Cervical Pre-Cancer vs Invasive Cancer: Molecular Differentiation with Potential to Improve Cervical Cancer Screening Worldwide

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Background

- Cervical cancer is the 4th most common cancer in in women[1].

- Persistent infection with high-risk human papillomavirus (hr-HPV) is an important co-factor in carcinogenesis and is associated with DNA methylation on both human and viral genes[2].

- Overtreatment of women who may otherwise clear infection is a problem in current screening programs.

- Cervical intraepithelial neoplasia (CIN) describes the degree of abnormality, with CIN1, CIN2 and CIN3 being classified as the pre-cancer stages. CIN3 is the most severe diagnosis before progression to cancer.

- The S5 DNA methylation classifier is based on target CpG sites of the human gene *EPB41L3*, and viral late gene regions of hr-HPV16, 18, 31 and 33[3,4].

Objectives

Test the performance of the S5 classifier to:

- Detect the high-grade disease samples;
- Quantify the degree of separation between histology negative, CIN3, cancer stages;
- Analyze classifier components individually.

Methods

Methylation status of the S5 selected CpG sites was tested in DNA extracted from exfoliated cervical cells from women diagnosed with: histology negative (Neg), CIN3, invasive cervical cancer FIGO stage I (CSI), stage II (CSII), stage III (CSIII) and stage IV (CSIV).

DNA-bisulfite conversion was carried out and followed by pyrosequencing for the 6 components of S5. Average methylation was calculated for each marker to define the S5 score.

Country	Neg N=220	CIN3 N=206	CSI N=256	CSII N=256	CSIII N=26	CSIV N=20
Bhutan	10	-	28	22	-	-
Colombia	25	50	21	25	-	-
Ethiopia	54	-	9	19	26	20
Georgia	-	-	40	2	-	-
India	10	-	12	38	-	-
Philippines	-	-	15	35	-	-
South Africa	-	-	2	48	-	-
Spain	21	50	28	22	-	-
UK	50	56	57	1	-	-
USA	50	50	44	44	-	-

Results
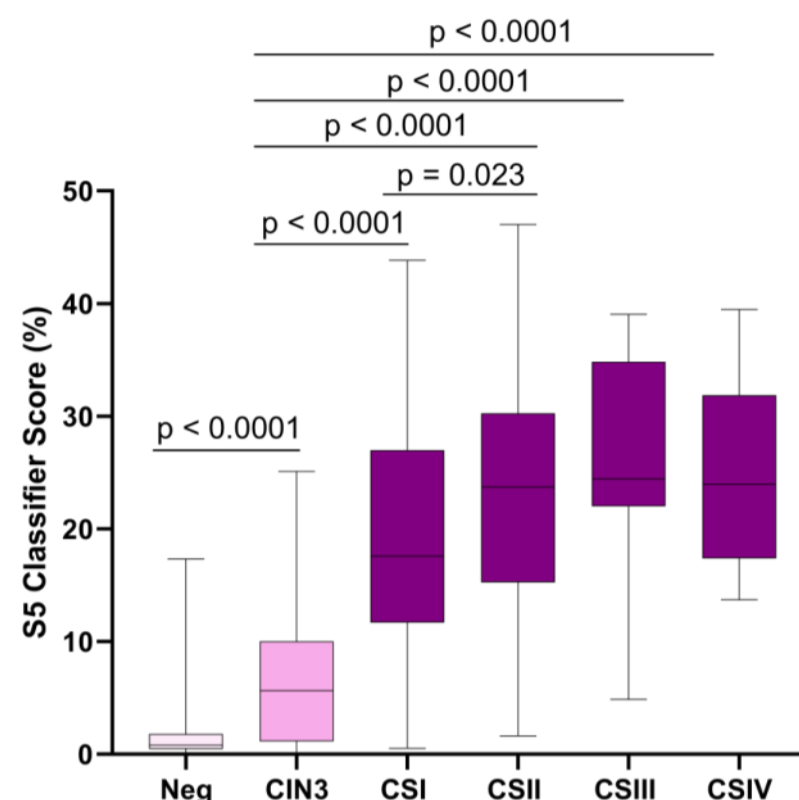
**S5 Classifier score distribution**

- S5 methylation score increased with disease severity with a Cuzick test for trend of z = 9.29 (p < 0.0001)

