Mermaid III – "The challenge of ovarian cancer: Screening, early diagnosis and identification of high-risk women".

Evaluation of the Scientific Progress Report for 2016 by the Independent Audit Committee (IAC).

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Mermaid III is the extension of very successfull research programmes – Mermaid I, which focused on ovarian cancer and contributed to a better understanding of the disease and its course and Mermaid II which produced significant results in terms of identification of virus types and their implication for development of cervical cancer and identification of women at high risk.

The Mermaid III project once again turn the focus to ovarian cancer with emphasis on early diagnosis, screening and identification of high risk groups.

Ovarian cancer is the most lethal of all gynecologic cancer types and accounts worldwide for app. 250.000 new cases per year. Denmark has a high prevalence of ovarian cancer and so far the prognosis after treatment in Denmark is worse compared to other nations although significant improvements in the treatment of this disease have been achieved over the last years.

Ovarian cancer prognosis is stage dependent and stage 1 disease has a very good prognosis with a 5-years survival of 90%. But low stage cases have very discreet symptoms and 2/3 of all patients with ovarian cancer are diagnosed with advanced disease and an inferior chance of cure. Attempts on population based screening programmes have so far not produced results which justifies introduction of a general screening for ovarian cancer.
A persistent effort to improve diagnosis at an earlier stage, continous search for identification of high risk groups and prognostic markers as well as exploring potential new etiological factors are therefore mandatory if prognosis should be improved considerably.

The Mermaid III project has a very ambitious set-up and research plan for improvement of ovarian cancer survival based on research in three main themes:

1. The study on early detection takes advantage of the already established screening programme for cervical cancer to explore the potential for early detection of ovarian cancer by means of DNA/RNA isolated from routinely collected cervical cytology samples. Data from national registers will be linked to the cohort in order to identify cases and controls and a panel of markers will be used to screen these samples. Moreover, analysis based on national registers on risk factors and previous ovarian abnormalities are undertaken to identify possible groups at increased risk.
2. Identification of new biomarkers for ovarian cancer or/and prognosis by means of new technology which has made it possible to elucidate the entire genetic map for each patients – the next-generation sequencing. These studies are founded on large biobanks combined with validated clinical databases.

3. A better understanding of the disease by exploring the infection theory based on the fact that ovarian cancer spreads within the abdominal cavity in a way similar to ascending infections from the vagina. By new technologies even small quantities of virus and bacterias can be detected. Potentially, establishment of infection as causal in the development of ovarian cancer opens a completely new scope for diagnosis, treatment and profylaxis.

The research team consists of experienced international recognized scientists. Together with their widespread international collaborators their organisations are equipped for performing the various analyzes as well as generating new tests. The research plan has the advantage of partly being based on already collected large numbers of tissue and cytology samples as well as clinical data. Moreover, the investigators use the national databases and registers. All together, the fundament for completing this ambitious research plan and add considerable new evidence to the diagnosis and treatment of ovarian cancer is present and realistic.

The progress report submitted by the three main investigators gives a comprehensive up-date on results in the different substudies obtained so far.

Early detection of ovarian cancer

Early detection/screening using a newly developed test - the PapGene test

A collaboration has been established with the laboratory at Johns Hopkins Hospital, Baltimore, Maryland, USA, who have the equipment for performing the PapGene test. After the test has passed the proof of principles testing, the next steps in the evaluation process progress as planned.

1a. Collection of cervical cytology speciments from ovarian cancer patients and controls for the evaluation process is running as scheduled and different algorithms for the PapGene test to improve the sensitivity and specificity have been tested. Preliminary results are encouraging.

1b. In a biobank comprising liquid based cytology samples from 40,000 women, patients who developed ovarian cancer after the enrollment in the cohort have been identified. Applications of the PapGene test on these cases and matched controls to establish the sensitivity and specificity of the test in detecting asymptomatic ovarian cancer patients are planned. The results will be correlated with clinical stage and histological subtype of ovarian cancer. The time from Pap smear until development of ovarian cancer in relation to the value of the PapGene test will be studied. The molecular genetic alterations detected by PapGene test will be validated in the corresponding ovarian cancer tissues.

1c Prospective collection of LBC samples to establish a Danish biobank is planned.
Normally, samples from women participating in cervical cancer screening in the region of Southern Denmark are destroyed. It is planned to collect and save a total number of 150.000 samples. The cohort will be linked to the Pathology Data Bank/the Danish Cancer Registry in order to identify women, who later develop ovarian cancer. The samples from these ovarian cancer cases as well samples from randomly selected women who did not develop ovarian cancer will be tested by the PapGene test.
Currently, approval for collection of samples have been obtained and logistics are in progress.

