Prospective Validation of the ADNEX Model for discrimination between benign and malignant adnexal masses in pregnancy: International Ovarian Tumour Analysis in pregnancy study (p-IOTA)

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1. STUDY SUMMARY

TITLE

Prospective Validation of the ADNEX Model for discrimination between benign and malignant adnexal masses in pregnancy: the International Ovarian Tumour Analysis in pregnancy study (p-IOTA).

DESIGN

Multicentre, prospective cohort observational study.

BACKGROUND

Adnexal masses are a common incidental finding in pregnancy. Whilst the majority are benign and resolve spontaneously, a proportion can exhibit suspicious features during pregnancy raising concern about an underlying malignancy. Correct classification of adnexal masses is particularly important during pregnancy given the potential foetal and maternal risks associated with surgical intervention. International Ovarian Tumour Analysis (IOTA) group have developed robust, ultrasound-based tools, including the ADNEX model to support the classification of adnexal masses. Ultrasound-based tools such the Modified Benign Simple Descriptors and ADNEX have been externally validated to aid in the classification of adnexal masses in non-pregnant women, but their use as a robust diagnostic tool in pregnancy remains to be demonstrated.

AIMS

The principal objective of this study is to prospectively investigate the ability of the ADNEX Model and a 2-step strategy (i.e. Modified Benign Simple Descriptors followed by ADNEX) to correctly discriminate between benign and malignant adnexal masses diagnosed in pregnancy.

PRIMARY OUTCOME MEASURE

False discovery rate (number of benign masses / number of masses classified as malignant) when using the ADNEX Model to discriminate between benign and malignant adnexal masses at 11-14 gestational weeks in pregnancy.

ELIGIBILITY

All women 18 years old and above with an adnexal mass found on ultrasound scan during pregnancy - irrespective of whether the mass known before pregnancy OR diagnosed for the first time on ultrasound scan during pregnancy.

DURATION

This study will be conducted over a minimum period of three years.

KEYWORDS

IOTA, ovarian mass, benign, malignant, ultrasound, pregnancy, post-partum

2. BACKGROUND

Ovarian masses

Ovarian masses are common in women of reproductive age and are often found incidentally on ultrasound in asymptomatic women. The majority of ovarian masses are benign, and thus do not require removal. Yet, 1 in 10 women will undergo surgery in their lifetime for the removal of an ovarian cyst[1]. The decision to proceed with surgical management is for two main reasons. First, because of concern regarding malignancy potential and second, to reduce the risk of or treat complications associated with adnexal masses such as cyst rupture or ovarian torsion.

In pregnancy, the integration of routine first trimester ultrasound as part of antenatal screening has led to an increased detection rate of adnexal masses. The prevalence of adnexal masses in pregnancy varies widely[2,3]. The vast majority of adnexal masses identified in pregnancy are benign physiological cysts which resolve spontaneously without any intervention. *Goh et al* analysed 24,868 pregnancies, identifying 1225 ovarian masses, of these only 171 (0.7%) had a persistent ovarian mass ≥5cm[2].

Cancer is estimated to complicate 1 in 1000 pregnancies, but is on the rise, largely driven by an increase in women choosing to delay starting a family into their thirties[4]. Ovarian malignancy is one of the top five cancers identified in pregnancy. A recent, large international cohort identified 1170 women diagnosed with primary cancer during pregnancy, of those 88 (7%) had ovarian cancer (primary invasive and borderline ovarian tumours - type not specified)[5]. The majority of women diagnosed with ovarian cancer in pregnancy had stage I disease (>70%). Ovarian cancer, although on the rise, remains uncommon in women of reproductive age. In a large US cohort consisting of 9375 adnexal masses identified in pregnancy, the rate of ovarian cancer (n=87) and BOTs (n=115) were 0.93% and 1.2% respectively[6]. It is worth highlighting that the definition of cancer Is not always precisely defined in each study.

IOTA: Ultrasound-based tools

The balance between surgical and expectant management is underpinned by confidence in correct characterization of ovarian masses. If one can confidently characterise the mass as benign, then expectant management is a safe strategy, given that the overall risk of torsion or cyst accident is low[7].

The use of ultrasound-based tools, such as Simple Descriptors, Simple Rules, ADNEX model, IOTA LR2 model and Simple Rules Risk estimation model, have been widely validated across multiple centres, in the hands of expert and non-expert ultrasound examiners[8-15]. These tools were validated to ensure their robustness to aid in the correct characterization of ovarian masses. ADNEX model incorporates six ultrasound characteristics: number of papillary projections (0, 1, 2, 3 or more than 3), number of cyst locules (≤10 vs. >10), presence of acoustic shadowing (yes/no), diameter of largest solid component (mm), largest diameter of adnexal lesion and ascites (yes/no). The ADNEX model is not only able to differentiate between benign and malignant, but also has the capability to sub-divide into the risks of specific types/stages of malignancy, including borderline, Stage I, Stage II-IV or metastases[15].

Simple rules method uses ten ultrasound features seen in benign masses (B-features) and malignant masses (M-features)[16]. The 'M' features include irregular solid tumour, presence of

ascites, 4 or more papillary projections, irregular multilocular solid tumour with largest diameter ≥100mm and very strong blood flow (Doppler colour score of 4). The 'B' features include unilocular mass, presence of solid components with largest diameter < 7mm, presence of acoustic shadowing, smooth multi-locular tumour with largest diameter < 100mm and no blood flow (Doppler colour score of 1)[16]. In cases where neither M or B rules apply, or where both are present, the mass is classified as 'unclassifiable' and further investigations are required.

Recognising the importance of correct characterization of ovarian masses, the Royal College of Obstetricians and Gynaecologist (RCOG) has integrated the 'Simple Rules' tool into national guidance for use in the management of ovarian masses in pre-menopausal women[1]. The IOTA group has also evaluated the behaviour of ovarian masses, characterised as benign, over a period of two years. The aim of the study was to further the overall understanding of the natural history of various adnexal mass sub-types and the incidence of complications such as malignant transformation, ovarian torsion and cyst accident. This was done to determine whether expectant management of adnexal masses represents a safe option. Overall, the data was reassuring with only 0.7% of masses displaying malignancy (i.e. false-negative results of initial scan or malignant transformation; 0.3% were borderline ovarian tumours), 0.4% ovarian torsion and 0.2% cyst rupture[7] (the reported incidences are cumulative). The low cumulative incidence of observed complications reinforces 'expectant' management as a safe, robust strategy for managing women with benign adnexal masses.

Tumour markers have some role in the characterisation of adnexal masses. Cancer antigen-125 (CA125) was identified as a screening/triage tool to identify women at risk of ovarian cancer in primary care. The aim was to enable prompt referral into tertiary care. It is a significant contributor to the Risk of Malignancy Index (RMI)[17,18]. CA125 has been shown to have more value in discriminating between benign and malignant masses in post-menopausal than pre-menopausal women[19,20]. CA125 is elevated in numerous gynaecological conditions including endometriosis and infections. In pregnancy, CA125 is produced by amnion cells and decidua during foetal development, so is typically elevated particularly in the first trimester. In early-stage ovarian cancer, 1 in 2 women will have a normal CA125[21]. Given CA125 is elevated in pregnancy and pregnant women typically present with stage I disease, it is likely to have limited clinical value as a tumour marker in pregnancy[22].

Adnexal Masses and Pregnancy

The natural history and behaviour of adnexal masses have not yet been explored in pregnancy and the post-partum period using large prospective patient cohorts. Pregnancy is a unique situation. First, it provides a group of women who will be scanned during the antenatal period, allowing for an opportunity to prospectively follow-up adnexal masses at distinct time points in pregnancy. Second, because of the potential risks of surgery (maternal and foetal) in pregnancy, women are more inclined to be managed conservatively. This generates a large cohort of women undergoing expectant management of adnexal masses. This gives us a possibility to improve our understanding of the natural history of adnexal masses during pregnancy.

The higher stakes in pregnancy (i.e. health of mother and baby) reinforce the importance of correct interpretation of ultrasound images. In particular, the potential value of using an ultrasound-based tool to aid in the characterisation of adnexal masses in pregnancy is evident. No ultrasound-based tools have yet been validated for use in pregnancy. Therefore, a clear need exists for a robust,

validated ultrasound tool to facilitate the correct characterization of adnexal masses in pregnancy. In turn, this will ensure appropriate management and importantly, surgical intervention only for women with a high index of suspicion of malignancy or clinical complications associated with an adnexal mass. In order to inform safe management of adnexal masses in pregnancy, it is first paramount that we further our understanding about the normal trajectory of adnexal masses during pregnancy and the postpartum period.

The majority of women identified as having an adnexal mass at 12-14 weeks have a pregnancy-associated physiological cyst, such as a follicular, corpus luteum or a haemorrhagic cysts[23]. The majority of cysts identified at first trimester ultrasound resolve spontaneously by 18-20 weeks[24]. Adnexal masses which persist beyond 16-20 weeks suggest an underlying pathology. The rate of spontaneous resolution was shown to be much lower if the adnexal mass was more than 5cm or complex in ultrasound appearance[23].

Dermoid cysts (mature teratomas) are the commonest ovarian masses seen in women beyond 16 weeks' gestation. Malignant transformation can occur, most commonly to an invasive squamous carcinoma[25,26,27]. Dermoid cysts have typical features on ultrasound including hyperechoic nodules, acoustic shadowing, hyperechoic lines or a fluid level[28]. Outside of pregnancy, dermoid cysts have been shown to be slow growing, with a mean growth rate of 1.67-1.8 mm per annum[29]. In a prospective study of dermoid cysts in pregnancy, they were not shown to significantly increase in size and no complications such as cyst rupture or torsion occurred[27].

Borderline ovarian tumours (BOTs) are more common in women of child-bearing age with a third diagnosed in women <40 years old[30]. BOTs account for up 3-8% of adnexal masses diagnosed in pregnancy[31]. They are the most frequent type of ovarian malignancy encountered in pregnancy. A multi-centre French retrospective study focusing on the diagnosis and management of BOTs in pregnancy showed that 90% of women underwent surgery. 20% were identified as having Stage II disease and were noted to have a higher incidence of invasive, aggressive patterns of disease than non-pregnant counterparts[32]. The ultrasound appearances of BOT can overlap with the changes that occur in endometriomas during pregnancy, making the distinction between benign and malignant masses more challenging in pregnancy[33].

Endometriomas represent 4-8% of ovarian cysts identified during routine sonography in the first trimester[31,34]. In pregnancy, the endometrium undergoes 'decidualisation'. This is a process associated with an increase in glandular epithelium and stromal vascularity, occurring to optimise endometrial receptivity in preparation for embryo implantation[35]. A similar process occurs within ectopic endometrial tissue within the walls of endometriomas. Decidualisation has the appearance of solid, vascular areas, within the wall of an endometrioma on ultrasound, which can raise a suspicion of malignant transformation and results in unnecessary surgical intervention in many cases[36].

Decidualisation is a transient, dynamic process that occurs during pregnancy and differs from the irreversible neoplastic changes that occur within malignant tumours. The decidualisation process does not occur in every endometrioma. A retrospective study of 34 endometriomas in pregnancy demonstrated it only occurred in 12% of endometriomas[35].

Furthermore, the solid areas within an endometrioma, which has undergone the process of decidualisation, can mimic the appearance of papillary projections. Papillary projections are features commonly associated with malignant tumours, particularly BOTs. Recognising the changes that pregnancy has on the ultrasound appearance of adnexal masses, *Mascilini et al* carried out a retrospective review of 34 women in pregnancy who underwent surgical removal of an ovarian cyst with papillary projections, but no other solid components. The study found that the presence of a smooth contour of papillations (79% vs 27%) and ground glass echogenicity (74% vs. 13%) were more commonly seen in benign than malignant adnexal masses[36]. Whilst *Mascilini et al* helped to improve the understanding of sonographic appearances of specific adnexal mass features, such as solid areas during pregnancy, a need exists for a prospective evaluation of adnexal masses at distinct time points during pregnancy in order to delineate the timing and pattern of change through pregnancy and into the post-partum period. A particular area of interest is the decidualisation process that occurs within endometriomas, and how the appearance of the solid areas may change during pregnancy. Understanding the pattern of 'physiological' changes within the ovary during pregnancy will facilitate the detection of an underlying malignant transformation process within an adnexal mass. This will, therefore, facilitate the distinction between a benign and malignant mass. Also of interest is the difference, if any, between the morphological appearance of invasive ovarian tumors, and metastases to the ovaries from primary tumours located somewhere else diagnosed during pregnancy[37,38].

