



**HAL**  
open science

## **Multiplex PCR assay to detect nasopharyngeal viruses in immunocompromised patients with acute respiratory failure.**

Alexis Maillard, Jérôme Le Goff, Mariame Barry, Virginie Lemiale, Séverine Mercier-Delarue, Alexandre Demoule, Linda Feghoul, Samir Jaber, Kada Klouche, Achille Kouatchet, et al.

► **To cite this version:**

Alexis Maillard, Jérôme Le Goff, Mariame Barry, Virginie Lemiale, Séverine Mercier-Delarue, et al.. Multiplex PCR assay to detect nasopharyngeal viruses in immunocompromised patients with acute respiratory failure.. Chest, 2023, 10.1016/j.chest.2023.07.4222 . hal-04180638

**HAL Id: hal-04180638**

**<https://hal.science/hal-04180638>**

Submitted on 29 Jan 2024

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# **Multiplex Polymerase Chain Reaction Assay to Detect Nasopharyngeal Viruses in Immunocompromised Patients With Acute Respiratory Failure**

Alexis Maillard <sup>1</sup>, Jérôme Le Goff <sup>2</sup>, Mariame Barry <sup>2</sup>, Virginie Lemiale <sup>1</sup>, Séverine Mercier-Delarue <sup>2</sup>, Alexandre Demoule <sup>1</sup>, Linda Feghoul <sup>2</sup>, Samir Jaber <sup>3</sup>, Kada Klouche <sup>4</sup>, Achille Kouatchet <sup>5</sup>, Laurent Argaud <sup>6</sup>, Francois Barbier <sup>7</sup>, Naike Bigé <sup>1</sup>, Anne-Sophie Moreau <sup>8</sup>, Emmanuel Canet <sup>9</sup>, Frédéric Pène <sup>10</sup>, Maud Salmona <sup>11</sup>, Djamel Mokart <sup>12</sup>, Elie Azoulay <sup>13</sup>

<sup>1</sup> Service de Médecine Intensive et Réanimation, Hôpital Saint-Louis, Assistance Publique des Hôpitaux de Paris, Paris.

<sup>2</sup> Service de Virologie, Hôpital Saint-Louis, Assistance Publique des Hôpitaux de Paris, Paris.

<sup>3</sup> Département Anesthésie et Réanimation B, Centre Hospitalier Universitaire de Montpellier, Hôpital Saint-Eloi.

<sup>4</sup> Département de Médecine Intensive et Réanimation, Hôpital Lapeyronie, Montpellier.

<sup>5</sup> Médecine Intensive et Réanimation, CHU d'Angers, Angers.

<sup>6</sup> Médecine Intensive-Réanimation, Hospices Civils de Lyon, Hôpital Edouard Herriot, Lyon.

<sup>7</sup> Unité de Soins Intensifs Médicaux, La Source Hospital, Centre Hospitalier Régional d'Orléans, Orléans.

<sup>8</sup> Pôle de Médecine Intensive Réanimation, Hôpital Roger Salengro, CHU Lille, Lille.

<sup>9</sup> Service de Médecine Intensive Réanimation, Centre Hospitalier Universitaire Hôtel-Dieu, Nantes.

<sup>10</sup> Service de Médecine Intensive-Réanimation, Hôpital Cochin, Assistance Publique des Hôpitaux de Paris, Paris.

<sup>11</sup> Laboratoire de Virologie, Hôpital Saint-Louis, Assistance Publique des Hôpitaux de Paris, Paris.

<sup>12</sup> Département d'Anesthésie-Réanimation, Institut Paoli Calmette, Marseille, France.

<sup>13</sup> Service de Médecine Intensive et Réanimation, Hôpital Saint-Louis, Assistance Publique des Hôpitaux de Paris, Paris. Electronic address: [elie.azoulay@aphp.fr](mailto:elie.azoulay@aphp.fr).

## **Keywords:**

acute respiratory failures; critical care; immunocompromised; respiratory viruses.

## **ABBREVIATIONS:**

ARF = acute respiratory failure; Ct = cycle threshold; hMPV = human metapneumovirus; HSCT = hematopoietic stem cell transplantation; IMV = invasive mechanical ventilation; PCR = polymerase chain reaction; PIV = human parainfluenza virus; RSV = respiratory syncytial virus; sHR = subdistribution hazard ratio; SOFA = Sequential Organ Failure Assessment

**CORRESPONDENCE TO:**

Elie Azoulay, MD, PhD;

*email:*

[elie.azoulay@aphp.fr](mailto:elie.azoulay@aphp.fr)

## Abstract

**Background:** In immunocompromised patients with acute respiratory failure (ARF), the clinical significance of respiratory virus detection in the nasopharynx remains uncertain.

**Research question:** Is viral detection in nasopharyngeal swabs associated with causes and outcomes of ARF in immunocompromised patients?

**Study design and methods:** This preplanned post hoc analysis of a randomized controlled trial enrolled immunocompromised patients admitted to 32 ICUs for ARF between May 2016 and December 2017. Nasopharyngeal swabs sampled at inclusion were assessed for 23 respiratory pathogens using multiplex polymerase chain reaction (PCR) assay. Causes of ARF were established by managing physicians and were reviewed by three expert investigators masked to the multiplex PCR assay results. Associations between virus detection in nasopharyngeal swabs, causes of ARF, and composite outcome of day 28 mortality, invasive mechanical ventilation (IMV), or both were assessed.

**Results:** Among the 510 sampled patients, the multiplex PCR assay results were positive in 103 patients (20.2%), and a virus was detected in 102 samples: rhinoviruses or enteroviruses in 35.5%, coronaviruses in 10.9%, and flu-like viruses (influenza virus, parainfluenza virus, respiratory syncytial virus, human metapneumovirus) in 52.7%. The cause of ARF varied significantly according to the results of the multiplex PCR assay, especially the proportion of viral pneumonia: 50.0% with flu-like viruses, 14.0% with other viruses, and 3.6% when no virus was detected ( $P < .001$ ). No difference was found in the composite outcome of day 28 mortality, IMV, or both according to positive assay findings (54.9% vs 54.7%;  $P = .965$ ). In a pre-established subgroup analysis, flu-like virus detection was associated with a higher rate of day 28 mortality, IMV, or both among recipients of allogeneic hematopoietic stem cell transplantation compared with those without detected virus.

**Interpretation:** In immunocompromised patients with ARF, the results of nasopharyngeal multiplex PCR assays are not associated with IMV or mortality. A final diagnosis of viral pneumonia is retained in one-third of patients with positive assay results and in one-half of the patients with a flu-like virus.

Tremendous advances in cancer management,<sup>1</sup> advances in solid organ transplantation,<sup>2</sup> and increased use of immunosuppressant medications<sup>3</sup> have made immunodeficiency a common condition in the critically ill.<sup>4-6</sup> In immunocompromised patients, acute respiratory failure (ARF) is the leading cause of admission to the ICU,<sup>7</sup> requires invasive mechanical ventilation (IMV) in more than one-half of patients, and is associated with a high fatality rate.<sup>8</sup> The diagnostic strategy is central, relying on a combination of invasive tests (eg, fiber-optic bronchoscopy with BAL) and noninvasive tests of sputum, nasopharyngeal aspirates, nasopharyngeal swabs, or blood. Despite an optimal early diagnostic investigation, the cause of ARF remains unknown in up to 30% of the patients,<sup>9</sup> for whom an undetermined cause has been associated with increased need for mechanical ventilation and mortality.<sup>8,10</sup>

Earlier studies in critically ill patients with hematologic disease reported that respiratory virus detection in nasopharyngeal swabs was associated with adverse outcomes.<sup>11,12</sup> However, although viral multiplex polymerase chain reaction (PCR) assays often are recommended in patients with ARF,<sup>9</sup> the clinical significance of virus detection in the upper respiratory tract has been investigated scarcely. We conducted a study to assess the clinical relevance of virus detection in nasopharyngeal swabs of critically ill immunocompromised patients admitted to the ICU for hypoxemic ARF.

