Chapter 7

High herpes simplex virus load in bronchoalveolar lavage fluid is related to poor outcome in critically ill patients

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Submitted

Abstract

Objective
The clinical relevance of detecting HSV-1 and -2 in bronchoalveolar lavage fluid (BALF) is unclear. The aim of this study was to evaluate the relationship between the HSV-1 and -2 loads in BALF and clinical outcome.

Study design
A total of 521 BALF samples from 461 patients were included. Patients were divided in three groups; i) patients admitted to the hospital <48 hours before lavage (Community), ii) patients admitted to the ICU >48 hours before lavage (ICU) and ii) the remaining patients (non-ICU group). HSV-1 and HSV-2 loads were determined by real-time polymerase chain reaction (PCR).

Results
HSV-1 DNA was detected in 4.3% (4/92) of the samples in the Community group, 15% (18/121) in the non-ICU group and in 32% (99/308) of the ICU group. In the age group <50 years HSV-1 DNA was less frequently isolated compared to patients in the age group ≥50 years (17/145 (12 %) versus 104/376 (28 %) respectively, OR=2.9; p<0.001). HSV-1 loads of $>10^5$ genome equivalents (ge)/ml in BALF were associated with an increased 14 day in-hospital mortality compared to patients with a BALF HSV-1 load $\leq 10^5$ ge/ml in BALF (41% versus 20% respectively, p-value=0.001). HSV-2 was detected in low quantities in only two non-ICU patients and was neither associated with morbidity nor mortality. HSV-1 pneumonia was histologically proven, at post-mortem examination, in two patients with a HSV-1 load exceeding 10^5 ge/ml.

Conclusion
HSV-1 occurs more in critically ill patients and high loads in BALF are associated with an increased mortality. The higher mortality observed in patients with HSV-1 load $>10^5$ ge/ml enforces its clinical relevance and the necessity to start randomized medical intervention studies.

Introduction

Human herpes simplex virus (HSV) belongs to the family of Herpesviridae and encompasses two subtypes, HSV-1 and HSV-2, both of which are highly prevalent and ubiquitously distributed[1,2]. A primary HSV infection usually involves mucocutaneous surfaces, with HSV-1 more frequently isolated from oral-facial sites, whereas HSV-2 usually affects genital sites[1]. In the immunocompetent adult host HSV infection usually has a benign course. Nevertheless, it is the most commonly identified cause of acute sporadic encephalitis[3].

As is the case with other herpesvirusses, the initial infection is followed by a lifelong latent infection, from which reactivation can occur. A variety of triggers for reactivation have been described such as injury to tissue innervated by the latently infected neurons, emotional or physical stress, hormonal imbalance, ultraviolet light, fever and immunosuppression[1].

Occasionally, HSV may cause pneumonia as a result of immunosuppression[4,5], with a high mortality. In earlier reports, HSV was isolated from respiratory secretions from patients with adult respiratory distress syndrome, burns and in other non-immunosuppressed patients without a history of lung disease[6-8]. Interestingly, Schuller *et al.*[9] showed that immunocompetent patients from which HSV-1 was recovered from the respiratory tract were more severely ill and had a worse outcome compared to immunocompromised patients. In a study by Bruynseels *et al.*[10], HSV-1 was detected in 16% of lower respiratory tract samples of patients admitted to the intensive care unit (ICU) as determined by viral culture. Patients with severe disease were found to be the most susceptible to HSV-1 infection or disease. Since this virus can severely affect pulmonary function in a wide range of patients and effective treatment with aciclovir is available, it is of the utmost importance to detect the infection accurately and early. Nevertheless, it has been described that HSV can also be isolated from the respiratory tract of asymptomatic adults (up to 5%)[1]. As a consequence, it may be crucial to distinguish asymptomatic carrier ship of HSV from symptomatic infection. To investigate whether this distinction can be made by determination of the HSV load in bronchoalveolar lavage fluid (BALF), we subjected a series of BALF samples to HSV-1 and HSV-2 specific quantitative, real-time PCR assays. Subsequently, the viral loads were correlated with clinical parameters and outcome.

