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Rapid detection of SARS-CoV-2 infection by multicapillary column coupled ion mobility spectrometry (MCC-IMS) of breath. A proof of concept study

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Abstract

There is an urgent need for screening patients of having a communicable viral disease to cut infection chains.
We could recently demonstrate that MCC-IMS of breath is able to identify Influenza-A infected patients. With decreasing Influenza epidemic and upcoming SARS-CoV-2 infections we extended our study to the analysis of patients with suspected SARS-CoV-2 infections.
51 patients, 23m, 28f, aged 64 ± 16 years, were included in this study.
Besides RT-PCR analysis of nasopharyngeal swabs all patients underwent MCC-IMS analysis of breath. 16 patients, 7m, 9f, were positive for SARS-CoV-2 by RT-PCR. There was no difference in gender or age according to the groups.
Stepwise canonical discriminant analysis could correctly classify the infected and non-infected subjects in 98% by cross-validation. Afterwards we combined the Influenza-A sub study and the SARS-CoV-2- sub study for a total of 75 patients, 34m, 41f, aged 64.8 ± 1.8 years, 14 positive for Influenza-A, 16 positive for SARS-CoV-2, the remaining 44 patients were used as controls. In one patient RT-PCR was highly suspicious of SARS-CoV-2 but inconclusive.
There was no imbalance between the groups for age or gender.
97.3% of the patients could be correctly classified to the respective group by discriminant analysis. Even the inconclusive patient could be mapped to the SARS-CoV-2 group applying the discrimination function.

Conclusion:
MCC-IMS is able to detect SARS-CoV-2 infection and Influenza-A infection in breath. As this method provides exact, fast non-invasive diagnosis it should be further developed for screening of communicable viral diseases.
Study registration: NCT04282135

Introduction:

To interrupt infections chains in SARS-CoV-2- disease screening methods are urgently needed. Diagnostic standard is reverse transcription polymerase chain reaction (RT-PCR) but deep nasopharyngeal swabs taken by trained personal are required and rapid PCR- techniques still take more than 30 minutes.[1] Especially for screening at airports or other sites where rapid screening of asymptomatic patients is demanded this method is too invasive and takes too long. Additionally especially in the scope of developing countries RT-PCR is far too expensive. So, there is still a need for a faster, really non- invasive screening tool.[2] A screening tool that can be easily used and prevents false negative results would fulfill this demand.

In classic antiquity without other diagnostic tools physicians had to rely on their basic senses, seeing, touching, hearing and smelling.[3] With improved technical possibilities these skills have been moved in the background. Only few scents physicians are taught in medical school like acetone for ketoacidosis and ammonia for liver disease. It is well known that different bacteria smell differently. *Pseudomonas aeruginosa* have a fruity scent while *Escherichia coli* smells faecal. Based on their better olfactory senses animals have been trained to smell infectious diseases but lack of reproducibility precludes wider application.[4]

The scents of infectious diseases are volatile organic compounds (VOC) that are released by the metabolism of the germ or the host. There are different technical approaches to discriminate pathogens or diseases based on VOCs but none has been used regularly in clinical practice.[5]

Gas chromatography coupled ion mobility spectrometry (GC-IMS) has proven to discriminate bacterial infections in vitro as in breath.[6–9]

Compared to bacteria viruses have no own metabolism. So using scents can only rely to the host response to the viral infection. Currently there are only few studies addressing this issue. Gas chromatography coupled mass spectrometry (GC-MS) was able to detect Influenza infection of cell cultures in vitro.[10]

As GC-MS is not feasible for point-of-care diagnostics there are attempts to train dogs to sniff for viral diseases, currently for SARS-CoV-2 associated disease.[11,12]

In a recent study we could demonstrate that Influenza-A infection can also be detected in the breath of Influenza-A infected patients by multicapillary-column-coupled ion mobility spectrometry (MCC-IMS).[13] Therefore, we extended this study to analyse whether MCC-IMS is also able to detect SARS-CoV-2- infection in breath.

Methods:

As already mentioned, our first study started in February with 24 patients, 11m, 13f, aged 66 ± 14.2 years, that were admitted to our hospital for suspected Influenza-A infection. With decreasing numbers of Influenza-A and increasing numbers of SARS-CoV-2 suspected patients we extended the study to also examine the breath of SARS-CoV-2 suspected patients (23m, 28f, aged 63 ± 16 years).

All patients underwent deep nasopharyngeal swabs for Influenza-A and SARS-CoV-2 PCR and were asked to participate in this study. History, Physical Examination, lab testing and imaging were done according to hospital policies.

