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Short communication

The clinical interest of HSV1 semi-quantification in bronchoalveolar lavage

Emilie Frobert[a,b,*], Geneviève Billaud[a], Jean-Sébastien Casalegno[a,b], Daniel Eibach[a], David Goncalves[a], Jean-Michel Robert[c], Bruno Lina[a,b], Florence Morfin[a,b]

[a] Virology Laboratory, Centre de Biologie et Pathologie Est, Hospices Civils de Lyon, Lyon, France
[b] Virology and Human Pathology, Université Lyon 1, EA 4610, Faculté de Médecine RTH Laënnec, Lyon, France
[c] Intensive Care Unit, Hôpital Edouard Herriot, Lyon, France

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ABSTRACT

Background: Detecting high herpes simplex (HSV) viral load in lower respiratory tract samples is reported to be significantly associated with the severity of the illness in critical patients, particularly in patients on mechanical ventilation. It may therefore be of interest to quantify HSV in bronchoalveolar lavage (BAL). Quantitative PCR for HSV is not commonly available in clinical routine. Real-time PCR tests are, however, used commonly and provide semi-quantitative information based on the cycle threshold (*Ct*).
Objectives: Our objectives were to determine the clinically significant threshold and to study the impact of viral load normalisation in relation to cell quantity in samples using real-time PCR.
Study design: During the period 2011–2012, 59 HSV1 positive BAL were included. HSV viral load was determined by a quantitative real-time PCR (R-gene, Argène BioMérieux, France) and compared to a semi-quantitative real-time PCR (SmartCycler®HerpesSimplex, Cepheid, USA). Viral load normalisation was determined using a real-time PCR targeting a cellular gene (Cc r-gene kit, Argène BioMérieux, France). The significant threshold was determined *versus* clinical features by statistical analysis (Epiinfo Software v3.5.1 CDC).
Results: A viral load of 10^4 copies/ml of BAL was significantly associated with admission to the intensive care unit ($p < 0.001$), mechanical ventilation ($p < 0.01$) and death ($p < 0.01$), with no influence of viral load normalisation in relation to cell quantity in the sample. This viral load was equivalent to a *Ct* value of 31 in the semi-quantitative technique.
Conclusions: As semi-quantitative techniques are currently used in many labs, determining this *Ct* value could be useful for interpreting the clinical advantages of detecting HSV in BAL.
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1. Background

In critically ill patients, detecting herpes simplex virus (HSV) in low respiratory samples raises the question of its clinical signification. It is effectively difficult to differentiate between (i) viral contamination of the lower respiratory tract from the throat or mouth, (ii) tracheobronchial excretion without parenchymal involvement and (iii) a real implication in pulmonary disease. Furthermore, the involvement of HSV in real pulmonary diseases or its role as a severity marker, in particular in bacterial ventilator-associated pneumonia, is currently a topic of discussion.[1] HSV is frequently isolated in the respiratory tracts of patients on long term mechanical ventilation, with a frequency of between 13.4% and 62% depending on studies and length of ventilation.[1–4] Even though viral reactivation without lung parenchymal involvement seems to be frequent, Luyt et al. reported up to 21% of patients with herpetic bronchopneumonia, defined by clinical pulmonary deterioration associated with HSV in bronchoalveolar lavage (BAL) and cytological or histological data. Herpetic bronchopneumonia mainly occurs during HSV reactivation, and is frequently preceded by oral lesions.[2] Dissemination from the oral area is the main physiopathology, favoured by respiratory manipulations, trauma and paralysis induced by mechanical ventilation. HSV can also be reactivated directly at the pulmonary level. Blood dissemination is described mainly in immuno-compromised patients. A number of publications show that HSV bronchopneumonitis seems to be significantly associated with admission to the intensive care unit, mechanical ventilation

Abbreviations: HSV, herpes simplex virus; BAL, bronchoalveolar lavage; ICU, intensive care unit; rtPCR, real-time PCR; *Ct*, cycle threshold.

* Corresponding author at: Laboratory of Virology, Centre de Biologie et Pathologie Est, Hospices Civils de Lyon, 59 Boulevard Pinel, F-69677 Bron Cedex, Lyon, France. Tel.: +33 4 72 12 96 17; fax: +33 4 72 12 95 00.
E-mail address: emilie.frobert@chu-lyon.fr (E. Frobert).

