgery
- disease stage: FIGO stage as stated in the files, calculation of revised stage according to the FIGO 2014 criteria where necessary on the basis of information regarding disease spread in the files and pathologic data base

- information on surgical treatment: date, surgery type (debulking, diagnostic, inoperable), timing (primary, interval), residual disease (coded according to the R0, R1, R2-system, where R0 means no residual macroscopic tumor, R1 stands for residual tumor smaller than 1cm in diameter and R2 means a residual tumor of 1cm or more in diameter, this is a common categorization, see for example (42))
- main surgery: As some of the patients were operated more than once, for every patient the main surgery of the first treatment line, e.g. the staging surgery if the patient had such, was identified based on the collected information on surgical treatment. The main surgery type was classified into three categories: *primary debulking surgery*, *interval surgery* after neo-adjuvant chemotherapy, or a *primary surgery that is hard to define* (e.g. primary inoperable/diagnostic). The last class includes cases with only diagnostic surgery in the first treatment line, and cases with attempted debulking but (partly) inoperable tumor, or emergency surgery with some degree of debulking, etc. The distinction between inoperable and suboptimal debulking was not clear in every case (see chapter 5.1).
- chemotherapy: dates and doses of each medication for the first three lines of treatment as well as first and last date and number of cycles for possible more treatment lines

Progress of clinical data collection: Data collection from the electronic data sources was done by data scientist Jani Salmi. It turned out that there was much more data available for newer cases, e.g. comorbidity data was available from 2005 on. For this reason, data collection from the paper archives started with the cases whose samples were taken after 1.1.2005. Of the 923 cases, 687 had samples taken after 1.1.2005[14]. Gynecologist Taru Tuomi started data collection from the archived paper files in the spring of 2018

[14] As the study time frame ended 31.8.2013 this means on average about 80 patients per year, which is close to the estimated 100 HGSC patients treated in HUS every year. However, for the time 1.1.1982 – 1.1.2005 the search identified much less cases per year, which might be due to the fact that the treatment of HGSC or ovarian cancer in general was not done centralized in HUS hospital during the whole time frame. Another explanation could be that the search algorithm identified newer samples better, because the search terms reflected more the language used in newer pathologic statements.

and collected information from 115 cases; I collected information from approximately 500 additional cases during summer 2018 and summer 2019.

The status of data collection from the paper archives at the time of writing this report is as follows (see also Figure 2): 113 of the 687 cases, for which paper files were ordered to the archive, have been excluded thus far. Cases were excluded due to information found in the paper files (no chemotherapy received, a non-high-grade serous diagnosis), or following the pathologists' screening of the tumor samples slides (not enough tissue, diagnosis other than HGSC). Patients who did not receive chemotherapy, usually very old patients with considerable comorbidities, were excluded from the cohort because one key goal of this project is to study tumor response and resistance to platinum-based chemotherapy. Clinical data has been collected for 516 cases, of which 320 now have complete information. For the remaining 196 cases, some data has been collected (usually surgery information), but part of the information is incomplete (usually chemotherapy information). There are three different reasons why the clinical information collection is not yet completed:

- Firstly, in 62 cases the patient came to the HUS hospital for surgery only and had chemotherapy at her home hospital, from which we do not (yet) have the corresponding information.
- Secondly, papers containing chemotherapy information or other clinical data were missing from the files (52 cases). For example, files for a patient with an active relapse at the time of data collection would be in use at the hospital and would therefore not be accessible in the archive. In addition, the type of data storage changed over time; from 2014 on, printouts of the patients' care reports were no longer included in the paper files, and the type of documentation for chemotherapy dates and dosages changed from handwritten forms to digital forms over the last years.
- Thirdly, detailed information about the chemotherapy is missing for a few cases due to a change in our data collection protocol (80 cases).

The research group has to decide which of the missing information is important to obtain. This could be accomplished by assessing the files again, searching for the missing documents, or requesting cooperation from the patients' home hospitals. One possibility is also that some of the clinical information is available only for some patients in the cohort and depending on the analyses one wants to conduct, one chooses the patients for which the corresponding information is available.

There are 35 cases for which data has not been collected yet, partly because their papers were not provided by the archive, and partly because of time constraints.

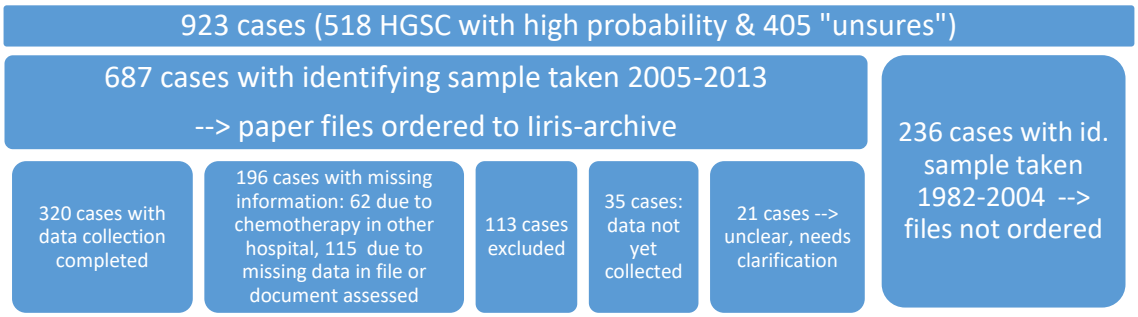

Figure 2: Clinical data collection from paper archives (status as of 12.10.2019)

4.3 Tissue samples and TMA formation

In order to test our research procedures, verify diagnoses and do preliminary testing for novel biological markers, an initial TMA consisting of tissue samples from 109 patients was constructed. For this first TMA, patients were selected according to the following criteria: they had received primary debulking surgery with tissue samples available from this surgery (this tissue was used for the TMA), they had stage III or IV disease at diagnosis, the HGSC diagnosis had been morphologically confirmed by a pathologist, they had received at least 6 cycles of platinum-taxane based chemotherapy following surgery and finally, they had a biochemically complete treatment response, meaning that the CA-125 value dropped below the threshold of 35 U/ml after starting treatment. This group contains patients with very different progression times, meaning the time after the end of the first chemotherapy treatment line to progression (as measured by a CA-125 value over 35 U/ml). The progression time ranged from 0 months, i.e. response after beginning of treatment but progression already before the end of the first treatment line, to over 18 months.

