Evaluation of cervical screening strategies using methylation markers in WLHIV

CJLM Meijer
Em. professor of pathology
Dept of Pathology
Amsterdam UMC, location VUMC
Amsterdam

cjlm.meijer@amsterdamumc.nl

Cervical cancer and HIV

- Cervical cancer incidence is six-fold greater among women living with HIV (WLHIV) [1]
  - Cervical cancer is a leading cause of cancer death in WLHIV [2]
- Disproportionally high burden of cervical cancer and HIV in developing countries
  - 85% of all cervical cancer cases [3]
  - 95% of global HIV infections [2]
- Effective cervical screening is uncommon in low and middle-income countries (LMIC) [4]
  - Low coverage, suboptimal screening tools, limited access to health care

➢ Need for objective screening tools, with high sensitivity and high specificity for cervical cancer and advanced cervical precursor lesions

1. Grulich, Lancet 2007; 2. Joint United National Program on HIV/AIDS (UNAIDS). The Gap Report. 2014;
3. GLOBOCAN 2012; 4. Gakidou, PLoS Med. 2008.

- What do we know about methylation markers?

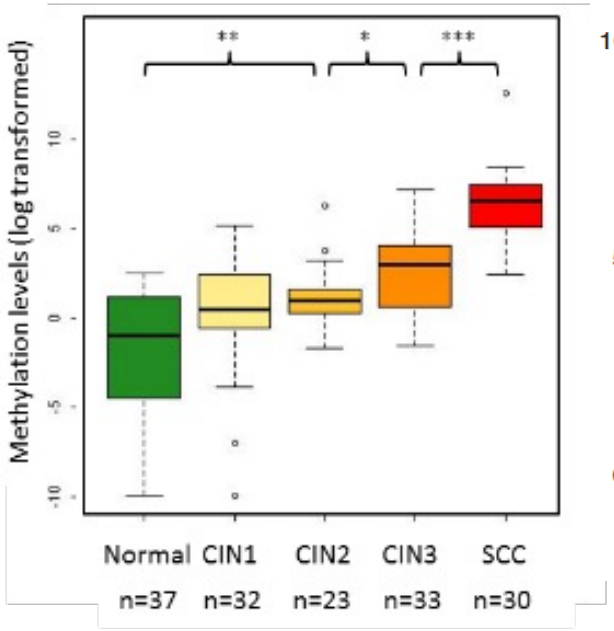
Methylation levels of genes involved in cervical carcinogenesis detect “advanced CIN lesions” and can be used as markers for progressive CIN disease

- Methylation-mediated silencing of tumor suppressor genes is involved in cervical carcinogenesis[1,2]
- Methylation levels of certain methylation markers i.e *FAM19A4, miR124-2, ASCL1, LHX8,CADM1, MAL, EPB41L3* increase with severity of underlying CIN lesion and duration of associated HPV infection (FAM19A4, CADM1) [3,4,6]
- Methylation analysis of these genes consistently identifies cervical cancer and CIN2-3 lesions with a longstanding (>5 years) persistent HPV infection, ie*. advanced lesions* [3-5]
- Methylation positive CIN lesions are mainly non-productive (HPV-E4 neg), transforming (p16/Ki-67 pos cervical lesions [6]
- FAM19A4/miR124-2 negative CIN lesions have a high regression rate[7]

1. Steenbergen, Nature Reviews Cancer 2014; 2. Wentzensen, Gynecol Oncol. 2009. 3. De Strooper, J. Clin. Pathol. 2014; 4. De Strooper, Cancer Prev. Res. 2014; 5.Luttmer, IJC 2015 6. Vink Int.J cancer 2021. 7. Kremer JCO 2022

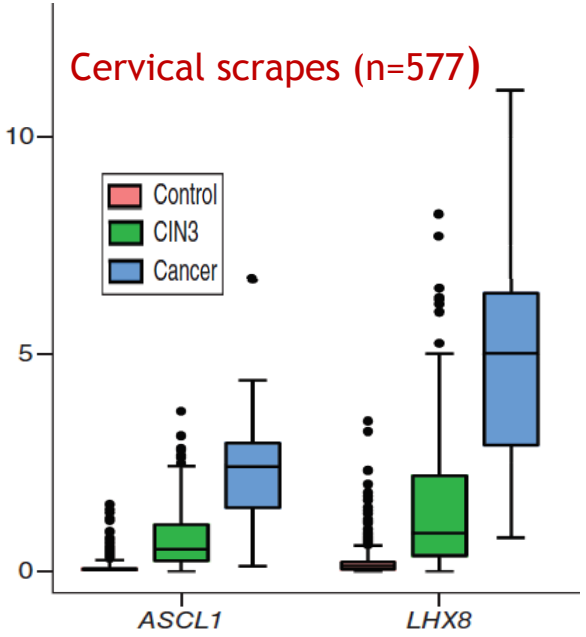
Methylation levels increase with CIN grade and are very high in CxCa

Cervical tissues (n=155)



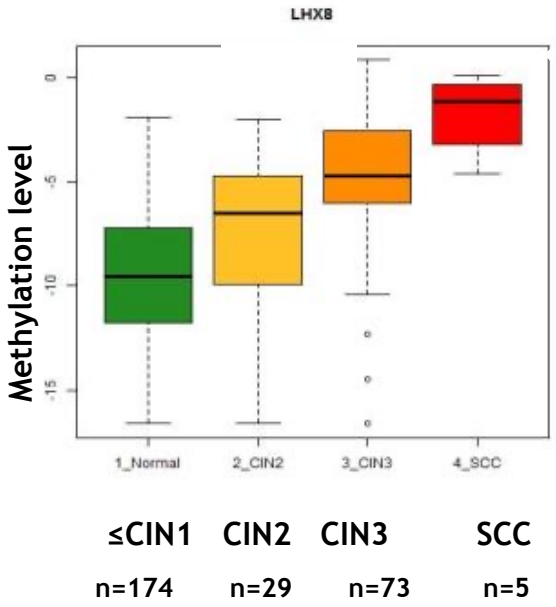
Verlaat *et al.*
Clinical Cancer Research 2017

