Respiratory Torque Teno Virus load at Emergency department visit predicts intensive care unit admission of SARS-CoV-2 infected patients

Jerome LeGoff[1], Linda Feghoul[1], Amandine Caillault[1], Olivier Peyrony[2], Maud Salmona[1], Marie-Laure Nere[1], Constance Delaugerre [1], E. Azoulay[1], and Sylvie Chevret[3]

[1]Hopital Saint-Louis
[2]Assistance Publique - Hopitaux de Paris
[3]Centre de Recherche en Epidemiologie et Statistiques Sorbonne Paris Cite

August 8, 2023

Abstract

Background: Accurate prediction of SARS-CoV-2 severity remains a challenge. Torque Teno Virus (TTV), recognized as a surrogate marker for cellular immunity in solid organ transplant recipients, holds potential for assessing infection outcomes. **Objectives**: We investigated whether quantifying TTV in nasopharyngeal samples upon emergency ward (ED) admission could serve as an early predictor of SARS-CoV-2 severity. **Study design**: Retrospective single-center study in the ED of Saint-Louis Hospital in Paris, France. TTV DNA was quantified in nasopharyngeal swab samples collected for SARS-CoV-2 testing. **Results**: Among 295 SARS-CoV-2 infected patients, 92 returned home, 160 were admitted to medical wards, and 43 to the intensive care unit. Among 295 SARS-CoV-2 patients, 92 were discharged, 160 hospitalized, and 43 admitted to the intensive care unit (ICU). Elevated TTV loads were observed in ICU patients (Median: 3.02 log copies/mL, interquartile range [IQR]: 2.215-3.825), exceeding those in discharged (2.215, [0; 2.962]) or hospitalized patients (2.24, [0; 3.29]) (p=0.006). Multivariate analysis identified diabetes, obesity, hepatitis, fever, dyspnea, oxygen requirement, and TTV load as predictors of ICU admission. A 2.91 log $_{10}$ copies/mL TTV threshold independently predicted ICU admission. **Conclusion**: Nasopharyngeal TTV quantification in SARS-CoV-2 infected patients is linked to the likelihood of ICU admission and might reflect respiratory immunosuppression.

INTRODUCTION

SARS-CoV-2 infection is associated with a significant rate of severe disease requiring intensive care (1,2). While various risk factors have been identified (3), severe illness can affect anyone, the potential for severe illness extends to a broad range of individuals, and reliable individual biological markers to predict unfavorable outcomes remain lacking. The severity depends, in particular, on an inefficient and unbalanced immune response. The severity of the disease is notably influenced by an inadequate and imbalanced immune response. Firstly, an insufficient interferon response contributes to uncontrolled SARS-CoV-2 replication (4), and secondly, an overactive proinflammatory response results in damage to respiratory epithelial cells (5).

Anelloviridae, a family of non-enveloped single-stranded DNA viruses, persistently inhabit various compartments within infected hosts, constituting a significant component of the human virome (6). Torque teno virus (TTV), belonging to the Alphatorquevirus genus, ranks among the most prevalent anelloviruses in humans (7). TTV is not associated with any specific disease. However, its presence in blood is indicative of cellular immune function, and TTV load is currently being investigated in clinical trials for adjusting immunosuppression in solid organ transplant cases to prevent opportunistic infections and graft rejection.

The respiratory tract stands as a hypothesized primary entry site for initial infection and serves as a frequent shedding source (8). TTV is detectable in respiratory samples from nearly all individuals. Elevated viral loads have been correlated with lung impairment in patients with respiratory conditions such as asthma (9) and linked to respiratory bacterial dysbiosis in lung transplant recipients (10).

Given its affinity for the respiratory mucosa and its connection with cellular immunity, our study aimed to ascertain whether the assessment of TTV levels in nasopharyngeal samples upon admission to the emergency ward could serve as a predictive indicator for identifying the potential risk of severe disease in patients infected with SARS-CoV-2.

