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# Performance of CADM1/MAL-methylation analysis for monitoring of women treated for high-grade CIN

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## HIGHLIGHTS

- First study to show the performance of methylation marker analysis for detecting recurrent high-grade CIN lesions (rCIN2/3) in post-treatment monitoring
- CADM1/MAL-methylation is associated with the severity of recurrent disease
- Post-treatment monitoring by CADM1/MAL-methylation analysis identifies women with an increased risk of rCIN2/3

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## ABSTRACT

*Introduction.* Recent studies have shown that CADM1/MAL-methylation testing detects high-grade CIN lesions with a high short-term progression risk for cervical cancer. Women treated for CIN2/3 are at risk of post-treatment disease, representing either persistent (incompletely treated) or incident (early onset) lesions. Here, we evaluated CADM1/MAL-methylation analysis as potential tool for detecting recurrent high-grade CIN lesions (rCIN2/3).

*Methods and materials.* A multicenter prospective clinical cohort study was conducted among 364 women treated for CIN2/3. Cervical scrapes were taken prior to treatment, and six and 12 months post-treatment and tested for cytology, hrHPV (plus genotype) and CADM1/MAL-methylation. When at six months either of these tests was positive, a colposcopy-directed biopsy was obtained. At 12 months, all women underwent an exit-colposcopy with biopsy. In case of rCIN2/3, re-treatment was done.

*Results.* We found 28 rCIN2 (7.7%) and 14 rCIN3 (3.8%), resulting in a total recurrence rate of 11.5%. All 14 women with rCIN3 and 15/28 (54%) with rCIN2 showed hrHPV type-persistence. Of these, 9/14 (64%) rCIN3 and 8/15 (53%) rCIN2 were CADM1/MAL-methylation positive. All incident rCIN2, characterized by hrHPV genotype-switch, were CADM1/MAL-methylation negative. All three carcinomas found after re-treatment were CADM1/MAL-methylation positive. CADM1/MAL-methylation positivity at both baseline and follow-up significantly increased the risk of ≥rCIN3 (from 0.7% to 18.4%), and ≥rCIN2 (from 8.2% to 36.8%), compared to a consistently CADM1/MAL-methylation negative result (p-value: <0.001).

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*Conclusion.* Post-treatment monitoring by CADM1/MAL-methylation analysis identifies women with an increased risk of rCIN2/3. Our results confirm previous data indicating that CADM1/MAL-methylation analysis provides a high reassurance against cancer.



## 1. Introduction

Long-term follow-up studies have indicated that women who undergo treatment for cervical intra-epithelial neoplasia grade 2 or 3 (CIN2/3) have an increased long term risk of recurrent CIN2 lesions or worse (≥rCIN2) compared to the general population [1,2]. Because of this long-term risk, gynecologists in many countries use surveillance strategies in order to detect recurrent disease. These surveillance strategies vary greatly in content and length. In many Western countries, women are monitored by cervical cytology at six, 12 and 24 months after treatment, and are referred to the routine screening program after three subsequent negative scrapes [3]. Recently, it has been shown that the addition of high-risk human papillomavirus (hrHPV) DNA testing to cytology at six months after treatment dramatically increases the sensitivity for ≥rCIN2 [2,4]. When at six months both test results are negative, testing at 12 months can be omitted without an increased ≥rCIN2 risk [2].

Recurrent CIN2/3 lesions are known to represent a group of heterogeneous diseases, comprising persistent lesions resulting from residual (i.e. incompletely treated) disease with persistence of the same HPV genotype, and incident (i.e. early onset) lesions. Incident lesions can either result from an infection with a hrHPV type different from the original type in the resected CIN2/3 lesion (incident rCIN), or a re-infection with the same hrHPV type as the original one [5]. Morphologically, persistent rCIN2/3 lesions cannot be distinguished from incident counterparts, and all rCIN2/3 are therefore treated. This may lead to overtreatment, which is (especially) harmful for women in their reproductive age since it may lead to adverse pregnancy outcomes [6]. For a more tailored management of women diagnosed with recurrent disease, studies on biomarkers that can detect women in immediate need of re-treatment are warranted. Ideally these biomarkers should distinguish persistent rCIN2/3 lesions, with likely a high short-progression risk to cancer (so-called advanced lesions) and therefore in need of immediate treatment, from incident, so-called early rCIN2/3 lesions, with a likely low short-term progression risk to cancer and for which a more conservative approach is acceptable.

