Respiratory Influenza viral load as a marker of poor prognosis in patients with severe symptoms

Charlotte Pronier, Arnaud Gacouin, Gisèle Lagathu, Yves Le Tulzo, Jean-Marc Tadié, V. Thibault

Respiratory Influenza viral load as a marker of poor prognosis in patients with severe symptoms

Charlotte Pronier[1], Arnaud Gacouin[2], Gisèle Lagathu[1], Yves Le Tulzo[2], Jean-Marc Tadié[2], Vincent Thibault[1]

[1] Virology Univ Rennes, CHU Rennes, Inserm, EHESP, Irset (Institut de recherche en santé, environnement et travail) - UMR_S 1085, F-35000 Rennes, France

[2] Maladies infectieuses et réanimation médicale, CHU Rennes, Inserm-CIC 1414, Biosit, F-35043 Rennes, France

Running title: Influenza viral load as a prognostic factor

Abstract word count: 246

Text word count: 2500

Corresponding author:

Vincent Thibault: vincent.thibault@chu-rennes.fr

Pr Vincent THIBAULT, Virology, CHU Pontchaillou, 2 rue Henri Le Guilloux, 35000 Rennes, FRANCE

tel: +33 299 289 059

fax: +33 299 284 159

Highlights

- Respiratory samples collected from ICU hospitalized patients are heterogeneous.
- The virus-over-cell ratio in respiratory samples allows some standardization of influenza virus load measurement.
- Standardized respiratory viral load could help predicting poor outcome in patients with acute respiratory distress syndrome.

Abstract

Background The link between influenza virus (IV) viral load (VL) in respiratory samples and disease severity is not clearly established. This study was designed to assess IV-VL in respiratory samples from flu patients admitted to intensive care unit (ICU).

Methods Patients admitted to ICU for IV infection, as documented by RT-PCR, with respiratory failure were included in the study during 5 flu-seasons (2014-2018). Routine ICU management parameters were recorded. Real-time amplification Ct-values for IV and cell GAPDH gene were measured in each respiratory sample collected at ICU admission.

Results Among 105 included patients, 59 (56.1%) presented an acute respiratory distress syndrome (ARDS). The overall mortality was 21%. IV-load assessed by amplification Ct-values and virus-over-cell ratio (expressed as $\log_{10}$) in each respiratory sample ranged from 20 to 40 and -5.2 to 3.7, respectively, and did not differ according to the type of sample and IV-A or -B type. Cell richness was higher in samples from ARDS patients compared to non-ARDS ($p=0.0003$) but no difference was noted for IV

Ct-values. In ARDS-patients, IV Ct-values (p=0.020) and the virus-per-cell ratio (p=0.038) were significantly higher in sample from patients who eventually died compared to those who survived. These 2 parameters remain independently associated with mortality with an odd-ratio of 1.21 and 2.19, respectively (p<0.05).

Conclusions While IV-VL does not seem to predict disease evolution in ICU flu-patients, normalized measurement of IV-VL in respiratory samples could be useful in ARDS patients to identify patients at higher risk of mortality.

Key words: real-time RT-PCR – Cell number – virus shedding – quantification

INTRODUCTION

Influenza A and B viruses (IV) are respiratory pathogens that cause regular seasonal epidemics, with every year thousands of death in the world [1,2]. Viral infection of the lower respiratory tract may exacerbate a chronic underlying lung disease, may cause pneumonia or acute respiratory distress syndrome (ARDS), all potentially leading to respiratory failure and mechanical ventilation (MV) requirement. Pathogenesis of infections with IV has been extensively described [3–5]. Disease severity and ARDS evolution could be explained by differences in epithelial-endothelial cell damage, alveolar epithelial cells involvement, immune cell recruitment, cell apoptosis, or cytokine production by endothelial and epithelial cells [6]. Disease severity is also linked to the epidemic viral strain as demonstrated for the pandemic 2009 A-H1N1 (H1N1pdm09) virus [3,5,7]. Diagnosis of IV infection relies nowadays mostly on genome detection through molecular amplification. Diagnosis accuracy largely depends on the sample collection quality. In the ICU setting, IV testing is done on nasopharyngeal swabs or nasal washes but also on tracheal aspirates or

bronchoalveolar lavage (BAL) especially in those receiving MV [8]. Because collection modes are different, samples are heterogeneous and comparable determination of IV loads is difficult in real-life settings. In clinical trials, quantification of IV load by RT-PCR has been used to assess the efficacy of antiviral therapies but standardization of nasopharyngeal sampling from all investigators was required, allowing more accurate viral load assessments [9–11]. Despite an absence of standardization, some data suggest a relationship between IV load and clinical outcomes including severity of symptoms, hospital length of stay, and protracted shedding [12]. Higher viral loads in patients who died from influenza than in those who survived was also reported for influenza A-H5N1 virus but such relationship seems less obvious with A-H1N1pdm09, A-H3N2, and B viruses [13]. A recent study from Granados et al. compared different IV loads according to the severity of symptoms and also failed to find a robust association between respiratory IV-A viral loads and severity [14]. Overall, it seems difficult to find a universal viral load threshold that would allow classifying an infection as severe or mild as it is highly impacted by the delay from the first symptoms and the patient's age [15]. The lack of viral load quantification standardization due to sample heterogeneity and technical diversity remains a true challenge.

We performed a retrospective study to assess the respiratory influenza viral load measured at admission to the ICU with the aim to determine whether high viral load could be associated with ICU outcomes, with a focus on patients presenting with an ARDS. To accurately compare viral load between heterogeneous respiratory samples, the amount of virus was determined against the total amount of cells.