Materials and methods

Patients included

This retrospective study was conducted at the University Hospital Maastricht. During a 72-month period (January 1999-December 2004) all consecutive BALF samples obtained from critically ill ICU patients clinically suspected of ventilator associated pneumonia (VAP) and all BALF samples from non-ICU patients, which were presented for viral culture were included. In the ICU patients clinical evolution was assessed by means of SOFA score (sequential organ failure assessment)[11]. Patients were devided in three groups; i) patients admitted to the hospital <48 hours before lavage were classified as Community, ii) patients admitted to the ICU >48 hours before lavage as ICU and ii) the remaining patients were included in the non-ICU group.

Clinical variables

Clinical suspicion of VAP was defined as described by Bonten *et al.*[12]. Microbiologically confirmed VAP was defined as admission to the ICU >48 hours prior to BALF collection, a total quantitative culture result of $\geq 10^4$ cfu/ml and/or ≥2% infected cells (IC)[13,14]. Mortality was measured as a dichotomous variable; death within 14 days after BAL-procedure (yes / no) in hospitalised patients.
Collected data included patients' demographic characteristics such as: age, gender and clinical data such as: admittance to ICU ward, smoking, length of ICU stay before the performance of BAL, total length of stay at ICU, total length of hospital stay, mortality, diagnosis at post-mortem examination and laboratory data concerning BALF: total cell count, differential cell count presence of ciliated epithelial cells, squamous epithelial cells, reactive pneumocytes type II (RPII)[15] and quantitative culture results.

Sampling technique

A fiberoptic bronchoscope (Pentax FB-15H/FB-15X, Pentax Medicals, Tokyo, Japan) was introduced and "wedged" into the affected segmental or subsegmental bronchus. Sterile saline (0.9% NaCl, room temperature) was instilled in four aliquots of 50 ml and immediately aspired and recovered. The samples were transported to the laboratory within 15 minutes after collection and processed immediately upon arrival at the microbiology laboratory.

Laboratory processing

The first fraction of BALF represents the bronchial fraction and was not used in this study. The remaining three fractions (alveolar fractions) were pooled. A total cell count was performed using a Fuchs Rosenthal haemocytometer chamber. Quantitative bacterial culture was performed as previously described[16]. Cytocentrifuged preparations[17] were made and stained with May-Grünwald Giemsa stain and Gram-stain. Differential cell count[18] was performed including infected cells (IC) and RPII[15]. From each sample 6 ml was centrifuged (250 g, 10 minutes), dividing the sample into cells and supernatant. The supernatant was stored in tubes of 1 ml at -80°C. The cells were resuspended in a mixture of Eagle's Minimal Essential Medium (EMEM) with 2% Dimethyl Sulfoxide (DMSO) and also stored in tubes of 1 ml at -80°C.

Virological assessment:

A total of 500 µl of the stored cell fraction was used for DNA isolation using the Wizard® Genomic DNA Purification Kit (Promega, Madison, USA). Purified DNA was resuspended in a final volume of 120 µl. Each sample was spiked with a control plasmid (murine cytomegalovirus glycoprotein B gene (MCMV gB)[19] before DNA isolation as an isolation and amplification control. An in-house real-time PCR was performed using primers and probes which target the glycoprotein G-(gG) gene (HSV-1) and the glycoprotein D- (gD) gene (HSV-2)[20]. Assays (both HSV-1 and HSV-2) were performed in a 96-well Optical Reaction plate (Applied Biosystems, Foster City, California) in a total volume of 50 µl which contained 0.2 µM forward primer, 0.6 µM reverse primer, 0.075 µM probe, 1×Taqman® Universal Master Mix (ABI) and 20 µl purified DNA. The PCR reactions for the internal control (MCMV gB) were carried out as described previously[19]. The PCR thermal profile consisted of an initial incubation of two minutes at 50°C, followed by activation of the AmpliTaq Gold® Polymerase during 10 minutes at 95°C. A total of 42 cycles of amplification were run consisting of 15 seconds at 95°C and 1 minute at 60°C. Amplification was performed using the ABI PRISM 7000 Sequence Detection System (ABI) and data were interpreted using the ABI Prism software. Quantification was done by extrapolation of data to standardcurves generated by amplification of quantified dilutions of plasmids containing the HSV-1gG gene and HSV-2 gDgene.

Clinical chemistry

Urea was measured in BALF (as a marker of dilution) and in serum using a commercially available kit (Urea, Beckman Coulter, Fullerton, California, USA).