As a first step, immunohistochemical staining for HGSC diagnosis verification was performed: aberrant TP53 and positive WT1 staining served as diagnostic criteria.

In the future, more TMAs are planned, e.g. those containing subsequent tissue samples from the patients in the first TMA, for those cases that such tumor tissue is available from later surgery or biopsies. Also, a TMA comparing pre-surgery and interval surgery

tissue samples will be interesting. In the end, the goal is to have tissue samples from all patients included in the study available in at least one TMA.

5 Results

As outlined in chapter 3 the aim of this report and research was to

- *divide the cohort into different outcome groups and study disease trajectories*
- *search for clinical information that can predict course of disease and outcome*

However, building the cohort took longer than originally planned and is not yet completed. A considerable proportion of the needed information, such as elements of the chemotherapy information, and the information on deaths for the years 2016-2018, is still missing. The confirmation of diagnoses by pathologists, and with that the inclusion of patients to the final study cohort, is still ongoing. Therefore, data analysis is not yet possible. Hence this chapter will first point out challenges in the clinical data collection and then give a preliminary description of some characteristics of the study cohort thus far.

5.1 Challenges in clinical data collection

It was challenging in some cases to classify certain information obtained from the archived files. For instance, identification of the surgery type was not always straightforward, e.g. to distinguish a primary debulking surgery from a primary diagnostic/inoperable surgery in a case where debulking had been attempted (and tumor mass was debulked to some degree) but the surgery report states that no further surgical measures are possible, and a substantial tumor mass remains. Consequently, the distinction between a surgery classified as “inoperable” (i.e. “hard to define”) and one classified as “debulking with residual tumor = R2” was in some cases too difficult to resolve and consequently this classification of the cases is not reliable. Future users of the data should be aware of this and the categorization should be revised and/ or reviewed by a gynecologic oncologist.

In contrast, information regarding whether the surgery took place before chemotherapy (primary surgery) or after chemotherapy (interval surgery) was readily available. Information about residual tumor size after surgery was clearly stated in some reports, but in others it was described only as “carcinosis” or “small lumps” of tumor, so the classification in terms of R1 vs R2 was not always straightforward. We classified residual tumor described as “carcinosis” as R2, unless it was clear from the description that it was less than 1 cm in diameter. In contrast, to distinguish an R0 result from surgery with some kind of residual tumor was easy, since complete cytoreduction was typically clearly stated in the surgical reports. The conclusion for future data users is that only the distinction between R0 and any other R (i.e. R1, R1-2, R2) is reliable. During data collection it also became evident that the R2 residual tumor class encompasses a wide variety of surgical outcomes. R2 disease ranges from one small tumor mass with a diameter of 1 cm, to thick carcinosis throughout the abdominal cavity, to a residual tumor measuring 15 cm or more. It would be informative to have a more precise measure for residual tumor. The inclusion of additional data, e.g. imaging data, could help. Another improvement could be to collect the pattern of residual tumor, e.g. how many patients present with carcinosis spread to a wide area.

Another observation was that the numbering of the treatment lines (first line, second line) as recorded in the archived files was not always uniform: sometimes a change of medication during a treatment line, i.e. because of allergy to paclitaxel, led to a new treatment line designation, while in other cases the treatment line number stayed the same, and the medication simply changed. As a result, the number of treatment lines as documented in the files is not necessarily a good measure of how many chemotherapy doses a patient has received.

5.2 Preliminary analysis of the cohort

The following descriptions refer to the cohort as of 10.10.2019, i.e. the cases for which clinical data were collected and which have not been excluded so far. This includes 516 cases (as described in chapter 4.2.); 320 cases with data collection completed plus the 196 cases with data on surgery completed but chemotherapy information partially

lacking. However, it is possible that some cases may still be excluded based on the pathologists' morphologic screening or the IHC staining, or that the surgery classification will be revised.

