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DNA METHYLATION ANALYSIS BY NASAL BRUSHING FOR EARLY DIAGNOSIS OF MALIGNANT TUMOUR OF THE SINO-NASAL CAVITY

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OBJECTIVES

Nasal and paranasal carcinomas are rare, constituting less than 5% of head and neck malignancies, affecting 0.556 per 100,000 individuals per year (1).

The nasal and paranasal region (NPNR) is composed of a wide range of different tissues, each giving rise to malignancies, the frequent being non-intestinal type adenocarcinoma (ITAC) and keratinizing squamous cell carcinomas (KSCC). NPNR tumours can present with non-specific symptoms often leading to delayed diagnosis, therefore prognosis is dismal due to late diagnosis and tumour aggressive biology (1).

Our group developed a non-invasive method to early detect oral squamous cell carcinoma and its precursor lesions, based on gentle brushing of the oral mucosa to collect cells evaluated by DNA-methylation profile of 13 genes (2,3,4,5). An algorithm of choice differentiating benign from potentially malignant or malignant lesions was identified (SG-OCRA)(3).

The method, validated on a large, multi-institutional series, demonstrated a sensitivity of 93.6% (CI 87.8–99.5), specificity of 84.9% (CI 76.2–93.6), PPV of 86.6% (CI 78.7–94.4), NPV of 92.8% (CI 86.2–99.4), and accuracy of 89.4%. Furthermore, ROC curve analysis using the calculated sample scores provided an AUC of 0.937 (5).

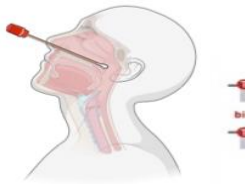
Aim of the present study was to evaluate the validity of the 13 gene-Meth analysis for the diagnosis of NPNR cancers.

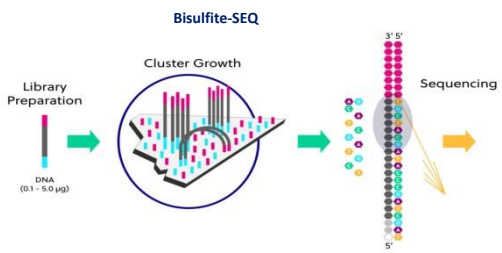
METHODS

Brushing specimens from patients with NPNR lesions were collected. In each patient nasal brushing was performed both on the lesion side and the contralateral side. Nasal brushing sample collection was performed using the same cytobrush (FLOQSwabs® 518CS01, Copan Brescia, Italy) in order to obtain exfoliated cells from the tumour and from clinically normal nasal mucosa. Each cytobrush sample was placed in a 2-ml tube containing 400 µl of DNA/RNA-Shield (Zymo Research, Irvine, CA) for nucleic acid preservation. Nasal brushing was performed before the pre-operative incisional biopsy. The following clinical informations were collected for all patients: age, sex, tumour location and histopathological data of the tumour, according to the WHO 2022 classification (7).

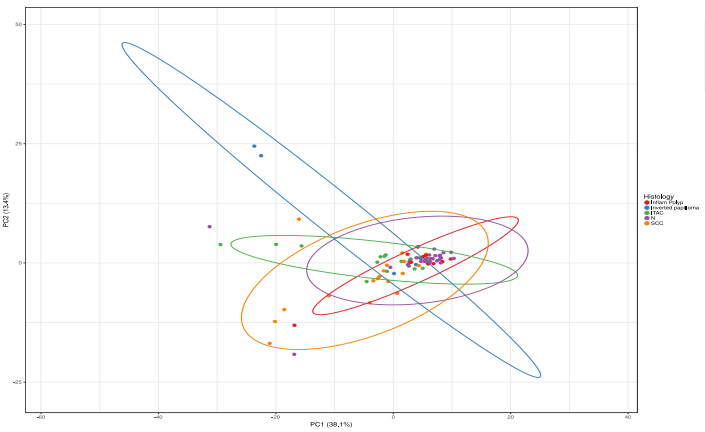
All brushing samples were analysed blindly of clinical and histological information.

DNA methylation analysis DNA was performed according to the methods previously described (2-6). A specific score for each sample was calculated using a linear discriminant analysis and values exceeding the threshold of 1.0615547 were considered positive.