METHODS

Patients and setting

This retrospective study was performed in a 21-bed ICU admitting mostly medical patients. Patients were identified through Rennes University Hospital ICU and virology laboratory databases. The study was approved by the hospital's ethics committee (N° 16.117). All patients aged over 18 years admitted to ICU between February 1, 2014 and March 30, 2018, for respiratory failure and with a documented positive respiratory sample for IV infection as assessed by RT-PCR detection were included in the study. Patients were classified according to the occurrence or not of ARDS as described in the supplementary file.

Data collection

Baseline characteristics of patients, severity score, ventilating support received during the ICU stay, and results of IV real-time RT-PCR Cycle threshold (Ct) values were collected prospectively, extracted from medical records through a standardized questionnaire and analyzed retrospectively. Collected biological data are provided in the supplementary file.

Samples and PCR methods

Respiratory samples were collected in universal transport medium. Up to January 2016, influenza viruses were detected using Argene (bioMérieux, Marcilly, France) and after this date, Seegene Allplex Respiratory panel (Eurobio, Les Ulis, France) became the routine method. Internal results and a recent publication indicate a good correlation between both techniques for the detection of influenza viruses [16]. The population tested with Argene (n=32) and Seegene (n=86) was not different for the tested parameters and the viral load and virus-over-cell ratios were not significantly different.

Retrospectively, on -80°C stored frozen samples, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene amount was quantified in each sample to normalize

for the number of cells contained in respiratory samples as previously described [17]. Cell DNA quantification was done against a standard curve based on standardized genomic DNA (Roche, Meylan, France). By using IV and GAPDH Ct values, the relative amount of virus over cellular DNA was calculated as proposed by Livak and Schmittgen and further described in the supplementary file [18].

Study endpoints

The main objectives were to assess IV load at admission and to determine whether viral load at admission was associated with ICU outcome in ARDS and non-ARDS patients.

Statistical analysis

Data are expressed as percentages, medians and interquartile ranges (IQR). As appropriate, qualitative data were compared using Chi-square test or Fisher exact test while quantitative data were compared using Mann-Whitney or Kruskal-Wallis test for comparisons between three groups or more. We used a logistic regression analysis to identify variables independently associated with mortality in the ICU as described in the supplementary file.

RESULTS

Patients

During the study period, 118 patients were positive for influenza infection as detected by IV molecular detection on respiratory samples obtained at admission to the ICU (Figure 1 and table 1). Type A IV was found for 82% (n=97) of patients. Fifty-five percent (n=65) of patients presented ARDS criteria. Among ARDS patients, all received neuromuscular blocking agents, 8 (14%) received iNO, 6 (10%) ECMO, and

29 (49%) prone positioning. Among non-ARDS patients, 22 patients (48%) received invasive MV, 5 (10%) received solely noninvasive ventilation (NIV), 6 (13%) only high-flow nasal oxygen therapy, and 13 (29%) standard oxygen therapy. Mortality for the entire cohort was 21% (n=25). As expected, patients with ARDS had statistically more often BAL than nasopharyngeal swab compared to non-ARDS patients (p=0.0017).

Results for RT-PCR and viral load assessment

Results for RT-PCR on respiratory samples are shown in table 2. Due to volume shortage, 13 samples (11%) could not be retrospectively tested for cell DNA content and were not included in the final analysis performed on 105 samples (Fig. 1). IV Ct values ranged from 20 to 40 and did not differ significantly according to the type of sample, endotracheal aspirates (n=70), BAL (n=22) or nasopharyngeal aspirates (n=13). Sample cellular richness, as assessed by GAPDH-DNA measurement, was quite broad ranging from 20 to 39 Ct, corresponding to approximately 10^2 to 10^7 cells/mL of sample and was independent from the sample type (Table 2). Noteworthy, the relative amount of detected virus-per-cell copy number was equal to 1 (virtually representing 1 virus per cell) in BAL and higher than in other samples (0.45 and 0.20 for nasopharyngeal and endotracheal aspirates, respectively). There was no correlation between viral load and cellular richness of the samples and the relative amount of virus-over-cell, expressed as log10 ranged from -5.2 to 3.7. A good correlation was observed between the absolute virus copy number and the virus-over-cell ratio (Spearman's -0.876) (figure 2).

When considering the entire population, there was a tendency (p=0.07) for a higher viral load in patients who eventually died, compared to those who survived, but no association with any other studied parameters such as the length of stay or additional

treatments was found. Cellular sample richness was higher for ARDS compared to non-ARDS patients, with a mean difference in Ct value of 2, corresponding to approximately 4 times more cells in ARDS patient samples (p=0.001).

As cellular richness was slightly less important in nasopharyngeal samples and to exclude a possible sampling bias, the same analysis was performed without considering nasopharyngeal samples and led to the same findings (Table S1 provided as supplementary file).

Mortality in the ICU

Overall mortality was 21% (n=25), 31% (n=20) for ARDS, and 9% (n=5) for non-ARDS patients (Table 1). Deaths in non-ARDS patients were attributed to preexisting comorbidities. Bilateral injury on chest-ray was associated with mortality after comparison on the entire population (p=0.02). SAPS II score calculated on admission differed significantly (p<0.001) between deceased patients and survivors after comparison on the entire population but also on each group of ARDS (p=0.004) and non-ARDS (p=0.01) patients. No other studied parameters varied significantly between dead and alive patients in the non-ARDS group. Noteworthy, mortality was lower (p=0.03) in ARDS-patients who received oseltamivir treatment (n=60) on the first day in ICU.

