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PCR-based diagnosis of respiratory virus in postsurgical septic patients

A preliminary study before SARS-CoV-2 pandemic

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Abstract

Respiratory viruses are part of the normal microbiota of the respiratory tract, which sometimes cause infection with/without respiratory insufficiency and the need for hospital or ICU admission. The aim of this study is to determine the prevalence of respiratory viruses in nontransplanted postoperative septic patients as well as lymphocyte count influence in their presence and its relationship to mortality. 223 nontransplanted postsurgical septic patients were recruited on the Intensive Care Unit (ICU) at Hospital Clínico Universitario de Valladolid prior to the SARS-COV-2 pandemic. Patients were split into 2 groups according to the presence/absence of respiratory viruses. Multivariate logistic regression analysis was used to identify independent factors related to positive respiratory virus PCR test. Respiratory viruses were isolated in 28.7% of patients. 28-day mortality was not significantly different between virus-positive and virus-negative groups. Logistic regression analysis revealed that lymphocyte count ≤ 928/μl is independently associated with a positive PCR result [OR 3.76, 95% CI (1.71–8.26), $P = .001$] adjusted by platelet count over 128,500/μL [OR 4.27, 95% CI (1.92–9.50) $P < .001$] and the presence of hypertension [OR 2.69, 95% CI (1.13–6.36) $P = .025$] as confounding variables. Respiratory viruses' detection by using PCR in respiratory samples of nontransplanted postoperative septic patients is frequent. These preliminary results revealed that the presence of lymphopenia on sepsis diagnosis is independently associated to a positive virus result, which is not related to a higher 28-day mortality.

Abbreviations: APACHE II = scale and chronic health evaluation, BAL = bronchoalveolar lavage, DNA = deoxyribonucleic acid, hCoV = human coronavirusHRV = rhinovirus, ICU = intensive care unit, OOP = optimal operating point, OR = odds ratio, PCR = polymerase chain reaction, RNA = ribonucleic acid, RSV = Respiratory Syncytial Virus, SARS-CoV-2 = Severe Acute Respiratory Syndrome Coronavirus 2, SOFA = Sequential Organ Failure Assessment.

Keywords: sepsis, viruses, polymerase chain reaction, surgery, intensive care units, postoperative period

1. Introduction

Sepsis represents a worrisome health problem worldwide, as it has a high incidence rate of 50–300 cases per 100,000 inhabitants per year and a mortality rate of up to 50–60% when associated with shock.[1–3] It is one of the main causes of emergency admission to the intensive care unit (ICU) and also one of the principal causes of mortality during the postoperative period in these special units.[4–6] For all of these reasons, sepsis represents one of the main causes of hospital expenses.[7,8]

Bacteria have been traditionally considered to be the only infective microorganisms involved in sepsis, with increasing attention given to fungi. However, prior to the emergence of the severe acute respiratory syndrome Coronavirus 2 (SARS-COV-2) pandemic, viruses were not commonly considered to be infectious agents implicated in sepsis. They were only considered in the case of sepsis secondary to community-acquired pneumonia and in that developed by patients receiving hematopoietic stem cell or solid organ transplants, immunosuppressed partly due to the drugs that are used in their treatment.[9–12]

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Competing interests: The authors have no competing interests to declare that are relevant to the content of this article.

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate: The study protocol was approved by the Ethics Committee for Clinical Research, Hospital Clínico Universitario de Valladolid, Valladolid, Spain (approval No. (PI18–1166)). This study followed current Spanish legislation for biomedical research, fulfilling the standards indicated by the Declaration of Helsinki. Written informed consent was obtained from patients, patients' relatives or their legal representative before enrollment.

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Lymphocytes are indispensable cells in the defence against intracellular pathogens such as viruses,[13] so their dysfunction or depletion can increase the risk of infection by this kind of microbes[14–16] and also the risk of patient death.[17]

Respiratory viruses are part of the normal microbiota of the respiratory tract,[18–22] which sometimes cause infection with/without respiratory insufficiency and the need for hospital or ICU admission.[23,24] The COVID-19 pandemic has highlighted the importance of virus infections.[25] However, the prevalence and impact of respiratory viruses in septic postoperative patients is still unknown, despite the fact that lymphocyte dysfunction and lymphopenia may exist secondary to the cytokine storm triggered by both surgery and sepsis.[16,26] For this reason, we think that is probable that respiratory viruses are present in nontransplanted postoperative septic patients and that their presence may have a negative influence on patients' outcomes.

