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COVID-19 patient variables associated with the detection of airborne SARS-CoV-2

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ABSTRACT

Background: Understanding the COVID-19 patient characteristics that impact environmental SARS-CoV-2 load is essential for improving infection risk management. In this study, we analyzed the influence of patient variables on airborne SARS-CoV-2 genome detection.
Methods: Sixty-nine COVID-19 patients were recruited across three independent studies with airborne SARS-CoV-2 genome assessed in individual hospital rooms using droplet digital PCR.
Results: In the bivariate analysis, the odds of airborne SARS-CoV-2 detection were significantly higher for patients with obesity, chronic respiratory diseases, pneumonia at admission, sampling, and discharge, and lower lymphocytes count. No significant associations were found between airborne SARS-CoV-2 detection and symptoms presence or duration, nor with the results of the most recent positive nasopharyngeal PCR test prior to air sampling. In the multivariate analysis, the best-fit model included patient age, type of admission, and symptoms duration. Patient age significantly contributed to the risk of airborne SARS-CoV-2 detection in the multivariate analysis.
Conclusions: Our findings highlight the variability in individual responses to SARS-CoV-2 infection and suggest that factors linked to COVID-19 severity, symptomatology, and immunocompetence influence the airborne SARS-CoV-2 detection. Our results may support the development of more precise preventive measures in healthcare settings.

Introduction

Airborne is the dominant transmission route of SARS-CoV-2 infection [1,2]. Since the COVID-19 outbreak, practical findings have been described in the knowledge of airborne SARS-CoV-2 transmission that are useful to mitigate the spread of the virus. Remarkably, it was described that bioaerosols containing SARS-CoV-2 travel up 4,8 m away from the emitter [3], that the median half-life of SARS-CoV-2 in aerosols is 1.1–1.2 h [4], and that the infectivity of the virus can be retained for up to 16 h [5]. As new scientific evidence on SARS-CoV-2 transmission is consolidated, this is translated into the prevention guidelines. Guidance of preventive measures applied to healthcare centers is intended to be exhaustive, since health care providers are highly exposed to being infected [6]. One of the major difficulties in stablishing general preventive measures is the high variability in the response to SARS-CoV-2 infection [7]. Therefore, it is still necessary to increase the understanding of the

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patient-related factors that influence the infection risk to better control it.

The infection risk from infected patients depends, in part, on several clinical variables of the emitter. Other dependent factors are the airborne SARS-CoV-2 dynamics in a particular indoor air and the immunocompetence of the persons that are at risk. The magnitude of the COVID-19 patient's viral load determines the infectivity. Specifically, the threshold SARS-CoV-2 concentration from which infectivity is significant has been estimated from samples of the respiratory tract [8,9]. Transmission-based precautions for patients with SARS-CoV-2 infection have mainly been drawn from studies in which biological samples were studied. The patient variables that are currently considered are the illness severity degree, immunocompetence, duration of symptoms, and the PCR test results [10]. Despite the successful advances, the infection risk is not fully controlled and, as an example, as of June 2024, according to the US Centers for Control Disease and Prevention, the exact criteria that determine which patients will shed replication-competent virus for longer periods than 20 days are not known [10]. Therefore, more studies are required to investigate which are the COVID-19 patient features that influence the infection risk.

The infection risk from infected patients also depends on the environmental SARS-CoV-2 concentration, which is in part determined by the individual viral concentration exhaled by a patient [11]. The airborne SARS-CoV-2 genome has been identified in air samples from hospital wards using RT-qPCR [3,6,12–19] and the infectivity of the virus collected from airborne samples has also been demonstrated [3]. SARS-CoV-2 load in exhaled air is, however, not detected in some individuals who tested positive in nasopharyngeal samples [20], meaning that it is required to address more studies in air samples to completely understand the risk for transmission from infected individuals.

The assessment of airborne SARS-CoV-2 levels entails several methodological challenges, including the requirement of a highly sensitive method approach, since the overall airborne viral concentration is expected to be very low. In our previous studies [16,18], we assessed the airborne SARS-CoV-2 levels with droplet digital PCR (ddPCR), a technique that is more sensitive than real time PCR and enables the absolute quantification. We detected the SARS-CoV-2 genome in 44.6 % of the air samples collected in individual patient rooms in the first two studies [16], and in 80 % of the samplings in the third study, in which we improved the efficiency of the protocol to collect bioaerosols and the sensitivity of the RT-ddPCR protocol to detect the SARS-CoV-2 genome [18]. In the present study, we aimed to identify the COVID-19 patient variables that are associated to the detection of airborne SARS-CoV-2 genome.

Materials and methods

Air sampling

Air samples were collected in patient rooms of the *Hospital Universitari Son Espases* (HUSE, Palma, Spain) in the presence of a diagnosed COVID-19 patient. Three different studies are included in this work, which differed in the criteria followed for patient recruitment and in the protocol to assess the airborne SARS-CoV-2 RNA levels. Even though we used the same methodology for SARS-CoV-2 detection in the three studies, several changes were introduced in the protocols for SARS-CoV-2 collection and quantification. The differences between the studies are depicted in Table 1.

Air samples were collected in patient rooms with different patient care (Table 1) belonging to the Pneumology Service. Samples were collected from September to November 2020 in Study I (n = 26), from November 2020 to May 2021 in Study II (n = 31), and from August to September 2021 in Study III (n = 12). Air sampling was performed in patient rooms of approximately 57 m^3. During sampling, door and window were closed, and patients did not wear a mask. To minimize the impact of environmental factors, such as room ventilation, air samples were collected under the same conditions. Air samples were collected using a SKC BioSampler® liquid impinger (SKC Inc, Pennsylvania, USA) at 12.5 L/min. This sampler was selected owing to the highest efficiency to collect particles of 1–3 μm diameter [21], high performance in collecting RNA virus [22], and better virus preservation [23]. The air volume sampled depends on the collection medium used, which was different in each study (Table 1). The sampler was placed between 1 and 1.5 m distance from the patient's face and at the patient's head height. Most of the rooms were sampled once, and in the case that more than one air sample was collected, the result with the highest concentration was selected.

Airborne SARS-CoV-2 RNA detection

Total RNA was extracted from 150–350 μL of collection medium with the TRItidy G™ protocol (Panreac AppliChem, Barcelona, Spain), except for the five first samples for which the MagMAX™ viral/pathogen RNA extraction kit (Applied Biosystems, Waltham, Massachusetts, USA) was used. RNA was extracted as described in detail in [16]. Total RNA was reverse-transcribed using the enzymes indicated in Table 1 and following the protocols previously described [16,18]. Superscript II reverse transcriptase (Invitrogen, Waltham, Massachusetts, USA) allowed the detection of a single RNA molecule with a high efficiency [18]. The SARS-CoV-2 genome was detected by ddPCR using a QX200 Droplet Reader (Bio-Rad, Hercules, California, USA), as previously described [16]. Forward primer, reverse primer, and probe sequences are, respectively, as follows: 5′-GGGGAACTTC TCCTGCTAGAAT-3′, 5′-CAGACATTTTGCTCTCAAGCTG-3′, and 5′-HEX-TTGCTGCTGCTTGACAGATT-TAMRA-3′ for the N gene; 5′-GACCCCAA AATCAGCGAAAT-3′, 5′-TCTGGTTACTGCCAGTTGAATCTG-3′, and 5′-FAM-ACCCCGCATTACGTTTGGTGGACC-BHQ1-3′ for the N1 gene; 5′- CCCTGTGGGTTTTACACTTAA-3′, 5′- ACGATTGTGCATCAGCTGA-3′, and 5′-HEX-CCGTCTGCGGTATGTGGAAAGGTTATGG-TAMRA-3′ for the ORF1ab and 5′ GTGARATGGTCATGTGTGGCGG-3′, 5′- CARATGT-TAAASACACTATTAGCATA-3′, and 5′-FAM- CAGGTGGAACCTCATCAG GAGATGC-BHQ1-3′ for the RdRP gene. Targets were amplified in multiplex reactions. RNA isolated from nasopharyngeal samples of COVID-19 patients were used as positive controls. Nuclease-free water (Panreac AppliChem, Barcelona, Spain) was used as negative control [16]. Biases due to poor sensitivity are unlikely to affect the analysis of the patient variables on SARS-CoV-2 detection since this procedure allows the detection of a single RNA molecule per reaction [18], with a limit of detection for the ORF1ab target of 0.7–1–1 cDNA copies per reaction [16].