After written consent breath samples were taken and analysed by MCC-IMS. The study was approved by the Ethics Committee of Erlangen University, Erlangen, Germany (Nr 426_18 B), and registered at ClinicalTrials.gov (NCT04282135). Patients were recruited between April 8th 2020 and May 7th 2020. Unfortunately, many patients could not be included as they were too sick and had to be transferred to the intensive care unit or were unable to provide written consent. As the time went on, measures of social distancing and segregation were successful leading to decreasing numbers of SARS-CoV-2 patients.

PCR

SARS-CoV-2 was tested by taking a deep nasopharyngeal swab applying the "Xpert® Nasopharyngeal Sample Collection Kit for Viruses" (Cepheid, Maurens-Scopont, France) and performing real time PCR by applying the Allplex 2019-nCoV Assay (Seegene, Seoul, South Korea) on the CFX 96 Real-Time Sytem (BioRad, Feldkirchen, Germany) after extracting RNA by using the StarMag 96 UniTube Kit (Seegene, Seoul, South Korea) on the SGPrep32 extraction system (Seegene, Seoul, South Korea). Due to shortage in supply RNA was in part alternatively extracted applying the QiaAmp DSP Virus Spin Kit (Qiagen, Hilden Germany) using the Qiacube automated extraction system (Qiagen, Hilden Germany). Influenza PCR was performed with the "Xpert® Xpress Flu/RSV" (Cepheid) on the "Infinity" (Cepheid).

Breath- Sampling and MCC-IMS

For the ion mobility spectrometry we used the MCC-IMS-device from STEP Sensortechnik und Elektronik, Pockau, Germany (STEP IMS NOO). The device is distributed as a medical device (In-Vitro-Diagnostic) in combination with an evaluation Software as "MultiMarkerMonitor®" by Graupner medical solutions GmbH, Geyer, Germany.

All patients were connected by a foam cuffed oxygen catheter (#01442958, Asid-Bonz, Herrenberg, Germany) via a 0.22µm Filter (Navigator Lab Instruments, Tinajin, China) and a Perfusor Line (B Braun, Melsungen, Germany) directly to the MCC-IMS.

Patients were instructed to take a deep breath and to exhale slowly through the nose. During the exhalation breath was sampled for 10s.

The STEP device directly draws the sample by an internal pump (200mL/min) into the analysing circuit without any pre analytical procedures.

In an inert sampling loop of 2 mL the sample is standardized for volume. Then the sample is pre-separated by isothermally heated multicapillary gas chromatography column (60°C) into single analytes, which enter the IMS unit based on their retention times. In the IMS unit the analytes are ionized by beta radiation of a tritium source below the free limit for radiation (99 MBq). Afterwards the generated ions are accelerated in a 50-mm-long drift-tube under the influence of an electric field (400 V/cm) towards the detector which is also tempered to 60°C. On their way the positive ions collide with air molecules from the drift gas (400 mL/min) flowing in the opposite direction and are separated depending on their ion mobilities and detected by the collector electrode sampled every 10µs. The received IMS spectra are stored internally in the device and later analysed offline.
The used IMS device is equipped with a circulation filter and internal gas circulation. Using a circulation pump, ambient air filtered by an activated carbon filter was provided as drift gas

and analysis gas (20 mL/min) to the device. Compared to other IMS devices there is no need for a special analysis gas.

Data analysis

The VOCs are characterized by their retention time in the MCC and the drift time in the IMS. One spectrum over 2048 measurement points every 10 µs (in total 20.48ms) is obtained every second for a total time of 240s.
These spectra can be visualized on a heatmap with retention time on the Y-axis and the drift time on the X-axis.

To decrease the complexity of the data we used a proprietary cluster analysis software using support vector machine (European Patent EP 2 729 801 B1).[14] After baseline correction for noise the software determines the clusters based on the signal threshold and categorizes them by retention time and drift time. Depending on these parameters the clusters are numbered assuming that every cluster represents a distinct VOC.

Statistical Analysis

Due to the small sample size and the lack of normal distribution Mann-Whitney-White U-Test and Kruskal-Wallis Test were applied for differences in patient characteristics.
Patients from both sub studies where neither Influenza-A nor SARS-CoV-2 was found in the PCR were combined as controls for the combined dataset.
To exclude cross-correlated clusters we performed a stepwise canonical discriminant analysis for optimal minimization of Wilks Lambda. For entering or removing variables from the model F significances of 0.05 and 0.1 were used.
For the statistical analysis we used IBM SPSS 22.0 (IBM, Armonk, NY).