The objectives of this study are to determine the prevalence of respiratory viruses in nontransplanted postoperative septic patients as well as lymphocyte count influence in their presence and its relationship to mortality.

2. Material and Methods

2.1. Study setting

This preliminary observational study was conducted at the post-surgical ICU of the Hospital Clínico Universitario de Valladolid, a Spanish tertiary referral center with 800 beds. The study was approved by the Institutional Review Board and conducted in accordance with guidelines established for experiments involving humans by the hospital´s Ethic Committee (PI18–1166) and the Code of Ethics of the Word Medical Association, Declaration of Helsinki. Written informed consent was obtained from patients or direct relatives when the former could not give consent due to deterioration of their physical condition.

2.2. Study design and patient selection

All adult patients with suspected sepsis during the postoperative period in the ICU were prospectively and consecutively included from January 2017 to December 2019. The definition of the different infections[27–30] is explained in Supplemental File 1, http://links.lww.com/MD/G911. They were treated as usual for this kind of patients, following the guidelines of good clinical practice. Respiratory, blood, urine, catheter and surgical site samples were collected and analyzed, looking for bacteria and fungi when sepsis was suspected.[31] In all septic patients, a swab of the posterior nasopharynx was taken in nonintubated patients and a bronchoalveolar lavage (BAL) through a bronchoscope was performed in intubated patients in order to detect respiratory viruses.

A retrospective analysis of data allowed us to divide the sample of patients into 2 groups: (i) the virus-positive group (n = 64), patients with a virus-positive respiratory sample; and (ii) the virus-negative group (n = 159), patients without a virus-positive respiratory sample. While analyzing results, we also decided to divide the virus positive group into 2 groups: (i) survivors (n = 43) and (ii) patients who died in the first 28 days after surgery (n = 21), to observe the tendency of the reached results.

The exclusion criteria were age under eighteen years old, to have undergone solid organ or stem cell transplantation, presence of any hematological malignancy, use of immunosuppressive drugs within the last 4 weeks, HIV positive status, use of antiviral drugs within the previous month or during hospital admission, death within the first 24 hours after ICU admission, and insufficient respiratory sample volume for virus analysis.

2.3. Data collection

Demographic data, comorbidity, the undergone surgery and the poorest clinical and laboratory data from the first 24 hours after sepsis diagnosis were prospectively recorded at the time of patients' inclusion. The severity of the disease was evaluated on the day of sepsis diagnosis by Sequential Organ Failure Assessment (SOFA)[32] and Scale and Chronic Health Evaluation (APACHE) II score.[33] After hospital discharge, data regarding the source of infection, isolated microorganisms, duration of ventilation therapy, ICU and total hospital stay, and mortality during the first 28 days after surgery were collected from patients' medical records.

2.4. Laboratory and Virological assessments

Procalcitonin and C-Reactive protein were measured in plasma (Supplemental File 1, http://links.lww.com/MD/G911).

For virological assessment, the swab of each patient was rinsed in 3 ml of Universal Tractor Medium (UTM) and RNA and DNA were extracted by Emag® (bioMérieux). The genetic material was then amplified through a multiplex PCR (polymerase chain reaction) technique using a battery of primers, and subsequently hybridized using microspheres labeled for detection in the MAGPIX system, using NxTAG-RPP reagents (Luminex®, Austin, TX, USA). These reagents detect the following respiratory viruses: adenovirus; bocavirus; human coronavirus (hCoV), hCoV-HKU1, hCoV-NL63, hCoV-OC43, hCoV-229E types; metapneumovirus; rhinovirus/enterovirus; influenzavirus A, A(H1N1)pdm09 and A(H3N2) subtypes; influenzavirus B; parainfluenza virus 1, 2, 3, and 4; and respiratory syncytial virus (RSV) A and B subtypes.

2.5. Statistical analysis

A preliminary study was carried out due to the inability to recruit more patients' respiratory samples in a safety way at the ends of 2019th. This was due to the emergence of the SARS-CoV-2

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pandemic, a new respiratory virus not previously found in patients, and whose behavior was still unknown.

