Clinical Significance of Upper Airway Virus Detection in Critically Ill Hematology Patients

Jérôme Legoff, Noémie Zucman, Virginie Lemiale, Djamel Mokart, Frédéric Pène, Jérôme Lambert, Achille Kouatchet, Alexandre Demoule, Francois Vincent, Martine Nyunga, et al.

AJRCCM Articles in Press. Published on 19-September-2018 as 10.1164/rccm.201804-0681OC

Clinical Significance of Upper Airway Virus Detection in Critically Ill Hematology Patients

Jérôme Legoff[1]*, Noémie Zucman[2]*, Virginie Lemiale[2], Djamel Mokart[3], Frédéric Pène[4], Jérôme Lambert[5], Achille Kouatchet[6], Alexandre Demoule[7], François Vincent[8], Martine Nyunga[9], Fabrice Bruneel[10], Adrien Contejean[2], Séverine Mercier-Delarue[1], Antoine Rabbat[4], Christine Lebert[11], Pierre Perez[12], Anne-Pascale Meert[13], Dominique Benoit[14], Carole Schwebel[15], Mercé Jourdain[16], Michael Darmon[2], Matthieu Resche-Rigon[5], Elie Azoulay[2]

* Jérôme LeGoff and Noémie Zucman contributed equally to this work.

[1]AP-HP, Virology Department, Saint Louis Teaching Hospital, Paris, FRANCE

[2]AP-HP, ICU, Saint Louis Teaching Hospital, Paris, FRANCE

[3]ICU, Paoli Calmette Institute, Marseille, FRANCE

[4]AP-HP, ICU, Cochin Teaching Hospital, Paris, France

[5]AP-HP, Statistics Department, Saint Louis Teaching Hospital, Paris, FRANCE

[6]ICU, Angers Teaching Hospital, Angers, FRANCE

[7] AP-HP, ICU, Pitié Salpêtrière Teaching Hospital, Paris, FRANCE

[8]AP-HP, ICU Avicennes Teaching Hospital, Bobigny, FRANCE

[9]ICU, Roubaix Regional Hospital Center, Roubaix, FRANCE

[10]ICU, Versailles Teaching Hospital, Le Chesnay, FRANCE

[11]ICU, District Hospital Center, La Roche sur Yon, FRANCE

[12]ICU, Brabois Teaching Hospital, Nancy, FRANCE

[13]ICU, Jules Bordet Institute, Brussels, BELGIUM

[14]ICU, Ghent University Hospital, Ghent, BELGIUM

[15]ICU, Grenoble Teaching Hospital, Grenoble, FRANCE

[16]ICU, Regional Teaching Hospital, Lille, FRANCE

Corresponding author: Professor Elie Azoulay, Medical Intensive Care Unit, APHP, Hôpital Saint-Louis, Famirea Study Group. ECSTRA team, and clinical epidemiology, UMR 1153 (Center of Epidemiology and Biostatistics Sorbonne Paris Cité, CRESS), INSERM, Paris Diderot Sorbonne University. E-mail: elie.azoulay@sls.aphp.fr

Contributions of each author

All authors substantially contributed to the conception and design of the work, as well as to the acquisition, analysis, and interpretation of data. All have worked on, revised and approved the submitted manuscripts. EA 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.

Sources of support

The study was funded by grant #PHRC AOM 08235 from the French Ministry of Health and French Intensive Care Society (FICS). MSD AVENIR has supported Saint-Louis hospital and the support was used to pay technicians and statisticians. GenMark Dx®, Carlsbad, CA, donated all ePlex® Respiratory Pathogen Panels and MSD had no role in designing, conducting, or interpreting the study or in writing the article.

Running head: Virus Detection in ICU Hematology Patients

Descriptor number: 4.5, Diagnostic techniques and monitoring

Word count: 3009

AJRCCM Articles in Press. Published on 19-September-2018 as 10.1164/rccm.201804-0681OC

At a glance commentary

Scientific knowledge on the subject

In critically ill hematology patients, identifying the cause of the acute illness while providing life-supporting interventions is the cornerstone of the initial management. PCR panels for the rapid detection of viruses are now available. However, the clinical significance of positive PCR results on upper respiratory tract samples remains unclear.

What this study adds to the field

Among critically ill hematology patients, one in six overall and one in four with acute respiratory failure (ARF) had a virus identified in a nasal swab at ICU admission. Presence of a virus was associated with lymphoproliferative disorders, hematopoietic stem cell transplantation, and treatment with steroids or other immunosuppressants. The most common viruses were rhinovirus/enterovirus (56.4%) and influenza/parainfluenza (PIV)/respiratory syncytial viruses (RSV) (30.7%). Virus detection was associated with higher ICU mortality, and this association was strongest for influenza/PIV/RSV. In patients with ARF, detection of any respiratory virus independently predicted ICU mortality.

Abbreviations

95%CI, 95% confidence interval

ARF, acute respiratory failure

CT, computed tomography

Grrr-OH, *Groupe de recherche respiratoire en réanimation en Onco-Hématologie*, a task force working on critical respiratory diseases in patients with cancer or hematological malignancies

HM, hematological malignancies

hMPV, human metapneumovirus

HSCT, hematopoietic stem-cell transplant

ICU, intensive care unit

IPA, invasive pulmonary aspergillosis

OR, odds ratio

PCR, polymerase chain reaction

PIV, parainfluenza *virus*

RRT, renal replacement therapy

RSV, respiratory syncytial virus

SOFA, Sequential Organ Failure Assessment

AJRCCM Articles in Press. Published on 19-September-2018 as 10.1164/rccm.201804-0681OC

ABSTRACT

Rationale: Noninvasive diagnostic multiplex molecular tests may enable the early identification and treatment of viral infections in critically ill immunocompromised patients.

Objectives: To assess the association between viral detection in nasopharyngeal swabs and ICU mortality in critically ill hematology patients.

Methods: Post-hoc analysis of a prospective cohort of critically ill hematology patients admitted to 17 ICUs. Nasal swabs sampled and frozen at ICU admission were tested using a multiplex PCR assay. Predictors of ICU mortality and assay positivity were identified.

Measurements and Main Results: Of the 747 patients (447 with acute respiratory failure [ARF]), 21.3% had a virus detected (56.4% rhinovirus/enterovirus and 30.7% influenza/parainfluenza [PIV]/respiratory syncytial viruses [RSV]). Overall ICU and hospital mortality rates were 26% and 37%, respectively. Assay positivity was associated with lymphoproliferative disorders, hematopoietic stem cell transplantation, treatment with steroids or other immunosuppressants, ARF (25.5% vs. 16.3%, *P*=0.004), and death in the ICU (28.9% vs. 19.3%, *P*=0.008). The association with ICU mortality was significant for all viruses and was strongest for influenza/PIV/RSV. In patients with ARF, detection of any respiratory virus was independently associated with ICU mortality (odds ratio, 2.07; 95% confidence interval, 1.22-3.50).

Conclusions: Respiratory virus detection in the upper airway by multiplex PCR assay is common in critically ill hematology patients. In patients with ARF, respiratory virus detection was independently associated with ICU mortality. Multiplex PCR assay may prove helpful for the risk stratification of hematology patients with ARF. Studies to understand whether respiratory tract viruses play a causal role in outcomes are warranted.

Abstract word count: 248

Keywords: Bone marrow transplantation, neutropenia, oxygen, influenza, parainfluenza virus, respiratory syncytial virus, mechanical ventilation, multiplex PCR

INTRODUCTION

Among patients with hematological malignancies (HM), up to 40% experience acute respiratory failure (ARF), for which the risk factors include prolonged neutropenia, complex immune deficiencies, and drug-related pulmonary toxicity (1–5). The causes of lung disease vary with the nature of underlying malignancy, level of immunosuppression, and treatment intensity. Early identification of the cause of ARF is associated with improved outcomes (6–8). Current diagnostic strategies combine semi-invasive tests (fiberoptic bronchoscopy with bronchoalveolar lavage [BAL], minimally invasive computed tomography [CT]-guided biopsies) with noninvasive PCR assays on sputum, nasopharyngeal aspirates, and nasal swabs. This combined strategy can be expected to identify the cause in about 80% of patients (9–11). Hospital mortality, which can reach 50%, is highest when the cause of ARF remains unknown (8, 12–14).

Whereas up to 40% of patients with hematological malignancies have one or more viruses detected in their respiratory samples, viruses, mainly diagnosed with the collection of upper respiratory tract samples, are much less considered than other pathogens such bacteria or Aspergillus as likely cause of ARF (12–15). A positive virus PCR test on nasopharyngeal aspirates or nasal swabs may reflect either upper or lower respiratory tract infection, the clinical consequences of which can differ substantially. However, a positive test may also indicate asymptomatic carriage, perhaps with an increased viral burden due to worsening immunosuppression, and with or without an impact on mucosal function and/or the local flora. When the virus is clinically relevant, as seen during H1N1 epidemics, its rapid detection can help to identify patients at risk for respiratory deterioration, determine that isolation and preventive measures are needed, and guide early antiviral therapy (19, 20).

AJRCCM Articles in Press. Published on 19-September-2018 as 10.1164/rccm.201804-0681OC

Multiplex assays on bronchoalveolar lavage fluid or nasal secretions are effective in detecting a wide range of pathogens (21–23), including several viruses known to cause pneumonia (e.g., influenza virus, respiratory syncytial virus, parainfluenza virus, human metapneumovirus) and others whose potential for causing lower respiratory tract disease is unclear (e.g., rhinovirus, coronavirus) (24, 25). Thus, whether the detection of viruses in respiratory samples has diagnostic, therapeutic, and/or prognostic relevance is unclear.

The objective of this study was to assess the clinical relevance of a positive PCR assay for viruses on respiratory samples of critically ill hematology patients with or without ARF. We retrospectively assessed prospectively collected nasal swabs using a multiplex assay and compared patients with and without positive results, notably regarding the presence of ARF and ICU mortality.

PATIENTS AND METHODS

Patients

We performed a post-hoc analysis of data from a prospective multicenter cohort study of 1011 critically ill patients with HM admitted to 17 ICUs between January, 1, 2010, to May, 1, 2011(14). The study was approved by the appropriate ethics committees. All patients/relatives gave written informed consent to study participation.