## Study Design and Methods

### Population

We performed a post hoc analysis of the High-Flow Nasal Oxygen vs Standard Oxygen Therapy in Critically Ill Immunocompromised Patients (HIGH) randomized clinical trial<sup>13</sup> that enrolled 776 adult immunocompromised patients with hypoxemic ARF (defined by PaO<sub>2</sub> < 60 mm Hg or saturation of peripheral oxygen (SpO<sub>2</sub>) < 90% on room air, or by tachypnea > 30/min or labored breathing or respiratory distress and need for oxygen ≥ 6 L/min), in 32 ICUs in France between May 19, 2016, and December 31, 2017. All patients had known immunosuppression, defined as the use of long-term (> 3 months) or high-dose (> 0.5 mg/kg/d) steroids, use of other immunosuppressant drugs, solid organ transplantation, solid tumor requiring chemotherapy in the last 5 years, hematologic malignancy regardless of time since diagnosis, or primary immune deficiency. Patients were randomized 1:1 to continuous high-flow oxygen therapy or to standard oxygen therapy. Mortality on day 28 and intubation rate were not significantly different between groups.

The original study protocol was approved by the CPP Ile de France IV St-Louis ethics committee (March 3, 2016; Identifier: NIRB00003835/ 2016/08) and French health authorities (Agence Nationale de Sécurité du Médicament et des Produits de Santé; Identifier: EudraCT2016-A00220-51). Written informed consent was obtained from all patients or their proxies.

### Molecular Assay for Respiratory Virus Detection

Nasopharyngeal swabs were collected at inclusion in 3 mL of Universal Transport Medium (Copan Diagnostics, Inc.) and stored at -80 °C until testing. In 2022, a multiplex molecular assay (QIAstat-Dx Respiratory SARS-CoV-2 Panel cartridge; QIAGEN, Inc.) was performed on a QIAstat-Dx Rise Instrument according to the manufacturer's instructions (QIAstat-Dx Analyzer; QIAGEN, Inc.).

This assay tests for 19 respiratory viruses (influenza viruses A, A-H1, A-H1N1, A-H3, and B; respiratory syncytial viruses [RSVs] A and B; human parainfluenza virus [PIV] types 1, 2, 3, and 4; human metapneumoviruses (hMPVs) A and B; rhinovirus, enterovirus, or both; coronaviruses 229E, HKU1, NL63, OC43, and SARS-CoV-2; adenovirus; and bocavirus) and four bacteria (*Bordetella pertussis*, *Chlamydophila pneumoniae*, *Legionella pneumophila*, and *Mycoplasma pneumoniae*).<sup>14</sup> The cycle threshold (Ct) values of real-time PCR amplification were obtained when a respiratory pathogen was detected. Samples showing positive results for rhinovirus or enterovirus were sequenced for the VP4 and VP2 coding region, as previously described.<sup>15</sup> The PCR products were sequenced using an ABI 3100 DNA Sequencer (Applied Biosystems, Life Technologies). Alignment and sequence comparison with published sequences from the National Center for Biotechnology Information were performed using Geneious 8.0.5 software (Biomatters) and MEGA version 7 software (CEMI). Positive QIAstat status was defined as the detection of at least one respiratory pathogen by the QIAstat-Dx Respiratory Panel. Detected viruses were categorized into flu-like viruses (influenza virus, PIV, hMPV, and RSV) and other viruses (rhinovirus or enterovirus, coronavirus, and adenovirus).

### **ARF Causes**

The final diagnoses for the cause of ARF were made by managing physicians using either invasive (fiber-optic bronchoscopy and BAL) or noninvasive tests, or both, according to the clinician's decisions. For each patient, up to three definite diagnoses could be retained and had to be ranked in order of importance. All etiologic diagnoses were reviewed by three expert investigators (D. M., V. L., and E. A.) based on clinical features, imaging, and standard biological testing including microbiological analyses, but not multiplex assays. In total, of the 510 patients with available nasal swabs, 63 cases of disagreement (12.3%) occurred and were resolved after discussion among the three expert investigators. When consensus could not be reached despite complete analysis of available data, patients were left with an undetermined diagnosis. After complete chart review, the available data were considered insufficient to establish an etiologic diagnosis for 19 patients, who were excluded from the diagnostic analysis (ie, missing etiologic diagnosis). Diagnoses were classified into 13 categories: (1) microbiologically documented bacterial pneumonia, (2) clinically documented bacterial pneumonia, (3) viral pneumonia, (4) invasive pulmonary aspergillosis, (5) *Pneumocystis jirovecii* pneumonia, (6) other opportunistic infections, (7) noninfectious infiltrative pneumonitis (ARF from pulmonary infiltration by the malignancy), (8) drug-related pneumonitis, (9) cardiogenic acute pulmonary edema, (10) pulmonary embolism, (11) extra-respiratory sepsis, (12) other causes, and (13) undetermined diagnosis. The arguments used to establish these diagnoses are detailed in [e-Appendix 1](#). The results of the QIAstat PCR assay were not available to clinicians or investigators when the diagnosis was made or reviewed. In the case of multiple diagnoses, the one that ranked first was considered as the main cause of ARF.

### **Statistical Analysis**

The primary outcome was the association between respiratory viral detection by QIAstat PCR and day 28 mortality, day 28 IMV (death or IMV within 28 days of inclusion), or both. The secondary outcome was the association between the detection of a respiratory virus using the QIAstat assay and the final etiologic diagnosis of ARF. We also assessed factors independently associated with 28-day mortality and 28-day IMV in a multivariable analysis.

Categorical variables were described as numbers (percentages) and quantitative variables were described as medians (interquartile ranges [IQRs]). Between-group comparisons were performed using Fisher exact tests or  $\chi^2$  tests for categorical variables, as appropriate, and the Wilcoxon rank-sum test for quantitative variables. ORs associated with day 28 mortality, IMV, or the combination of day 28 mortality and IMV were computed using a logit-regression model. Subdistribution hazard ratios (sHRs) for day 28 IMV were computed using a Fine and Gray competitive risk model, with death as the competitive risk. In addition to the QIAstat assay, multivariable models included all factors associated with outcomes in the univariate analysis as well as other clinically meaningful variables, as detailed in [e-Appendix 1](#). Patients with a decision not to intubate were excluded from the multivariable analysis assessing the risk of day 28 IMV. Multivariable models were subjected to variable selection using a backward stepwise procedure, with a stopping rule based on the Akaike criterion as detailed in [e-Appendix 1](#). No missing data were imputed. All tests were two-sided with a  $\alpha$  0.05. Analyses were performed using R version 4.1.1 software (R Foundation for Statistical Computing).

## Results

Among the 776 immunocompromised patients with ARF, 510 patients underwent nasopharyngeal swab testing by QIAstat PCR and were included in the analysis ([Fig 1, e-Tables 1, 2](#)). Compared with patients who were not sampled, patients with available nasopharyngeal swabs were more likely to have a Sequential Organ Failure Assessment (SOFA) score of  $> 7$  (16.3% vs 10.0%;  $P = .041$ ) and a higher heart rate (115 beats/min [IQR, 90-135 beats/min] vs 109 beats/min [IQR, 86-127 beats/min];  $P = .028$ ). Among the sampled patients, neither the day 28 mortality (34.3% vs 38.7%;  $P = .249$ ) nor the composite outcome of day 28 mortality or IMV rate (54.7% vs 55.3%;  $P = .943$ ) differed from those of non-sampled patients.

The baseline characteristics of the study population are presented in [Table 1](#). The median age was 64 years (IQR, 57-71 years), and the median SOFA score was 4 (IQR, 1- 6). Almost half of the patients, 224 (44.8%), were treated for hematologic malignancies, 179 patients (35.7%) were treated for solid tumors, and 57 patients (11.4%) were solid organ transplantation recipients. The multiplex PCR assay identified 111 pathogens in 103 patients (20.2%), including 95 patients (18.6%) with a single pathogen and eight patients (1.6%) with two pathogens ([e-Fig 1, e-Table 3](#)). One sample showed positive results for *M pneumoniae*, and all other 102 samples showed positive results for respiratory viruses: 39 samples for rhinovirus or enterovirus (38.2%), 22 samples for influenza virus (21.6%), 14 samples for PIV (13.7%), 12 samples for coronavirus (11.8%), 11 samples for RSV (10.8%), 11 samples for hMPV (10.8%), and one sample for adenovirus (1.0%).