METHODS

Patients

We conducted a retrospective, single-center study within the Emergency Department (ED) of Saint-Louis Hospital (Assistance Publique - Hôpitaux de Paris, Paris, France) spanning from March 9th, 2020, to May 6th, 2020. The study encompassed all patients diagnosed with SARS-CoV-2 infections. We categorized the patients into three groups: (i) individuals with acute respiratory distress syndrome attributed to COVID-19, who were subsequently transferred to the medical intensive care unit; (ii) patients admitted to a non-intensive care unit for COVID-19 treatment; and (iii) patients displaying no indications of severity and subsequently discharged from the hospital. Qualified trained medical or nursing personnel conducted the collection of nasopharyngeal secretions using standardized methods, employing nylon flocked swabs which were then placed in 3 ml of Universal Transport Medium (Copan Diagnostics Inc.). The swabs were gently inserted along the nasal septums into the nasopharynx until a sense of resistance was encountered, as described (11).

TTV detection and quantification.

TTV DNA was quantified in nasopharyngeal swabbing samples used for SARS-Cov-2 RT-PCR. DNA was extracted using the QiaSymphony instrument (Qiagen, Hilden, Germany) from 200 µl of Universal Transport Medium. Quantification was carried out by real-time PCR (TTV R-Gene, bioMérieux, Marcy l'étoile, France) on an ABI 7500 instrument (ThermoFisher, Waltham, MA) according to manufacturer instructions. The limit of quantification is 250 copies per mL.

Data collection

All data were retrospectively collected using emergency department clinical records, including demographic data (age and sex), comorbidities (HIV status, diabetes, hypertension, cancer, hemopathy, graft, obesity, chronic lung disease, cardiopathy, immunosuppressive treatment, corticoid treatment, chronic renal insufficiency), clinical signs related to COVID-19 (fever, dyspnea, cough, myalgia, anosmia/ageusia, headaches, rhinorrhea, fatigue, confusion, discomfort, chest pain and abdominal pain), lymphocyte counts, date of symptom onset, pulse oximetry (SpO2) at triage.

Ethics

The study was approved by the Institutional Review Board of the French Speaking society for respiratory medicine – Société de Pneumologie de Langue Française (number CEPRO 2020-014). Data analyses were conducted using an anonymized database.

Statistical analysis

Results are presented as summary statistics, namely median (interquartile range) or absolute frequency (percentage) unless specified otherwise. Comparisons between baseline groups were based on the Mann-Whitney test or the exact Fisher exact test, respectively. Univariable logistic regression models were used to identify factors associated with Intensive Care Unit (ICU) admission. The log-linearity of TTV load effect was checked using splines. The discriminatory performance of the TTV load was measured using the area under the curve (AUC) of the ROC curve, with the optimal cut-off point defined according to the Youden index. Variables associated with the outcome at the 0.05 level in univariable analyses were included

in a multivariable model on complete cases, with variable selection based on the Akaike criterion (AIC). Final model was confirmed after multiple imputation by chained equations (MICE) of missing data, with a predictive mean matching method for quantitative variables and logistic regression models (binomial, ordinal, or multinomial) for categorical variables (12). Results are presented as pooled Odds Ratio (OR) from 30 imputed datasets, with 95% confidence intervals (CI). Statistical analyses were performed using R 4.1.1 (*https://www.R-project.org/*). All p-values were two-sided and values of 0.05 or less were considered statistical significant.

RESULTS

Between March 9th and May 6th, 2020, a total of 1370 patients presented at the Emergency Department (ED) of Saint-Louis Hospital. Out of these, 1364 underwent SARS-CoV-2 testing via PCR, and 370 were found to have a positive nasopharyngeal sample for SARS-CoV-2. Leftover samples were available for 295 patients, comprising 92 individuals who were discharged to their homes, 160 who were admitted to a medical ward, and 43 who were admitted to the medical intensive care unit ()ICU.

Patients differed across the different admission groups with regard to age, comorbidities, duration since the onset of initial symptoms, presence of dyspnea, and requirement for oxygen (Table 1). The TTV load in nasopharyngeal samples obtained upon ED admission exhibited a notably higher median value among patients admitted to the ICU (Median: 3.02 log10 copies/mL) compared to those who were discharged home (2.22) or were admitted to a medical ward (2.24) (p=0.006) (Table 1).

In the univariate analysis, factors such as diabetes, obesity, hepatitis, fever, dyspnea, oxygen requirement, and TTV load were identified as predictors of ICU admission. A subsequent multivariate analysis utilizing model selection based on Akaike's information criterion (AIC) and incorporating multiple imputation by chained equations (MICE) yielded the same contributing factors (Table 2).

