

Sentinel node in ovarian cancer

The SONAR study



(Sentinel nOde iN ovArian canceR)

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List of abbreviations and relevant definitions

| | |
|------|---|
| EOC | Epithelial ovarian cancer |
| FIGO | International Federation of Gynecology and Obstetrics |
| SAE | Serious adverse event |

Summary

Rationale:

As most cancers, ovarian cancer also spreads to regional lymph nodes. The concept of sentinel lymph node surgery is to see whether the cancer has spread to the very first lymph node or sentinel node. If the sentinel node does not contain cancer, then there is a high likelihood that the cancer has not spread to other lymph nodes. This means that, at least theoretically, a radical lymphadenectomy could be omitted and thus the associated morbidity. The sentinel node technique has been proven to be effective in other cancers such as breast cancer and malignant melanoma. In the gynaecological field it has been shown to be effective in vulvar cancer. Currently sentinel node studies are done for cervix and uterine cancer.

Objective:

- 1) To determine whether or not a sentinel node procedure in patients with ovarian cancer is feasible when the tracers are injected in the ovarian ligaments.
- 2) To determine the anatomical localisations of the sentinel nodes.
- 3) To determine the incidence of false negative sentinel nodes.

Study design: phase I feasibility study.

Study population: the following patients will be included:

- Patients with a high suspicion of an ovarian malignancy in whom a median laparotomy and a frozen section analysis is planned.
- Patients with endometrial cancer in whom a staging laparotomy is planned.

Intervention (if applicable): After opening the abdomen, before starting the surgical staging procedure, blue dye and the radioactive-colloid will be injected in the ligamentum ovarium proprium and the ligamentum infundibulo-pelvicum. This will be done on the ovary with suspicion of malignancy (patients with an ovarian tumour) or one of the normal ovaries (patients with endometrial cancer).

Main study parameters: percentage of patients in whom it is feasible to identify sentinel nodes. Other study parameters are the anatomical localisation of the sentinel node(s) and the incidence of false negative lymph nodes.

Nature and extent of the burden and risks associated with participation, benefit and

group relatedness: the surgery is prolonged with 20-25 minutes due to the required incubation time after injection of the blue dye and radioactive isotope. A scintigram will be performed 24 hours after the surgery to determine whether residual radioactive lymph nodes

can be detected. The scintigram will only be performed if the patient is capable to be transported to the nuclear department. No extra blood samples will be taken, no extra visits, physical examinations or other tests are necessary. There is no risk of tumour dissemination by injecting the tracers in the ovarian ligaments. There is a 0,07 to 2,7% risk of an allergic reaction to the blue dye. The dose of radioactive isotope given does not give adverse side effects, either to the patients or the personnel present in the operating theatre.

1. Introduction and rationale

Epidemiology

Epithelial ovarian cancer (EOC) remains the tumour with the most unfavourable prognosis within the field of the gynaecological oncology. The incidence of ovarian cancer in the Netherlands in 2008 was 14.5 per 100.000, with 12.3 deaths per 100.000 [1]. In the US in 2007 the incidence was 13.0 per 100.000 and there were 8.2 deaths per 100.000 [2]. The high mortality rate is partially due to the fact that approximately 75% of patients is diagnosed with advanced stage EOC.

Background

EOC can metastasize through three different ways [3,4]: intraperitoneal (in the abdominal cavity), lymphogenous and haematogenous. Concerning the lymphogenous spread, it is clear that lymphatic metastases of EOC mainly occur in the para-aortic lymph nodes [5]. It is believed that the tumour cells follow the lymph vessels that accompany the ovarian artery and vein in the infundibulopelvic ligament up to the high para-aortic region and renal vein. Nevertheless, pelvic lymph node metastases are also frequently found [6-16]. These tumour cells probably follow a different route, following the para-uterine vessels in the broad ligament towards the uterine artery and vein and further on to the iliac vessels. In some case reports isolated inguinal node metastases are also described [17-20]. The exact mechanism of this route of metastasis is still unclear, but the metastatic cells might follow the course of the round ligament towards the inguinal lymph nodes, or might follow the iliac vessels towards the femoral vessels. The incidence of lymph node metastasis in clinical stage I-II ovarian carcinoma is 14.2% [21].