Data collection

In each center, an investigator used a standardized electronic case-report form to prospectively collect the study data. The Sequential Organ Failure Assessment (SOFA) score was computed at admission. ARF was defined as oxygen saturation <90% or PaO_2 <60 mmHg on room air combined with a respiratory rate >30/min and/or clinical signs of respiratory distress (14, 26). A comprehensive diagnostic assessment was performed including a physical examination and CT followed by bronchoscopy and BAL and/or noninvasive diagnostic tests (27, 11). Etiological diagnoses were made by consensus among the managing physicians (intensivists, hematologists, and consultants). Patients with ARF were classified into four diagnostic categories (8) (infectious pneumonia as defined by a clinically or microbiologically documented lower respiratory tract infection; non-infectious lung involvement; opportunistic infection; and undetermined diagnosis).

Molecular assay for respiratory virus detection

Nasopharyngeal flocked swabs were collected at admission in 3 mL of Universal Transport Medium (UTM) (Copan Diagnostics Inc, Murrieta, CA) in patients admitted on weekdays and stored at -80°C until testing. For the present study, a multiplex molecular assay (ePlex® Respiratory Pathogen Panel, GenMark Diagnostics Inc., Carlsbad, CA) was performed according to the manufacturer's instructions. This assay tests for 20 respiratory

AJRCCM Articles in Press. Published on 19-September-2018 as 10.1164/rccm.201804-0681OC

viruses (influenza viruses A–H1, A-H1N1, A-H3, and B; respiratory syncytial viruses [RSV] A and B; parainfluenza viruses [PIV] types 1, 2, 3, and 4; human metapneumovirus [hMPV]; rhinovirus and/or enterovirus; coronaviruses 229E, HKU1, NL63, OC43, and MERS; adenovirus, and bocavirus) and for four bacteria (*Bordetella pertussis, Chlamydophila pneumoniae, Legionella pneumophila,* and *Mycoplasma pneumoniae*).

Samples positive for rhinovirus/enterovirus, underwent sequencing the VP4/VP2 coding region as previously described (28). PCR products were sequenced on an ABI 3100 DNA Sequencer (Applied Biosystems, Life Technologies, Carlsbad, CA). Alignment and sequence comparison to NCBI published sequences were carried out using the Geneious 8.0.5 software (Biomatters, Auckland, NZ) and MEGA version 7 (CEMI, Tempe, AZ).

Statistical analysis

The primary outcome was the rate of respiratory viral infections in patients with ARF and those without. Secondary outcomes were the association between respiratory viral infections and death in ICU and risk factors of death in patients with ARF. Several groups were compared: patients with respiratory viral infections and those without, patients with and without ARF. ePlex® positive status was defined as the detection of at least one pathogen by the ePlex® respiratory panel

Categorical variables were described as numbers and percentages and quantitative variables as medians (25^{th}-75^{th} quartiles). Between-group comparisons were with Fisher's exact test for categorical variables and the Wilcoxon rank sum test for quantitative variables.

Factors associated with ICU mortality were assessed by logistic regression. In addition to the ePlex® assay result, the model included both the variables associated with the outcome in the original study (14) and the clinically meaningful variables (underlying malignancy, poor ECOG performance status, Charlson comorbidity index, allogeneic

hematopoietic stem cell transplant [HSCT] recipient, complete or partial remission, time from hospital to ICU admission <24 h, SOFA score, organ infiltration by the malignancy, and invasive pulmonary aspergillosis). Variable selection was with a backward stepwise procedure with a stopping rule based on the Akaike criterion. Odds ratios (ORs) of variable effects in the final model are reported with their 95% confidence intervals (95%CIs). Goodness-of-fit of the final model was checked using the le Cessie–van Houwelingen test. All tests were two-sided at the 0.05 level. Analyses were performed using the R statistical package (http://www.R-project.org).

AJRCCM Articles in Press. Published on 19-September-2018 as 10.1164/rccm.201804-0681OC

RESULTS

Patients

Among the 1011 critically ill hematology patients, 747 had nasal swabs tested by ePlex® (Figure 1). None of the collected variables were significantly different between the 747 included patients and the 264 patients without nasal swabs.

Table 1 reports the patient characteristics at admission. Median age was 60 [49;70] years. Almost half the patients had a lymphoid malignancy and one-third had myeloid malignancies (Table 1). The HM was newly diagnosed in 280 (37.5%) patients and in partial or complete remission in 174 (23.3%) patients. There were 190 (25.4%) HSCT recipients. In the previous month, 423 (56.6%) patients had received corticosteroids or other immunosuppressants. The main reason for ICU admission was ARF (n=447, 59.8%). Shock was present in 316 (42.4%) patients. Invasive mechanical ventilation was provided to 351 (33.6%) patients and vasoactive drugs to 370 (49.5%) patients. ICU and hospital mortality rates were 26% and 37.1%, respectively.

Results of molecular viral detection

The ePlex® Respiratory Pathogen Panel identified 179 pathogens in 163 (21.8%) patients, including 149 (83.2%) with a single pathogen and 14 with more than one pathogen (12 with two and 2 with three pathogens). Four samples were positive for *Legionella pneumophila.* All other samples were positive for respiratory viruses (Table 2).

The most prevalent virus was rhinovirus/enterovirus (n=92/163, 56.4%), followed by coronavirus (n=22, 13.5%), influenza virus (n=20, 12.3%), RSV (n=18, 11.0%), and PIV (n=12, 7.4%). Adenovirus (n=5, 3.1%), hMPV (n=4, 2.5%), and bocavirus (n=2, 1.2%) were less frequently detected. The virus type was determined for 71 of the 92

rhinovirus/enterovirus-positive samples, with the following results: rhinovirus species A, n=35; rhinovirus species B, n=9; rhinovirus species C, n=24; and enterovirus D68, n=3. Of the respiratory viruses, 66.8% were detected during the winter and spring. There was a seasonal variation of samples positive for respiratory viruses (Table 3). Influenza and RSV were significantly more detected during winter ($p<0.0001$) while rhinoviruses were slightly more frequent during summer and autumn ($p=0.02$) (Table S1).

Overall, respiratory viruses were more frequently detected in patients with ARF (25.5% vs. 16.3%, $P=0.004$) and in patients who died in the ICU (28.9% vs. 19.3%, $P=0.008$) (Table 2). Influenza-like viruses (influenza/PIV/RSV) were particularly prevalent in patients with ARF (10.1% vs. 1.7%, $P<0.0001$) and in patients who died in the ICU (11.3% vs 5.1%, $P=0.004$). Rhinovirus/enterovirus, coronavirus, adenovirus, hMPV, and bocavirus were found in similar proportions of patients across subgroups with vs. without ARF and in ICU survivors vs. nonsurvivors. Viral-viral or viral-bacterial coinfections were not associated with ARF but were more prevalent in patients who died in the ICU (5.7 vs. 0.5%, $P<0.0001$).

Characteristics and outcomes of patients with a positive viral assay

As shown in Table 3, neither SOFA score on day 1 nor presence of shock differed between patients with vs. without detected viruses. Respiratory viruses were more frequently detected in HSCT recipients than in other patients (42.9% vs. 20.7%, $P<0.0001$) and in patients on steroids (46% vs. 36%, $P=0.026$) or other immunosuppressants (23.0% vs. 16.6%, $P=0.043$). Presence of respiratory symptoms on day 1 (including fever, cough, dyspnea, chest pain, wheezing, rhinitis, and/or myalgia) with or without signs of ARF was significantly more common in patients with a positive ePlex® (85.9% vs. 72.2%, $P=0.0005$). However, 23 (14.1%) patients with a positive ePlex® had no respiratory symptoms on day 1

AJRCCM Articles in Press. Published on 19-September-2018 as 10.1164/rccm.201804-0681OC

but none had any Influenza-like virus (Table S2). In patients with no respiratory symptoms, there was no difference of mortality in ICU between patients with a positive ePlex® result and those with a negative ePlex® result (Table S3).

Overall there was no difference in of the presence of rhinovirus in patients who died in ICU compare to those who were discharged alive (Table 2). However, death was observed more frequently in patients with a detection of rhinovirus Species C (11/24, 45.8%) than in those with a detection of rhinovirus Species A or B (9/44, in 20.5%) (Fisher exact test, p.value=0.049) (Table 2). In addition, within the cohort of patients with ARF (447 patients), we observed a higher proportion of death among patients with Rhinovirus species C (9/17, 52.9%) than among patients with Rhinovirus Species A or B (p.value=0.049).

Noninvasive ventilation was used significantly more often in virus-positive patients (39.9% vs. 29.6%, *P*=0.017). For invasive mechanical ventilation, the difference was nearly significant (54.0% vs. 45.0%, *P*=0.053). ICU mortality was higher in virus-positive patients (34.3% vs. 23.6%, *P*=0.008). No significant difference was found for hospital mortality (41.1% vs. 36%, *P*=0.27) or mortality on day 90 (47.7% vs. 45.2%, *P*=0.69). There were no differences in the use of vasoactive drugs or renal replacement therapy (RRT) or in the rate of ICU-acquired infections (Table 3).

Characteristics and outcomes associated with respiratory virus detection in patients with acute respiratory failure (ARF)

In the group with ARF (Table 4), the clinical presentation at admission was similar in the virus-positive and virus-negative patients. Although there was no difference in maximal oxygen flow in patients breathing spontaneously, the PaO_2/FiO_2 ratio tended to be lower in the virus-positive patients who required mechanical ventilation (118 [71-206] vs. 150 [96-258], *P*=0.052).

The chest radiograph was more often normal in the virus-positive patients (17.9% vs. 9.9%, *P*=0.039). In patients who underwent CT of the chest, the only finding that differed between groups was pleural effusion, which was significantly less common in the virus-positive group (12.3% vs. 24.8%, *P*=0.009).

Among virus-positive patients with ARF, 27.2% had clinically or microbiologically documented bacterial pneumonia. Documented bacterial pneumonia was less common among virus-positive than virus-negative patients. Invasive pulmonary aspergillosis (IPA) was diagnosed in 9.7% of virus-positive and 3.9% of virus-negative patients (Table 4).

