Combined promoter methylation analysis of CADM1 and MAL: an objective triage tool for high-risk human papillomavirus DNA positive women

Running title: Methylation markers to triage hrHPV-positive women

Albertus T. Hesselink1, Daniëlle A.M. Heideman1, Renske D.M. Steenbergen1, Veerle M.H. Coupé2, Renee M. Overmeer1, Dorien Rijkaart1, Johannes Berkhof2, Chris J.L.M. Meijer1, Peter J.F. Snijders1*
Departments of Pathology1, and Epidemiology and Biostatistics2, VU University Medical Center, P.O. Box 7057, 1007 MB Amsterdam, the Netherlands.

Corresponding author and address for reprints:
Prof. P.J.F. Snijders, PhD
Dept. of Pathology, VU University medical center
De Boelelaan 1117, 1081HV Amsterdam, The Netherlands
Phone: +31-204444023, Fax: +31-204442964
Email: pjf.snijders@vumc.nl

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Conflict of interest
C.J.L.M. Meijer is a member of the advisory board of Qiagen, Gaithersburg, USA, and received lecture fees from GSK. P.J.F. Snijders provided occasional consultation to Roche and Gen-Probe. Qiagen, Gen-Probe and Roche are companies involved with HPV diagnostics. C.J.L.M.Meijer, P.J.F. Snijders, D.A.M. Heideman, and R.D.M. Steenbergen have relationships with Self-screen, The Netherlands. All other authors declared no conflict of interest.
Translational relevance

High-risk HPV (hrHPV) DNA testing is now considered an attractive primary cervical cancer screening tool yielding a superior protection against cervical (pre)cancer compared to cytology. However, its relatively low specificity for high-grade cervical (pre)malignant lesions requires secondary, triage testing to distinguish those hrHPV positive women with clinically relevant cervical disease in need of colposcopic examination. This asks for a triage biomarker assay that is easily applicable to physician-taken cervical scrapings as well as self-collected (cervico-)vaginal specimens. The objective triage assay developed and validated in this study (methylation analysis of CADM1 and MAL genes) allows a completely objective, non-morphological molecular-based test system (i.e. hrHPV followed by molecular triage testing with a CADM1/MAL methylation marker panel) for cervical screening.
Abstract

Purpose

Screening women for high-grade cervical intraepithelial neoplasia or cervical cancer (CIN3+) by high-risk human papillomavirus (hrHPV) testing has as side-effect the detection of hrHPV-positive women without clinically relevant lesions. Here, we developed an objective assay assessing the methylation status of the promoter regions of CADM1 and MAL to triage hrHPV-positive women for CIN3+.

Experimental design

In a training set (51 women with CIN3+ and 224 without CIN2+) panels consisting of one to four quantitative methylation-specific PCR (qMSP) assays (CADM1-m12,CADM1-m18,MAL-m1,MAL-m2) were analyzed. Cross-validated receiver-operating characteristics (ROC) curves were constructed and the panel with highest cross-validated area-under-the-curve (AUC) was used for validation in an independent set of 236 consecutive hrHPV-positive women from a screening cohort. In the validation set the ROC-curve of the panel was compared to CIN3+ sensitivity and specificity of cytology and of cytology combined with HPV16/18 genotyping.

Results

In the training set CADM1-m18 combined with MAL-m1 was the best panel (cross-validated partial AUC=0.719). In the validation set this panel revealed CIN3+ sensitivities ranging from 100% (95%CI:92.4-100) to 60.5% (95%CI:47.1-74.6), with corresponding specificities ranging from 22.7% (95%CI:20.2-25.2) to 83.3% (95%CI:78.4-87.4). For cytology these were 65.8% (95%CI:52.3-79.0) and 78.8% (95%CI:73.7-83.1) and for cytology/HPV16/18 these were 84.2% (95%CI:72.0-92.7) and 54.0% (95%CI:49.2-58.7), respectively. The point-estimates of both cytology and cytology/HPV16/18 were equal to the values of the ROC-curve of CADM1-m18/MAL-m1.