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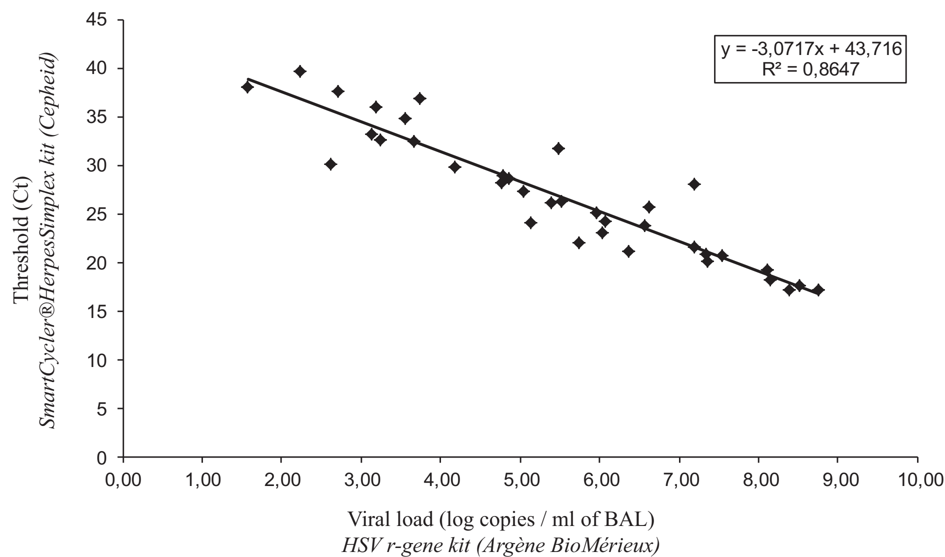

Fig. 1. Correlation between HSV1 viral load (log copies/ml of BAL) and HSV1 semi-quantification (*Ct*).

and a poorer outcome.[2,5] In particular, a high viral load in BAL was significantly associated with these features, with two thresholds commonly reported, 80,000 copies/10^6 cells[2] and 10^5 copies/ml of BAL, respectively.[3,5–7]

2. Objectives

The aims of the present study were to (1) assess the significant threshold of HSV viral load associated with clinical features, (2) study the advantages of normalising viral load in relation to cell quantity in samples, as viral load may depend on the volume of BAL recovered, and (3) establish a correlation with a clinically significant *Ct* value determined using semi-quantitative real-time PCR.

3. Study design

During the period 2011–2012, 1338 BAL were received for virological analyses. A total of 59 patients presenting with HSV1 positive BAL, detected by either PCR or culture were studied retrospectively. The patients had been admitted to the intensive care unit (ICU) ($n = 37$), pneumonology unit ($n = 7$), internal medicine unit ($n = 7$), infectious diseases unit ($n = 4$) and clinical haematology unit ($n = 4$). Overall, 23 patients were considered as immunocompromised (9 haemopathies, 4 HIV, 4 solid cancers, 3 bone marrow transplants, 2 kidney transplants and one lung transplant). HSV DNA was extracted from 200 μl of BAL using the automated NucliSens easyMAG system (Biomerieux, SA, Marcy l'Etoile, France) and elution of the extracted nucleic acids was performed in 70 μl. HSV viral load was determined using real-time PCR (rtPCR) (HSV r-gene, Argène BioMérieux, France) with a quantification range of 1000–5,000,000 copies/ml. The quantity of cells in the samples was indirectly estimated by the rtPCR targeting the HPRT1 cellular gene (Cc r-gene, Argène BioMérieux, France). Normalisation of the HSV1 viral load according to the quantity of cells was calculated as previously described.[8] The significant threshold was determined by statistical analysis (Epiinfo Software v3.5.1 CDC) analysing the connections with an age >50 years, immune status, the presence of pulmonary signs, co-morbidity factors, co-infections, admission to the ICU, mechanical ventilation and death. Finally, the correlation between HSV1 quantification (*i.e.* viral load in copies/ml of BAL using the HSV R-gene kit, Argéne BioMérieux, France) and HSV1 semi-quantification was established (*i.e.* the *Ct* value of rtPCR using the SmartCycler®HerpesSimplex kit, Cepheid, Sunnyvale, CA, USA) on 36 samples. This retrospective study was carried out in compliance with French laws and Hospices Civils de Lyon guidelines, and in accordance with the ethical standards of the Declaration of Helsinki.

4. Results

In line with previous studies, our results confirmed that there was a significant association between an HSV1 viral load >100,000 copies/ml of BAL and admission to the ICU ($p < 0.0001$), mechanical ventilation ($p < 0.001$) and death ($p < 0.01$) (Table 1). No significant difference was found for an age >50 years, immune status, presence of pulmonary signs, co-morbidity factors or co-infections. The threshold was then reassessed based on these 3 significant parameters (ICU, ventilation and death). With the HSV1 viral load threshold at 10,000 copies of HSV/ml of BAL, the association was still significant ($p = 0.00012$), whereas the threshold of 1000 copies/ml of BAL was no longer significant ($p = 0.19$) (Table 2). Normalising the HSV1 viral load in relation to cell quantity did not reveal any differences with a mean *Ct* value of cellular gene in samples at 22.8 ± 2. The threshold of 10,000 copies/ml of BAL remained significant with normalised viral loads (Table 2). The correlation between the quantitative and semi-quantitative techniques showed that a viral load of 10,000 copies/ml of BAL was related to a *Ct* value threshold of 31 in the Cepheid technique, with a linear regression coefficient (R^2) of 0.8647 (Fig. 1).