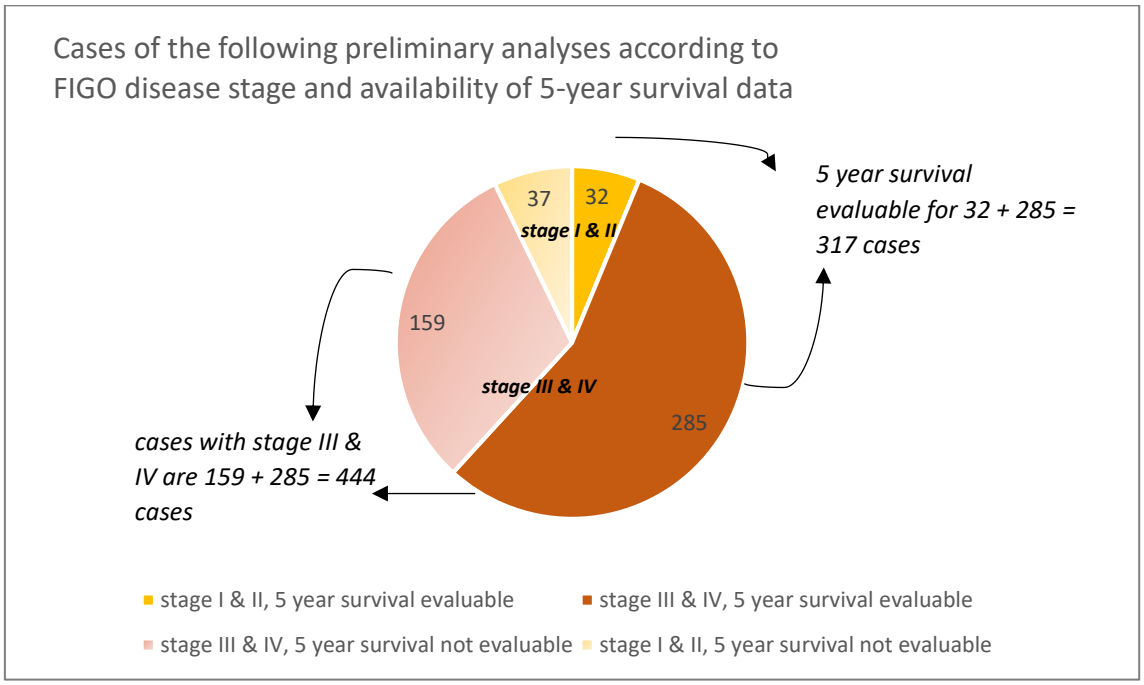

Figure 3: Number of cases according to FIGO disease stage and availability of 5-year survival information (n=513, 3 cases with missing information on stage)

When looking at 5-year overall survival, 317 of the 516 cases can be evaluated because they were diagnosed in 2010 or earlier, as information regarding patient deaths is currently available up to 2015. When looking only at the patients with spread of the disease outside the pelvis, i.e. stages III and IV, the numbers are 444 cases in total, with 285 cases diagnosed 2010 or earlier. Figure 3 gives an overview of the different groups used for the following preliminary descriptive analyses. The number of cases used in a certain graph can be somewhat smaller if there are missing values for the variable studied, i.e. if some information was not available in the papers assessed.

Similar to findings in other studies, most of the patients in our cohort presented with advanced stage disease. 86.5% of them were stage III or IV at diagnosis, with stage IIIc being the most frequent stage (57.9% of patients). For comparison, a study from Peres and colleagues (mentioned in chapter 2.8) found 77.9% of patients diagnosed with stage

III or IV and 22% with stage I or II (12). Table 4 presents the frequencies of different disease stages at diagnosis in our preliminary sample[15].

Table 4: Patients' FIGO disease stage at diagnosis, n=513[16]

Stage at diagnosis according to FIGO 2014		Frequency	Percent	
Stage I	1a	11	2.1	7,0 %
	1b	2	.4	
	1c	1	.2	
	1c1	7	1.4	
	1c2	3	.6	
	1c3	11	2.1	
	1 NOS	1	.2	
Stage II	2a	17	3.3	6,4 %
	2b	16	3.1	
Stage III	3a1	17	3.3	67,6 %
	3a2	6	1.2	
	3b	26	5.1	
	3c	297	57.9	
	3 NOS	1	.2	
Stage IV	4a	33	6.4	18,9 %
	4b	62	12.1	
	4 NOS	2	.4	
Total		513	100.0 %	

The mean age of patients at diagnosis was 64.3 years (SD 9.7 years); the youngest patient being 34 years old at the time of diagnosis and the oldest 87. Figure 4 shows the distribution of patients' age at diagnosis, showing that most of the patients were between 60 and 70. The mean age was about the same when patients were grouped according to their disease stage at diagnosis (see table 5).

Table 5: Mean age at diagnosis by disease stage

FIGO stage	Mean age at diagnosis	n	sd
I	64.9 yrs	36	10.1
II	62.9 yrs	33	11.4
III	64.5 yrs	347	9.8
IV	64.0 yrs	97	9.0
Total	64.3 yrs	513	9.7

[15] IBM SPSS Statistics 25 software was used for all analyses.
[16] For 3 cases information for stage at diagnosis was missing, for this reason n=513 in this table.

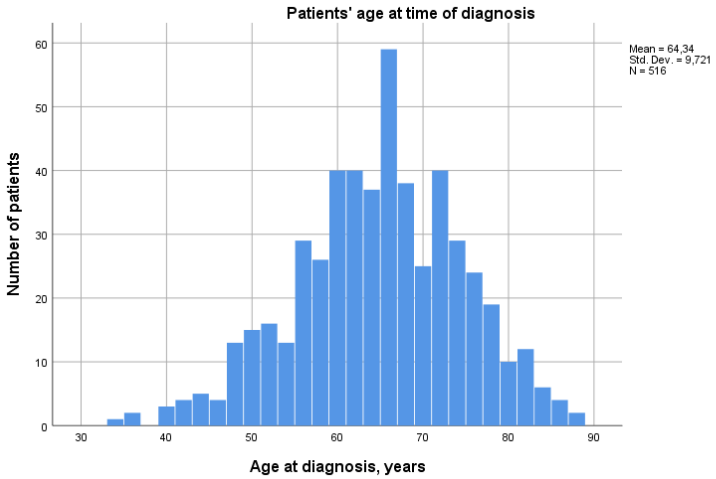

Figure 4: Distribution of patients' age at diagnosis

Similar to findings in other studies the 5-year overall survival is clearly higher in patients with stage I or II disease as compared to stage III and IV disease, e.g. 78.6% of patients with disease stage I were alive after 5 years compared with 16.7% of patients with stage IV disease (see table 6).

Table 6: 1-year and 5-years overall survival in patients grouped by FIGO disease stage at diagnosis (Chi-Square-Test for differences between stage groups significant for 5-year-overall survival)

FIGO stage	OS 1 year	OS 5 years
I	100.0% (n=14)	78.6% (n=11)
II	100.0% (n=18)	72.2% (n=13)
III	95.0% (n=208)	32.0% (n=70)
IV	100.0% (n=66)	16.7% (n=11)
Total	96.5% (n=306)	33.1% (n=105)

Comparing the mean age at diagnosis of those patients who are alive 5 years after diagnosis and those who are not, one finds a significant, but possibly not very important, difference: Those alive after 5 years are slightly younger (61.6 vs. 64.7 years, mean; t-test for independent samples significant (2-tailed) $p < 0.05$) – a preliminary finding that is in concordance with the findings reported in chapter 2.8 on age as a prognostic factor.