Early detection focusing on ovarian serous borderline tumors

2a. Borderline tumors are no-malignant ovarian tumors. Never the less they may be associated with peritoneal implants. Based on a previous established database, clinicopathologic features and behavior of ovarian serous borderline tumors as evaluated by the mutational status of BRAF, KRAS, and p53 are analysed to determine the molecular genetic features of peritoneal tumor implants and elucidate their relationship to the primary serous borderline tumor. Results are correlated to clinical outcome.
By January 2017 molecylar analyses and data follow-up are in progress after pathology review of all primary ovarian tumors/ extra-ovarian implants by gynecologic pathologists from Johns Hopkins hospital, Baltimore, USA. A total of more than 1000 Serous Borderline Tumors have been identified and implants have been categorized. Further informations including FIGO stages on this cohort have been achieved by linking the cohort with the Danish National Patient Registry.

2b. The Danish national registers have been searched for women diagnosed with benign ovarian tumors, during 1978-2012, to explore the association between benign ovarian tumors and subsequent risk for borderline ovarian tumors and for ovarian cancer, overall and for different histological subtypes (serous and mucinous).
Two cohorts have been identified - women with benign tumors (N ~ 160,000) of the ovary and women with ovarian borderline tumors (N ~ 4,500). These cohorts have been linked to the Danish Cancer Registry and the Pathology Data Bank to identify subsequent diagnoses of ovarian borderline tumor and/or cancer. The statistical analyses are performed, and the scientific papers are in preparation.

2c. Ovarian serous borderline tumors is a non-invasive tumor suspected to be a precursor for some types of serous ovarian cancer. The literature is inconsistent regarding risk factor profile in Borderline tumors (SBTs) and serous ovarian cancer. In a case-control approach and a cohort (follow-up) approach, factors potentially related to the risk of ovarian cancer or borderline tumors like pelvic inflammatory disease, infertility, PCOS have been evaluated in order to elucidate whether similar risk factor profiles exist. The results have been extensively published.

Long-term survival of ovarian cancer

An analysis of women with long-term survival (>10 years) after a diagnosis of ovarian or tubal cancer in Denmark from 1978 and onwards, and their characteristics associated with being a long-term survivor of ovarian or tubal cancer (clinical factors, lifestyle factors, histopathologic factors, and socioeconomic factors) have been performed combining an extensive number of national registers - the Danish Cancer Registry, The Pathology Data Bank, The Danish Civil Registration System, The Danish Causes of Death Registry, The Medical Birth Registry. The In Vitro Fertilization Registry, The Prescription Database and The Integrated Database for Labor Market Research.

So far, a total of 16 (9 in 2016) publications have been published from these substudies.

Establishment of new biomarkers

Collection of biological specimens as basis for the project

The project is based on blood and tissue samples from the ongoing Pelvic Mass project initiated in 2004. More than 3600 patients have been included in Pelvic Mass I+II and several studies, including PhD, have been completed, are in progress or planned on this biobank.
A comprehensive database including both clinical and prognostic variables has been established on all patients. From 2015 the types of specimens were expanded with collection of ascites and endocervical brush/fluid samples in the new Pelvic Mass III study. Protocols have in 2016 been finalized for exceeding

the sample collection with selected samples for cell cultures and stool for studies on the microbiome. The newest sample collection started in 2015/2016 has been effective with an inclusion rate by more than 85%. The results have been obtained after allocation of a designated MERMAID study nurse to overcome the complicated and time consuming inclusion procedures and logistics associated with the many different sample types. These large biobanks with an excellent coverage are unique and enables studies on both usual and rare ovarian cancer types.

Translational biochemical and molecular studies

A range of analyses have been established and planned according to the 5 year study plan in the MERMAID protocol and budget. At the moment the analyses are progressing rapidly and are almost one year ahead of the time-frame in the MERMAID III protocol.
The global and selected miRNA expression levels have been demonstrated to reflect relapse after platin based chemotherapy more or less than 6 months and the results are submitted for publication.
At the moment different expression levels of selected miRNAs as predictor for prognosis and treatment issues are studied. Findings so far indicate that 8-10 miRNA combined are able to predict chemo resistense, time to progression and overall survival.
Moreover, miRNA levels can be detected in blood. This is promising as miRNA levels of a selected number of miRNA seem to be able to differentiate between early stages of ovarian cancer and benign ovarian pelvic masses.
Analyses (HE4, micro-RNA (miRNA), messenger- RNA (mRNA), tetranectin, PAPP-A proteolytic activity, endothelial cell CD31 protein expressions) have been established and follow the time schedule. Tissue microarrays (TMAs) tests use immunohistochemical staining and FISH/CISH analyses which are a fast and economical way to examine a high number of well-characterized primary tumors compared to the traditional methods.
Planned analysis of DNA methylation changes for Epigenetics studies is under establishment.
New panels of both P53 autoantibodies, p53 immunohistochemistry and P53 mutation status have in a preliminary phase demonstrated promising results in detection of CA125 negative ovarian cancers and therefore indicate a very positive potential role in future marker panels for detection of early stage ovarian cancer.