The use of antibiotics and of life-supporting interventions (mechanical ventilation, vasoactive drugs, RRT) in the group with ARF was not significantly different between virus-positive and virus-negative patients. As in the overall population, a positive viral assay was associated with higher ICU mortality in the ARF group (40.3% vs. 24.3%, P=0.002). The hospital and day-90 mortality rates were not different between the virus-positive and virus-negative groups. Changes in the daily SOFA score from admission to day 7 were not different between virus-positive and virus-negative patients (Figure S1) and or patients with vs. without detection of influenza-like viruses (Figure S1).

By multivariable analysis, detection of a virus was independently associated with ICU mortality (OR, 2.07; 95%CI, 1.22-3.50, *P*=0.006). Other independent risk factors for ICU mortality were IPA (OR, 2.43; 95%CI, 1.23-4.81; *P*=0.01), poor ECOG performance status (OR, 1.89; 95%CI, 1.09-3.27; *P*=0.024), and SOFA score >7 at admission (OR, 1.27; 95%CI, 1.20-1.36; *P*<0.001) (Figure 2, Table S4). Steroid therapy was not associated with a worse prognosis in patients with ARF (Table S5 and S6).

Impact of influenza-like viruses

AJRCCM Articles in Press. Published on 19-September-2018 as 10.1164/rccm.201804-0681OC

In the overall population, patients with influenza-like viruses had higher ICU mortality (44.4% vs. 24.5%, P=0.002). They more often presented with ARF (88.9% vs. 57.6%, P<0.0001) and more often required invasive or noninvasive mechanical ventilation (75.9% vs. 58.7%, P=0.014) (Figure 3).

DISCUSSION

In this study we assessed the clinical relevance of a positive multiplex PCR assay for viruses on respiratory samples of critically ill hematology patients. We analyzed nasopharyngeal swabs collected at admission to ICU in patients with or without respiratory symptoms, enabling to better estimate the clinical significance of detection of respiratory viruses. In this large multicenter prospective cohort study of 747 critically ill patients with HMs, respiratory virus detection was associated with both ARF and higher ICU mortality. Both associations were largely due to the influenza-like viruses (Influenza/PIV/RSV). Species C of rhinovirus was associated with a higher proportion of death among patients in the whole cohort and in patients with ARF than rhinovirus Species A or B.

Although there is no recommendation for routine molecular testing, multiplex PCR is widely used to identify the cause of ARF, in combination with clinical, radiological, and standard microbiological evaluations. However, due to the high sensitivity of molecular assays and high prevalence of asymptomatic carriage of respiratory viruses, a positive molecular assay is not proof that the detected virus is causing disease. Immunosuppression may increase the burden of viruses not conventionally associated with significant disease, so that a positive PCR assay may be merely a marker for poor immune function (29). In addition, presence of a virus can alter the bacterial flora in the upper respiratory tract, an effect that might in turn promote bacterial pneumonia (30). Finally, respiratory viruses may enhance bacterial adhesion to, and invasion of, the respiratory mucosa (30). In studies of patients with community-acquired bacterial pneumonia, viral coinfection was associated with a worse prognosis (31–33). Thus, the effects of respiratory viruses are complex, and whether virus detection by PCR in an upper airway sample from a critically ill hematology patient should lead to antiviral treatment is unclear. As a first step toward obtaining clarification, we routinely sampled the upper airway of unselected, critically ill, hematology

AJRCCM Articles in Press. Published on 19-September-2018 as 10.1164/rccm.201804-0681OC

patients at ICU admission, regardless of whether respiratory symptoms were present. The results were analyzed at a distance from ICU admission. Presence of a virus in this setting might indicate a direct contribution of the virus to the critical illness (e.g., viral pneumonia), an indirect contribution of the virus via alterations in respiratory tract ecology and mucosal function, asymptomatic carriage with no role for the virus in the symptoms but a possible viral load increase due to immunosuppression, or a false-positive test result. Antiviral treatment would be in order only in the first two cases. The lack of lower respiratory tract samples, such as broncho-alveolar lavages, hampered direct evaluation of the impact on respiratory tract ecology and mucosal function. We sought to determine whether any of our findings were in favor of a direct or indirect causal role for detected viruses in the clinical illness.

Half the pathogens detected in our patients were rhinovirus/enterovirus. With coronaviruses, rhinoviruses are the leading cause of rhinitis, and less often cause pneumonia (29, 34–38) . The higher prevalence of these viruses than previously reported in hematology patients may be due to the very high sensitivity of ePlex®, particularly for rhinovirus species C. Several studies have recently shown that rhinovirus could contribute to severe lower respiratory tract infections in immunocompromised hosts (39–41). Severe rhinovirus infections presented with similar clinical features and same overall mortality than Influenza-like viruses In our study including patients with or without respiratory symptoms at admission, the prevalence of rhinovirus did not differ neither between patients with ARF and those nor between who died in ICU or were alive at ICU discharge. Rhinovirus is classified into three species A, B and C. Some studies reported that species C was associated with more severe infections, with more frequent lower respiratory tract infections (42, 43) but some others found no difference between the three species (41, 44). In addition recent studies suggest that Species C targets specifically ciliated epithelial cells while species A and

B may infect non epithelial cells (45). The analysis of rhinovirus as one group only may thus be not appropriate. In this series, we found that Species C was associated with a higher mortality in the whole cohort and in patients with ARF compare to species A and B. These results suggest that the impact of rhinovirus infection may differ according to the species. This observation needs to be confirmed in other studies and deserves further research to understand underlying mechanisms.

Other viruses were detected in similar proportions of patients after adjustment for age and season. In keeping with this fact, the higher frequencies of ARF and ICU death in the virus-positive group was largely ascribable to the patients with influenza-like viruses (Influenza/PIV/RSV, hMPV). Others have reported similar results (46).

Thus, in agreement with other studies, our results emphasize the need for specific antiviral therapy in critically ill patients with hematological malignancies when a molecular multiplex assay is positive for influenza in an upper respiratory tract sample Early oseltamivir therapy improves the outcome in critically ill patients with influenza: a propensity analysis (47–49).

Among patients with ARF, respiratory virus detection was a predictor of ICU mortality that was nearly as strong as IPA. While IPA is a well-known risk factor for mortality in critically ill hematology patients, our study is the first to report that respiratory viruses were also independently associated with ICU mortality. Three hypotheses may explain this association. One involves a double hit, in which patients developing a respiratory virus infection shortly before ICU admission would be then more vulnerable to bacterial infection, leading to a critical illness. The lower PaO_2/FiO_2 ratio in virus-positive patients may seem to support this possibility, but the more often normal chest radiographs and similar SOFA scores over the first week compared to virus-negative patients do not. Another hypothesis is that bacterial or fungal respiratory tract infection would promote viral

AJRCCM Articles in Press. Published on 19-September-2018 as 10.1164/rccm.201804-0681OC

infection via weakening of the local defense mechanisms (50). The viral infection may then worsen the initial disease. A recent work suggested that the alteration of microbial ecology after antibiotic exposure was associated with a higher risk of viral lower respiratory tract infection with following PIV, RSV, and hMPV (51). Viral infections and invasive fungal infections share common risk factors (52–54) that are common in hematology patients, such as allogeneic HSCT and treatment with corticosteroids or other immunosuppressants. Finally, a positive viral assay might be a marker for immunosuppression or other vulnerability factors.

The contribution of PCR assays to the etiological diagnosis of ARF is probably modest. Nevertheless, our results suggest that rapid routine multiplex molecular testing for upper respiratory tract viruses in hematology patients admitted to the ICU may contribute to identify patients at highest risk for mortality. Such finding could be delineated into different ways. Namely, based on a positive virus in the nasal swab, these high risk patients should be admitted earlier to the ICU/HDU to undergo noninvasive management and close monitoring. Moreover, these patients should be considered as more severely immunocompromised and may also be eligible for preventive strategies such as antifungal prophylaxis. However, for such practices to be implemented, we need to validate these concepts in future observational and interventional studies. Although antiviral drugs available to treat respiratory viral infections are currently limited to influenza, our results identify patients who should benefit to new antiviral drugs or included in trials evaluating treatments under development.

Regarding the association with IPA, antifungal treatment might be in order in patients with ARF who do not respond promptly to antibacterial treatment and whose multiplex test for upper respiratory tract viruses is positive. In patients whose test is positive for influenza, early antiviral treatment and preventive measures to limit nosocomial

transmission may be warranted. Our results suggest that critically ill hematology patients may be a population of interest for evaluating new drugs targeting RSV and PIV.

This study has several limitations. For feasibility reasons, 264 patients (26.1% of the initial cohort) admitted on weekends were not included. However, the baseline characteristics and ICU mortality of these 264 patients were not significantly different from those in the included patients. We had no information on the occurrence of hospital-acquired pneumonia or ventilator-associated pneumonia during the ICU stay. The study design does not allow conclusions about the nature of the link between the detection of viruses and ICU mortality. Indeed, although mortality related to infection is usually high in this setting, ascription of death to infection was not assessed due to the level of uncertainty. Future studies involving the collection of biomarkers and specific patient phenotypes in this hematology population are warranted. Also, lower respiratory tract samples and autopsy studies of the lung lesions might shed light on the role for viruses versus other causes of lung involvement, document undetected pathogens, and provide information on resident and recruited alveolar cells and their relationship to severity and mortality.

In conclusion, a respiratory virus was detected in the upper airway of one in six critically ill hematology patients overall and of one in four of those with ARF. Virus detection was more common in patients who died in the ICU, and this association was chiefly due to influenza/PIV/RSV. In patients with ARF, however, detection of any respiratory virus was an independent predictor of ICU mortality. Routine PCR screening of upper airway samples in critically ill hematology patients may help to identify patients at high risk for respiratory deterioration and mortality. However, whether these patients are potential candidates for immunomodulation, new antiviral treatments, early ICU admission, or antifungal therapy remains unclear. These results encourage a trial of routine screening for respiratory viral detection by PCR at ICU admission in hematology patients. Therapeutic

AJRCCM Articles in Press. Published on 19-September-2018 as 10.1164/rccm.201804-0681OC

indications must be appraised. This high-risk population may be a good target for evaluating the antiviral treatments that are currently being developed.

