Breath analysis: identification of potential volatile biomarkers for non-invasive diagnosis of chronic kidney disease (CKD)

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Background:

Breath analysis (BA) is a non-invasive approach relying on the chemical speciation of volatile organic compounds (VOCs) in exhaled breath (EB) that in turn reflects the volatile composition of the bloodstream and airways and thus the health status of the whole organism. In fact, the concentrations of VOCs in breath samples can provide information about metabolic status, allowing a distinction between healthy and sick clinical conditions, due to an instantaneous equilibrium established between the pulmonary blood and the air in the alveoli of the lung[1]. Recently, VOCs determination in exhaled breath has been growing interest due to its promising potential in diagnosis of different pathological conditions including chronic kidney diseases (CKD)[3]. Therefore, the aim of this study is to explore the role of breath analysis in the early detection of CKD and in the non-invasive monitoring of CKD and dialysis patients.

Table 1: Clinical characteristics of the study population

Total Samples n: 30	CTRL (n = 10)	CKD (DIAL + G)		
		DIAL (n =10)	CKD pazients stage G3 (n = 7)	CKD pazients stage G2 (n = 3)
Age	63 (49 - 81)	67 (54 - 82)	75 (60 - 83)	66 (65 - 68)
M : F	6:4 (60 % vs 40%)	6:4 (60% vs 40%)	6:1 (86 % vs 14%)	2:1 (67% vs 33%)
BMI mean	25	25	25	27
diabetes	0	2	2	1
hypertension	4	3	3	2
hypercholesterolemia	3	1	2	0

Materials and methods:

In the period from April to December 2023, in the framework of the study approved by the Italian Institutional Ethic Committee (Prot. n. 990.90.2022), a total of 30 subjects aged between 49 and 81 years were enrolled. More specifically, the breath samples were collected from: a) n. 10 dialyzed patients before undergoing hemodialysis treatment (DIAL); b) 10 non-dialyzed patients affected by CKD (G) including 3 patients in stages G2 (mild renal functional impairment) and 7 patients in stage G3 (moderate renal functional impairment) [3] and c) 10 healthy controls (CTRL). Clinical characteristics of the study population are reported in Table 1. For each one volunteers, an end-tidal exhaled breath sample and an ambient air sample were collected at the same time on two sorbent tube (biomonitoring, Markes) by an automated sampling system (Mistral) and analyzed by Thermal Desorption-Gas Chromatography-Mass Spectrometry (TD-GC/MS -TD Markes Unity 2 - GC Agilent 7890/MS Agilent 5975) [4].

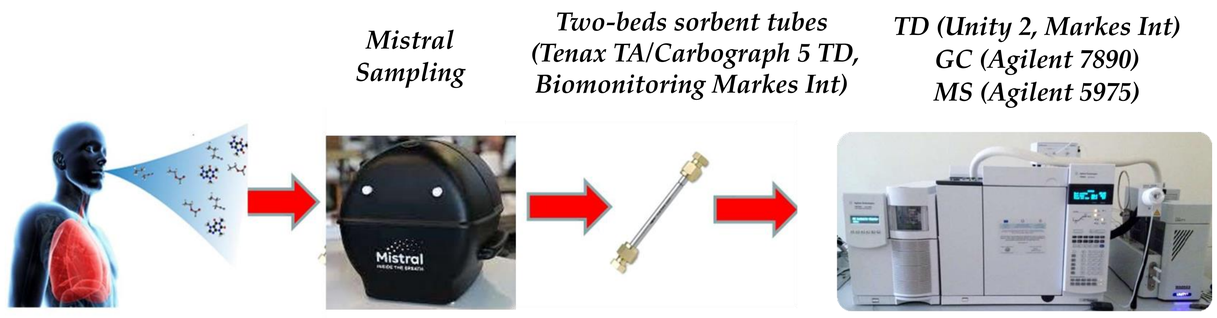


Figure 1: Instrumental setup

Results and Discussions

Nonparametric test as Wilcoxon/Kruskal Wallis tests (R version 3.5.1) allowed to identify the most weighting variables to discriminate among DIAL, G and CTRL breath samples, excluding the variables related to ambient air. Considering p-values lower than 0.05 reported in Table 2, a multivariate statistical approach was applied at collected data.