The Kolmogorov-Smirnov test was used to check the normality of the data distribution. Quantitative data were expressed as medians and 25–75 interquartile ranges (Q1-Q3) and qualitative data as absolute numbers and percentages.

Categorical variables were compared using the chi-square test or Fischer exact test, when appropriate. Quantitative variables were compared using the Mann–Whitney U test. Kaplan-Meier curves were constructed to assess the impact of respiratory virus presence on survival. Multivariate logistic regression analysis was used to identify independent factors related to positive respiratory virus PCR test, using cut-off values of absolute lymphocytes as principal variable. Potential confounding clinical factors that yielded $P \leq .1$ in the univariate analysis were used for adjusting the model.

The normal range of absolute lymphocytes according to our laboratory is 0.9 to 5.2 lymphocytes × 10^3/μL. The normal range for platelets is 150 to 400 platelets × 10^3/μL. The optimal cut-off values of absolute lymphocyte and platelet counts for predicting a positive PCR test for respiratory viruses in the studied population were obtained by using the optimal operating point (OOP), the value for which the point on the ROC curve had the minimum distance to the upper left corner (where sensitivity = 1 and specificity = 1).[34] By Pythagoras' theorem this distance is:

$$\text{OOP} = \sqrt{(1 - \text{sensitivity})^2 + (1 - \text{specificity})^2}$$

Results are summarized as adjusted odds ratios (aORs) and 95% confidence intervals (CIs). The diagnostic performance of the model was confirmed using a receiver operating characteristic (ROC) curve. Results derived from the multivariate logistic regression analysis were validated by the bootstrapping method, using 1000 random samples. The level of significance was set at $P \leq .05$. Statistical analysis was performed using SPSS 24.0 software.

Table 1

Clinical characteristics of patients.

VARIABLE	Virus-negative sample n = 159 (71.3%)	Virus-positive sample n = 64 (28.7%)	*P* value
Age (yr)	71 [63–77]	71 [64–76]	0.934
Gender			0,489
Male	99 (62.26)	43 (67.18)	
Female	60 (37.73)	21 (32.81)	
Comorbidity			
Smoker	46 (28.9)	14 (21.9)	0.516
Diabetes mellitus	29 (18.2)	20 (31.3)	0.282
Arterial hypertension	86 (54.1)	42 (65.6)	**0.034**
Chronic renal insufficiency	18 (11.3)	4 (6.3)	0.115
COPD	24 (15.1)	6 (9.4)	0.251
Chronic hepatic disease	12 (7.5)	3 (4.7)	0.295
Intraoperative data			
Main scheduled surgery			0.393
Cardiac surgery	94 (59.1)	29 (45.0)	
Abdominal surgery	50 (31.5)	23 (36.0)	
Vascular surgery	7 (4.4)	5 (8.0)	
Other surgeries	8 (5.0)	7 (11.0)	
Surgery duration (min)	210 [145–300]	205 [150–275]	0.687
Postoperative data			
APACHE II Score	15 [12–17]	15 [13–18]	0.110
SOFA score	8 [6–10]	8 [6–10]	0.481
Creatinine (mg/dl)	1.40 [0.92–2.29]	1.60 [0.98–2.50]	0.630
Procalcitonine (ng/ml)	2.86 [0.68–14]	4.69 [0.77–18.00]	0.383
C-reactive protein (mg/dl)	260 [136–344.7]	212 [138–311]	0.290
Lactate (mmol/L)	2.83 [1.78–4.38]	2.70 [1.78–4.12]	0.969
Leukocytes (U/μl)	11,130 (9100–15,940)	8560 (4850–17,820)	0.402
Lymphocytes (U/μl)	1100 [610–1660]	800 [610–1150]	**0.028**
Platelets (U/μl)	98,000 [21,000–170,000]	142,000 [70,000–215,000]	**0.037**
PaO_2/FiO_2	191.24 [120.00–221.00]	176.00 [126.00–230.00]	0.208
Other postoperative data			
Bacteremia	46 (28.9)	15 (23.4)	0.405
Central venous catheter infection	66 (41.5)	22 (34.4)	0.365
Surgical Site Infection	51 (32.1)	15 (23.4)	0.201
Pneumonia/Tracheobronchitis	40 (25.2)	22 (34.4)	0.116
Peritonitis	51 (32.1)	23 (35.9)	0.638
Septic Shock	60 (37.7)	33 (51.6)	0.058
Mechanical ventilation (days)	3 [0-7]	3 [0–5]	0.335
Mechanical ventilation > 48 hours	54 [34.0]	21 (32.8)	0.869
Transfusion	62 (39.0)	23 [35.9]	0.671
Total stay in ICU (days)	11 [6–18]	9 [5–17]	0.397
Total stay at hospital (days)	26 [16–42]	25 [17–43]	0.924
Mortality at 28th postoperative day	49 (30.8)	21 (32.8)	0.772