References

1. Chaoui D, Legrand O, Roche N, Cornet M, Lefebvre A, Peffault de Latour R, Sanhes L, Huchon G, Marie J-P, Rabbat A. Incidence and prognostic value of respiratory events in acute leukemia. *Leukemia* 2004;18:670–675.

2. Azoulay E, Schlemmer B. Diagnostic strategy in cancer patients with acute respiratory failure. *Intensive Care Med* 2006;32:808–822.

3. Moreau A-S, Peyrony O, Lemiale V, Zafrani L, Azoulay E. Acute Respiratory Failure in Patients with Hematologic Malignancies. *Clin Chest Med* 2017;38:355–362.

4. Bird GT, Farquhar-Smith P, Wigmore T, Potter M, Gruber PC. Outcomes and prognostic factors in patients with haematological malignancy admitted to a specialist cancer intensive care unit: a 5 yr study. *Br J Anaesth* 2012;108:452–459.

5. Mokart D, van Craenenbroeck T, Lambert J, Textoris J, Brun J-P, Sannini A, Chow-Chine L, Hamouda S, Fouché L, Ettori F, Faucher M, Blache J-L. Prognosis of acute respiratory distress syndrome in neutropenic cancer patients. *Eur Respir J* 2012;40:169–176.

6. Rano A, Agusti C, Jimenez P, Angrill J, Benito N, Danes C, Gonzalez J, Rovira M, Pumarola T, Moreno A, others. Pulmonary infiltrates in non-HIV immunocompromised patients: a diagnostic approach using non-invasive and bronchoscopic procedures. *Thorax* 2001;56:379–387.

7. Azoulay É, Thiéry G, Chevret S, Moreau D, Darmon M, Bergeron A, Yang K, Meignin V, Ciroldi M, Le Gall J-R, Tazi A, Schlemmer B. The Prognosis of Acute Respiratory Failure in Critically Ill Cancer Patients: *Medicine (Baltimore)* 2004;83:360–370.

8. Contejean A, Lemiale V, Resche-Rigon M, Mokart D, Pène F, Kouatchet A, Mayaux J, Vincent F, Nyunga M, Bruneel F, Rabbat A, Perez P, Meert A-P, Benoit D, Hamidfar R, Darmon M, Jourdain M, Renault A, Schlemmer B, Azoulay E. Increased mortality in hematological malignancy patients with acute respiratory failure from undetermined

AJRCCM Articles in Press. Published on 19-September-2018 as 10.1164/rccm.201804-0681OC

etiology: a Groupe de Recherche en Réanimation Respiratoire en Onco-Hématologique (Grrr-OH) study. *Ann Intensive Care* 2016;6:102.

9. Rabbat A, Chaoui D, Lefebvre A, Roche N, Legrand O, Lorut C, Rio B, Marie J-P, Huchon G. Is BAL useful in patients with acute myeloid leukemia admitted in ICU for severe respiratory complications? *Leukemia* 2008;22:1361–1367.

10. Azoulay E, Mokart D, Rabbat A, Pene F, Kouatchet A, Bruneel F, Vincent F, Hamidfar R, Moreau D, Mohammedi I, Epinette G, Beduneau G, Castelain V, de Lassence A, Gruson D, Lemiale V, Renard B, Chevret S, Schlemmer B. Diagnostic bronchoscopy in hematology and oncology patients with acute respiratory failure: Prospective multicenter data*: *Crit Care Med* 2008;36:100–107.

11. Azoulay É, Mokart D, Lambert J, Lemiale V, Rabbat A, Kouatchet A, Vincent F, Gruson D, Bruneel F, Epinette-Branche G, Lafabrie A, Hamidfar-Roy R, Cracco C, Renard B, Tonnelier J-M, Blot F, Chevret S, Schlemmer B. Diagnostic Strategy for Hematology and Oncology Patients with Acute Respiratory Failure: Randomized Controlled Trial. *Am J Respir Crit Care Med* 2010;182:1038–1046.

12. Villa F, Coppadoro A, Bellani G, Foti G, Fumagalli R, Pesenti A. Etiology of respiratory failure is related to mortality in critically ill patients affected by a hematological malignancy: a retrospective study. *Minerva Anestesiol* 2010;76:7–12.

13. Burghi G, Lemiale V, Seguin A, Lambert J, Lacroix C, Canet E, Moreau A-S, Ribaud P, Schnell D, Mariotte E, Schlemmer B, Azoulay E. Outcomes of mechanically ventilated hematology patients with invasive pulmonary aspergillosis. *Intensive Care Med* 2011;37:1605–1612.

14. Azoulay E, Mokart D, Pène F, Lambert J, Kouatchet A, Mayaux J, Vincent F, Nyunga M, Bruneel F, Laisne L-M, Rabbat A, Lebert C, Perez P, Chaize M, Renault A, Meert A-P, Benoit D, Hamidfar R, Jourdain M, Darmon M, Schlemmer B, Chevret S,

Lemiale V. Outcomes of Critically Ill Patients With Hematologic Malignancies: Prospective Multicenter Data From France and Belgium—A Groupe de Recherche Respiratoire en Réanimation Onco-Hématologique Study. *J Clin Oncol* 2013;31:2810–2818.

15. Martino R, Porras RP, Rabella N, Williams JV, Rámila E, Margall N, Labeaga R, Crowe JE, Coll P, Sierra J. Prospective Study of the Incidence, Clinical Features, and Outcome of Symptomatic Upper and Lower Respiratory Tract Infections by Respiratory Viruses in Adult Recipients of Hematopoietic Stem Cell Transplants for Hematologic Malignancies. *Biol Blood Marrow Transplant* 2005;11:781–796.

16. van Elden LJR, van Kraaij MGJ, Nijhuis M, Hendriksen KAW, Dekker AW, Rozenberg-Arska M, van Loon AM. Polymerase chain reaction is more sensitive than viral culture and antigen testing for the detection of respiratory viruses in adults with hematological cancer and pneumonia. *Clin Infect Dis Off Publ Infect Dis Soc Am* 2002;34:177–183.

17. van Kraaij MGJ, van Elden LJR, van Loon AM, Hendriksen KAW, Laterveer L, Dekker AW, Nijhuis M. Frequent detection of respiratory viruses in adult recipients of stem cell transplants with the use of real-time polymerase chain reaction, compared with viral culture. *Clin Infect Dis Off Publ Infect Dis Soc Am* 2005;40:662–669.

18. Schnell D, Legoff J, Mariotte E, Seguin A, Canet E, Lemiale V, Darmon M, Schlemmer B, Simon F, Azoulay É. Molecular detection of respiratory viruses in immunocopromised ICU patients: Incidence and meaning. *Respir Med* 2012;106:1184–1191.

19. Rodríguez A, Díaz E, Martín-Loeches I, Sandiumenge A, Canadell L, Díaz JJ, Figueira JC, Marques A, Alvarez-Lerma F, Vallés J, Baladín B, García-López F, Suberviola B, Zaragoza R, Trefler S, Bonastre J, Blanquer J, Rello J, H1N1 SEMICYUC Working Group. Impact of early oseltamivir treatment on outcome in critically ill patients with 2009

AJRCCM Articles in Press. Published on 19-September-2018 as 10.1164/rccm.201804-0681OC

pandemic influenza A. *J Antimicrob Chemother* 2011;66:1140–1149.

20. Choi S-M, Boudreault AA, Xie H, Englund JA, Corey L, Boeckh M. Differences in clinical outcomes after 2009 influenza A/H1N1 and seasonal influenza among hematopoietic cell transplant recipients. *Blood* 2011;117:5050–5056.

21. Poritz MA, Blaschke AJ, Byington CL, Meyers L, Nilsson K, Jones DE, Thatcher SA, Robbins T, Lingenfelter B, Amiott E, Herbener A, Daly J, Dobrowolski SF, Teng DH-F, Ririe KM. FilmArray, an Automated Nested Multiplex PCR System for Multi-Pathogen Detection: Development and Application to Respiratory Tract Infection. In: Costa C, editor. *PLoS ONE* 2011;6:e26047.

22. Ruggiero P, McMillen T, Tang Y-W, Babady NE. Evaluation of the BioFire FilmArray Respiratory Panel and the GenMark eSensor Respiratory Viral Panel on Lower Respiratory Tract Specimens. *J Clin Microbiol* 2014;52:288–290.

23. Gadsby NJ, Russell CD, McHugh MP, Mark H, Conway Morris A, Laurenson IF, Hill AT, Templeton KE. Comprehensive Molecular Testing for Respiratory Pathogens in Community-Acquired Pneumonia. *Clin Infect Dis* 2016;62:817–823.

24. Milano F, Campbell AP, Guthrie KA, Kuypers J, Englund JA, Corey L, Boeckh M. Human rhinovirus and coronavirus detection among allogeneic hematopoietic stem cell transplantation recipients. *Blood* 2010;115:2088–2094.

25. Self WH, Williams DJ, Zhu Y, Ampofo K, Pavia AT, Chappell JD, Hymas WC, Stockmann C, Bramley AM, Schneider E, Erdman D, Finelli L, Jain S, Edwards KM, Grijalva CG. Respiratory Viral Detection in Children and Adults: Comparing Asymptomatic Controls and Patients With Community-Acquired Pneumonia. *J Infect Dis* 2016;213:584–591.

26. Lemiale V, Mokart D, Resche-Rigon M, Pène F, Mayaux J, Faucher E, Nyunga M, Girault C, Perez P, Guitton C, Ekpe K, Kouatchet A, Théodose I, Benoit D, Canet E, Barbier

F, Rabbat A, Bruneel F, Vincent F, Klouche K, Loay K, Mariotte E, Bouadma L, Moreau A-S, Seguin A, Meert A-P, Reignier J, Papazian L, Mehzari I, *et al.* Effect of Noninvasive Ventilation vs Oxygen Therapy on Mortality Among Immunocompromised Patients With Acute Respiratory Failure: A Randomized Clinical Trial. *JAMA* 2015;314:1711–1719.

27. Azoulay É, Schlemmer B. Diagnostic strategy in cancer patients with acute respiratory failure. *Intensive Care Med* 2006;32:808–822.

28. 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;47:3958–3967.