Cervical scrapes (n=577)



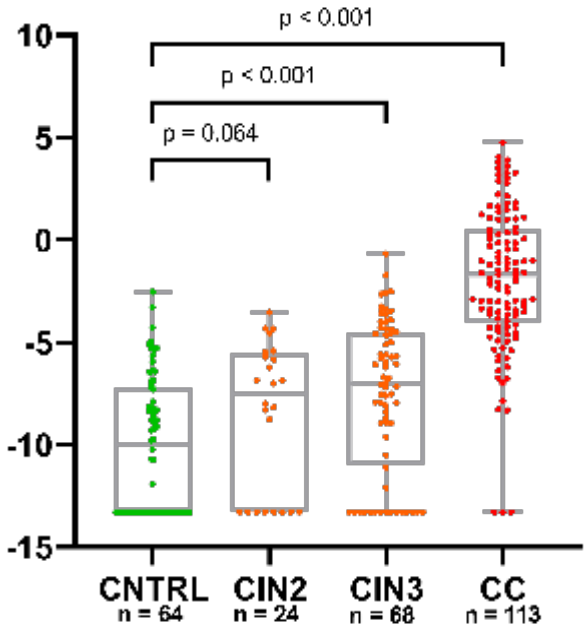
Dick, Verhoef *et al.* Epigenomics 2020

Cervicovaginal brush



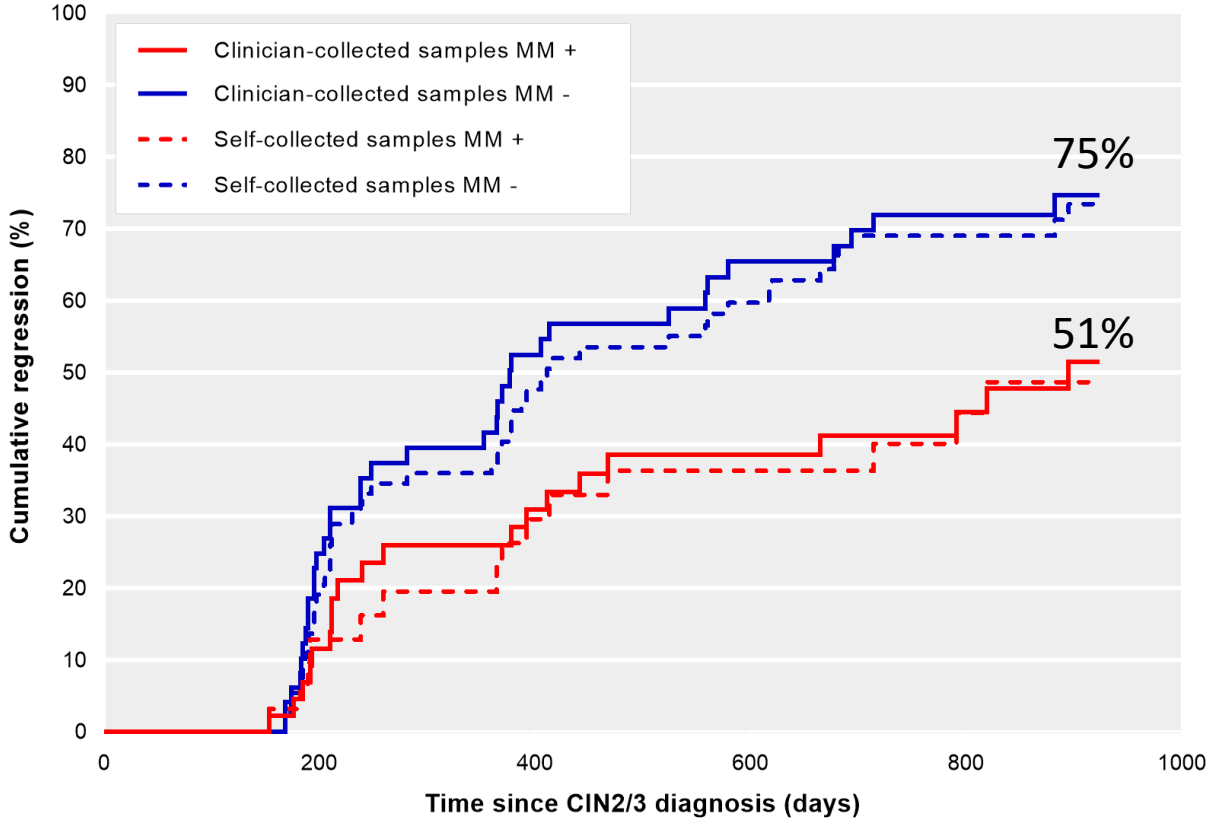
Verlaat et al. CCR 2018

urine



Van den Helder et al. CCR 2022

Regression analysis: Methylation



→ FAM19A4/miR124-2 M-negative CIN2/3 showed more regression compared to FAM19A4/miR124-2 M-positive CIN2/3 (p=0.013)

→ Result
Cervical scrapes = self collected samples

See: Use of methylation markers in guidance for CIN2/3 management | Eurogin 2022 Düsseldorf | S. Dick Tuesday 12 April CS-05 room14

Kremer and Dick JCO in press

Background and aim of the studies in SA:

- B: Presently opportunistic screening is done by cytology (threshold≥HSIL) and women with ASC-US are asked to come back.
- No recall system. Loss to F-up high.