Results

In our Influenza-A sub study there was a slight gender imbalance between Influenza-A PCR-positive and negative patients. Age and infection markers were not different between both groups. In the SARS-CoV-2 subgroup there was no gender disparity. The only difference was the reduced leukocyte count in the SARS-CoV-2- positive population (Tab. 1).

	Influenza			SARS-CoV-2			
	Negative	Positive	Total	Negative	Positive	Uncertain	Total
Male / Female	7 / 3	4 / 10	11 / 13	16 / 17	7 / 10	0 / 1	23 / 28
	X^2=4.03, p=0.045			n.s.			
Age (yrs)	61.6 ± 14.8 36–80, 61	69.9 ± 13.2 43–89, 70.5	66.4 ± 14.2 36–89, 66.5	63.5 ± 18.3 23–87, 64.5	65.8 ± 10.8 40–90, 65	56	64 ± 16 23–90, 64.5
	n.s.			n.s.			
Leukocytes [G/L]	7.3 ± 3.7 1.5–11.5, 8.3	7.8 ± 2.7 2.2–12.1, 8.4	7.6 ± 2.9 1.5–12.1, 8.4	10.1 ± 4.4 0.7–22.7, 9.9	6.6 ± 2.6 3.6–13.5, 6.1	11.6	8.9 ± 4.2 0.7–22.7, 7.9
	n.s.			X^2=3.341, p=0.0008			
CRP [mg/L]	38.5 ± 28 4–66, 45.5	76 ± 64 1–240, 75.5	65 ± 57 1–240, 60.5	75 ± 91 1–352, 32	65 ± 56 1–175, 37	165	74 ± 80 01–352, 37
	n.s.			n.s.			
PCT [ng/L]	2.2 ± 1.8 1–5.6, 1.7	3.7 ± 9.1 0.7–35.4, 1.3	3.3 ± 7.6 0.7–35.4, 1.3	16 ± 58 0.3–282, 1.1	1.4 ± 1.5 0.2–5.5, 0.95	0.4	1.0 ± 4.4 0.2–28.2, 1.0
	n.s.			n.s.			

Table 1
Patient characteristics for the two sub studies. For the continuously measurable parameters in the first row mean and SD, in the second row range and median are reported. n.s. = not significant.

Though the patients were triaged as suspected by the symptoms at presentation there is significant more fever, cough, dyspnea and pathological chest X-rays in the SARS-CoV-2-

positive group. Only gastrointestinal symptoms did not differ between the groups (Tab. 2). The pathological X-ray results in the SARS-CoV-2- negative group were caused by bacterial pneumonias.

	Control	SARS-CoV-2
Fever	11/44	12/16
	X^2= 4.90, p= 0.027	
Dyspnea	11/44	13/16
	X^2= 10.42, p= 0.001	
Cough	7/44	10/16
	X^2= 8.51, p= 0.004	
Gastrointestinal symptoms	4/44	3/16
	X^2= 0.44, p = 0.507, n.s.	
Pathologic X–Ray	8/44	15/16
	X^2= 14.43, p< 0.001	

Table 2
Patient symptoms for SARS-CoV-2– study. n.s. = not significant.

In one patient SARS-CoV-2- PCR was inconclusive. In nasopharyngeal swabs there was twice a highly suspicious finding for SARS-CoV-2 but it could not really be confirmed. This patient is described as uncertain.

As the nasal sampling and MCC-IMS diagnostics did not differ between the two sub studies they were subsequently merged into a combined dataset. Here only the difference in the leukocyte count was statistically significant.

Combined	Control	Influenza	SARS-CoV-2
Male/Female	23/21	4/10	7/9
Age yrs	63 ± 17.5 23–87, 63.5	69.9 ± 13.2 43–89, 70.5	65.8 ± 10.8 40–90, 65
		ns.	
Leukocytes [G/L]	9.6 ± 4.4 0.7–22.7, 9.5	7.8 ± 2.7 2.2 – 12.1, 8.4	6.6 ± 2.6 3.6–13.5, 6.1
		X^2=10.22, p=0.0060	
CRP [mg/L]	69 ± 85 1–352, 33	76 ± 64 1 –240, 75.5	65 ± 56 1–175, 37
		n.s.	
PCT [ng/L]	13 ± 52 0.3–282, 1.4	3.7 ± 9.1 0.7–35.4, 1.3	1.4 ± 1.5 0.2–5.5, 0.95
		n.s.	

