One Step Nucleic Acid Amplification (OSNA) versus ultrastaging to detect sentinel lymph node metastasis in endometrial cancer: a randomized, multicenter, controlled trial (SENT-OSNA study).

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PURPOSE OF THE STUDY

The objective of this study is to compare the ability to detect sentinel lymph node metastasis between OSNA (One Step Nucleic Acid Amplification) method and ultrastaging in the lymph nodes of women with apparent early-stage endometrial cancer.

BACKGROUND

Endometrial cancer is the most frequent gynecological cancer in developed countries [1]. The incidence of this malignancy has raised in the last years with an estimated increase of 1% every year [2]. It is known that lymph node metastasis represents a major prognostic factor, and it is an indication to adjuvant chemo-radiotherapy [3]. Sentinel node (SLN) mapping is now widely used in the staging process for apparent uterine-confined endometrial cancer. This is supported by large literature evidence [4,5] and consequently, incorporated into international guidelines [3,6]. Accuracy and sensitivity of SLN in endometrial cancer has been shown to be promising not only in low and intermediate risk cases, but more recently, also in high-risk patients [7,8]. It is well known that ultrastaging protocol of SLN allows the diagnosis of a higher number of low volume metastases [9]. Nevertheless, there is a large heterogeneity in literature regarding ultrastaging standardization with not universally accepted protocol [10].

More recently, the One Step Nucleic Acid Amplification (OSNA) method has been proposed to diagnose lymph node metastasis. OSNA is a rapid assay, able to detect the presence of cytokeratin 19-mRNA in SLN, consisting of a short homogenization followed by amplification of cytokeratin 19 mRNA directly from the lysate [11]. The choice of CK19 derived by its high prevalence in endometrial cancer tissue (98% of primary tumors in a study by Nagai et al [16]); therefore, the CK19 gene was selected as a suitable marker for OSNA assay to target lymph node metastasis. Different studies have described the use and the accuracy of OSNA for the detection of nodal metastases in endometrial cancer [12-15]. Nevertheless, the evidence supporting the use of OSNA in endometrial cancer is still poor, particularly when compared with other malignancies [17].

Recently, a prospective, multicentric, interventional study including patients with apparent early-stage endometrial cancer who underwent primary surgical staging with SLN mapping was published. SLNs were serially sectioned with 2-mm slices perpendicular to the longest axis of the node: the odd slices were submitted to ultrastaging, whereas the even slices to the OSNA analysis. Diagnostic performance was calculated taking ultrastaging as referral standard. The study concluded that OSNA method had high specificity and high accuracy in detecting SLN metastasis in apparent early-stage endometrial cancer. The advantage of the OSNA method could be represented by the possibility to analyze the entire lymph node thus eliminating sampling bias [18]. Nevertheless, a randomized study comparing the ability of OSNA versus ultrastaging to detect SLN metastases in endometrial cancer has not been performed yet and might represent the only way to definitively understand the role of OSNA in endometrial cancer.

STUDY OBJECTIVES

Primary:

To compare the ability to detect sentinel lymph node metastasis between OSNA and ultrastaging.

Secondary:

- Outcomes of SLN metastasis according to molecular classification
- 3 and 5-year disease-free survival in OSNA versus ultrastaging negative patients
- 3 and 5-year overall survival in OSNA versus ultrastaging negative patients
- Total Tumor Load (=sum of CK19 copies from each SLNs) and correlation with prognosis and other clinico-pathologic characteristics (LVI, T, molecular classification etc.)
- GTL (= The maximal CK19 mRNA copy number among valued sentinel nodes)
- Dimension of SLN metastases and other histological parameters (multifocality of tumoral deposits, extranodal spread, neoplastic emboli in extranodal tissue) at ultrastaging and correlation with prognosis

ENDPOINTS

Primary:

Incidence of SLN metastasis in OSNA versus ultrastaging groups

Secondary:

- Outcomes of SLN metastasis according to molecular classification
- 3 and 5-year disease-free survival in OSNA versus ultrastaging negative patients
- 3 and 5-year overall survival in OSNA versus ultrastaging negative patients
- Incidence of endosalpingiosis in ultrastaging arm
- Total Tumor Load (=sum of CK19 copies from each SLNs) and correlation with prognosis and other clinico-pathologic characteristics (LVI, T, molecular classification etc.)
- GTL (= The maximal CK19 mRNA copy number among valued sentinel nodes)
- Dimension of SLN metastases and other histological parameters (multifocality of tumoral deposits, extranodal spread, neoplastic emboli in extranodal tissue) at ultrastaging and correlation with prognosis

STUDY DESIGN

Prospective, multicenter, non-inferiority, randomized study. It concerns an interventional study and post-market analysis.