Table 7: 5-year overall survival according to performance status (ASA-class), n= 308[17], Chi-Square test significant p<0,05

Performance status	5-year OS
ASA1, 2 or 2-3	42.2% (n=54)
ASA3, 4 or 3-4	27.2% (n=49)
Total	33.4% (n=103)

Performance status seems to have prognostic value as well, which was also noted in other studies (see chapter 2.8). Looking at all disease stages together, 42.2% of patients with ASA class 1, 2 or 2-3 were alive 5 years after diagnosis, compared with 27.2% of patients with ASA class 3, 4 or 3-4 (see table 7).

The distribution of the main surgery type is displayed in table 8. 60.5% of cases had primary debulking surgery as the main surgery, 20.2% an interval surgery, and in 19.3% the primary surgery type was hard to define. When analyzing only cases with stage III or IV disease, the percentage of interval surgery is somewhat higher and the percentage of primary debulking surgery somewhat less, which is due to the fact that nearly all patients with stage I or II disease are able to undergo primary debulking surgery (see table 8).

Table 8: Distribution of main surgery type[18]

Type of main surgery	all stages	only stages III & IV
interval surgery	103 (20.2%)	97 (22.1%)
primary debulking surgery	308 (60.5%)	246 (56.0%)
primary surgery that is hard to define	98 (19.3%)	96 (21.9%)
Total	509 (100%)	439 (100.0%)

Regarding residual disease at the conclusion of the main surgery, 30.3% of the preliminary cohort patients had a complete cytoreduction (R0), 10.9% had residual disease measuring less than 1 cm diameter (R1), and 58.8% had residual disease greater than 1 cm (R2, see table 9). As mentioned previously, the distinction between R1 and R2 was not always possible with the available records, so one can only reliably distinguish

[17] There were 9 cases with no information on ASA class, therefore n=308

[18] for all stages n= 509, 7 cases with information on main surgery type missing, and for stage III & IV only n=439, 5 cases with information on main surgery type missing

between R0 and non-R0 in this dataset. When looking at the cases with stage III or IV disease, the percentage of patients with complete cytoreductions drops to about 20% because R0 is much more likely in a patient with stage I or II disease (see table 9).

Table 9: Distribution of residual disease after main surgery in the first treatment line[19]

Residual tumor	all stages	only stages III & IV
R0	153 (30.3%)	89 (20.5%)
R1	55 (10.9%)	52 (12.0%)
R2 (including R1-2)	297 (58.8%)	293 (67.5%)
Total	505 (100.0%)	434 (100.0%)

When looking at the residual disease by surgery type, it seems that a larger percentage of interval surgeries had an optimal result, i.e. R0, as compared to primary debulking cases (46.0% vs 34.2% across all stages and 43.6% vs. 19.6.% for stages III & IV, see tables 10 and 11).

Table 10: Distribution of residual disease according to different main surgery types for all stages[20]

Main surgery type	R0	R1	R2 & R1-2	Total
interval surgery	46.0% (n=46)	26.0% (n=26)	28.0% (n=28)	100.0% (n=100)
primary debulking surgery	34.2% (n=105)	9.1% (n=28)	56.7% (n=174)	100.0% (n=307)
primary hard to define	1.0% (n=1)	1.0% (n=1)	97.9% (n=94)	100.0% (n=96)
Total	30.2% (n=152)	10.9% (n=55)	58.8% (n=296)	100.0% (n=503)

This could be due to the fact, that NACT reduces tumor size and the tumor is then easier to resect. Another explanation for this difference could be that the pre-operative determination of the likelihood of achieving an optimal cytoreduction influenced the decision to do an interval surgery[21] more than it influenced the decision to undertake a

[19] for all stages n= 505, 11 cases with missing information on tumor residual; and for only stages III & IV n=434, 10 cases with missing information on residual tumor

[20] n = 503, 13 cases with missing information on main surgery type and/or residual tumor size

[21] For example patients treated with NACT, whose tumor did not shrink in response to the treatment, might not have had an interval surgery. So those patients would have the primary diagnostic surgery as their "main surgery type", which would be classified as "primary hard to define" in this study. Or for patients, that did not have surgical treatment at all, i.e. patients, who did not have primary diagnostic surgery, and interval surgery was planned but not done because of the tumor not responding to the neo-adjuvant chemotherapy; these patients would not be included in our cohort, because one criteria for inclusion was surgical treatment.

primary debulking surgery. Also the difficulties with classifying patients' surgery type, i.e. the difficulty to distinguish *primary debulking* and *primary hard to define*, influence the results. In general, the changing surgical treatment protocol over the time span of the study cohort also influences the results.

Table 11: Distribution of residual disease by main surgery type for patients with stage III or IV disease[22]

Main surgery type	R0	R1	R2 & R1-2	Total
interval surgery	43.6% (n=41)	27.7% (n=26)	28.7% (n=27)	100.0% (n=94)
primary debulking surgery	19.6% (n=48)	10.2% (n=25)	70.2% (n=172)	100.0% (n=245)
primary hard to define	0.0% (n=0)	1.1% (n=1)	98.9% (n=93)	100.0% (n=94)
Total	20.6% (n=89)	12.0% (n=52)	67.4% (n=292)	100.0% (n=433)

When comparing 5-year overall survival between patients according to their surgical outcome, the more favorable outcome of patients with complete cytoreduction compared to patients with a residual tumor of R1 or R2 seems evident (61.3% compared to 27.8% and 23.1.% across all stages and 51,9% compared to 25.7% and 22,8% for stages III & IV, see table 12). This is in congruence with earlier research findings mentioned in chapter 2.8.