Immunofluorescent staining of cytocentrifuged preparations

From each BALF sample cytospin centrifuged preparations were stored at -20°C until the PCR results were available. In case of a positive PCR result, the slides were thawed and an immunofluorescent staining technique was performed. First the cytospin preparations were fixated using acetone at +4°C for 10 minutes. Hereafter a monoclonal antibody based test for the detection of and the differentiation between HSV-1 and HSV-2 (Pathfinder® Herpes Simplex Virus Types 1 and 2, Bio-Rad laboratories, Hercules, California, USA).
was performed on the cytospin preparations.

Statistical analysis

In order to compare the quantitative real-time PCR results between recovered BALF samples, the quantity was converted to those present in the epithelial lining fluid (ELF) using the formula described by Wiedermann *et al.*[21], which is based upon urea concentrations in BALF and in serum:

$$[X]ELF = ([X]BALF \times \text{urea serum})/\text{urea BALF}$$

Geometrical mean viral loads were calculated as log10 of HSV-positive BALF samples. Groups were compared by t-test statistics and Chi square statistics when appropriate. Risk factors for HSV-1 shedding (yes/no) were identified by univariate logistic regression analysis (Table 7.1). Clinical outcome measures were defined as in-hospital death within 14 days, presence of pneumonia and/or and presence of HSV-pneumonitis at post mortem examination.

Results

Patient population

In the period January 1999 until December 2004, 1033 BALF samples (from 866 patients) were eligible for inclusion in our study. This included 456 samples retrieved from ICU patients and 577 samples retrieved from non-ICU patients. Insufficient material for PCR was available for 148 and 55 BALF samples respectively, therefore these patients were excluded. Viral culture was requested on 132 out of the 522 (25%) samples from non-ICU patients. This resulted in a total of 521 BALF samples from 461 patients to be included. The study population consisted of 92 (18%) samples from 88 Community patients, 121 (23%) samples from 113 non-ICU patients and 308 (59%) samples from 260 ICU patients (Figure 7.1).

Table 7.1 Clinical and demographic variables of patients with and without HSV-1 DNA in BALF

		HSV + (n=121)	HSV - (n=400)	Crude OR	p-value
Gender					0.354
Male		79 (65%)	279 (70%)		
Female		42 (35%)	121 (30%)		
VAP					
No		77 (20%)	309 (80%)		
Yes		44 (33%)	91 (67%)		
Age				2.60	<0.001
<50 years	145	17 (12%)	128 (88%)		
>50 years	376	104 (28%)	272 (72%)		
Patient group					<0.001
Community	92	4 (4.3%)	88 (95.7%)	1.0	
Non-ICU	103	18 (15%)	103 (85%)	3.7	<0.001
ICU	308	99 (32%)	209 (68%)	9.6	<0.001
Smoking					0.614
Non-smoker	136	31 (23%)	105 (77%)		
Not active smoker	58	10 (17%)	48 (83%)		
Active smoker	115	26 (23%)	89 (77%)		
SOFA score at admission					0.101
≤7	93	22 (24%)	71 (76%)		
>7	80	28 (35%)	52 (65%)		
Length of hospitalisation		121	400		
Mean days (SEM)		50.8 (3.7)	46.2 (2.5)		
Length of stay at the ICU		121	400		
Mean days (SEM)		32.5 (2.8)	27.9 (2.1)		

HSV: Herpes simplex virus, OR: odds ratio, BALF: bronchoalveolar lavage fluid, SOFA: sequential organ failure score, ICU: intensive care unit, SEM: standard error of the mean. VAP: ventilator-associated pneumonia

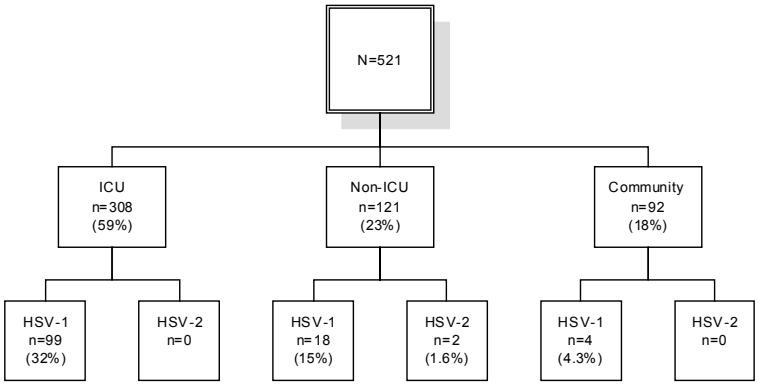

Figure 7.1 Prevalence of HSV-1 and HSV-2 in BALF samples.
HSV-1: Herpes simplex virus type 1, HSV-2: Herpes simplex virus type 2, ICU: intensive care unit, BALF: bronchoalveolar lavage fluid.