Staging

In case of a clinical early stage ovarian cancer, the Dutch guideline [1] recommends a staging laparotomy with adequate lymph node sampling, with an absolute minimum of ten lymph nodes removed. In the same guideline, a footnote is made stating that a larger number of removed lymph nodes will increase the chance of finding metastases. These lymph nodes also need to be sampled from different anatomical regions, of which the most important are the

para-aortic and paracaval region between the renal vein and inferior mesenteric artery, the common, internal and external iliac vessels and the obturator fossa.

The clinical significance of staging

According to the International Federation of Gynecology and Obstetrics (FIGO), EOC with lymph node metastases is classified as FIGO stage IIIC disease, even in the absence of peritoneal metastases [22]. In contrast to patients with FIGO stage I ovarian cancer after a comprehensive staging procedure, patients with a FIGO stage III ovarian cancer obtain adjuvant chemotherapy. Therefore, the recognition of lymph node metastases is of utmost importance.

Surgical staging of EOC and the extent of lymph node dissection differs greatly from centre to centre [23].

A review published in 2011 showed an incidence of 14.2% for lymphnode metastasis in early EOC [21]. The incidence is higher in the grade 3 tumours (20.0%) and the serous histological subtype (23.3%). Whereas in grade 1 and mucinous tumours this is respectively 4.0% and 2.6%.

Methods for identification of lymph node metastases

Radiological imaging

The assessment through radiological techniques (CT scan, MRI, PET) alone is not feasible. The sensitivity and specificity for detection of lymph node metastases in ovarian cancer of a PET scan are 73.2% and 96.7% respectively, of a CT scan 42.6% and 95.0%, and of a MRI 54.7% and 88.3%. [25].

During surgery

Removal of lymph nodes:

- systematic lymphadenectomy / lymph node sampling

Approaches range from taking some lymph nodes only (lymph node sampling) to systematic lymphadenectomy [22]. A systematic lymphadenectomy can be seen as the golden standard. However, such a radical procedure gives more late morbidity than lymph node sampling. These include the formation of lymphocyst (up to 13.5%), nerve and vessel injury (up to 4%) and increased blood loss and operating time [26, 27].

- removal of sentinel node(s)

Through a sentinel node procedure the first node that receives primary lymphatic flow can be identified (the so called sentinel node). The pathological examination is an indication of the nodal status of the remaining nodes. If the sentinel node is negative, the patient may be spared a radical lymphadenectomy and thus the morbidity that can be caused by it [28].

The sentinel node technique has been proven effective in other cancers such as breast cancer and malignant melanoma. In the gynaecological field it has been shown to be effective in vulvar cancer. Currently sentinel node studies are done for cervix en uterine cancer. Studies done for sentinel node in ovarian cancer are very limited. One article that describes the role of sentinel lymph node sampling in gynaecological cancers, only briefly mentions ovarian cancer. The authors state that the procedure is difficult due to the often bulky mass ovarian tumours. Furthermore, with injecting in the ovary there is a possible risk of tumour dissemination [28].

Nyberg et al. have done a study in 16 patients with high-risk uterine cancer in which technetium and blue dye were injected in the right or left ovary [29]. They used an incubation time of 15 minutes. In this study patients were included with a high-risk uterine carcinoma because these patients obtain the same operation as patients with clinical early stage ovarian cancer: a total abdominal hysterectomy (TAH) with bilateral salpingo-oophorectomy (BSO) and a pelvic and para-aortic lymphadenectomy or lymph node sampling. In 15 out of 16 patients the sentinel node was detected. Another study used activated charcoal solution to identify ovarian lymphatics in 11 patients. The charcoal was injected in the cortex of the ovary. The charcoal was deposited in the lymph nodes of all patients [12].

These studies all inject in the ovary, which gives a risk of tumour dissemination. In our feasibility study we are planning to inject in the ligaments of the ovary, not in the cortex itself. Therefore, besides patients with high risk endometrial cancer, we also can include patients with an enlarged ovary without risk of tumour dissemination.