Expectant versus Surgical management during pregnancy

Traditionally, because of the overall concern about the potential risks of complications relating to persistent ovarian cysts in pregnancy, routine surgical removal was recommended. Practice has now changed in favour of expectant management of adnexal masses which appear benign on ultrasound. This is primarily because of the maternal/foetal risks associated with undergoing surgery during pregnancy, combined with a low incidence of complications, such as ovarian torsion, cyst rupture or malignant potential during pregnancy. Hence, expectant management appears to be a logical management strategy of adnexal masses in pregnancy.

Surgical intervention may be required acutely if suspected torsion/cyst accident. Pregnancy is thought to increase the risk of ovarian torsion due to ovarian displacement secondary to uterine enlargement, (19.9% in pregnancy vs. 9% non-pregnancy)[39,40]. The rate of torsion varies widely but does appear to be proportional to the size of the adnexal mass, with larger masses more likely to undergo torsion or rupture[24]. Torsion tends to occur between 10 and 17 weeks and rarely beyond 20 weeks[24]. The hypothesis here is that the gravid uterus restricts the mobility of the adnexa.

Despite a low risk of an ovarian malignancy in pregnancy, a large number of women undergo surgery during pregnancy because of the concern for a potential malignant adnexal mass[41]. A retrospective study by *Yen et al* evaluated the risk of malignancy in a cohort of women who were identified as having an adnexal mass and underwent surgical management during pregnancy or at the time of delivery. The frequency of ovarian cancer within the cohort was 2.3%, which is reassuringly low[42]. In a study by *Schmeler et al,* of the 63 patients diagnosed with a mass >5cm, 17 required surgery during pregnancy, 13 due to suspicious ultrasound findings and 4 due to acute pain suggestive of torsion[43]. Four patients had a histological diagnosis of ovarian cancer but the remainder had a benign diagnosis, most commonly a dermoid cyst[43].

The use of ultrasound as first line imaging modality in pregnancy was reinforced by *Moro et al,* who demonstrated that the ultrasound features of malignant ovarian masses in women (n=22) scanned prior to surgical intervention of a mass were consistent to those seen outside of pregnancy[37]. Surgical intervention is preferably avoided in pregnancy due to the potential maternal and foetal risks[44]. The rate of non-obstetric surgery was 0.73% in a large retrospective cohort study of 6.5 million pregnancies[45]. Although surgery is perceived as relatively safe in pregnancy, a recent retrospective cohort study by *Balinskaite et al,* observed an increase in low birth weight (<2.5Kg), preterm delivery (<37 weeks) and foetal growth restriction in women who underwent surgery compared to those that did not[45]. Women who underwent laparoscopic procedures, which required hospitalisation were shown to have a higher rate of miscarriage than those who underwent open abdominal surgery[46]. A laparoscopic approach, however, is generally preferred, due to better post-op recovery, less opioid use, reduced basal lung atelectasis, minimal uterine manipulation and a lower incidence of venous thromboembolism (VTE) events[24].

Taking the potential maternal and foetal risks of surgery into account, combined with the inherent low risks of complications in association with adnexal masses, expectant management is the preferred management approach for adnexal masses in pregnancy. In addition, it is important to highlight that assessing the adnexal mass at 11-14 weeks gestation allows us to plan for minimally invasive surgery if necessary. Surgery before this period would coincide with a time in gestation where risk of miscarriage is at its highest[45]. After 11-14 weeks, risk of miscarriage falls. Therefore, if surgery is deemed necessary, it is preferably performed between 14 and 20 weeks. Risks of surgery increase after 20 weeks, although if necessary, surgery is feasible throughout pregnancy[45].

3. STUDY OBJECTIVES

Primary objective

- To prospectively validate the ability of the ADNEX Model to discriminate between benign and malignant adnexal masses at 11-14 weeks gestation.

Secondary objectives

- To prospectively validate the ability of the 2-step strategy (i.e. Modified Benign Simple Descriptors followed by ADNEX) to discriminate between benign and malignant adnexal masses at 11-14 weeks gestation;
- To prospectively validate the ability of the ADNEX Model and the 2-step strategy (i.e. Modified Benign Simple Descriptors followed by ADNEX) to discriminate between benign and malignant adnexal masses when first detected at any time point in pregnancy (irrespective of whether the mass was known before pregnancy or detected for the first time during pregnancy);
- To longitudinally examine the development of an adnexal mass during pregnancy with description of final outcome, based on scans performed at pre-specified time points and scans added as a result of a clinical decision.

Tertiary objectives

- To describe the evolution of endometriomas during gestation;
- To study the occurrence of complications such as rupture, torsion, or malignancy during pregnancy in patients with conservatively treated masses;
- To compare the performance of the ADNEX model with CA125 with that of ADNEX without CA125;
- To suggest an IOTA triaging system on the basis of the results of the study which may be applied to guide management during pregnancy;
- To collect images, clips and volumes for quality control, illustration of changes during pregnancy, educational purposes.

4. STUDY DESIGN AND SETTING

4.1.Study Centres

The following type of unit/centre will be included:

- ***Expert centres*** for ultrasound (with expertise as per definition described below) **with or without** an oncology department attached to it;
- ***Early Pregnancy Units*** (EPUs) but only if capable on deciding management without referring to a tertiary referral centre (EPUs are defined as units which offer a service for women with symptoms such as pain or bleeding in early pregnancy <14 weeks and are run by gynaecologists).

There will be:

- A named individual in each participating Study centre responsible for coordinating the project locally and reporting directly to the p-IOTA Management Committee.

- Regular inspection by the Chief Investigator (Dr Srdjan Saso) and the IOTA Study Manager (Mr Matt Malecki), will be carried out ensuring that both the service (communication, documentation, supervision and logistics) and facilities (ultrasound equipment and space) are of high standards.

4.2 Study design

Multicentre prospective cohort observational study

4.3 Duration

The study will be conducted over a minimum of three years.

5. PATIENTS

5.1 Inclusion Criteria

- Consecutive patients with non-physiological adnexal masses or physiological cysts measuring 5cm or more in largest dimension;
- In case of more than one mass seen, only most suspicious mass to be included OR in case of two similar masses, the one with the largest dimension or most easily accessible with ultrasound;
- Previously recruited patient presenting with a different mass in subsequent pregnancy;
- Age 18 years and above.

5.2 Exclusion Criteria

- Cysts deemed to be clearly physiological WHEN smaller than 5 cm (largest diameter);
- Non-adnexal masses, e.g. peritoneal inclusion cysts (when diagnosis is certain) and peritoneal carcinomatosis with no adnexal mass;
- The denial or withdrawal of written informed consent;
- Same cyst already recruited for p-IOTA in a previous pregnancy.
- Age < 18 years

5.3 Withdrawal Criteria

Patients may withdraw from the study at any stage and all data captured in relation to their participation may be destroyed at their request.

5.4 Consent and Information leaflet

All participating patients will be consented prior to enrolment and will receive a full explanatory information leaflet. Signed participant consent will be obtained. The right of the participant to decline participation without giving reasons will be respected. After the participant has entered the study the clinician remains free to propose management different from the one specified in the protocol at any stage if he/she feels it is in the participant's best interest. The reasons for doing so should be recorded. In these cases, the participants remain within the study for the purposes of follow-up and data analysis. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

6. METHOD and DATA COLLECTION

6.1 General Process

We plan an initial recruitment period of 36 months. Women will be recruited to p-IOTA if found to have an adnexal mass (either newly diagnosed or previously known) at an ultrasound examination performed **at any gestational age.**

Patients will be managed as per local clinical guidelines specific to the case, with all clinical decisions taken by lead clinicians according to current policy and practice. If a patient consents to taking part in the study, the number of scans will be determined by the p-IOTA protocol.

For patients managed surgically, no follow-up ultrasound examinations after surgery will be required. Follow-up for these women is arranged by their clinicians as per clinical practice.

6.2 Study flow

Figure 1-3 illustrate the study flow.

First arm (***primary objective - figure 1***)**:** Adnexal mass scanned at 11-14 weeks. If the decision is to manage the adnexal mass conservatively, the patient is to be re-scanned once more: 0 - 90 days postpartum. In addition, the patient will be asked to enter into the third arm of the study (see below) knowing that she can opt out from that arm at any point. If she consents to participate in the third arm, she will participate in a series of longitudinal examinations as described for the third arm.

All patients with a mass detected before 11 weeks will also be scanned at 11-14 weeks and can enter all three arms of the study. In this scenario, if more than one scan is performed during pregnancy before 11 weeks, only the first of several scans before 11 weeks will be included for the second and third arms of the study (see below).

Second arm (***secondary objective - figure 2***)**:** Adnexal mass is seen *for the first time* during that pregnancy at any gestation. If the decision is to manage the adnexal mass conservatively, the patient is to be re-scanned once more: 0 - 90 days postpartum. For patients with a mass detected before 11 weeks, see also above (they will be re-scanned at both 11-14 weeks and at 0-90 days postpartum).

In addition, the patient will be asked to enter into the third arm of the study (see below) knowing that she can opt out from that arm at any point. If she consents to this, she will participate in a series of longitudinal examinations as described for the third arm.

Third arm (**secondary objective - figure 3**): Patients from the first and second arms who agree to enrol into the third arm i.e. longitudinal examination of an adnexal mass during pregnancy. For the purposes of the longitudinal evaluation, the time points for the ultrasound examination are:

- initial presenting scan if <11 weeks;
- 11-14 weeks;
- 16 week scan - endometriomas ONLY;
- second trimester routine scan (18-22 weeks);
- 30-34 weeks;
- any additional scans during pregnancy which resulted in either conservative management with extra scans or surgical intervention (with reason for the extra scan or for surgery documented);
- 0-90 days postpartum.

If the decision is to operate after the scan at which the mass was first detected during pregnancy with no follow-up scans planned before surgery, the patient is included in the study with the final outcome being the histopathological diagnosis of the surgically removed mass (see next section).

If the adnexal mass is not seen at a subsequent follow-up scan but has been replaced with a normal ovary, there is no need for further follow-up scans. The final outcome is benign.

Patients enrolled into the study but who suffer miscarriage, ectopic pregnancy or intra-uterine death can be included in the study using same criteria as above.

Definition of final outcome

The final outcome, for the purpose of the analysis, will be based on the pathology result after surgery. If surgery is not performed, the diagnosis assigned by the ultrasound examiner using subjective assessment at the 0-90 days postpartum scan will be used. If neither surgery nor a postpartum scan are performed, the final outcome is defined as 'uncertain'. We describe this in more detail below.

The final outcome will be benign or malignant (borderline or invasive) and will be based on:

1) Histological diagnosis of the mass, if surgery performed during pregnancy OR within 120 days of the postpartum ultrasound scan

If surgery is performed more than 120 days after the postpartum scan the histological diagnosis will be the final diagnosis only if the histological outcome is benign.

If surgery is performed before a scheduled postpartum scan, there is no need for a postpartum scan for the purpose of the study.