Conclusions

We developed an objective methylation marker panel that was equally discriminatory for CIN3+ as cytology or cytology with HPV16/18 genotyping in hrHPV-positive women. This
opens the possibility for complete cervical screening by objective, non-morphological molecular methods.
Introduction

Recent randomized-controlled cervical cancer screening trials have shown that high-risk human papillomavirus (hrHPV) testing yields a superior protection against cervical precancerous lesions and cervical cancer compared to cytology (1-7). Therefore, hrHPV testing is a more attractive primary cervical screening tool than cytology (2,8). Moreover, in combination with self-sampling hrHPV testing has shown to improve screening attendance in developed countries (9,10) and to increase access to cervical screening in low/intermediate resource settings (7,11,12). A drawback of hrHPV testing is the lower specificity (approximately 4-6%) for CIN3 or cervical cancer (CIN3+) compared to cytology. Introduction of a less specific screening test may lead to a substantial increase in the burden of health care resources, particularly in countries that currently have relatively low colposcopy referral rates. To control the number of colposcopy referrals, hrHPV positive women should not be offered colposcopy immediately but should be further stratified by means of secondary (i.e. triage) tests to guide referral for colposcopy and minimize over-diagnosis and -treatment (3,13,14).

At present cytology is considered an appropriate triage tool for hrHPV-positive women (13). Immunostaining of cytology slides for p16INK4a with or without Ki-67 staining potentially yields better results than cytology (15,16). However, given their subjective nature, cytology-based methods are not fully compatible with the advantages of hrHPV testing in terms of reproducibility, quality (17,18), and sample flexibility, the latter hampering their performance on self-collected cervico-vaginal samples (19). In order to reach a higher sensitivity of triage testing for hrHPV positive women, cytology may be complemented with genotyping for HPV16 and -18 (HPV16/18) to cover the excess risk of cytomorphologically normal women who harbour one or both of these types (20-22). Indeed, the American Society for Colposcopy and Cervical Pathology (ASCCP) 2006 consensus guidelines include a recommendation of viral genotyping for HPV16 and 18 as a triage tool for colposcopy referral of hrHPV positive women of 30 years or older with normal cytology (23). Ideally, one type of
A triage method with an objective readout should be available that might be applicable to self-sampled specimens as well.

It is well known that following a hrHPV infection specific (epi)genetic alterations of host cell genes are compulsory extra hits for the development of cervical (pre)cancer. Recently, we identified the CADM1 (cell adhesion molecule 1) gene, originally referred to as TSLC1 (tumor suppressor in lung cancer-1), and MAL (T-lymphocyte maturation associated protein) as novel tumor suppressor genes functionally involved in cervical carcinogenesis (24-26). Promoter methylation showed to be the main mode of inactivation of these genes (25,26). Subsequent studies using quantitative methylation-specific PCR (qMSP) analysis on tissue specimens revealed that the application of two qMSP assays, representing CADM1 and MAL each, was sufficient to reach the highest positivity rates for CIN3 lesions (97%) and carcinomas (99%), when scoring the sum of these assays *. Hence, when applicable to cervical scrapes a combination of these methylation markers could represent a promising candidate triage tool for hrHPV-positive women.

In this study, we evaluated the potential of assessing promoter methylation of these two genes as an objective triage tool for hrHPV positive women in case of cervical screening by primary hrHPV testing. We composed, trained and validated a CADM1- and MAL-based qMSP marker panel to stratify hrHPV-positive women for CIN3+. Training and validation of the markers were performed on two large, independent sets of hrHPV-positive scrapes that were collected during population-based cervical screenings studies.