COVID-19 patient information

Data from COVID-19 patients were collected to analyze their influence on the detection of airborne SARS-CoV-2. This research was approved by the Research Ethics Committee of the Balearic Islands (IB4503/21PI). The patient data collected were gender, age, presence of obesity (IMC ≥ 25 Kg/m^2) and previous chronic diseases (respiratory diseases, cancer), presence of symptoms (headache, nausea or vomiting, diarrhea, general malaise, dyspnea, arthromyalgia, fever, and coughing), number of days from symptoms onset, type of health care (regular or intermediate/intensive), the Ct value of the diagnostic PCR test, number of days from a positive diagnostic PCR test, pneumonia severity at different time-points (day of hospital admission, day of air sampling, and day of discharge), fatalities, treatment received (corticosteroids, Remdesivir, Tocilizumab), and blood parameters.

Table 1
Differences between studies in the COVID-19 patient selection criteria and the protocols to quantify environmental SARS-CoV-2 in patient rooms.

	Study I	Study II	Study III
COVID-19 patient selection criteria			
Time lapse since COVID-19 diagnose to air sampling	Not applicable	Not applicable	Positive PCR test within 10 days before air collection
Time lapse since symptoms onset to air sampling (days)	Not applicable	< 12	Not applicable
Patient care	Ward, intermediate	Ward, intermediate, intensive	Intermediate. All patients required high-flow oxygen therapy (40–80 L/min)
Changes in the protocol to quantify airborne SARS-CoV-2			
Volume of collected air (L)	250–375	500–750	500
Collection medium	5 mL deionized sterile water	5 mL viral transport medium	1–1.5 mL mineral oil (SKC, Valley View, Pennsylvania, USA)
RNA isolation protocol	MagMAX™ viral/pathogen RNA extraction kit (Applied Biosystems, Waltham, Massachusetts, USA) and Phenol method	Phenol method	Phenol method
Reverse transcriptase	M-MuLV TRANSCRIPTME (BLIRT, Dunzig, Poland)	M-MuLV TRANSCRIPTME	Superscript II (Invitrogen, Waltham, Massachusetts, USA)
SARS-CoV-2 genome targets	N, N1, RdRP, and ORF1ab	N, N1, RdRP, and ORF1ab	N1 and ORF1ab

Statistical analysis

A descriptive analysis was conducted to compare the general demographic characteristics and COVID-19 patient data collected across studies I, II, and III. Then, we investigated the impact of patient data on the airborne SARS-CoV-2 genome detection using a bivariate analysis. For these purposes, the Compare Groups package in R [24] was employed to assess significance. The normality of each variable in each study was assessed performing a bootstrapped Kolmogorov-Smirnov test. Continuous baseline characteristics with a normal distribution were compared using Pearson's test, while non-normally distributed continuous variables were assessed using Spearman's test. Categorical baseline characteristics were analyzed via the chi-squared test.

In the second stage, we applied a multivariable risk prediction model using the generalized linear model (GLM) with a binomial distribution and logit link function. The response variable was presence/absence of airborne SARS-Cov-2 RNA detection and all of the variables included in the analysis can be observed in Table 2. To assess for multicollinearity, variance inflation factors (VIF) were calculated for all variables, with only variables showing VIF values below a strict threshold of 3 included. We developed three models: a null model (Model 1); a second model that included patient variables in accordance or related with the CDC criteria (Model 2): pneumonia degree at the day of air sampling, lymphocytes count, duration of symptoms, and the result of the last positive diagnostic PCR test before air sampling; and a final model utilizing a backward stepwise approach to optimize the selection of patient data variables (Model 3). All analyses were performed using the GLM function in R Stats Package 4.3.2, Vienna, Austria).

Results

Characteristics of the different studies and rate of airborne SARS-CoV-2 positive samples

A total of 69 COVID-19 patients were recruited among the three independent studies. The airborne SARS-CoV-2 genome was detected in 54 %, 44 %, and 83 % of the air samples of the studies I, II, and III, respectively (Table 2). Collectively, the rate of positive cases was 54 %. The airborne SARS-CoV-2 positivity rate was close to being significantly higher in Study III ($p = 0.051$). An important difference in the design of the studies is the period lapsed from the last positive diagnostic PCR test to air sampling, which was delimited in Study III. However, the differences between the three studies did not reach statistical significance ($p = 0.096$). On the other hand, only in Study II, we recruited patients with a recent onset of symptoms, being such period significantly shorter than that of the studies I and III ($p < 0.001$). Patients without symptoms were recruited only in study I. Moreover, there were also significant differences between studies regarding the type of inpatient admission and in the degree of pneumonia presented at the day of air sampling. Thus, all the patients of the Study III were admitted in an intensive care unit (ICU) or intermediate respiratory care unit (IRCU) and there was a significantly higher frequency of cases with severe pneumonia than that reported in the patients of the studies I and II. There were no significant differences between studies regarding patient gender, the presence of previous diseases, pneumonia degree at discharge, or lymphocyte and platelet concentrations (Table 2). Finally, the SARS-CoV-2 variant was not identified in studies I and II, whereas in Study III it could be identified in 75 % of the patients and corresponded to the delta variant in all of them.

To analyze the influence of patient variables in the detection of airborne SARS-CoV-2 genome, the three studies were treated independently given the differences in the experimental design and the output.

Influence of individual variables on airborne SARS-CoV-2 genome detection

COVID-19 patient sex did not influence the detection of airborne SARS-CoV-2 genome in any study (Table 3). However, patient age showed a clear trend in Study II ($p = 0.054$), in which patients within the positive cases were older than those in the negative ones (Fig. 1). When the cases of the three studies were pooled, the mean patient age in the positive cases was 63.4 years old, whereas in the negative ones was 55.2 years old. Such a difference did not reach statistical significance, despite the clear the trend persisted ($p = 0.074$).

Both the history of previous respiratory diseases and the presence of obesity influenced the detection of airborne SARS-CoV-2 genome in Study I (Table 3). Thus, there was a higher relative frequency of positive cases within the patients with previous respiratory diseases ($p < 0.05$), as well as within patients with obesity ($p < 0.05$). Such effects were not statistically significant in the other studies.

Influence of viral load estimation on airborne SARS-CoV-2 genome detection

To analyze the influence of the estimation of patient viral load, the Ct values of nasopharyngeal PCR tests were divided into two groups according to the median value (Ct = 25) as a proxy of viral

Table 2
Descriptive table of variables collected for each of the patients.