Of the 39 samples showing positive results for rhinovirus or enterovirus, the species could be identified for 29 samples using PCR amplification. Most were rhinovirus species A ( $n = 18$ ) or rhinovirus species C ( $n = 9$ ), with rhinovirus species B being found in only two samples. Of the 110 respiratory viruses, 55 viruses (50.0%) were detected in winter, and the rate of assay positivity varied from 9.7% in the summer to up to 39.9% in the winter ( $P < .001$  for seasonal variation) ([e-Fig 1, e-Table 4](#)). Influenza virus was almost only detected in winter (20 of 22 samples;  $P < .001$ ), and both RSV and coronaviruses were predominant in autumn and winter ( $P < .001$  and  $P = .002$ , respectively).

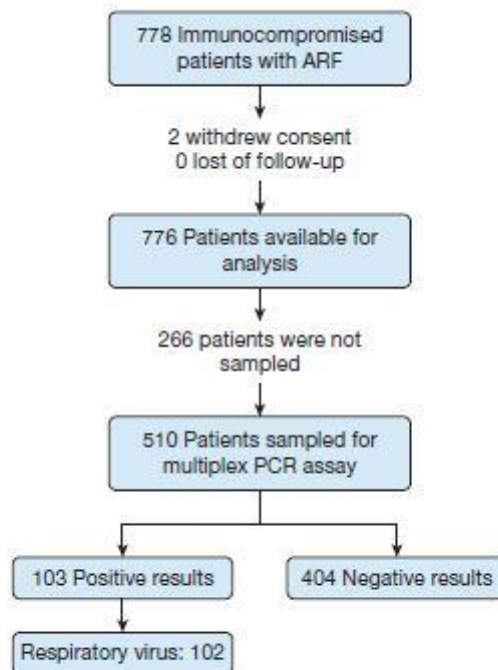


Figure 1 – Flow chart of the study. Further details are provided in the original article reporting the HIGH randomized controlled trial.<sup>13</sup> HIGH = High-Flow Nasal Oxygen vs Standard Oxygen Therapy in Critically Ill Immunocompromised Patients; PCR = polymerase chain reaction.

As shown in [Tables 1](#) and [2](#), patients with a detected respiratory pathogen showed no significantly different SOFA or Simplified Acute Physiology Score - II (SAPS-II) scores, but did show a lower rate of vasopressor use (3.9% vs 11.8%;  $P = .029$ ). The multiplex assay more frequently showed positive results in hematopoietic stem cell transplantation (HSCT) recipients (34.8% vs 18.0%;  $P = .001$ ) and less commonly showed positive results in patients with solid tumors (13.4% vs 23.8%;  $P = .006$ ) or those with poor performance status (14.4% vs 23.4%;  $P = .017$ ).

On admission, the median time from the onset of acute respiratory symptoms was 1 day (IQR, 0-3 days). Neither respiratory symptoms (eg, cough, chest pain, rhinitis, or myalgia) nor chest radiography patterns differed significantly according to the multiplex PCR assay results ([Table 2](#)). Among the 340 patients (66.7%) with available baseline CT scans, only 4.7% of scans were interpreted as demonstrating normal findings. Almost half of the patients, 230 (45.2%), showed diffuse or localized alveolar condensation, 233 patients (45.7%) showed ground-glass opacities, and 226 patients (44.3%) showed pleural effusion. Nodular opacities were found in 118 patients (23.2%), interlobular septal thickening was found in 59 patients (17.4%), and a reticular interstitial pattern was found in 19 patients (5.6%). Scannographic patterns did not differ significantly according to virus detection, except for non-flu-like viruses that were associated with less frequent diffuse ground-glass opacities than in other patients ([Table 2](#)). The main microbiological procedures performed for etiologic diagnosis are presented in [e-Table 5](#). Viral testing of nasopharyngeal swabs and aspirates was reported by attending physicians using local PCR assays in 179 patients (35.1%), which showed positive results in 33 patients (18.4%), as detailed in [e-Table 6](#).



As shown in [Table 3](#), the most prevalent cause of ARF was bacterial pneumonia for 232 patients (47.2%), followed by disease-related infiltrates for 57 patients (11.4%), viral pneumonia for 46 patients (9.6%; caused by a respiratory virus in 40 patients and cytomegalovirus/herpes simplex virus pneumonia in six patients), *P jirovecii* pneumonia for 44 patients (9.0%), and lung involvement in extrapulmonary sepsis for 47 patients (9.6%). Patients with a virus detected in nasopharyngeal swabs were more likely to receive a diagnosis of viral pneumonia (33.3% vs 3.3%;  $P < .001$ ) and less likely to receive a diagnosis of infiltrative pneumonitis (4.1% vs 13.5%;  $P = .013$ ). The cause considered as primarily responsible for ARF varied widely according to the results of the multiplex assay ([Fig 2](#), [e-Table 7](#)). Among the patients with flu-like viruses (influenza virus, PIV, hMPV, or RSV), the two main causes were viral pneumonia for 18 patients (33.3%) and bacterial pneumonia for 20 patients (37.1%). For patients with a non-flu-like virus (rhinovirus, coronavirus, or adenovirus), bacterial pneumonia was the most frequent main diagnosis 27 patients (62.8%), with only two patients (4.7%) categorized as having viral pneumonia. Finally, for patients who showed negative results for respiratory viruses, viral pneumonia was rare—only five patients (1.3%) showed such findings (one demonstrated cytomegalovirus and four demonstrated respiratory viruses)—and bacterial pneumonia remained the main cause of ARF for 209 patients (42.6%). When considering whether positive assay findings were indicative of viral pneumonia, the number of cycles required for the fluorescent signal to cross the positive threshold (ie, the Ct, inversely proportional to the amount of target nucleic acid in the sample) was not correlated significantly with the main ARF cause ([e-Fig 2](#)). Similarly, we found no association between the time from the onset of respiratory symptoms and Ct values of the detected viruses ([e-Fig 3](#)). Among patients with a clinical or microbiological documented bacterial pneumonia, 13 patients showed low Ct values ( $< 25$ ), including six patients with flu-like viruses ([e-Table 8](#)), raising questions about the causative agent of pneumonia. Among the 66 patients with an undetermined cause of ARF despite investigations, 10 patients (15.2%) showed positive multiplex PCR assay results: six showed flu-like viruses (one showed influenza virus, one showed hMPV, one showed RSV, and three showed PIV), three patients showed rhinovirus or enterovirus, and one patient showed coronavirus ([e-Table 7](#)).

Compared with patients who showed negative test results, those with a respiratory virus assay demonstrated a similar 28-day mortality rate (33.3% vs 34.5%;  $P = .827$ ) and a similar composite outcome of day 28 mortality or IMV rate (54.9% vs 54.7%;  $P = .882$ ) ([Table 4](#), [e-Table 9](#), [e-Fig 4](#)). Outcomes did not differ across patients with a flu-like virus and those with another virus or no virus. The PCR cycle threshold was not associated significantly with the outcomes ([e-Fig 5](#)). In a preplanned subgroup analysis according to the cause of immunosuppression ([e-Figs 6, 7](#)), the detection of a flu-like virus was associated with a higher day 28 mortality or IMV rate in allogeneic HSCT recipients (OR, 1.51; 95% CI, 1.03- 2.22). Multiplex PCR Ct values of the virus detected also were significantly lower among HSCT recipients who died at day 28 compared with those who remained alive ([e-Fig 8](#)). Among patients with a diagnosis of bacterial pneumonia, those with a detected respiratory virus showed a similar clinical and radiologic presentation and similar outcomes as those with negative multiplex PCR assay findings ([e-Tables 10-12](#)).