The lymphatic mapping can be done with blue dyes as well as radioactive isotopes; both can be injected in the ovarian ligaments which contain the main routes of lymph drainage. After the incubation time the sentinel nodes should be visualized by either colorization (blue lymph nodes can be identified) and / or by the radioactive tracer [29]. Although in breast cancer radioactive isotope compared to blue dye shows a higher detection rate of the sentinel node [30], it has been shown that the detection rate is the highest when both radioactive isotope and blue dye are combined, instead of using them alone [30, 34].

The blue dyes can cause an allergic reaction, which can present itself from hypotension with urticaria and erythema to severe cardiovascular collapse with bronchospasm [28]. However, the incidence of an allergic reaction from blue dye is very low and varies from 0.07 to 2.7% [31].

The radioactive isotope is safe for the patient as well as the health care workers [35]. No allergic reactions are described due to the radio-active isotope.

Conclusion

This study is social relevant because in patients with clinical early stage ovarian cancer lymph node metastasis are found in 14.2%. A sentinel node procedure can prevent unnecessary radical lymph node dissection with its associated morbidity. If this procedure proves to be effective, it is also more accurate than the at random sampling which is currently done in the Netherlands.

This study is scientific relevant since the research done for sentinel node procedure in ovarian cancer is very limited and so far limited to injection of tracers in the ovarian cortex with the possibility of tumour spread. Injection of tracer in the ovarian ligaments has, to our knowledge, never been published.

2. OBJECTIVES

Primary Objective

To determine whether or not a sentinel node procedure in patients with ovarian cancer is feasible by injecting the tracers into the ovarian ligaments instead of into the ovary itself.

In this feasibility study we will include both patients with (suspicion of) ovarian cancer as well as patients with a high-risk uterine carcinoma. The latter group of patients can also be included because these patients obtain the same surgical procedure; a total abdominal hysterectomy (TAH) with bilateral salpingo-oophorectomy (BSO) and a pelvic and para-aortic lymphadenectomy of lymph node sampling.

Secondary Objective(s)

- The anatomical location(s) and number of the sentinel node(s):
 - visualized at surgery regarding blue dye
 - recorded by the gammaprobe at surgery radiocolloid
 - visualized at scintigraphy 24 hours after surgery regarding possibly remaining lymph nodes
- Incidence of false negative lymph nodes.

3. STUDY DESIGN

Observational feasibility study to detect sentinel nodes: mapping the lymphatic drainage of the ovaries.

The study will end after 20 patients, of which at least 10 patients with ovarian cancer, are included in which the complete sentinel node procedure has been performed. The expected inclusion period is estimated to be two years. If the detection rate of the sentinel node is less than 50%, or when less 5 patients are included in a year, the study will end prematurely.

4. STUDY POPULATION***Population (base)***

The study will be performed in the Maastricht University Medical Centre.

1) Patients with a high suspicion of a malignant ovarian tumour planned for exploratory laparotomy will be asked to participate in the study. Only when a malignancy is confirmed by frozen section, the sentinel nodes will be removed prior to proceeding with a complete staging procedure.

2) Patients with endometrial cancer in whom a staging laparotomy is planned.

Inclusion criteria

- Patients with a high suspicion of a malignant ovarian tumour planned for exploratory laparotomy.
- Patients with high-risk endometrial cancer in whom a staging laparotomy is planned.
- Age between 18 and 85 years.

Exclusion criteria

- Previous surgery of both ovaries.
- Previous vascular surgery of the aorta, caval vein, and/or iliac vessels.
- Previous lymphadenectomy of lymph node sampling in the iliac or para-aortal region.
- History of a malignant lymphoma.
- History of a malignant tumour in the abdominal cavity.
- Previous allergic reaction to blue dye.
- Pregnant or lactating patients.
- An allergy for human albumin.

Sample size calculation

Between 24 and 28 patients will be included in the study.