Study	I	II	III	p.overall
	N = 26	N = 31	N = 12	
Airborne SARS-CoV-2 RNA detection				0.051
-	12 (46.2 %)	18 (58.1 %)	2 (16.7 %)	
+	14 (53.8 %)	13 (41.9 %)	10 (83.3 %)	
Age	57.0 [45.0;77.2]	65.0 [45.2;77.0]	57.5 [43.0;64.5]	0.441
Sex				0.282
Female	8 (30.8 %)	13 (41.9 %)	7 (58.3 %)	
Male	18 (69.2 %)	18 (58.1 %)	5 (41.7 %)	
Previous respiratory diseases				0.938
NO	18 (69.2 %)	22 (73.3 %)	9 (75.0 %)	
YES	8 (30.8 %)	8 (26.7 %)	3 (25.0 %)	
Cancer				0.309
NO	24 (92.3 %)	30 (100 %)	12 (100 %)	
YES	2 (7.69 %)	0 (0.00 %)	0 (0.00 %)	
Obesity				1
NO	20 (76.9 %)	23 (76.7 %)	10 (83.3 %)	
YES	6 (23.1 %)	7 (23.3 %)	2 (16.7 %)	
Result of the last positive diagnostic PCR test before air sampling				0.33
Ct > 25	12 (50.0 %)	7 (30.4 %)	4 (57.1 %)	
Ct ≤ 25	12 (50.0 %)	16 (69.6 %)	3 (42.9 %)	
Days lapsed from the last positive diagnostic test to air sampling				0.096
≤3	13 (50.0 %)	24 (77.4 %)	5 (71.4 %)	
> 3	13 (50.0 %)	7 (22.6 %)	2 (28.6 %)	
Presence of symptoms				0.006
Asymptomatic	6 (23.1 %)	0 (0.00 %)	0 (0.00 %)	
Symptomatic	20 (76.9 %)	31 (100 %)	12 (100 %)	
Presence and duration of symptoms				< 0.001
Asymptomatic	6 (23.1 %)	0 (0.00 %)	0 (0.00 %)	
< 10 days	5 (19.2 %)	28 (90.3 %)	7 (58.3 %)	
≥10 days	15 (57.7 %)	3 (9.68 %)	5 (41.7 %)	
Duration of symptoms (days)	12.5 [9.75;28.2]	4.00 [4.00;6.00]	9.00 [6.00;11.8]	< 0.001
Dyspnea				0.484
NO	12 (46.2 %)	11 (36.7 %)	3 (25.0 %)	
YES	14 (53.8 %)	19 (63.3 %)	9 (75.0 %)	
Cough				0.234
NO	12 (46.2 %)	12 (40.0 %)	2 (16.7 %)	
YES	14 (53.8 %)	18 (60.0 %)	10 (83.3 %)	
Dyspnea and cough				0.125
NO	10 (38.5 %)	6 (20.0 %)	1 (8.33 %)	
YES	16 (61.5 %)	24 (80.0 %)	11 (91.7 %)	
Nausea and/or vomits				0.111
NO	25 (96.2 %)	23 (76.7 %)	10 (83.3 %)	
YES	1 (3.85 %)	7 (23.3 %)	2 (16.7 %)	
Diarrhea				0.15
NO	22 (84.6 %)	20 (66.7 %)	7 (58.3 %)	
YES	4 (15.4 %)	10 (33.3 %)	5 (41.7 %)	
Fever				0.51
NO	8 (30.8 %)	11 (36.7 %)	2 (16.7 %)	
YES	18 (69.2 %)	19 (63.3 %)	10 (83.3 %)	
Cephalea				0.252
NO	23 (88.5 %)	23 (76.7 %)	8 (66.7 %)	
YES	3 (11.5 %)	7 (23.3 %)	4 (33.3 %)	
Arthromyalgia				0.456
NO	21 (80.8 %)	20 (66.7 %)	8 (66.7 %)	
YES	5 (19.2 %)	10 (33.3 %)	4 (33.3 %)	
General malaise				0.019
NO	17 (65.4 %)	10 (33.3 %)	3 (25.0 %)	
YES	9 (34.6 %)	20 (66.7 %)	9 (75.0 %)	
Type of inpatient admission				< 0.001
Hospital ward	25 (96.2 %)	28 (90.3 %)	0 (0.00 %)	
IRCU or ICU	1 (3.85 %)	3 (9.68 %)	12 (100 %)	
Pneumonia at hospital admission				0.392
NO	6 (23.1 %)	3 (9.68 %)	1 (8.33 %)	
YES	20 (76.9 %)	28 (90.3 %)	11 (91.7 %)	
Pneumonia degree at hospital admission				0.251
NO	6 (23.1 %)	3 (9.68 %)	1 (8.33 %)	
Mild	7 (26.9 %)	13 (41.9 %)	1 (8.33 %)	
Moderate	9 (34.6 %)	11 (35.5 %)	8 (66.7 %)	
Severe	4 (15.4 %)	4 (12.9 %)	2 (16.7 %)	
Pneumonia at the day of air sampling				0.392
NO	6 (23.1 %)	3 (9.68 %)	1 (8.33 %)	
YES	20 (76.9 %)	28 (90.3 %)	11 (91.7 %)	

(*continued on next page*)

Table 2 (*continued*)

Study	I	II	III	p.overall
Pneumonia degree at the day of air sampling				0.002
NO	6 (23.1 %)	3 (9.68 %)	1 (8.33 %)	
Mild	5 (19.2 %)	10 (32.3 %)	1 (8.33 %)	
Moderate	14 (53.8 %)	11 (35.5 %)	2 (16.7 %)	
Severe	1 (3.85 %)	7 (22.6 %)	8 (66.7 %)	
Pneumonia at discharge				0.392
NO	6 (23.1 %)	3 (9.68 %)	1 (8.33 %)	
YES	20 (76.9 %)	28 (90.3 %)	11 (91.7 %)	
Pneumonia degree at discharge				0.216
NO	6 (23.1 %)	3 (9.68 %)	1 (8.33 %)	
Mild	2 (7.69 %)	3 (9.68 %)	1 (8.33 %)	
Moderate	12 (46.2 %)	13 (41.9 %)	2 (16.7 %)	
Severe	6 (23.1 %)	12 (38.7 %)	8 (66.7 %)	
Fatalities				0.584
NO	23 (88.5 %)	29 (93.5 %)	12 (100 %)	
YES	3 (11.5 %)	2 (6.45 %)	0 (0.00 %)	
Lymphocytes per μL	1200 [755;1808]	1175 [838;1608]	990 [648;1652]	0.78
Platelets per μL	188,000 [151,500;250,250]	166,000 [142,500;212,000]	204,500 [187,000;231,000]	0.298
Corticosteroids				0.543
NO	9 (34.6 %)	10 (33.3 %)	2 (16.7 %)	
YES	17 (65.4 %)	20 (66.7 %)	10 (83.3 %)	
Remdesivir				0.041
NO	23 (88.5 %)	23 (76.7 %)	6 (50.0 %)	
YES	3 (11.5 %)	7 (23.3 %)	6 (50.0 %)	
Tocilizumab				0.001
NO	23 (88.5 %)	30 (100 %)	7 (58.3 %)	
YES	3 (11.5 %)	0 (0.00 %)	5 (41.7 %)	

load. The frequency of positive and negative airborne SARS-CoV-2 samples was not significantly different between patients with a high or low Ct value in any study (Table 3). Subsequently, we analyzed the influence of the time lapsed from the latest positive nasopharyngeal PCR test prior to air sampling. The cases were divided into two groups according to the median day number (3 days). The number of days lapsed from the positive nasopharyngeal PCR test did not affect the airborne SARS-CoV-2 detection in any study.

