Detection of DNA Methylation Markers in Urine of Cervical Cancer Patients: a feasibility study

W.F. Rurup¹, N.E van Trommel², A.P. van Splunter³, L.I. Segerink¹, G.G. Kenter², D.A.M. Heideman³, M. van Gent⁴, H.M. Pinedo⁵, P.J.F. Snijders², A. van den Berg¹, R.D.M. Steenbergen³

¹ MESA+ Institute for Nanotechnology, University of Twente, Enschede, The Netherlands
² Department of Gynecologic Oncology, Center of Gynecologic Oncology Amsterdam, The Netherlands.
³ Department of Pathology, VU University Medical Center Amsterdam, The Netherlands.

Introduction

- Current cervical screening programs use cervical cytology, and will be replaced by primary high-risk HPV (hrHPV) testing in the next few years.
- Cytology has a relatively low sensitivity (50-80%), which is largely improved by hrHPV testing. hrHPV testing however requires further triage testing.
- Analysis of DNA methylation of host cell tumor suppressor genes in cervical scrapes provides a promising triage strategy for hrHPV-positive women to detect (pre)cancerous lesions in need of treatment (Steenbergen et al., Nature Reviews Cancer 2014).
- Since urine collection is expected to increase the uptake of cervical screening programs, and hrHPV testing in urine appears promising, this study aimed to test the feasibility of DNA methylation analysis in urine to detect cervical cancer.

Materials & Methods

- Thirty-five urine specimens and cervical scrapes were collected from cervical cancer patients visiting the Anthoni van Leeuwenhoek hospital Amsterdam. As a reference urine samples were collected from 30 healthy controls.
- Patient samples were analysed for the presence of hrHPV DNA using the HPV-Risk assay (Self-screen BV). Patient and control samples were tested for DNA methylation of 6 genes (A-F) by multiplex quantitative methylation specific PCR (qMSP; Snellenberg et al., BMC Cancer 2012)

Results I

- Patient characteristics and HPV test results are shown in Table 1. An 95% concordance is found between HPV test results in urine and in cervical scrapes.

Results II

- DNA methylation levels of all 6 genes were significantly (p<0.001) increased in cervical cancer patients compared to controls (Figure 1).

Conclusions

- DNA methylation analysis testing in urine is feasible.
- DNA methylation analysis in urine shows a high accuracy to detect cervical cancer.
- Further studies are warranted to explore the clinical performance of methylation markers in urine-based cervical screening programs.

Table 1: Methylation levels of markers A-F in urine samples of healthy controls and cervical cancer patients.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Controls (N=30)</th>
<th>Cc patients (N=21)</th>
<th>p-value</th>
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<tbody>
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<td>A</td>
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HR-pos: high-risk HPV positive; (16): hrHPV16; (18): hrHPV18; (other) non-hrHPV16/18 high-risk type
** SCC: Squamous cell carcinoma; AC: Adeno carcinoma; ASC: Adenosquamous carcinoma, NA: not applicable

Figure 1: Methylation levels of markers A-F in urine samples of healthy controls and cervical cancer patients. (* p<0.001)

Figure 2: ROC curve analysis of control versus cervical cancer patient derived urine samples for markers A-F.