Viral load results available for 105 patients were compared between deceased patients and survivors over the entire population and in the subgroups of ARDS and non-ARDS patients (Table 3). Considering the entire population, IV Ct-values were significantly lower, and therefore viral loads higher, in patients who died compared to those who survived (p=0.03). However, when IV Ct-values were normalized to cell DNA content, there was only a tendency (p=0.07) for a higher virus-over-cell ratio in patients who

eventually died (Figure 3). The same conclusions were reached when considering only lower respiratory samples (n=92) (Table S2 provided as supplementary file).

Subgroup of ARDS patients

When considering only ARDS-patients (n=59), IV loads but also virus-over-cell ratios were significantly higher, p=0.01 and p=0.02, respectively, in patients who ultimately died (n=19) compared to those who survived. When considering the relative virus-over-cell index, patients who died had more than 5 times virus-per-cell than those who survived (1 vs. 0.17, p=0.02). These differences were not observed in non-ARDS patients (n=46) although the number were limited (n=5 deaths in this subgroup) to draw firm conclusions. Restraining the analysis merely on lower respiratory samples (n=57) led also to the same observation (Table S2).

The variables included in adjusted analyses included age, oseltamivir treatment started on the first day at ICU admission, SAPS-II score (table 2) in addition to IV Ct-values. Adjusted analyses showed that IV Ct-value (1-point decrement, OR = 1.21, 95% CI 1.04-1.40, p= 0.009), log virus-over-cell ratio difference (1-point increment, OR = 2.19, 95% CI 1.23-3.91, p= 0.008), remained independently associated with mortality in the ICU.

DISCUSSION

In this retrospective study performed on patients admitted to the ICU with a diagnosis of acute respiratory failure due to influenza virus, higher viral load as assessed either by RT-PCR Ct-values or by normalized virus-over-cell ratios on admission to the ICU was associated with mortality. More specifically, this association was highly significant in patients presenting with ARDS at ICU admission.

We hypothesized that high IV loads could be associated with poor prognosis in ICU patients admitted with respiratory failure, because they could reflect high replication level and large amount of infected cells in the lung. Indeed, viral load has been shown to be predictive of different clinical outcomes in respiratory tract infections [12,14,15,19,20]. Persistent detection of virus by RT-PCR is often considered to determine whether combination of new antivirals might be more effective than oseltamivir alone in the treatment of flu and viral load reduction was used to assess the efficacy of baloxavir marboxil in the treatment of patients with uncomplicated influenza [9,10]. To our knowledge, few studies have previously assessed the usefulness of respiratory viral load measurement in the prognosis of flu patients. It has been reported that in patients with avian influenza-A H5N1-virus infection, non-survivors had significantly higher viral load on pharyngeal and nasal swabs than survivors [13]. Clark et al. also demonstrated that in patients with influenza-induced acute respiratory illness, as in patients with non-influenza viral-induced acute respiratory illness, high viral loads were strongly associated with a longer duration of hospitalization but not with hospital mortality [12].

In our study, high viral load on admission appears associated with bad prognosis in ARDS but not in non-ARDS patients, while viral load did not differ significantly between the two groups of patients. Influenza-mediated upper and lower airway damages, lung injury and pneumonia certainly result from viral pathogenicity but also from the host response [21]. Immunologic mechanisms involved in influenza-induced ARDS, including cytokines production, alterations in neutrophils and macrophages functions, or apoptosis of epithelial cells differ from those noted in non-influenza ARDS and from those present in patients without ARDS [5–7]. Clark et al suggested that clinical illness severity following respiratory viral infection could be predicted by viral load magnitude,

kinetics and persistence [12]. Our results could suggest that in patients with acute respiratory failure severe enough to require ICU admission, mortality appears more associated with a great inoculum of influenza virus than with an excessive immune response. Reduction of viral load through antiviral treatment might therefore be a desirable goal, particularly for ARDS patients. Of note, oseltamivir treatment was associated with decreased mortality in ARDS but not in non-ARDS patients. Measurement of cellular DNA highlighted a higher cell content in samples from ARDS compared to non-ARDS patients. Unfortunately, this study was not designed to identify the type of recruited cells. Yet, this observation could set the path for qualitative studies looking at either transcriptomics or precise identification of recruited cells in such samples.

Reflecting our standard procedures, respiratory samples from our patients were heterogeneous due to upper or lower airway collection. Calculating a virus-over-cell ratio index is an attempt to normalize as much as possible the inherent variability resulting from sample heterogeneity. Although imperfect, this index based on virus and cellular DNA Ct-values through the validated "delta-Ct method" [$2^{-\Delta Ct}$], remains at least a homogeneous marker between patients [18]. This method provides a standardized measurement of the amount of virus-over-cell genome equivalent, independently of the sample quality. Using this approach, we demonstrate a high heterogeneity of sample cellular richness ranging from 100 to more than 10^7 cell/mL, in agreement with the recent work from Piralla et al. that nicely demonstrated the wide range of DNA amount in unselected nasal swabs from clinical practice [22]. Our findings would support the use of such standardized measurement to assess its relevance in virally induced lung pathologies.

Several assets of our study can be highlighted. First, it encompasses several flu seasons with the circulation of different strains; noteworthy, no difference of viral loads or virus-over-cell ratio was observed according to the viral strain type. The second is the relative homogeneity of the unselected population recruited in a single center with standardized procedures of care and stringent disease classification. The third is the standardization of respiratory samples by assessing their cellular DNA load and the measurement of viral loads through standardized and commercialized procedures. Importantly, all sub-analyses performed after removal of nasopharyngeal samples led to the same conclusions, strengthening our findings and eliminating a potential bias due to sample heterogeneity. Obviously, some limitations might be raised such as its retrospective nature and the relative limited number of patients, particularly when performing subgroup analyses. Unfortunately, an extensive validation of this proof of principle was not performed because of the lack of samples and resources. Complementary efforts should be done to confirm this preliminary set of data.