Influence of COVID-19 patient symptoms on airborne SARS-CoV-2 genome detection

The elapsed time since COVID-19 symptoms onset has been taken into consideration in the design of preventive measures, as the infection risk was expected to be very low after 12 days of symptoms onset [10]. In Study I, patients were recruited regardless of the elapsed period since symptoms onset. There, we detected airborne SARS-CoV-2 in the rooms of patients whose symptoms had been initiated a long time before air sampling, even up to more than 30 days in the case of three patients (Fig. 2). Hence, the mean period was 18.8 days within the positive cases, which is not different from the mean of 17.5 days found in the negative ones. In Study II, in which, by contrast, a period shorter than 12 days since the onset of symptoms was applied as an inclusion criterion, there were no significant differences between positive and negative cases in this variable. Likewise, no significant differences were found in Study III and in the pooled studies.

There were no differences in airborne SARS-CoV-2 detection between the symptomatic and asymptomatic patients of the study I (Table 3). Regarding the influence of specific symptoms, there were no significant differences between positive and negative cases in any study for any of the reported symptoms (Table 3). In the pooled studies, the risk of detecting airborne SARS-CoV-2 was 3.6 times higher in the patients that reported both dyspnea and cough than in those not reporting such symptoms, despite the odds ratio did not reach statistical significance ($p = 0.05$).

Impact of disease severity on the detection of airborne SARS-CoV-2 genome

To explore the potential relation between COVID-19 disease severity and airborne SARS-CoV-2 detection, we first analyzed the influence of the type of admission (Table 3). COVID-19 patients were admitted in hospital wards or ICU/IRCU depending on their medical requirements. In the pooled studies, the odds ratio of detecting airborne SARS-CoV-2 was 3.2 times higher in the patients admitted in an ICU or IRCU than in those admitted in hospital wards, although it was not statistically significant ($p = 0.095$). We also analyzed the influence of developing pneumonia and its degree (Table 3). In Study I, the detection of airborne SARS-CoV-2 was significantly influenced by the degree of pneumonia severity presented at admission, being a mild or moderate severity more frequent in the positive cases ($p < 0.05$). The influence of the degree of pneumonia severity was maintained at subsequent points, which are at the day of air sampling and at discharge. In the latter case, the risk of detecting airborne SARS-CoV-2 was 18 times higher in the patients who presented moderate pneumonia ($p < 0.05$). The use of corticosteroids, immunosuppressants, or antiviral treatment to COVID-19 patients had no influence on airborne SARS-CoV-2 detection (data not shown).

Impact of blood parameters of COVID-19 patients on the detection of airborne SARS-CoV-2 genome

The significant associations found between the detection of airborne SARS-CoV-2 genome and the degree of pneumonia severity prompted us to analyze whether the blood concentration of molecular and cellular indicators of patient disease could also influence the detection of airborne SARS-CoV-2. We analyzed the influence of leucocytes, lymphocytes, platelets, ESR, D-dimer, c-reactive protein, ferritin, lactate dehydrogenase, and procalcitonin, known as risk factors for COVID-19 severity [25]. Interestingly, the positive cases of the study I presented a significantly lower lymphocyte concentration than the negative ones ($p < 0.05$). Moreover, such a difference

Table 3
COVID-19 patient categorical variables in relation to environmental SARS-CoV-2 detection. The influence of individual variables, patient viral load estimation, symptoms, and disease severity indicators on airborne SARS-CoV-2 detection was analyzed. (+): Airborne SARS-CoV-2 RNA detected, (-): Airborne SARS-CoV-2 RNA not detected. OR: odds-ratio. Overall *p* value is indicated. Categorical baseline characteristics were analyzed via the chi-squared test.

	STUDY I				STUDY II				STUDY III				ALL			
	- *N = 12*	+ *N = 14*	OR	p value	- *N = 18*	+ *N = 13*	OR	p value	- *N = 2*	+ *N = 10*	OR	p value	- *N = 32*	+ *N = 37*	OR	p value
Individual variables																
Sex				1				0.439				1				0.465
Female	4 (33.3 %)	4 (28.6 %)	Ref.		6 (33.3 %)	7 (53.8 %)	Ref.		1 (50.0 %)	6 (60.0 %)	Ref.		11 (34.4 %)	17 (45.9 %)	Ref.	
Male	8 (66.7 %)	10 (71.4 %)	1.24 [0.21;7.21]		12 (66.7 %)	6 (46.2 %)	0.44 [0.09;1.95]		1 (50.0 %)	4 (40.0 %)	0.69 [0.01;32.2]		21 (65.6 %)	20 (54.1 %)	0.62 [0.23;1.65]	
Previous respiratory diseases				0.036				1				0.455				0.435
NO	11 (91.7 %)	7 (50.0 %)	Ref.		13 (72.2 %)	9 (75.0 %)	Ref.		1 (50.0 %)	8 (80.0 %)	Ref.		25 (78.1 %)	24 (66.7 %)	Ref.	
YES	1 (8.33 %)	7 (50.0 %)	9.17 [1.21;270]		5 (27.8 %)	3 (25.0 %)	0.88 [0.14;4.79]		1 (50.0 %)	2 (20.0 %)	0.29 [0.01;14.5]		7 (21.9 %)	12 (33.3 %)	1.76 [0.59;5.54]	
Cancer				1				/				/				1
NO	11 (91.7 %)	13 (92.9 %)	Ref.		18 (100 %)	12 (100 %)			2 (100 %	10 (100 %)			31 (96.9 %)	35 (97.2 %)	Ref.	
YES	1 (8.33 %)	1 (7.14 %)	0.85 [0.02;35.9]		0 (0.00 %)	0 (0.00 %)			0 (0.00 %)	0 (0.00 %)			1 (3.12 %)	1 (2.78 %)	0.89 [0.02;35.6]	
Obesity				0.017				0.669				1				0.361
NO	12 (100 %)	8 (57.1 %)			13 (72.2 %)	10 (83.3 %)	Ref.		2 (100 %)	8 (80.0 %)			27 (84.4 %)	26 (72.2 %)	Ref.	
YES	0 (0.00 %)	6 (42.9 %)			5 (27.8 %)	2 (16.7 %)	0.55 [0.06;3.32]		0 (0.00 %)	2 (20.0 %)			5 (15.6 %)	10 (27.8 %)	2.03 [0.62;7.48]	
Patient viral load estimation																
Result of the last positive diagnostic PCR test before air sampling				1				1				0.429				0.752
Ct > 25	6 (54.5 %)	6 (46.2 %)	Ref.		4 (28.6 %)	3 (33.3 %)	Ref.		0 (0.00 %)	4 (66.7 %)			10 (38.5 %)	13 (46.4 %)	Ref.	
Ct ≤ 25	5 (45.5 %)	7 (53.8 %)	1.38 [0.26;7.50]		10 (71.4 %)	6 (66.7 %)	0.80 [0.12;5.70]		1 (100 %)	2 (33.3 %)			16 (61.5 %)	15 (53.6 %)	0.73 [0.24;2.18]	
Days lapsed from the last positive diagnostic test to air sampling				0.694				1				1				0.543
≤ 3	7 (58.3 %)	6 (42.9 %)	Ref.		14 (77.8 %)	10 (76.9 %)	Ref.		1 (100 %)	4 (66.7 %)			22 (71.0 %)	20 (60.6 %)	Ref.	
> 3	5 (41.7 %)	8 (57.1 %)	1.81 [0.37;9.47]		4 (22.2 %)	3 (23.1 %)	1.00 [0.73;1.37]		0 (0.00 %)	2 (33.3 %)			9 (29.0 %)	13 (39.4 %)	1.57 [0.55;4.64]	
Symptoms																
Presence of symptoms				0.365				/				/				0.405
NO	4 (33.3 %)	2 (14.3 %)	Ref.		0 (0.00 %)	0 (0.00 %)			0 (0.00 %)	0 (0.00 %)			4 (12.5 %)	2 (5.41 %)	Ref.	
YES	8 (66.7 %)	12 (85.7 %)	2.80 [0.41;27.0]		18 (100 %)	13 (100 %)			2 (100 %)	10 (100 %)			28 (87.5 %)	35 (94.6 %)	2.39 [0.41;20.4]	
Dyspnea				0.122				1				1				0.258
NO	8 (66.7 %)	4 (28.6 %)	Ref.		7 (38.9 %)	4 (33.3 %)	Ref.		0 (0.00 %)	3 (30.0 %)			15 (46.9 %)	11 (30.6 %)	Ref.	
YES	4 (33.3 %)	10 (71.4 %)	4.58 [0.89;28.5]		11 (61.1 %)	8 (66.7 %)	1.25 [0.27;6.48]		2 (100 %)	7 (70.0 %)			17 (53.1 %)	25 (69.4 %)	1.98 [0.73;5.51]	