Table 3.
Patient characteristics for the combined dataset. For the continuously measurable parameters in the first row mean and SD, in the second row range and median are reported. n.s. = not significant.

Using the cluster analysis, we could find 155 clusters that were used for the multivariate analysis afterwards.

As previously reported stepwise canonical discrimination analysis could classify patients with and without Influenza-A with 100% sensitivity and 100% specificity in the cross- validation using leave-one-out.[13]

Using only the dataset from the SARS-CoV-2 study stepwise discrimination analysis was able to correctly classify controls and Patients with SARS-CoV-2 in 98% with only one false negative result in the cross-validation.

In the combined dataset 97.3% of the cases were correctly classified, even between Influenza-A and SARS-CoV-2. There was only one control misclassified as Influenza-A and one as SARS-CoV-2 respectively. There were no false negatives in this analysis.

Classification Results[a,c]

			Predicted Group Membership			
Group			Control	Influenza A	SARS CoV-2	Total
Original	Count	Control	44	0	0	44
		Influenza A	0	14	0	14
		SARS CoV-2	0	0	16	16
		Ungrouped cases	0	0	1	1
	%	Control	100.0	0.0	0.0	100.0
		Influenza A	0.0	100.0	0.0	100.0
		SARS CoV-2	0.0	0.0	100.0	100.0
		Ungrouped cases	0.0	0.0	100.0	100.0
Cross-validated[b]	Count	Control	43	0	1	44
		Influenza A	0	13	1	14
		SARS CoV-2	0	0	16	16
	%	Control	97.7	0.0	2.3	100.0
		Influenza A	0.0	92.9	7.1	100.0
		SARS CoV-2	0.0	0.0	100.0	100.0

a. 100.0% of original grouped cases correctly classified.
b. Cross validation is done only for those cases in the analysis. In cross validation. each case is classified by the functions derived from all cases other than that case.
c. 97.3% of cross-validated grouped cases correctly classified.

Table 4
Classification results for the canonical discriminant analysis of the combined dataset

Two discriminant functions are able to explain 100% of the variance. In the scatter plot of these two discriminant functions all 3 groups are separated nicely (Fig. 1). Interestingly the patient with the inconclusive PCR result is mapped to the SARS-CoV-2 group.