2) If no surgery performed - subjective assessment of the ultrasound image by the ultrasound examiner at postpartum scan:

The subjective assessment of the mass will include:

- A: Benign or Malignant (borderline or invasive)

- B: Probability of malignancy (level of certainty / confidence with the diagnosis as being benign or malignant and managing it as that):

1 = benign
2 = probably benign
3 = uncertain/not possible to classify
4 = probably malignant (borderline / invasive)
5 = malignant (borderline / invasive);

- C: *Self impression:* presumed histological diagnosis (e.g. dermoid, serous cystadenoma, endometrioma, decidualised endometrioma, abscess…);
- D: *Management:* conservative management with ultrasound follow-up, surgery by general gynaecologist, surgery by oncological surgeon, other.

The final outcome is defined as described below

- Ovary normal at postpartum scan, then **outcome** = benign
- Not operated on and level of certainty/confidence at postpartum scan = 1-2, then **outcome** = benign
- Not operated on and level of certainty/confidence at postpartum scan = 5, then **outcome** = malignant
- Not operated on and level of certainty/confidence at postpartum scan = 3-4, then **outcome** = uncertain
- Operated and histology = benign, then **outcome** = benign (even if surgery > 120 days after postpartum scan)
- Operated within 120 days since postpartum scan and histology = malignant, then **outcome** = malignant
- Operated > 120 days since postpartum scan AND histology = malignant AND level of certainty/confidence at postpartum scan is 4-5, then **outcome** = malignant
- Operated > 120 days since postpartum scan AND histology = malignant AND level of certainty/confidence at postpartum scan is 1-3, then **outcome** = uncertain
- Neither surgery nor postpartum scan (within 0-90 days) performed, then **outcome** = uncertain.

<u>Additional information</u>

At every scan, irrespective of gestation or which arm of the study the patient falls under, the following are recorded in this specific order:

1) Subjective assessment (A-D as described above) and the following additional E-G criteria:

- E: *Any subjective ultrasound change to adnexal mass from previous scan (five options):* 1) yes, change noted; 2) no change noted; 3) adnexal mass no longer detectable (no ovary seen); 4) adnexal mass no longer detectable with only a normal ovary seen; 5) unsure (comment to explain why).
- F: *Colour score of ovarian mass:* score 1, 2, 3, 4

- G: *If papillations present:* 1) number of papillations; 2) three orthogonal diameters of largest papillary projection i.e. height, base 1 and base 2; 3) smooth rounded papillations - yes / no; 4) colour in papillation - score 1, 2, 3, 4 (choose worst case scenario in case of multiple papillations).

2) Modified Benign Descriptors and ADNEX model variables.

Modified Benign Simple Descriptors and ADNEX model variables will be recorded for all arms of the study but will not be used when deciding on management (Appendix 1).

Images of all four descriptors variables are shown and selected by ticking. The ultrasound ADNEX model variables are listed and filled in as per IOTA website and application. To ensure minimum inference, they will be recorded on a separate page from the subjective assessment on the computer software programme (Clinical Data Miner, CDM).

Modified Benign Descriptors and ADNEX model will not be used to guide management - only subjective assessment of the expert scanner. Therefore, participating ultrasound examiners are asked NOT to calculate the risk of malignancy using ADNEX, because the management decision should not be influenced by the results of ADNEX.

CA125 must be measured at the 11-14 week scan and-at the inclusion scan if done before 11 weeks OR after 14 weeks. It should be done within two weeks (+/- two weeks) of the scan - this must be prior to surgery, if surgery has been arranged.

If found to be pregnant again at the 0-90 days postpartum scan, the outcome will be recorded in the same manner as described above. The patient can be recruited again as a separate case, but only if the patient has presented with a new cyst.

Retrospectively from the notes and patient's history, complications such as torsion and rupture that may have occurred will also be recorded.

6.3 Definition of level of ultrasound experience

The level of ultrasound experience is based on the number of gynaecological scans in non-pregnant women that the examiner has performed at the start of the study. Basic experience is defined as <500 scans, intermediate experience as 500-5000 scans, and expert experience as >5000. In addition, we also record other information related to experience: the EFSUMB classification[47] and the number of ovarian masses that the examiner has examined with ultrasound during the past year (classified as <50 (< 1 per week); 50-200 (up to 4 per week); >200 (> 4 per week)).

Only those patients where the management decision was made based on the findings of an expert ultrasound examiner will be included in the study. The management decision itself need not be made by the expert ultrasound examiner.

We advise that all scans are done by the same ultrasound examiner. If not possible, as a minimum, the images from all scans of the same patient should be ***assessed*** by the same ultrasound examiner.

6.4 Surgical intervention

Surgery will be performed according to local protocols and the lead clinician for the patient. The reason for surgery, for example, symptoms, suspected torsion or cyst rupture or concern for malignant potential, will be recorded as part of the study. The tumour should be removed as per clinical practice. All histology will be reported and collected as per clinical practice and histology results will be documented for the study (**Appendix 5**).

6.5 Study Closure

We aim to recruit approximately 855 pregnant patients from 19 tertiary referral centres, with a minimum study duration of three years (please refer to section 7).

6.6 Documentation and Communication

Clinical data, patient demographics and ultrasound findings will be recorded prospectively in a computer software programme, acting as a web-based database (Clinical Data Miner, CDM - **Appendices 2-5**). This is a secured data collection software, dedicated for p-IOTA downloaded on password secured hospital computers. Data after entry are locked and cannot be changed.

The flow will be as follows: patient history, initial ultrasound scan, follow-up ultrasound scans, mass complications during pregnancy and surgical findings if applicable.

General procedures

Upon inclusion in the study, written informed consent is obtained from the patient. First, clinical information about the patient is entered into CDM, including information related to gestational age at time of scan and whether the mass was known before pregnancy. Second, the ultrasound examiner provides a diagnosis based on subjective assessment and enters the Modified Benign Simple Descriptors and ADNEX model variables directly after. The Modified Benign Simple descriptors and the ADNEX models are not used to decide on management.

Clinical Information

As the type of centre (oncological vs non-oncological centre) is a predictive variable in the ADNEX model, this information will be filled in. An oncological centre is a tertiary referral centre with a specific gynaecological oncology unit.

The ultrasound system used, ultrasound modality (transabdominal and/or transvaginal) and the name of the ultrasound examiner will be recorded. All ultrasound examiners must have passed the IOTA certification test.

Appendix 2 outlines the specific general patient characteristics which will be recorded at the inclusion scan.

CA125 will be analysed and recorded at the inclusion scan and at the 11-14 week scan but must not be used to guide clinical management.

Ultrasound examination

We recognise that most patients will present with a solitary mass. However, a minority will have more than one on one ovary or bilaterally. Therefore, only the most suspicious non-physiological adnexal mass or physiological cyst (if the largest diameter measures 5cm or more) should be included. The chosen mass should have a unique identifier in the form of a numeral (1,2,3..) with letter 'L' or 'R' to signify side (for example, 1L or 2R) should be given to the mass so that it can be identified throughout pregnancy and hence avoid errors. That label should also be written on the ultrasound image so as not to confuse masses in the same ovary with each other at follow-up scans. In case of two similar masses, the one with the largest dimension or most easily accessible with ultrasound should be selected for inclusion.

A standardised transvaginal (supplemented with transabdominal if transvaginal is not sufficient) examination is performed. When a colour Doppler ultrasound examination is performed, the pulse repetition frequency should be 0.3-0.6 KHz. The colour Doppler gain should be increased until colour Doppler artefacts appear and then lowered until just below the reappearance of colour Doppler artefacts. Ultrasound frequency and "priority" (grey scale or colour/Power Doppler) must also be optimised when using colour/power Doppler. Doppler ultrasound should be used with all masses included, irrespective of gestational age.

Collection of ultrasound images and ultrasound volumes

All participants will be asked to store a sufficient number of high-quality transvaginal ultrasound images/volumes of all masses. Images must be stored if they are suggestive of a malignancy, decidualisation and masses with papillary projections.

7. STUDY OUTCOME MEASURES

7.1 Primary Outcome Measures

- Estimation of the false discovery rate when the ADNEX Model is applied at 11-14 weeks.

7.2 Secondary Outcome Measures

- Estimation of the false discovery rate when the 2-step strategy (i.e. Modified Benign Simple Descriptors followed by ADNEX) is applied at 11-14 weeks.
- Estimation of the false discovery rate when the ADNEX Model and the 2-step strategy (i.e. Modified Benign Simple Descriptors followed by ADNEX) are applied at any time point during pregnancy;
- Estimation of the ability of the ADNEX model and of the 2-step strategy (sensitivity, specificity, C-index, calibration intercept, calibration slope, clinical utility) to discriminate between benign and malignant adnexal masses at 11-14 weeks gestation.
- Estimation of the ability of the ADNEX model and the 2-step strategy (sensitivity, specificity, C-index, calibration intercept, calibration slope, clinical utility) to discriminate between benign and malignant adnexal masses when detected at any time point in pregnancy;
- Evaluation of change in morphology of ovarian masses throughout pregnancy based on subjective assessment;
- Evaluation of change throughout pregnancy and postpartum in number, size, colour score, and morphology of papillations based on subjective assessment.

7.3 Tertiary Outcome Measures

- Change in the ultrasound appearance of endometriomas during gestation;
- Occurrence of complications such as rupture, torsion, or malignancy during pregnancy in patients with conservatively treated masses (cumulative incidence illustrated by 'reverse' Kaplan-Meier curves).
- Comparison of the performance of ADNEX with CA125 and that of ADNEX without CA125.

8. STUDY POPULATION AND SAMPLE SIZE

In an ideal scenario, the results of this study will point to a very low false positive rate when applying ADNEX and Modified Benign Simple Descriptors to masses in pregnancy. Ideally, all participating centres should have been 'tried and tested' by IOTA already to minimize issues with data collection and missing data.

Our sample population will come from Early Pregnancy Units and from Tertiary referral centres for ultrasound **with or without** an oncology department attached to it. The sample size estimation is based on data from Tertiary referral centres.

The table below gives the sensitivity, specificity, and false discovery rate (defined as 1 minus positive predictive value) based on ADNEX thresholds as estimated in surgically and conservatively managed masses in the IOTA 5 study (non-pregnant patients).[43] It assumes a prevalence of malignancy in pregnant women presenting in tertiary referral centers of 8% (based on a survey of a number of IOTA recognized centers; unpublished data). At a risk threshold of 10% (risk of malignancy calculated by ADNEX), the false discovery rate may be too high (65%) because of the low prevalence of malignancy in pregnant women. A risk threshold of 25% to 40% (Dutch standard in non-pregnant women) appears to offer a good balance between sensitivity (detection of malignant masses) and false discovery rates (false positive results may lead to intervention during pregnancy in benign masses).

threshold	**sensitivity**	**specificity**	**false discovery rate**
(risk of malignancy according to ADNEX)	**100*(number classified as cancer/number of cancer)**		**100*(number benign/number classified as cancer)**
1	99.1	12.0	91.1
3	94.7	59.7	83.0
5	93.3	76.4	74.4
10	91.2	85.3	65.0
15	87.3	89.0	59.2
20	83.5	91.5	53.9
25	80.2	93.1	49.7
30	77.3	94.4	45.4
40	73.0	95.8	39.8
50	66.7	97.1	33.3

The table below assumed an average of 15 pregnant women per year per tertiary referral center and assumed a sensitivity of 73% and a specificity of 95.8%. With 19 centers included in the data collection, the expected total sample size in three years is 855 pregnant women and 68 malignancies. This allows us to estimate the specificity, sensitivity, and false discovery rate with a precision (width 95% CI/2) of 1%, 11% and 11%, respectively.