**TABLE 1 ] Characteristics at Inclusion**

Characteristic	Overall	QIAsat Results		P Value
		Negative	Positive	
No. of patients	510	407	103	NA
<b>Demographic</b>				
Age, y	64.0 (56.7-71.3)	64.1 (57.2-71.1)	63.7 (53.4-73.6)	.663
Sex, female	167 (32.7)	124 (30.5)	43 (41.7)	.039
Weight, kg <sup>a</sup>	74.0 (62.0-85.0)	74.6 (63.0-85.0)	71.0 (59.0-81.5)	.072
BMI, kg/m <sup>2b</sup>	25.0 (22.2-28.1)	25.0 (22.3-28.1)	24.8 (21.6-27.6)	.431
<b>Underlying conditions</b>				
Charlson comorbidity index <sup>c</sup>	5 (3-7)	5 (4-7)	5 (3-7)	.370
Poor performance status, 3 or 4 <sup>d</sup>	201 (42.3)	172 (45.1)	29 (30.9)	.017
<b>Chronic<sup>e</sup></b>				
Respiratory (restrictive or obstructive disease)	158 (31.5)	134 (33.4)	24 (23.8)	.081
Asthma	12 (2.4)	9 (2.2)	3 (3.0)	.950
COPD	49 (9.8)	44 (11.0)	5 (5.0)	.102
Emphysema	16 (3.2)	15 (3.7)	1 (1.0)	.276
Diffuse interstitial pneumonitis	19 (3.8)	15 (3.7)	4 (4.0)	1.000
Restrictive respiratory disease	10 (2.0)	7 (1.7)	3 (3.0)	.697
Heart failure	29 (5.8)	23 (5.8)	6 (5.9)	1.000
Liver disease	66 (13.1)	57 (14.2)	9 (8.9)	.213
Kidney disease	102 (20.3)	76 (19.0)	26 (25.7)	.168
Cancer	397 (77.8)	322 (79.1)	75 (72.8)	.214
Hematologic malignancies <sup>f</sup>	224 (44.8)	172 (43.2)	52 (51.0)	.195
Solid tumors <sup>g</sup>	179 (35.7)	155 (38.8)	24 (23.5)	.006
Time since last chemotherapy, < 1 mo	177 (46.5)	144 (48.8)	33 (38.4)	.113
Immunosuppressive drugs <sup>h</sup>	186 (37.2)	143 (35.8)	43 (42.6)	.256
After solid organ transplantation	57 (11.4)	41 (10.3)	16 (15.7)	.173
Non-transplant-related reasons	135 (27.0)	106 (26.6)	29 (28.7)	.758
Time since diagnosis of underlying condition, mo <sup>i</sup>	6.7 (1.0-34.5)	5.9 (0.9-29.6)	13.9 (2.4-40.2)	.013
<b>Stem cell transplantation<sup>j</sup></b>				
Autologous	31 (6.1)	18 (4.5)	13 (12.6)	.004
Allogeneic	37 (7.3)	26 (6.5)	11 (10.7)	.208
<b>Clinical severity at inclusion</b>				
Time between inclusion and ICU admission, d	0 (0-1)	0 (0-1)	0 (0-1)	.550
Simplified Acute Physiology Score II score <sup>k</sup>	37 (27-48)	37 (27-47)	38 (28-50)	.362
SOFA score <sup>l</sup>	4 (1-6)	4 (1-6)	4 (1-6)	.788
> 7 <sup>m</sup>	71 (16.3)	60 (17.3)	11 (12.4)	.335
<b>Hemodynamic status</b>				
Vasopressors	52 (10.2)	48 (11.8)	4 (3.9)	.029
Systolic BP <sup>n</sup>	104.0 (86.0-138.0)	104.0 (86.0-138.0)	102.0 (81.5-138.0)	.574
Heart rate <sup>o</sup>	115.0 (90.0-134.5)	115.0 (91.0-134.2)	112.0 (86.0-134.5)	.523
<b>Respiratory status</b>				
Respiratory rate, /min <sup>p</sup>	32.0 (28.0-38.0)	32.0 (27.0-38.0)	33.0 (28.0-38.0)	.488
Oxygen flow, L/min <sup>q</sup>	10.0 (6.0-15.0)	10.0 (6.0-15.0)	10.0 (6.0-15.0)	.530
PaO <sub>2</sub> to FiO <sub>2</sub> ratio <sup>r</sup>	118.0 (84.0-166.8)	117.0 (82.0-166.0)	125.0 (92.5-175.5)	.215
<b>Neurologic status</b>				
Glasgow score <sup>s</sup>	15 (15-15)	15 (15-15)	15 (15-15)	.793
Lactate, mM <sup>t</sup>	1.7 (1.0-2.8)	1.8 (1.1-2.9)	1.6 (1.0-2.8)	.565

Data are presented as No. (%) or median (interquartile range), unless otherwise indicated. NA = not applicable; SOFA = Sequential Organ Failure Assessment.

<sup>a</sup>Data missing for 17 patients.

<sup>b</sup>Data missing for 28 patients.

<sup>c</sup>Data missing for 11 patients.

<sup>d</sup>Data missing for 35 patients.

<sup>e</sup>Data missing for 9 patients.

<sup>f</sup>Data missing for 10 patients.

<sup>g</sup>Data missing for 8 patients.

<sup>h</sup>Data missing for 4 patients.

<sup>i</sup>Data missing for 142 patients.

<sup>j</sup>Data missing for 26 patients.

<sup>k</sup>Data missing for 74 patients.

<sup>l</sup>Data missing for 19 patients.

<sup>m</sup>Data missing for 37 patients.

<sup>n</sup>Data missing for 29 patients.

<sup>o</sup>Data missing for 52 patients.

<sup>p</sup>Data missing for 15 patients.

<sup>q</sup>Data missing for 83 patients.



**TABLE 2 ] Clinical and Radiologic Presentation**

Variable	Overall	No Virus Detected	Flu-like Viruses	Other Viruses	P Value
No. of patients	510	408	56	46	NA
Clinical presentation					
Temperature <sup>a</sup>	38.0 (37.0-38.9)	38.0 (37.0-39.0)	38.2 (37.4-38.8)	37.8 (37.0-38.3)	.381
Cough <sup>b</sup>					.057
None	270 (54.3)	225 (56.7)	21 (37.5)	24 (54.5)	
Dry	116 (23.3)	92 (23.2)	16 (28.6)	8 (18.2)	
Productive	11 (22.3)	80 (20.2)	19 (33.9)	12 (27.3)	
Purulent sputum <sup>b</sup>	70 (14.1)	51 (12.8)	10 (17.9)	9 (20.5)	.268
Rhinorrhea <sup>b</sup>	6 (1.2)	3 (0.8)	3 (5.4)	0 (0.0)	.010
Chest pain <sup>c</sup>	28 (5.6)	22 (5.5)	3 (5.4)	3 (6.8)	.934
Lung auscultation					
Localized crackles	166 (32.5)	126 (30.9)	20 (35.7)	20 (43.5)	.195
Squeaks	10 (2.0)	6 (1.5)	1 (1.8)	3 (6.5)	.064
Myalgia <sup>d</sup>	12 (2.4)	8 (2.0)	2 (3.6)	2 (4.5)	.488
Abdominal pain <sup>d</sup>	48 (9.7)	40 (10.2)	7 (12.5)	1 (2.3)	.186
Diarrhea <sup>e</sup>	47 (9.5)	37 (9.4)	7 (12.5)	3 (6.8)	.621
Time from the onset of respiratory symptoms, d <sup>f</sup>	1.0 (0.0-3.0)	1.0 (0.0-3.0)	1.0 (0.0-4.0)	1.0 (0.0-4.2)	.452
Blood tests					
Leucocytes count, G/L <sup>g</sup>	9.3 (3.2-16.1)	10.2 (3.6-16.7)	3.2 (1.0-9.6)	11.9 (6.2-15.4)	< .001
Platelets count, /mm <sup>3g</sup>	130.5 (49.0-239.0)	132.0 (49.0-240.8)	99.5 (37.8-186.5)	162.0 (63.0-236.5)	.195
Total bilirubin, μmol/L <sup>h</sup>	12.0 (7.0-22.9)	12.0 (7.4-22.9)	11.0 (6.9-27.5)	9.0 (6.8-18.5)	.455
Chest radiography pattern <sup>i</sup>					
Normal	25 (5.4)	19 (5.1)	2 (3.8)	4 (9.3)	.451
Unilateral alveolar condensations	57 (12.2)	42 (11.3)	7 (13.5)	8 (18.6)	.370
Bilateral alveolar condensations	141 (30.3)	118 (31.8)	12 (23.1)	11 (25.6)	.343
Interstitial opacities	202 (43.3)	161 (43.4)	24 (46.2)	17 (39.5)	.810
Nodular opacities	10 (2.1)	8 (2.2)	1 (1.9)	1 (2.3)	.990
Pleural effusion	68 (14.6)	51 (13.7)	6 (11.5)	11 (25.6)	.092
Excavated opacities	4 (0.8)	4 (1.0)	0 (0.0)	0 (0.0)	.603
CT scan findings <sup>j</sup>					
Normal	16 (4.7)	12 (4.4)	2 (5.6)	2 (6.1)	.887
Ground-glass opacities					
Localized	70 (20.6)	53 (19.6)	8 (22.2)	9 (27.3)	.566
Diffuse	86 (25.3)	76 (28.0)	8 (22.2)	2 (6.1)	.021
Nodular opacities					
Centrilobular	25 (7.4)	18 (6.6)	5 (13.9)	2 (6.1)	.281
Peribronchovascular	15 (4.4)	11 (4.1)	2 (5.6)	2 (6.1)	.817
Subpleural	18 (5.3)	16 (5.9)	1 (2.8)	1 (3.0)	.609
Other patterns	31 (9.1)	24 (8.9)	4 (11.1)	3 (9.1)	.907
Alveolar condensations					
Localized	105 (30.9)	79 (29.2)	14 (38.9)	12 (36.4)	.382