The sample size calculation is based on the fact that:

- with a group of 20 evaluable patients the study group is large enough to give an answer whether sentinel node(s) can be detected related to the ovarian lymphatic flow when the tracers are injected in the ovarian ligaments. For this purpose both patients with an ovarian and endometrial malignancy, planned for a staging laparotomy can and will be included.
- patients with a benign result on frozen section of the suspicious ovary will be excluded from the study because no lymph node(s) will be resected in these patients. These patients will be documented and described in the report after the study.
- based on retrospective data, in 60% of the patients with a suspicious ovary, frozen section will confirm a ovarian malignancy.
- to calculate one of the secondary endpoints (incidence of false negative sentinel lymph node) at least 10 patients with ovarian cancer should be part of the 20 evaluable patients.

5. METHODS***Main study parameter***

To determine whether or not a sentinel node procedure in patients with ovarian cancer is feasible by injecting the tracers into the ovarian ligaments instead of into the ovary itself.

The lymphatic drainage of the ovary follows the lymph vessels that accompany the ovarian artery and vein in the infundibulopelvic ligament up to the high para-aortic region and renal vein. The exact mechanism of drainage to pelvic area is still unclear. It may follow the lymph vessels adjacent to the uterus towards the pelvic lymph nodes.

Through a sentinel node procedure the first node(s) that receives primary lymphatic flow should be identified (the so-called sentinel node(s)). In case of lymphatic spread, this lymph node will be the first lymph node obtaining a metastasis.

In this feasibility study we will include both patients with (suspicion of) ovarian cancer as well as patients with a high-risk uterine carcinoma. The latter group of patients can also be included because these patients obtain the same surgical procedure; a total abdominal hysterectomy (TAH) with bilateral salpingo-oophorectomy (BSO) and a pelvic and para-aortic lymphadenectomy of lymph node sampling.

Secondary study parameters

In order to test the reliability of the sentinel node detection by injection in the ovarian ligaments, some secondary study parameters are also important:

- The anatomical location(s) and number of the sentinel node(s), visualized at surgery by either blue dye or the radioactive tracer
- The incidence of false negative lymph nodes, i.e. in contrast to the sentinel lymph node, one of the resected non-sentinel lymph nodes contains tumor cells. To calculate the percentage of false negative lymph nodes: the number of patients with negative sentinel lymph node(s) AND positive non-sentinel lymph node(s) is the numerator, the true amount of negative lymph nodes is the denominator."

The golden standard would be to perform a complete pelvic and para-aortic lymphadenectomy. However, the Dutch guideline advises a comprehensive lymph node sampling as standard treatment. We feel such a golden standard can be considered in subsequent study, when this study provides evidence that a sentinel node procedure in this way indeed is feasible. Nevertheless, a possible false negative result obtained in this study is important and therefore will be documented.

- The incidence of unrecognized sentinel nodes

Recognition of sentinel nodes with blue dye and the gamma probe during surgery is less reliable than by making a scintigram. Therefore ideally, as in breast and vulvar cancer, a scintigram is made before surgery to recognize the sentinel nodes so that during surgery no sentinel nodes will be missed. However, this principle cannot be accomplished in patients with ovarian cancer, because the tracers cannot be injected at the appropriate sides without opening the abdomen. However, we expect that missing sentinel nodes in patients with ovarian cancer occurs less frequent because the area where the sentinel nodes can reside is restricted to well described areas. Nevertheless, by making a scintigram 24 hours after surgery, at least an impression is obtained on the incidence of unrecognized sentinel lymph nodes.

Study procedures

Informed consent procedure

Eligible patients will be asked to take part in the study at the time the diagnosis and the suggested surgical therapy are discussed at the outpatient services (either high suspicion of a malignant ovarian tumour planned for exploratory laparotomy or high-risk endometrial cancer planned for staging laparotomy). The gynaecological oncologist will explain the outline of the study and gives the patient the information brochure. Of course it will be stressed that the patient remains free to withdraw at any time from protocol investigations without giving reasons and without prejudicing any further treatment. After admittance to the hospital (one day before surgery) the gynecologist who will perform the surgery, once more discusses the study and, when the patient agrees to take part in the study, obtain a written informed consent.