Number of centers	n	n malignancy	True +ve	False -ve	True -ve	False +ve	Precision sensitivity	Precision false discovery rate	Precision specificity
20	900	72	53	19	793	35	11%	11%	1%
19	855	68.4	50	18	754	33	11%	11%	1%
18	810	64.8	47	17	714	31	11%	11%	2%
17	765	61.2	45	17	674	30	12%	12%	2%
16	720	57.6	42	16	635	28	12%	12%	2%
15	675	54	39	15	595	26	12%	12%	2%
14	630	50.4	37	14	555	24	13%	13%	2%
13	585	46.8	34	13	516	23	13%	13%	2%
12	540	43.2	32	12	476	21	14%	14%	2%
11	495	39.6	29	11	436	19	15%	14%	2%
10	450	36	26	10	397	17	15%	15%	2%
9	405	32.4	24	9	357	16	16%	16%	2%
8	360	28.8	21	8	317	14	17%	17%	2%
7	315	25.2	18	7	278	12	18%	18%	2%
6	270	21.6	16	6	238	10	20%	20%	3%
5	225	18	13	5	198	9	22%	21%	3%
4	180	14.4	11	4	159	7	24%	24%	3%
3	135	10.8	8	3	119	5	28%	27%	4%
2	90	7.2	5	2	79	3	33%	33%	5%
1	45	3.6	3	1	40	2	42%	42%	8%

The table below repeats the same calculation, now assuming sensitivity of 80.2% and specificity of 93.1%. Including 19 centers allows us to estimate the specificity, sensitivity, and false positive rate with a precision (width 95% CI/2) of 2%, 11% and 10%, respectively.

Centre Number	n	n malignancy	True +ve	False -ve	True -ve	False +ve	Precision sensitivity	Precision false discovery rate	Precision specificity
20	900	72	58	14	771	57	11%	10%	2%
19	855	68.4	55	14	732	54	11%	10%	2%
18	810	64.8	52	13	694	51	11%	10%	2%
17	765	61.2	49	12	655	49	12%	11%	2%
16	720	57.6	46	11	617	46	12%	11%	2%
15	675	54	43	11	578	43	12%	11%	2%
14	630	50.4	40	10	540	40	13%	12%	2%
13	585	46.8	38	9	501	37	13%	12%	2%
12	540	43.2	35	9	463	34	14%	13%	2%
11	495	39.6	32	8	424	31	15%	13%	2%
10	450	36	29	7	385	29	15%	14%	3%
9	405	32.4	26	6	347	26	16%	15%	3%
8	360	28.8	23	6	308	23	17%	15%	3%
7	315	25.2	20	5	270	20	18%	17%	3%

6	270	21.6	17	4	231	17	20%	18%	3%
5	225	18	14	4	193	14	22%	20%	4%
4	180	14.4	12	3	154	11	24%	22%	4%
3	135	10.8	9	2	116	9	28%	25%	5%
2	90	7.2	6	1	77	6	33%	30%	6%
1	45	3.6	3	1	39	3	42%	39%	9%

In addition, below are the minimum required sample sizes to demonstrate that IOTA-ADNEX has clinical utility in pregnant women at a range of thresholds for the IOTA-ADNEX model.[48]

Threshold	Assumed Standardized Net Benefit	Assumed sNB treat all	sNB treat none	Target precision sNB (width 95% CI/2)	N required (pregnant patients with eligible mass)
5%	0.790	0.395	0	0.395	28
25%	0.538	<0	0	0.518	57
40%	**0.408**	**<0**	**0**	**0.388**	**165**
50%	0.334	<0	0	0.314	325

With a sample size of 855 patients and assuming a C-statistic of 0.90, we can calculate the C-statistic with a precision (width of CI/2) of 0.04.[48] With respect to calibration, with 855 patients we have enough data to detect if 54 cancers were predicted instead of 68 observed (O/E=1.26), and to detect if 86 cancers predicted instead of the 68 observed (O/E=0.80). We do note that the sample size is insufficient to detect an O/E of 1.10 or an O/E of 0.90. To detect miscalibration-in-the-large of this magnitude would require 4389 patients.

We conclude that with 855 patients, this study will be sufficiently powered to obtain an estimate of the primary outcome, the false discovery rate of ADNEX at a risk threshold of 40%, with a precision of 11%. The study is also sufficiently powered to study the following secondary outcomes with sufficient precision: diagnostic accuracy (sensitivity and specificity), the C-statistic, and clinical utility (quantified by the Net Benefit) of ADNEX. The study is powered to detect moderate to large differences between (average) predicted and (average) observed risks, but underpowered to detect minor miscalibration.

Moreover, we will validate the IOTA-ADNEX model at 10%, 25%, 40%, and 50% risk thresholds. Intervening when the risk of malignancy is 10% or higher is a very sensitive approach with few missed malignancies, but may lead to too many unnecessary interventions during pregnancy. In contrast, intervening when the risk of malignancy is 50% or higher will yield a much more specific approach, with fewer interventions during pregnancy, but may lead to more missed cancers

Finally, data from adnexal masses encountered in two early pregnancy units will be collected and analysed separately to examine the distribution of predicted risks of malignancy and referral rates to tertiary care if ADNEX were used for triage (referral based on clinical decision at early pregnancy unit). In a period of three years and with an expected 5000 deliveries per center per year, we expect 30,000 pregnant women. With an expected prevalence of adnexal masses of 6%, we expect data on 1800 masses and at a BOT rate of 3-5% when an adnexal mass is picked up in pregnancy, between 50-90 malignancies (i.e. BOTs and invasive).

8.1 Summary

Validation of diagnostic models will occur in subgroups according to type of center (referral center v. early pregnancy unit), as the prevalence of malignancy will likely influence performance of IOTA-ADNEX and the Modified Benign Simple Descriptors.

Validation of diagnostic models refers to:

1) prospective validation of the false discovery rate of IOTA ADNEX and the two-step strategy at 11-14 weeks GA (False Discovery Rate in this context is how many pregnancies with a benign mass would be referred to tertiary care);

2) prospective validation of IOTA ADNEX and the two-step strategy in terms of sensitivity/specificity/c-index/calibration/net benefit at 11-14 weeks GA;

3) prospectively validation of IOTA ADNEX and the two-step strategy to discriminate between benign and malignant adnexal masses when detected at any point during pregnancy, in terms of false discovery rate/sensitivity/specificity/c-index/calibration/net benefit.

9. STUDY SUPERVISION AND MONITORING

This study does not involve any risk to the participants.

Central supervision: The IOTA Steering Committee is responsible for the protocol, quality control, advice on progress and final analysis and reporting of the study.

The study will be co-ordinated by Dr Srdjan Saso, to ensure timely progress and regular participation.

Local supervision: Principal Investigators are responsible for the data collection at their centres and communication with Dr Srdjan Saso.

10. REGULATORY ISSUES

PUBLICATION POLICY

The IOTA steering committee is responsible for publication of the data in scientific journals and will be listed as co-authors if they fulfil the requirements of authorship of the International Committee of Medical Journal Editors and in accordance with the requirements of the respective medical journal.

The chief statistician is responsible for data analysis, contributes to study design (protocol draft) and will be listed as a co-author. Principal Investigators are also co-authors, according to the number of patients they contributed to the study (depending on the journal's restriction of the number of co-authors) on condition that they contribute to writing the papers, read and approve the final version, and agree to be accountable for all aspects of the work, as defined by the International Committee of Medical Journal Editors and in accordance with the requirements of the respective medical journal.

INTELLECTUAL PROPERTY

The KU Leuven VAT number BE 419.052.173, represented by its department K.U. LEUVEN RESEARCH & DEVELOPMENT, having its office in 3000 Leuven, Waaistraat 6, Belgium, and represented by its university hospital, UNIVERSITAIRE ZIEKENHUIZEN LEUVEN holds all intellectual property rights that might result from the IOTA project (including, amongst others, this p-IOTA). Each participating centre shall remain the owner of its source data and may utilise such data AFTER the main scientific papers of the multicentre study have been published. 2D ultrasound images, clips and 3D ultrasound volumes may be used for educational purposes outside the study, as well as in other studies.

ETHICS AND REGULATORY APPROVALS

The multicentre project is first approved by the central Ethics Committee of UZ Leuven, Belgium and afterwards approved by the Ethics Committee in each investigating centre. The study will be performed in accordance with the terms of the protocol, generally accepted standards of Good Clinical Practice and the investigators will adhere to all applicable laws and regulations governing the conduct of clinical trials, including but not limited to the ICH Harmonised Tripartite Guidelines for Good Clinical Practice and the Declaration of Helsinki.

Each Participating centre shall obtain an informed consent (oral or written, depending on the applicable local legislation and local Ethics Committee requirements) for all patients prior to their enrolment and participation in the Study in compliance with all applicable laws, regulations and the approval of the (local) Ethics Committee.

The Investigator shall treat all information and data relating to the Study as confidential and shall not disclose such information to any third parties or use such information for any purpose other than the performance of the Study. The collection, processing and disclosure of encoded personal data, such as patient health and medical information is subject to compliance with applicable personal data protection and the processing of personal data and/or the privacy laws applicable to the Participating centre's location).

Sponsor is subject to the rights and obligations as 'data controller' set forth under the European Data Protection Directive (95/46/EC) (and upon its entry into force the General Data Protection Regulation 2016/679 ("GDPR")) in relation to the processing of Personal Data.
Each participating centre is subject to the rights and obligations as 'data processor' set forth under the European Data Protection Directive (95/46/EC) (and upon its entry into force the GDPR) in relation to the processing of Personal Data.
Parties' rights and obligations with respect to Personal Data (as defined in Appendix 6) are further detailed in Appendix 6.

INSURANCE
Each participating centre is fully responsible for patient care within its own hospital in agreement with local laws. Each centre is also responsible for all legal aspects of patient care and for its own insurance for all matters related to this study as required by local laws.

For Belgian participating centres
Art. 29 of the Belgian Law relating to experiments on human persons dated May 7th, 2004 applies. Prior to the start of the study, the Sponsor shall enter into an insurance contract in order to adequately cover study participants from Belgian sites in accordance with art. 29 of the said law.
For non-Belgian participating Sites
Each Participating centre shall have and maintain in full force and effect during the term of this study (and for a reasonable period following termination of the study, adequate insurance coverage for other possible damages resulting from the study at the participating centre, as required by local law. Each such insurance coverage shall be in amounts appropriate to the conduct of the services of the participating centre under this study. The participating centre and Sponsor shall be solely responsible for any deductible or self-insured retention under any such policies.

FINANCIAL ASPECTS
Gynaecological ultrasound and clinical evaluations as well as treatment and pathological diagnosis are performed in the framework of routine clinical practice and require no additional expenses. There is no financial compensation for patients, including the postpartum scan, which is standard clinical practice.

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12. APPENDICES

Appendix 1 - Ultrasound examination IOTA terms and definitions

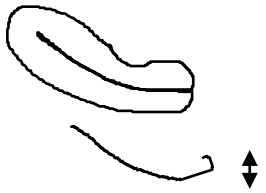
A standardised ultrasound examination following the IOTA protocol is carried out. All ultrasound variables are included in the dedicated software. In the database 0 always means NO and 1 always means YES.

The adnexal lesion is that part of an ovary or of an adnexal mass that is judged by ultrasonography to be not consistent with normal physiology. This can be a persistent unilocular cyst, surrounded by normal looking ovarian stroma with some follicles. In this case the whole ovary containing the cyst is the 'ovary', whereas the unilocular cyst is the 'lesion'. Both are measured and the cyst is described as being 'unilocular' and not 'unilocular-solid'. In other cases the lesion is separate from the ovary (e.g. hydrosalpinx). Again, both ovary and lesion are measured separately. In other cases no normal ovarian stroma is seen. In these cases the lesion and the ovary are undistinguishable and the measurement of lesion and ovary will be the same.

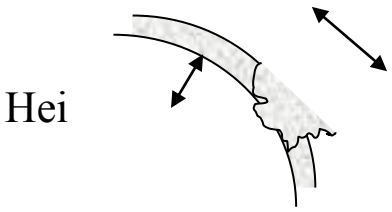
Measurements (in mm): The ovary in two perpendicular planes
The lesion in two perpendicular planes

The volume of the tumour is calculated from the three diameters in two perpendicular planes by the software.

- The presence of ascites (*i.e. fluid outside* the pouch of Douglas) is noted (yes/no).
- Fluid in the pouch of Douglas is measured in the sagittal plane (the largest anteroposterior diameter is given).