(*continued on next page*)

Table 3 (*continued*)

	STUDY I				STUDY II				STUDY III				ALL			
	- *N = 12*	+ *N = 14*	OR	p value	- *N = 18*	+ *N = 13*	OR	p value	- *N = 2*	+ *N = 10*	OR	p value	- *N = 32*	+ *N = 37*	OR	p value
Cough				0.122				0.709				1				0.103
NO	8 (66.7 %)	4 (28.6 %)	Ref.		8 (44.4 %)	4 (33.3 %)	Ref.		0 (0.00 %)	2 (20.0 %)			16 (50.0 %)	10 (27.8 %)	Ref.	
YES	4 (33.3 %)	10 (71.4 %)	4.58 [0.89;28.5]		10 (55.6 %)	8 (66.7 %)	1.56 [0.34;8.02]		2 (100 %)	8 (80.0 %)			16 (50.0 %)	26 (72.2 %)	2.55 [0.94;7.26]	
Dyspnea and cough				0.105				0.358				1				0.05
NO	7 (58.3 %)	3 (21.4 %)	Ref.		5 (27.8 %)	1 (8.33 %)	Ref.		0 (0.00 %)	1 (10.0 %)			12 (37.5 %)	5 (13.9 %)	Ref.	
YES	5 (41.7 %)	11 (78.6 %)	4.69 [0.87;31.8]		13 (72.2 %)	11 (91.7 %)	3.71 [0.47;109]		2 (100 %)	9 (90.0 %)			20 (62.5 %)	31 (86.1 %)	3.59 [1.13;13.1]	
Nausea and/or vomits				1				1				0.318				1
NO	12 (100 %)	13 (92.9 %)			14 (77.8 %)	9 (75.0 %)	Ref.		1 (50.0 %)	9 (90.0 %)	Ref.		27 (84.4 %)	31 (86.1 %)	Ref.	
YES	0 (0.00 %)	1 (7.14 %)			4 (22.2 %)	3 (25.0 %)	1.17 [0.18;6.96]		1 (50.0 %)	1 (10.0 %)	0.15 [0.00;8.55]		5 (15.6 %)	5 (13.9 %)	0.87 [0.21;3.57]	
Diarrhea				1				0.139				1				0.435
NO	10 (83.3 %)	12 (85.7 %)	Ref.		14 (77.8 %)	6 (50.0 %)	Ref.		1 (50.0 %)	6 (60.0 %)	Ref.		25 (78.1 %)	24 (66.7 %)	Ref.	
YES	2 (16.7 %)	2 (14.3 %)	0.84 [0.08;9.30]		4 (22.2 %)	6 (50.0 %)	3.31 [0.67;18.3]		1 (50.0 %)	4 (40.0 %)	0.69 [0.01;32.2]		7 (21.9 %)	12 (33.3 %)	1.76 [0.59;5.54]	
Fever				0.401				0.266				1				1
NO	5 (41.7 %)	3 (21.4 %)	Ref.		5 (27.8 %)	6 (50.0 %)	Ref.		0 (0.00 %)	2 (20.0 %)			10 (31.2 %)	11 (30.6 %)	Ref.	
YES	7 (58.3 %)	11 (78.6 %)	2.48 [0.44;16.6]		13 (72.2 %)	6 (50.0 %)	0.40 [0.08;1.89]		2 (100 %)	8 (80.0 %)			22 (68.8 %)	25 (69.4 %)	1.03 [0.36;2.95]	
Cephalea				0.58				1				1				1
NO	10 (83.3 %)	13 (92.9 %)	Ref.		14 (77.8 %)	9 (75.0 %)	Ref.		1 (50.0 %)	7 (70.0 %)	Ref.		25 (78.1 %)	29 (80.6 %)	Ref.	
YES	2 (16.7 %)	1 (7.14 %)	0.42 [0.01;5.88]		4 (22.2 %)	3 (25.0 %)	1.17 [0.18;6.96]		1 (50.0 %)	3 (30.0 %)	0.46 [0.01;22.1]		7 (21.9 %)	7 (19.4 %)	0.86 [0.26;2.91]	
Arthromyalgia				1				1				0.515				0.811
NO	10 (83.3 %)	11 (78.6 %)	Ref.		12 (66.7 %)	8 (66.7 %)	Ref.		2 (100 %)	6 (60.0 %)			24 (75.0 %)	25 (69.4 %)	Ref.	
YES	2 (16.7 %)	3 (21.4 %)	1.32 [0.17;13.3]		6 (33.3 %)	4 (33.3 %)	1.01 [0.19;4.92]		0 (0.00 %)	4 (40.0 %)			8 (25.0 %)	11 (30.6 %)	1.31 [0.45;3.98]	
General malaise				0.429				1				1				0.852
NO	9 (75.0 %)	8 (57.1 %)	Ref.		6 (33.3 %)	4 (33.3 %)	Ref.		0 (0.00 %)	3 (30.0 %)			15 (46.9 %)	15 (41.7 %)	Ref.	
YES	3 (25.0 %)	6 (42.9 %)	2.15 [0.40;13.9]		12 (66.7 %)	8 (66.7 %)	0.99 [0.20;5.21]		2 (100 %)	7 (70.0 %)			17 (53.1 %)	21 (58.3 %)	1.23 [0.47;3.27]	
Disease severity indicators																
Type of inpatient admission				0.462				0.558				/				0.095
Hospital ward	11 (91.7 %)	14 (100 %)			17 (94.4 %)	11 (84.6 %)	Ref.		0 (0.00 %)	0 (0.00 %)			28 (87.5 %)	25 (67.6 %)	Ref.	
IRCU or ICU	1 (8.33 %)	0 (0.00 %)			1 (5.56 %)	2 (15.4 %)	2.82 [0.21;96.1]		2 (100 %)	10 (100 %)			4 (12.5 %)	12 (32.4 %)	3.24 [0.97;13.2]	
Pneumonia at hospital admission				0.065				1				1				0.17
NO	5 (41.7 %)	1 (7.14 %)	Ref.		2 (11.1 %)	1 (7.69 %)	Ref.		0 (0.00 %)	1 (10.0 %)			7 (21.9 %)	3 (8.11 %)	Ref.	
YES	7 (58.3 %)	13 (92.9 %)	7.81 [0.95;236]		16 (88.9 %)	12 (92.3 %)	1.40 [0.10;47.7]		2 (100 %)	9 (90.0 %)			25 (78.1 %)	34 (91.9 %)	3.04 [0.74;16.1]	