5. Discussion

Overall, an HSV1 viral load of 10,000 copies/ml of BAL was significantly associated with admission to the ICU, mechanical ventilation and death. This threshold was similar, even one log lower on average to that of other studies.[2,3] The different quantification methods used between studies might explain the slight difference regarding the 10,000 copies/ml threshold determined in this study. Furthermore, as shown by recent kinetic analyses, the quantity of HSV in BAL is not static.[4] The delay between the beginning of the infection and the BAL sampling may thus have an impact on the viral load results. Unfortunately, we did not have any sequential samples in this study.

In clinical practice, the correlation between quantitative (10,000 copies/ml) and semi-quantitative ($Ct = 31$) data may be useful. The

Table 1
HSV1 detection in bronchoalveolar lavage specimens and association with clinical features.

Features	Total number (%)	Viral load		Relative risk	*P* value
		<100,000 copies/ml BAL	>100,000 copies/ml BAL		
Number of BAL	59				
Age > 50	48 (81%)	19 (40%)	26 (60%)	1.27 [0.3–48]	0.48
Pulmonary signs	46 (78%)	18 (39%)	28 (61%)	1.33 [0.4–4.6]	0.44
Immunosuppression	23 (39%)	11 (48%)	12 (52%)	0.62 [0.2–1.8]	0.27
Comorbidities	46 (78%)	20 (43%)	26 (57%)	0.58 [0.2–2.2]	0.31
Coinfections	48 (82%)	30 (63%)	18 (37%)	2 [0.5–7.5]	0.24
Admission to ICU	37 (63%)	7 (19%)	30 (81%)	14 [4–53]	<0.0001
Mechanical ventilation	33 (56%)	6 (18%)	27 (82%)	10 [3–34.1]	<0.001
Death	22 (37%)	3 (14%)	19 (86%)	8.3 [2.1–39.2]	<0.01

Table 2
Reassessment of HSV1 viral load, with and without normalisation according to the quantity of cells, in association with admission to ICU, mechanical ventilation and death.

Features	Viral load		
	>1000 copies/ml BAL	>10,000 copies/ml BAL	>100,000 copies/ml BAL
Admission to ICU			
Without normalisation according to the quantity of cells	$p = 0.19$	$p = 0.00012$	$p = 0.000007$
With normalisation according to the quantity of cells	$p = 0.19$	$p = 0.00012$	$p = 0.000057$
Mechanical ventilation			
Without normalisation according to the quantity of cells	$p = 0.35$	$p = 0.0017$	$p = 0.00008$
With normalisation according to the quantity of cells	$p = 0.35$	$p = 0.0017$	$p = 0.00027$
Death			
Without normalisation according to the quantity of cells	$p = 0.27$	$p = 0.008$	$p = 0.001$
With normalisation according to the quantity of cells	$p = 0.27$	$p = 0.008$	$p = 0.002$

Ct value is provided by all the real-time PCR assays and more complex quantitative PCR does not need to be set up. We have shown in this study that the *Ct* value can easily identify a viral load above or below 10,000 copies/ml of BAL. When determined, it may help in the clinical interpretation of HSV1 detection in low respiratory samples and make it possible to identify reactivation, probably with no clinical consequences. As the correlation we propose depends on the type of PCR used, the *Ct* may change if another commercial or laboratory-developed HSV PCR is used. For this reason, the *Ct* value threshold of 31 is only valid for the assay used in this study. Each lab should determine its own significant *Ct* value. Developing a gold standard method or using a conversion factor, as done for CMV quantification, would make it possible to determine a significant "universal" *Ct* value.

The number of cells remained more or less constant in the 59 BAL included, and we did not reveal any advantage to HSV1 viral load normalisation in relation to the quantity of cells. As the number of cells in certain BALs can be significantly lower, this point should be confirmed by a larger study and compared with cytological analysis of the BAL. Nevertheless, avoiding normalisation would allow make a prompt virological diagnosis possible.

Previous studies using virus isolation were unable to provide absolute clinical evidence for either the direct pathogenicity of HSV or the effects of antiviral therapy.[4,9] Nevertheless, we would expect culture efficiency to be higher in samples with a viral load of >10,000 copies/ml. The results we obtained did not confirm this point as almost all the samples (all but 7) were culture positive (data not shown). We thus cannot conclude that if HSV1 is detected in a BAL by culture, it correlates with a viral load of more than 10,000 copies/ml. This point needs further investigation, taking into account both the kind of samples (swabs *versus* aspirates) and the routing time to labs as this may have an influence on culture efficiency.

In conclusion, the *Ct* value determined by a semi-quantitative real-time PCR, with no viral load normalisation in relation to the quantity of cells, would make possible a rapid virological diagnosis of real clinical interest. Without a gold standard method, virological labs should determine a significant *Ct* value with their own techniques and then be able to release it in their reports to clinicians and provide the threshold for interpretation. The validity of such a threshold should be further evaluated by large clinical studies investigating the impact of a systematic acyclovir treatment, particularly in patients admitted to the ICU and on mechanical ventilation.

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Competing interests

No competing interests.

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