Analysis of the probability of ICU admission, using splines to alleviate the constraint of log-linearity, exhibited a plateau characterized by a similar threshold (Figure 1). Employing ROC curve analysis, we established the optimal cutoff value for TTV load that predicts ICU admission to be 2.91 log10 copies/mL, yielding a sensitivity of 0.605 and a specificity of 0.69. This cutoff value was employed to apply the previously selected multivariate model in predicting ICU admission based on the dichotomization of TTV load. Consequently, factors such as obesity, hepatitis, time since first symptoms, body temperature, dyspnea, and a TTV load exceeding 2.91 $\log_{10}$ copies/mL emerged as predictors of ICU admission (Figure 2).

DISCUSSION

Anelloviruses, and Torque Teno virus (TTV) in particular, are now considered potential surrogate markers of cellular immunity in solid organ transplant recipients. Interestingly, recent reports found that high levels of TTV in blood are predictive of a poor response to SARS-CoV-2 vaccination in lung and kidney transplant recipients (13–15).

In this study, we observed that the TTV load in nasopharyngeal samples could predict the need for ICU admission in patients infected with SARS-CoV-2. We established a threshold value as an independent predictor. This analysis was conducted during the initial wave of the SARS-CoV-2 pandemic in France and involved patients who were infected with the virus for the first time. Subsequent to the first wave, point-of-care testing using nasopharyngeal dry swabs was directly implemented in the emergency ward to enhance patient care and optimize bed management (16). Consequently, this prevented the possibility of conducting further analyses on the same sample. It is imperative to corroborate these findings using current SARS-CoV-2 variants and after SARS-CoV-2 vaccination or prior infection, as well as in the context of other acute respiratory viral infections.

Prior studies have documented the correlation between the detection and quantification of TTV and viral respiratory tract infections caused by SARS-CoV-2 and other viruses. In a study conducted before the COVID-19 pandemic, involving children with acute respiratory diseases, Maggi et al. noted that TTV was more frequently detected and at higher levels in nasal secretions of patients with bronchopneumonia compared

to those with milder symptoms (8). In an investigation into the respiratory microbiome and virome signatures associated with the severity of COVID-19 infection, Merenstein et al. discovered that within a week of admission, anelloviridae were more prevalent and present in higher titers in patients with severe disease (17). Furthermore, a recent study also observed that viruses from the anelloviridae family were significantly more abundant in samples from deceased and hospitalized patients in comparison to those from ambulatory individuals (18). Conversely, in a study that compared TTV plasma loads over a two-week period between severe and mild-moderate SARS-CoV-2 cases, Solis et al. demonstrated that a TTV DNA load lower than 700 copies/mL was linked to a higher risk of severe COVID-19. However, this association was observed at a time when lymphocyte counts were notably lower in severe cases during the second week after symptom onset (19).

Collectively, the growing body of evidence suggests that anellovirus reactivation is linked to an inadequate cellular antiviral response, and the replication of TTV within the respiratory tract might reflect respiratory immune deficiency. Detecting and quantifying TTV could serve as markers for predicting the severity of acute viral respiratory infections.

REFERENCES

1. Wu Z, McGoogan JM. Characteristics of and Important Lessons From the Coronavirus Disease 2019 (COVID-19) Outbreak in China: Summary of a Report of 72 314 Cases From the Chinese Center for Disease Control and Prevention. JAMA. 7 avr 2020;323(13):1239-42.

2. Havers FP, Pham H, Taylor CA, Whitaker M, Patel K, Anglin O, et al. COVID-19-Associated Hospitalizations Among Vaccinated and Unvaccinated Adults 18 Years or Older in 13 US States, January 2021 to April 2022. JAMA Intern Med. 1 oct 2022;182(10):1071-81.

3. Vardavas CI, Mathioudakis AG, Nikitara K, Stamatelopoulos K, Georgiopoulos G, Phalkey R, et al. Prognostic factors for mortality, intensive care unit and hospital admission due to SARS-CoV-2: a systematic review and meta-analysis of cohort studies in Europe. Eur Respir Rev Off J Eur Respir Soc. 31 dec 2022;31(166):220098.

4. Hadjadj J, Yatim N, Barnabei L, Corneau A, Boussier J, Smith N, et al. Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. Science. 7 aout 2020;369(6504):718-24.