- An incomplete septum (as seen in hydrosalpinges) is defined as a thin strand of tissue running across the cyst cavity from one internal surface to the contralateral side, but is not complete in some scanning planes. If a cyst only has incomplete septa, it is unilocular, despite the fact that in certain sections the cyst appears to be multilocular.
- Solid means echogenicity suggesting the presence of tissue (e.g. the myometrium, the ovarian stroma, myomas, fibromas). Blood clots can be distinguished from solid tissue by looking for internal movement when gently pushing the structure with the transducer. The presence of blood flow (with the appropriate colour Doppler settings) is diagnostic for solid tissue. The absence of flow is not diagnostic. In cases of doubt the lesion should be classified as solid.
- Solid papillary projections are defined as any solid projections into the cyst cavity from the cyst wall or septum greater than or equal to 3 mm in height

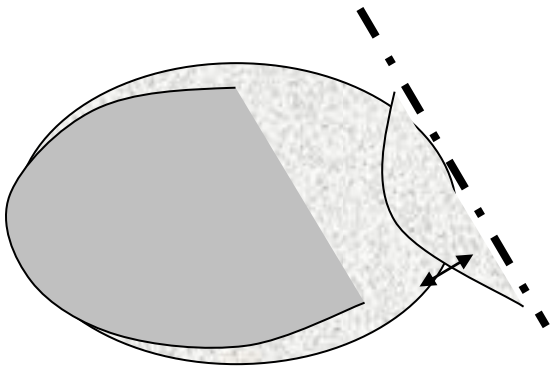


If it is uncertain whether a solid papillary projection or an incomplete septum is present, the 'worse case scenario' is used. For example, 'cogwheel excrescences' and 'beads-on-a-string' (as seen in hydrosalpinges) should be classified as papillary excrescences if their height is greater than or equal to 3 mm. The 'white ball' in a dermoid (i.e. Rokitansky node) should not be classified as a solid papillary projection.

The 'sludge' on the internal walls of endometriotic cysts is not regarded as a papillary projection. In these cases the internal walls are usually 'irregular'.

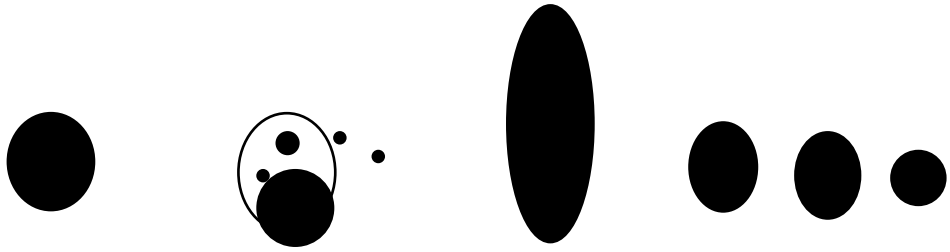
- The number of separate papillary projections is noted (1/2/3/more).
- The presence of flow within some of these projections is noted (yes/no).
- Solid papillary projections are described as being 'smooth' or 'irregular' (e.g. cauliflower-like - this variable will be added to CDM).

In some cases, it is difficult to judge whether it is a papillary projection and from which point to measure the projection. In these cases ,it may be helpful to use an imaginary line as shown in the schematic drawing:



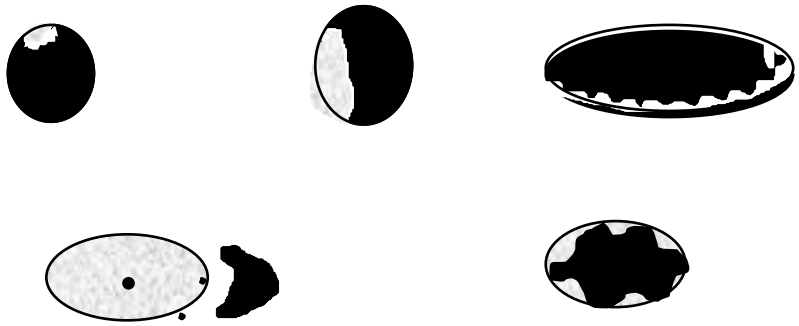
All lesions are qualitatively classified into one of five categories:

1. <u>unilocular</u> (a unilocular cyst without septa and without solid parts or papillary structures). Normal ovarian stroma is not regarded as a 'solid component' (e.g. a peritoneal cyst with one cyst locule, containing a normal ovary, is unilocular and not unilocular-solid).

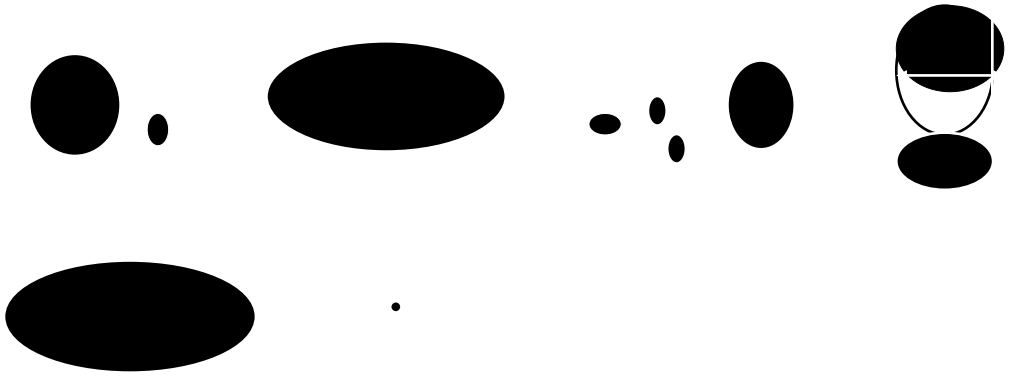


incomplete septum; e.g. in hydrosalpinx)

2. <u>unilocular cyst with solid component, i.e. a unilocular solid cyst</u> (a unilocular cyst with a measurable solid component or at least one papillary structure). This category may include pyo- or hydrosalpinges with the so-called 'beads-on-a-string' or 'cogwheel' appearance if the solid protrusions are ≥ 3mm.

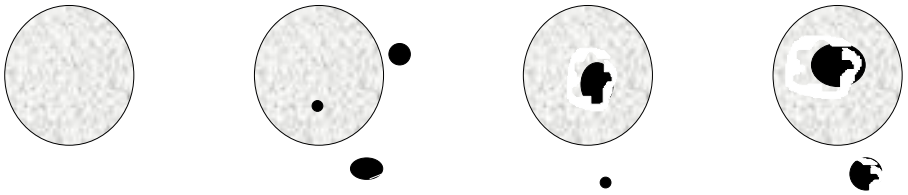


3. <u>multilocular</u> (a cyst with at least one septum but no measurable solid components or papillary projections). The 'lesion' is measured as indicated by the arrows.



4. multilocular with solid component, multilocular solid tumour (a multilocular cyst with a measurable solid component or at least one papillary structure)

5. solid (a tumour where the solid components comprise 80% or more of the tumour when assessed in a two-dimensional section).



(solid tumour with an irregular internal cyst wall)

A solid tumour may contain papillary projections protruding into cysts.

Quantitative assessment of morphology

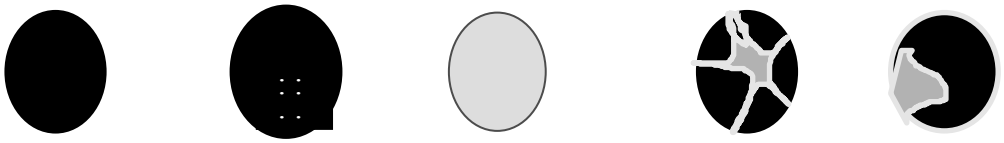
- In cystic-solid tumours the largest solid component is measured separately (in three perpendicular planes). The solid component is noted as being smooth or irregular (i.e. cauliflower-like). In some cases a solid papillary projection is the largest solid component and thus the papillary projection is recorded both as papillary projection and as solid component.
- The internal wall is also noted as being smooth or irregular.

Smooth Smooth Irregular

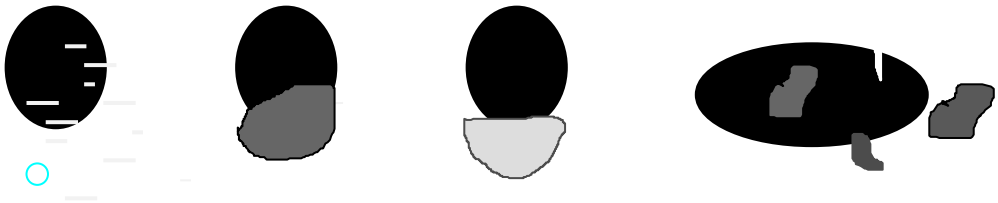
If there is a solid papillary projection, then the wall is irregular by definition.

- The external walls of tumours are not described unless they the tumour is solid.

- In case of solid tumours the description of the internal wall being smooth or irregular is usually not applicable but the outline of the tumour is described as smooth or irregular.
- If there is any irregularity in either the inner wall of any cyst or in the outer wall of a solid tumour or on the surface of a solid component, the lesion is described as 'irregular'.
- The dominant feature of the cystic contents is described as anechoic (black), low-level echogenic (homogeneous low level echogenic as seen in mucinous tumours), 'ground glass' appearance (homogeneously dispersed echogenic cystic contents, as often seen in endometriotic cysts), haemorrhagic (with internal thread-like structures, representing fibrin strands; it is possible to describe the echogenicity as star-shaped, cobweb-like or jelly-like) or mixed echogenic (as often seen in teratomas) (see images attached).



Anechoic | Low level | Ground glass | Haemorrhagic | Haemorrhagic



Mixed | Mixed (old blood-fluid level or fat-fluid level) | Mixed (e.g. abscess)

- The presence of acoustic shadows, defined as loss of acoustic echo behind a sound-absorbing structure, is noted. Solid tumours are identified by the appearance of the internal texture, by the absence of internal movement when moving the transducer or by colour Doppler imaging (presence of central flow).
- 'Ovarian crescent sign', defined as the presence of normal ovarian tissue adjacent to an adnexal tumour.
- Ultrasound evidence of metastases (e.g. "omental cake" or peritoneal tumoural implants). ("absent" or "present", mandatory new variable for phase 3 and 5)

For quality control, at least one 2D greyscale and one 2D colour representative image, as well as 3D volume must be saved. In addition, one greyscale and one colour representative video clip must also be saved. Preferably, these should be stored digitally. Photographs or video are acceptable as well.

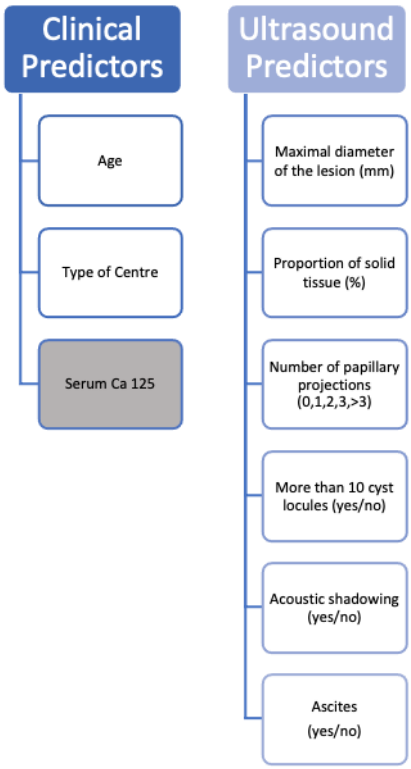
Modified Benign Simple Descriptors

1. Unilocular cyst with ground-glass echogenicity in premenopausal woman with largest diameter < 10 cm
2. Unilocular cyst with mixed echogenicity and acoustic shadows in pre-menopausual woman with largest diameter < 10 cm
3. Unilocular anechoic cyst with smooth internal cyst walls and largest diameter < 10 cm
4. All other unilocular cysts with smooth internal cyst walls and largest diameter < 10 cm

ADNEX model

ADNEX model[15] includes three clinical predictors and six ultrasound predictors. The clinical predictors are: age (years), serum CA125 (U/mL) and type of centre to which the patient has been referred for ultrasound examination. Type of centre has been divided into oncology centres versus other centres.