TARGET POPULATION

Endometrial cancer patients undergoing SLN biopsy

INCLUSION CRITERIA

- Histologically confirmed endometrial cancer
- Apparent (pre-operative) FIGO stage I-II
- Radical surgery
- Attempt of SLN mapping

- obtaining informed consent/consent to the processing of personal data

EXCLUSION CRITERIA

- Uterine sarcoma (including endometrial stromal sarcoma)
- Fertility sparing surgery
- Dedifferentiated histology
- Undifferentiated histology
- Neoadjuvant therapy
- Previous surgery to pelvic lymph nodes
- Lymph nodes with short axis >15 mm at pre-operative imaging [19]
- inability to provide informed consent/consent to the processing of personal data

STUDY DURATION

4 years of accrual (and 3-years follow-up for a total of 7 years)

STUDY PROCEDURES

All patients undergo pre-operative workup including hysteroscopic biopsy, transvaginal ultrasound scan, abdominal magnetic resonance imaging scan and/or computed tomography scan (to be performed within 60 days from the surgery for endometrial cancer). Surgical staging consists of total hysterectomy, bilateral salpingo-oophorectomy and SLN mapping algorithm [20].

If SLN is detected and retrieved, patient can be spared the same side systematic pelvic lymphadenectomy. A side-specific lymphadenectomy in case of failed SLN mapping is recommended. If empty lymph node packet (no evidence of lymph node tissue documented in the specimen) at final histology is found, patient will be reoperated with lymphadenectomy only if uterine factors/retrieved SLN (in one side) are not sufficient to define the potential need for adjuvant treatment.

Patients will be randomized pre-operatively (after signing the consent form) to SLN analysis by OSNA versus ultrastaging (Figure 1). SLN undergoing OSNA analysis will not be analyzed during the surgery (no action taken intra-operatively according to OSNA results).

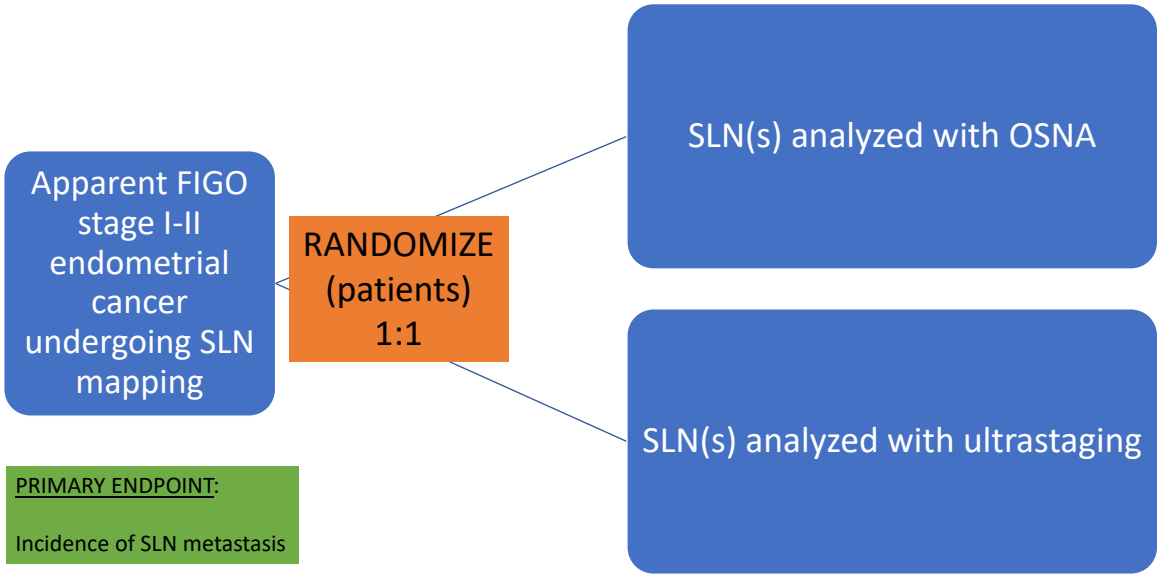


Figure 1. Study protocol scheme.