29. Wolfromm A, Porcher R, Legoff J, Peffault de Latour R, Xhaard A, de Fontbrune FS, Ribaud P, Bergeron A, Socié G, Robin M. Viral respiratory infections diagnosed by multiplex PCR after allogeneic hematopoietic stem cell transplantation: long-term incidence and outcome. *Biol Blood Marrow Transplant J Am Soc Blood Marrow Transplant* 2014;20:1238–1241.

30. Bosch AATM, Biesbroek G, Trzcinski K, Sanders EAM, Bogaert D. Viral and bacterial interactions in the upper respiratory tract. *PLoS Pathog* 2013;9:e1003057.

31. Voiriot G, Visseaux B, Cohen J, Nguyen LBL, Neuville M, Morbieu C, Burdet C, Radjou A, Lescure F-X, Smonig R, Armand-Lefèvre L, Mourvillier B, Yazdanpanah Y, Soubirou J-F, Ruckly S, Houhou-Fidouh N, Timsit J-F. Viral-bacterial coinfection affects the presentation and alters the prognosis of severe community-acquired pneumonia. *Crit Care Lond Engl* 2016;20:375.

32. Cawcutt K, Kalil AC. Pneumonia with bacterial and viral coinfection. *Curr Opin Crit Care* 2017;23:385–390.

33. McCullers JA. Insights into the interaction between influenza virus and

AJRCCM Articles in Press. Published on 19-September-2018 as 10.1164/rccm.201804-0681OC

pneumococcus. *Clin Microbiol Rev* 2006;19:571–582.

34. Jain S, Self WH, Wunderink RG, Fakhran S, Balk R, Bramley AM, Reed C, Grijalva CG, Anderson EJ, Courtney DM, Chappell JD, Qi C, Hart EM, Carroll F, Trabue C, Donnelly HK, Williams DJ, Zhu Y, Arnold SR, Ampofo K, Waterer GW, Levine M, Lindstrom S, Winchell JM, Katz JM, Erdman D, Schneider E, Hicks LA, McCullers JA, *et al.* Community-Acquired Pneumonia Requiring Hospitalization among U.S. Adults. *N Engl J Med* 2015;373:415–427.

35. Van Rijn AL, Claas EC, von dem Borne PA, Kroes ACM, de Vries JJC. Rhinovirus viremia in adult patients with high viral load in bronchoalveolar lavages. *J Clin Virol Off Publ Pan Am Soc Clin Virol* 2017;96:105–109.

36. Lu X, Schneider E, Jain S, Bramley AM, Hymas W, Stockmann C, Ampofo K, Arnold SR, Williams DJ, Self WH, Patel A, Chappell JD, Grijalva CG, Anderson EJ, Wunderink RG, McCullers JA, Edwards KM, Pavia AT, Erdman DD. Rhinovirus Viremia in Patients Hospitalized With Community-Acquired Pneumonia. *J Infect Dis* 2017;216:1104–1111.

37. Piñana JL, Madrid S, Pérez A, Hernández-Boluda JC, Giménez E, Terol MJ, Calabuig M, Navarro D, Solano C. Epidemiologic and Clinical Characteristics of Coronavirus and Bocavirus Respiratory Infections after Allogeneic Stem Cell Transplantation: A Prospective Single-Center Study. *Biol Blood Marrow Transplant J Am Soc Blood Marrow Transplant* 2018;24:563–570.

38. Campbell AP, Guthrie KA, Englund JA, Farney RM, Minerich EL, Kuypers J, Corey L, Boeckh M. Clinical outcomes associated with respiratory virus detection before allogeneic hematopoietic stem cell transplant. *Clin Infect Dis Off Publ Infect Dis Soc Am* 2015;61:192–202.

39. Choi S-H, Huh JW, Hong S-B, Lee JY, Kim S-H, Sung H, Do K-H, Lee S-O, Kim

M-N, Jeong J-Y, Lim C-M, Kim YS, Woo JH, Koh Y. Clinical characteristics and outcomes of severe rhinovirus-associated pneumonia identified by bronchoscopic bronchoalveolar lavage in adults: comparison with severe influenza virus-associated pneumonia. *J Clin Virol Off Publ Pan Am Soc Clin Virol* 2015;62:41–47.

40. Seo S, Waghmare A, Scott EM, Xie H, Kuypers JM, Hackman RC, Campbell AP, Choi S-M, Leisenring WM, Jerome KR, Englund JA, Boeckh M. Human rhinovirus detection in the lower respiratory tract of hematopoietic cell transplant recipients: association with mortality. *Haematologica* 2017;102:1120–1130.

41. Jacobs SE, Lamson DM, Soave R, Guzman BH, Shore TB, Ritchie EK, Zappetti D, Satlin MJ, Leonard JP, van Besien K, Schuetz AN, Jenkins SG, George KS, Walsh TJ. Clinical and molecular epidemiology of human rhinovirus infections in patients with hematologic malignancy. *J Clin Virol Off Publ Pan Am Soc Clin Virol* 2015;71:51–58.

42. Cox DW, Bizzintino J, Ferrari G, Khoo SK, Zhang G, Whelan S, Lee WM, Bochkov YA, Geelhoed GC, Goldblatt J, Gern JE, Laing IA, Le Souëf PN. Human rhinovirus species C infection in young children with acute wheeze is associated with increased acute respiratory hospital admissions. *Am J Respir Crit Care Med* 2013;188:1358–1364.

43. Lee W-M, Lemanske RF, Evans MD, Vang F, Pappas T, Gangnon R, Jackson DJ, Gern JE. Human rhinovirus species and season of infection determine illness severity. *Am J Respir Crit Care Med* 2012;186:886–891.

44. Choi S-H, Hong S-B, Kim T, Kim S-H, Huh JW, Do K-H, Lee S-O, Kim M-N, Lim C-M, Kim YS, Koh Y, Woo JH, Choi S-H, Sung H. Clinical and molecular characterization of rhinoviruses A, B, and C in adult patients with pneumonia. *J Clin Virol Off Publ Pan Am Soc Clin Virol* 2015;63:70–75.

45. Griggs TF, Bochkov YA, Basnet S, Pasic TR, Brockman-Schneider RA, Palmenberg AC, Gern JE. Rhinovirus C targets ciliated airway epithelial cells. *Respir Res* 2017;18:84.

AJRCCM Articles in Press. Published on 19-September-2018 as 10.1164/rccm.201804-0681OC

46. Carrat F, Leruez-Ville M, Tonnellier M, Baudel J-L, Deshayes J, Meyer P, Maury E, Galimand J, Rouzioux C, Offenstadt G. A virologic survey of patients admitted to a critical care unit for acute cardiorespiratory failure. *Intensive Care Med* 2006;32:156–159.

47. Hernu R, Chroboczek T, Madelaine T, Casalegno J-S, Lina B, Cour M, Argaud L, On behalf the "Flu in Lyon ICUs" Study Group. Early oseltamivir therapy improves the outcome in critically ill patients with influenza: a propensity analysis. *Intensive Care Med* 2018;44:257–260.

48. Kumar A. Early versus late oseltamivir treatment in severely ill patients with 2009 pandemic influenza A (H1N1): speed is life. *J Antimicrob Chemother* 2011;66:959–963.

49. Muthuri SG, Venkatesan S, Myles PR, Leonardi-Bee J, Al Khuwaitir TSA, Al Mamun A, Anovadiya AP, Azziz-Baumgartner E, Báez C, Bassetti M, Beovic B, Bertisch B, Bonmarin I, Booy R, Borja-Aburto VH, Burgmann H, Cao B, Carratala J, Denholm JT, Dominguez SR, Duarte PAD, Dubnov-Raz G, Echavarria M, Fanella S, Gao Z, Gérardin P, Giannella M, Gubbels S, Herberg J, *et al.* Effectiveness of neuraminidase inhibitors in reducing mortality in patients admitted to hospital with influenza A H1N1pdm09 virus infection: a meta-analysis of individual participant data. *Lancet Respir Med* 2014;2:395–404.

50. Sajjan US, Jia Y, Newcomb DC, Bentley JK, Lukacs NW, LiPuma JJ, Hershenson MB. H. influenzae potentiates airway epithelial cell responses to rhinovirus by increasing ICAM-1 and TLR3 expression. *FASEB J Off Publ Fed Am Soc Exp Biol* 2006;20:2121–2123.

51. Ogimi C, Krantz EM, Golob JL, Waghmare A, Liu C, Leisenring WM, Woodard CR, Marquis S, Kuypers JM, Jerome KR, Pergam SA, Fredricks DN, Sorror ML, Englund JA, Boeckh M. Antibiotic Exposure Prior to Respiratory Viral Infection Is Associated with Progression to Lower Respiratory Tract Disease in Allogeneic Hematopoietic Cell Transplant Recipients. *Biol Blood Marrow Transplant J Am Soc Blood Marrow Transplant*

2018;doi:10.1016/j.bbmt.2018.05.016.

52. White PL, Parr C, Barnes RA. Predicting invasive aspergillosis in haematology patients by combining clinical and genetic risk factors with early diagnostic biomarkers. *J Clin Microbiol* 2017;doi:10.1128/JCM.01122-17.

53. Wauters J, Baar I, Meersseman P, Meersseman W, Dams K, De Paep R, Lagrou K, Wilmer A, Jorens P, Hermans G. Invasive pulmonary aspergillosis is a frequent complication of critically ill H1N1 patients: a retrospective study. *Intensive Care Med* 2012;38:1761–1768.

54. Martino R, Piñana JL, Parody R, Valcarcel D, Sureda A, Brunet S, Briones J, Delgado J, Sánchez F, Rabella N, Sierra J. Lower respiratory tract respiratory virus infections increase the risk of invasive aspergillosis after a reduced-intensity allogeneic hematopoietic SCT. *Bone Marrow Transplant* 2009;44:749–756.

AJRCCM Articles in Press. Published on 19-September-2018 as 10.1164/rccm.201804-0681OC

FIGURE LEGENDS

Figure 1

Patient flow diagram

* Reasons for not sampling or testing patients included weekend admissions (n=241) and unusable samples (n=23).

Abbreviations: ARF, acute respiratory failure; ICU, intensive care unit.