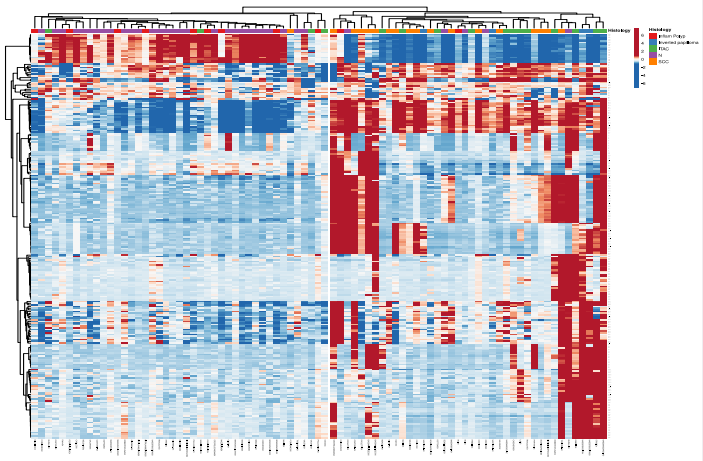




PCA: Unit variance scaling is applied to rows; SVD with imputation is used to calculate principal components. X and Y axis show principal component 1 and principal component 2 that explain 38.1% and 13.4% of the total variance, respectively. Prediction ellipses are such that with probability 0.95, a new observation from the same group will fall inside the ellipse. N = 83 data points.



Cluster Analysis: Rows are centered; unit variance scaling is applied to rows. Imputation is used for missing value estimation. Both rows and columns are clustered using correlation distance and average linkage. 244 rows, 83 columns. ITAC and cancers clustered on the right, while normal and inflammatory polyps on the left side.



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RESULTS

A total of 37 patients were enrolled, comprising 30 patients with malignant tumours (in 3 patients the tumour was bilateral at the time of presentation), 2 patients with inverted papillomas and 6 patients with inflammatory polyps. Results are summarized in tables 1-3.

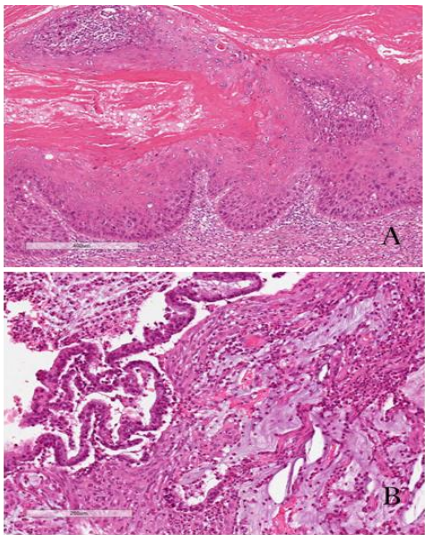

Figure 1. Hematoxylin and eosin staining relative to keratinizing squamous cell carcinoma (200x of magnification) (A) and to intestinal-type sinonasal adenocarcinoma (200X of magnification) (B)

Clinical features	
N. of patients	38
Sex	
Male	25
Female	13
Age in year	18-86 (mean: 63; median:66)

Table 1. Main Clinicopathological Patient Features.

Tumour type	N. Positive test / N. of cases
ITAC	14/15 (93.3%)
Squamous Cell Carcinoma	4/4 (100%)
Mucosal melanoma	3/3 (100%)
Neuroblastoma	1/2 (50%)
Teratocarcinosarcoma	1/1 (100%)
Rhabdomyosarcoma	1/1 (100%)
Ewing Sarcoma	1/1 (100%)
Anaplastic Lymphoma	1/1 (100%)
Sinonasal Undifferentiated Carcinoma	1/1 (100%)
SWI/SNF complex deficient Sinonasal Carcinoma	1/1 (100%)

Table 2. Brushing results in malignant tumours.

	N. Positive test / N. of cases
Malignant Tumours (main site)	28/30 (93,3%)
Contralateral mucosa in cases of Bilateral malignant tumours	2/3 (66,6%)
Clinically normal mucosa contralateral to the main malignant tumour	1/27 (3,7%)
Inverted papilloma	2/2 (100%)
Clinically normal mucosa contralateral to inverted papilloma	0/2 (0%)
Benign inflammatory polyps	0/6 (0%)
Clinically normal mucosa contralateral to benign inflammatory polyps	0/6 (0%)

Table 3. Brushing results in malignant tumours, control cases and contralateral mucosa.

CONCLUSIONS

The present data indicate that the 13-gene-Meth analysis can be useful in the early diagnosis of malignancies of the NPNR region. The method here applied was positive not only on epithelial malignancies, but also on malignant tumours of other tissues (lymphoma, sarcoma and melanoma).

The 13-gene-Meth analysis was positive on inverted polyps, while always negative on inflammatory polyps.

The preliminary data here shown are promising on the application of the 13-gene-Meth analysis as first approach in the diagnosis of tumours of the NPNR.

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