5. McElvaney OJ, McEvoy NL, McElvaney OF, Carroll TP, Murphy MP, Dunlea DM, et al. Characterization of the Inflammatory Response to Severe COVID-19 Illness. Am J Respir Crit Care Med. 15 sept 2020;202(6):812-21.

6. Arze CA, Springer S, Dudas G, Patel S, Bhattacharyya A, Swaminathan H, et al. Global genome analysis reveals a vast and dynamic anellovirus landscape within the human virome. Cell Host Microbe. 11 aout 2021;29(8):1305-1315.e6.

7. van Rijn AL, Roos R, Dekker FW, Rotmans JI, Feltkamp M. Torque teno virus load as marker of rejection and infection in solid organ transplantation - A systematic review and meta-analysis. Rev Med Virol. 3 sept 2022;e2393.

8. Maggi F, Pifferi M, Fornai C, Andreoli E, Tempestini E, Vatteroni M, et al. TT virus in the nasal secretions of children with acute respiratory diseases: relations to viremia and disease severity. J Virol. fevr 2003;77(4):2418-25.

9. Pifferi M, Maggi F, Andreoli E, Lanini L, Marco ED, Fornai C, et al. Associations between nasal torquetenovirus load and spirometric indices in children with asthma. J Infect Dis. 1 oct 2005;192(7):1141-8.

10. Young JC, Chehoud C, Bittinger K, Bailey A, Diamond JM, Cantu E, et al. Viral metagenomics reveal blooms of anelloviruses in the respiratory tract of lung transplant recipients. Am J Transplant Off J Am Soc Transplant Am Soc Transpl Surg. janv 2015;15(1):200-9.

11. Marty FM, Chen K, Verrill KA. How to Obtain a Nasopharyngeal Swab Specimen. N Engl J Med. 28 mai 2020;382(22):e76.

12. van Buuren S, Groothuis-Oudshoorn K. mice: Multivariate Imputation by Chained Equations in R. J Stat Softw. 12 dec 2011;45(3):1-67.

13. Solis M, Benotmane I, Gallais F, Caillard S, Fafi-Kremer S. Torque teno virus viral load predicts SARS-CoV-2 vaccine response in kidney transplant recipients. J Med Virol. juill 2023;95(7):e28936.

14. Graninger M, Stumpf J, Bond G, Gorzer I, Springer DN, Kessel F, et al. Prediction of humoral and cellular immune response to COVID-19 mRNA vaccination by TTV load in kidney transplant recipients and hemodialysis patients. J Clin Virol Off Publ Pan Am Soc Clin Virol. mai 2023;162:105428.

15. Roberto P, Cinti L, Napoli A, Paesani D, Riveros Cabral RJ, Maggi F, et al. Torque teno virus (TTV): A gentle spy virus of immune status, predictive marker of seroconversion to COVID-19 vaccine in kidney and lung transplant recipients. J Med Virol. fevr 2023;95(2):e28512.

16. Baron A, Peyrony O, Salmona M, Mahjoub N, Ellouze S, Anastassiou M, et al. Impact of Fast SARS-CoV-2 Molecular Point-Of-Care Testing on Patients" Length of Stay in an Emergency Department. Microbiol Spectr. 2022;10:e0063622.

17. Merenstein C, Liang G, Whiteside SA, Cobian-Guemes AG, Merlino MS, Taylor LJ, et al. Signatures of COVID-19 Severity and Immune Response in the Respiratory Tract Microbiome. mBio. 31 aout 2021;12(4):e0177721.

18. Iša P, Taboada B, García-López R, Boukadida C, Ramírez-González JE, Vázquez-Pérez JA, et al. Metagenomic analysis reveals differences in the co-occurrence and abundance of viral species in SARS-CoV-2 patients with different severity of disease. BMC Infect Dis. 19 oct 2022;22(1):792.

19. Solis M, Gallais F, Garnier-Kepka S, Lefebvre N, Benotmane I, Ludes PO, et al. Combining predictive markers for severe COVID-19: Torquetenovirus DNA load and SARS-CoV-2 RNAemia. J Clin Virol Off Publ Pan Am Soc Clin Virol. mars 2022;148:105120.

Table 1. Patient Characteristics. Data are number of patients with percentage (%) or median with interquartile range [IQR].

