Methylation of promoters of the genes ASTN1, DLX1, ITGA4, RXFP3, SOX17 and ZNF671 in patients conized by CIN2/3 and its relationship with the histological results of the conization specimen

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Objectives

The detection of a hypermethylation pattern in the promoters of ASTN1, DLX1, ITGA4, RXFP3, SOX17 and ZNF671 through a specific PCR test (GynTect®) represents a promising diagnostic tool in patients with cervical lesions caused by HPV. We have studied in a

group of patients older than 25 years with HSIL/CIN2+ cervical lesions who are going to undergo cervical conization the relationship between the detection of the hypermethylation pattern and the anatomopathological results of the specimen.

Methods

In the prospective, observational, non-randomized, multicenter trial MethylCON, 136 patients who attended the cervical pathology and colposcopy units of five hospitals in Madrid (Spain) were recruited between July 2020 and January 2022. The sample for methylation test was taken by cervical brushing prior to conization and preserved for study in Thinprep medium. Methylation testing was performed according to the GynTect IFU. Results were collected in an encrypted database, with restricted access to collaborating researchers. The pathologists and collaborating researchers were unaware of the result of the methylation test. Statistical analysis was performed with IBM SPSS Statistics v.29.0.

Results

Of the 136 participants 132 could be included, of which 66 were methylation-positive, the other 66 methylationnegative. Whereas none of the cases showing no disease, and two of the 10 cases with low-grade lesions in the coni were methylation-positive, all four cancer cases were methylation-positive. Among the CIN2/3 cases, a majority (61 of 107) were methylation-positive (see Table 1). In 103 of 130 cases with status of the edges of the conization piece known, disease-free margins were observed. 21 of the 27 cases with margins showing disease were methylationpositive (see Table 2). The methylation status may provide a hint to the severity of the disease and thus be included in the therapy decision.

Histopathology result of	DNA Methylation (GynTect) result			
conization	Negative		Positive	
	Ν	%	N	%
CIN1/Condyloma (n=10)	8	80	2	20
CIN2/3 (n=107)	46	43	61	57.0
Invasive Cancer (n=3)			3	100
Normal (n=12)	12	100		
Total (n=132)	66	50	66	50

In the follow-up up to 2 years after conization 8 patients underwent reconization and all these patients initially were methylation-positive. These made up 12.5% of the methylation-positive group (see Table 3).

Conclusions

 Prior detection of a hypermethylation pattern in patients undergoing conization for CIN2+ is statistically significantly associated with a higher rate of detection of CIN2+ in the conization specimen and a higher likelihood of involvement of the specimen margins, which is a risk factor for persistence of cervical lesions and HPV infection after conization. **Table 1:** DNA methylation in relation to overall histopathology results obtained from analysis of the conized tissue. The differences observed between the groups were statistically significant.

Histopathology result of conization: resection margins	DNA Methylation (GynTect) result			
	Negative		Positive	
	Ν	%	Ν	%
Affected (n=27)	6	22.2	21	77.8
Free (n=103)	58	56.3	45	43.7
Total (n=130)	64	49.2	66	50.8

Table 2: DNA methylation in relation to resection margins observed in the removed tissue. The differences observed between the groups were statistically significant.

Necessity of	DNA Methylation (GynTect) result			
reconization	Negative	Positive	Total	

• Detection of a pattern of hypermethylation in patients with CIN2+ cervical lesions to be treated by cervical conization could help to identify the group of patients at highest risk of relapsing.

	Ν	%	Ν	%	Ν	% ov.
No reconization	65	100	56	87.5	121	93.8
Reconization			8	12.5	8	6.2
Total	65	100	64	100	130	100

Table 3: Reconization during the 2-year post-conization follow-up period. All reconizations occured among the cases. The differences observed between the groups were statistically significant.