Figure 2

Multivariable analysis for factors independently associated with ICU mortality among patients with ARF

Abbreviations: ICU, intensive care unit; ARF, acute respiratory failure; SOFA, Sequential Organ Failure Assessment.

Figure 3

Associations linking influenza-like virus infections or infections with other viruses to ARF and death or mechanical ventilation

Influenza-like viruses include influenza viruses, parainfluenza viruses, respiratory syncytial viruses and human metapneumovirus. Other viruses include rhinovirus and coronavirus.

Abbreviations: MV, mechanical ventilation; ARF, acute respiratory failure.

Table 1

Baseline characteristics at ICU admission

The data are numbers and percentages unless otherwise specified.

* Some patients were admitted for more than one reason.

Abbreviations: ICU, intensive care unit; IQR, interquartile range (25th and 75th quartiles) for quantitative data

Table 2

Comparison of ePlex® results according to presence or absence of ARF and ICU mortality

Categorical data are reported as numbers and percentages.

* Viral-viral and viral-bacterial co-infections were considered.

Abbreviations: ARF, acute respiratory failure; ICU, intensive care unit; HRV, human rhinovirus; EV, enterovirus; HRV-A, human rhinovirus species A; HRV-B, human rhinovirus species B; HRV-C, human rhinovirus species C; FLU, influenza virus; RSV, respiratory syncytial virus; PIV, parainfluenza virus; hMPV, human metapneumovirus

Table 3

Comparison of patients with a positive versus a negative ePlex® result

Quantitative data are reported as median [25th and 75th quartiles] and categorical data as numbers and percentages.

Abbreviations: IQR, interquartile range; HSCT, hematopoietic stem cell transplantation; ICU, intensive care unit; ARF, acute respiratory failure

AJRCCM Articles in Press. Published on 19-September-2018 as 10.1164/rccm.201804-0681OC

Table 4

Among patients with acute respiratory failure, comparison of patients with a positive versus a negative ePlex® result

Quantitative data are reported as median [25th and 75th quartiles] and categorical data as numbers and percentages.

Abbreviations: ARF, acute respiratory failure; IQR, interquartile range; PaO_2/FiO_2, ratio of partial pressure of arterial oxygen over fraction of inspired oxygen; SOFA, Sequential Organ Failure Assessment; CT, computed tomography; ICU, intensive care unit

Table 1

Variable	N	%
Age, years, median [IQR]	60 [49;70]	
Male gender	454	60.8
Chronic respiratory insufficiency	189	25.3
Charlson comorbidity index, median [IQR]	4 [3;6]	
Poor performance status (>2)	150	20.1
Underlying malignancy		
Lymphoid malignancy	367	49.1
Non-Hodgkin lymphoma	235	31.5
Chronic lymphocytic leukemia	60	8
Acute lymphoblastic leukemia	53	7.1
Hodgkin lymphoma	19	2.5
Myeloid malignancy	250	33.5
Acute myeloid leukemia	200	26.8
Myelodysplastic syndrome	35	4.7
Chronic myeloid leukemia	15	2
Other	130	17.4
Multiple myeloma	96	12.8
Other hematological malignancy	34	4.6
Time between diagnosis and ICU admission, days, median [IQR]	174 [6;865]	
Malignancy status at admission		
Newly diagnosed	280	37.5
Complete or partial remission	174	23.3
Progression	270	36.2
Unknown	23	3
Hematopoietic stem cell transplantation	190	25.5
Autologous	84	11.3
Allogeneic	106	14.2
Corticosteroids in the previous month	284	38.2
Other immunosuppressants in the previous month	136	18.2
Time from hospital to ICU admission, days, median [IQR]	4 [1;18]	
Reason for ICU admission *		
Acute respiratory failure	447	59.8
Shock	316	42.4

AJRCCM Articles in Press. Published on 19-September-2018 as 10.1164/rccm.201804-0681OC

Table 2

N (%)	**Total** N=747	**ARF** N=447	**No ARF** N=300	*P* value	**Patients who died in the ICU** N=194	**Patients alive at ICU discharge** N=553	*P* value
Positive ePlex®	163 (21.8)	114 (25.5)	49 (16.3)	0.004	56 (28.9)	107 (19.3)	0.008
HRV/EV	92	62 (13.9)	30 (10)	0.14	32 (16.5)	60 (10.9)	0.053
HRV-A	35	22	13		8	27	
HRV-B	9	6	3		1	8	
HRV-C	24	17	7		11	13	
FLU-like	54	48 (10.7)	6 (2.0)	<0.0001	24 (12.4)	30 (5.4)	0.002
FLU	20	20	0		12	8	
RSV	18	15	3		7	11	
PIV	12	10	2		3	9	
HMPV	4	3	1		2	2	
Coronavirus	22	11 (2.5)	11 (3.7)	0.46	9 (4.6)	13 (2.4)	0.17
Adenovirus	5	2 (0.5)	3 (1)	0.65	1 (0.5)	4 (0.7)	1
hMPV	4	3 (0.7)	1 (0.3)	0.91	2 (1.0)	2 (0.4)	0.6
Bocavirus	2	1 (0.2)	1 (0.3)	1	1 (0.5)	1 (0.2)	1
Co-infections*	14	12 (2.7)	2 (0.7)	0.086	11 (5.7)	3 (0.5)	<0.0001

Table 3

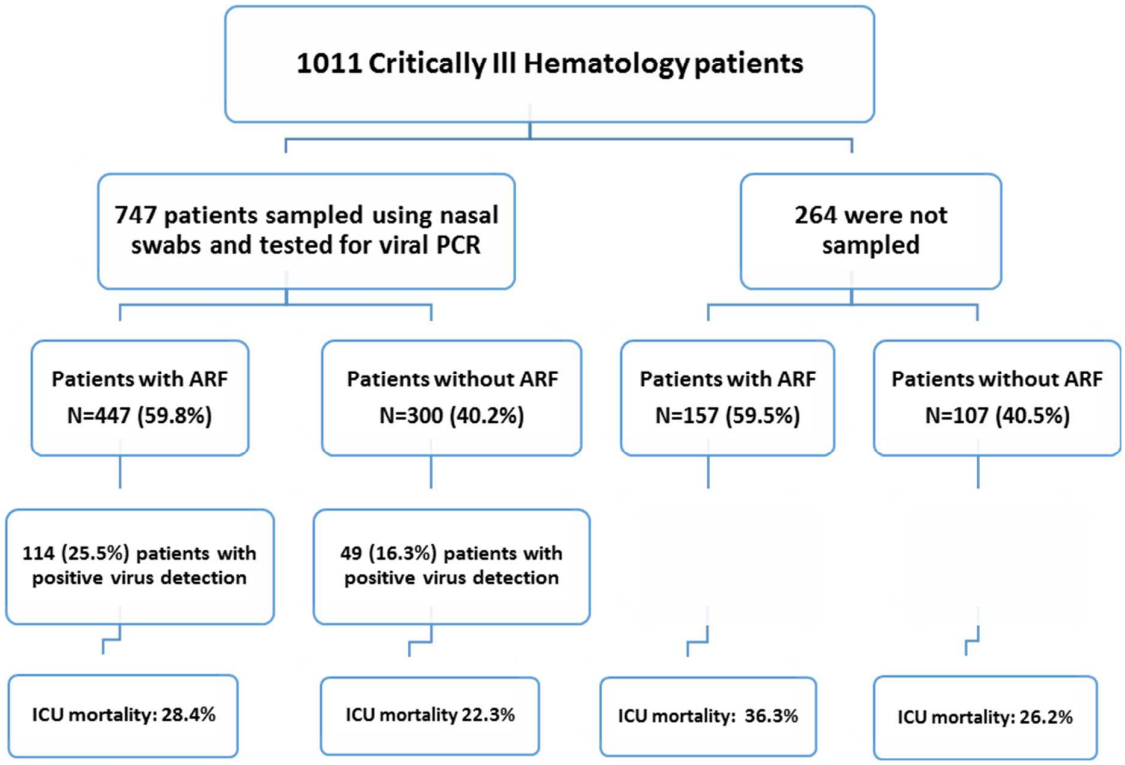
N (%) - median [IQR]	Negative ePlex® N=584	Positive ePlex® N=163	*P* value
Baseline characteristics			
Age, years	61 [49;70]	58 [47;67]	0.11
Underlying malignancy			
Myeloid malignancy	209 (35.8)	41 (25.1)	0.029
Lymphoid malignancy	280 (47.9)	87 (53.4)	
Other	95 (16.3)	35 (21.5)	
Hematopoietic stem cell transplantation			
No	463 (79.3)	92 (57.1)	<0.0001
Yes, autologous	57 (9.7)	27 (16.8)	
Yes, allogeneic	64 (11.0)	42 (26.1)	
Corticosteroids in the previous month	209 (36.0)	75 (46.0)	0.026
Other immunosuppressants in the previous month	97 (16.6)	39 (23.9)	0.043
Season at ICU admission			
Winter	191 (32.7)	72 (44.2)	0.035
Spring	202 (34.6)	42 (25.8)	
Summer	82 (14.0)	24 (14.7)	
Autumn	109 (18.7)	25 (15.3)	
Reason for ICU admission			
Acute respiratory failure	333 (57.0)	114 (69.9)	0.004
Shock	245 (42.0)	71 (43.6)	0.79
Respiratory symptoms on day 1			
Respiratory symptoms with ARF	308 (52.7)	111 (68.1)	0.0005
Respiratory symptoms without ARF	114 (19.5)	29 (17.8)	
No respiratory symptoms	162 (27.8)	23 (14.1)	
SOFA >7	229 (41.5)	77 (49.7)	0.084
Interventions and outcomes			
Chemotherapy	164 (28.1)	31 (19.0)	0.025
Anti-infectious therapies			
Antibacterial therapy	452 (77.9)	137 (84.6)	0.083
Antifungal therapy	224 (38.4)	63 (38.7)	1.00
Antiviral therapy	241 (41.3)	81 (49.7)	0.067
Life-supporting interventions			
Noninvasive ventilation	173 (29.6)	65 (39.9)	0.017
Mechanical ventilation	263 (45.0)	88 (54.0)	0.053
Vasoactive drugs	284 (48.6)	86 (52.8)	0.4
Renal replacement therapy	149 (26.2)	45 (28.1)	0.7
Outcomes			
Hospital-acquired infection	96 (16.4)	30 (18.4)	0.64
ICU mortality	138 (23.6)	56 (34.4)	0.008
Hospital mortality	210 (36.0)	67 (41.1)	0.27
Overall mortality on day 90	248 (45.2)	73 (47.4)	0.69