Variable	Group 1 ICU N=43	Group 2 Medical ward N= 160	Group 3 Discharged N=92	p-value
Age	63 [54.5 ;68.5]	66 [54 ;80]	55 [44 ;65.25]	<0.0001
Sexe F	15 (34.88 %)	62 (38,75 %)	42 (45.65 %)	0.41
M	28 (65.12 %)	98 (61,25 %)	50 (54.35 %)	
Chronic comorbidities				
Diabete (292)[1]	15 (34.88 %)	37 (23.27 %)	10 (11.11 %)	0.005
Active Cancer (292)[1]	4 (9.3 %)	16 (10.06 %)	8 (8.89 %)	0.95
Hemopathy (292)[1]	8 (18.6 %)	24 (15.09 %)	5 (5.56 %)	0.042
Transplantation (292)[1]	3 (6.98 %)	8 (5.03 %)	3(3.33 %)	0.64
Use of immunosuppressive Drugs (288)[1]	5 (11.9 %)	13(8.28 %)	5 (5.62 %)	0.45
Use of corticoïde (292)[1]	5 (11.63 %)	13 (8.18 %)	5 (5.56 %)	0.47
HIV (292)[1]	1 (2.33 %)	8 (5.03 %)	6 (6.67 %)	0.57

Variable	Group 1 ICU N=43	Group 2 Medical ward N= 160	Group 3 Discharged N=92	p-value
Pulmonary history (292)[1]	11 (25.58 %)	36 (22.64 %)	12 (13.33 %)	0.14
Hearth Disease (292)[1]	23 (53.49 %)	82 (51.57 %)	23 (25.56 %)	0.0001
Obesity (287)[1]	13 (30.95 %)	24 (15.38 %)	11 (12.36 %)	0.023
Dialysis (289)[1]	4 (9.52 %)	21 (13.38 %)	3 (3.33 %)	0.037
Hepatitis (289)	4 (9.52 %)	3 (1.91 %)	3 (3.33 %)	0.056
Symptoms				
Fever (294)[1]	37 (86.05 %)	116 (72.5 %)	51 (56.04 %)	0.0009
Dyspnea (294)[1]	33 (76.74 %)	93 (58.13 %)	32 (35.16 %)	<0.0001
Cough (292)[1]	34 (79.07 %)	103 (65.19 %)	52 (57.14 %)	0.045
Rhinorrhea (286)[1]	3 (6.98 %)	14 (8.92 %)	6 (6.98 %)	0.84
Myalgia (292)[1]	17 (39.53 %)	34 (21.38 %)	39 (43.33 %)	0.0006
Anosmia/ Ageusia (280)[1]	10 (25.64 %)	16 (10.46 %)	18 (20.45 %)	0.023
Headache (289)[1]	8 (18.6 %)	10 (6.37 %)	13 (14.61 %)	0.026
Digestive symptoms (291)[1]	12 (27.91 %)	40 (25.32 %)	23 (25.56 %)	0.94
Chest pain (291)[1]	2 (4.65 %)	10 (6.33 %)	10 (11.11 %)	0.29
Confusion (294)[1]	3 (6.98 %)	13 (8.12 %)	0 (0%)	0.022
Malaise (288)[1]	2 (4.76 %)	9 (5.77 %)	5 (5.56 %)	0.97
Asthenia (189)[1]	27.39 % (26.19 %)	43 (27.39 %)	15 (16.67 %)	0.15
Hemoptysis (293)[1]	1 (2.33 %)	5 (3.14 %)	3 (3.3 %)	0.95
Temperature	37.8 [37.35;38.35]	37.5 [36.8;38.1]	37 [36.6;37.9]	0.0008
spo2	98 [95;100]	100 [95;100]	100 [99;100]	0.0004
0xygen supply	24 (55.81 %)	76 (47.5 %)	3 (3.26 %)	<0.0001
Oxygen volume (L)	2 [2;3	2 [2;3]	3.5 [2.25;4.75]	0.16
WBC (10^9/L)	7.58 [4.71;9.97]	6.55[4.38;8.46]	5.96[4.59;7.69]	0.24
Lymphocytes (10^9/L)	0.95 [0.54;1.24]	0.97 [0.62;1.44]	1.08 [0.79;1.56]	0.10
TTV Viral Load ($\log_{10}$/mL)	3.02 [2.21;3.82]	2.24 [0;3.29]	2.21 [0;2.96]	0.006
Time since first symptoms (days)	4 [1;8]	7 [3;10]	7 [3;10]	0.019

[1] (N) number of individuals with available data.