TABLE 2 ] (Continued)

Variable	Overall	No Virus Detected	Flu-like Viruses	Other Viruses	P Value
Diffuse	49 (14.4)	38 (14.0)	5 (13.9)	6 (18.2)	.810
Interlobular septal thickening	59 (17.4)	46 (17.0)	5 (13.9)	8 (24.2)	.492
Halo sign	2 (0.6)	2 (0.7)	0 (0.0)	0 (0.0)	.774
Excavated opacities	7 (2.1)	7 (2.6)	0 (0.0)	0 (0.0)	.403
Pleural effusion	151 (44.4)	124 (45.8)	11 (30.6)	16 (48.5)	.200
Air bronchogram	42 (12.4)	29 (10.7)	6 (16.7)	7 (21.2)	.158
Reticular interstitial pattern	19 (5.6)	17 (6.3)	2 (5.6)	0 (0.0)	.334
Bronchia wall thickening	41 (12.1)	32 (11.8)	4 (11.1)	5 (15.2)	.842
Vascular enlargement	11 (3.2)	11 (4.1)	0 (0.0)	0 (0.0)	.235
Thoracic tumoral mass	6 (1.8)	5 (1.8)	0 (0.0)	1 (3.0)	.618
Mediastinal lymph node enlargement	49 (14.4)	36 (13.3)	9 (25.0)	4 (12.1)	.158

Data are presented as No. (%) or median (interquartile range), unless otherwise indicated. Only blood tests included in the Sequential Organ Failure Assessment score or the Simplified Acute Physiology Score (SAPS) II score were collected systematically. NA = not applicable.

<sup>a</sup>Data missing for 37 patients.

<sup>b</sup>Data missing for 13 patients.

<sup>c</sup>Data missing for 10 patients.

<sup>d</sup>Data missing for 16 patients.

<sup>e</sup>Data missing for 17 patients.

<sup>f</sup>Data missing for 22 patients.

<sup>g</sup>Data missing for 24 patients.

<sup>h</sup>Data missing for 83 patients.

<sup>i</sup>Data missing for 44 patients.

<sup>j</sup>CT scan was available for 340 patients.

By multivariable analysis (Fig 3, e-Tables 13-16), factors associated with day 28 mortality were a low PaO<sub>2</sub> to FIO<sub>2</sub> ratio (OR, 0.69 for an increase of 100; 95% CI, 0.49-0.97), high non-respiratory SOFA score (OR, 1.10 per 1-point increase; 95% CI, 1.02-1.18), poor health status (assessed by a performance status of  $\leq$  3; OR, 1.98; 95% CI, 1.22- 3.20), and an undetermined ARF cause (OR, 2.49; 95% CI, 1.22-5.08). Factors associated with day 28 IMV using death as a competitive risk were the non-respiratory SOFA score (sHR, 1.09 per 1-point increase; 95% CI, 1.04-1.14), high heart rate (sHR, 1.09 per 10-beats/min increase; 95% CI, 1.03-1.14), oxygen flow of > 10 L/min (sHR, 1.47; 95% CI, 1.08-1.99), and a final diagnosis of *P jirovecii* pneumonia (sHR, 2.02; 95% CI, 1.31-3.11).

## Discussion

In this multicenter prospective cohort study including 510 critically ill immunocompromised patients with ARF, we found that the results of nasopharyngeal multiplex PCR assays were not associated with the need for IMV or mortality, but with the causes of ARF. Because of the possibility of asymptomatic carriage or benign upper respiratory tract infections,<sup>16-21</sup> the detection of a virus in nasopharyngeal swabs is not sufficient to establish its involvement in ARF.<sup>12,17</sup> Beyond direct pathogenicity, a respiratory virus also can promote bacterial infections<sup>22</sup> and can worsen their outcomes.<sup>23-25</sup> In addition, they have been shown to favor subsequent invasive aspergillosis in critically ill patients.<sup>26-31</sup> In our study, which included patients admitted to the ICU with ARF, those with evidence of upper respiratory tract virus showed a clinical and radiologic presentation similar to the presentations of those with negative results. As opposed to a previous study conducted in 2010 and 2011 in critically ill



patients with hematologic disease,<sup>12</sup> the post hoc detection of respiratory viruses in nasopharyngeal swabs was not associated with outcomes. However, our study was not limited to hematologic malignancies, but included patients with different types of immune dysfunction, all of whom had ARF. Notably, a higher proportion of flu-like viruses was found in the present study (52.7%) than previously reported in patients with hematologic disease who were admitted to the ICU for ARF or other causes (33.2%;  $P = .040$ ). We also found that flu-like viruses were associated with higher day 28 mortality or IMV in recipients of allogeneic HSCT. In addition, viral loads, as estimated by PCR Ct values, were higher in autologous and recipients of allogeneic HSCT who died than in those who did not die. Taken together, these results suggest that systematic rapid screening in recipients of allogeneic HSCT can be used as a surrogate for imminent deterioration and prompt initiation of antiviral treatment. In other patients, outcomes equally were poor with and without detection of respiratory viruses (Table 4, e-Table 12), highlighting the potential morbidity of respiratory viruses in immunocompromised patients associated with similar outcomes to bacterial pneumonia and other causes of ARF.

**TABLE 3 ] Causes of Hypoxemic Acute Respiratory Failure**

Variable	Overall	No Virus Detected	Flu-like Viruses	Other Viruses	P Value
No. of patients <sup>a</sup>	491	394	54	43	NA
<b>Infectious</b>					
<b>Bacterial pneumonia</b>					
Microbiologically documented	140 (28.5)	109 (27.7)	14 (25.9)	17 (39.5)	.237
Clinically documented	92 (18.7)	71 (18.0)	10 (18.5)	11 (25.6)	.483
Viral pneumonia	46 (9.4)	13 (3.3)	27 (50.0)	6 (14.0)	< .001
<b>Opportunistic infections</b>					
<i>P. jirovecii</i> pneumonia	44 (9.0)	38 (9.6)	2 (3.7)	4 (9.3)	.357
Invasive aspergillosis	17 (3.5)	13 (3.3)	4 (7.4)	0 (0.0)	.130
Other opportunistic infection <sup>b</sup>	5 (1.0)	4 (1.0)	0 (0.0)	1 (2.3)	.526
<b>Noninfectious lung involvement</b>					
Specific infiltrative pneumonitis	57 (11.6)	53 (13.5)	0 (0.0)	4 (9.3)	.013
Drug-related pneumonitis	8 (1.6)	8 (2.0)	0 (0.0)	0 (0.0)	.367
Cardiogenic pulmonary edema	26 (5.3)	22 (5.6)	3 (5.6)	1 (2.3)	.661
Pulmonary embolism	12 (2.4)	12 (3.0)	0 (0.0)	0 (0.0)	.220
Other causes	12 (2.4)	10 (2.5)	2 (3.7)	0 (0.0)	.484
Extrarespiratory sepsis	47 (9.6)	43 (10.9)	4 (7.4)	0 (0.0)	.059
Undetermined	66 (13.4)	56 (14.2)	6 (11.1)	4 (9.3)	.581
> 1 cause	75 (15.3)	53 (13.5)	17 (31.5)	5 (11.6)	.002

Data are presented as No. (%), unless otherwise indicated. NA = not applicable.

