

Classification of high-grade CIN by p16^{ink4a}, Ki-67, HPV E4 and *FAM19A4/miR124-2* methylation status demonstrates considerable heterogeneity with potential consequences for management

Supplementary material

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† Prof. Karl Ulrich Petry passed away on April 21st, 2020

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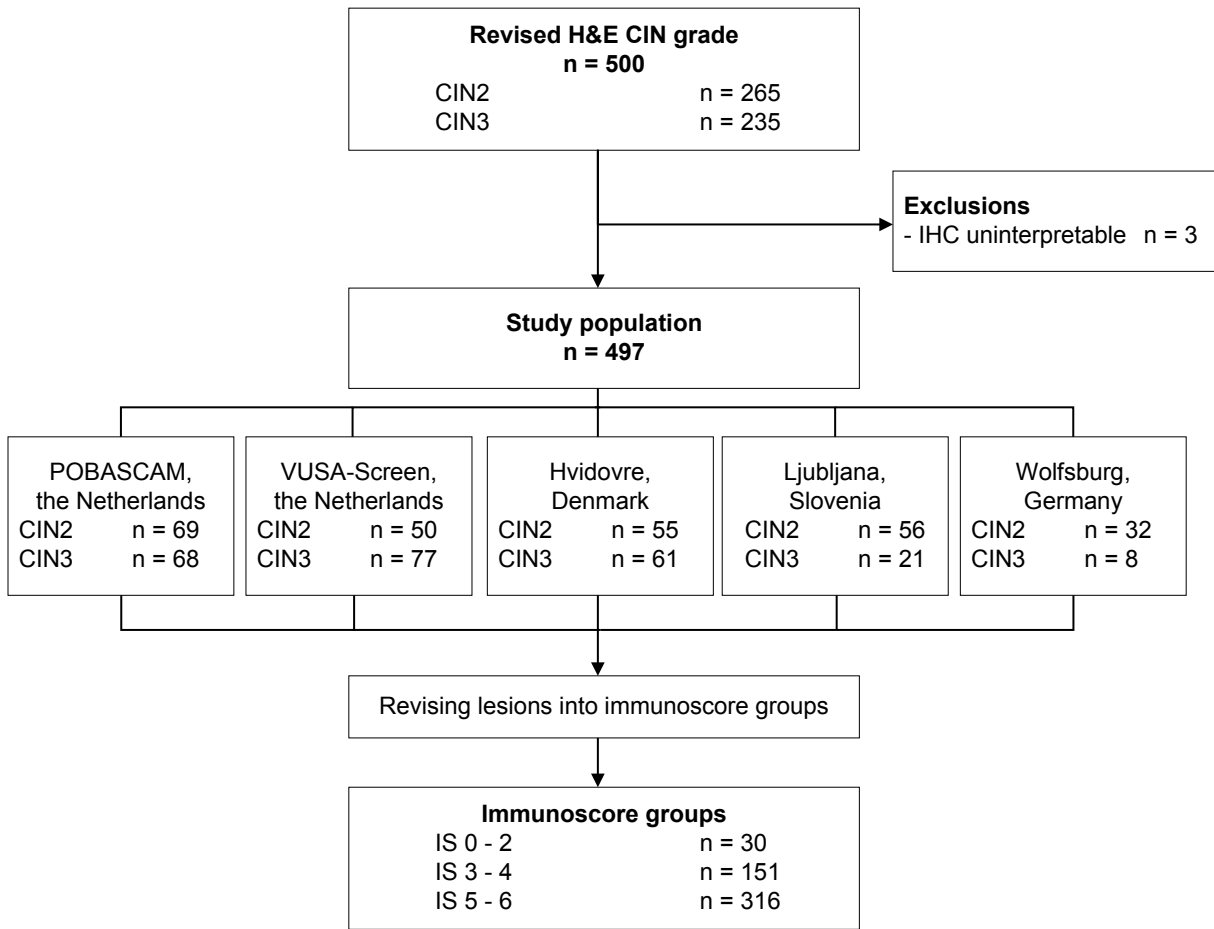
Supplementary Table 1. Cohort information by participating centre

Country of origin	the Netherlands		Denmark	Slovenia	Germany
Laboratory of origin	Department of Pathology Amsterdam UMC, VU University Medical Center, Amsterdam		Department of Pathology, Hvidovre Hospital, Denmark	Institute of microbiology and Immunology, University of Ljubljana, Slovenia	Department of Obstetrics and Gynecology, Klinikum Wolfsburg, Germany
Cohort origin	POBASCAM study* ³¹	VUSA-screen study* ³²	Routine national screening program	the Slovenian HPV prevalence study ³³ and routine screening samples	the Wolfsburg primary HPV screening project WOLPHSCREEN ³⁴ and routine screening samples
Number of included samples	137	127	116	77	40
Mean age in years (range)	36.0 (29 - 55)	37.4 (29 - 60)	32.6 (21 - 64)	35.6 (21 - 76)	33.3 (22 - 51)
Cervical scrape collection	Screening samples that led to colposcopy referral			Screening sample that led to colposcopy referral or additional sample taken before colposcopy	
Cervical scrape collection media	PBS with 0.5% thiomersal	UCM	SurePath	ThinPrep	ThinPrep
DNA extraction platform	Hamilton Star Robot	Hamilton Star Robot	MagNApure 96	Biorobot EZ1 [#]	Manual isolation
DNA extraction kit	NucleoMag tissue	NucleoMag tissue	Roche DNA & VIRAL NA	QIAamp DNA mini [#]	QIAamp DNA mini
HPV assay	GP5+/6+ PCR-enzyme immunoassay	HC2 (QIAGEN)	Onclarity (BD) and CLART HPV2 (Genomica)	RealTime High Risk HPV Assay (Abbott)	HC2 (QIAGEN)
Methylation test	Prototype version of QIASure methylation test	QIASure methylation test®	QIASure methylation test®	QIASure methylation test®	QIASure methylation test®