In conclusion, we report here the first study comparing standardized respiratory IV load at admission to the ICU, in patients with acute respiratory failure according to the development of ARDS or not. A high viral-over-cell content in patients with ARDS was associated with a poor prognosis. This measurement could help to identify patients at higher risk of mortality and justifying closer management and treatment. In patients without ARDS criteria, such link between IV load and clinical outcome or length of stay was not as robust. Our results suggest that viral load reduction through antiviral treatment might be an important goal to achieve, particularly in ARDS patients.

Credit-Author-Statement

Charlotte Pronier, Arnaud Gacouin, Gisèle Lagathu, Yves Le Tulzo, Jean-Marc Tadié, Vincent Thibault

- Conceptualisation: AG, GL, VT
- Methodology: AG, GL, VT
- Validation: CP, AG, GL, VT
- Formal analysis: AG, VT
- Investigation: CP, YLT, JMT, AG, GL, VT
- Resources: CP, AG, GL, VT
- Data Curation: AG, VT
- Writing - Original Draft: AG, VT
- Writing - Review & Editing: CP, AG, GL, VT
- Visualization: CP, YLT, JMT, AG, GL, VT
- Supervision: AG, VT

Acknowledgments

REFERENCES

[1] W. Fiers, M. De Filette, A. Birkett, S. Neirynck, W. Min Jou, A “universal” human influenza A vaccine, Virus Res. 103 (2004) 173–176. https://doi.org/10.1016/j.virusres.2004.02.030.

[2] GBD 2017 Influenza Collaborators, Mortality, morbidity, and hospitalisations due to influenza lower respiratory tract infections, 2017: an analysis for the Global Burden of Disease Study 2017, Lancet Respir. Med. 7 (2019) 69–89. https://doi.org/10.1016/S2213-2600(18)30496-X.

[3] W.-J. Shieh, D.M. Blau, A.M. Denison, M. Deleon-Carnes, P. Adem, J. Bhatnagar, J. Sumner, L. Liu, M. Patel, B. Batten, P. Greer, T. Jones, C. Smith, J. Bartlett, J. Montague, E. White, D. Rollin, R. Gao, C. Seales, H. Jost, M. Metcalfe, C.S. Goldsmith, C. Humphrey, A. Schmitz, C. Drew, C. Paddock, T.M. Uyeki, S.R. Zaki, 2009 pandemic influenza A (H1N1): pathology and pathogenesis of 100 fatal cases in the United States, Am. J. Pathol. 177 (2010) 166–175. https://doi.org/10.2353/ajpath.2010.100115.

[4] T. Mauad, L.A. Hajjar, G.D. Callegari, L.F.F. da Silva, D. Schout, F.R.B.G. Galas, V.A.F. Alves, D.M.A.C. Malheiros, J.O.C. Auler, A.F. Ferreira, M.R.L. Borsato, S.M. Bezerra, P.S. Gutierrez, E.T.E.G. Caldini, C.A. Pasqualucci, M. Dolhnikoff, P.H.N. Saldiva, Lung pathology in fatal novel human influenza A (H1N1) infection, Am. J. Respir. Crit. Care Med. 181 (2010) 72–79. https://doi.org/10.1164/rccm.200909-1420OC.

[5] K.R. Short, E.J.B.V. Kroeze, R.A.M. Fouchier, T. Kuiken, Pathogenesis of influenza-induced acute respiratory distress syndrome, Lancet Infect. Dis. 14 (2014) 57–69. https://doi.org/10.1016/S1473-3099(13)70286-X.

[6] J.V. Camp, C.B. Jonsson, A Role for Neutrophils in Viral Respiratory Disease, Front. Immunol. 8 (2017) 550. https://doi.org/10.3389/fimmu.2017.00550.

[7] S. Herold, C. Becker, K.M. Ridge, G.R.S. Budinger, Influenza virus-induced lung injury: pathogenesis and implications for treatment, Eur. Respir. J. 45 (2015) 1463–1478. https://doi.org/10.1183/09031936.00186214.

[8] T.M. Uyeki, H.H. Bernstein, J.S. Bradley, J.A. Englund, T.M. File, A.M. Fry, S. Gravenstein, F.G. Hayden, S.A. Harper, J.M. Hirshon, M.G. Ison, B.L. Johnston, S.L. Knight, A. McGeer, L.E. Riley, C.R. Wolfe, P.E. Alexander, A.T. Pavia, Clinical Practice Guidelines by the Infectious Diseases Society of America: 2018 Update on Diagnosis, Treatment, Chemoprophylaxis, and Institutional Outbreak Management of Seasonal Influenzaa, Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am. 68 (2019) e1–e47. https://doi.org/10.1093/cid/ciy866.

[9] J.H. Beigel, Y. Bao, J. Beeler, W. Manosuthi, A. Slandzicki, S.M. Dar, J. Panuto, R.L. Beasley, S. Perez-Patrigeon, G. Suwanpimolkul, M.H. Losso, N. McClure, D.R. Bozzolo, C. Myers, H.P. Holley, J. Hoopes, H.C. Lane, M.D. Hughes, R.T. Davey, IRC003 Study Team, Oseltamivir, amantadine, and ribavirin combination antiviral therapy versus oseltamivir monotherapy for the treatment of influenza: a multicentre, double-blind, randomised phase 2 trial, Lancet Infect. Dis. 17 (2017) 1255–1265. https://doi.org/10.1016/S1473-3099(17)30476-0.