Table 2 . Model predicting ICU admission after MICE and selection

	Odds Ratio	95% Confidence Interval	p-value
Obesity	2.79	1.23 – 6.32	0.01
Hepatitis	5.69	1.21 – 26.83	0.03
Time since first symptoms	0.92	0.85 – 0.99	0.03
Fever	2.94	1.12 – 7.73	0.03
Dyspnea	3.42	1.50 – 7.80	0.00
TTV load	1.31	1.08 – 1.57	0.01

Legend

Figure 1. Probability of ICU admission according TTV load. Probability of ICU admission according to TTV load (expressed in $\log_{10}$ copies/mL) in nasopharyngeal samples collected at admission in the emergency ward. The vertical dotted blue line indicates the best cutoff value of TTV load predicting ICU admission as defined with a ROC curve (with a sensitivity of 0.605 and specificity of 0.69).

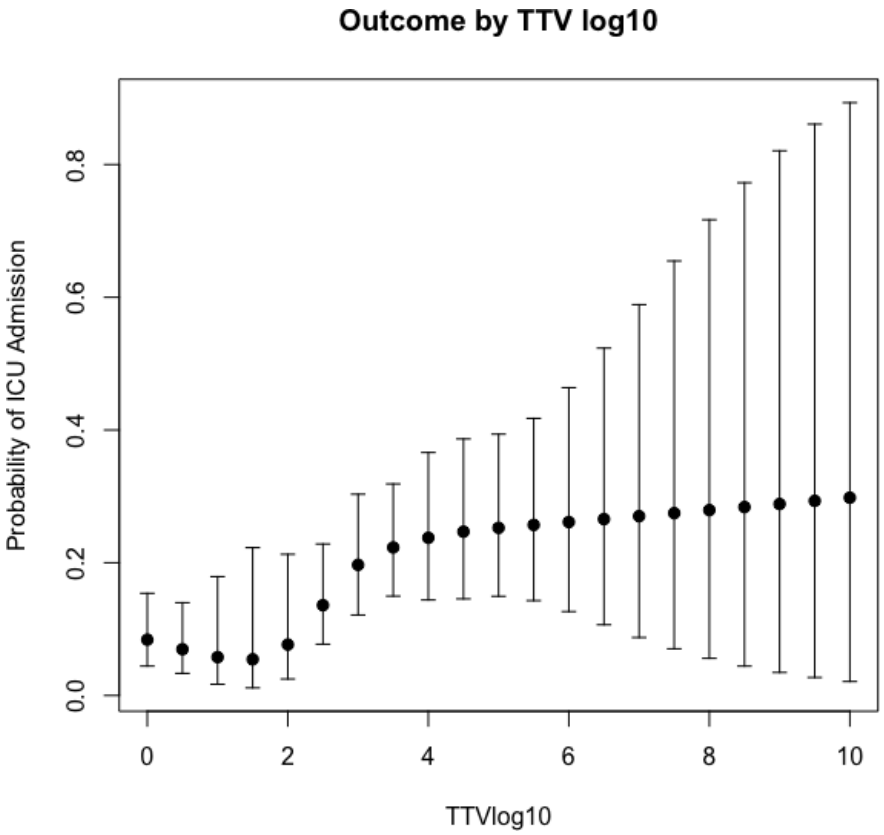

Figure 2. Predictors of ICU admission. Clinical and biological predictors of ICU admission, model with TTV log10 dichotomized, based on Youden index predicting ICU admission after selection.

Variable	N	Odds ratio	p
Obesity	259	2.88 (1.20, 6.76)	0.016
Hepatitis	259	8.35 (1.57, 45.61)	0.012
TimeSince1stSymptoms	259	0.92 (0.85, 0.99)	0.049
Temperature	259	1.47 (1.00, 2.17)	0.051
Dyspnea	259	2.14 (0.88, 5.53)	0.101
Oxygen	259	2.22 (1.00, 5.07)	0.053
TTV.above	259	2.88 (1.35, 6.31)	0.007