Table 12: 5-year overall survival by residual tumor amount[23]

Residual tumor	5-year OS, all stages	5-year OS, stages III & IV
R0	61.3% (n=49)	51.9% (n=27)
R1	27.8% (n=10)	25.7% (n=9)
R2	23.1% (n=45)	22.8% (n=44)
Total	33.2% (n=104)	28.6% (n=80)

When comparing the outcome of patients with the different types of major surgery, the 5-year overall survival is higher in the primary debulking group as compared to the

[22] n = 433; 11 cases with missing information on main surgery type and/or residual tumor size, Chi-square test significant $p<0.05$

[23] for all stages: 6 cases with missing information on residual tumor, Chi-square test significant $p<0,05$; for only cases with stage III or IV disease: 5 cases with missing information on residual tumor, Chi-square test significant $p<0.05$.

interval surgery group (41.2% vs. 29.5.% for all stages and 35.2% vs. 28.3% for stages III and IV, see table 13). When comparing 5-year overall survival in patients with advanced disease and surgical outcome R0 there is no statistical significant difference between the primary debulked group and the interval surgery group (48.3% vs. 56.5%, see table 14), however the sample might be too small to show a possible difference.

Table 13: 5-year overall survival according to major surgery type[24]

Main surgery type	5-year OS, all stages	5-year OS, stages III & IV
interval surgery	29.5% (n=18)	28.3% (n=17)
primary debulking	41.2% (n=80)	35.2% (n=58)
primary hard to define	8.8% (n=5)	8.8% (n=5)
Total	33.0% (n=103)	28.4% (n=80)

However, one cannot draw conclusions regarding the question of whether a primary or interval surgery is better in general, because the patients in the primary debulking group were more likely to be patients with tumor that was resectable, which could mean that their disease had a better prognosis regardless of the timing of their surgery (see discussion on surgery types in chapter 2.8).

Table 14: 5-year overall survival according to major surgery type for patients with stage III or stage IV disease AND residual tumor of R0, Chi-square test **not** significant, p>0.05.

	5-year overall survival
stage III or IV & interval surgery & R0	48.3% (n=14)
stage III or IV & primary debulking & R0	56.5% (n=13)

One goal of building the cohort was to divide patients into different outcome groups and try to find explanations for patients´ very different disease trajectories. Figure 5, which Jani Salmi kindly provided from his presentation, shows, that the collected data can be used to study disease trajectories on the level of the individual patient. While the three patients had similar performance status, stage and first line chemotherapy, their

[24] for all stages: 5 cases with missing information on major surgery type, Chi-square test significant p<0.05, and for only cases with stage III or IV disease: 3 cases with missing information on major surgery type, Chi-square test significant p<0.05.

outcomes are still very different. The picture demonstrates the considerable difference in survival between patients, which could be due to biological differences in patients` carcinomas[25]. Further research with this cohort, once it is ready, will try to find such biological differences.

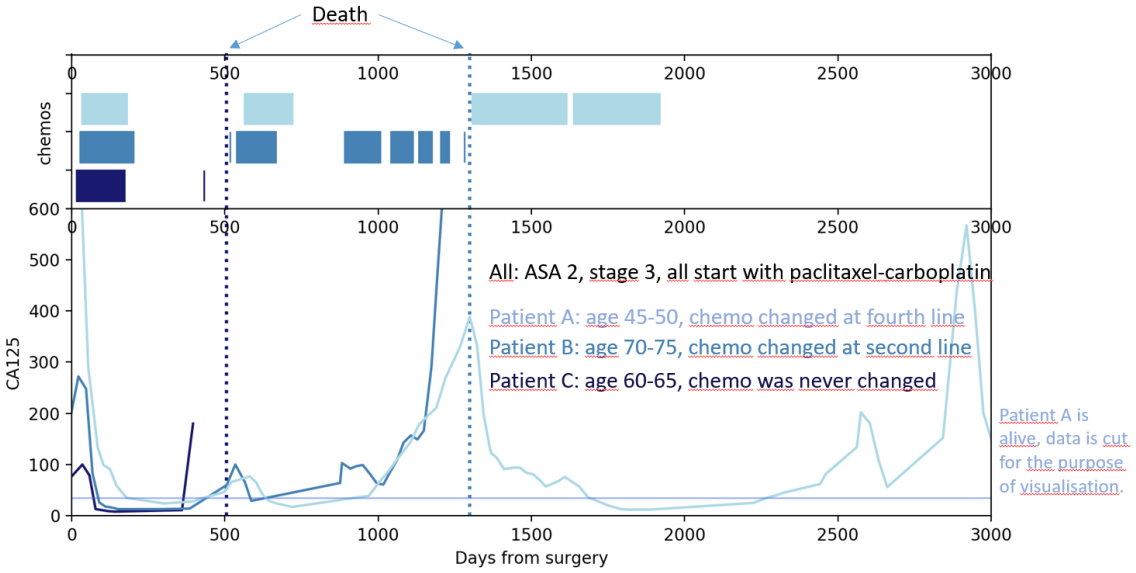

Figure 5: Disease trajectories of three patients with same performance status, stage and first line chemotherapy treatment but different outcomes, picture from Jani Salmi

6 Conclusions and prospects

High-grade serous ovarian cancer is an important and interesting disease to study. Chapter 2 introduced the topic and gave an overview of the research findings in this field. Despite progress in the understanding of the underlying biological processes, the prognosis of patients has not improved significantly during the last decades, though the new PARP-inhibitors might be about to change this.

The primary goal of the research project covered in this report is to create a retrospective cohort of HGSC patients with tissue samples and clinical follow-up data, which can be used to study different research questions (for example studying the response and resistance to platinum-based chemotherapy) and can also be provided to

[25] The younger age of patient A could indicate the higher possibility of a BRCA-mutation. This will have to be checked. The BRCA-mutation is one of the very few, if not the only, known biological marker in HGSC, that is used in the clinic with consequences for the choice of the treatment, e.g. the application of PARP-inhibitors and also genetic counselling for family members of the patient.

collaborating research groups, such as for the testing of potential biomarkers. The current status of the cohort formation was described in chapter 4. There were initially over 900 patients identified as potential cases for inclusion in the cohort. The process of confirming diagnoses and other requirements (i.e. only patients who received surgical and chemotherapeutic treatment are included) is ongoing. There were some challenges with the collection and categorization of clinical data, such as with the characterization of the surgery type. These challenges were discussed in chapters 4 and 5. Important information on potential problems with some of the collected clinical information has been added to the description in the appendix, so that future users of the dataset can understand the nature and limitations of the data.