Surgical approach consists of laparoscopic or robot-assisted according to body mass index.

Patients with intra-operative evidence of enlarged lymph nodes and/or with extrauterine disease will not be included in the final analysis but will be considered in the intention to treat.

Adjuvant therapy will be administered according to final histology risk factors.

SLN found to be metastatic at OSNA and ultrastaging will receive adjuvant chemo-radiotherapy in case of micro- and macro-metastases. ITCs will be considered as negative lymph nodes and will not undergo adjuvant therapy (unless for uterine factors).

Molecular profile will be also performed on hysterectomy specimens.

POSSIBLE RISKS & BENEFITS

This study will explore the sensitivity and specificity of OSNA in identifying metastases in the lymph nodes. The test will compare the existing standard (ultrastaging) with the OSNA.

The OSNA device is CE-marked and has been proven to be inherently safe and reliable in other cancers including endometrial. All testing is ex-vivo and hence the patient safety will not be

affected. The lymph nodes used in this study are dissected following the routine surgical procedure and will be analysed by ultra-staging or OSNA.

It is possible that this study will show that the clinical performance of OSNA is non-inferior to ultra-staging.

The results of the study will be widely disseminated as it is likely to influence future patient care.

Benefits to the institution: Moreover, the user will be informed before the study about the correct use of the devices and reagents. This is accomplished via user trainings that provide specific information on how to use the device, reagents and other ancillary material. These informational trainings are also supplemented by the instructions for use provided with the kits, which include warnings and precautions for the user, as well as known limitations of the device. OSNA could be in the position to provide analysis results of the whole lymph node faster and more accurately and standardised than the reference test thereby saving time to process and expedite results for patients as well as resulting in significant cost saving to the health authorities.

DESCRIPTION DEVICE

Identification

RD-210 and LYNOAMP CK19E, hereinafter referred to as OSNA.

Manufacturer

Sysmex Corporation, 1 Chome-5-1 Wakinohamakaigandōri, Chūō-ku, Kōbe-shi, Hyōgo-ken 651-0073, Japan

Intended purpose

OSNA for detection and quantification of CK19 mRNA in surgically removed lymph node(s) lysate of breast, colorectal, gastric cancer patients using the automated Gene Amplification Detector RD-210. Use in diagnosis of lymph node metastasis and aid to diagnose the size of metastasis and metastases burden in the lymph node(s). To be used by health care professionals and properly trained personnel.

Analyte or marker

The marker is cytokeratin 19 mRNA (CK19), which is present in positive lymph nodes of

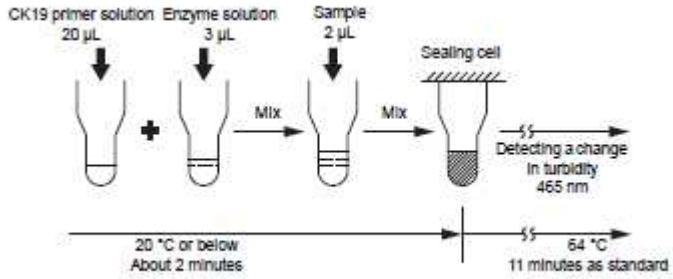
endometrial cancer patients.