Material and Methods

Study populations

For the training set we selected baseline cervical scrapings of 300 of a total of 1,102 hrHPV-positive women participating in the intervention arm of the population-based, randomized-controlled screening trial POBASCAM (2,27). The training set was used to build a prediction model that enables distinguishing precancer cases from controls. Specifically, we selected 60 women with a histologically-confirmed CIN3+ lesion detected within the first screening round and 240 women without evidence of CIN2 lesions or worse (CIN2+) after two screening rounds (≥5 years of follow-up). Since CIN2 is a poor proxy of precancer and often represents a misclassified CIN1 or CIN3 we on purpose did not include CIN2 cases in the model building phase. In the intervention arm of the POBASCAM trial, the referral policy at the baseline and subsequent screening round was based on a combination of hrHPV presence, as determined by hrHPV GP5+/6+-PCR, and/or abnormal cytology. Detailed descriptions of this trial, including the referral policy and follow-up procedures have been published previously and the trial is registered as an International Standard Randomised Controlled Trial, number ISRCTN20781131 (2,27). Of the 300 hrHPV-positive women 284 had sufficient sample material left for qMSP and 275 of these revealed a valid qMSP result according to the criterion detailed below and were further used in our analysis (Supplementary Figure 1). These consisted of samples of 51 women with CIN3+ (including 4 squamous cell carcinomas (SCCs) and 1 adenocarcinoma), of whom 18 had normal and 33 abnormal cytology at baseline and 224 women without evidence of CIN2+, of whom 169 had normal cytology and 55 abnormal cytology.

For the validation set we collected the scrapings of the first 250 hrHPV-positive women derived from a group of 3,135 women, participating in the population based screening programme in the same region and using the same referral protocol (co-testing with hrHPV and cytology) as in the intervention arm of the POBASCAM study. The intake of these women took place between April 2003 and April 2004 (28). Of 239 of the 250 hrHPV-
positive women sufficient sample material was left to assess the promoter methylation status and from 236 we obtained valid qMSP results. 38 women (16.1%) had a CIN3+ lesion and three of them had a SCC, and one an adenocarcinoma in situ (ACIS). An additional 20 women had CIN2 (8.5%) and 178 (75.4%) either had histologically no CIN or CIN1, or remained cytologically normal and displayed hrHPV clearance at follow-up.

Colposcopy-directed biopsies were taken for histological examination according to standard procedures in the Netherlands (29). Biopsy specimens were histologically classified as normal, CIN grade 1, 2, or 3, invasive cancer, adenocarcinoma in situ, or adenocarcinoma according to international criteria (30).

Detection of HPV DNA was done on cervical scrapes by GP5+/6+-PCR followed by enzyme immunoassay detection of 14 high-risk types (ie, HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) with a cocktail of oligonucleotide probes (31). Reverse line blot (RLB) analysis was performed to genotype the hrHPV infection as described previously (32).

Informed consent was obtained from all study participants and this study followed the local ethical guidelines of the medical center.

**DNA extraction, bisulfite treatment and quantitative methylation-specific PCR**

DNA was isolated from cervical scrapes using NucleoSpin 96 Tissue kit (Macherey-Nagel, Germany) and a Microlab Star robotic system (Hamilton, Germany) according to manufacturers' instructions. Extracted DNA was subjected to bisulfite treatment using the EZ DNA Methylation Kit (Zymo Research, USA) as described previously (25,26). Primers, probes, and reaction conditions for qMSP on an ABI 7500 real-time PCR system (Applied Biosystems, USA) to detect promoter hypermethylation of two regions (m12 and m18) in the CADM1 and two regions (m1 and m2) in the MAL promoter were done as described previously (25,33). In addition, a PCR for the bisulfite converted housekeeping gene β-actin was performed as a reference. All qMSP assays were run in separate reactions. Cycle threshold (Ct) values were measured at a fixed fluorescence threshold (i.e. 0.01). Ct ratios
between the Ct values of the β-actin and target were used to quantify the level of methylation, as calculated by the following formula: \[ \frac{2^{(Ct \text{ (β-actin)} - Ct \text{ (target)})}}{100} \]. Ct signals >40 were considered to represent a negative test result, whereas samples with Ct values for β-actin >32 were considered invalid and therefore excluded from analysis because of an indication of poor DNA quality or recovery after bisulfite treatment. In pilot experiments all qMSP assays displayed good reproducibility. Spearman correlation coefficients were 0.86 for β-actin, 0.88 for CADM1-m12, 0.74 for CADM1-m18, 0.82 for MAL-m1, and 0.83 for MAL-m2 when samples were retested according to conditions outlined above (Supplementary Figure 2).