The second and third research aims, as stated in chapter 3, were to divide the cohort into different outcome groups and study disease trajectories in order to search for clinical information that may predict the disease course and patient outcome. Because the cohort building took more time than was planned and is not yet complete, chapter 5 presents only some preliminary descriptions of the cohort. These descriptions seem to be in concordance with other research findings, e.g. patients with lower disease stage (stage I and II) had a considerably better 5-year overall survival than patients with disease stages III or IV at diagnosis; the distribution of disease stages was similar to other studies. Additionally, patients with complete cytoreduction (R0) had a better prognosis. These preliminary findings are reassuring; the cohort will be an appropriate group of patients in which to study the disease.

The next steps in the cohort formation will be to complete the diagnosis confirmation and clinical data collection for those cases which were not assessed, e.g. the cases with the diagnostic samples taken before 2005. Then the research team needs to decide which missing information to continue to search for (see chapter 3). Another step will be to begin analyzing the chemotherapy information; data scientist Jani Salmi has started working on this. It is also planned to study progression free survival; for now, the CA-125 levels are used to identify relapses. However, additional reports, such as radiology information, will need to be assessed in some patients in order to reliably identify relapses. Patient comorbidities will also be analyzed.

In addition, the cohort will be used by other researchers and research groups. Cyclin E expression will be analyzed by biologist Noora Andersson using RNA in-situ-hybridization as well as standard immunohistochemistry. Both techniques are being used in order to validate the relatively novel RNA in-situ-hybridization technique. The research question here is whether cyclin E expression is increased in patients with shorter progression free survival. Further, it is planned to test additional markers using this same TMA, e.g. Liisa Kauppi´s group will stain the TMA for cytokeratin 7 expression to distinguish tumor cells from stroma cells and then evaluate gamma-H2AX expression, which is a new marker of DNA double-strand breaks. Also, expression of fusion proteins such as MLL1 will be tested. Pathologist Anna Laury will also use the TMA in her project involving image analysis of histological slides.

To conclude, the building of such a large cohort comprising both tissues samples and a large amount of clinical information, which comes from various electronic databases as well as from archived paper files, is a long process. Despite modern technologies to gather large amounts of data, bottlenecks remain. For instance, knowledge of the reporting practices as well as clinical expertise both in gynecological oncology and in pathology are vital for the successful execution of such a project. Diagnostic criteria and clinical recommendations on surgery and chemotherapy evolve over time, which poses a challenge for dissecting the role of biological characteristics in the disease outcome. However, as a conclusion, the Finnish biobank infrastructure, electronic medical record system and legislation provide unique opportunities for collection of cohorts combining clinical information and biological specimens. Once the cohort is ready it will be a unique and invaluable tool for studying diverse research questions in the field of high-grade ovarian carcinoma, that might someday help improve patients' prognosis.

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Appendix

Description of clinical data collected from paper archives

The following information in Finnish was used as a guide for the collection of clinical data from archived paper files.

1. Tarkista diagnoosi

Tavoitteena on, että kohortti rajoittuisi ovario-, tuuba- tai peritoneum-peräisiin high-grade serooseihin syöpiin (HGSC). Ei kerätä tietoja, jos diagnoosi on muu epiteelimunasarjasyöpä kuin seroosi (esim. Ca endometrioides, kirkassoluinen jne.) tai jos kyseessä on vanhan diagnostiikan mukaiset seroosit G1 karsinoomat. G2 seroosit otetaan mukaan. High-grade mixed type voi tulla mukaan (on vanhaa luokittelua, nämä ovat usein high-grade serous), sen sijaan Ca serosum endometroides jää pois.
Kaikki potilaat, joiden osalta päätetään, ettei kerätä tietoja, kirjoitetaan omaan listaan. Heille kuten kaikille muillekin patologi tekee tarvittaessa vielä morfologian ja IHC-värjäyksen perusteella diagnoosin tarkistuksen.

2. Yleiset tiedot

pvm	päivämäärä, jolloin tiedot kirjoitetaan Exceliin
aika	kellonaika, esim. 9:10 - 9:45
kuka	lyhenne henkilöstä, joka kerää tietoja

Potilaan yleiset tiedot

hetu		
nimi		
dg pvm	päivämäärä, milloin potilas tulee ensimmäistä kertaa poliklinikalle (jos sitä ei ole saatavilla, niin esim. ensimmäisen kohonneen CA-125-mittauksen päivämäärä tai esim. ensimmäisen leikkauksen päivämäärä, jossa syöpä oli sivulöydöksenä etc. tai ensimmäisen kemokuurin päivämäärä jos oli NACT)	
paino	paino hoidon alussa: jos paino löytyy ensimmäisen leikkauksen anestesiakaavakkeesta, otetaan sieltä, muuten esitiedoista.	kg
pituus		cm
ASA	Jos luokka vaihtelee, otetaan luokka ensimmäisessä leikkauksessa. Jos ensimmäisen leikkauksen ASA-luokka ei lue papereissa, otetaan toisen leikkauksen ASA-luokka jos sellainen on.	
erityistä	Merkitään esim. jos potilas on siirtynyt muualta tänne.	