Specimen type

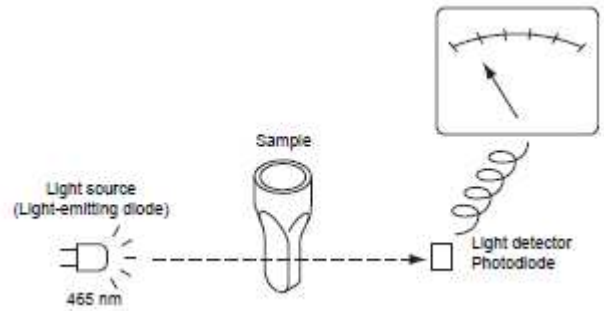
OSNA is performed using lymph nodes.

Technical and functional features

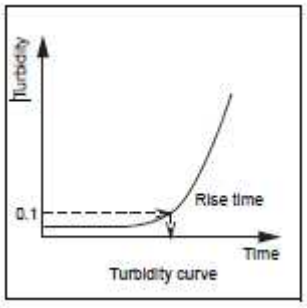
A certain amount of primer solution, enzyme solution and sample is pipetted by the pipetting unit of the RD-210 into the respective detection cell in the reaction block of the instrument. The reaction solution is mixed and the reaction block subsequently heats up to a defined reaction temperature (64 °C). A first reverse transcription reaction is followed by the amplification reaction of target genes (CK19 mRNA), if any, in the sample.



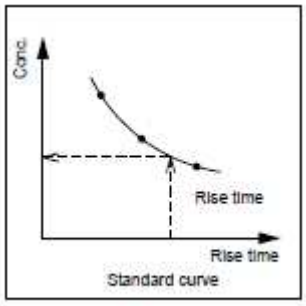
The detection principle is based on turbidity measurement. Magnesium pyrophosphate, which is a by-product of the amplification reaction, is a complex that causes turbidity and leads to a change in the transmitted light. Transmission of light is measured in the detection cell at a wavelength of 465 nm.



The reaction is monitored in real time. The measuring point, which is defined as rise time, is the point where the reaction curve reaches a turbidity of 0.1.



The concentration of the sample is calculated from the obtained rise time and a calibration curve (three different calibrators with three different concentrations of CK19 mRNA) which has been set up prior to sample analysis.



Specimen collection and storage

Excised lymph nodes shall be immediately refrigerated (2 to 8 °C) or stored on ice (0 to 4 °C) without drying to avoid RNA degradation. Use sterile biopsy sample containers, tightly fastened to avoid tissue dehydration. Recommended transport time between surgery and laboratory is within 15 minutes. If OSNA cannot be performed within eight hours after resection, human lymph nodes shall be stored at -80 °C.

The weight of lymph nodes for homogenisation shall be within a range of 25 to 600 mg.

For lymph nodes exceeding 600 mg, dissection of the node to sub-samples is mandatory.

Create as few sub samples as necessary (25 to 600 mg) in order for all of the tissue to be analysed.

Samples

The samples that will be included are SLNs from women with endometrial cancer. The SLNs will be analysed by using ultra-staging and the OSNA method. For this purpose, indocyanine green will

be applied as tracer.

Proficiency panel

Only trained personnel who have passed the proficiency test for the usage of the RD-210, LYNOAMP CK19 and all required equipment will be allowed to use OSNA in this study.

Measures to minimise bias

Care must be taken to avoid introducing a spectrum bias. This can be achieved by a strict consecutive recruitment of cases.

In addition, the laboratory personnel performing the OSNA method must be blinded against the results of the reference standard and vice versa.

Test procedure

SLNs deriving from SLNB will be dissected carefully from the surrounding adipose tissue and submitted to ultra-staging or shock frozen in liquid nitrogen and stored at – 80˚C for further OSNA analysis.

SLN undergoing ultra-staging:

The SLN will be fixed in 10% buffered formalin, embedded in paraffin and submitted to permanent sections as follows. From each paraffin block (slice), levels at 150 µm interval will be sliced. At each level, three adjacent sections will be cut and the first section of each level will be stained with H&E. If no tumour can be detected, then the second section will be stained with IHC using the anti-cytokeratin AE1:AE3 in order to detect a low volume metastatic disease by using a light microscope. The third is kept as a spare section and can be used to repeat staining if necessary. If upon IHC no micro- or macrometastasis are detected, then the node will be judged as negative. Conversely, if IHC detects a metastasis greater than 0.2 mm, then it will be judged as positive (macrometastasis: > 2.0 mm; micrometastasis: > 0.2 mm but ≤ 2.0 mm). The presence of ITCs (clusters of tumour cells no greater than 0.2 mm) should be documented in the results table, but

the node will be considered negative. *The dimension of SLN metastases will be documented in mm.*