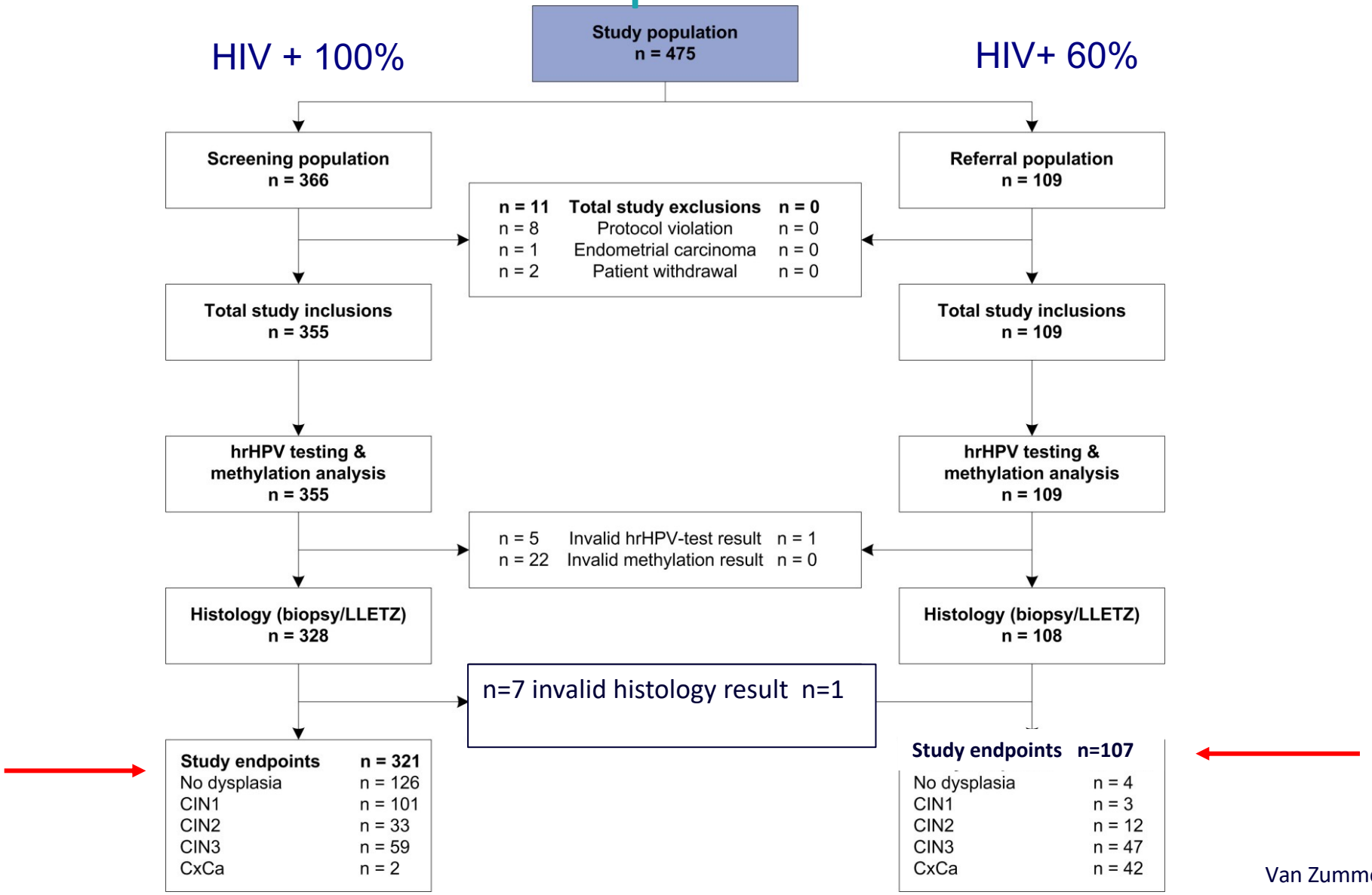
- Q: To evaluate new molecular strategies with high sensitivities and good specificities which can be implemented in SA

- M: HPV test with partial genotyping and Methylation marker testing

Methylation assays and markers involved

- QIAsure®
  Genes: *FAM19A4/miR124-2.* Assay: qMSP multiplex.
- Gyntect®
  Genes: *AST1, DLX1, ITG4, RXFP3, SOX17, ZNF671* and 2 controls. Assay: qPCR.
- S5 classifier
  Genes: EPB41L3, HPV16L1.3, HPV16L2, HPV18L2, HPV31L1, HPV33L2. Assay: pyrosequencing.
- Care Me
  Genes: EPB41L3, HPV16 and HPV18. Assay: pyrosequencing.
- Condifence marker™
  Genes: POU4F3. Assay: qMSP.
- Cervi-M®
  Genes: PAX1. Assay: qMSP.

1a. Study population in Steve Biko Academic district Hospital and Tswane Hospital Pretoria



Van Zummeren 2017

CADM1, MAL, mir124-2 DNA methylation marker analysis in total population (n=428, screening n=321, referrals n=107)

DNA methylation scored positive if at least 1 of 3 markers was above the set threshold

	CIN0		CIN1		CIN2		CIN3		SCC/AdCa		Total	
DNA methylation	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Negative	92	70%	65	62%	18	40%	22	21%	0	0%	197	46%
Positive	39	30%	40	38%	27	60%	83	79%	42	100%	231	54%

- DNA methylation scored positive if at least 1 of 3 markers was above the set threshold
- 79% of CIN3 are methylation positive
- 30% of CIN0 and 38% of CIN1 are methylation positive

- *Methylation positivity increases with CIN grade, all carcinomas test methylation positive*

Van Zummeren AIDS 2017

Performance of HPV testing, **CADM1, MAL en miR124-2 methylation** in screening population of WLHIV (n=321)

Screening method	CIN3+ sensitivity[a]	95%CI	*n1/N1*	CIN3+ specificity	95%CI	*n2/N2*
Cohort of WLHIV (*n* = 321)						
HrHPV testing	83.6%	74.3–92.9	51/61	67.7%	62.0–73.4	176/260
Methylation analysis	85.2%	76.3–94.1	52/61	49.6%	43.5–55.7	129/260
HrHPV testing with reflex methylation analysis	73.8%	62.7–84.8	45/61	81.5%	76.8–86.3	212/260

n1: number of screen pos disease cases, N1: total number of disease cases

n2:number of screen negative non-disease cases; N2: total number of non-disease cases