3. Stage

Stage (papereissa)	tässä kirjoitetaan stage, joka lukee papereissa
Uusi stage	Jos stagen määritelmä on tehty ennen 2014: luokitellaan stage uuden 2014 FIGO:n luokituksen mukaan

Luokittelun uudistus 2014 pitää sisällään 4 asiaa:

- IC on alaluokiteltu numeroin 1-3 sen mukaan, onko tuumori puhjennut leikkauksessa, ennen leikkausta, tai onko askiteksen sytologia positiivinen. Tuumorin puhkeaminen pitäisi olla mainittu leikkauskertomuksessa.
- IIC on kokonaan poistettu (tämä on varmasti harvinainen stage).
- Stage IIIA on muutettu siten, että siinä on mukana imusolmukkeisiin metastasoineet syövät ilman peritoneaalista metastasointia (IIIA1) ja mikroskooppisen peritoneaalisen metastasoinnin

kanssa (IIIA2**). Aiemmin imusolmukemetastasointi teki stagen aina IIIC:ksi.** Uudistus tehtiin sen takia, että isoloituun imusolmukemetastasointiin liittyy parempi ennuste kuin vanhaan stage IIIC:hen. Uusi stage IIIA on harvinainen, mutta pitäisi olla jäljitettävissä leikkauskertomuksesta ja PAD-lausunnosta. IIIA1(i) ja IIIA1(ii) eivät luultavasti ole aina erotettavissa toisistaan, koska erottelu perustuu imusolmukemetastaasin kokoon, jota ei taida olla useinkaan mainittu PAD-lausunnossa.

- IV on saanut alaluokat A (pleura) ja B (maksa, perna, ekstra-abdominaaliset).

→ Leikkauskertomuksesta ja kuvantamislöydöksistä pitäisi selvitä paikat, missä kasvainta on. Kuvantaminen on keskeinen lähinnä stage IV:n toteamisessa. Epikriisissä on tällöin yleensä yhteenveto kuvantamislöydöksistä.
→ Munasarjasyövän, tubakarsinooman ja peritoneaalikarsinoosin staging-luokitus on sama. Peritoneaalikarsinoosin stage on lähtökohtaisesti aina vähintään II.

FIGO Ovarian Cancer Staging Effective Jan. 1, 2014
(Changes are in italics.)

STAGE I: Tumor confined to ovaries				
OLD			NEW	
IA	Tumor limited to 1 ovary, capsule intact, no tumor on surface, negative washings/ascites.		IA	Tumor limited to 1 ovary, capsule intact, no tumor on surface, negative washings.
IB	Tumor involves both ovaries otherwise like IA.		IB	Tumor involves both ovaries otherwise like IA.
IC	Tumor involves 1 or both ovaries with any of the following: capsule rupture, tumor on surface, positive washings/ascites.		*IC Tumor limited to 1 or both ovaries*	
			IC1	*Surgical spill*
			IC2	*Capsule rupture before surgery or tumor on ovarian surface.*
			IC3	*Malignant cells in the ascites or peritoneal washings.*

STAGE II: Tumor involves 1 or both ovaries with pelvic extension (below the pelvic brim) or primary peritoneal cancer				
OLD			NEW	
IIA	Extension and/or implant on uterus and/or Fallopian tubes		IIA	Extension and/or implant on uterus and/or Fallopian tubes
IIB	Extension to other pelvic intraperitoneal tissues		IIB	Extension to other pelvic intraperitoneal tissues
IIC	IIA or IIB with positive washings/ascites.			

Old stage IIC has been eliminated

STAGE III: Tumor involves 1 or both ovaries with cytologically or histologically confirmed spread to the peritoneum outside the pelvis and/or metastasis to the retroperitoneal lymph nodes				
OLD			NEW	
IIIA	Microscopic metastasis beyond the pelvis.		*IIIA (Positive retroperitoneal lymph nodes and /or microscopic metastasis beyond the pelvis)*	
			IIIA1	*Positive retroperitoneal lymph nodes only*
			IIIA1(i)	*Metastasis ≤ 10 mm*
			IIIA1(ii)	*Metastasis > 10 mm*
			IIIA2	*Microscopic, extrapelvic (above the brim) peritoneal involvement ± positive retroperitoneal lymph nodes*
IIIB	Macroscopic, extrapelvic, peritoneal metastasis ≤ 2 cm in greatest dimension.		IIIB	*Macroscopic, extrapelvic, peritoneal metastasis ≤ 2 cm ± positive retroperitoneal lymph nodes. Includes extension to capsule of liver/spleen.*
IIIC	Macroscopic, extrapelvic, peritoneal metastasis > 2 cm in greatest dimension and/or regional lymph node metastasis.		IIIC	*Macroscopic, extrapelvic, peritoneal metastasis > 2 cm ± positive retroperitoneal lymph nodes. Includes extension to capsule of liver/spleen.*

STAGE IV: Distant metastasis excluding peritoneal metastasis				
OLD			NEW	
IV	Distant metastasis excluding peritoneal metastasis. Includes hepatic parenchymal metastasis.		*IVA*	*Pleural effusion with positive cytology*
			IVB	Hepatic and/or *splenic parenchymal* metastasis, metastasis to extra-abdominal organs (including inguinal lymph nodes and lymph nodes outside of the abdominal cavity)

Other major recommendations are as follows:

- Histologic type including grading should be designated at staging
- Primary site (ovary, Fallopian tube or peritoneum) should be designated where possible
- Tumors that may otherwise qualify for stage I but involved with dense adhesions justify upgrading to stage II if tumor cells are histologically proven to be present in the adhesions

4. Leikkaukset

Tässä haetaan tietoja vatsaontelon sisälle tehdyistä leikkauksista, joiden syynä on ollut syöpäkudoksen pienentäminen syövän parantamiseksi, tuumorin pienentämiseksi/diagnostiikan varmentamiseksi ja levinneisyyden selvittämiseksi. Huom: ei palliatiiviset leikkaukset (esim. aivometastaasien leikkaaminen).