Quantitative data are expressed as median with interquartile rank (IQR = [Q1-Q3]) and qualitative data as absolute number and percentage. The significance of the bold values in the Tables is that is a significant *P* value.

APACHE II = Acute Physiology and Chronic Health Evaluation II, COPD = Chronic Obstructive Pulmonary Disease, FiO_2 = fraction of inspired oxygen, ICU = intensive care unit, PaO_2 = arterial partial pressure of oxygen, SOFA = Sequential Organ Failure Assessment.

3. Results

3.1. Characteristics of septic patients

A total of 223 septic patients were recruited for this study. Respiratory viruses were isolated in 28.7% of them (n = 64). Patients' characteristics, intra- and postoperative data are expressed in Table 1. Demographic data of the patients were similar between the groups, as well as their comorbid conditions, except for hypertension which was significantly more prevalent in the virus-positive group (P = .034). In the first 24 hours of sepsis diagnosis, absolute lymphocyte count was significantly lower in the virus-positive group (P = .028) while platelet count was higher in this group of patients (P = .037). Virus-positive patients showed more frequently septic shock status, but this difference was not significant (P = .058). There were no differences in pneumonia development (P = .116) nor in days of mechanical ventilation (P = .335). No differences were found in survival rates at 28 days between the groups (P = .772). A Kaplan-Meier curve is shown in Supplemental Figure 1, http://links.lww.com/MD/G911.

3.2. Respiratory samples

The isolated microbes identified in the respiratory samples are summarised in Table 2.

Eight different kinds of respiratory viruses were identified in the virus-positive patients. The most common isolated virus was rhinovirus/enterovirus (32.8%). Coinfection by 2 different species of virus was observed in 11 (17.2%) patients at sepsis diagnosis.

Bacteria and fungi were also isolated from the respiratory samples. There was co-infection of the respiratory sample by bacteria and fungi in 31.3% and 18.8% of virus-positive patients, respectively. The most common co-infecting microbe was *Candida albicans* (15.6%), followed by *Haemophilus influenzae* (7.8%). In respiratory samples from the virus-negative group, bacteria were more often isolated (42.1%) while fungi were less frequently present (7.5%) than in those from the virus-positive group. Bacterial diversity was also higher in virus-negative group. The most common microbe in the virus-negative group was *Haemophilus influenzae* (10.1%).

Table 2

Viruses, bacteria, and fungi isolated in respiratory samples.

MICROORGANISM	Virus-negative patients n = 159	Virus-positive patients n = 64
Virus		
Rhinovirus/enterovirus	0 (0.0)	21 (32.8)
Bocavirus	0 (0.0)	13 (20.3)
Metapneumovirus	0 (0.0)	12 (18.8)
Influenza A virus	0 (0.0)	10 (15.6)
Parainfluenza virus	0 (0.0)	7 (10.9)
Adenovirus	0 (0.0)	4 (6.3)
Respiratory syncytial virus	0 (0.0)	6 (9.4)
Coronavirus	0 (0.0)	2 (3.1)
Gram negative bacteria		
Acinetobacter baumanii	9 (5.7)	0 (0.0)
Enterobacter cloacae	2 (1.3)	0 (0.0)
Enterobacter sakazakii	2 (1.3)	0 (0.0)
Escherichia coli	1 (0.6)	2 (3.1)
Haemophilus influenzae	16 (10.1)	5 (7.8)
Haemophilus parainfluenzae	8 (5.0)	4 (6.3)
Klebsiella oxytoca	1 (0.6)	0 (0.0)
Klebsiella pneumoniae	5 (3.1)	2 (3.1)
Morganella morganii	1 (0.6)	0 (0.0)
Proteus mirabilis	3 (1.9)	0 (0.0)
Pseudomonas aeruginosa	6 (3.8)	4 (6.3)
Stenotrophomonas maltophilia	2 (1.3)	1 (1.6)
Gram-positive bacteria		
Staphylococcus aureus	6 (3.8)	2 (3.1)
Staphycoloccus epidermidis	3 (1.9)	0 (0.0)
Staphycoloccus hominis	1 (0.6)	0 (0.0)
Streptococcus pneumoniae	1 (0.6)	0 (0.0)
Fungi		
Candida albicans	9 (5.7)	10 (15.6)
Other *Candida* species	3 (1.9)	2 (3.1)