- *Full molecular screening is feasible: HPV testing with methylation triage testing detects all carcinomas with acceptable CIN3+ sensitivity and specificity*

Van Zummeren AIDS 2017

1b. Study cohort Tswane Hospital, Pretoria, SA
FAM19A4/miR124-2 methylation performance

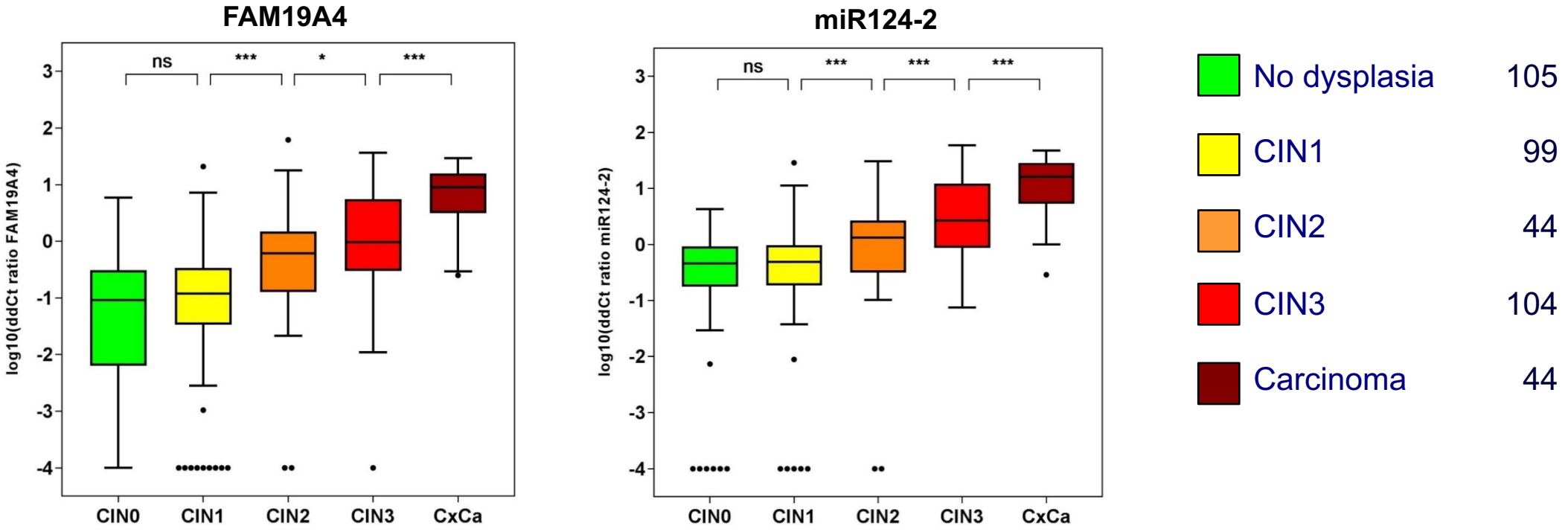
- 396 samples from a prospective observational cohort study from Pretoria, South Africa, were used [1]:
  - 289 WLHIV who were *invited for cervical screening*,
    HPV pos : 42% (n=135)
    24% (n=30) no dysplasia; 31% (n=31) CIN1; 70% (n=23)CIN2; 83% (n=49) CIN3; 100% (n=2) CxCa
  - 107 women (60% HIV+) *from a gynaecological referral population*

- High-risk HPV status and histologic endpoint were available for all subjects

- Bisulphite converted DNA from cervical scrapes collected from all patients was tested for DNA methylation of FAM19A4 and miR124-2 by the QIAsure Methylation Test®

Kremer AIDS 2019.

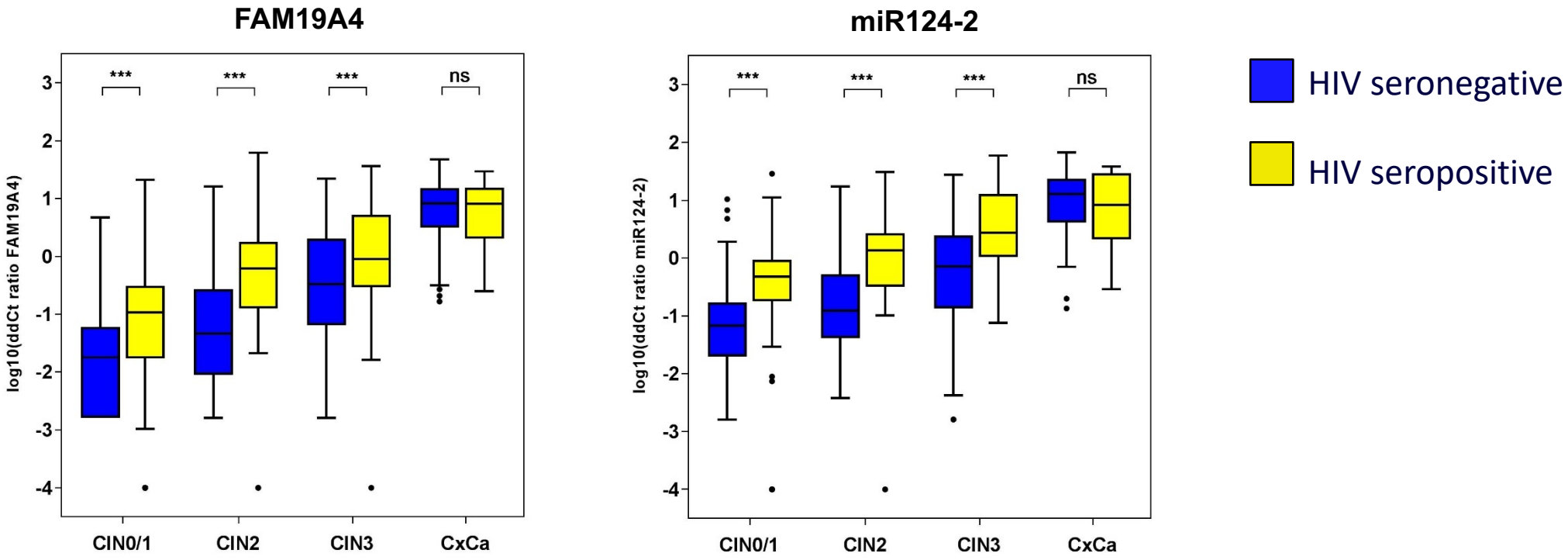
Methylation levels in the total study population (n = 396)