(*continued on next page*)

Table 3 (*continued*)

	STUDY I				STUDY II				STUDY III				ALL			
	- *N = 12*	+ *N = 14*	OR	p value	- *N = 18*	+ *N = 13*	OR	p value	- *N = 2*	+ *N = 10*	OR	p value	- *N = 32*	+ *N = 37*	OR	p value
Pneumonia degree at hospital admission				0.04				0.948				/				0.405
NO	5 (41.7 %)	1 (7.14 %)	Ref.		2 (11.1 %)	1 (7.69 %)	Ref.		0 (0.00 %)	1 (10.0 %)			7 (21.9 %)	3 (8.11 %)	Ref.	
Mild	1 (8.33 %)	6 (42.9 %)	18.8 [1.36;804]		7 (38.9 %)	6 (46.2 %)	1.59 [0.10;58.5]		0 (0.00 %)	1 (10.0 %)			8 (25.0 %)	13 (35.1 %)	3.55 [0.73;21.8]	
Moderate	3 (25.0 %)	6 (42.9 %)	7.87 [0.75;273]		7 (38.9 %)	4 (30.8 %)	1.09 [0.07;41.7]		2 (100 %)	6 (60.0 %)			12 (37.5 %)	16 (43.2 %)	2.95 [0.65;17.0]	
Severe	3 (25.0 %)	1 (7.14 %)	1.58 [0.03;77.1]		2 (11.1 %)	2 (15.4 %)	1.77 [0.07;86.7]		0 (0.00 %)	2 (20.0 %)			5 (15.6 %)	5 (13.5 %)	2.20 [0.34;16.5]	
Pneumonia at the day of air sampling				0.065				1				1				0.17
NO	5 (41.7 %)	1 (7.14 %)	Ref.		2 (11.1 %)	1 (7.69 %)	Ref.		0 (0.00 %)	1 (10.0 %)			7 (21.9 %)	3 (8.11 %)	Ref.	
YES	7 (58.3 %)	13 (92.9 %)	7.81 [0.95;236]		16 (88.9 %)	12 (92.3 %)	1.40 [0.10;47.7]		2 (100 %)	9 (90.0 %)			25 (78.1 %)	34 (91.9 %)	3.04 [0.74;16.1]	
Pneumonia degree at the day of air sampling				0.04				0.802				1				0.253
NO	5 (41.7 %)	1 (7.14 %)			2 (11.1 %)	1 (7.69 %)	Ref.		0 (0.00 %)	1 (10.0 %)			7 (21.9 %)	3 (8.11 %)	Ref.	
Mild	2 (16.7 %)	3 (21.4 %)			6 (33.3 %)	4 (30.8 %)	1.25 [0.08;48.6]		0 (0.00 %)	1 (10.0 %)			8 (25.0 %)	8 (21.6 %)	2.22 [0.42;14.3]	
Moderate	4 (33.3 %)	10 (71.4 %)			5 (27.8 %)	6 (46.2 %)	2.16 [0.14;81.7]		0 (0.00 %)	2 (20.0 %)			9 (28.1 %)	18 (48.6 %)	4.36 [0.94;25.8]	
Severe	1 (8.33 %)	0 (0.00 %)			5 (27.8 %)	2 (15.4 %)	0.80 [0.04;34.9]		2 (100 %)	6 (60.0 %)			8 (25.0 %)	8 (21.6 %)	2.22 [0.42;14.3]	
Pneumonia at discharge				0.065				1				1				0.17
NO	5 (41.7 %)	1 (7.14 %)	Ref.		2 (11.1 %)	1 (7.69 %)	Ref.		0 (0.00 %)	1 (10.0 %)			7 (21.9 %)	3 (8.11 %)	Ref.	
YES	7 (58.3 %)	13 (92.9 %)	7.81 [0.95;236]		16 (88.9 %)	12 (92.3 %)	1.40 [0.10;47.7]		2 (100 %)	9 (90.0 %)			25 (78.1 %)	34 (91.9 %)	3.04 [0.74;16.1]	
Pneumonia degree at discharge				0.023				1				/				0.235
NO	5 (41.7 %)	1 (7.14 %)	Ref.		2 (11.1 %)	1 (7.69 %)	Ref.		0 (0.00 %)	1 (10.0 %)			7 (21.9 %)	3 (8.11 %)	Ref.	
Mild	1 (8.33 %)	1 (7.14 %)	3.87 [0.07;229]		2 (11.1 %)	1 (7.69 %)	1.00 [0.02;57.8]		0 (0.00 %)	1 (10.0 %)			3 (9.38 %)	3 (8.11 %)	2.19 [0.25;21.3]	
Moderate	2 (16.7 %)	10 (71.4 %)	18.0 [1.72;640]		7 (38.9 %)	6 (46.2 %)	1.59 [0.10;58.5]		0 (0.00 %)	2 (20.0 %)			9 (28.1 %)	18 (48.6 %)	4.36 [0.94;25.8]	
Severe	4 (33.3 %)	2 (14.3 %)	2.22 [0.13;87.4]		7 (38.9 %)	5 (38.5 %)	1.34 [0.08;50.1]		2 (100 %)	6 (60.0 %)			13 (40.6 %)	13 (35.1 %)	2.23 [0.48;13.0]	
Fatalities				1				1				/				1
NO	11 (91.7 %)	12 (85.7 %)	Ref.		17 (94.4 %)	12 (92.3 %)	Ref.		2 (100 %)	10 (100 %)			30 (93.8 %)	34 (91.9 %)	Ref.	
YES	1 (8.33 %)	2 (14.3 %)	1.70 [0.12;58.7]		1 (5.56 %)	1 (7.69 %)	1.40 [0.03;58.3]		0 (0.00 %)	0 (0.00 %)			2 (6.25 %)	3 (8.11 %)	1.29 [0.18;11.7]	

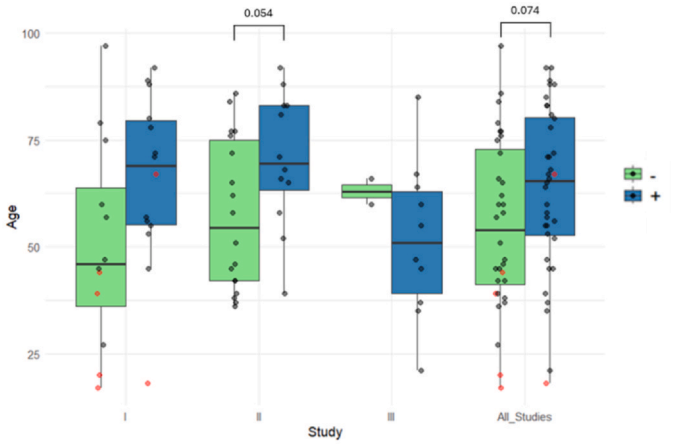

Fig. 1. COVID-19 patient age in relation to the environmental SARS-CoV-2 detection. The influence of age on airborne SARS-CoV-2 detection was analyzed. (+): Airborne SARS-CoV-2 RNA detected, (-): Airborne SARS-CoV-2 RNA not detected. Black points represent symptomatic patients, while red points represent asymptomatic patients. Age was compared using Pearson's test or Spearman's test, depending on the normality of the data in each study.