Surgical procedures

Patients with a high suspicion of a malignant ovarian tumour

After making the median incision and opening the abdomen, before starting with removal of the enlarged and suspicious adnex, blue dye and the radioactive isotope will be injected in the ligamentum ovarii proprium (median side) and the ligamentum infundibulo-pelvicum (lateral side), close to the ovary and just below the peritoneum. Four syringes will be made containing 0,2 ml patent blue and 0,15 ml (20-MBq) radioactive isotope (99mTc-nanocolloid or Nanocoll®, GE Healthcare, Eindhoven, The Netherlands, see appendix for information leaflet) will be given. Mixing patent blue and radioactive isotope does not affects the quality of the two substances, nor does it give extra or different side effects for the patient [36]. The

99mTc-nanocolloid is prepared by trained laboratory staff on the nuclear lab in a sterile 'class A' Laminar Air Flow (LAF) hood. Afterwards patent blue is added and the syringe is mixed. This is done 30 minutes before the start of the surgery.

A 15-minute interval will be planned after injection. The exact time interval for detection of positive nodes is unclear. Common practice is to wait for 15 minutes, if in this time period the node is not detected, it is considered negative. In daily practice a wait of more than 15 minutes during surgery is not workable. At 5 and 10 minutes the radioactivity will be measured along the lymphatic tract to document whether or not perhaps sentinel node(s) can be identified at a shorter time-interval in future studies. A Geiger counter measures the radioactivity. The Geiger counter will be the same device in all surgeries.

After the 15 minutes time-interval the adnex will be removed and admitted to the pathologist for a frozen section. If the result is benign, no further actions will be performed in these patients. If the result is malignant, the sentinel node(s) will be identified either (once more) by the radioactive tracer and / or visually (blue dye) after opening the retroperitoneal space.

After removal of the sentinel node(s) a complete standard staging procedure will be performed including a comprehensive at random sampling of other lymph nodes at the different locations.

Surgical steps related to the study (standard or extra):

- Median laparotomy (standard care)
- Injection of blue dye and radioactive isotope (extra for the study)
- 15 minute waiting time during the surgery (extra for the study)
- Removal of the adnex and admittance to the pathologist for a frozen section analysis. (standard care)
- If the result is benign, no further actions will be performed in these patients and the procedure ends at this time. (standard care)
- If the result is malignant, the sentinel nodes will be identified either by the radioactive tracer and / or visually (blue dye). (extra for the study)
- The sentinel nodes will be removed. (standard or extra for the study, depending on whether or not the lymph node(s) would have been removed as part of the standard lymph node sampling)
- Subsequently a complete staging procedure will be performed. (standard care)

Patients with endometrial cancer in whom a staging laparotomy is planned

After making the median incision and opening the abdomen, blue dye and the radioactive isotope will be injected in the ligamentum ovarii proprium (median side) and the ligamentum infundibulo-pelvicum (lateral side) of one of the ovaries, in the same manner as described above in patients with a high suspicion of an ovarian malignancy. The choice of the ovary (left or right side) will switch between left and right by each patient who is included in the study.

A 15-minute interval will be planned after injection. In this time period at 5 and 10 minutes the radioactivity will be measured along the lymphatic tract to document whether or not perhaps sentinel node(s) can be identified at a shorter time-interval in future studies. The radioactivity is measured by a Geiger counter. The Geiger counter will be the same device in all surgeries.

After the 15 minutes time-interval the sentinel node(s) will be identified either (once more) by the radioactive tracer and / or visually (blue dye) after opening the retroperitoneal space. After removal of the sentinel node(s) a complete standard staging procedure will be performed including a comprehensive at random sampling of other lymph nodes at the different locations.

Surgical steps related to the study (standard or extra):

- Median laparotomy (standard care)
- Injection of blue dye and radioactive isotope (extra for the study)
- 15 minute waiting time during the surgery (extra for the study)
- The sentinel nodes will be identified either by the radioactive tracer and / or visually (blue dye) (extra for the study)
- The sentinel nodes will be removed. (standard or extra for the study, depending on whether or not the lymph node(s) would have been removed as part of the standard lymph node sampling)
- Subsequently a complete standard staging procedure will proceed as normal (standard care).