➢ *Methylation levels increase with severity of the underlying cervical disease*

Kremer AIDS 2019

Influence of HIV-status on methylation levels



- *Methylation levels are higher in HIV+ women compared to HIV- women for all CIN grades, except for women with cervical carcinomas*

Kremer AIDS 2019

Table 1. Accuracy and diagnostic efficiency of screening strategies to detect cervical intraepithelial neoplasia grade 3 or worse.

No.	Strategy	Sensitivity (95% CI)	*n1/N1*	Specificity (95% CI)	*n2/N2*	PPV	NPV	Referral rate	Referrals needed to detect one CIN3+	Number of tests/1000 women screened
Cytology-based screening										
1	Cytology (≥HSIL)	59.3% (46.8–71.9)	35/59	91.6% (88.0–95.2)	207/226	64.8%	89.6%	18.9%	1.5	1000
2	Cytology (≥HSIL) with *FAM19A4/miR124-2* triage of ASC-US/LSIL	67.8% (55.9–79.7)	40/59	85.0% (80.3–89.6)	192/226	54.1%	91.0%	26.0%	1.9	1095
3	*FAM19A4/miR124-2* with cytology (≥ASC-US) triage	62.7% (50.4–75.1)	37/59	87.2% (82.2–91.5)	197/226	56.1%	90.0%	23.2%	1.8	1674
HPV-based screening										
4	HPV	83.1% (73.5–92.6)	49/59	66.4% (60.2–72.5)	150/226	39.2%	93.8%	43.9%	2.6	1000
5	HPV with *FAM19A4/miR124-2* triage	72.9% (61.5–84.2)	43/59	76.1% (70.5–81.7)	172/226	44.3%	91.5%	34.0%	2.3	1440
6	HPV16/18 with *FAM19A4/miR124-2* triage of non16/18HPV+	79.7% (69.4–89.9)	47/59	74.8% (69.1–80.4)	169/226	45.2%	93.4%	36.5%	2.2	1315

n1: number of screen pos disease cases, N1: total number of disease cases

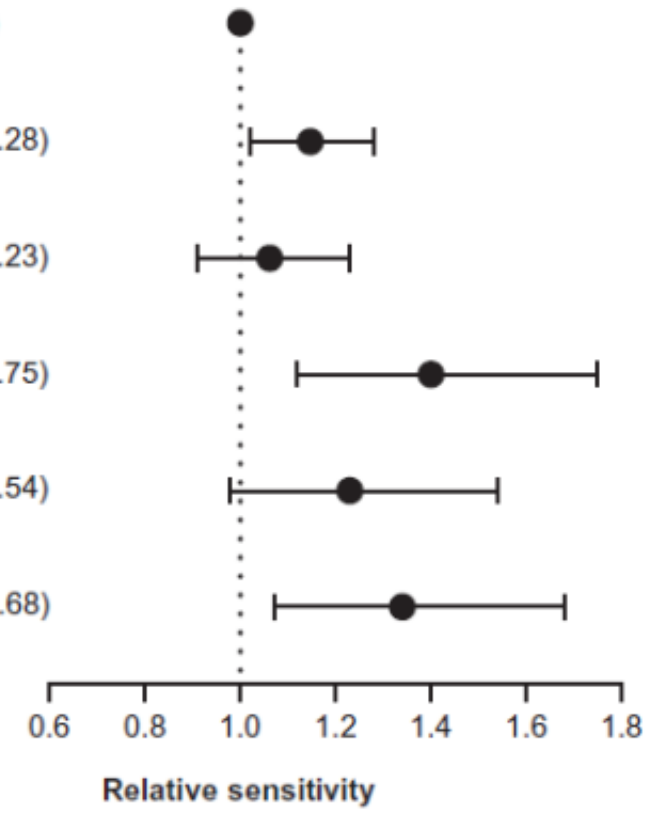
n2:number of screen negative non-disease cases; N2: total number of non-disease cases

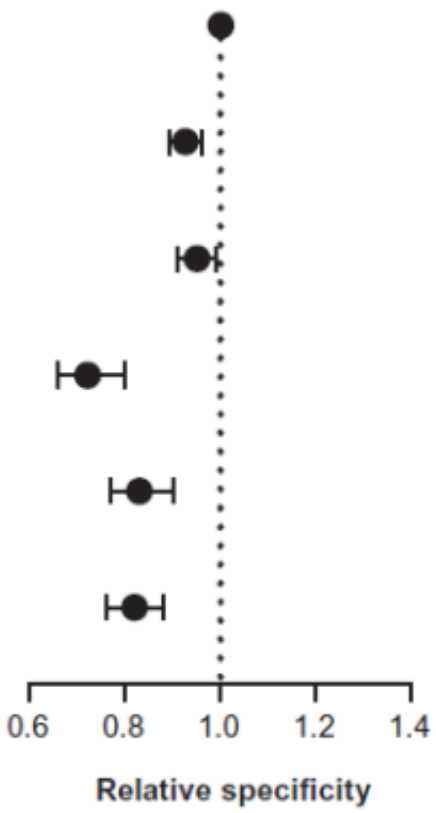
- *Cytology strategies have lower sens. and higher spec than HPV strategies*
- *Cytology (≥HSIL)with metyhylation triage testing of ASC-US has good accuracy*
- *HPV testing with methylation triage testing or HPV testing with triage by HPV 16/18 + and methylation testing of non-16/18 HPV perform well*