Data are expressed as absolute number and percentage. The significance of the bold values in the Tables is that is a significant P value.

3.3. Association between lymphocyte count and presence of viral infection

Logistic multivariate regression analysis revealed that lymphocyte count OOP (lymphocyte count ≤ 928/μL) is independently associated with a positive virus result [Odds Ratio (OR) 3.76, 95% CI (1.71–8.26), P = .001], adjusted by platelet count OOP (platelet count > 128500/μL) [OR 4.27, 95% CI (1.92–9.50) P < .001] and the presence of hypertension [OR 2.69, 95% CI (1.13–6.36) P = .025] as confounding variables (Table 3). Results from the multivariate regression analysis were confirmed in the validation analysis (Table 4).

3.4. Mortality

Sociodemographic, intra- and postoperative data from patients with a positive PCR test for respiratory a virus are shown in Table 5, for both those who were alive and those who had died by the 28th postoperative day. There were no significant differences in preoperative variables. Patients from the virus-positive group who died had undergone significantly more abdominal surgeries than those who survived, in whom cardiac surgery was the most prevalent. There were no significant differences in the absolute lymphocyte count in the first 24 hours of sepsis diagnosis between survivors and patients who died (P = .909), although those who died were more often in septic shock (P = .005).

4. Discussion

This preliminary study revealed that in nontransplanted septic patients during the postoperative period i) Respiratory viruses were isolated in 28.7% of them by PCR technique; ii) to reach lymphocyte absolute values ≤ 928/μL the first day of sepsis diagnosis is independently associated to a higher presence of respiratory viruses; iii) mortality at 28 postoperative days was not significantly different between patients with and without respiratory viruses at sepsis diagnosis.

Table 3

Multivariate regression analysis for evaluating lymphocyte count association to the presence of viruses in respiratory samples.

	OR	95% CI	*P* value
Arterial hypertension	2.69	1.13–6.36	**0.025**
Platelets > 128500/μl	4.27	1.92–9.50	**< 0.001**
Lymphocytes < 928/μl	3.76	1.71–8.27	**0.001**

The significance of the bold values in the Tables is that is a significant P value.
CI = confidence interval, OR = odds ratio.

Table 4

Validation of the multivariate regression analysis by Bootstrapping method using 1000 random samples.

	B	95% CI	*P* value
Arterial hypertension	0.99	0.19–1.96	**0.013**
Platelets > 128500/μl	1.45	0.63–2.39	**0.001**
Lymphocytes < 928/μl	1.33	0.57–2.25	**0.003**

The significance of the bold values in the Tables is that is a significant P value.
CI = confidence interval.

Table 5

Characteristics of survivors and nonsurvivors of virus-positive group.