Potential biomarker tests that can discriminate between early and advanced stages of cervical disease involve tests assessing DNA promoter methylation of certain host cell tumor suppressor genes involved in cervical carcinogenesis [7–10]. Cell adhesion molecule 1 (*CADM1*) and myelin and lymphocyte (*MAL*) belong to the most frequently methylated genes in cervical carcinoma [8,11]. Silencing of these genes by DNA promoter methylation is a common and functionally relevant event in cervical cancer development [12–14]. We have previously shown that the extent of DNA promoter methylation of *CADM1* and *MAL* genes increases with the severity of cervical disease and that these epigenetic changes are considered to reflect the presence of a more advanced high-grade CIN lesion with a longer duration of existence [9,11,15]. This is supported by the finding that levels of *CADM1* and *MAL* promoter methylation are extremely high in cervical cancer and significantly increased in CIN3 lesions of women with a hrHPV infection that has persisted over a long time period (≥5 years) compared to early onset CIN3 lesions resulting from a recently acquired hrHPV infection [7].

In this study, we have evaluated the performance of CADM1/MAL-methylation analysis as a potential tool to monitor women treated for CIN2/3 for recurrent disease.

## 2. Methods and materials

### 2.1. Study population

This study was designed as a multicenter prospective clinical cohort study conducted in six outpatient clinics in The Netherlands. The study flowchart is depicted in Fig. 1. The participating centers were VU University Medical Center, Erasmus MC University Medical Center Rotterdam, University Medical Center Utrecht, Flevo Hospital Almere, Sint Antonius Hospital Nieuwegein and Onze Lieve Vrouwen Gasthuis West Amsterdam. Between April 2010 and June 2012, all women aged 18 years and older, who were scheduled for treatment of a biopsy confirmed CIN2/3 lesion by Large Loop Excision of the Transformation Zone (LLETZ), were asked to participate in this study. Also women who were treated directly without prior biopsy and in whom the presence of a CIN2/3 or an adenocarcinoma in situ (AIS) lesion was confirmed in the LLETZ specimen (see-and-treat), were enrolled. The study was approved by the Medical Ethical Committee (METC) of the VU medical center (METC-VUmc 2009/285) and endorsed by all other participating clinics and registered in The Netherlands Trial Register (NTR1964). All women gave written informed consent prior to any study procedure.

### 2.2. Study procedures

Prior to treatment, a cervical scrape was taken with a Cervex brush® (Rovers medical devices B.V., Oss, the Netherlands). Scrapes were stored in Thinprep (Hologic, Marlborough MA, USA) and used for hrHPV detection and CADM1/MAL-methylation analysis. At six months post-treatment, two cervical scrapes were obtained from each study participant. The first specimen was collected for cytology according to local protocols (conventional slide or stored in Thinprep) of the participating hospital. The second specimen was collected in Thinprep medium for hrHPV and CADM1/MAL-methylation analysis. HrHPV and methylation tests were performed in a reference laboratory (Department of Pathology, VU University Medical Center, Amsterdam, The Netherlands). Women were referred for colposcopy when at least one of the three tests was positive, i.e., borderline or mild dyskaryosis or worse cytology (≥BMD; comparable with ≥ASC-US; see classification details below) and/or hrHPV positive and/or positive for CADM1/MAL-methylation. At 12 months post-treatment, one cervical scrape was collected in Thinprep medium and analyzed in the reference laboratory for cytology, hrHPV presence and methylation status. After taking the cervical scrape, an exit-colposcopy with mandatory biopsy was also performed at this 12 month visit. Primary outcome measure was ≥rCIN3 and ≥rCIN2.

### 2.3. Cytology reading

The Dutch CISOE-A classification was used to report the cytological results. The results can easily be translated into the Bethesda 2001 classification [16]. Cytological results were grouped as normal, BMD or ≥BMD. Cytology results classified in CISOE-A as ≥S2, E3 or O3, comparable to ≥BMD were considered abnormal.

### 2.4. DNA isolation and hrHPV DNA testing

DNA was isolated from cervical scrapes using the Nucleo-Spin 96 Tissue kit (Macherey-Nagel, Germany) and Microlab Star robotic system (Hamilton, Germany), according to the recommendations of the