Statistical analyses

The training cohort was used to compose a panel out of four qMSPs markers (CADM1-m12, CADM1-m18, MAL-m1, MAL-m2). The panel to be selected should best discriminate women with CIN3+ from other hrHPV-positive women. To that end, we considered panels of 1, 2, 3 and 4 markers (in total 15 marker panels). Methylation outcomes were dichotomized (positive/negative) using Ct ratios. The outcome was considered positive if at least one of the markers in a panel had a Ct ratio above its threshold. The thresholds were estimated by maximizing the CIN3+ sensitivity at a given, predefined specificity. The CIN3+ sensitivity maximization was determined at specificity levels of ≥20%, ≥30%, ≥40%, ≥50%, ≥60%, ≥70%, and ≥80%, which yielded for each marker panel a receiver-operating (ROC) curve. To compare the performance of the different marker panels, we carried out 10-fold cross-validation and measured the performance of each marker panel by the partial cross-validated area under the ROC (AUC). In case two panels had similar partial cross-validated AUC, the panel with the fewest markers was selected. Regarding the calculations, we first computed cross-validated CIN3+ sensitivities at predefined specificity levels and used those values to construct the cross-validated ROC and to compute the partial cross-validated AUC.

In the validation cohort qMSP testing was performed blinded to clinical data using the Ct ratios as thresholds that were estimated in the training set. ROC curves (end-points
CIN3+ and CIN2+) were computed for the selected marker panel (that is, the marker panel with the highest cross-validated partial AUC in the training data). The ROC curve was compared to the CIN3+ and CIN2+ sensitivities and specificities of cytology and of cytology combined with HPV16/18 genotyping (i.e. presence of HPV16 and/or 18). The threshold used for cytology positivity was borderline dyskaryosis (i.e. ASCUS).

In subsequent logistic regression analyses, the association between age and methylation was studied. The maximization of the CIN3+ sensitivity and the calculation of the cross-validated AUC were performed in the R package (version 2.8.1.). Other calculations were performed in SPSS (version 15.0).
Results

Training set analysis

We considered panels of one to four markers (in total 15 marker panels) out of four qMSPs markers (CADM1-m12, CADM1-m18, MAL-m1, MAL-m2) to discriminate amongst 275 hrHPV-positive women those with CIN3+. The composition of training set cohort, which included 51 women with CIN3+ and 224 women without evidence of CIN2+, is outlined in Supplementary Figure 1. For each panel a cross-validated ROC curve was constructed by optimizing CIN3+ sensitivity at predefined specificity levels of ≥20%, ≥30%, ≥40%, ≥50%, ≥60%, ≥70%, and ≥80%. The cross-validated ROC curve and corresponding partial cross-validated AUC of the marker panels are shown in Figure 1. In Supplementary Figure 3 an example of the prediction values of the sensitivity and specificity from the 10-fold cross validation is presented for one of the marker panels (i.e. CADM1-m18/MAL-m1). Roughly, two clusters of ROC curves were found, with cluster 1 comprising a total of seven marker combinations with partial AUC values of over 0.68 (Figure 1). One of these combinations contained all four markers (CADM1-m12, CADM1-m18, MAL-m1, and MAL-m2), three contained three markers (CADM1-m12, CADM1-m18 and MAL-m1; CADM1-m12, CADM1-m18 and MAL-m2; CADM1-m18, MAL-m1 and MAL-m2), and three contained two markers (CADM1-m12 and CADM1-m18; CADM1-m18 and MAL-m1; CADM1-m18 and MAL-m2). CADM1-m18 was present in all seven marker combination of cluster 1. Of the panels containing only two markers the combination CADM1-m18 and MAL-m1 had the highest partial AUC (i.e. 0.719; Figure 1). Since adding additional qMSP markers did not yield markedly higher AUC values we selected the CADM1-m18/MAL-m1 panel for further evaluation of its clinical performance in the independent validation set.