Variable	Survivors n = 43 (67.2%)	Nonsurvivors at 28th day n = 21 (32.8%)	*P* value
Age (yr)	72 [64–78]	68 [65–73]	0.252
Gender			0.529
Male	30 (69.8%)	13 (61.9)	
Female	13 (30.2%)	8 (38.1)	
Comorbidity			
Smoker	11 (25.6)	3 (14.3)	0.356
Diabetes mellitus	13 (30.20)	7 (33.3)	0.802
Arterial hypertension	26 (60.5)	16 (76.2)	0.214
Chronic renal insufficiency	1 (2.3)	3 (14.3)	0.099
COPD	5 (11.6)	1 (4.8)	0.646
Chronic hepatic disease	2 (4.7)	1 (4.8)	1.00
Intraoperative data			
Main scheduled surgery			**0.042**
Cardiac surgery	25 (58.1)	4 (19.0)	
Abdominal surgery	13 (30.2)	10 (47.6)	
Vascular surgery	2 (4.7)	3 (14.3)	
Other surgeries	3 (7.0)	4 (19.0)	
Surgery duration (min)	210 [150–250]	195 [135–358]	0.972
Postoperative data			
APACHE II Score	14 [13–16]	17 [15–20]	**0.003**
SOFA score	4 [2–8]	9 [6–11]	**0.003**
Creatinine (mg/dl)	1.31 [0.89–2.14]	2.50 [1.54–3.16]	**0.015**
Procalcitonine (ng/ml)	1.25 [0.15–7.10]	4.69 [0.33–16.50]	0.165
C-reactive protein (mg/dl)	198.50 [136.00–322.50]	222.00 [158.00–293.00]	0.737
Lactate (mmol/L)	1.99 [1.00–3.69]	2.70 [1.09–3.90]	0.433
Leukocytes (U/µl)	14,495 [11,770–19,640]	19,000 [13,680–23,990]	0.186
Lymphocytes(U/µl)	800 [500–1410]	800 [670–1030]	0.909
Platelets (U/µl)	163,500 [114,500–212,500]	89,000 [35,000–215,000]	0.395
PaO_2/FiO_2	191.24 [120.00–221.00]	176.00 [126.00–230.00]	0.079
Other postoperative data			
Bacteremia	7 (16.3)	8 (38.1)	0.066
Central venous catheter infection	12 (27.9)	10 (47.6)	0.119
Surgical site infection	8 (18.6)	7 (33.3)	0.197
Pneumonia/tracheobronchitis	26 (60.5)	17 (81.0)	0.101
Peritonitis	14 (32.6)	10 (47.6)	0.280
Septic shock	16 (37.2)	17 (81.0)	**0.005**
Mechanical ventilation (days)	1 [0–4]	3 [1–6]	0.093
Mechanical ventilation > 48 hours	11 (25.6)	10 (47.6)	0.078
Transfusion	12 (27.9)	11 (52.4)	0.055
Total stay in ICU (d)	9 [5–17]	12 [5–18]	0.856
Total stay at hospital (d)	27 [18–47]	25 [8–36]	0.210

Quantitative data are expressed as median with interquartile rank (IQR = [Q1-Q3]) and qualitative data as absolute number and percentage. The significance of the bold values in the Tables is that is a significant *P* value.

APACHE II = Acute Physiology and Chronic Health Evaluation II, COPD = Chronic Obstructive Pulmonary Disease, FiO_2 = fraction of inspired oxygen, ICU = intensive care unit, PaO_2 = arterial partial pressure of oxygen, SOFA = Sequential Organ Failure Assessment.

Emerging pathogens are a global public health challenge, as evidenced by the COVID-19 pandemic, highlighting the importance of virus infections.[25] Prevalence of respiratory viruses in adult patients admitted to the ICU described in the literature is very variable (18–32%), perhaps due to the great variability of the studied populations.[35–37] In our study, the prevalence of respiratory viruses was at the high end of this range (28.7%), although we were unable to find any study exclusively performed in postoperative nontransplanted adult patients with sepsis of any origin to properly compare our results.

Rhinovirus/enterovirus, followed by bocavirus and metapneumovirus, were the viruses most commonly detected in the respiratory samples of our virus-positive patients. Rhinovirus (HRV) belongs to the *Picornaviridae* family. They are single-stranded negative-sense RNA viruses, which previously formed a genus of their own, but have been reclassified by the International Committee on Taxonomy and now belong to the genus enterovirus.[38,39] These viruses are found in different settings and in both symptomatic and asymptomatic patients.[19,35,36,40–43] Their capability to cause asymptomatic carrier state,[44] which do not lead to rejection of patients for surgery, together with a long period of viral shedding,[38,44] may be the reason why HRVs were the viruses most commonly found in our virus-positive patients. It is difficult to compare the relative frequency of the other viruses detected here with that observed in other studies due to the great variability of the populations studied and the large degree of interpersonal microbiome diversity, even in the absence of disease.[44–47]