<sup>a</sup>Data on etiologic diagnosis were missing for 19 patients, with no significant difference between groups ( $P = .576$ ).

<sup>b</sup>Other opportunistic pathogens were *Leishmania* species ( $n = 1$ ), *Geotrichum* species ( $n = 1$ ), *Candida* species ( $n = 1$ ), *Scedosporium* species ( $n = 1$ ), and *Cryptococcus* species ( $n = 1$ ).

Detection of a respiratory virus was associated with viral and bacterial causes of ARF. Among the 66 patients with an undetermined diagnosis in our study, a respiratory virus was detected by multiplex PCR assay in 15%, and most of these were flu-like viruses. In addition, high loads of flu-like respiratory viruses were detected in 5% of patients with clinically or microbiologically documented pneumonia. These results suggest that a multiplex PCR assay of nasopharyngeal swabs could contribute to reducing the proportion of undetermined etiologic diagnoses, which remains associated with poorer prognosis. We can speculate that it also may prevent the use of bronchoscopy, which has been associated with worse outcomes.<sup>32</sup> Beyond detection, the critical question remains: is the detected virus a pathogen or just a so-called witness (not involved in the ARF)? A possible approach to explore this

question could be to combine transcriptomic host responses in respiratory samples with PCR assay results.

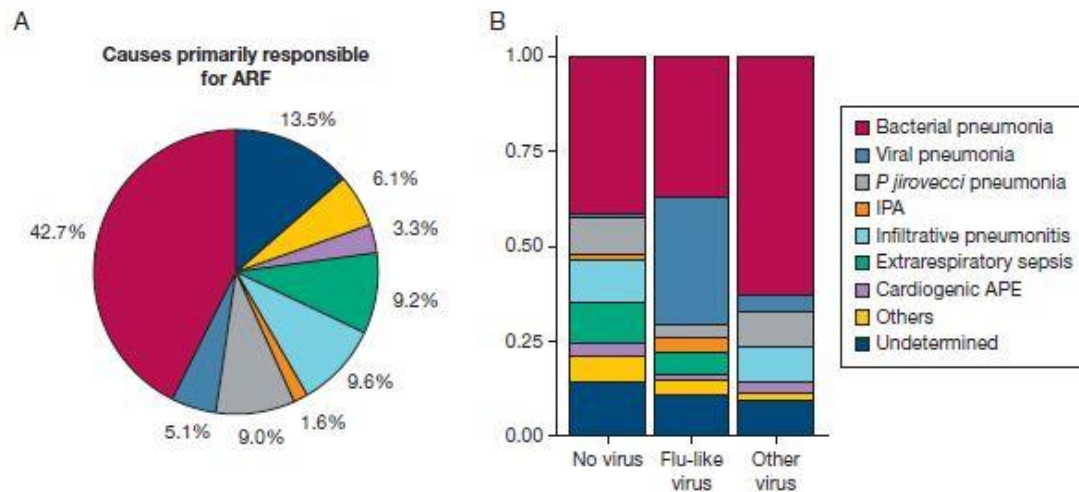


Figure 2 – Graphs showing causes primarily responsible for hypoxemic ARF. Among the 510 patients who had an available nasal swab tested with the QIAstat-Dx respiratory panel, a flu-like virus (influenza, parainfluenza, respiratory syncytial virus, or human metapneumovirus) was detected in 56 patients, and other viruses were detected in 46 patients. A, Pie graph showing causes considered to be primarily responsible for hypoxemic ARF among all included patients. B, Bar graph showing causes considered primarily responsible for hypoxemic ARF according to the results of the QIAstat assay. APE = acute pulmonary edema; ARF = acute respiratory failure; IPA = invasive pulmonary aspergillosis.

Transcriptomic analysis could reveal pathogen-induced gene expression changes, as well as dynamic immune evasion specific to respiratory virus infections.<sup>33</sup> We can hypothesize that the host response to a pathogenic virus would be different from the response to a “witness” virus. This type of approach has been used successfully in a proof-of-concept study of lower respiratory tract infections in critically ill immunocompetent adults<sup>34</sup> and is worth exploring in immunocompromised patients.

This study has several limitations. First, although managing clinicians were asked to provide a nasopharyngeal swab for each patient enrolled, 264 patients (34.0%) were not sampled and were not included in this analysis. We cannot exclude selection bias in the analysis, although outcomes and clinical presentation were similar. Second, diagnostic strategies were not standardized across centers. Because of likely heterogeneous practices in centers regarding viral assays and testing, some cases of viral pneumonia may have been misclassified as bacterial pneumonia. In particular, the high viral load in some patients with a diagnosis of bacterial pneumonia (e-Fig 2, e-Table 8) could indicate either coinfection or misclassification. Furthermore, of the 231 patients with a diagnosis of bacterial pneumonia, 82 patients (35.5%) had no available documentation. In addition, we were not able to assess the correlation between viruses detected in BAL fluid and nasopharyngeal swabs. Also, therapeutic management of patients was not standardized across centers, and that could have biased the analysis of outcomes. We did not have precise information about patients’ previous vaccinations, nor about the type of immunosuppressive chemotherapy they had received. Finally, the observational design precludes conclusions regarding causal associations.

**TABLE 4 ] Intervention and Outcomes**

Variable	Overall	No Virus Detected	Flu-like Viruses	Other Viruses	P Value
No. of patients	510	408	56	46	NA
<b>Interventions (until day 28)</b>					
Chemotherapy	75 (14.7)	65 (15.9)	2 (3.6)	8 (17.4)	.043
<b>Anti-infectious therapies</b>					
Antibacterial therapy <sup>a</sup>	447 (96.1)	356 (96.2)	49 (94.2)	42 (97.7)	.675
Delay from admission to ICU, d <sup>b</sup>	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	.522
Initiate before ICU	44 (9.8)	36 (10.1)	5 (10.2)	3 (7.1)	
Initiated within 24 h of admission	306 (68.5)	237 (66.6)	35 (71.4)	34 (81.0)	
Initiated > 24 h after admission	97 (21.7)	83 (23.3)	9 (18.4)	5 (11.9)	
Antiviral therapy	152 (29.8)	104 (25.5)	31 (55.4)	17 (37.0)	< .001
Oseltamivir	35 (7.5)	15 (4.1)	15 (28.8)	5 (11.6)	< .001
Delay from admission to ICU, d <sup>c</sup>	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	.407
Initiate before ICU <sup>c</sup>	1 (2.9)	0 (0.0)	1 (6.7)	0 (0.0)	
Initiated within 24 h of admission <sup>c</sup>	30 (85.7)	12 (80.0)	13 (86.7)	5 (100.0)	
Initiated > 24 h after admission <sup>c</sup>	4 (11.4)	3 (20.0)	1 (6.7)	0 (0.0)	
Antifungal therapy	169 (33.1)	140 (34.3)	18 (32.1)	11 (23.9)	.359
Antipneumocystis therapy	118 (25.4)	97 (26.2)	8 (15.4)	13 (30.2)	.181
Delay from admission to ICU, d	0.0 (0.0-0.0)	0.0 (0.0-0.0)	0.0 (-0.8 to 1.0)	0.0 (0.0-0.0)	.673
Initiate before ICU	16 (12.5)	12 (11.4)	2 (20.0)	2 (15.4)	
Initiated within 24 h of admission	83 (64.8)	69 (65.7)	5 (50.0)	9 (69.2)	
Initiated > 24 h after admission	29 (22.7)	24 (22.9)	3 (30.0)	2 (15.4)	
<b>Other therapies</b>					
Corticosteroid boluses <sup>d</sup>	161 (33.0)	132 (33.8)	16 (29.6)	13 (30.2)	.768
Diuretics	225 (44.1)	172 (42.2)	31 (55.4)	22 (47.8)	.152
G-CSF	37 (7.3)	30 (7.4)	4 (7.1)	3 (6.5)	.978
Vasoactive drugs <sup>e</sup>	239 (48.9)	193 (49.6)	27 (49.1)	19 (42.2)	.643
<b>Life-supporting interventions (until day 28)</b>					
Randomization group: HFNO vs standard oxygen therapy	251 (49.2)	203 (49.8)	28 (50.0)	20 (43.5)	.716
IMV	207 (40.7)	167 (41.1)	25 (44.6)	15 (32.6)	.441
Noninvasive ventilation <sup>f</sup>	69 (13.6)	53 (13.1)	9 (16.1)	7 (15.2)	.785
Renal replacement therapy <sup>g</sup>	82 (16.9)	69 (17.9)	8 (14.5)	5 (11.1)	.460
Prone positioning <sup>h</sup>	43 (8.8)	31 (7.9)	8 (14.8)	4 (8.9)	.245
Extracorporeal membrane oxygenation <sup>i</sup>	4 (0.8)	2 (0.5)	1 (1.9)	1 (2.2)	.323
<b>Outcomes</b>					
Death at day 28	176 (34.5)	141 (34.6)	20 (35.7)	15 (32.6)	.947
Death or mechanical ventilation at day 28	279 (54.7)	223 (54.7)	33 (58.9)	23 (50.0)	.665
ICU mortality	149 (29.2)	123 (30.1)	14 (25.0)	12 (26.1)	.647
Hospital mortality <sup>j</sup>	200 (39.4)	161 (39.6)	20 (35.7)	19 (42.2)	.789

