The breath analysis for early detection of malignant pleural mesothelioma (MPM) and management of asbestos exposure subjects

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INTRODUCTION

Malignant Pleural Mesothelioma (MPM) is a rare neoplasm cancer with still a poor prognosis and mainly caused by previous both occupational and environmental asbestos exposure. The management of MPM is challenging due to the long latency period between the exposure and the diagnosis and due to symptoms appearing only at an advanced stage [1].

Recently, the chemical characterization of Volatile Organic Compounds (VOCs) in human breath has been recognized as non-invasive and promising approach for the early detection of neoplastic diseases [2] .

This study aimed to explore the breath analysis potentialities in early detection of MPM and the identification of metabolic alterations in subjects with previous asbestos exposure.

METHODS

A cross-sectional study approved by the Italian Institutional Ethic Committee (Prot. n. 679 C.E.) **was carried out recruiting a total of 60 subjects :**

- **24** patients affected by **MPM**
- **24** healthy controls (**HC**)
- **10** former asbestos-exposed individuals (**EXP**)
- **2** patients in follow-up after first-line chemotherapy (**FU**)

Clinical characteristics of the study population are reported in Table 1

	MPM	HC	EXPOSED	FU
Subject N.	24	24	10	2
Median age (years)	66	42	66	76
Sex Ratio (M : F)	15:9 (62% vs 38%)	9:15 (38% vs 62%)	8:2 (80% vs 20%)	1:1 (50% vs 50%)
Tobacco smoking	15 NO / 9 EX	4 YES / 19 NO /1 EX	10 NO	1 NO / 1 EX

Table 1: Study population features.

For each volunteer, an end-tidal exhaled breath sample and an ambient air (AA) sample were collected on two sorbent tube (Biomonitoring steel tubes, Markes International) by an automated sampling system (Mistral – Predict srl). Breath and AA samples (n. 116) were thermally desorbed (Unity Ultra-xr Markes) and analyzed by Gas Chromatography/Mass Spectrometry (GC Agilent 7890/MS Agilent 5975, Figure 1).

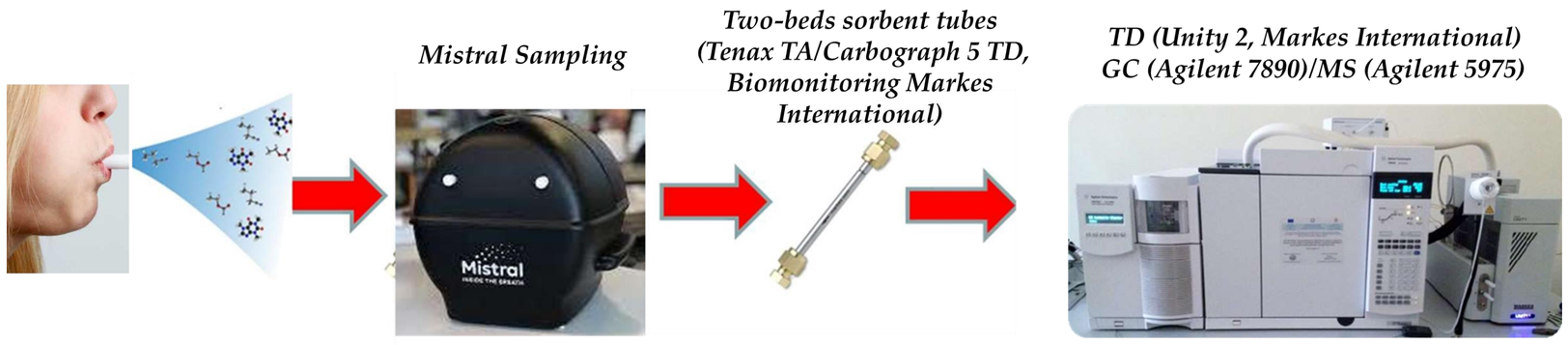

Figure 1: Instrumental setup

Experimental data were statistically processed by non-parametric Wilcoxon signed rank test (R version 3.5.1) in order to identify the most weighting variables in the discrimination between MPM and HC breath samples. Based on the outcomes of the preliminary statistical treatment, Principal Components Analysis (PCA) and Linear Discriminant Analysis (LDA) were applied to the dataset to validate breath analysis-based methodology in the discrimination between MPM, HC and EXP subjects.

RESULTS AND DISCUSSION

A total of 84 VOCs were detected in breath samples but only 35 compounds showed levels significatively different respect to AA. Nonparametric test as Wilcoxon signed rank tests allowed to identify the seven most weighting variables able to discriminate between end-tidal breath samples of patients with MPM and of HCs (variables with p-values < 0.05, Table 2).