Factors associated with HSV-1 shedding in BALF

Patient variables like gender and smoking were not associated with the presence of HSV-1 in BALF. However, patients over the age of 50 years did significantly shed HSV-1 more often in their BALF samples when compared to patients younger than 50 years (28% versus 12 %; Table 7.1). Among the clinical variables, including SOFA-score, length of hospitalisation, length of stay at the ICU, concomitant presence of a bacterial pneumonia and admittance to the ICU, only the latter was significantly associated with HSV-1 in BALF (Table 7.1). HSV-1 was found in 32% (99/308) of ICU samples, whereas it was only detected in 15% (18/121) of the non ICU samples and 4.3% (4/92) of the Community samples (Figure 7.1). None of the cytological parameters of BALF, including total cell count, differential cell count including IC and RPII were found to be associated with the presence of HSV-1 in BALF. Multivariate analysis showed that hospital admittance and age over 50 years were independent predictors for the presence of HSV-1 in BALF with odds ratios of 3.7 (95 % confidence interval (CI): 1.2-11) for the non-ICU, 9.6 (95% CI: 3.4–27) for the ICU and 2.6 (95 % CI: 1.5–4.6) for age over 50.

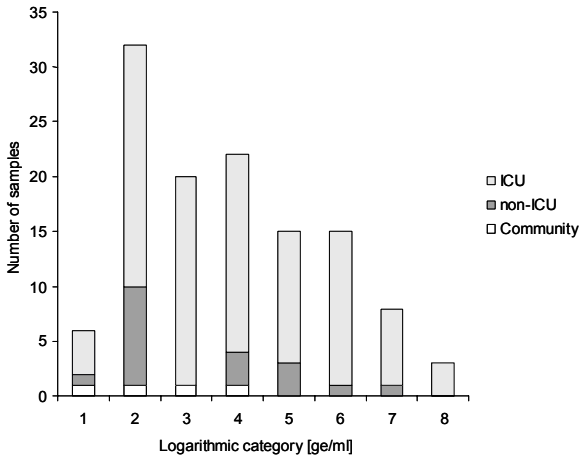

Figure 7.2 Distribution of quantitative HSV-1 loads in BALF. Each bar is divided into ICU, non-ICU and Community patients. The y-axis represents the number of samples whereas the x-axis represents the HSV-1 load in logaritmic categories.
BALF: Bronchoalveolar lavage fluid, ICU: intensive care unit, HSV-1: herpes simplex virus type 1, PCR: polymerase chain reaction, ge/ml: genome equivalents per milliliter.

Quantitative PCR results

The BALF samples in which HSV-1 was identified, were divided into logarithmic categories, based upon quantitative PCR results (10 ge/ml=log 1, 100 ge/ml= log 2 and so forth). Figure 7.2 shows the number of BALF samples with HSV-1

present divided into logarithmic categories. All patients in the Community group had a viral load in BALF of 10^4 ge/ml or less. Among the ICU and non-ICU group 36% (36/99) and 28% (5/18) had a BALF viral load $\geq 10^5$ ge/ml respectively. In one patient, BAL was performed twice within a period of five days, the first BALF showed 10^5 ge/ml, the second BALF 10^7 ge/ml. The patient died four days after the second BAL. Post mortem examination revealed an extended pneumonia caused by HSV-1.

Establishing a clinical relevant cut-off value for HSV-1 viral load

To assess a cut-off value for the HSV-1 viral load, the in-hospital mortality within 14 days after collection of BALF was selected as relevant clinical parameter. At each possible cut-off value, the in-hospital mortality was compared to the in-hospital mortality of patients with a non-reactive HSV-1 PCR. Up to the cut-off value of 10^5 ge/ml HSV-1 in BALF, the group above the cut-off value showed a significant higher mortality (Table 7.2). At higher cut-off values, the group above and below the cut-off value both showed significant differences. This indicated that the test was not differentiating anymore between the group below and above the cut-of value (Table 7.2). Therefore the cut-off value for HSV-1 load in BALF was set at 10^5 ge/ml. This resulted in an in- hospital death within 14 days of 20% below compared to 41% above the cut-off value.