Data are presented as No. (%) or median (interquartile range), unless otherwise indicated. IMV = invasive mechanical ventilation; G-CSF = granulocyte colony stimulating factor; HFNO = high-flow nasal oxygen; NA = not applicable.

<sup>a</sup>Data missing for 45 patients.

<sup>b</sup>Data missing for 63 patients.

<sup>c</sup>For oseltamivir.

<sup>d</sup>Data missing for 22 patients.

<sup>e</sup>Data missing for 21 patients.

<sup>f</sup>Data missing for 3 patients.

<sup>g</sup>Data missing for 24 patients.

<sup>h</sup>Data missing for 20 patients.

<sup>i</sup>Data missing for 2 patients.



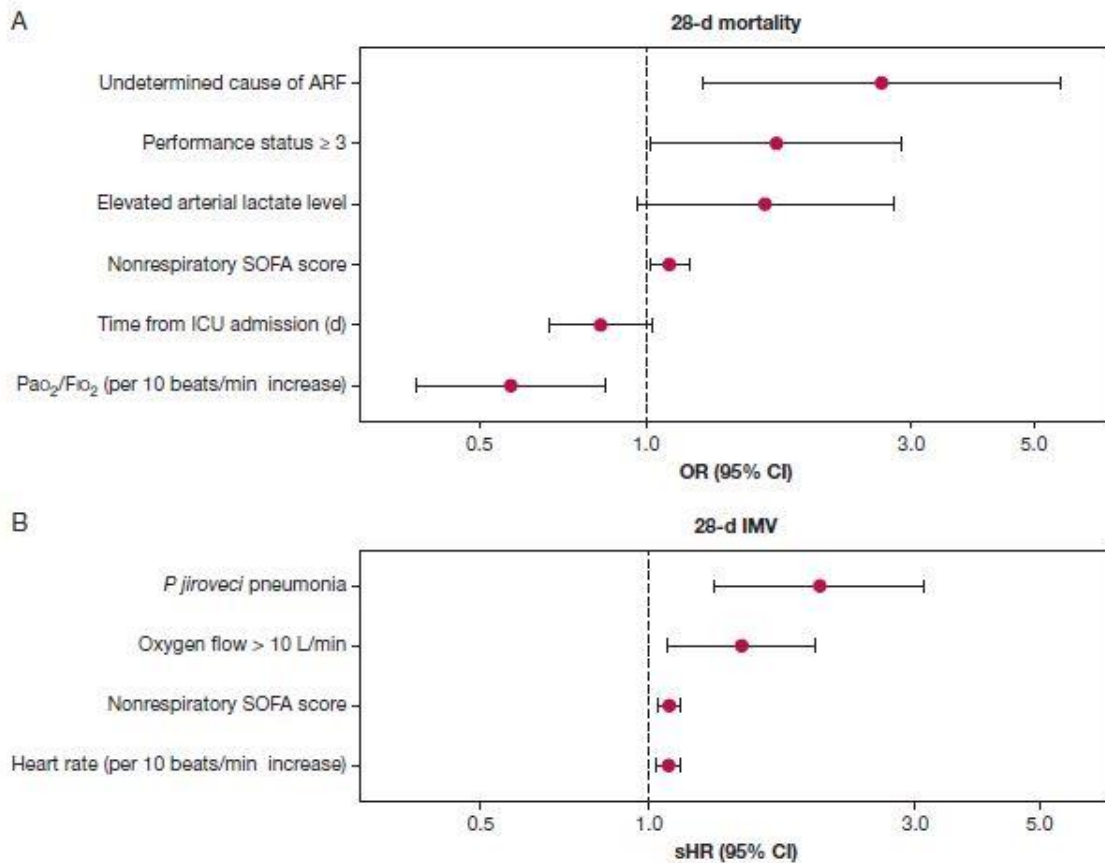


Figure 3 – A, B, Graphs showing multivariable analysis for factors independently associated with 28-day mortality (A) and IMV (B). IMV = invasive mechanical ventilation; sHR = subdistribution hazard ratio; SOFA = Sequential Organ Failure Assessment.

## Interpretation

A respiratory virus was detected in the upper respiratory airways in one of five critically ill immunocompromised patients with hypoxemic ARF. The detection of respiratory viruses was associated poorly with clinical and radiologic presentations and was not predictive of day 28 mortality or IMV, except in HSCT recipients. Virus detection by multiplex PCR assay is associated with ARF causes, which could help to reduce the proportion of ARF diagnoses misclassified or of undetermined origin.

## Funding/Support

Funded by the French Ministry of Health and French Intensive Care Society [Grant P150912 HIGH]. QIAGEN donated all QIAstat-Dx Respiratory SARSCoV-2 Panel cartridges and a QIAstat-Dx Rise instrument.

## Financial/Nonfinancial Disclosures

The authors have reported to CHEST the following: J. L. reports receiving personal fees from Qiagen and Abbott Rapid Diagnostics SAS. V. L. received support for article research from Assistance Publique -Hôpitaux de Paris and is treasurer of the GRRR-OH research group, which has received fees from Pfizer, Fisher-Paykel, Gilead, Astellas, Sanofi, and Alexion. F. B. reports personal fees from Merck Sharp and Dohme and BioMérieux outside the submitted work. A. D. reports grants from the French Ministry of Health; grants and personal fees from



Philips, Fisher & Paykel, Respinor, and Lungpacer; personal fees from Baxter, Getinge, Gilead, and Lowenstein; and nonfinancial support from Fisher & Paykel outside the submitted work. E. A. has received fees for lectures from MSD, Pfizer, and Alexion; his institution and research group have received support from Baxter, Jazz Pharmaceuticals, Fisher & Paykel, Gilead, Alexion, and Ablynx; and he received support for article research from Assistance Publique - Hôpitaux de Paris. None declared (A. M., M. B., S.M.-D., L. F., S. J., K. K., A. K., L. A., N. B., A.-S. M., E. C., F. P., M. S., D. M.).

## Acknowledgments

### Author contributions:

A. M., J. L., M. B., V. L., S. M.-D., A. D., L. F., S. J., K. K., A. K., L. A., F. B., N. B., A.-S. M., E. C., F. P., M. S., D. M., and E. A. contributed substantially to the conception and design of the work and to the acquisition, analysis, and interpretation of data. All authors have worked on, revised, and approved the submitted manuscript. E.A. is the guarantor of the study, had full access to all the data, and takes responsibility for the integrity of the data and the accuracy of the data analysis.

### Role of sponsors:

The funders or sponsors had no role in the design and conduct of the study; collection, management, analysis, or interpretation of the data; preparation, review, or approval of the manuscript; or decision to submit the manuscript for publication.

### Disclaimer:

This study was performed on behalf of the Groupe de Recherche Respiratoire en Réanimation Onco-Hématologique (GRRR-OH).