After surgery

One day after the surgery a scintigram will be taken if possible (depending on mobility of the patient) at the nuclear department to detect any remained radioactive hot spots. A scintigram is done in a similar way as a CT-scan. If there is any rest radioactivity this may indicate that

sentinel nodes have not been identified during surgery and therefore gives an indication of the reliability of identifying sentinel nodes during surgery.

Data collection

Patient characteristics. The following information will be recorded:

- Record number
 - Age
 - Checked inclusion and exclusion criteria
 - Checked informed consent
 - Histology: tumour type (ovarian or endometrial), differentiation grade, numbers of lymph node removed including sentinel node(s) including histological results specified per sentinel node
 - FIGO stage
-
- Surgical findings: tumour side, injections side, result frozen section, time between injection and detection, sentinel node positive or not, number of sentinel nodes, first sentinel node blue, first sentinel node gamma detection, amount of gamma counts, complications during surgery, side effects during surgery.
 - Postoperative scintigram, residual nodes, location of residual nodes.
 - Result pathology nodes removed: benign or malignant.

The surgeon has to register the number and location of both sentinel nodes resected. For this purpose the surgeon has to draw the location of the lymph nodes in an anatomical drawing (see Figure 1 - page 20). Ten different locations will be used to report where the sentinel nodes were found:

1. high para-aortic
2. low para-aortic
3. interaortic-caval
4. para-caval
5. iliaca communis left
6. iliaca communis right
7. external iliaca left
8. external iliaca right
9. obturator left
10. obturator right

The surgeon also has to register the location of the lymphatic tissue removed, related to the lymph node sampling following the removal of the sentinel node(s).

Withdrawal of individual subjects and replacement

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons. Also patients with an ovarian tumour and benign result at frozen section during surgery will be excluded from the study.

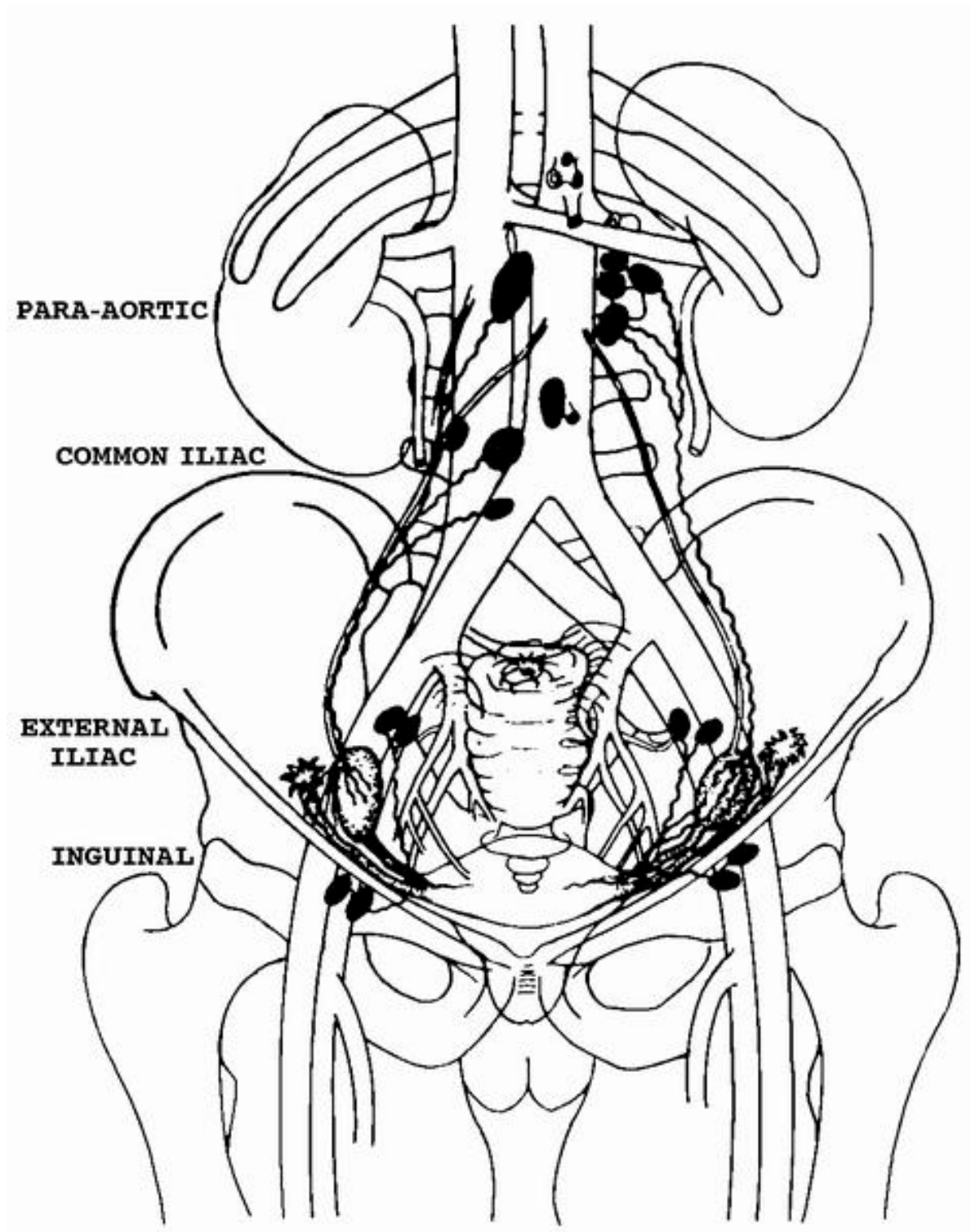
These patients will be replaced by a new study subject until 20 completed sentinel node procedures are performed.