[10] F.G. Hayden, N. Sugaya, N. Hirotsu, N. Lee, M.D. de Jong, A.C. Hurt, T. Ishida, H. Sekino, K. Yamada, S. Portsmouth, K. Kawaguchi, T. Shishido, M. Arai, K. Tsuchiya, T. Uehara, A. Watanabe, Baloxavir Marboxil Investigators Group, Baloxavir Marboxil for Uncomplicated Influenza in Adults and Adolescents, N. Engl. J. Med. 379 (2018) 913–923. https://doi.org/10.1056/NEJMoa1716197.

[11] J. Dunning, J.K. Baillie, B. Cao, F.G. Hayden, International Severe Acute Respiratory and Emerging Infection Consortium (ISARIC), Antiviral combinations for severe influenza, Lancet Infect. Dis. 14 (2014) 1259–1270. https://doi.org/10.1016/S1473-3099(14)70821-7.

[12] T.W. Clark, S. Ewings, M.-J. Medina, S. Batham, M.D. Curran, S. Parmar, K.G. Nicholson, Viral load is strongly associated with length of stay in adults hospitalised with viral acute respiratory illness, J. Infect. 73 (2016) 598–606. https://doi.org/10.1016/j.jinf.2016.09.001.

[13] M.D. de Jong, C.P. Simmons, T.T. Thanh, V.M. Hien, G.J.D. Smith, T.N.B. Chau, D.M. Hoang, N.V.V. Chau, T.H. Khanh, V.C. Dong, P.T. Qui, B.V. Cam, D.Q. Ha, Y. Guan, J.S.M. Peiris, N.T. Chinh, T.T. Hien, J. Farrar, Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia, Nat. Med. 12 (2006) 1203–1207. https://doi.org/10.1038/nm1477.

[14] A. Granados, A. Peci, A. McGeer, J.B. Gubbay, Influenza and rhinovirus viral load and disease severity in upper respiratory tract infections, J. Clin. Virol. 86 (2017) 14–19. https://doi.org/10.1016/j.jcv.2016.11.008.

[15] D.R. Feikin, W. Fu, D.E. Park, Q. Shi, M.M. Higdon, H.C. Baggett, W.A. Brooks, M. Deloria Knoll, L.L. Hammitt, S.R.C. Howie, K.L. Kotloff, O.S. Levine, S.A. Madhi, J.A.G. Scott, D.M. Thea, P.V. Adrian, M. Antonio, J.O. Awori, V.L. Baillie, A.N. DeLuca, A.J. Driscoll, B.E. Ebruke, D. Goswami, R.A. Karron, M. Li, S.C. Morpeth, J. Mwaba, J. Mwansa, C. Prosperi, P. Sawatwong, S.O. Sow, M.D. Tapia, T. Whistler, K. Zaman, S.L. Zeger, K.L. O' Brien, D.R. Murdoch, PERCH Study Group, Is Higher Viral Load in the Upper Respiratory Tract Associated With Severe Pneumonia? Findings From the PERCH Study, Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am. 64 (2017) S337–S346. https://doi.org/10.1093/cid/cix148.

[16] M. Chan, S.H. Koo, B. Jiang, P.Q. Lim, T.Y. Tan, Comparison of the Biofire FilmArray Respiratory Panel, Seegene AnyplexII RV16, and Argene for the detection of respiratory viruses, J. Clin. Virol. Off. Publ. Pan Am. Soc. Clin. Virol. 106 (2018) 13–17. https://doi.org/10.1016/j.jcv.2018.07.002.

[17] A.D. Medhurst, D.C. Harrison, S.J. Read, C.A. Campbell, M.J. Robbins, M.N. Pangalos, The use of TaqMan RT-PCR assays for semiquantitative analysis of gene expression in CNS tissues and disease models, J. Neurosci. Methods. 98 (2000) 9–20.

[18] K.J. Livak, T.D. Schmittgen, Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2−ΔΔCT Method, Methods. 25 (2001) 402–408. https://doi.org/10.1006/meth.2001.1262.

[19] J.A. Fuller, M.K. Njenga, G. Bigogo, B. Aura, M.O. Ope, L. Nderitu, L. Wakhule, D.D. Erdman, R.F. Breiman, D.R. Feikin, Association of the CT values of real-time PCR of viral upper respiratory tract infection with clinical severity, Kenya, J. Med. Virol. 85 (2013) 924–932. https://doi.org/10.1002/jmv.23455.

[20] P.-X. Lu, Y.-Y. Deng, G.-L. Yang, W.-L. Liu, Y.-X. Liu, H. Huang, Y.-X.J. Wang, Relationship between respiratory viral load and lung lesion severity: a study in 24 cases of pandemic H1N1 2009 influenza A pneumonia, J. Thorac. Dis. 4 (2012) 377–383. https://doi.org/10.3978/j.issn.2072-1439.2012.08.02.

[21] V.N. Petrova, C.A. Russell, The evolution of seasonal influenza viruses, Nat. Rev. Microbiol. 16 (2018) 60. https://doi.org/10.1038/nrmicro.2017.146.

[22] A. Piralla, F. Giardina, F. Rovida, G. Campanini, F. Baldanti, Cellular DNA quantification in respiratory samples for the normalization of viral load: a real need?, J. Clin. Virol. 107 (2018) 6–10. https://doi.org/10.1016/j.jcv.2018.07.010.