1. leikkaus, pvm		
1.leikkaus, tyyppi	• primary diagnostinen • primary debulking • primary inoperable/diagnostinen • interval debulking • interval diagnostinen • interval inoperable/diagnostinen • muu leikkaus: vastaa primary diagnostinen/inoperable • muu leikkaus: vastaa primary debulking • debulking: hoidetaan uusiutunut tauti • second look Leikkaus merkitään interval-leikkaukseksi aina kun se on tehty sytostaattihoidon alkamisen jälkeen. HUOM: - tyyppi inoperable/diagnostinen ei ollut alusta asti mukana, siksi diagnostista tyyppiä ja tyyppiä inoperable/diagnostinen ei pysty erottamaan toisistaan. Pelkästään diagnostisia leikkauksia oli vähän. - primary debulking & R0: on myös laitettu jos on löydetty esim. stage-1-tauti ja silloin poistettu vaikka vain munasarjat ja munanjohtimet. - erotus inoperable/diagnostinen ja debulking & R2 välillä on ollut välillä erittäin hankalaa, ei ole saatu hyvin eroteltua.	
1.leikkaus jäännös	Jos leikkauksen tarkoitus oli debulking, kirjoitetaan jäännöskasvaimen koko (löytyy leikkauskertomuksista) • R0 = no residual disease, • R1 = minimal residual disease (deposits of residual tumour <1 cm), • R2 = gross residual disease (deposits of residual tumour >=1 cm). Mitataan yksittäisten kasvainmuutosten läpimitta: Jos jää parikymmentä 0,5 cm:n tuumoria, kyseessä on R1. Jos taas jää yksi 2 cm:n tuumori, kyseessä on R2. Leikkauskertomukseen merkityllä jäännöskasvaimen koolla tarkoitetaan suurimman pesäkkeen läpimittaa siten, että tämän kokoisia tai tätä pienempiä pesäkkeitä voi olla useita. Esim. jos jäännöskasvaimeksi on merkitty 1 cm, tämän kokoisia pesäkkeitä voi olla useita, ja lisäksi voi olla pienempiä pesäkkeitä. Jos ei selvästi kerrota, ovatko jäljelle jäävät kasvaimet R1 vai R2, käytetään määritelmää R1-2. Jos jäännöskasvaimena on mainittu vain karsinoosi ilman kokoa tai mainitaan että karsinoosi on todella pieni, kirjoitetaan R2.	• R0 • R1 • R2 • (R1-2)
1.leikkaus karsinoosi	Tänne kirjoitetaan silloin, kun jäännöskasvain on karsinoosi, teksti siitä, miten karsinoosi on kuvattu leikkauskertomuksessa (esim. ”jäännöskasvaimeksi jää laaja karsinoosi”) HUOM: tämä kategoria ei ollut koko ajan mukana, on ollut välillä mukana kun yritettiin löytää parempaa mittaria jäännöskasvaimen luokitteluun.	
1. leikkaus kommentti		

Vastaavasti muut leikkaukset.

<u>Myöhemmin lisätty: main surgery</u>

main_surgery type of the first treatment line → Valitaan jokaiselle potilaalle ensimmäisen hoitolinjan „pääleikkaus“, eli jos on tehty staging leikkaus / debulking leikkaus, niin sitten se on „pääleikkaus“; jos on vain diagnostinen leikkaus tai inoperable, niin sitten se on „pääleikkaus“ —> tämä tehtiin sen takia, että osalla potilaita on enemmän kuin yksi leikkaus ensimmäisen hoitolinjan aikana ja kun vertaillaan leikkaustyyppejä, jäännöskasvaimia jne., niin tarvitaan per potilas yksi „pääleikkaus“, johon tyyppi, jäännöskasvain jne. viittaa. Eli käytännössä katsotaan, mitkä leikaukset potilaalle on tehty ja jos on enemmän kuin yksi, niin identifioidaan pääleikkaus ja sitten valitaan yksi seuraavista kolmesta tyypistä:

- *PDS = primary debulking surgery*
    - *Ei kemoterapiaa ennen leikkausta*
    - *Voi olla että on ollut diagnostinen leikkaus ennen PDS:ää*
- *interval surgery*
    - *Kemoterapia ennen pääleikkausta: voi olla että kemoterapia ennen leikkausta ja sen jälkeen tai vain ennen leikkausta*
    - *Voi olla että ennen kemoterapiaa on ollut diagnostinen leikkaus*
- *primary_hard-to-define*
    - *Jos pääleikkaus on primaarileikkaus ja esim. diagnostinen/inoperable tai muu vastaava*

Pääleikkauksen päivämäärä (date of main surgery)

Pääleikkauksen jäänöskasvain (residual tumour): R0, R1, R2

Age at diagnosis: laskettu syntymäpäivämäärän (hetusta) ja diagnoosipäivämäärän erotuksena

5. Hoitolinjat

Tässä kerätään tietoa, milloin mitä sytostaattia tai muuta syöpälääkettä on annettu ja millä annoksella. Annokset kirjoitetaan karboplatiinilla AUC:na, Avastinille mg/kg:na ja muille sytostaateille mg/m2:na. Tarkoitus on, että annoksia voidaan verrata eri potilaiden välillä.

Hoitolinjat 1 – 3 kirjoitetaan tarkemmin, muut hoitolinjat ilman annosta.

Hoitolinjat 1 – 3: Tässä sovittiin, että kirjoitetaan aloitus- ja loppupäivämäärä sekä kaikki annokset.

Hetu	Hoitolinja	Hoitosykli	Pvm	1. Lääkeaine	Annos	2. Lääkeaine	Annos	3. Lääkeaine	Annos

Hoitolinjat 4 – x

Hetu	Hoitolinja	Lääkeaine	Pvm aloitus	Pvm loppu	Kuurien lukumäärä

6. Skannataan

- Leikkauskertomukset & loppuarvio
- Anestesiakaavake
- Esitietokaavake
- CA125-markkeri/hoitolinjakaavake
- Patologiset näytteet -kaavake (leikkausnäytteet)