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

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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.

1	SCC	2B	HR-pos (other)	HR-pos (other)
2	SCC	2A+2B	NA	HR-pos (16)
3	SCC	2A+2B	HR-pos (other)	HR-pos (other)
4	SCC	IB	invalid	NA
5	SCC	IvB	HR-pos (16)	NA
6	SCC	IvA	HR-pos (16)	NA
7	SCC	2A+2B	HR-pos (other)	NA
8	SCC	IB	invalid	NA
9	SCC	Ib1	HR-pos (other)	invalid
10	SCC	2A+2B	HR-pos (other)	NA
11	SCC	Ib1	HR-pos (16)	HR-pos (16)
12	AC	Ib2	HR-pos (16)	HR-pos (16)
15	SCC	IB1	HR-pos (other)	HR-neg
16	SCC	1B1	HR-pos (18)	HR-pos (18)
17	SCC	2B	HR-pos (other)	HR-pos (other)
19	AC	Ib1	HR-neg	HR-neg
20	SCC	2A+2B	NA	HR-pos (16)
21	SCC	Ib1	HR-pos (16)	HR-pos (16)
22	SCC	2A+2B	HR-pos (other)	HR-pos (other)
23	SCC	Ib1	HR-pos (16)	HR-pos (16)
24	SCC + AC	IB1	HR-pos (16, 18)	HR-pos (16, 18)
26	PCC + ASC	Ib1	HR-pos (18)	HR-pos (18)
27	SCC	2b	HR-pos (18)	HR-pos (18)
28	AC	Ib1	HR-pos (16)	HR-pos (16)
29	SCC	Ib2	HR-pos (other)	HR-pos (other)
30	SCC	IIA1	HR-pos (16)	HR-pos (16)
31	AC	recidief	HR-pos (16)	HR-pos (16)
33	SCC	IIIb	HR-pos (16)	HR-pos (16)
34	SCC	2b	HR-pos (16)	HR-pos (16)
35	SCC	Ib1	HR-pos (other)	HR-pos (other)

HR-pos: high-risk HPV positive; (16): HPV16; (18) HPV18; (other) nonHPV16/18 high-risk type
** SCC: Squamous cell carcinoma; AC: Adeno carcinoma; ASC: Adenosquamous carcinoma, NA: not applicable

Results II

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

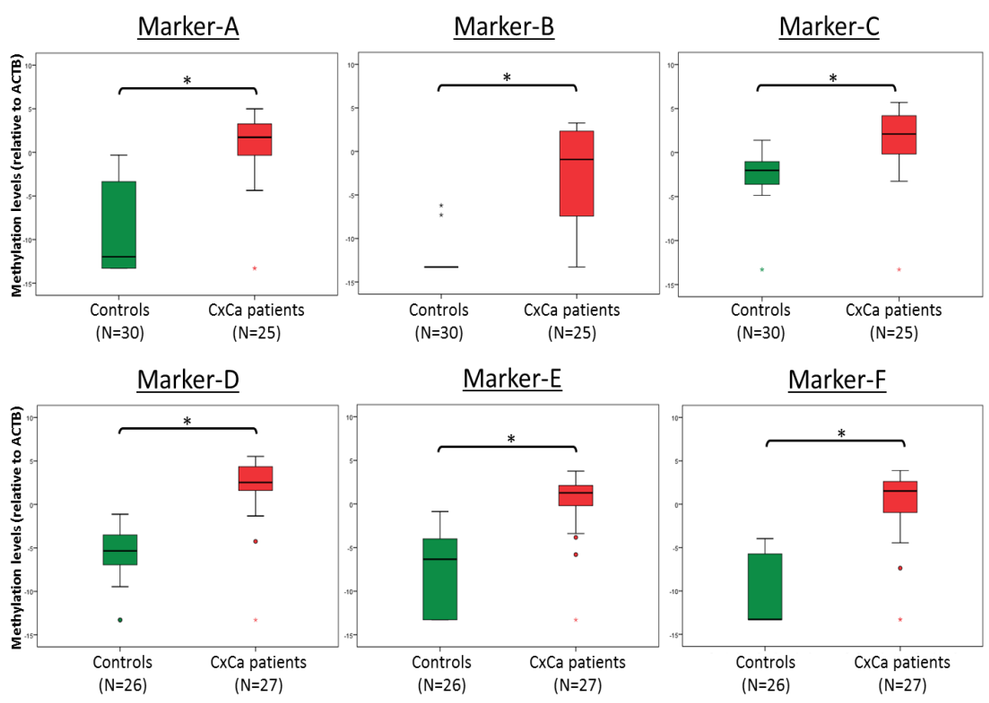

Figure 1: Methylations levels of markers A-F in urine samples of healthy controls and cervical cancer patients. (* p<0,001)

- Receiver operating curve analysis of the 6 methylation markers showed AUCs ranging from of 0.88 to 0.95 (**Figure 2**)

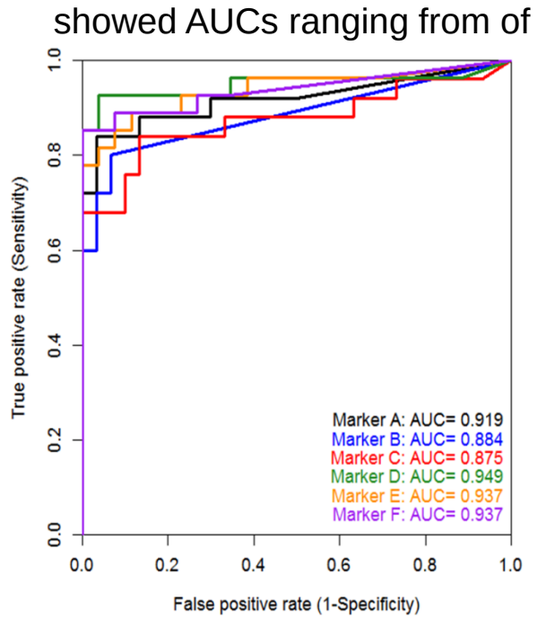

Figure 2: *ROC curve analysis of control versus cervical cancer patient derived urine samples for markers A-F*

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.

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