Cervical cancer diagnostics – the challenge

The introduction of Pap testing was followed by an enormous decrease regarding the incidence and mortality of cervical cancer. Since this development is stagnant for at least a decade, more sophisticated screening and triage tools are needed. Therefore testing for high-risk types of human papillomaviruses (HPV) has become an important part of cervical cancer screening, management and treatment. Although highly sensitive, hrHPV testing has only limited specificity. Consequently, triage methods following a positive hrHPV test result are needed to avoid unnecessary follow-up of women who are infected without having clinically relevant disease.

The new PCR-based assay GynTect® can be used as a triage test for PAP-abnormal and/or HPV-positive women, allowing to identify those with cervical (pre)cancer.

The rationale for GynTect®

Persistent infection with a high-risk HPV type leads to genetic instability of infected cervical cells due to the expression of active viral oncogenes E6 and E7. Consequently, different genomic changes occur in the course of a normal cell developing into a cancer cell. Among these changes are epigenetic modifications involving the hypermethylation of specific genes, also called DNA methylation.

GynTect® detects DNA methylation of six human marker gene regions which specifically arises during carcinogenesis. As these genes are reliably found methylated in cervical (pre)cancer cells, GynTect® detects malignant host cell transformation instead of viral infection.

Clinical Performance of GynTect®

The clinical performance of GynTect® was evaluated in a clinical trial using 280 cervical scrapes collected for routine testing in the colposcopy clinic. Histopathology results were available for 28% of the samples: in 2 cases CIN1, in 20 cases CIN2, in 47 cases CIN3 and in 5 cases cervical carcinoma (CxCa) was diagnosed (see Table 2). 72% of all samples had a normal cytology finding (PAP I), thus no biopsy was taken.

GynTect® detected all cervical carcinomas. Only 1.7% of the samples with normal cytology (PAP I) were GynTect®-positive in the older age group and none in the younger group, which could significantly reduce unnecessary colposcopies (see Table 1). The clinical performance is depicted in Table 1. GynTect® showed high sensitivity and specificity for the detection of CIN1+ in HPV-positive women in both age groups. In younger women (<30 years of age), GynTect® performed less sensitive regarding the detection of CIN1 and CIN2. This finding has to be seen in the light that especially in younger women many CIN lesions regress spontaneously¹. Therefore, future work may provide more information that a certain proportion of CIN1 cases may not need to be detected yet.

Based on the current data, GynTect® is suitable for triaging PAP-abnormal and/or HPV-positive tested women. Besides, it is intended as an aid in the diagnosis of cervical (pre)cancer.

Clinical performance of GynTect®: Sensitivity CIN3(+) 68.6% 62.5% 66.7% Specificity CIN3+ 93.8% 96.8% 94.2% GynTect® performance (all ages)

Sample Collection via STM™ (Qiagen) or PreservCyt® (Hologic)

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Table 1: Detection rates and study cohort stratified for histopathology finding & age groups. All samples were tested in total, 276 had valid GynTect® results.

<table>
<thead>
<tr>
<th></th>
<th>women ≥ 30 years</th>
<th>women &lt; 30 years</th>
<th>total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAP I</td>
<td>1.7% (3/174)</td>
<td>0.0% (0/25)</td>
<td>1.5% (3/199)</td>
</tr>
<tr>
<td>CIN1</td>
<td>33.3% (2/6)</td>
<td>0.0% (0/1)</td>
<td>28.6% (3/7)</td>
</tr>
<tr>
<td>CIN2</td>
<td>50.0% (7/14)</td>
<td>20.0% (1/5)</td>
<td>42.1% (8/19)</td>
</tr>
<tr>
<td>CIN3</td>
<td>63.3% (19/30)</td>
<td>62.5% (10/16)</td>
<td>63.0% (29/46)</td>
</tr>
<tr>
<td>carcinoma</td>
<td>100% (5/5)</td>
<td>n/a</td>
<td>100% (5/5)</td>
</tr>
</tbody>
</table>

Table 2: Clinical performance (CIN3+, CIN3 for women <30y) stratified for age groups.