Two different genera of respiratory viruses were simultaneously detected in 17.2% of virus-positive patients, which is a greater prevalence than that described in the literature (4.8–11%)[36,48,49]; however, a lesser degree and variety of bacterial coinfection was found in the virus-positive group compared to the virus-negative group. A primary function of any ecosystem of microorganisms is to create a symbiotic state, blocking the uncontrolled growth of germs that can provoke an infection latter, in such a way that the more variety of microorganism, the greater respiratory health the person will have.[18]

Respiratory virus infection after stem cell or solid organ transplant has been associated with higher mortality rates[50,51]; however, there is controversy surrounding whether the detection of respiratory viruses in nontransplanted patients during the postoperative period is associated with poorer prognosis.[52–54]

During the postoperative period, nontransplanted septic patients may be immunosuppressed secondary to the cytokine storm that occurs in sepsis or when undergoing surgery, causing a decrease in the absolute number and function of circulating lymphocytes, thereby increasing patients' susceptibility to secondary infections and virus reactivations.[16,55,56] In agreement with this, the absolute lymphocyte count was lower in our virus-positive group; however, we did not find a significant difference in mortality between our virus-positive and virus-negative patients. We also did not find significant differences between these groups in terms of pneumonia development (P = .116) or days of mechanical ventilation (P = .335). Again, this leads us to question whether our virus-positive patients may not have had an active viral infection at the time of the respiratory sample collection, despite a positive PCR test, since as Motley *et al* said that the PCR test detects genetic material of both infective and inactive virus, so a positive PCR respiratory virus test does not necessarily indicate the presence of replicating virus.[57] We think that it would be interesting for future studies to use viral cultures to determine if the presence of an active infection is positively associated with mortality during the postoperative period in nontransplanted septic patients. Thus, appropriate use of antivirals could be implemented when an active viral infection is actually present and not merely in the presence of virus genetic material. This would improve the prognosis of these patients.

Our study has several limitations. First, the study was conducted at a single center as it was a preliminary study, which limits the generalisability of our results. Second, as we did not evaluate immunosuppressive patients, testing for reactivation of herpes simplex virus or cytomegalovirus, which are well known for their ability to cause pneumonia, was not performed. Third, we did not separately consider elective from emergent surgery because of the limited sample size, which may have an impact on viral detection and mortality. Fourth, the PCR test did not allow us to detect genetic material from respiratory viruses not previously characterized in laboratories, as is possible with other diagnostic tests such as those based on next generation sequencing, not available in our hospital.[58] Fifth, absolute lymphocyte count was only determined on the first day of sepsis diagnosis for logistical reasons, which is also shortly following the surgery.

5. Conclusions

Considering all of our results, we can conclude that the detection of respiratory viruses using a PCR-based technique in respiratory samples from nontransplanted postoperative septic patients is frequent. These preliminary results revealed that the presence of lymphopenia on sepsis diagnosis is independently associated to a positive virus result. However, a positive PCR test for respiratory virus is not associated with higher 28-day mortality. Further studies are needed in order to determine the role of infective virus in these patients, as the PCR technique does not distinguish between infective and inactive virus.

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Author contributions

Conceptualization, M.H-R., S.B-A., E.T. and E.G-S.; Methodology, M.H-R., S.B-A., M.M-F., A.T-V., E.T. and E.G-S.; Formal Analysis, M.M-F. and A.T-V; Investigation, M.H-R., S.B-A., M-L-L., E-G-P., P.J-M., S.R-R., L.S-P., I.S-M., J.M-E., P.M-P., H.G-B., A.T-V., M.M-F., P.S-C., E.T. and E.G-S.; Validation, M.H-R. and S.B-A.; Writing—Original Draft Preparation, M.H-R., S.B-A., M-L-L., E-G-P., P.J-M., J.M-E., M.M-F., P.S-C., E.T. and E.G-S.; Writing—Review & Editing, M.H-R., S.B-A., M-L-L., E-G-P., P.J-M., S.R-R., L.S-P., I.S-M., J.M-E., P.M-P., H.G-B., A.T-V., M.M-F., P.S-C., E.T. and E.G-S.; Visualization, M.H-R., S.B-A., M.M-F. and A.T-V.; Supervision, E.T. and E.G-S.; Project Administration, E.T. and E.G-S.; Funding Acquisition, M.H-R.

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