### Other contributions:

The authors thank QIAGEN for their donation of all QIAstat- Dx Respiratory SARS-CoV-2 Panel cartridges and the provision of a QIAstat-Dx Rise instrument and the reviewers for their detailed insightful comments that greatly helped to make this study more interesting for readers.

### Additional information:

The e-Appendix, e-Figures, and e-Tables are available online under “Supplementary Data.”

## References

1. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2021;71(3):209-249.

2. Israni AK. OPTN/SRTR 2020 annual data report: introduction. *Am J Transplant*. 2022;22(S2):11-20.
3. Wallace BI, Kenney B, Malani PN, Clauw DJ, Nallamotheu BK, Waljee AK. Prevalence of immunosuppressive drug use among commercially insured US adults, 2018-2019. *JAMA Netw Open*. 2021;4(5):e214920.
4. Harpaz R, Dahl RM, Dooling KL. Prevalence of immunosuppression among US adults, 2013. *JAMA*. 2016;316(23): 2547-2548.
5. Azoulay E, Schellongowski P, Darmon M, et al. The Intensive Care Medicine research agenda on critically ill oncology and hematology patients. *Intensive Care Med*. 2017;43(9):1366-1382.
6. Soares M, Bozza FA, Azevedo LCP, et al. Effects of organizational characteristics on outcomes and resource use in patients with cancer admitted to intensive care units. *J Clin Oncol*. 2016;34(27): 3315-3324.
7. Azoulay E, Russell L, Van de Louw A, et al. Diagnosis of severe respiratory infections in immunocompromised patients. *Intensive Care Med*. 2020;46(2): 298-314.
8. Azoulay E, Pickkers P, Soares M, et al. Acute hypoxemic respiratory failure in immunocompromised patients: the Efraim Multinational Prospective Cohort Study. *Intensive Care Med*. 2017;43(12): 1808-1819.
9. Azoulay E, Mokart D, Kouatchet A, Demoule A, Lemiale V. Acute respiratory failure in immunocompromised adults. *Lancet Respir Med*. 2019;7(2):173-186.
10. Contejean A, Lemiale V, Resche-Rigon M, et al. Increased mortality in hematological malignancy patients with acute respiratory failure from undetermined etiology: a Groupe de Recherche en Réanimation Respiratoire en Onco-Hématologique (Grrr-OH) study. *Ann Intensive Care*. 2016;6(1):102.
11. Lee J, Kim SC, Rhee CK, Lee J, Lee JW, Lee D-G. Prevalence and clinical course of upper airway respiratory virus infection in critically ill patients with hematologic malignancies. *PloS One*. 2021;16(12): e0260741.
12. Legoff J, Zucman N, Lemiale V, et al. Clinical significance of upper airway virus detection in critically ill hematology patients. *Am J Respir Crit Care Med*. 2019;199(4):518-528.
13. Azoulay E, Lemiale V, Mokart D, et al. Effect of high-flow nasal oxygen vs standard oxygen on 28-day mortality in immunocompromised patients with acute respiratory failure: the HIGH randomized clinical trial. *JAMA*. 2018;320(20): 2099-2107.
14. Leber AL, Lisby JG, Hansen G, et al. Multicenter evaluation of the QIAstat-Dx Respiratory Panel for detection of viruses and bacteria in nasopharyngeal swab specimens. *J Clin Microbiol*. 2020;58(5): e00155-20.

15. Wisdom A, Leitch ECM, Gaunt E, Harvala H, Simmonds P. Screening respiratory samples for detection of human rhinoviruses (HRVs) and enteroviruses: comprehensive VP4-VP2 typing reveals high incidence and genetic diversity of HRV species C. *J Clin Microbiol*. 2009;47(12):3958-3967.
16. Self WH, Williams DJ, Zhu Y, et al. Respiratory viral detection in children and adults: comparing asymptomatic controls and patients with community-acquired pneumonia. *J Infect Dis*. 2016;213(4): 584-591.
17. Das D, Le Floch H, Houhou N, et al. Viruses detected by systematic multiplex polymerase chain reaction in adults with suspected community-acquired pneumonia attending emergency departments in France. *Clin Microbiol Infect*. 2015;21(6): 608.e1-e8.
18. Marinelli T, Wee LYA, Rowe E, et al. Respiratory viruses cause late morbidity in recipients of hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2020;26(4):782-788.
19. Milano F, Campbell AP, Guthrie KA, et al. Human rhinovirus and coronavirus detection among allogeneic hematopoietic stem cell transplantation recipients. *Blood*. 2010;115(10): 2088-2094.
20. Bridevaux P-O, Aubert J-D, Soccac PM, et al. Incidence and outcomes of respiratory viral infections in lung transplant recipients: a prospective study. *Thorax*. 2014;69(1):32-38.
21. Mombelli M, Lang BM, Neofytos D, et al. Burden, epidemiology, and outcomes of microbiologically confirmed respiratory viral infections in solid organ transplant recipients: a nationwide, multi-season prospective cohort study. *Am J Transplant*. 2021;21(5):1789-1800.
22. McCullers JA. Do specific virus-bacteria pairings drive clinical outcomes of pneumonia? *Clin Microbiol Infect*. 2013;19(2):113-118.
23. Voiriot G, Visseaux B, Cohen J, et al. Viral-bacterial coinfection affects the presentation and alters the prognosis of severe community-acquired pneumonia. *Crit Care*. 2016;20(1):375.
24. Liu Y, Ling L, Wong SH, et al. Outcomes of respiratory viral-bacterial co-infection in adult hospitalized patients. *eClinical Medicine*. 2021;37. *Lancet website*. Accessed December 15, 2022. [https://www.thelancet.com/journals/eclinm/article/PIIS2589-5370\(21\)00235-2/fulltext](https://www.thelancet.com/journals/eclinm/article/PIIS2589-5370(21)00235-2/fulltext)
25. Cawcutt K, Kalil AC. Pneumonia with bacterial and viral coinfection. *Curr Opin Crit Care*. 2017;23(5):385-390.
26. Schauwvlieghe AFAD, Rijnders BJA, Philips N, et al. Invasive aspergillosis in patients admitted to the intensive care unit with severe influenza: a retrospective cohort study. *Lancet Respir Med*. 2018;6(10):782-792.
27. Verweij PE, Rijnders BJA, Brüggemann RJM, et al. Review of influenza-associated pulmonary aspergillosis in ICU patients and proposal for a case definition: an expert opinion. *Intensive Care Med*. 2020;46(8): 1524-1535.

28. Gangneux J-P, Dannaoui E, Fekkar A, et al. Fungal infections in mechanically ventilated patients with COVID-19 during the first wave: the French multicentre MYCOVID study. *Lancet Respir Med.* 2022;10(2):180-190.
29. White PL, Parr C, Barnes RA. Predicting invasive aspergillosis in hematology patients by combining clinical and genetic risk factors with early diagnostic biomarkers. *J Clin Microbiol.* 2018;56(1): e01122-17.
30. Martino R, Piñana JL, Parody R, et al. Lower respiratory tract respiratory virus infections increase the risk of invasive aspergillosis after a reduced-intensity allogeneic hematopoietic SCT. *Bone Marrow Transplant.* 2009;44(11): 749-756.
31. Apostolopoulou A, Clancy CJ, Skeel A, Nguyen MH. Invasive pulmonary aspergillosis complicating noninfluenza respiratory viral infections in solid organ transplant recipients. *Open Forum Infect Dis.* 2021;8(11):ofab478.
32. Bauer PR, Chevret S, Yadav H, et al. Diagnosis and outcome of acute respiratory failure in immunocompromised patients after bronchoscopy. *Eur Respir J.* 2019;54(1): 1802442.
33. Feys S, Heylen J, Carvalho A, Van Weyenbergh J, Wauters J. Variomic Study Group, A signature of differential gene expression in bronchoalveolar lavage fluid predicts mortality in influenza-associated pulmonary aspergillosis. *Intensive Care Med.* 2023;49(2):254-257.
34. Langelier C, Kalantar KL, Moazed F, et al. Integrating host response and unbiased microbe detection for lower respiratory tract infection diagnosis in critically ill adults. *Proc Natl Acad Sci.* 2018;115(52): E12353-E12362.