Kremer AIDS 2019

Relative sensitivity and specificity of **FAM19A4/miR124-2** for the detection of CIN3+ of different screening strategies in WLHIV compared to cytology (threshold ≥ HSIL)

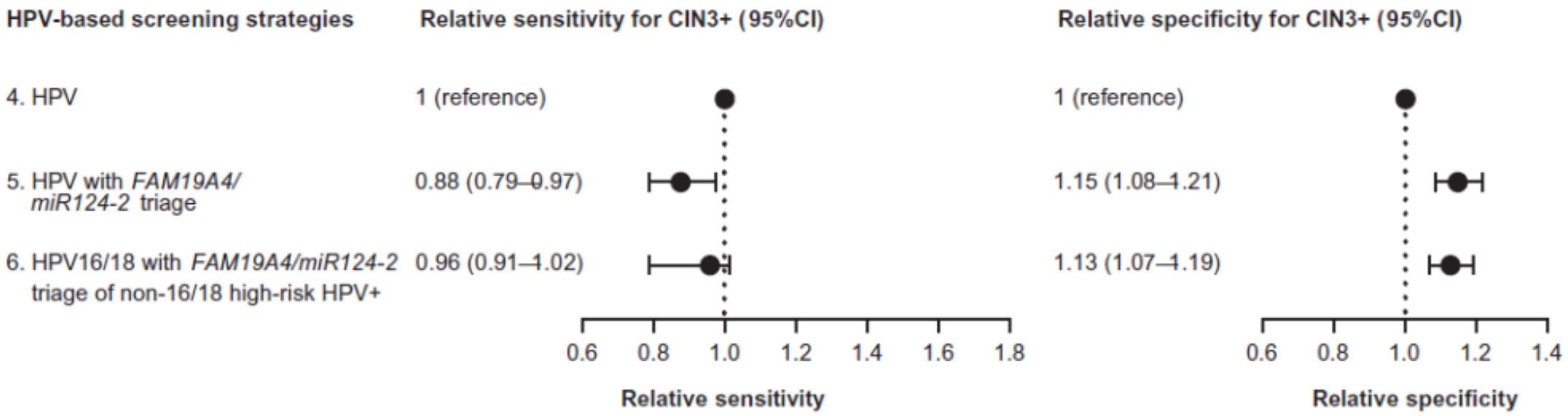
Screening strategies	Relative sensitivity for CIN3+(95%CI)	Relative specificity for CIN3+(95%CI)
1. Cytology (≥HSIL)	1 (reference)	1 (reference)
2. Cytology (≥HSIL) with *FAM19A4/miR124-2* triage of ASC-US/LSIL	1.14 (1.02–1.28)	0.93 (0.89–0.96)
3. *FAM19A4/miR124-2* with cytology (≥ASC-US) triage	1.06 (0.91–1.23)	0.95 (0.91–0.99)
4. HPV	1.40 (1.12–1.75)	0.72 (0.66–0.80)
5. HPV with *FAM19A4/miR124-2* triage	1.23 (0.98–1.54)	0.83 (0.77–0.90)
6. HPV16/18 with *FAM19A4/miR124-2* triage of non-16/18 high-risk HPV+	1.34 (1.07–1.68)	0.82 (0.76–0.88)





Kremer AIDS 2019

Relative sensitivity and specificity of **FAM19A4/miR124-2** for the detection of CIN3+ of different screening strategies in WLHIV compared to HPV testing (threshold ≥ HSIL)



Kremer AIDS 2019

Conclusion I

- Methylation levels In WLHIV are significantly higher compared to HIV negative women.
- In both WLHIV and HIV neg women methylation levels of FAM19A4 and miR124-2 increase with increasing CIN grade

- Strategies based on molecular methods (HPV alone or HPV with molecular triage) have higher sensitivities and acceptable specificity compared to cytology

- These strategies have equal sensitivity with higher specificity compared to sole HPV testing resulting in significantly less referrals than sole HPV testing

- All cervical carcinomas in the screening population (n = 2) and in the gynaecological referral population (n = 42) tested positive for QIAsure® Methylation Test and CADM1,MAL/miR124-2

➢ *HPV testing followed by Qiasure® triage is a feasible cervical screening strategy for LMIC*

2. DiaVACCS study[1]

- *Post hoc* analysis of *ASCL1* and *LHX8* methylation within the HIV-positive women of the DiaVACCS study

- A South African observational multicentre cohort study designed to evaluate primary HPV testing and several triage algorithms for cervical cancer screening in South Africa

1. Dreyer *et al*. S Afr Med J 2022

Study population

- 411 HIV-positive women
- Recruited from public outpatient clinics and ART clinics
- Methylation analysis on DNA isolated from cervical scrapes
- Comparison of
  primary methylation screening with
  primary HPV testing and
  primary cytology testing

Table 1. Baseline study population characteristics

	n	%
Cytology		
NILM	243	59,1%
ASC-US	13	3,2%
LSIL	27	6,6%
AGUS	1	0,2%
ASC-H	17	4,1%
HSIL	87	21,2%
Ca/suspicious/malignant cells	9	2,2%
Inadequate	14	3,4%
Histology		
Lost to follow-up	22	5,40%
No histology, double screen negative	79	19,2%
No CIN	88	21,4%
CIN1	73	17,8%
CIN2	71	17,3%
CIN3	71	17,3%
Squamous cell carcinoma	7	1,7%
HPV		
Positive	199	48,4%
HPV16/18 positive	72	17,5%
Non-1618 positive	127	30,9%
Negative	212	51,6%
Origin of sample		
Tshwane	328	79,8%
Cape Town	31	7,5%
Kalafong	52	12,7%
Median age in years (range)	40	(25 - 64)
Total	**411**	**100%**