Table 1: Baseline characteristics of patients

	All patients N= 118			ARDS patients N= 65			Non-ARDS patients N= 53		
	Alive N= 93	Dead N= 25	P value	Alive N= 45	Dead N= 20	P value	Alive N= 48	Dead N= 5	P value
Age, years	60 (50-68)	66 (57-73)	0.13	61 (50-65)	66 (59-73)	0.07	59 (49-69)	66 (50-72)	0.82
Male gender, n (%)	55 (59)	14 (56)	0.77	25 (55)	10 (50)	0.68	30 (62)	4 (80)	0.64
SAPS II score, points	40 (29-56)	64 (44-73)	<0.001	46 (34-59)	64 (47-72)	0.004	35 (24-49)	64 (41-73)	0.01
Vaccination status, n (%)									
Vaccinated	15 (16)	4 (16)	0.26	4 (9)	2 (10)	0.26	11 (23)	2 (40)	0.51
Non vaccinated	70 (75)	16 (64)		38 (84)	14 (70)		32 (67)	2 (40)	
Unknown	8 (9)	5 (20)		3 (7)	4 (20)		5 (10)	1 (20)	
At least one factor associated with increased morbidity and mortality from influenza, n (%)*	76 (82)	23 (92)	0.19	37 (82)	18 (90)	0.41	39 (81)	5 (110)	0.57
Oseltamivir treatment started on the first day in ICU	87 (95)	21 (84)	0.13	44 (98)	16 (80)	0.03	43 (90)	5 (100)	>0.99
Bilateral injury on chest X-ray, (%)	69 (74)	24 (96)	0.02	45 (100)	20 (100)	>0.99	24 (50)	4 (80)	0.35
Associated bacteria, n (%)	38 (41)	11 (44)	0.78	19 (42)	9 (45)	0.84	19 (40)	2 (40)	>0.99
Neutrophils, 10^9/L	7.3 (4.0-11.3)	8.4 (4.3-10.7)	0.98	5.5 (3.3-10.6)	8.4 (4.1-11.9)	0.40	8.8 (6.2-11.9)	7.4 (5.1-9.6)	0.60
Lymphocytes, 10^9/L	0.54 (0.35-0.84)	0.66 (0.27-1.16)	0.12	0.46 (0.32-0.77)	0.49 (0.26-0.83)	0.39	0.72 (0.47-1.03)	1.33 (0.74-1.44)	0.88

Influenza virus, n (%)									
A	76 (78)	21 (88)	0.40	39 (87)	18 (90)	>0.99	34 (71)	4 (80)	>0.99
B	20 (22)	3 (12)		6 (13)	2 (10)		14 (80)	1 (20)	

*See text for definition; continuous variables are presented with (interquartile ranges)

Table 2: Results for RT-PCR values obtained from bronchoalveolar lavages and from nasopharyngeal and endotracheal aspirates

	All patients	Nasopharyngeal aspirates	Endotracheal aspirates	Bronchalveolar lavages	P value
	n= 105	n= 13	n= 70	n= 22	
Influenza virus, n (%)					0.12
H3N2 A	52 (50)	5 (38)	34 (48)	13 (59)	
H1N1pdm09 A	33 (31)	4 (31)	20 (29)	9 (41)	
B	20 (19)	4 (31)	16 (23)	0 (0)	
Cell DNA Ct-value (IQR)	25 (23-27)	26 (25-29)	25 (23-27)	25 (24-27)	0.52
IV Ct-value (IQR)	27 (22-34)	34 (28-35)	27 (22-34)	26 (22-29)	0.07
Virus-over-cell ratio (IQR)	0.222 (0.005-5.517)	0.451 (0.005-0.518)	0.204 (0.003-6.288)	1.032 (0.097-6.364)	0.08
$\log_{10}$ Virus/Cell (IQR)	- 0.661 (-2.332-0.575)	- 1.135 (-2.365- -0.490)	- 0.730 (-2.470-0.770)	0.005 (-1.010-0.800)	0.08

IQR, Interquartile Range

Table 3: Comparisons of CT values between patients who died and those who survived the ICU stay

	Alive	Dead	P value
All Patients	n= 81	n= 24	
Cell DNA Ct-value (IQR)	25 (24 - 27)	24 (23 - 26)	0.15
IV Ct-value (IQR)	28 (23 - 34)	25 (20 - 28)	0.03
Virus-over-cell ratio (IQR)	0.189 (0.004– 3.714)	1.129 (0.044– 26.278)	0.10
log_{10} Virus/Cell (IQR)	-0.722 (-2.446 – 0.570)	0.051 (-1.422 – 1.368)	0.07
ARDS Patients	n= 40	n= 19	
Cell DNA Ct-value (IQR)	24 (23 - 26)	24 (23 - 26)	0.71
IV Ct value (IQR)	28 (24 - 33)	24 (20 - 27)	0.01
Virus-over-cell ratio (IQR)	0.186 (0.003 – 0.698)	2.144 (0.100 – 32.297)	0.02
log_{10} Virus/Cell (IQR)	-0.730 (-2.475 – -0.160)	0.331 (-1.003 – 1.475)	0.02
Non-ARDS patients	n= 41	n= 5	
Cell DNA Ct-value (IQR)	27 (24 - 28)	25 (24 -28)	0.64
IV Ct-value (IQR)	29 (21 - 35)	31 (23 -35)	0.94
Virus-over-cell ratio (IQR)	0.227 (0.004 – 23.997)	0.021 (0.005 – 35.59)	0.76
log_{10} Virus/Cell (IQR)	-0.644 (-2.409 – 1.377)	-1.668 (-2.326 – 0.594)	0.94

IQR: Interquartile Range.