SLN undergoing the OSNA analysis:

The SLN will be immediately refrigerated (2 to 8 °C) or stored on ice (0 to 4 °C) without drying to avoid RNA degradation. Use sterile biopsy sample containers that are tightly fastened to avoid tissue dehydration. Recommended transport time between surgery and laboratory is within 15 minutes, however if the nodes are stored in the refrigerator or on ice without drying, the time can be prolonged to up to eight hours until final storage at – 80˚C for further OSNA analysis. Throughout the evaluation, samples should be tested with OSNA according to the 'Test Procedure' described in the instructions for use supplied with the reagents and shown in Figure 2.

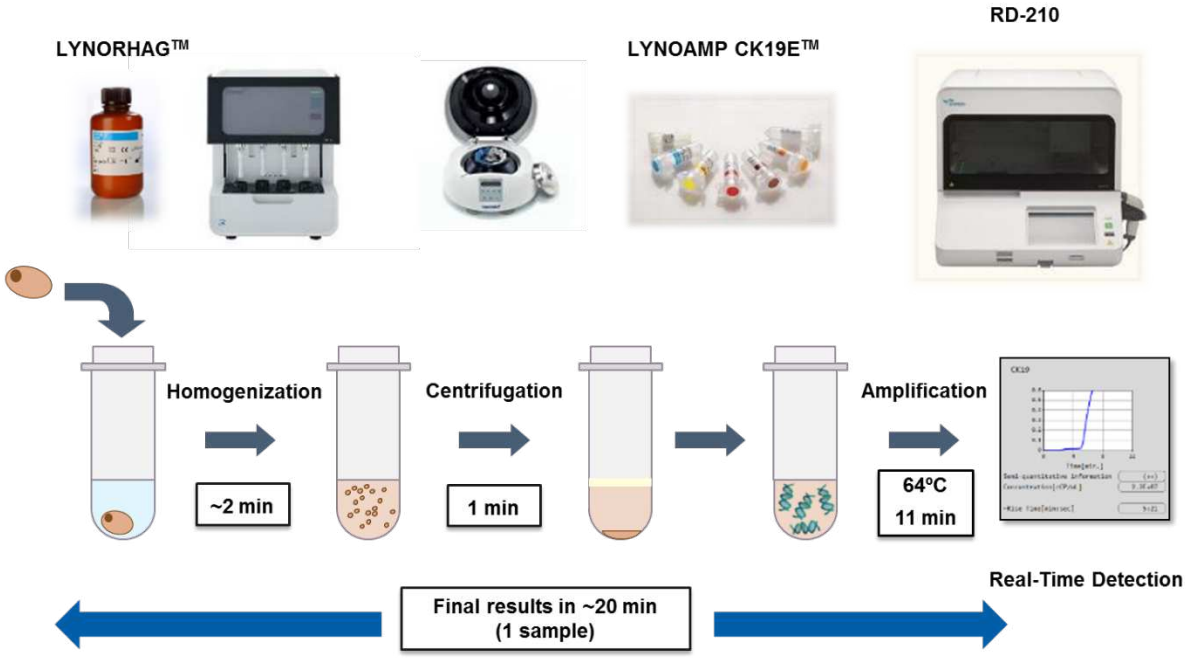


Figure 2: Scheme describing the OSNA workflow

Briefly, frozen samples are homogenised in 4 ml of lysing buffer for 90 s at 25,000 rpm using stainless steel blades or for 60 s at 10,000 rpm using LYNOPREP Blade Set and centrifuged for one minute at 10,000 x g. Subsequently, Cytokeratin 19 mRNA is amplified by Reverse Transcription Loop-mediated Isothermal Amplification (RT-LAMP) in the RD-210. Automated amplification with a ready-to-use reagent kit is performed directly from the sample lysate, with no RNA purification necessary, according to the manufacturer's instructions. Lymph nodes are

defined as 'negative' or 'positive' according to established cut-off values[21]. Therefore, negative nodes are classified for Cytokeratin 19 mRNA ccP/µl as less than 250. Lymph nodes with possible ITCs are classified for Cytokeratin 19 mRNA ccP/µl as 160 – 249. Lymph nodes positive for micrometastases (+) show mRNA Cytokeratin 19 levels of 250-4,999 ccP/µl and those nodes with macrometastases (++) show 5,000 mRNA ccP/µl or more. In Figure 3, an example of a result table is shown.

