GynEC-DX: A new test to determine endometrial cancer from endometrial aspirates in patients with Abnormal Uterine Bleeding


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Introduction

Endometrial cancer (EC) is the second most frequent gynaecological cancer worldwide and the most common in Europe and the USA. Over 280,171 new cases are diagnosed every year, accounting for 9.4 % of the worldwide incidence of cancer in women. While EC is generally considered to have a good prognosis, almost 74,000 women die each year of this disease1, and early diagnosis is key to reduce mortality rates. Several works have described different biomarkers for endometrial cancer but to date, there is no molecular screening test for diagnosis this disease. Here, we report the successful translation of a profile of 5 biomarkers identified previously in a genome wide gene expression study2 to the clinical setting.

Current Diagnostics Options

• Endometrial sampling (biopsy)
  – Unsusceptible sample
  – Additional costs
• Transvaginal ultrasound
• Endometrial aspirate (biopsy)
  – Accessible
  – Accessible
• Endometrial aspirate (biopsy)
  – Accessible
• Immunohistochemistry
• Histology
• Reliability of high failure rate
  – Low cost

Endometrial sampling (biopsy)
• High sensitivity
• High specificity
• Minimally invasive office sampling
  – No dependence on Pathologists skills
• Relative high failure rate
  – Cost

Immunohistochemistry
• High specificity and sensitivity
• More invasive
• Increased risk and discomfort
  – No proximity for endometrial biopsy
  – Increased risk and discomfort

Histology
• High sensitivity and specificity
• More invasive
• Increased risk and discomfort
  – More costly

Current Clinical EC Diagnosis Algorithm

Objective

To evaluate the diagnostic accuracy of a new tool for endometrial cancer based on molecular analysis by RT-PCR of uterine aspirates

Methods

Study design
• Type: Double blind prospective study
• Size: 519 patients recruited between December 2009- September 2010
• Current diagnostic algorithm to achieve final diagnosis includes TVS and/or biopsy by aspiration and/or hysteroscopy and/or surgery, with anatopathological analysis.
• GynEC-Dx analysis is added to the current diagnostic algorithm and performed on the remnants of the aspirate sample obtained for anatopathological analysis.
• Inclusion criteria: women of 45 years and older with Abnormal Uterine Bleeding (AUB)
• Exclusion criteria: previous treatment for gynaecological cancer / HJV or haematosis

Sample collection
Clinicians collected endometrial aspirate samples using a curette from Gynetics Medical Products. The remnants from the material used for histology were deposited in apartment with an RNA preserving solution. Samples were stored at 4°C for a maximum of eight hours. After that, tubes were centrifuged at 12000 rpm to pellet the tissue sample. The pellets were frozen and sent on dry ice from the clinics to the central laboratory at Oryzon where they were processed and analysed.

Development of the prototype qRT-PCR assay
Previously, we reported a retrospective case control study in which we identified biomarkers that discriminated endometrial cancer from normal endometrial tissue (from surgery) using a microarray based genome wide expression study. Not only did we validate the identified biomarkers using Taqman low density arrays (LDAs), we also performed a feasibility study that showed the possibility to translate the analysis of primary tumour tissue to biopsies obtained by endometrial aspiration, a minimally invasive sampling method. To develop our assay prototype, the biomarkers identified previously in higher content assays were translated to an effective qRT-PCR format designed to analyze a small profile consisting of the best performing individual markers on the Roche lightcycler instrument.

Results

Development of classification models selected for clinical validation: calibration study

Classification models were generated to translate the levels of expression of the five biomarkers assayed by qRT-PCR into a categorical EC cancer score. Training of the models was done on the prototype qRT-PCR assay data obtained from the analysis of a set of samples characterized previously in a case-control study. The sample set included aspirates from 71 patients, 23 of which were diagnosed with endometrial cancer diagnosis. Selected biomarker combinations were used to generate the diagnosis predictors. The five best performing classifiers with the lowest error rates were selected for a final clinical validation in a new prospective study.

Table 1: Demographic and pathology data

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| Patient ID | Age (years) | Type of EC | Type of EGC | Endometriosis | Epithelial 

Comparison of GynEC-DX with classical diagnostic methods

• In this study, the qRT-PCR assay was able to detect 8 EC cases missed by the previous diagnostic methods with a sensitivity of 79% and a specificity of 93%.
• The combination of GynEC-DX with AP biopsy obtained an NPV value of 99% and a specificity of 96% with a sensitivity of 92%.

Conclusions

• GynEC-DX identifies endometrial cancer with excellent NPV (97%), excellent specificity (95%) and good sensitivity (80%). The test detects both Type I and Type II tumours.
• The combination of GynEC-Dx and AP biopsy on aspirate had an excellent NPV value of 99% and a specificity of 96% with a sensitivity of 92%.

References

www.neigofine.com www.oryzon.com