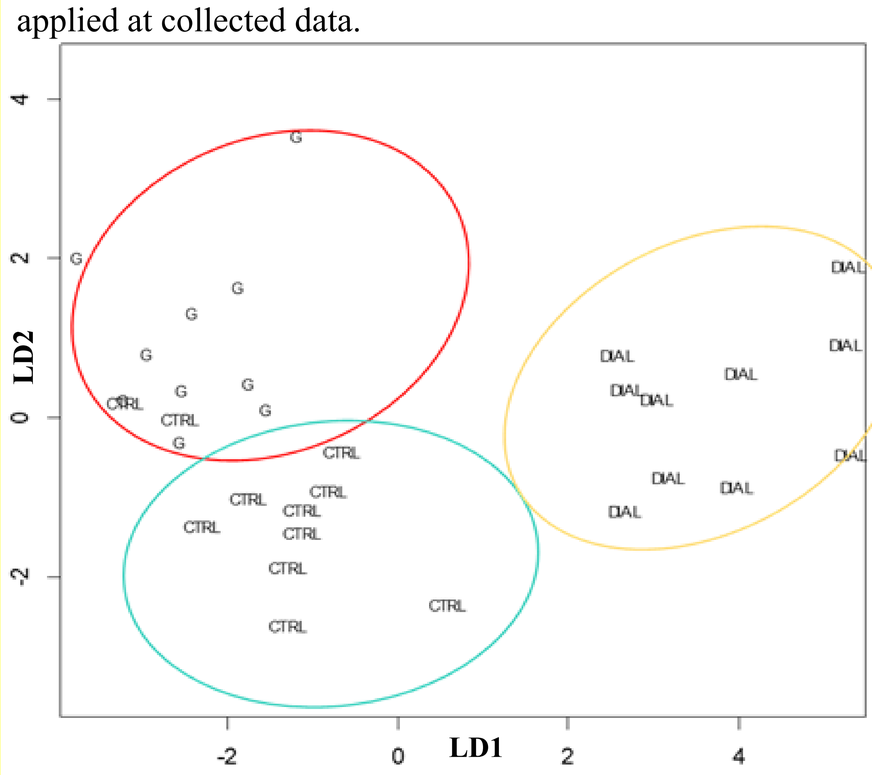


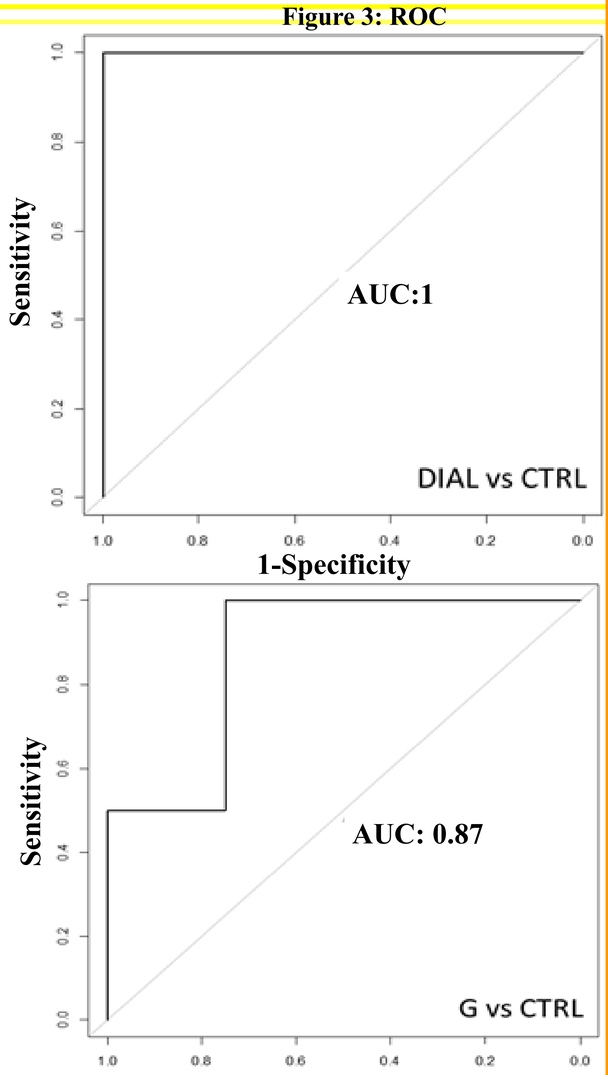
Figure 2: Score plot of LDA output

Table 2: p-values of diagnostic VOCs

VOC	p-value	VOC	p-value	VOC	p-value
Acetophenone	0.01	Benzene	0.02	Undecane	0.01
Nonanal	0.01	Toluene	0.01	Butandione	0.003
Pentane	0.03	Ethylbenzene	0.0004	Ethylhexanol	0.0003
Trioxane	0.01	o-Xylene	0.0006	Octane	0.02
Eptene	0.0005	Benzaldehyde	0.02	Pentanone	0.02

To assess the discrimination efficiency among the different groups, i.e. CTRL, G and DIAL, 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. As shown in Figure 2, a complete separation of the three groups was achieved and leave-one-out cross validation resulted in a recognition ability of 89% with only two CTRL misclassified as G (false positive). Using the predicted outcomes, a receiver operating characteristic (ROC) curves were constructed (using p-ROC package - R version 3.5.1) both for the discrimination between G and CTRL and between DIAL and CTRL. ROC analysis reported promising results showing the diagnostic accuracy of 87% and 100% when G vs CTRL and DIAL vs CTRL were considered, respectively (Figure 3).

Figure 3: ROC



Conclusions

Although the limited number of data collected, the results of this study are very promising. The advancement of a non-invasive breath test enabling the early diagnosis of chronic kidney disease and the follow-up of patients affected by this pathology can result in less severe damage and simpler treatment options for the patients, leading to better outcomes and reducing healthcare costs.

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