- CIN3 and cancers differentiation from histology negative samples was highly significant (Mann Whitney test, all p < 0.0001)
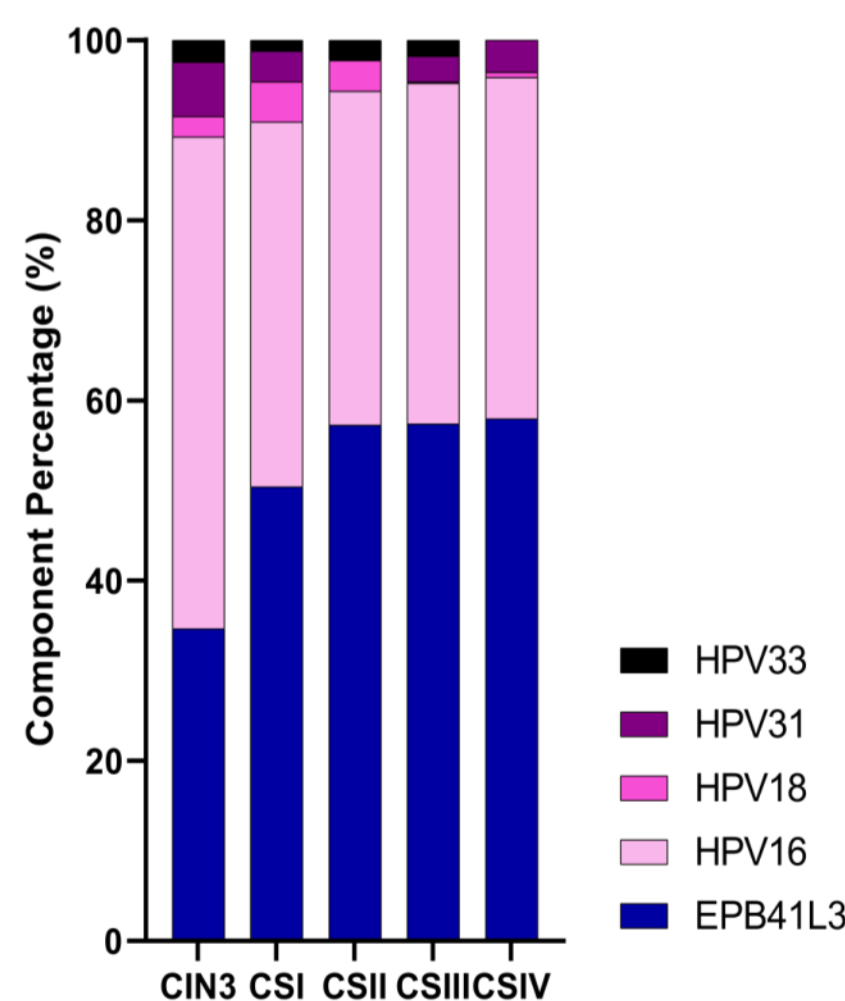
S5 component breakdown in cancers

- Host *EPB41L3* methylation increased with disease severity and plateau at CSII (Mann Whitney test, all p < 0.0001)

- HPV16 methylation decreases (Mann Whitney test, all p < 0.0001)
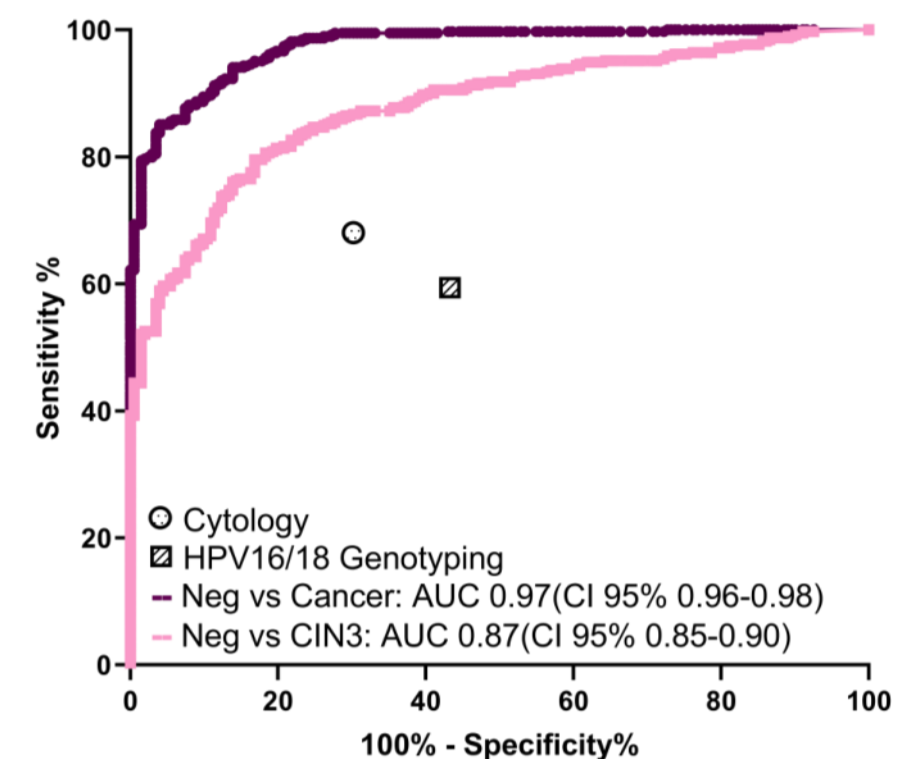
Diagnostic potential of S5

	Sensitivity (CI 95%, p<0.0001)	Specificity (CI 95%, p<0.0001)
Cancer	94.09% (91.28%-96.03%)	86.14% (80.70%-90.23%)
CIN3	76.02% (71.55%-79.98%)	86.14% (80.70%-90.23%)

S5 is better than cytology (66% sensitivity, 65% specificity) and HPV16/18 genotyping (54% sensitivity, 52%specificity) routinely used in screening programs.

Conclusions

- Excellent differentiation between S5 scores of histology negative, CIN3 and cervical cancer samples.

- Potential for use of S5 as stratification tool in current screening programs to avoid over-diagnosis.

- Host methylation events between CIN3 and CSI might drive pre-cancer to cancer transition.

References

1. www.globocan.iarc.fr (WHO) 2018
2. Lorincz A, *et al.,* 2016
3. Cuschieri K. *et al.* 2018
4. Nedjai B., *et al.* 2018