Study population

- 411 HIV-positive women
  - 79 no histology endpoint
  - 88 no CIN
  - 73 CIN1
  - 71 CIN2
  - 71 CIN3
  - 7 squamous cell carcinoma

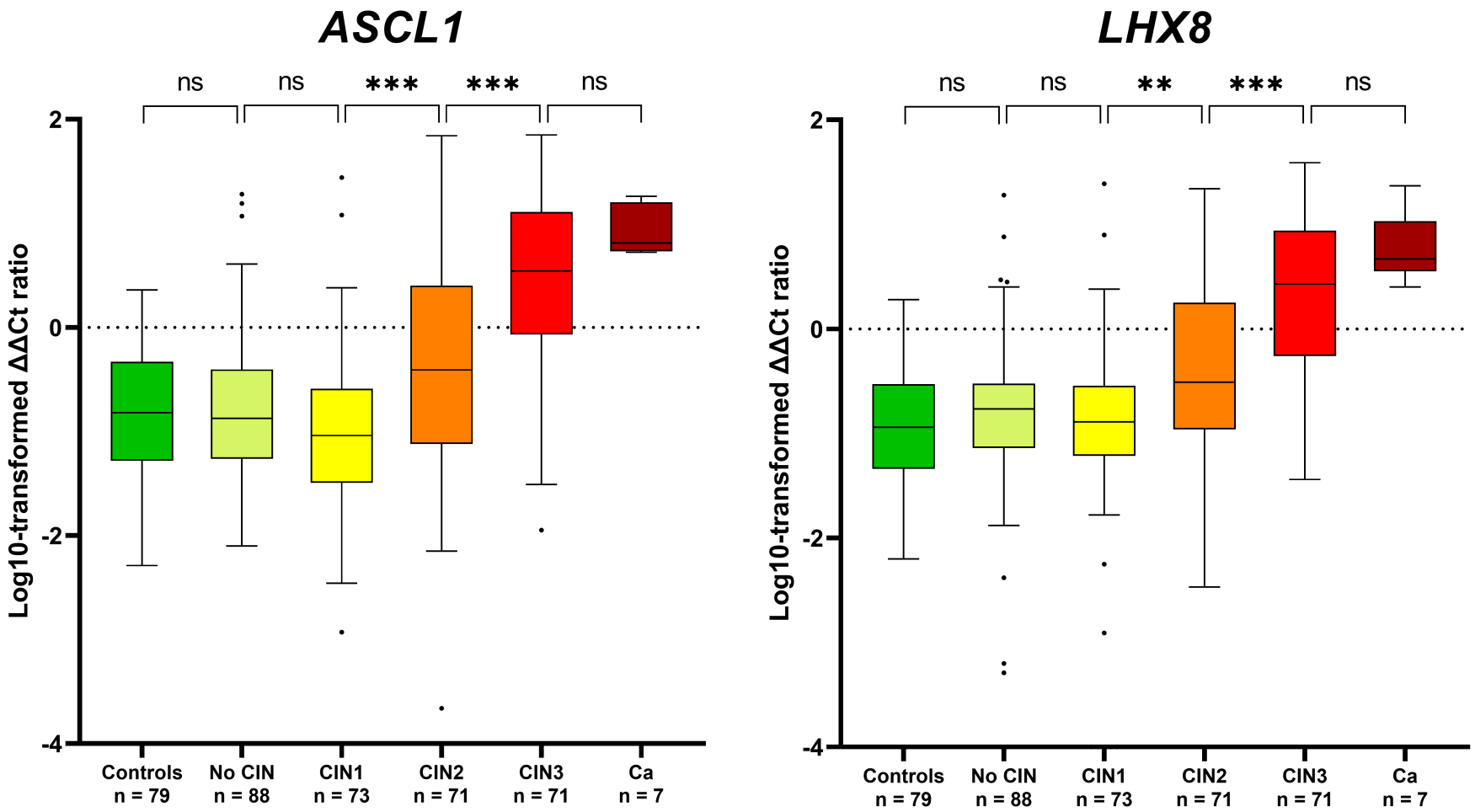
% HPV positivity

36.4%
27.4%
80.3%
93.0%
100%

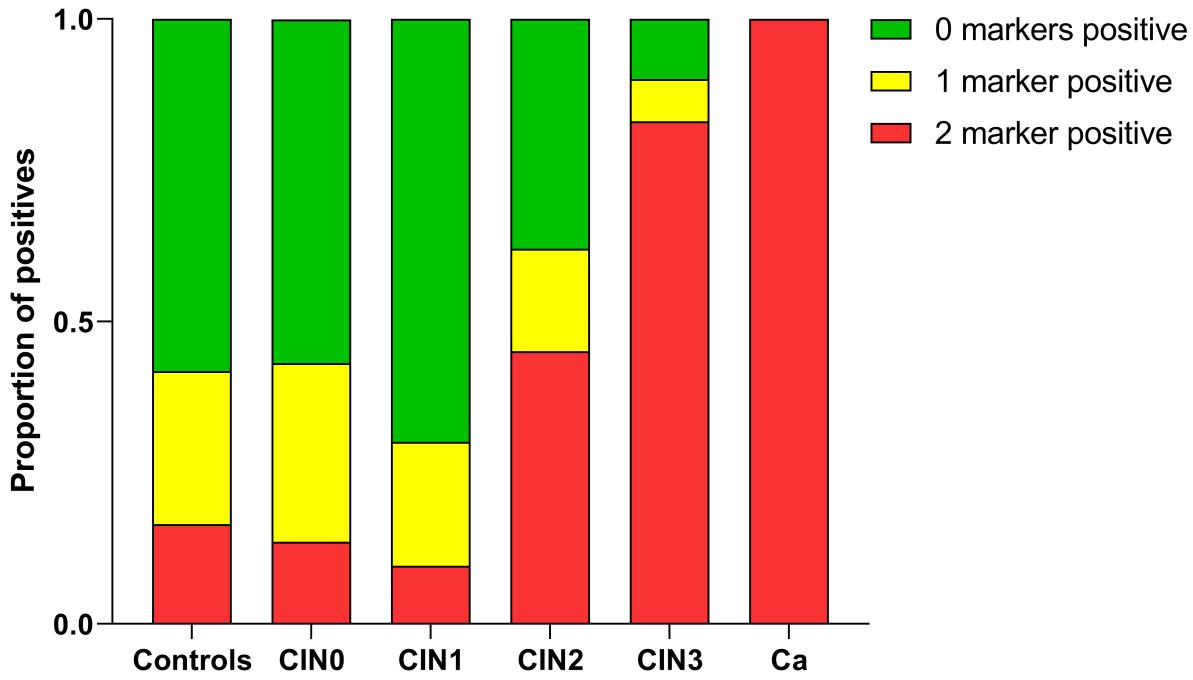
- Methylation analysis on DNA isolated from cervical scrapes