The ultrasound predictors are the maximal diameter of the lesion (mm), proportion of solid tissue (%), number of papillary projections (0, 1, 2, 3, > 3), presence of more than 10 cyst locules (yes/no), acoustic shadows (yes/no), and presence of ascites (yes/no). The proportion of solid tissue is defined as the ratio of the maximal diameter of the largest solid component and the maximal diameter of the lesion.



Appendix 2 - Data recorded at inclusion scan

General information

- Type of centre where scan performed: Tertiary Referral Centre / Oncology Centre with ultrasound attached / Early Pregnancy Unit
- Patient referred from: self-referral, family doctor/GP, local/district hospital, Early Pregnancy Unit, obstetrics ultrasound department, private gynaecologist

- Family history:
  Number of first-degree relatives with ovarian or breast cancer (0-20)

- Medical history:
  Age (years)
  Previous oophorectomy (Yes/No); if yes, which side? (Right/Left)
  Is this pregnancy a result of fertility treatment? (Yes/No); Which type (in vitro fertilisation, intracytoplasmic sperm injection, intrauterine insemination)?

- Adnexal mass-related (the index mass of this study):
  Diagnosed with this mass before pregnancy (Yes/No)
  If yes - how many months prior to today?

Ultrasound data

- Name of the ultrasound examiner
- Date of scan
- Gestational age according to ultrasound (Weeks…. Days….)

- Ultrasound system used
- Which modality used - transabdominal or transvaginal or transrectal
- Ovarian or tubal or para-ovarian or uncertain? Left or right?

Subjective assessment
After ultrasonographic examination of the mass the investigator gives his/her subjective assessment of the mass:

- A: Benign or Malignant (borderline / invasive);
- B: Probability of malignancy (level of certainty / confidence with the diagnosis as being benign or malignant and managing it as that):

1 = benign
2 = probably benign
3 = uncertain/not possible to classify
4 = probably malignant (borderline / invasive)
5 = malignant (borderline / invasive);

- C: *Self impression:* presumed histological diagnosis (e.g. functional cyst, haemorrhagic corpus luteum cyst, pregnancy-associated cyst, theca-lutein cyst, teratoma, serous cystadenoma, mucinous cystadenoma, serous cystadenofibroma, mucinous cystadenofibroma, fibroma, endometrioma, decidualised endometrioma, abscess, hydrosalpinx, invasive - specify type, borderline - specify type; granulosa cell tumour, dysgerminoma, other);
- D: *Management:* conservative management with ultrasound follow-up, surgery by general gynaecologist, surgery by oncological surgeon, other;
- E: (*If mass diagnosed and scanned prior to pregnancy, proceed with question; otherwise non-applicable) Any subjective ultrasound change to adnexal mass from previous scan (five options):* 1) yes, change noted; 2) no change noted; 3) adnexal mass no longer detectable (no ovary seen); 4) adnexal mass no longer detectable but replaced by a normal ovary; 5) unsure (comment to explain why).
- F: *Colour score of ovarian mass:* score 1, 2, 3, 4
- G: *If papillations present:* 1) number of papillations; 2) three orthogonal diameters (mm) of largest papillary projection i.e. height, base 1 and base 2; 3) smooth rounded papillations - yes / no; 4) colour in papillation - score 1, 2, 3, 4 (choose worst case scenario in case of multiple papillations).

ADNEX Variables

- Age
- Oncology Centre (yes/no)
- Maximum diameter of the lesion (mm)
- Maximum diameter of largest solid component
- Number of papillary projections (0, 1, 2, 3, > 3)
- Presence of more than 10 cyst locules (yes/no)
- Acoustic shadows (yes/no)
- Presence of ascites (yes/no)
- CA125 level (must be measured at 11-14 week scan and at the inclusion scan if done before 11 weeks OR after 14 weeks; it should be done within two weeks (+/- two weeks) of the scan - this must be prior to surgery, if surgery has been arranged)

Modified Benign Simple Descriptors (tick images)

1. Unilocular cyst with ground-glass echogenicity in premenopausal woman with largest diameter < 10 cm
2. Unilocular cyst with mixed echogenicity and acoustic shadows in premenopausual woman with largest diameter < 10 cm
3. Unilocular anechoic cyst with smooth internal cyst walls and largest diameter < 10 cm
4. All other unilocular cysts with smooth internal cyst walls and largest diameter < 10 cm

Ultrasound features suggestive of a complication of adnexal mass: no /yes

- pop-up list (you can select more than one):
    - Suspected torsion
    - Infection/abscess
    - Haemorrhage
    - Rupture
    - Other, please specify: ….

Management data

- Suggested management: "What type of management do you propose for this patient based on ultrasound subjective assessment?"

    - Conservative management with one further ultrasound performed 0-90 days post-partum;
    - Conservative management with longitudinal follow-up as specified in the protocol (third arm of study - if patient consents to this);
    - Conservative management but with additional scans to the one listed in third arm of study with reason (pop-up: change in morphology, change in size, suspicion of malignancy; symptoms; other: specify);
    - Surgery by a gynaecologist or general surgeon;
    - Surgery by an oncological surgeon;
    - Other, specify

Appendix 3 - Data recorded at follow-up scan

General information

- Type of centre where scan performed: Tertiary Referral Centre / Oncology Centre with ultrasound attached / Early Pregnancy Unit

Ultrasound data

- Name of the ultrasound examiner
- Date of scan
- Gestational age according to ultrasound (Weeks…. Days….)

- Ultrasound system used
- Which modality used - transabdominal or transvaginal or transrectal
- Mass detectable (yes/no; and if answer is 'yes') - pop-up: no ovary seen; ovary seen)
- Ovarian or tubal or para-ovarian or uncertain? Left or right?

Subjective assessment

After ultrasonographic examination of the mass the investigator gives his/her subjective assessment of the mass:

- A: Benign or Malignant (borderline or invasive)
- B: Probability of malignancy (level of certainty / confidence with the diagnosis as being benign or malignant and managing it as that):

1 = benign
2 = probably benign
3 = uncertain/not possible to classify
4 = probably malignant (borderline / invasive)
5 = malignant (borderline / invasive);

- C: *Self impression:* presumed histological diagnosis (e.g. functional cyst, haemorrhagic corpus luteum cyst, pregnancy-associated cyst, theca-lutein cyst, teratoma, serous cystadenoma, mucinous cystadenoma, serous cystadenofibroma, mucinous cystadenofibroma, fibroma, endometrioma, decidualised endometrioma, abscess, hydrosalpinx, invasive - specify type, borderline - specify type; granulosa cell tumour, dysgerminoma, other);
- D: *Management:* conservative management with ultrasound follow-up, surgery by general gynaecologist, surgery by oncological surgeon, other.
- E: *Any subjective ultrasound change to adnexal mass from previous scan (five options):* 1) yes, change noted; 2) no change noted; 3) unsure (comment to explain why).
- F: *Colour score of ovarian mass:* score 1, 2, 3, 4
- G: *If papillations present:* 1) number of papillations; 2) three orthogonal diameters (mm) of largest papillary projection i.e. height, base 1 and base 2; 3) smooth rounded papillations - yes / no; 4) colour in papillation - score 1, 2, 3, 4 (choose worst case scenario in case of multiple papillations).

ADNEX Variables

- Age
- Oncology Centre (yes/no)
- Maximum diameter of the lesion (mm)
- Maximum diameter of largest solid component
- Number of papillary projections (0, 1, 2, 3, > 3)
- Presence of more than 10 cyst locules (yes/no)
- Acoustic shadows (yes/no)
- Presence of ascites (yes/no)
- CA125 level (NOT TO BE MEASURED AT FOLLOW-UP SCANS)

Modified Benign Simple Descriptors (tick images)

1. Unilocular cyst with ground-glass echogenicity in premenopausal woman with largest diameter < 10 cm
2. Unilocular cyst with mixed echogenicity and acoustic shadows in premenopausual woman with largest diameter < 10 cm
3. Unilocular anechoic cyst with smooth internal cyst walls and largest diameter < 10 cm
4. All other unilocular cysts with smooth internal cyst walls and largest diameter < 10 cm

Ultrasound features suggestive of a complication of adnexal mass: no /yes

- pop-up list (you can select more than one):
  - Suspected torsion
  - Infection/abscess
  - Haemorrhage
  - Rupture
  - Other, please specify: ….

Management data

- Suggested management: "What type of management do you propose for this patient based on ultrasound subjective assessment?"

  - Conservative management with one further ultrasound performed 0-90 days post-partum;
  - Conservative management with longitudinal follow-up as specified in the protocol (third arm of study - if patient consents to this);
  - Conservative management but with additional scans to the one listed in third arm of study with reason (pop-up: change in morphology, change in size, suspicion of malignancy; symptoms; other: specify);
  - Surgery by a gynaecologist or general surgeon;
  - Surgery by an oncological surgeon;
  - Mass is no longer present and only a normal ovary is seen - end of follow-up and completion of study;
  - Other, specify.

Appendix 4 - Data recorded at 0-90 days postpartum scan

General information

- Type of centre where scan performed: Tertiary Referral Centre / Oncology Centre with ultrasound attached / Early Pregnancy Unit
- Date of scan
- Outcome of pregnancy: Live birth, stillbirth, miscarriage, ectopic
- Date of delivery
- In case of miscarriage - date when miscarriage was diagnosed
- In case of an ectopic - date ectopic pregnancy was diagnosed

- If ectopic or miscarriage – expectant, medical or surgical management?
- Delivery mode: Spontaneous vaginal delivery, instrumental vaginal delivery, C/Section

- Number of days from delivery
- In case of miscarriage/ectopic - number of days from diagnosis

Ultrasound data

- Name of the ultrasound examiner
- Ultrasound system used
- Which modality used - transabdominal or transvaginal or transrectal
- Mass detectable (yes/no; and if answer is 'yes') - pop-up: no ovary seen; ovary seen)
- Ovarian or tubal or para-ovarian or uncertain? Left or right?

Subjective assessment

After ultrasonographic examination of the mass the investigator gives his/her subjective assessment of the mass:

- A: Benign or Malignant (borderline or invasive)
- B: Probability of malignancy (level of certainty / confidence with the diagnosis as being benign or malignant and managing it as that):

1 = benign
2 = probably benign
3 = uncertain/not possible to classify
4 = probably malignant (borderline / invasive)
5 = malignant (borderline / invasive);

- C: *Self impression:* presumed histological diagnosis (e.g. functional cyst; haemorrhagic corpus luteum cyst, pregnancy-associated cyst, theca-lutein cyst, teratoma, serous cystadenoma, mucinous cystadenoma, serous cystadenofibroma, mucinous cystadenofibroma, fibroma, endometrioma, decidualised endometrioma, abscess, hydrosalpinx, invasive - specify type, borderline - specify type; granulosa cell tumour, dysgerminoma, other);
- D: *Management:* conservative management with ultrasound follow-up, surgery by general gynaecologist, surgery by oncological surgeon, other.
- E: *Any subjective ultrasound change to adnexal mass from previous scan (five options):* 1) yes, change noted; 2) no change noted; 3) unsure (comment to explain why).
- F: *Colour score of ovarian mass:* score 1, 2, 3, 4

- G: *If papillations present:* 1) number of papillations; 2) three orthogonal diameters (mm) of largest papillary projection i.e. height, base 1 and base 2; 3) smooth rounded papillations - yes / no; 4) colour in papillation - score 1, 2, 3, 4 (choose worst case scenario in case of multiple papillations).