<table>
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<tr>
<td>Sensitivity CIN3(+)</td>
<td>68.8%</td>
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</tr>
</tbody>
</table>

Step 1: Gynecologist visit
Abnormal Pap smear (Pap-III, Pap-IIID)? And positive HPV test result? Your patient is uncertain and wants further clarification? GynTect® offers you as a gynecologist the option to further clarify the status of your patient: GynTect® allows to distinguish between cervical lesions that require invasive diagnostics and eventually surgery, and those that may heal without treatment.

Step 2: Cervical sampler
GynTect® is performed using conventional cervical smear material, which is transferred to a specific transport medium. ThinPrep PreservCyt® medium (Hologic) or ST™ medium (Glagen) can be used for taking the smear. Please contact your diagnostic lab or oncognostics via the website, by email, fax or phone in order to receive a cervical sample.

Step 3: Sampling and delivery to laboratory
Use the cervical brush from the sample and take a smear, transfer the smear material to the transport medium. The sample may be sent by normal mail, and should arrive at the diagnostic lab or oncognostics within 7 days. The cost for GynTect® is incurred directly to the patient, who will either send it to her health insurance or pay it by herself.

Step 4: Performance of GynTect®
The laboratory performs GynTect® within a work day. The sample is prepared in the laboratory, and the GynTect® markers are detected by the PCR-based test. You receive a result within a few days.

Step 5: Clear decision
You discuss the results with your patient. In case of a negative result your patient should not have any severe lesion that may proceed to carcinoma. You may observe your patient following a watchful waiting strategy, with repetition of the HPV test in order to determine when the infection clears.

In case of a positive GynTect® result a colposcopy and biopsy should provide clarity, where at the cervix the disease is located. This allows a specifically-targeted intervention.

GynTect® – assay principle and workflow
The GynTect® assay principle is based on the detection of DNA methylation of human marker gene regions. DNA methylation is a process during which methyl groups are added to the DNA, more specifically, to cytosines followed by guanines, so-called CpG dinucleotides.

The analysis of a patient sample comprises two steps. First, the methylation status of the human DNA in a cervical sample is “fixed” by a so-called bisulfite treatment. In the second step the bisulfite-converted DNA is analyzed in the regions of interest by applying several sensitive real-time PCR reactions. Using specific PCR primers, only the marker regions originally methylated in tumour DNA are selectively amplified, and a PCR product can be detected. Therefore, this procedure is called methylation-specific PCR (MSP). The GynTect® assay is rated positive, if a defined set of markers is amplified.

For a highly reliable workflow, the assay includes several internal sample quality control markers. Additionally, separate positive and negative control materials are provided with the kit.

Benefits of GynTect®
As a triage test based on cervical cancer cell biomarkers, GynTect® was developed to identify those individuals among PAP-abnormal and/or HPV-positively tested women who have clinically relevant cervical disease. Consequently, one may recommend colposcopy-guided diagnostics if the test is positive. A negative test results indicates the absence of a severe lesion or carcinoma and allows the continuation of a non-invasive screening algorithm, certainly with a shorter screening interval due to the initial positive HPV test result. Generally, DNA methylation-based biomarker tests may be applied in different cervical cancer screening settings, e.g. as triage in an HPV test-based screening scenario or for the clarification of minor cytological cervical lesions. GynTect® is easily incorporated in current gynecologists’ practices as it may be performed using residual material from e.g. the HPV test or liquid based cytology.

Positive GynTect® test result
» Indicates the presence of a clinically relevant cervical disease
» colposcopy-guided diagnostics with biopsy sampling for histopathology, if necessary

Negative GynTect® test result
» Indicates the absence of a severe lesion or carcinoma
» continuation of a non-invasive screening algorithm with shorter interval due to the initial positive HPV/PAP test

GynTect®: advantages for the gynecologist
» objective test result can be provided to the patient
» visit of a specialist is not needed in any case
» next steps can be recommended directly, watchful waiting can be avoided
» no long-term uncertainties between first diagnosis and clarification
» avoid unnecessary invasive diagnostics and surgeries
» classify an HPV/PAP-positive test result
» no change in the current practice

GynTect®: advantages for the patient
» no change in the current practice