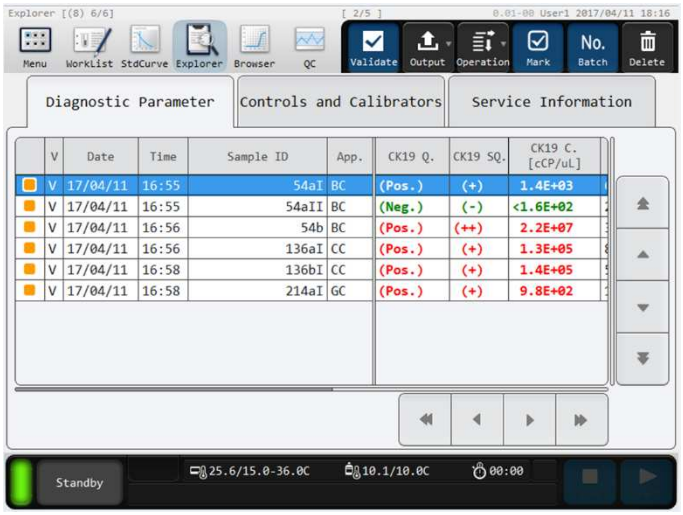


Figure 3: Scheme showing an example of the OSNA outcome

The OSNA lysates will be stored by the study sites for further molecular analysis.

DATA MANAGEMENT

Data and results recording

All submitted data will be anonymised and no patient identifiable information will be collated during the course of the study except locally to answer any queries that may arise. The results of OSNA assay and ultra-staging will be reported on electronic study report form (eSRF). Upon completion of the both assays, the Principal Investigator and/or Study Coordinator reviews the recorded data for completeness, accuracy and legibility.

The eSRF together with the clinical performance study report will be made available to Sysmex upon finalisation of the testing.

To protect the subject's or patient's privacy, no personal data shall appear anywhere on the eSRF.

Data and results management plan

All data will be filed in eSRF by each study site. Data will be stored for at least five years. All laboratory results are strictly confidential.

Principal Investigator and Study Coordinator will check the eSRF, and corrections or clarifications of data entered in each eSRF will be resolved by sending a query to the responsible investigator. The errors are corrected in the original database by the Principal Investigator. Then, the cleaned database (= final database) is signed and locked (= included as pdf file in Annex of the CPSR) by the Principal Investigator.

All data will be summarised in a final report in the English language by the Principal Investigator, including a material and methods section, results tables, discussion and conclusion per item. The final report will be authorised and signed by the Principal Investigator.

The data should be clearly legible and marked with the name of the laboratory and initialled by the responsible investigator.

TRANSLATIONAL SUB-STUDIES:

- Molecular profile including POLE on tumor tissue
- Molecular profile including POLE on lymph node metastases
- Circulating tumor-free DNA taken at following timepoints: pre-operatively, 1-month PO, 12 months PO

  Aim:
  - Correlation between free CT DNA and OSNA versus ultrastaging SLN metastasis

- Complete blood count and CRP taken at following timepoints: pre-operatively, 1-month PO, 12 months PO
- Microbiome correlation with SLN metastasis

STATISTICAL CONSIDERATIONS

SAMPLE SIZE CALCULATION:

This is a non-inferiority randomized trial with the incidence of patients with metastatic SLN as primary endpoint. Based on recent literature, incidence of patients with metastatic SLN detected by ultrastaging has an average value of 11% [21-23]. Assuming a -4% as the maximum allowable difference in detection rate to declare non-inferiority, a power of 80% and a significance level of 2.5% (one-side) a sample size of 1922 (961 per arm) is needed. Two interim analyses are planned, one after the first 640 and the second after 1280 patients. At the first interim analysis null hypothesis (inferiority of OSNA with respect to ultrastaging) will be rejected at a significance level <0.00010 otherwise the study will go on and at the second analysis the null hypothesis will be rejected at a significance level of 0.0059. The final analysis will use 0.0189 as significance threshold. The O'Brien Fleming alpha spending function was used to define these values.