Follow-up of subjects withdrawn from treatment

The reasons why patients have left the study after initial inclusion will be documented in an anonymous way.

Premature termination of the study

- When after 1 year <5 patients have been included.
- When in < 50% of patients the sentinel nodes are identified (interim analysis after 10 procedures).

Figure 1: location of lymph nodes

6. SAFETY REPORTING

Section 10 WMO event

In accordance to section 10, subsection 1, of the WMO, the investigator will inform the subjects and the reviewing accredited METC if anything occurs, on the basis of which it appears that the disadvantages of participation may be significantly greater than was foreseen in the research proposal. The study will be suspended pending further review by the accredited METC, except insofar as suspension would jeopardise the subjects' health. The investigator will take care that all subjects are kept informed.

Adverse and serious adverse events

Because both the blue dye and the radioactive tracer are injected outside the ovary, there is no risk of spread of tumour cells.

An allergic reaction can occur due to the blue dye. The clinical presentation can range from urticaria, skin rash, blue hives, erythema, hypotensive episodes to an anaphylactic shock. For breast cancer minor reactions are reported in 0.9% of the sentinel node procedures. Severe allergic reactions, requiring administration of vasopressor occurred in 4 of 5853 procedures (0.07%). Anaphylactic shock after administration of blue dye is considered a medical emergency [30]. The anaesthesiologist is informed of the use of dye during the surgery, before the start of the surgery. Adequate fluid supply and if necessary resuscitation can be given in the setting of the surgery. Epinephrine injections can be given to treat hypotension, salbutamol spray for broncospasms [31].

All SAEs will be reported through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 15 days after the sponsor has first knowledge of the serious adverse reactions.

SAEs that result in death or are life threatening should be reported expedited. The expedited reporting will occur not later than 7 days after the responsible investigator has first knowledge of the adverse reaction. This is for a preliminary report with another 8 days for completion of the report.