Validation set analysis

In the validation cohort qMSP testing was performed blinded to clinical data. ROC curves (end-points CIN3+ and CIN2+) were computed for the selected CADM1-m18/MAL-m1 marker panel using the thresholds as determined in the training set. The ROC curve was
compared to the CIN3+ and CIN2+ sensitivities and specificities of cytology and of cytology combined with HPV16/18 genotyping (i.e. presence of HPV16 and/or 18). The threshold used for cytology positivity was borderline dyskaryosis (i.e. ASCUS). Sensitivities of the CADM1-m18/MAL-m1 panel for CIN3+ ranged from 100% (95%CI 92.4-100) to 60.5% (95%CI 47.1-74.6), with corresponding specificities ranging from 22.7% (95%CI 20.2-25.2) to 83.3% (95%CI 78.4-87.4; Table 2 and Figure 2a). The three SCCs and the ACIS in this cohort were detected at the thresholds that gave rise to a CIN3+ sensitivity of 81.6% (95% CI, 69.0-91.0) or higher. By comparison, cytology revealed sensitivity and specificity values for CIN3+ of 65.8% (25/38, 95%CI 52.3-79.0) and 78.8% (156/198, 95%CI 73.7-83.1), respectively. Cytology combined with HPV16/18 positivity resulted in a sensitivity for CIN3+ of 84.2% (32/38; 95%CI 72.0-92.7) and specificity of 54.0% (107/198; 95%CI 49.2-58.7) (Table 2). Of interest, the point estimates of both cytology and the cytology/HPV16/18 combination were equal to respective values deduced from the ROC curve generated from the CADM1-m18/MAL-m1 panel for CIN3+ (Figure 2a).

When considering CIN2+ as outcome measure, similar results were obtained, with overall slightly higher specificity values against slightly lower sensitivity values (Figure 2b and Supplementary Table 1). Furthermore, the sensitivity and specificity values for CIN2/3+ of the CADM1-m18/MAL-m1 panel, cytology, and cytology combined with genotyping did not change meaningfully when validation set analysis was restricted to women ≥30 years of age. For instance for women aged 30-60 years the corresponding CIN3+ sensitivities were 83.3% (95%CI: 70.6-92.3; when using the thresholds giving rise to ≥50% training set specificity), 63.9% (95%CI: 50.0-77.7) and 83.3% (95%CI: 70.6-92.3), respectively, and corresponding specificity values were 49.4% (95%CI: 44.7-54.2), 77.5% (95%CI: 72.0-82.2), and 53.4% (95%CI: 48.3-58.4), respectively. Logistic regression analysis showed that age was not a confounder in the relation between the methylation panel and CIN3+ (ORs were, e.g. at the thresholds corresponding to 50% specificity in training set, 5.7 (95%CI 2.3-14.2) versus 6.1 (95%CI 2.4-15.3), with and without age added to the logistic regression model, respectively).
Discussion

Using a training/validation set approach we composed and analyzed a CADM1/MAL-specific methylation marker panel to be used for triage testing of hrHPV-positive women. When applying assay thresholds corresponding with 75.3% specificity (resulting in 68.4% sensitivity) or 52.5% specificity (resulting in 84.2% sensitivity), this panel was equally discriminatory for CIN3+ as cytology or the cytology/HPV16/18 genotyping combination in hrHPV-positive women.

Several previous studies already have indicated that methylation analysis of host cell genes in cervical scrapings holds great promise for detecting high-grade CIN lesions and cervical cancer (25,26,34-37). However, these studies were conducted on selected populations, such as outpatient referral populations or screening populations of women who were tested with cytology solely. This hampered comparison of methylation markers with cytology in a screening setting since cytology negative women had no follow-up. The strength of this study is the availability of cervical samples from prospective population-based screening studies conducted with hrHPV testing and with referral for colposcopy based on triage by cytology and/or hrHPV. This allowed comparison of the methylation markers with sole cytology or cytology/HPV16/18 genotyping for the triage of hrHPV-positive women. However, a possible verification bias in favor of cytology cannot be excluded, since women were mainly referred on the basis of abnormal cytology (2). Because loss to follow-up was most pronounced at the 18 month visit, the only moment at which women were referred on the basis of sole hrHPV testing (2), this could have resulted in a slight underestimation of the sensitivity of the methylation marker panel.