- Comparison of primary methylation screening with primary HPV testing and primary cytology testing

Methylation levels



Methylation levels increase with disease severity



The proportion of both markers testing positive increases with disease severity

Performance CIN3+

	Strategy	Positivity in Ca	Positivity in CIN3	Positivity in CIN2	Sensitivity CIN3+ (95% CI)	Specificity ≤CIN1 (95% CI)	PPV CIN3+	NPV CIN3+	Referral rate
1	*ASCL1*	100.0%	84.5%	53.5%	85.9% (78.2 - 93.6)	72.9% (67.3 - 78.5)	39.4% (21.3 - 46.8)	95.0% (92.1 - 97.9)	43.7%
2	*LHX8*	100.0%	88.7%	53.5%	89.7% (83.0 - 96.5)	75.0% (69.5 - 80.5)	41.7% (34.2 - 49.1)	96.4% (93.9 - 98.8)	43.2%
3	*ASCL1* and *LHX8*	100.0%	83.1%	45.1%	84.6% (76.6 - 92.6)	86.7% (82.4 - 91.0)	50.8% (42.2 - 59.4)	95.4% (92.8 - 97.9)	33.4%
4	HPV	100.0%	93.0%	80.3%	93.6% (88.2 - 99.0)	78.3% (73.1 - 83.5)	40.1% (33.0 - 47.2)	97.6% (95.5 - 99.7)	46.8%
5	Cytology* (≥HSIL)	100.0%	71.4%	40.6%	74.0% (64.2 - 83.8)	91.0% (87.3 - 94.7)	53.8% (44.3 - 63.3)	92.7% (89.6 - 95.8)	28.0%

ASCL1 and *LHX8* combination (Strategy #3)

- High sensitivity (84.6%) and high specificity (86.7%), no carcinomas missed

Conclusions

- Combined analysis of *ASCL1* and *LHX8* is objective full molecular strategy applicable on cervical scrapes and cervicovaginal self-samples

- Useful alternative to primary cytology or primary HPV screening, without the need for triage testing

Conclusions

- Methylation markers (FAM19A4/miR124-2, ASCL1/LHX8) can be used on cervical scrapes for CxCA screening in WLHIV. They have very high sensitivity for CxCa and advanced CIN2/3

- Dependent on the methylation markers used they can be used for primary (ASCL1/LHX8) or secundary screening (FAM19A4/miR124-2, ASCL1/LHX8) with high sensitivity and good specificity. *Full molecular screening*

- Methylation markers for CxCa screening can be used on Self-collected vaginal- and urine samples *but validation is key,* since Methylation markers, which perform well on scrapes often do not perform equally well on self-samples

- Results of a given Methylation marker on self-samples or urine should be

- Automation of bisulphite conversion will facilitate CxCa screening in WLHIV and is ongoing

Acknowledgements

VU University Medical Center

Dep. of (Molecular) Pathology

Wieke Kremer

Marjolein van Zummeren

Frederique Vink

Renske Steenbergen

Daniëlle Heideman

Wina Verlaat

Putri Novianti

All research staff and technicians of the unit of Molecular Pathology Vumc

University of Pretoria, South Africa

Dep. of Obstetrics and Gynaecology

Greta Dreyer

MC van Aardt

Judy van Aardt

Erika Breytenbach

Brenda Pethla

ARV Clinic Tshwane District Hospital

Tabitha Motsei

Dep. of Virology

Karin Richter

Stellenbos University

M.Hendrk.Botha

Acknowledgements

All collaborators, general practitioners, pathologists, clinicians, and participants in the studies

Amsterdam UMC location VUmc

Department of Pathology

- Maaike Bleeker
- Danielle Heideman
- Renske Steenbergen
- Peter Snijders †
- HPV research team members

Department of Clinical Epidemiology and Data Science

- J. Berkhof
- Data Science research team members


thank you
danke
謝謝
gracias
merci
obrigado
dziękuję
bedankt
teşekkür ederim
go raibh maith agat
mochchakkeram
tapadh leat
ngiyabonga
спасибо
рахмат
Баярлалаа
dank je
hvala
mauruuru
sukriya
kop khun krap
terima kasih
grazie
arigatô
takk
dakujem
merci
мерси
ευχαριστώ
감사합니다
xièxie
dankie
kiitos
faafetai lava
vinaka
blagodaram
misaotra
matondo
paldies
grazzi
mahalo
barka
welalin
tack
mersi
spas
kia ora
tapadh leat
хвала
asante
manana
obrigada
murakoze
tanki
chokran
mamnun
дякую
djere dieuf
tau
akun
dankon
aciu
gracias
sulpáy
chnorakaloutioun
gratias ago
dekuji
sobodi
mesi
sagolun
najis tuke
rahmat
tanemirt
rahmet
dhanyavadagalu
shukriya
merce
trugarez
diolch
enkosi
bayarlalaa
gracie
nandri
nanni
dhanyavad
köszönöm
ありがとう
তোমাকে ধন্যবাদ
kam sah hamnida
didi madloba