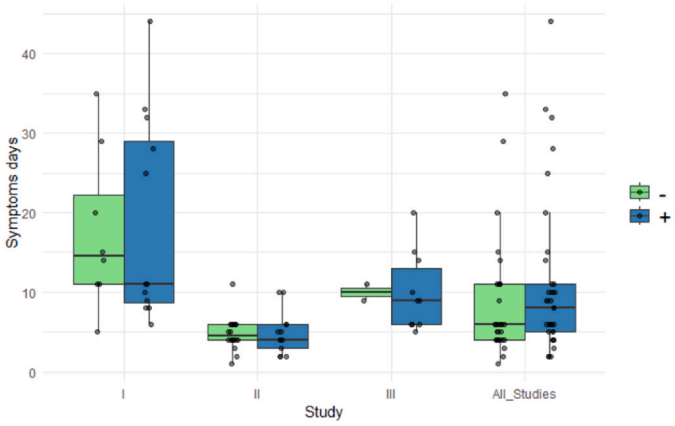

Fig. 2. Time lapsed from the onset of COVID-19 symptoms to air sampling in relation to the environmental SARS-CoV-2 detection. The influence of the number of days since symptoms onset on airborne SARS-CoV-2 detection was analyzed. (+): Airborne SARS-CoV-2 RNA detected, (-): Airborne SARS-CoV-2 RNA not detected. The number of days with symptoms was compared using Pearson's test or Spearman's test, depending on the normality of the data in each study.

persisted when the cases of all studies were pooled ($p < 0.05$). Regarding the platelet concentration, positive cases in each study presented lower concentration than the negative cases. This difference was close to reaching statistical significance when the studies were pooled ($p = 0.064$) (Fig. 3). No statistically significant results were found for the remaining parameters analyzed (data not shown).

Predicting the risk of airborne SARS-CoV-2 detection in individual hospital rooms

A multivariate analysis was performed to predict the risk of detecting the airborne SARS-CoV-2 genome (Table 4). All the patient variables previously studied in the bivariate analysis were included in the generalized linear model. First, we constructed a model in which we incorporated the variables that are considered in the CDC criteria or related with them: degree of pneumonia severity at the day of air sampling, presence and duration of symptoms, lymphocyte count, and the outcome of the most recent PCR test prior to air sampling (GLM model 2, AIC = 83.95). On the other hand, the best-fit model identified three independent factors: patient age, duration of symptoms, and type of admission (GLM model 3, AIC = 78.43). According to the best-fit model, the risk of detecting airborne SARS-CoV-2 was significantly higher as the age of the patient increased ($p < 0.05$). According to this model, the risk of detecting SARS-CoV-2 is associated with a combination of advanced patient age, prolonged symptom duration, and the need for ICU admission.

Discussion and conclusions

Implementing preventive measures is the most effective strategy for reducing the risk of SARS-CoV-2 transmission in healthcare settings. To prevent infections from hospitalized patients, management protocols are based on specific patient features with sufficient scientific evidence linking them to infection risk. However, to fully understand the risk of airborne SARS-CoV-2 transmission, further studies should be conducted, specifically in air samples. In this study, we analyzed the potential association between airborne SARS-CoV-2 detection in hospital rooms and COVID-19 patient variables across three independent studies. The use of highly sensitive procedure with which a single SARS-CoV-2 genome copy per reaction can be detected [16,18] allows the analysis of the influence of external factors. We found a significant association for patient variables currently included in guidelines for controlling COVID-19

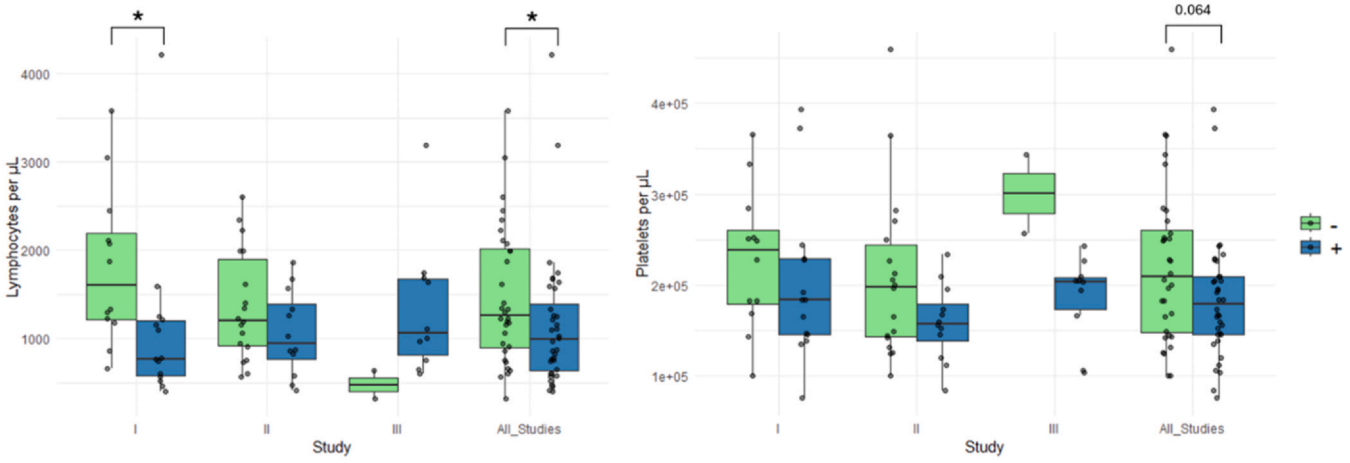

Fig. 3. Blood parameters in relation to the environmental SARS-CoV-2 detection. The influence of lymphocyte and platelet concentration on airborne SARS-CoV-2 detection was analyzed. (+): Airborne SARS-CoV-2 RNA detected, (-): Airborne SARS-CoV-2 RNA not detected. Lymphocytes and platelets counts were compared using Pearson's test or Spearman's test, depending on the normality of the data in each study.

Table 4
Multivariable prediction risk models. Model 1 is the null model. Model 2 included patient variables indicative of or associated with disease severity, symptomatology, and immunocompetence. Pneumonia degree was referred to patients without pneumonia. The duration of symptoms was referred to asymptomatic patients. The Ct values of PCR tests ≤25 were referenced by Ct values > 25. Model 3 is the best-fit model and included age, duration of symptoms, and type of admission.

		Estimate	Std. Error	z value	Pr(> |z|)	AIC
Model 1						97.29
	(Intercept)	0.145	0.241	0.601	0.548	
Model 2						83.95
	(Intercept)	−0.252	1.603	−0.157	0.875	
	Pneumonia degree (mild)	0.458	1.108	0.413	0.679	
	Pneumonia degree (moderate)	1.249	1.106	1.129	0.259	
	Pneumonia degree (severe)	0.113	1.296	0.087	0.931	
	Lymphocytes (per uL)	-4×10^{-4}	4×10^{-4}	−1.100	0.271	
	Duration of symptoms (≤10 days)	0.168	1.381	0.121	0.903	
	Duration of symptoms (> 10 days)	0.600	1.469	0.409	0.683	
	PCR test (Ct≤25)	−0.077	0.642	−0.120	0.905	
Model 3						78.43
	(Intercept)	−2.78	1.239	−2.245	0.025	
	Age	0.034	0.016	2.055	0.040	
	Duration of symptoms	0.044	0.032	1.358	0.174	
	Type of admission (IRCU or ICU)	1.188	0.773	1.537	0.125	

transmission in healthcare centers [10], as well as with additional variables not yet considered. Our findings are relevant for refining the understanding of the COVID-19 infection risk and could support adjustments to preventive measures.