Table 7.2 Odds ratios (OR) for different cut-off values as compared to 14 days in-hospital mortality for non-reactive HSV-1 PCR samples (reference).

Category	N (%)	OR	95% CI	p-value
Reference	62 (17)	1	-	-
≤ Log 3	11 (22)	1.39	0.67 – 2.86	0.37
> Log 3	25 (36)	2.73	1.56 – 4.78	<0.001[a]
≤ Log 3.5	13 (22)	1.42	0.72 – 2.79	0.31
> Log 3.5	23 (37)	2.90	1.62 – 5.20	<0.001[a]
≤ Log 4	17 (24)	1.55	0.84 – 2.85	0.16
> Log 4	19 (39)	3.12	1.65 – 5.89	<0.001[a]
≤ Log 4.5	20 (25)	1.67	0.94 – 2.97	0.08
> Log 4.5	16 (39)	3.15	1.59 – 6.24	0.001[a]
Log 5	22 (26)	1.72	0.98 – 3.00	0.057
Log 5	14 (41)	3.28	1.58 – 6.80	0.001[a]
≤ Log 5.5	26 (28)	1.88	1.11 – 3.19	0.019[a]
> Log 5.5	10 (39)	3.08	1.33 – 7.09	0.008[a]
≤ Log 6	29 (29)	2.01	1.21 – 3.35	0.007[a]
> Log 6	7 (35)	2.65	1.02 – 6.91	0.046[a]

[a] Statistically significant

Post-mortem examination results

A total of 152 patients died during hospitalisation. Out of these 152 patients 93 died within 14 days after BAL (Community: 6, non-ICU: 11 and ICU: 76). These 93 patients could be divided into patients from whom the BALF showed a HSV-1 load $\leq 10^5$ ge/ml (n=79) and patients with a HSV-1 load in BALF exceeding 10^5 ge/ml (n=14). In 37 patients, post-mortem examination was performed (Community: 4, non-ICU: 5, ICU: 28). In the group patients with a HSV-1 load $\leq 10^5$ ge/ml 32 (32/79, 41%) post-mortem examinations were performed. In 70% (26/37) of these patients, the post-mortem examination revealed pneumonia as the cause of death. Out of the 14 patients with $>10^5$ ge/ml, post-mortem examination was available in five (5/14, 36%). All five post-mortem examination reports stated pneumonia as the cause of death, including two patients (2/5, 40%) who were diagnosed with HSV-1 associated pneumonia, showing the presence of specific herpetic nuclear inclusions in the lungs at post-mortem examination which stained positive in the HSV-1 immunofluorescent stain. In the group of patients with a HSV-1 load in BALF $\leq 10^5$ ge/ml this diagnosis was never attributed.

Concomitant presence of VAP

Out of the 308 episodes of suspected VAP in 260 patients, 135 episodes (44%) in 125 patients were microbiologically confirmed VAP. The causative organisms were: *Pseudomonas aeruginosa* (n=37), *Staphylococcus aureus* (n=27), Enterobacteriaceae (n=38), *Haemophilus influenzae* (n=4), *Streptococcus pneumoniae* (n=3) and others (*e.g.* *Stenotrophomonas maltophilia*, *Moraxella catarrhalis*, n=26). In 44 (33%) episodes of VAP, the quantitative PCR for HSV-1 was positive. In 13 (10%) cases the HSV-1 load in BALF exceeded 10^5 ge/ml. There were no significant differences in type of bacteria causing VAP between the HSV-1 positive and –negative group.

Results of HSV-2 detection in BALF

HSV-2 was detected in only 2 out of 520 examined BALF with viral loads of 253 ge/ml and 104 ge/ml, respectively. Both BALF samples were from non-ICU patients who were admitted to the department of internal medicine (Figure 7.2). One patient was diagnosed with Morbus Wegener, the other with a bacterial pneumonia. In neither of the patients the recovery of HSV-2 was associated with morbidity or mortality.