AJRCCM Articles in Press. Published on 19-September-2018 as 10.1164/rccm.201804-0681OC

Table 4

N (%) - median [IQR]	Negative ePlex® N=333	Positive ePlex® N=114	*P* value
Clinical presentation on day 1			
Respiratory rate (/min)	32 [26;38]	32 [26;36]	0.68
Temperature (°C)	38.2 [37.3;39.1]	38.3 [37.4;39.2]	0.31
Cough	122 (36.6)	46 (40.4)	0.64
Purulent sputum	34 (10.4)	7 (6.3)	0.28
Myalgia	10 (3.1)	3 (2.7)	1.00
Rhinorrhea	4 (1.2)	3 (2.7)	0.52
Chest pain	43 (13.1)	8 (7.2)	0.13
Maximal oxygen flow (L/min)	8 [4;15]	9 [4;15]	0.91
PaO_2/FiO_2 in patients requiring mechanical ventilation	150 [96;258]	118 [71;206]	0.052
SOFA >7	139 (44.4)	58 (52.3)	0.19
Chest radiography pattern			
Normal	32 (9.9)	20 (17.9)	0.039
Alveolar condensations	203 (61.0)	63 (55.3)	0.34
Interstitial opacities	89 (27.6)	39 (34.8)	0.18
Nodular opacities	15 (4.6)	1 (0.9)	0.13
Pleural effusion	86 (26.6)	19 (17.0)	0.054
Computed tomography			
Normal	5 (1.5)	2 (1.8)	1
Alveolar condensations	77 (23.1)	23 (20.2)	0.6
Ground-glass opacities	53 (15.9)	18 (15.8)	1
Nodular patterns	36 (10.8)	14 (12.3)	0.8
Pleural effusion	82 (24.8)	14 (12.4)	0.009
Etiologies of pulmonary involvement			
Infectious			<0.0001
Clinically documented bacterial infections	52 (15.6)	9 (7.9)	
Microbiologically documented bacterial infections	55 (16.5)	22 (19.3)	
Opportunistic infections			
Invasive pulmonary aspergillosis	13 (3.9)	11 (9.7)	
Pneumocystis jirovecii infections	10 (3)	5 (4.4)	
Noninfectious lung involvement			
Cardiac pulmonary edema	39 (11.7)	14 (12.3)	
Undetermined	48 (14.4)	12 (10.5)	
Interventions and outcomes			
Anti-infectious therapies			
Antibacterial therapy	282 (85.5)	106 (93.0)	0.054
Antifungal therapy	151 (45.4)	50 (43.9)	0.87
Antiviral therapy	151 (45.4)	64 (56.1)	0.06
Life-supporting interventions			
Noninvasive ventilation	144 (43.2)	57 (50)	0.25
Mechanical ventilation	173 (52.0)	67 (58.8)	0.25
Vasoactive drugs	174 (52.3)	70 (61.4)	0.11
Renal replacement therapy	78 (24.2)	32 (28.6)	0.42
Outcomes			
Hospital-acquired infection	59 (17.7)	21 (18.4)	0.98
ICU mortality	81 (24.3)	46 (40.4)	0.002
Hospital mortality	123 (36.9)	52 (45.6)	0.13
Overall mortality on day 90	146 (46.8)	55 (51.4)	0.48

Figure 1.



AJRCCM Articles in Press. Published on 19-September-2018 as 10.1164/rccm.201804-0681OC

Figure 2.

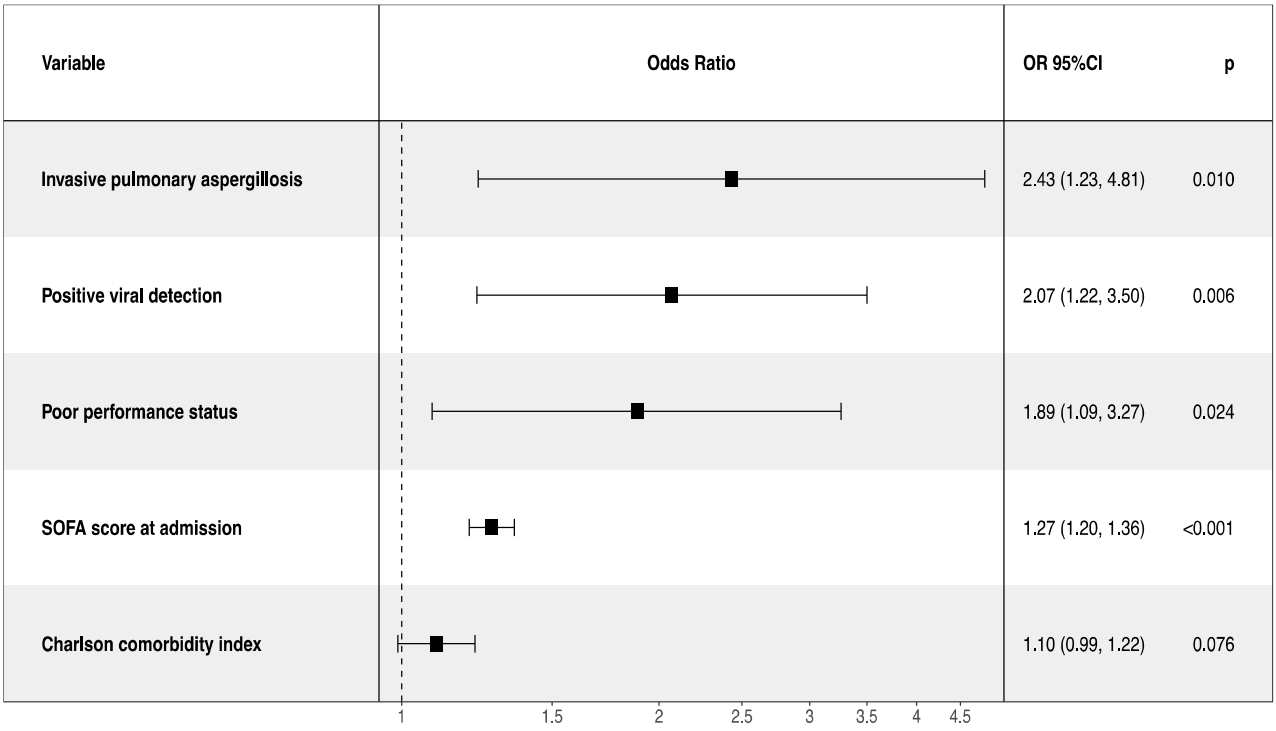
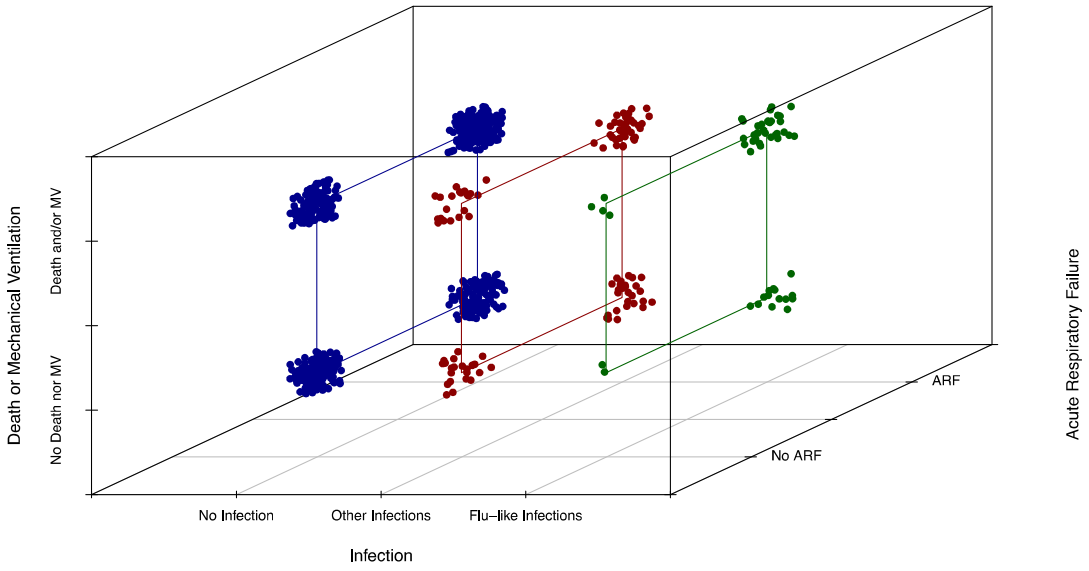


Figure 3.



AJRCCM Articles in Press. Published on 19-September-2018 as 10.1164/rccm.201804-0681OC

Supplementary data

Clinical Significance of Upper Airway Virus Detection in Critically Ill Hematology Patients

Jérôme Legoff[1]*, Noémie Zucman[2]*, Virginie Lemiale[2], Djamel Mokart[3], Frédéric Pène[4], Jérôme Lambert[5], Achille Kouatchet[6], Alexandre Demoule[7], François Vincent[8], Martine Nyunga[9], Fabrice Bruneel[10], Adrien Contejean[2], Séverine Mercier-Delarue[1], Antoine Rabbat[4], Christine Lebert[11], Pierre Perez[12], Anne-Pascale Meert[13], Dominique Benoit[14], Carole Schwebel[15], Mercé Jourdain[16], Michael Darmon[2], Matthieu Resche-Rigon[5], Elie Azoulay[2]

* Jérôme LeGoff and Noémie Zucman contributed equally to this work.