Another issue that deserves attention is that not only scrapes of women with CIN2 but also a small subset of women with CIN3 showed relatively low methylation levels. When for example the thresholds were applied that gave rise to a 81.6% CIN3+ sensitivity and at which all carcinomas and ACIS in the validation set were detected seven CIN3 lesions scored negative because of lower methylation levels. For endpoint measure CIN2+ cytology and the combination of cytology and HPV 16/18 genotyping performed slightly better than the
methylation panel. Possible reasons for low methylation levels in these cases could be sampling error, the presence of early onset CIN3 lesions with few chromosomal abnormalities (38), or CIN2 lesions representing productive hrHPV infections with a low risk to progress to invasive cancer till the next screening round after 5 years.

An important finding in the validation procedure was that blinded testing of the methylation panel displayed similar performances in both the training and validation sets. This indicates the robustness of the panel as it yielded reproducible results in independent screening cohorts of women, irrespective of possible confounding factors like age, storage time of samples, and the use of different collection media.

Despite the fact that, given the quantitative nature of the assay many threshold settings are possible we advocate to use the qMSP thresholds giving rise to CIN3+ sensitivity and specificity values equalling those of the combination of cytology and HPV 16/18 genotyping (i.e 84.2% and 52.5%, respectively) so that no SCCs and adenocarcinomas are missed and relevant high-grade CIN lesions are detected.

In conclusion, we established and validated a CADM1-m18/Mal-m1 marker panel, based on qMSP technology, that can be used as an objective triage tool for hrHPV-positive women. This could result in a completely objective, non-morphological molecular-based test system for cervical screening.

**Acknowledgements**

The authors are grateful to Anna Zwier, Muriel Verkuijten, and Nathalie Fransen-Daalmeijer for performing methylation analyses.
Legends to the Figures, Supplementary Figures and Supplementary Table.

Figure 1. Cross-validated ROC curves and partial AUC for CIN3+ in hrHPV-positive women of the training cohort for 14 possible combinations of CADM1-m12 (m12), CADM1-m18 (m18), MAL-m1 (mal1), and MAL-m2 (mal2). Cluster 1 contains the following 7 marker panels: CADM1-m12/CADM1-m18/ MAL-m1/MAL-m2; CADM1-m12/CADM1-m18/MAL-m1; CADM1-m12/CADM1-m18/MAL-m2; CADM1-m18/MAL-m1/MAL-m2; CADM1-m12/CADM1-m18; CADM1-m18/MAL-m1; CADM1-m18/MAL-m2. For the single marker MAL-m2 no ROC curve could be generated since the MAL-m2 Ct ratios were 0 in more than 80% of the samples and therefore any Ct ratio threshold above 0 automatically results in a specificity >80%, which falls outside the specificity range of 20% to 80% used to generate the ROC curves. At a Ct ratio threshold of 0 the sensitivity value for MAL-m2 was 32.1% and the specificity 81.3%.

Figure 2. ROC curve of the panel CADM1-m18/MAL-m1 and the point estimates of cytology and cytology combined with HPV16/18 positivity for CIN3+ (Figure 2a) and CIN2+ (Figure 2b) in hrHPV-positive women of the validation set.

Supplementary Figure 1. Consort diagram indicating the composition of the training set. This set was selected from a total of 1,102 hrHPV positive cervical scrapings of women participating in the POBASCAM trial (2,27).

Supplementary Figure 2. Reproducibility of the β-actin, CADM1-m12, CADM1-m18, MAL-m1, MAL-m2 qMSP assays.

Supplementary Figure 3. Prediction values of the sensitivity and specificity from the 10-fold cross validation and average ROC curve for the CADM1-m18/MAL-m1 marker panel.
Supplementary Table 1. Sensitivity, specificity, positive and negative predictive value for CIN2+ and referral percentage among hrHPV-positive women of the CADM1-m18/MAL-m1 marker panel at different cut-offs, cytology, and cytology combined with HPV16/18 positivity.
Table 1. Characteristics of the hrHPV-positive women with valid qMSP results of the validation cohort stratified for study endpoint.