STATISTICAL ANALYSIS:

The primary endpoint is defined as the proportion of patients with positive SLN over the total of randomized patients. This proportion will be reported together with the 95% confidence interval to better identify the range of inferential values.

Incidence of isolated tumor cells, micro- and macro-metastasis will be calculated.

Number of copies of CK19 mRNA to define volume of metastases will be adapted from previous reports [12].

To describe the sample, quantitative variables will be summarized using median and interquartile range while categorical items will be reported as absolute counts and percentages. Patients will be described according the arm they were randomized to. A first analysis will be performed on an Intent-To-Treat basis, considering all randomized patients. A secondary analysis will be performed on the Per-Protocol population.

EVALUATION PHASES

Study site initiation

An initiation meeting shall be conducted (in person or remotely) and documented by the Study Coordinator at the beginning of the clinical performance study.

Names, initials, signatures and functions shall be documented on the training log.

Prerequisites for initiation:

A. Signed clinical performance study plan; copies shall be provided to all parties involved
B. Instructions for use of the IVD product provided
C. Required number of IVD devices are available at the investigation site
D. Any financial arrangements between the study sites and Sysmex are documented in separate agreements
E. Any required application(s) to begin the clinical performance study in a given country have been submitted to the appropriate regulatory authority(ies) for review, acceptance or permission
F. Ethics Committee's approval/favourable opinion has been obtained and documented where required
G. Evidence of GCP training of all study personnel

Training

Only trained and experienced persons can perform the assays for this evaluation.

Training of all involved parties shall be documented in the training log and should include training of the CPSP and instructions for use.

The 'Test Procedure' described in the instructions for use supplied with the reagents should be used throughout the evaluation. A proficiency panel should be tested by all persons involved in conducting the clinical performance study tests, test results shall be attached to the training log.

The CPSP should be clearly understood by all members of the study team.

The training log is sent to the Study Coordinator before starting the study.

Conduct of the study

Testing of the samples must be done by a trained person whose name and function shall be put on the eSRF.

Any issues observed during the testing phase shall be reported to the Study Coordinator and

discussed.

Monitoring activities will be performed to verify compliance with the CPSP, issue reporting, adequate study site resources, storage and accountability of the IVD devices, and to ensure that records are accurate, complete, up-to-date, and stored and maintained appropriately.

Quality assurance

For quality assurance purposes, submission of all specimens (preferably in digital form) and full pathology report (preferably in digital form, original language and English translation), will be required at least from two patients per centre (randomly selected by the Study Coordinator or Principal Investigator) and reviewed centrally at Policlinico Agostino Gemelli IRCCS. Additional cases will be reviewed in case a major discrepancy is found from the protocol. The centre may be closed and the patients from the respective centre withdrawn for the final analysis if critical discrepancies or repeated major discrepancies are identified.

Centers will be selected according to:

- Availability of OSNA device
- Adherence to ESGO quality indicators for endometrial cancer items (Appendix 1) [24]: QI6, QI8, QI9, QI19, QI20

End of the study

The following documentation shall be available at end of the study:

- Completed and signed eSRFs
- Clinical performance study report, signed by the investigator(s).

Clinical performance study plan amendments and deviations

Any deviation from the clinical performance study plan will be reported to the Principal Investigator and Study Coordinator and documented in the clinical performance study report.

Amendments to the clinical performance study plan shall be agreed between the Sysmex, Study

Coordinator and the Principal Investigator. The amendment will not be implemented before the approval is obtained from all involved parties.

Statements of compliance and ethical principles

The study shall be conducted in accordance with ethical principles that have their origin in the Declaration of Helsinki, with GCP and any regional or national regulations.