[1]AP-HP, Virology Department, Saint Louis Teaching Hospital, Paris, FRANCE
[2]AP-HP, ICU, Saint Louis Teaching Hospital, Paris, FRANCE
[3]ICU, Paoli Calmette Institute, Marseille, FRANCE
[4]AP-HP, ICU, Cochin Teaching Hospital, Paris, France
[5]AP-HP, Statistics Department, Saint Louis Teaching Hospital, Paris, FRANCE
[6]ICU, Angers Teaching Hospital, Angers, FRANCE
[7] AP-HP, ICU, Pitié Salpêtrière Teaching Hospital, Paris, FRANCE
[8]AP-HP, ICU Avicennes Teaching Hospital, Bobigny, FRANCE
[9]ICU, Roubaix Regional Hospital Center, Roubaix, FRANCE
[10]ICU, Versailles Teaching Hospital, Le Chesnay, FRANCE
[11]ICU, District Hospital Center, La Roche sur Yon, FRANCE
[12]ICU, Brabois Teaching Hospital, Nancy, FRANCE
[13]ICU, Jules Bordet Institute, Brussels, BELGIUM
[14]ICU, Ghent University Hospital, Ghent, BELGIUM
[15]ICU, Grenoble Teaching Hospital, Grenoble, FRANCE
[16]ICU, Regional Teaching Hospital, Lille, FRANCE

Corresponding author: Elie Azoulay, Medical Intensive Care Unit, Hôpital Saint-Louis, 1 avenue Claude Vellefaux, 75010 Paris, FRANCE

E-mail: elie.azoulay@aphp.fr

Methods

Step wise model.

In the step wise model, we considered variables already known as prognostic factors in the princeps study based on this cohort (Azoulay, E., Mokart, D., Pène, F., Lambert, J., Kouatchet, A., Mayaux, J., Vincent, F., Nyunga, M., Bruneel, F., Laisne, L.M. and Rabbat, A., 2013. Outcomes of critically ill patients with hematologic malignancies: prospective multicenter data from France and Belgium—a groupe de recherche respiratoire en reanimation onco-hematologique study. Journal of Clinical Oncology, 31(22), pp.2810-2818). In practice we considered the following factors: poor ECOG performance status (`Performance status`), Charlson comorbidity index (`Charlson`), allogeneic hematopoietic stem cell transplant [HSCT] recipient (`Allograft`), complete or partial remission (`Remission`), time from hospital to ICU admission <24 h (`Time to ICU <24h`), SOFA score (`SOFA`), organ infiltration by the malignancy (`Organ infiltration`), and invasive pulmonary aspergillosis (`Aspergillus`). Admission after cardiac arrest was not considered due to the too small number of patients with admission after cardiac arrest in this subcohort. Underlying malignancies was added in initial model as immunocompromised patients are more susceptible for severe viral infection (`Underlying malignancies`). Nevertheless, this variable was not kept by the variable selection procedure. Variable selection was a backward stepwise procedure (backward and forward) with a stopping rule based on the Akaike criterion. Please find the complete procedure below, that we have added in supplementary data.

AJRCCM Articles in Press. Published on 19-September-2018 as 10.1164/rccm.201804-0681OC

The linear predictor considered for the initial logistic model was the following:

ICU death ~ Underlying malignancies + Performance status + Charlson + Allograft + Remission + Time to ICU <24h + SOFA + Organ infiltration + Aspergillus + Viral detection

The complete selection process is described below. For all model the Akaike Criteria (AIC) is given:

Start: AIC=420.19

ICU death ~ Underlying malignancies + Performance status + Charlson + Allograft + Remission + Time to ICU <24h + SOFA + Organ infiltration + Aspergillus + Viral detection

	Df	Deviance	AIC
- Underlying malignancies	2	398.13	418.13
- Allograft	1	396.20	418.20
- Organ infiltration	1	396.32	418.32
- Time to ICU <24h	1	396.41	418.41
- Remission	1	396.96	418.96
<none>		396.19	420.19
- Charlson	1	398.73	420.73
- Performance status	1	399.37	421.37
- Aspergillus	1	401.12	423.12
- Viral detection	1	402.08	424.08
- SOFA	1	471.05	493.05

Step: AIC=418.13

ICU death ~ Performance status + Charlson + Allograft + Remission + Time to ICU <24h + SOFA + Organ infiltration + Aspergillus + Viral detection

	Df	Deviance	AIC
- Allograft	1	398.19	416.19
- Organ infiltration	1	398.34	416.34
- Time to ICU <24h	1	398.36	416.36
- Remission	1	398.60	416.60
<none>		398.13	418.13
- Charlson	1	401.20	419.20
- Performance status	1	401.42	419.42
- Aspergillus	1	403.12	421.12
- Viral detection	1	403.92	421.92
- SOFA	1	472.52	490.52

Step: AIC=416.19

```
ICU death ~ Performance status + Charlson + Remission + Time to ICU
<24h + SOFA + Organ infiltration + Aspergillus + Viral detection
                                Df      Deviance    AIC
- Time to ICU <24h              1       398.41      414.41
- Organ infiltration            1       398.41      414.41
- Remission                     1       398.81      414.81
<none>                                  398.19      416.19
- Performance status            1       401.42      417.42
- Charlson                      1       401.71      417.71
- Aspergillus                   1       403.13      419.13
- Viral detection               1       403.92      419.92
- SOFA                          1       472.53      488.53

Step:  AIC=414.41
ICU death ~ Performance status + Charlson + Remission + SOFA + Organ
infiltration + Aspergillus + Viral detection
                                Df      Deviance    AIC
- Organ infiltration            1       398.59      412.59
- Remission                     1       399.05      413.05
<none>                                  398.41      414.41
- Charlson                      1       401.79      415.79
- Performance status            1       401.92      415.92
- Aspergillus                   1       403.44      417.44
- Viral detection               1       404.02      418.02
- SOFA                          1       472.61      486.61

Step:  AIC=412.59
Icu death ~ Performance status + Charlson + Remission + SOFA +
Aspergillus + Viral detection
                                Df      Deviance    AIC
- Remission                     1       399.27      411.27
<none>                                  398.59      412.59
- Charlson                      1       401.98      413.98
- Performance status            1       402.06      414.06
- Aspergillus                   1       403.50      415.50
- Viral detection               1       404.09      416.09
- SOFA                          1       472.69      484.69

Step:  AIC=411.27
Icu death ~ Performance status + Charlson + SOFA + Aspergillus +
Viral detection
                                Df      Deviance    AIC
<none>                                  399.27      411.27
- Performance status            1       402.75      412.75
```

AJRCCM Articles in Press. Published on 19-September-2018 as 10.1164/rccm.201804-0681OC

- Charlson	1	402.98	412.98
- Viral detection	1	404.26	414.26
- Aspergillus	1	404.43	414.43
- SOFA	1	473.71	483.71

The coefficient obtained with the final model is:

Coefficients:

(Intercept)	-3.7521
Performance status	0.5387
Charlson	0.1040
SOFA	0.2411
Aspergillus	0.8552
Viral detection Pos	0.6199

Degrees of Freedom: 419 Total (i.e. Null); 414 Residual

Null Deviance: 504.4

Residual Deviance: 399.3 AIC: 411.3

Results

Table S1. Frequencies of Influenza virus, Respiratory Syncitial Virus, and Rhinovirus according to the season.

	Influenza		RSV		Rhinovirus	
	Neg	Pos	Neg	Pos	Neg	Pos
Winter	247	16	247	16	234	29
Spring	241	3	243	1	223	21
Summer	106	0	106	0	86	20
Autumn	133	1	133	1	112	22
p value[a]		0.0007		<0.0001		0.02

[a] Fisher exact test

Table S2. Pathogens detected by ePlex in in patients with no respiratory.

Pathogen	Number of positive samples
Adenovirus	1
Adenovirus-CoronavirusOC43	1
Coronavirus229E	4
CoronavirusHKU1	1
CoronavirusNL63	1
CoronavirusOC43	1
CoronavirusOC43-Rhinovirus	1
Legionnella pneumophila-CornavirusOC43-Rhinovirus	1
Rhinovirus	12

Table S3. ICU mortality in patients with no respiratory symptoms with a positive ePlex result or a negative ePlex result.

ICU mortality	ePlex negative	ePlex positive
No	131	19
Yes	31	4
p value[a]	1	

[a] Fisher exact test

AJRCCM Articles in Press. Published on 19-September-2018 as 10.1164/rccm.201804-0681OC

Table S4. Multivariable analysis for factors independently associated with ICU mortality among all patients admitted to ICU with a ePlex test.

	OR	CI95 Low	CI95 High	p.value
Invasive pulmonary aspergillosis	2.36	1.23	4.51	0.0094
Positive Eplex	1.64	1.06	2.54	0.026
Poor performance status	2.30	1.50	3.53	0.00014
SOFA score at admission	1.28	1.22	1.34	3.2e-23
Charlson comorbidity index	1.14	1.05	1.24	0.0015

Table S5. Impact of steroid therapy during ICU stay in patients with ARF. Multivariable analysis for factors independently associated with ICU mortality among patients with ARF. When adjusted on factors associated with mortality, steroid therapy was not significantly associated with a worse prognosis.

	OR	CI95 low	CI95 high	p.value
Invasive pulmonary aspergillosis	2.42	1.23	4.79	0.011
Positive Eplex	2.05	1.21	3.48	0.0075
Poor performance status	1.85	1.06	3.22	0.029
SOFA score at admission	1.28	1.20	1.36	6.2e-15
Charlson comorbidity index	1.10	0.99	1.22	0.072
Steroid therapy during ICU stay	**1.32**	**0.81**	**2.15**	**0.27**

Table S6.

Impact of steroid therapy during ICU stay in patients with ARF and a positive viral detection. Multivariable analysis for factors independently associated with ICU mortality among patients with ARF. When adjusted on factors associated with mortality, steroid therapy was not significantly associated with a worse prognosis.

	OR	CI95 low	CI95 high	p.value
Invasive pulmonary aspergillosis	7.66	2.15	33.34	0.0031
Poor performance status	1.43	0.52	3.91	0.48
SOFA score at admission	1.32	1.17	1.52	3.3e-05
Charlson comorbidity index	1.04	0.85	1.29	0.69
Steroid therapy during ICU stay	**1.03**	**0.41**	**2.63**	**0.95**

Figure S1. Daily SOFA score according to the detection of any respiratory virus (A) or of influenza, parainfluenza, and respiratory syncytial viruses (B).

Abbreviations: SOFA, Sequential Organ Failure Assessment; PIV, parainfluenza virus; RSV, respiratory syncytial virus; D, day