Follow-up of adverse events

All adverse events will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

Data safety Monitoring Board (DSMB)

The principle of sentinel nodes is familiar for several malignancies, among others breast and vulvar cancer. With respect to ovarian cancer the knowledge is limited and so far only studied by injecting the tracers in the ovarian cortex with the possible risk of tumour dissemination. The method described in this protocol (injection in the ovarian ligaments) has no risk of tumour dissemination. No new medicaments or substances are tested. Both the blue dyes as well as the radioactive tracers are known substances used for detecting sentinel nodes related to other malignancies. Therefore no DSMB is involved in this feasibility study.

7. STATISTICAL ANALYSIS***Descriptive statistics***

Registered items:

- Sentinel nodes
 - Number per patient
 - Location(s) per patient
 - Number(s) of sentinel nodes containing a metastasis per patient
 - Location of sentinel nodes containing a metastasis per patient
- Non-sentinel lymph nodes removed during the subsequent staging procedure
 - Number per patient
 - Location(s) per patient
 - Number(s) containing a metastasis per patient
 - Location of non sentinel nodes containing a metastasis per patient

Based on the above registered items the median and mean number of sentinel nodes and its main locations can be determined. By combining the data of the sentinel en non-sentinel nodes, cases in which de sentinel node is false negative can be identified

Interim analysis.

For the surgeons there will be probably a learning curve for applying the injection and detecting the nodes, therefore initially the detecting rate of the sentinel node(s) in this pilot study should not be set to high. Nevertheless, an interim analysis will be done once we have included 10 patients in whom the sentinel node procedure could be performed. This will be done by one of the investigators. If less than 50% of the cases no sentinel node(s) could be detected the study will prematurely end.

8. ETHICAL CONSIDERATIONS***Regulation statement***

The study will be conducted according to the principles of the Declaration of Helsinki (see for the most recent version: www.wma.net) and in accordance with the Medical Research Involving Human Subjects Act (WMO).

Recruitment and consent

The recruitment will be done by the gynaecologic oncologists based in the Maastricht University Medical Centre. The patient will be informed by the doctor who sees her in the out patient clinic. This is not always the doctor who will do the surgery. A patient information letter will be given. The patient has at least three days to consider their decision. The patient information letter and the consent form are two separate documents. When the patient returns for a routine visit, the patient is asked whether or not she wants to participate in the study.

Objection by minors or incapacitated subjects (if applicable)

No minors or incapacitated subjects will be included in the study.

Benefits and risks assessment, group relatedness

The risk of the study are low, a small chance of 0.07-2.7% of an allergic reaction to the blue dye. The surgery will be prolonged with 20-25 minutes, which is without extra risk for the patient.

Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7, subsection 6 of the WMO.

The sponsor has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO and the Measure regarding Compulsory Insurance for Clinical Research in Humans of 23th June 2003). This insurance provides cover for damage to research subjects through injury or death caused by the study.

1. € 450.000,-- (i.e. four hundred and fifty thousand Euro) for death or injury for each subject who participates in the Research;
2. € 3.500.000,-- (i.e. three million five hundred thousand Euro) for death or injury for all subjects who participate in the Research;
3. € 5.000.000,-- (i.e. five million Euro) for the total damage incurred by the organisation for all damage disclosed by scientific research for the Sponsor as ‘verrichter’ in the meaning of said Act in each year of insurance coverage.

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

9. ADMINISTRATIVE ASPECTS AND PUBLICATION***Handling and storage of data and documents***

Each patient will be given a unique identification number (= inclusion number) to ensure the privacy of the patient. Reference to the case will only be made by means of an identification number. The acquired data will be recorded anonymously in a Microsoft Access file. This file contains the patient characteristics and surgical findings (see Study Design – data collection) and will be saved for 15 years. The members of the study group have access to the data. In a separate file, owned by the principal investigator, the patient can be identified by correlating the inclusion number with the patient’s hospital number.

Annual progress report

The investigator will submit a summary of the progress of the trial to the accredited METC after one year. Information will be provided on the date of inclusion of the first subject,

numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

End of study report

The investigator will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit.

In case the study is ended prematurely, the investigator will notify the accredited METC, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

Public disclosure and publication policy

At the end of the trial there a meeting will be planned to discuss the final data which will be published in a peer reviewed paper. At least submission of the manuscript will be done within 6 months after the study has ended.

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