The study shall not begin until the Ethics Committee's approval, favourable opinion or waiver is obtained.

The investigator will perform the clinical performance study in accordance with the applicable laws and regulations, including the applicable privacy laws and recognised ethical principles laid down in the Declaration of Helsinki. The study will be carried out in restricted laboratories in accordance with the applicable laws and in conformity with the European recognised quality standards.

Adverse device effects and device deficiencies

All devices applied in this study are already CE marked according to 98/79/EC except for the use in endometrial cancer. Only trained personnel shall use the device.

Any adverse device effect or observed device deficiency as defined below that is encountered during the clinical performance study will be recorded and reported to the Study Coordinator and documented in the clinical performance study report.

- Adverse device effect: adverse event related to the use of an IVD medical device under evaluation. This includes any adverse event resulting from insufficient or inadequate instruction for use, installation operation, or any malfunction of the IVD medical device under evaluation. Additionally, any event resulting from the use error or from intentional misuse of the device under evaluation.
- Any serious adverse device effect or device deficiency that could have led to a serious adverse device effect, as defined below, shall be reported without unjustified delay to the Study Coordinator; this information shall be promptly followed by a detailed written report.
- Serious adverse device effect: adverse device effect that has resulted in any of the

consequences characteristic of a serious adverse event that has led to death, to serious deterioration in health or to foetal distress, foetal death, or a congenital abnormality or birth defect.

Suspension or premature termination of the Clinical Performance Study

The Principal Investigator can decide to immediately suspend or prematurely terminate the clinical performance study in case of:

- occurrence of serious adverse device effects/device deficiencies
- suspicion of unexpected risk to the users or other persons involved in the study
- monitoring identifies serious deviations from the CPSP

The Study Coordinator will justify the decision for suspension or premature termination of the CPS in writing to the Principal Investigator. All routine end of study activities shall be conducted.
After risk assessment, it can be concluded to resume the suspended CPS. The Study Coordinator will inform the Principal Investigator with the decision including the rationale and the relevant supporting data.

Clinical Performance Study Report and publication of the results

The clinical performance study report will be written by the Principal Investigator and shall contain documented information on the clinical performance study plan, results and conclusions of the clinical performance study, including negative findings.
The results and conclusions shall be transparent, free of bias and clinically relevant. The report shall contain sufficient information to enable it to be understood by an independent party without reference to other documents. The report shall also include, if appropriate, any clinical performance study plan amendments or deviations and data exclusions with the appropriate rationale.
Upon completion of the multi-centric study project, the study sites and the Principal Investigator shall cooperate in publishing the results in a scientific journal relevant to the therapeutic area within maximum 12 months of the completion of the study project and subject to the provisions set forth

under this section.

- The order of authorship will be based on the number of patients recruited by the study site.
- The first and last authors will be authors from the sponsor institution, independent of the size of the recruited cohort at this site.
- Only study sites which have contributed to the study with ≥2% of the total patients will be listed as co-authors; the remaining study sites will be listed in the acknowledgment section of the paper. Every further 5% of randomized patients, will grant an extra co-author from that institution.

For this section, Principal Investigator shall provide Sysmex with a draft of any manuscript, presentation material or other written or printed document regarding the results and findings of the multi-centric study project at least thirty (30) days prior to its submission. Within twenty (20) days thereafter, Sysmex may request the exclusion of its proprietary information (including, but not limited to its confidential information).

Data and findings gained at the individual study sites shall be presented in national and international congresses and published in scientific journals relevant to the therapeutic area of the study, subject to the provisions set forth under this section. For the avoidance of doubt, with the prior written consent of Sysmex and Principal Investigator, study sites shall have the right to publish or present their results and findings exclusively and firstly as a study site is an academic entity, provided that such publication is conducted within 24 months of the completion of the study project. For this section, study site and the study project responsible shall provide Sysmex and the Principal Investigator with a draft of any manuscript, presentation material or other written or printed document regarding the results and findings at least thirty (30) days prior to its submission. Within twenty (20) days thereafter, Sysmex may request the exclusion of its proprietary information (including, but not limited to its confidential information).

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