ADNEX Variables

- Age
- Oncology Centre (yes/no)
- Maximum diameter of the lesion (mm)
- Maximum diameter of largest solid component
- Number of papillary projections (0, 1, 2, 3, > 3)
- Presence of more than 10 cyst locules (yes/no)
- Acoustic shadows (yes/no)
- Presence of ascites (yes/no)
- CA125 level (NOT TO BE MEASURED AT FOLLOW-UP SCANS)

Modified Benign Simple Descriptors (tick images)

1. Unilocular cyst with ground-glass echogenicity in premenopausal woman with largest diameter < 10 cm
2. Unilocular cyst with mixed echogenicity and acoustic shadows in premenopausual woman with largest diameter < 10 cm
3. Unilocular anechoic cyst with smooth internal cyst walls and largest diameter < 10 cm
4. All other unilocular cysts with smooth internal cyst walls and largest diameter < 10 cm

Ultrasound features suggestive of a complication of adnexal mass: no /yes

- pop-up list (you can select more than one):
  - Suspected torsion
  - Infection/abscess
  - Haemorrhage
  - Rupture
  - Other, please specify: ….

Management data

- Suggested management: "What type of management do you propose for this patient based on ultrasound subjective assessment?"
  - Conservative management with continued follow-up as per decision of clinician (data not to be used for this study);
  - Surgery by a gynaecologist or general surgeon;
  - Surgery by an oncological surgeon;
  - Mass is no longer present and only a normal ovary is seen - end of follow-up and completion of study;
  - Other (specify).

Appendix 5 – Outcomes (non-ultrasound)

General

- Patient decides to stop participating in the study (please specify why: …..) (no other pop-up)
- Patient withdraws her consent (data cannot be used for statistical analysis and no reason is asked) (no other pop-up)
- Lost to follow-up

Complications from the mass during pregnancy?

Yes/No (pop up list) with number of episodes (one pop up per episode):

- Acute pain
- Chronic pain
- Suspected torsion
- Pelvic infection
- Cyst rupture with haemorrhage (ultrasound diagnosis)
- Cyst rupture without haemorrhage (ultrasound diagnosis)
- Underwent surgery for a mass-related incident but mass not removed

  If Yes - type of surgery and findings:
- Other, please specify: ….

Surgery (give option for more than one option)

- Gestational age at surgery or number of days postpartum when surgery done

- Type of operation:
  - Laparotomy with vertical incision
  - Laparotomy with horizontal incision
  - Operative laparoscopy
  - Diagnostic laparoscopy
  - Robotic surgery
  - Biopsy followed by neo-adjuvant chemotherapy (NACT)
  - Other (specify.....)

- Surgery performed:
  - Cyst drainage
  - Cystectomy/tumour resection
  - Oophorectomy
  - Salpingectomy
  - Salpingo-oophorectomy
  - Hysterectomy with cystectomy/tumour resection
  - Hysterectomy with salpingo-oophorectomy
  - Hysterectomy with oophorectomy
  - Hysterectomy with salpingectomy
  - Adhesiolysis

  - Biopsy
  - Other: specify.....

- Indication for operation (more than one possibility may be ticked):
  - Suspicion of malignancy based on ultrasound
  - Suspicion of malignancy based on other information; if so, which?
  - Acute / chronic pain
  - Suspected torsion
  - Suspected cyst rupture
  - Patient request
  - Increase in size of the tumour
  - Indicated by other imaging technique (suspicion of malignancy or torsion/haemorrhage/rupture)
  - Opportunistic removal of the mass when patient was operated for another indication
  - Opportunistic removal of the mass during a C/Section
  - Other: Specify: ….

- Findings at operation (more than one option may be ticked):
  - No tumour found
  - No complications of the tumour
  - Torsion of mass
  - Rupture of mass
  - Inflammation/Infection
  - Adhesions
  - Bleeding from tumour
  - Disseminated carcinosis
  - Omental cake
  - Other complications of tumour: specify: ….
  - Other non-gynaecological pathology (e.g. appendicitis): specify: ….

- Complications (intra-operative, 0-30 days, >30 days post-operative; more than one option may be ticked) with specification on when complication occurred:
  - Conversion from laparoscopy to laparotomy
  - Bowel perforation
  - Bleeding requiring transfusion
  - Pulmonary embolism or deep venous thrombosis
  - Wound-related issues
  - Urine infection
  - Chest infection
  - Pre-term labour and delivery
  - Fetal complications (specify)
  - Other: Specify: ….

- Histological diagnosis (pop-up list as before) with open text area (“Details”):

  <u>Benign tumour:</u>

- o Normal adnexa
- o Simple cyst
- o Functional cyst
- o Haemorrhagic corpus luteum cyst
- o Endometrioma
- o Teratoma (benign)
- o Fibroma
- o Thecoma
- o Serous cystadenoma
- o Mucinous cystadenoma
- o Serous cystadenofibroma
- o Mucinous cystadenofibroma
- o Paraovarian/parasalpingeal cyst
- o Hydrosalpinx
- o Other: .....

Rare benign tumour:

- o Struma ovarii
- o Brenner tumour (benign)
- o Schwannoma
- o Other: .....

Infectious (acute/chronic):

- o Abscess
- o Salpingitis
- o Other: .....

Uterine lesion:

- o Fibroid
- o Other: .....

Borderline tumours:

- o Serous borderline
- o Mucinous endocervical borderline
- o Mucinous gastrointestinal borderline
- o Other: .....

- FIGO stage:
  - o I
  - o II
  - o III
  - o IV
  - o Not known
  - o Not applicable

Primary invasive malignant tumour:

Epithelial ovarian cancer
o Serous HGSOC (high grade serous ovarian cancer) OR LGSOC (low grade serous ovarian cancer)
o Mucinous
o Endometrioid
o Clear cell
o Small cell carcinoma
o Other: .....
FIGO stage:
o I
o II
o III
o IV
o Not known
o Not applicable

Malignant germ cells tumour of the ovary
o Immature teratoma
o Dysgerminoma
o Choriocarcinoma
o Yolk sac/ Endodermial sinus tumour
o malignant mixed germ cell tumor
o embryonal carcinoma
o Other: .....
FIGO stage:
o I
o II
o III
o IV
o Not known
o Not applicable

Stromal and sex-cord and steroid cell tumours of the ovary
o Granulosa-adult
o Granulosa-juvenile
o Sertoli
o Sertoli-Leydig
o Sclerosing stromal cell tumour
o steroid cell tumour NUD
o Leydig
FIGO stage:
o I
o II
o III
o IV

o Not known
o Not applicable

Other:
o Fibrosarcoma
o Carcinosarcoma
o Other:.....
FIGO stage:
o I
o II
o III
o IV
o Not known
o Not applicable

Tubal cancer:
o Serous
o Mucinous
o Endometrioid
o Clear cell
o Small cell carcinoma
o Other: .....
FIGO stage:
o I
o II
o III
o IV
o Not known
o Not applicable

Metastatic malignant tumour:
o Krukenberg
o Metastasis from breast cancer
o Metastasis from gastrointestinal tumour
o Lymphoma
o Other:.....

Very rare malignant tumour:
- Malignant struma ovarii
- Or Specify.....

FIGO stage:
o I
o II
o III
o IV

- Not applicable

- Not known

Appendix 6 – Data Processing Agreement (DPA)

This data processing agreement, including any annexes hereto, (together the "Data Processing Agreement") is an integrated part of this Protocol.

All defined terms within the Agreement shall have the same meaning when used in this Data Processing Agreement, unless explicitly defined otherwise in this Data Processing Agreement.

1. SCOPE OF THE DATA PROCESSING AGREEMENT

1.1 Each participating centre acts as a data processor as defined under article 4, 8) of the Regulation (EU) 2016/679 ("**Data Processor**") for the Sponsor who acts as data controller as defined under article 4, 7) of the Regulation (EU) 2016/679 ("**Data Controller**"), as each participating centre processes Personal Data for the Sponsor as set out in Annex 1.

1.2 **"Applicable Law"** means any applicable data protection or privacy laws, including

(a) the Regulation (EU) 2016/679 also referred as the General Data Protection Regulation ("GDPR"),

(b) other applicable laws that are similar or equivalent to or that are intended to or implement the laws that are identified in (a) of this definition,

1.3 **"Personal Data"** means any information relating to an identified or identifiable natural person ('Data Subject'), including without limitation pseudonimized information, as defined in Applicable Law and described in Annex 1.

2. PROCESSING OF PERSONAL DATA

2.1 Instructions: The Data Processor is instructed to process the Personal Data for the term of this Data Processing Agreement and only for the purposes of providing the data processing tasks set out in Annex 1. The Data Processor may not process or use Personal Data for any purpose other than a Data Subject's medical records, or other than provided in the instructions, including with regard to transfers of personal data to a third country or an international organization, unless the Data Processor is required to do so according to Union or Member State law. In that case, the Data Processor shall inform the Data Controller in writing of that legal requirement before processing, unless that law prohibits such information on important grounds of public interest.

2.2 Data Processor shall at all times maintain a record of processing of Personal Data in accordance with Applicable Law and if the Data Processor considers an instruction from the Data Controller to be in violation of the Applicable Law, the Data Processor shall promptly inform the Data Controller in writing about this.

3. THE DATA PROCESSOR'S OBLIGATIONS

3.1 The Data Processor must ensure that persons authorized to process the Personal Data have committed themselves to confidentiality or are under an appropriate statutory obligation of confidentiality.

3.2 The Data Processor shall implement appropriate technical and organizational measures to prevent that the Personal Data processed is:

i. accidentally or unlawfully destroyed, lost or altered,

ii. disclosed or made available without authorization, or

iii. otherwise processed in violation of Applicable Law.

3.3 The Data Processor must also comply with the special data security requirements of Annex 1.

3.4 The appropriate technical and organizational security measures must be determined with due regard for:

i. the current state of the art,

ii. the cost of their implementation, and

iii. the nature, scope, context and purposes of processing as well as the risk of varying likelihood and severity for the rights and freedoms of natural persons.

3.5 The Data Processor shall upon request provide the Data Controller with sufficient information to enable the Data Controller to ensure that the Data Processor's obligations under this Data Processing Agreement are complied with, including ensuring that the appropriate technical and organizational security measures have been implemented.

3.6 Taking into account the nature of the processing, the Data Processor shall assist the Data Controller, by means of appropriate technical and organizational measures, insofar as this is possible, in fulfilling its obligation to respond to requests from Data Subjects pursuant to laws and regulations in the area of privacy and data protection (such as, the right of access, the right to rectification, the right to erasure, the right to restrict the processing, the right to data portability and the right to object).

3.7 The Data Controller is entitled to appoint at its own cost an independent expert, reasonably acceptable to Data Processor, who shall have access to the Data Processor's data processing facilities and receive the necessary information for the sole purpose of auditing whether the Data Processor has implemented and maintained said technical and organizational security measures. The expert shall upon the Data Processor's request sign a non-disclosure agreement provided by the Data Processor, and treat all information obtained or received from the Data Processor confidentially, and may only pass on, after conferral with Data Processor, the findings as described under article 3.9, (ii) below to the Data Controller.

3.8 The Data Processor must give authorities who by Union or Member State law have a right to enter the Data Controller's or the Data Controller's processors' facilities, or representatives of the authorities, access to the Data Processor's physical facilities against proper proof of identity and mandate, during normal business hours and upon reasonable prior written notice.

3.9 The Data Processor must without undue delay in writing notify the Data Controller about:

i. any request for disclosure of Personal Data processed under the Agreement by authorities, unless expressly prohibited under Union or Member State law,

ii. any finding of (a) breach of security that results in accidental or unlawful destruction, loss, alteration, unauthorized disclosure of, or access to, Personal Data transmitted, stored or otherwise processed by the Data Processor under the Agreement, or (b) other failure to comply with the Data Processor's obligations under Clause 3, or

iii. any request for access to the Personal Data (with the exception of medical records for which the Data Processor is considered data controller) received directly from the data subjects or from third parties.