Virus-over-cell ratio virtually represents the amount of virus per detected cell in each sample

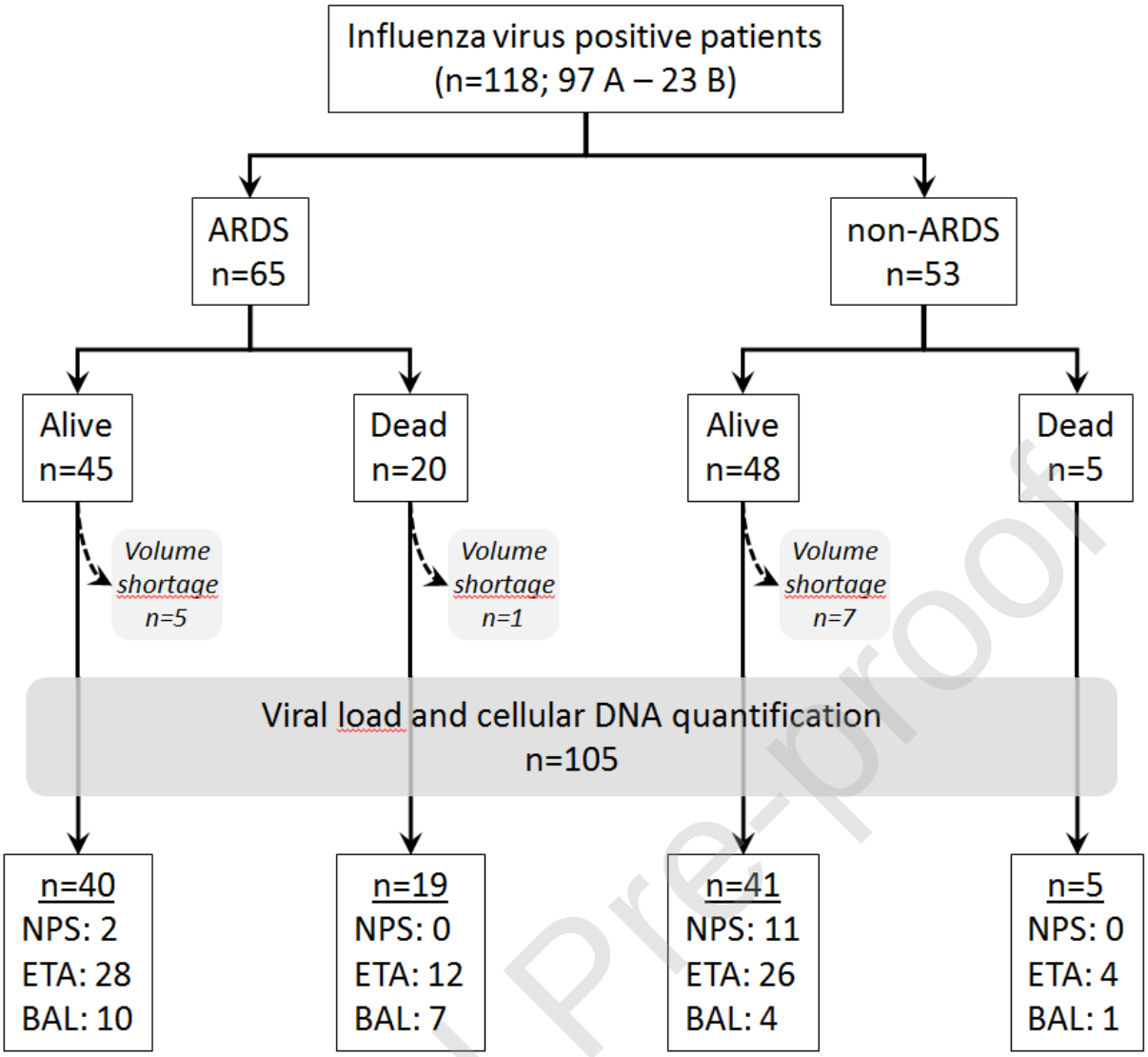

Figure 1: Sample disposal for the 118 patients presenting with influenza virus infection.

(NSP: nasopharyngeal swab; ETA: endotracheal aspirate; BAL: broncho-alveolar lavage)