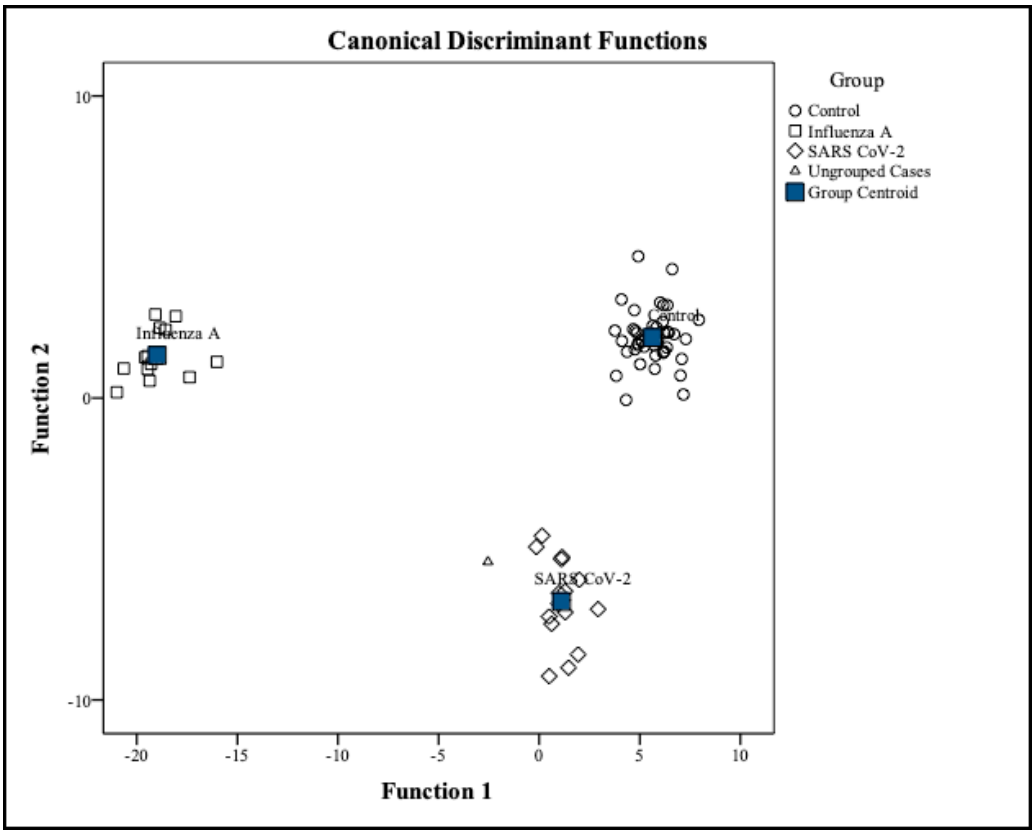

Figure 1.
Results of the canonical discrimination analysis for the combined dataset. Controls as circles, Influenza-A as squares, SARS-CoV-2 as diamonds and the only unclassified as triangle.

Discussion:

Today RT-PCR is considered the gold standard in the diagnosis of SARS-CoV-2- infection but even in trained hands a false negative rate of about 25% and a false positive rate of 2.3-6.9% has to be expected.[15]

According to the Editorial in Lancet Infectious Diseases May 2020 SARS-CoV-2 infections are rising in developing countries while declining in most of the western countries. In underdeveloped countries neither trained personal nor financial power to afford RT-PCR for mass screening can be assumed.[2]

To our best knowledge our study is the first showing that breath-analysis is able to discriminate SARS-CoV-2 infected patients and controls. It has to be noticed that we could also tell Influenza-A from SARS-CoV-2 infection by this method. Even one suspected but not clearly PCR- confirmed patient could be assigned to the SARS-CoV-2 group. These are encouraging results.

We used MCC-IMS because of the ease of application. The STEP-IMS device does not need any preanalytic procedures or test gases. So, no shortage of swabs, tubes or reagents has to be faced in the scope of a pandemic.

The device draws the breath into the system by an internal pump. This simplifies the sampling compared to other IMS devices where absorption/desorption tubes are needed.[9]

The only task to be fulfilled by less trained personal is to introduce the foam-cuffed catheter into a nostril of a spontaneously breathing individual and to hit a key to start the measurement.

For our study written consent of the patient was a prerequisite. Therefore, we had to exclude demented, delirious or too severely ill patients not able to consent but this should be no constraint for the method in real life.

It could be argued that IMS only provides peaks according to retention time and drift time while mass spectrometry (MS) is able to chemically describe the VOCs but also in MS not all peaks are clearly assigned to a chemical substance and are therefore also only numbered or characterised by the time of flight.[6]

We think that not knowing exactly the chemical structure of the VOC is no detriment as another attempt to screen for SARS-CoV-2 is by the smell of trained dogs.[12] Like smelling dogs, a fingerprint of peaks should enable the classification of the odour of infected patients.

As the scent of the breath does not rely on the virus itself but on the host response to the infection cross-reactivity of breath analysis with other viral infections has to be expected. As MCC-IMS could differentiate between SARS-CoV-2 and Influenza-A infection we assume that different viruses cause different host responses and therefore produce different fingerprints of IMS spectra.

Similar to antibody tests an overlap of the VOCs with other corona virus infections has to be anticipated. But this is a constraint every analysis of metabolomics has to face.

Compared to other breath analysis studies we did not require fasting before the sampling. Though fasting state may reduce interferences with other metabolism it will not be feasible for screening on a grand scale.

Another necessity for a further progress of breath analysis into screening is the extension of the study to other ethnicities and civilisations to allow for different host reactions to the same virus.

One drawback of our study is the limited number of patients. As pointed out already many patients with SARS-CoV-2 were not able to give informed consent. Another point is the weakening of the SARS-CoV-2 wave in Germany end of April as this led to a slowing of accrual.

We therefore assess this study as a proof-of-concept and encourage other researches to further investigate breath analysis by MCC-IMS for the detection of SARS-CoV-2 infections.

Conclusion:
Breath analysis using MCC-IMS is able to discriminate between Influenza-A, SARS-CoV-2-infections and not infected controls in a few minutes.
As this method is completely non-invasive and does not need any reagents or preanalytic procedures it seems promising for a screening device even in underdeveloped countries.
We encourage further trials to use this technique in different patient settings.

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Gunther Becher holds parts of the patent for the cluster analysis (EP 2 729 801 B1)
All other authors state that there is no conflict of interests.

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Authors Contribution

Claus Steppert:	Literature search, study design, data collection, data analysis, data interpretation, writing, review
Isabel Steppert:	Data analysis, writing, review
Gunther Becher:	Device setup, writing, review
William Sterlacci:	Review
Thomas Bollinger:	Data analysis, writing, review

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