<table>
<thead>
<tr>
<th>Validation cohort (n=236)</th>
<th>≤CIN1</th>
<th>CIN2</th>
<th>CIN3+</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>178 (75.4%)</td>
<td>20 (8.5%)</td>
<td>38 (16.1%)</td>
</tr>
<tr>
<td>Median age (range) *</td>
<td>40 (19-62)</td>
<td>34 (24-49)</td>
<td>36 (25-61)</td>
</tr>
<tr>
<td>median follow-up time (range) †</td>
<td>15 (1-36)</td>
<td>3 (1-33)</td>
<td>6 (0-33)</td>
</tr>
<tr>
<td>Cytology at baseline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>149 (63.1%)</td>
<td>7 (3.0%)</td>
<td>13 (5.5%)</td>
</tr>
<tr>
<td>Abnormal</td>
<td>29 (12.3%)</td>
<td>13 (5.5%)</td>
<td>25 (10.6%)</td>
</tr>
</tbody>
</table>

*) in years
†) in months
Table 2. Sensitivity, specificity, positive and negative predictive value for CIN3+ and referral percentage among hrHPV-positive women of the CADM1-m18/MAL-m1 marker panel at different cut-offs, cytology, and cytology combined with HPV16/18 positivity.

<table>
<thead>
<tr>
<th>Triage marker</th>
<th>Sensitivity (in total 38 CIN3+)</th>
<th>Specificity (in total 198 &lt;=CIN2)</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
<th>Referral %</th>
</tr>
</thead>
<tbody>
<tr>
<td>CADM1-m18 combined with MAL-m1 for thresholds:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>specificity ≥20%</td>
<td>100% (92.4-100)</td>
<td>22.7% (20.2-25.2)</td>
<td>19.9% (14.2-25.6)</td>
<td>100% (99.0-100)</td>
<td>80.9% (75.9-85.9)</td>
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<td>specificity ≥30%</td>
<td>97.4% (86.2-99.9)</td>
<td>32.3% (28.9-35.9)</td>
<td>21.6% (15.4-27.8)</td>
<td>98.5% (95.5-99.9)</td>
<td>72.5% (66.8-78.2)</td>
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<td>specificity ≥40%</td>
<td>86.8% (75.0-94.4)</td>
<td>43.4% (39.2-47.8)</td>
<td>22.8% (16.0-29.6)</td>
<td>94.5% (89.8-99.2)</td>
<td>61.4% (55.2-67.6)</td>
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<tr>
<td>specificity ≥50%</td>
<td>84.2% (72.0-92.7)</td>
<td>52.5% (47.7-57.3)</td>
<td>25.0% (17.5-32.5)</td>
<td>94.4% (90.1-98.7)</td>
<td>54.2% (47.8-60.6)</td>
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<tr>
<td>specificity ≥60%</td>
<td>81.6% (69.0-91.0)</td>
<td>62.6% (57.5-67.5)</td>
<td>29.5% (20.8-38.2)</td>
<td>94.7% (90.9-98.5)</td>
<td>44.5% (38.2-50.8)</td>
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<td>specificity ≥70%</td>
<td>68.4% (50.0-81.1)</td>
<td>75.3% (70.0-79.9)</td>
<td>34.7% (23.9-45.5)</td>
<td>92.5% (88.4-96.6)</td>
<td>31.8% (26.9-37.7)</td>
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<td>specificity ≥80%</td>
<td>60.5% (47.1-74.6)</td>
<td>83.3% (78.4-87.4)</td>
<td>41.8% (28.8-54.8)</td>
<td>91.7% (87.7-95.7)</td>
<td>23.3% (17.9-28.7)</td>
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<td>Cytology</td>
<td>65.8% (52.3-79.0)</td>
<td>78.8% (73.7-83.1)</td>
<td>37.3% (25.7-48.9)</td>
<td>92.3% (88.3-96.3)</td>
<td>28.4% (22.6-34.2)</td>
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<tr>
<td>Cytology combined with HPV16 and 18 genotyping</td>
<td>84.2% (72.0-92.7)</td>
<td>54.0% (49.2-58.7)</td>
<td>26.0% (18.2-33.8)</td>
<td>94.7% (90.6-98.8)</td>
<td>52.1% (45.7-58.5)</td>
</tr>
</tbody>
</table>
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