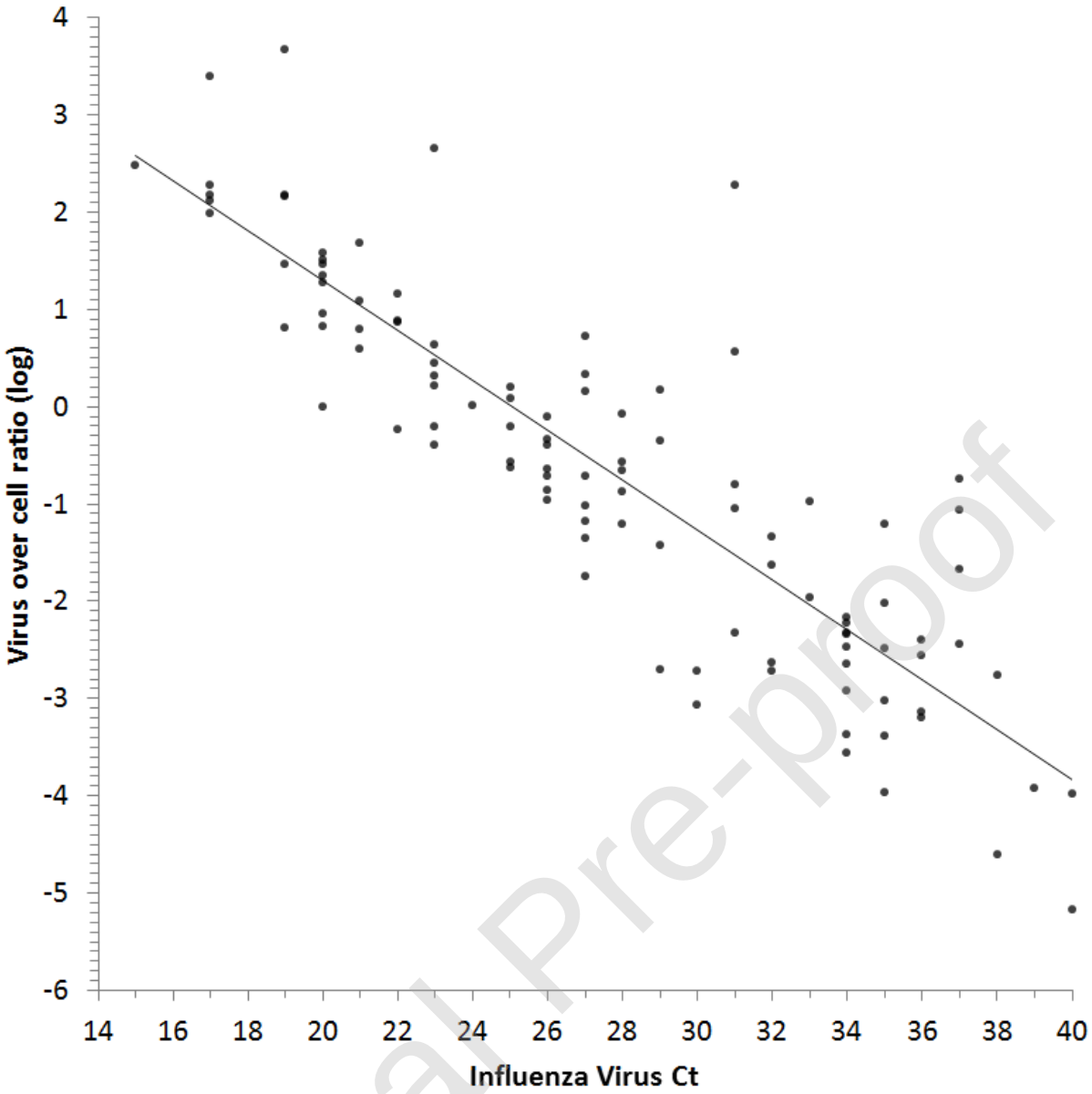

Figure 2: Correlation between Influenza virus Ct values and virus over cell ratio as expressed as log10.

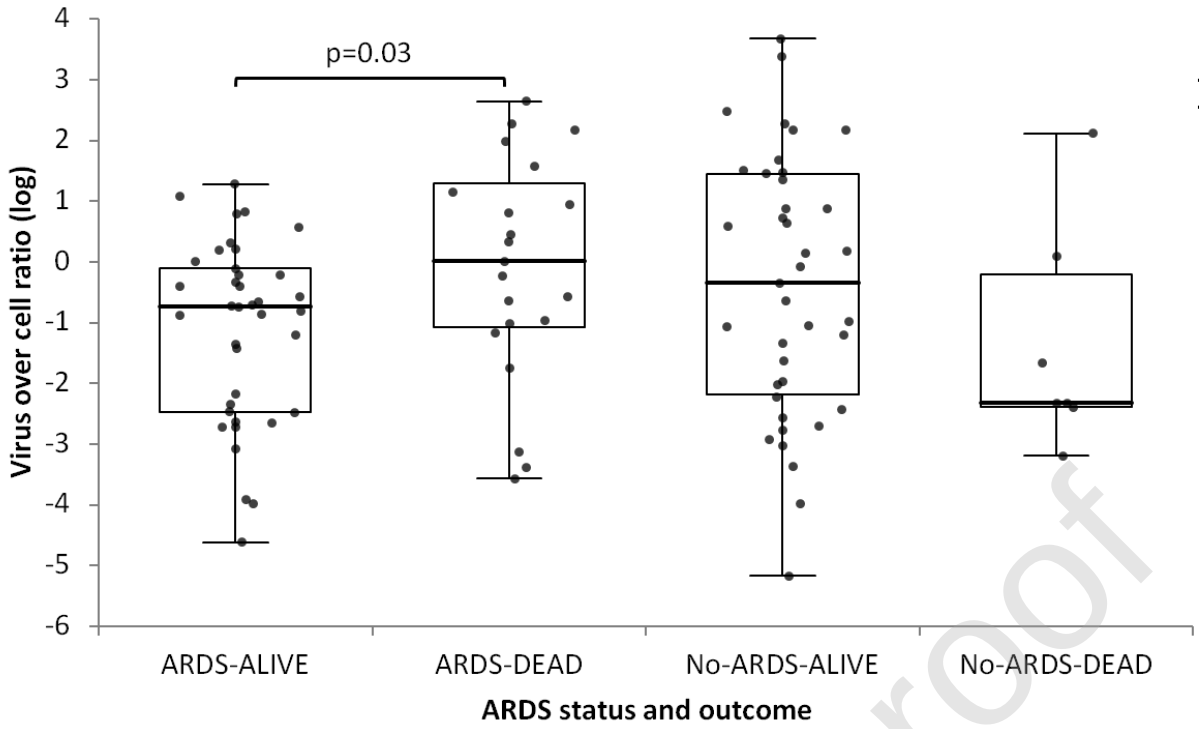

Figure 3: box-plot representing virus over cell ratio expressed as log according to patient's outcomes and ARDS.