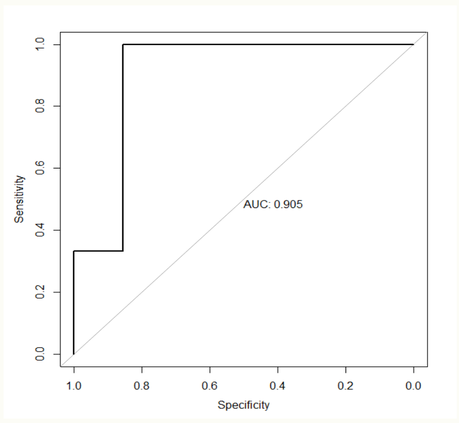

Figure 2: ROC

To identify the feature variables with the best discrimination between groups, i.e. MPM and HC, a multivariate analysis of normalized data was carried out by LDA (R version 3.5.1 – MASS package) and two different discriminant functions were computed accounting 89% and 10% of the total variance of data, respectively.

Leave-one-out cross-validation method was applied to calculate the prediction accuracy obtaining sensitivity equal to 92% and diagnostic accuracy equal to 90% (ROC AUC: 0.905, Figure 2).

Moreover, to explore the potentiality of the developed model in detection of metabolic alterations linked to previous asbestos exposure, a more extensive data set including asbestos exposed participants (EXP) has been processed by means Linear Discriminant Analysis (LDA) (R version 3.5.1 – MASS package, Figure 3). Two discriminating functions LD1 and LD2 explained 99% of the total variance of the data (accounting 58% and 41%, respectively) and leave-one-out cross validation resulted in four out of 10 EXP subjects misclassified as MPM. Hence, it could be useful a further and more frequent clinical surveillance for the follow up of these subjects.

Furthermore, two breath samples collected from MPM patients in follow up (FU), after first-line chemotherapy, were exploratively analyzed, processed by the validated statistical method as blinded samples and classified as MPM. Good agreement was found with patients' outcomes because the two MPM–FU subjects were in disease progression.

VOCs	MPM vs HC (p-value)
Butanal	0.030
Eptanal	0.032
Octanal	0.025
Nonanal	0.045
Decanal	0.045
Acetaldehyde	0.045
Acetonitrile	0.008

Table 2: Wilcoxon signed-rank test outcomes: p-values

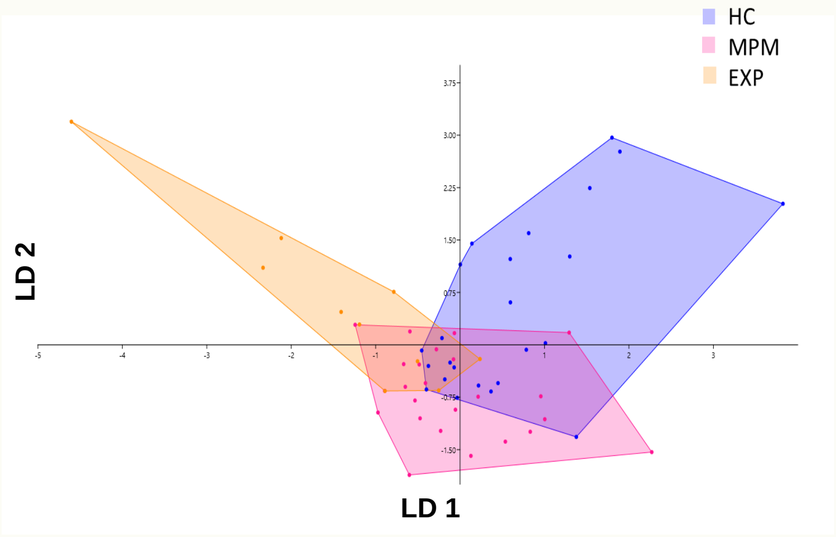

Figure 3: Linear Discriminant Analysis (LDA) score plot

CONCLUSIONS

The combination of non-parametric tests with a supervised classification method enabled to detect metabolites in human breath with discriminant power between healthy subjects and MPM patients.

Despite the promising results, the size and the homogeneity of the sample population deserves further investigation to validate breath analysis as a helpful tool in the screening and clinical management of MPM as well as to possibly integrate this procedure into future screening programs for at-risk, previously asbestos-exposed population.

REFERENCES

[1] Catino *et al.*, *Cancers* **2019** , *11* , 831.
[2] Allard-Coutu *et al.*, *Tumor Discovery* **2024**, 3 (2), 2061.