Data registration, validation, selection and description of study cohorts
Valid clinical data are crucial for clinical and translational studies. Data for the MERMAID III projects are based on the national Danish Gynecological Cancer Database (DGCD) with a coverage on nearly 100%. DGCD registration is compulsory and data are registered online by the treating doctors and nurses, which gives a high validity. DGCD data are linked to the National Patient Register (NPR), the National Cause of Death register, The Danish Pathology Register and the Danish Cancer Biobank for continuous supplement. Thus, detailed descriptive and prognostic statistics can be performed and special patient courses as well as specific biochemical and molecular patterns in the paired biological samples can be studied.

The ongoing extensive data validation on the MERMAID patient cohorts has resulted in several studies published. The validation is mandatory for the translational studies ahead.

So far, 16 publications (9 in 2016) publications have been published in the Biomarker substudies.

The infectious theory

1. Collection of biological specimens as basis for the project and DNA extraction for HPV and CMV virus detection

Tissue samples from the ongoing 'Pelvic Mass' project together with the comprehensive database including both clinical and prognostic variables on all patients are the basis for the study. DNA for the HPV and CMV viral studies has been extracted from ovarian cancer tissue as well as for the rest of the study. Results on HPV have been published and CMV data are ready for publication. Identification of an control group is under consideration as normal ovaries are not frequently surgically removed.

2. Next generation Sequencing

The time and the technological development have made these analysis) affordable. By using this technique it is possible to look for genetic material from microbes (virus or bacteria) in ovarian cancer tissue. The DNA for this study has been extracted and the equipment is under testing and soon ready for the NGS to be performed. A panel is being put together (virus and bacteria) creating the highest possibility for a positive outcome.

3 .Improved survival from ovarian cancer

A: Hypertherm IntraPEritoneal Chemotherapy - HIPEC
Complete cytoreductive surgery (CRS) combined with adjuvant systemic platinum based chemotherapy is considered "Golden Standard" for optimal treatment of advanced ovarian cancer with spread to peritoneal surfaces. However, the risk of intraabdominal recurrence is still high. Hypertherm intraperitoneal chemotherapy (HIPEC) consists of intra-operative perfusion of the abdominal cavity with a heated solution containing a cytotoxic agent. Addition of HIPEC to CRS improves survival in some other peritoneal surface malignancies and an initial study evaluating feasibility, morbidity and mortality of cytoreductive surgery combined with HIPEC in patients with advanced stage ovarian, tubal or primary peritoneal cancer revaled that the procedure could be performed without any deaths and with an acceptable rate of severe or life-threatening complications. The study is at the moment extended with an absorption study. A large scale multicenter randomized study is planned.

B: Endometriosis as risk factor for ovarian cancer
Endometriosis resembles malignant diseases ie may develop distant and local foci, invasion and damage of other tissue and angiogenesis. Whether the ectopic foci undergo malignant transformation or endometriosis and certain cancers share some of the same preceding mechanisms or predisposing factors, e.g. genetic, epigenetics, inflammation, and immune responses has been hypothesized.
A study on women with endometriosis, who develop ovarian cancer, to identify their risk profile compared to those with ovarian cancer but no endometriosis is planned. So far a literature review has been performed and published and collaboration with international partners has been established.

So far, three publications have been published in the Infectious theory substudies.

In conclusion
The research plan is followed and the Mermaid III projects provides new evidence and data on ovarian cancer both in terms of risk factors, screening and early detection, new markers, prognostic factors and potential new treatment options. The very robust scientific environments combined with international collaborators form a promising background for continuous progress ahead.
The projects in the Mermaid III projects have already published several papers indicating that all the parts in projects show expected progress.

The results may provide very important evidence within the field of personalized treatment in the future.

On behalf of the Independent Audit Committee

Randers 21/3 2017 Lone Kjeld Petersen