3.10 Such a notification from the Data Processor to the Data Controller with regard to a breach of security as meant in Clause 3.9 (ii)(a) will contain at least the following information:

i. The nature of the Personal Data breach, stating the categories and (by approximation) the number of Data Subjects concerned, and stating the categories and (by approximation) the number of the personal data registers affected (datasets);

ii. The likely consequences of the Personal Data breach;

iii. A proposal for measures to be taken to address the Personal Data breach, including (where appropriate) measures to mitigate any possible adverse effects of such breach.

The Data Processor shall document (and shall keep such documentation available for the Data Controller) any Personal Data breaches, including the facts related to the Personal Data breach, its effects and the corrective measures taken. After consulting with the Data Controller, the Data Processor shall take any measures needed to limit the (possible) adverse effects of Personal Data breaches (unless such consultation cannot be awaited due to the nature of the Personal Data breach).

3.11 The Data Processor must promptly and reasonably assist the Data Controller (with the handling of (a) responses to any breach of security as described in 3.9 (ii) above and (b) any requests from Data Subjects under Chapter III of the GDPR, including requests for access, rectification, blocking or deletion. The Data Processor must also reasonably assist the Data Controller by implementing appropriate technical and organizational measures for the fulfilment of the Data Controller's obligation to respond to such requests. Any reasonable documented costs and expenses pre-approved in writing by the Data Controller related to the above will be reimbursed by the Data Controller to the extent such costs and expenses are not related to any requirements according to Applicable Law imposed on the Data Processor or due to any breach of this Appendix 6 or the Agreement by Data Processor.

3.12 The Data Processor must reasonably assist the Data Controller with meeting the other obligations that may be incumbent on the Data Controller according to Union or Member State law where the assistance of the Data Processor is implied, and where the assistance of the Data Processor is necessary for the Data Controller to comply with its obligations. This includes, but is not limited to, at the request to provide the Data Controller with all necessary information about an incident under Clause 3.9 (ii), and all necessary information for an impact assessment in accordance with Article 35 and Article 36 of the GDPR. Any reasonable documented costs and expenses pre-approved in writing by the Data Controller related to the above will be reimbursed by the Data Controller to the extent such expenses are not related to any requirements according to Applicable Law imposed on the Data Processor or due to breach of this Appendix 6 or the Agreement by Data Processor.

4. SUBPROCESSORS

4.1 The Data Processor may only engage a subprocessor, with prior specific or general written consent from the Data Controller. At the time of this Data Processing Agreement, the Data Processor uses the subprocessor listed in Annex 2. The Data Processor

undertakes to inform the Data Controller of any intended changes concerning the addition or replacement of a subprocessor by providing a reasonable prior written notice to the Data Controller. The Data Controller may reasonably and in a duly substantiated manner object to the use of a subprocessor. The Data Processor must inform the Data Controller in writing of the discontinued use of a subprocessor.

4.2 Prior to the engagement of a subprocessor, the Data Processor shall conclude a written agreement with the subprocessor, in which at least the same data protection obligations as set out in this Data Processing Agreement shall be imposed on the subprocessor, including obligations to implement appropriate technical and organizational measures and to ensure that the transfer of Personal Data is done in such a manner that the processing will meet the requirements of the Applicable Law.

4.3 The Data Controller has the right to receive a copy of the relevant provisions of Data Processor's agreement with the subprocessor related to data protection obligations. The Data Processor shall remain fully liable to the Data Controller for the performance of the subprocessor obligations under this Data Processing Agreement. The fact that the Data Controller has given consent to the Data Processor's use of a subprocessor is without prejudice for the Data Processor's duty to comply with this Data Processing Agreement.

5. CONFIDENTIALITY

5.1 The Data Processor shall keep Personal Data confidential.

5.2 The Data Processor shall not disclose the Personal Data to third parties or take copies of Personal Data unless strictly necessary for the performance of the Data Processor's obligations towards the Data Controller according to this Data Processing Agreement, and on condition that whoever Personal Data is disclosed to is under the responsibility of a professional subject to the obligation of professional secrecy under Union or Member State law or rules established by national competent bodies or by another person also subject to an obligation of secrecy under Union or Member State law or rules established by national competent bodies.

5.3 The Data Processor shall ensure that its employees comply with this Data Processing Agreement.

5.4 The Data Processor shall limit the access to Personal Data to employees for whom access to said data is necessary to fulfil the Data Processor's obligations towards the Data Controller.

5.5 The obligations of the Data Processor under Clause 5 shall continue until such time as provided by Applicable Law and regardless of whether the cooperation of the parties has been terminated.

6. TERM AND TERMINATION OF THE DATA PROCESSING AGREEMENT

6.1 Regardless of the expiry or termination, for whatever reason, of the Agreement, this Data Processing Agreement remains in force and applicable as long as the Data Processor processes the Personal Data for the Data Controller under the Agreement.

6.2 In case of termination of the Agreement, the Data Processor must provide the necessary transition services to the Data Controller. The Data Processor is obliged to reasonably assist Data Controller at Data Controller's expense.

Data Processor shall have appropriate procedures in place for the archiving of the Personal Data after the end of the Study in accordance with Applicable Law and at the end of the legally mandated archiving period ensure the destruction of the Personal Data and promptly inform Data Controller of this same.

6.3 If the Data Processor is required based on Union or Member State law to retain all or part of the Personal Data for a longer period than is possible based on the period mentioned in the Data Processing Agreement, the Data Processor shall immediately communicate this to the Data Controller, stating the basis, term and scope of such obligation. Once compliance with the obligation is no longer impeded by Union or Member State law, the Data Processor shall as yet erase the data in accordance with the provisions in the Data Processing Agreement.

Annexes:

Annex 1: Instructions

Annex 2: Subprocessors

Annex 1 – Instructions

This Annex 1 constitutes the Data Controller's instruction to the Data Processor in connection with the Data Processor's Personal Data processing for the Data Controller, and is an integrated part of the Data Processing Agreement.

Contact details of the Data Controller (including its Data Protection Officer, if applicable): dpo@uzleuven.be

Contact details of the Data Processor (including its Data Protection Officer, if applicable):

[insert DPO per each participating centre]

The processing of Personal Data

a) Purpose and nature of the processing operations

Performance of Clinical Study services under the Agreement and for the purpose of mandatory safety monitoring– as specifically described in the Protocol.

i. Transfer of Personal Data to another country: Yes
ii. If YES to I., transfer outside the EU AND EEA: No

b) Categories of Data Subjects

i. Former, current or future persons and/or patients who voluntarily enrolled in the Study, and/or their relatives, and/or
ii. Data described in this Protocol

c) Categories of Personal Data

Re b) I: Date of birth and/or age, initials, personal identification number assigned to Data Subjects participating in the Study, description of characteristics of physical features of the body

Re b) II: Data described in this Protocol.

d) Special categories of Personal Data

Re b) I: Health information including past medical history and medical test information (such as blood samples results from scans and biopsies), data revealing racial or ethnic origin, genetic data and/or social security number

e) Addresses of all locations of all participating centres where the processing will be performed are listed above in the first pages of the Protocol.

f) Specific security requirements

The following requirements reflect the minimum data processing requirements expected of the Data Processor. It is a condition that other agreed documents, legislation or industry standards laying down requirements of the processing of Personal Data in connection with Study/ /mandatory safety monitoring are complied with as well.

1. The Personal Data may only be used for the Study and/or mandatory safety monitoring.

2. The collection, registration and other processing of Personal Data must be legally authorized under Applicable Law, or applicable policies issued of the supervisory authorities.

3. Any person who takes part in the processing of Personal Data must be familiar with these requirements.

4. Premises used for the storage and other processing of Personal Data must be arranged in such a way as to prevent unauthorized access.

5. Appropriate security measures must be implemented to protect data against accidental or unlawful destruction, loss or impairment. Furthermore, it must be ensured that no incorrect or misleading Personal Data is processed. Incorrect or misleading data, or data processed in contravention of the above Applicable Law, policy of the supervisory authority or these requirements, shall be rectified or erased.

6. Personal Data may not be stored in a way that makes it possible to identify the Data Subjects for longer than is necessary for the achievement of the Study and/or mandatory safety monitoring.

7. The publication of results from clinical studies must take place in such a way that it is impossible to identify individual persons.

8. It is a condition that other legislation laying down requirements of the processing of Personal Data in connection with Study and/or mandatory safety monitoring is complied with.

Electronic data

9. Identification data must be encrypted or replaced by a code number or similar. Alternatively, all data stored can be encrypted. Encryption keys, code keys, etc. must be stored securely and separately from the Personal Data. This also applies to Personal Data that is stored on portable devices such as laptop PCs, tablets, etc.

10. Data may only be accessed by using a unique user name and a confidential password. The password must be renewed at least once a year and when otherwise necessary in order to ensure the secure processing of the data.

11. On the transfer of Personal Data via the internet or other external networks, the necessary security measures must be taken to ensure that the Personal Data does not come to the knowledge of any unauthorized persons. This includes that encryption is required if sensitive Personal Data is transferred via the internet (or other open networks), and security of authenticity (identities of transmitter and recipient) and integrity (the authenticity of the transmitted Personal Data) must be appropriately ensured by the use of suitable security measures. On using internal networks, it must be ensured that no unauthorized persons can gain access to the data.

12. Removable storage media, safety copies of Personal Data, etc. must be stored securely and under lock and key, so that unauthorized access is prevented.

Manual ("paper") data

13. Manual material, including print-outs, error and control lists, etc. with Personal Data, must be stored securely under lock and key, and in such a way as to prevent unauthorized access.

Biobank and biological material

14. Samples with biological material and biological material in biobanks must be stored securely under lock and key so as to prevent unauthorized access, and in such a way as to ensure that the material is not lost, impaired, or accidentally or illegally destroyed.

15. Biological material collected for the purpose of the Study and marked with a civil registration number or name must be stored subject to special safety requirements.

16. Internal guidelines must be laid down within the Data Processor's organization regarding the project for the storage of biological material.

Information to be given to the clinical Studyparticipant/Data Subject

17. Where the Personal Data is obtained from the clinical Study participant/Data Subject (via interviews, questionnaires, clinical or para-clinical examination, treatment, observation, etc.), more detailed information concerning the clinical Study/testing/safety monitoring shall be distributed/forwarded to the Data Subject in accordance with Article 13 of the GDPR. The clinical Study participant must, via the notification or informed consent form (as applicable) as approved by the relevant ethics committee and /or relevant authorities, be informed of the name of the Data Controller, the purpose of the trial/testing/safety monitoring, the fact that it is voluntary to participate in the trial/testing, the identity of any recipients of Personal Data, and the purpose of the disclosure of Personal Data, as well as any further information which is necessary for the clinical Study participant / Data Subject to be able to safeguard his/her interests. The Data Subject has been informed about the right of access to the Personal data that is processed concerning the person in question.

Disclosure

18. Disclosure/issue of Personal Data to other parties may take place to the extent that this is legally authorized under Applicable Law.

On the conclusion of the project

19. At the latest on the conclusion of the trial/testing/safety monitoring the Personal Data (including biological material) shall be erased, made anonymous, or destroyed, unless Union or Member State law requires continued storage of the Personal Data. In accordance with Belgian Law as defined in the Agreement the Data Processor shall be allowed to store the medical records for 30 years. It must not subsequently be possible to identify individuals participating in the clinical Study/testing/safety monitoring. The deletion of Personal Data must be properly documented.

20. Alternatively, the Personal Data may be transferred for further storage in archives according to the Data Controller's instructions. Any costs related to such transfer and further storage of Personal Data shall be borne by the Data Controller.

21. Erasure of Personal Data from electronic media shall take place in such a manner that it is impossible to recover the Personal Data and such erasure must be properly documented.

Annex 2 – Sub-processors

The Data Controller agrees that the Data Processor – subject to compliance with Clause 4 of the Data Processing Agreement – engages the parties listed below as subprocessors.

None