We conducted three independent studies with variations in the experimental design and outcomes. Although there were no significant differences in positivity rate between studies, the results may have been influenced by variations in several methodological aspects (Table 1). In all three studies, bioaerosols collection was performed using the same liquid impinger and the SARS-CoV-2 genome quantification was conducted via ddPCR. However, in Study III [18], we introduced changes to the protocols for collecting and detecting airborne SARS-CoV-2, including the use of oil as the collection medium and a more efficient reverse transcriptase, which may have influenced the positivity rate compared to Studies I and II. Additionally, Study III included patients with a recent COVID-19 diagnosis who were receiving high-flow oxygen therapy, a criterion not applied in Studies I and II, which may have further impacted the positivity rate. Indeed, according to the multivariate analysis, the patient respiratory support influenced the risk of airborne SARS-CoV-2 detection, since all patients admitted in ICU or IRCU required an oxygen supply. Another key difference is that Study II included patients with a recent onset of symptoms, which, contrary to expectations [26], did not result in a higher positivity rate compared to Study I. In the bivariate analysis, the risk of detecting airborne SARS-CoV-2 genome was significantly higher in patients with preexisting respiratory diseases, obesity, pneumonia at admission and discharge, and low lymphocyte counts. Conversely, this risk was not significantly associated with the presence or duration of symptoms, or the time since the last positive nasopharyngeal PCR test. In the multivariate analysis, patient age, symptom duration, and the type of admission emerged as the most influential factors.

The lack of association between airborne SARS-CoV-2 genome detection and symptom duration in the bivariate analysis may partly stem from differences in the experimental designs of the three studies. In study II, patients with symptom onset ≥12 days prior to air sampling were not recruited, reducing the variability of this parameter and limiting the likelihood of observing significant differences. However, this criterion was not applied in Study I, where no significant differences were observed either. This finding contrasts with the widely accepted notion that the likelihood of infectiousness in COVID-19 patients with mild-to-moderate illness is low beyond 10 days of symptoms onset [26] and with the observed association between environmental SARS-CoV-2 detection and symptom onset [11,20]. In many patients the viral load in throat swabs peaks around day 4 after symptom onset [27] and then declines [9,28]. However, viral replication and bioaerosol shedding vary significantly between individuals [7]. Additionally, the detection of airborne SARS-CoV-2 RNA even after the development of a neutralizing IgG response [29] suggests a potential risk of infection post-seroconversion. In Study I, we detected 5 positive airborne SARS-CoV-2 samples in patients whose symptoms had begun 20 days or more before air sampling. This may be due to differences in SARS-CoV-2 dynamics between airborne samples and throat swabs, similar to the observed persistence in sputum samples compared to upper respiratory tract samples [30]. Viral shedding likely does not depend solely on symptom presence or duration since no differences in viral load have been found between symptomatic, presymptomatic, and asymptomatic individuals [28,30,31]. In the multivariate analysis, the duration of symptoms positively contributed to the risk of detecting airborne SARS-CoV-2 in the best-fit model, suggesting that prolonged symptom duration is linked to an increased airborne SARS-CoV-2 detection, and potentially higher infection risk from patients with persistent symptoms and more severe disease. Regarding specific symptoms, dyspnea and cough showed the most consistent trend, aligning with previous studies [9], though statistical significance was not reached ($p = 0.05$). As with other variables, the limited sample size may have influenced these non-significant trends.

Differences in experimental design may have contributed to the lack of significant association between airborne SARS-CoV-2 detection and PCR test results. Specifically, only in Study III, we recruit patients who had a positive PCR test within 10 days prior to air sampling. We observed no significant differences in airborne SARS-CoV-2 detection based on the time elapsed from the last positive PCR test or the Ct value in Study III, and similarly no significant associations were found in studies I and II, where this inclusion criterion was not applied. Patient viral load was estimated by RT-PCR, which, although useful, is not necessarily a reliable marker for assessing the infectious status of COVID-19 patients [32]. Indeed, there are considerable limitations to using RT-PCR Ct values as predictors of infectivity [33].

Interestingly, we identified variables indicatives of or associated with COVID-19 severity, such as the type of admission, pneumonia severity, obesity [34–36], and lymphocyte count [25,37] that were positively associated with airborne SARS-CoV-2 genome detection. A low platelet count, also linked to COVID-19 severity [25], showed a trend towards association ($p = 0.064$). Altogether, these findings corroborate that managing patients with severe disease carries a higher infection risk. Consistent with this, SARS-CoV-2 quantification in exhaled air via face masks was positively associated with other indicators of severity [20].

Along with symptomatology and disease severity, immunocompetence is an important criterion in the management of COVID-19 patients [10]. Immunosenescence, a hallmark of aging, is characterized by a decline in lymphocyte concentration. Consistent with the immunocompetence criterion, both lower lymphocyte count and an advanced age were associated with the airborne SARS-CoV-2 detection. Importantly, patient age emerged as the most significant factor in the multivariate predictive model, suggesting that immunosenescence plays a predominant role in the infection risk.

Our results emphasize the heterogeneity in the response to SARS-CoV-2 infection, particularly in viral replication and bioaerosol shedding [7]. COVID-19 was characterized by superspreading events driven by high inter-individual variability in transmission potential [38]. Superspreaders are individuals who transmit the virus to more people than expected. It is estimated that around 80 % of SARS-CoV-2 secondary transmissions were caused by a small fraction of infectious individuals (10–19 %) [39]. This highlights significant inter-individual variability [40], and underscores the need to identify factors that contribute to this variation and assess their impact on infection risk.

In conclusion, our findings underscore the potential value of assessing airborne SARS-CoV-2 to enhance understanding of infectivity. They suggest that COVID-19 severity and indicators of immunosenescence are associated with airborne SARS-CoV-2 detection. These insights could aid in developing more targeted preventive measures in healthcare settings to reduce SARS-CoV-2 transmission risk.

Abbreviations

CDC	center for disease control
Ct	cycle threshold
ddPCR	droplet digital polymerase chain reaction
ESR	erythrocyte sedimentation rate
GLM	generalized linear model
ICU	intensive care unit
IRCU	intermediate respiratory care unit
N	nucleocapside
OR	odds-ratio
ORF1ab	open reading frame 1ab
PCR	polymerase chain reaction
RdRP	RNA-dependent RNA polymerase
RT-qPCR	reverse transcription and quantitative polymerase chain reaction
VIF	variance inflation factors

CRediT authorship contribution statement

Conceptualization: J.M.B. E.S.L. A.C.F. M.D.F.; methodology: J.T.V. G.E.G. A.C.F. J.M.B.; validation: J.T.V. G.E.G. A.C.F. J.M.B.; formal analysis: G.E.G. L.A.G. A.C.F.; investigation: J.T.V. N.T.P. J.S.R. P.A.F.R. E.S.L.; data curation: J.T.V. G.E.G. L.A.G. N.T.P., J.M.B.; writing-original draft: J.M.B. J.S.R. M.D.F.; writing-review and editing: J.M.B. J.S.R. E.S.L. A.D.S; visualization: J.T.V. G.E.G. L.A.G. A.D.S, J.M.B.; supervision: J.M.B. A.C.F.; resources: E.S.L.; project administration: H.G.B. J.M.B.; funding acquisition: H.G.B., J.M.B.

Ethical approval

This research was approved by the Research Ethics Committee of the Balearic Islands (IB4503/21PI).

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Data availability

Access to the raw data used and analyzed during the current study is available upon request by contacting the authors.

Declaration of Competing Interest

The corresponding author on behalf of all authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this article.

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