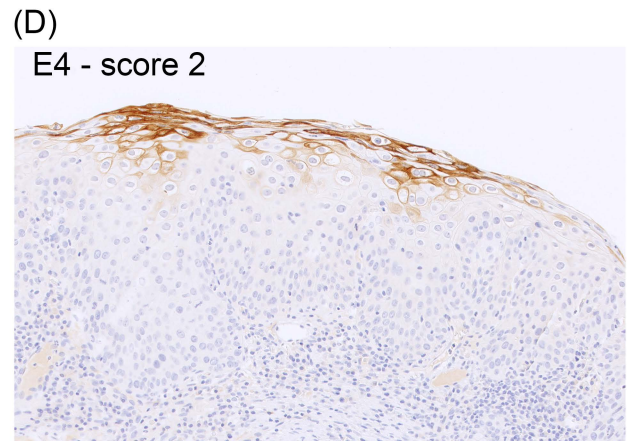
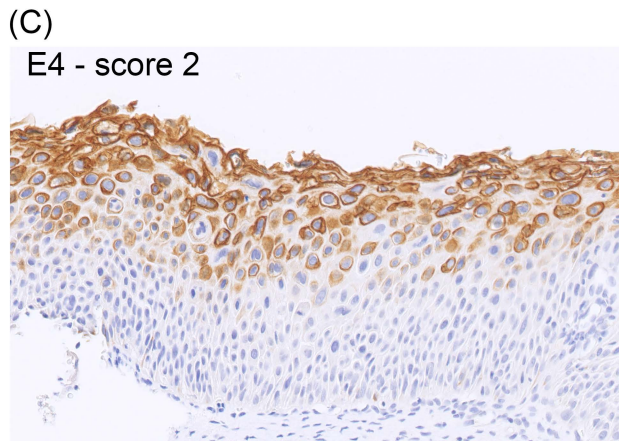
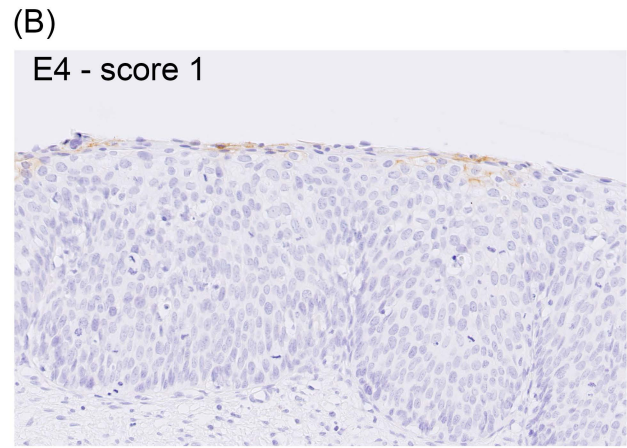
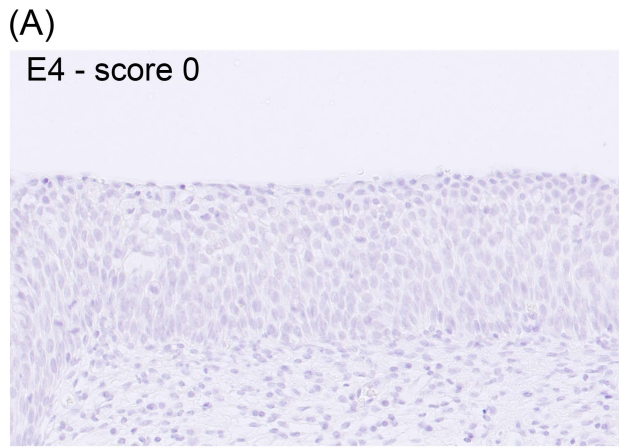
* FFPE material from the POBASCAM and VUSA-screen trials was localized and retrieved from the local histopathological laboratories through the nationwide network and registry of histo- and cytopathology in the Netherlands (PALGA)³⁰.

[#] Extraction was either done automatically with the Biorobot EZ1 (QIAGEN) or manually using the QIAamp DNA mini kit.

Abbreviations: PBS: Phosphate-Buffered Saline, UCM: Universal Collection Medium, HC2: Hybrid Capture 2



Supplementary Figure 1. Study flowchart. Abbreviations: CIN: cervical intraepithelial neoplasia, IHC: immunohistochemistry, FFPE: formalin-fixed paraffin embedded, H&E: haematoxylin-eosin, IS: immunosome.



Supplementary Figure 2.

Scoring of HPV E4 immunostaining.

E4 staining is scored as negative staining (score 0) in (A), focally positive staining (limited staining of some cells restricted to the superficial layer of the epithelium, score 1) in (B), extensive widespread membranous and/or cytoplasmic staining in the superficial layers of the epithelium extending to half of the epithelial width, score 2) in a methylation-negative lesion in (C) and extensive mainly membranous staining in the superficial epithelial layers of a methylation-positive lesion in (D).