Results of Direct Immunofluorescent staining of cytospin preparations

Since a direct immunofluorescent HSV-1 staining of a cytospin preparation from a BALF would produce results more rapidly compared to the real-time PCR, we compared the performance of these assays on PCR positive samples. From 101 out of 120 (84%) HSV-1 PCR positive samples, cytospin centrifuged preparations were available for HSV-1 immunofluorescent staining. This included samples with a viral load below 10^5 ge/ml (n=65), of 10^5 ge/ml (n=11), 10^6 ge/ml (n=14) and 10^7 ge/ml (n=11). None of the samples with a HSV-1 viral load below 10^7 ge/ml stained positive, whilst 64% (7/11) samples exceeding 10^7 ge/ml stained positive.

Discussion

In this study, HSV-1 DNA was detected in 32% of BALF samples from ICU patients compared to 4.3% BALF obtained from the open population and 15% obtained from non-ICU patients. From the clinical and demographic variables that we studied, including age, gender, smoking, ICU admittance, concomitant VAP and SOFA score, only age above 50 years and the patient group (ICU, non-ICU and community) of the patient appeared to be independent predictive factors for HSV-1 shedding. A significantly higher 14-day in-hospital mortality was observed in patients with HSV-1 loads $>10^5$ ge/ml compared to patients with HSV-1 loads $\leq 10^5$ ge/ml in BALF, 41% versus 20% respectively. HSV-1 viral loads in BALF exceeding 10^5 ge/ml reflected serious disease and was associated with an increased mortality rate of 21% in this study.

Sero-epidemiological studies have shown a worldwide variation in HSV seroprevalence according to socio-economic status, geographical region and sexual activity with measured rates of 50-80% and of 13-41% for HSV-1 and HSV-2 respectively[22].The most common manifestations are self-limited oral and genital lesions. Although rare, the most severe presentation of HSV infection is encephalitis in immunocompetent patients[23]. Visceral infections of oesophagus, liver and lung are considered to be extremely rare except in severely immunosuppressed patients[24,25]. However, animal models, case reports and pilot studies showed that a HSV-1 pneumonia is not exclusively linked to a state of immunosuppression[5,26,27]. Moreover, two recent reports, with a large number of patients included, detected HSV-1 in ICU patients that were not considered to be primarily immunocompromised[10,28]. In both studies a significant adverse effect of HSV-1 shedding in the respiratory tract on clinical outcome was established. In the first study, HSV-1 detection was performed with viral culture, a method that is not quantitative and has a sensitivity far

below that of PCR. In the second study, a nested PCR assay was used. However this assay, which was HSV-1 specific, was not quantitative, and did not include controls for monitoring the process from nucleic acid extraction through amplification.

Since HSV can be detected in the pharynx of as much as 5 % of adults in the general population[1] we applied a quantitative real-time PCR on BALF in order to establish a cut-off value for the HSV-1 and -2 loads that may be predictive for a poor outcome. HSV-1 DNA was detected in 4.3% of BALF samples obtained from the open population, 15% of BALF samples obtained from non-ICU patients and 32% of BALF samples from ICU patients. Overall detection rate in samples obtained from the hospital population was 27%. The detection rate found in this study is similar to the 27% that has been reported in a pilot study using BALF and PCR in ICU patients[28]. In another study, a lower prevalence was found (16%). This study, however used viral culture instead of the more sensitive PCR technique to detect HSV[10]. However, a third study showed a much higher prevalence of up to 64%[29], probably because their study population consisted only of severely ill ICU patients who were on ventilation for more than five days. From the clinical and demographic variables that we studied, including age, gender, smoking, ICU admittance, concomitant VAP and SOFA score, only age above 50 years and ICU admittance of the patient appeared to be independent predictive factors for HSV-1 shedding with odds ratios of 3.7 for non-ICU patients and 9.6 for ICU-patients as compared to Community patients.

In our studied group, an overall mortality of 35% was observed. The 14 day in-hospital mortality in patients with a HSV-1 load in BALF $\leq 10^5$ ge/ml was 20% compared to 41% in patients with a load $>10^5$ ge/ml. Thus, a HSV-1 viral load of $>10^5$ ge/ml was associated with 21% increase in mortality. A recent study by Luyt *et al.*[29] showed the patients they classified as having bronchopneumonitis had BALF viral loads of 10^2 to 10^8 copies/10^6 cells present in BALF. In this study, only ICU patients with prolonged ventilation (>5 days) were included. They classified their patients as having bronchopneumonitis by clinical suspicion of lower respiratory tract infection associated with the presence of HSV-1 in the lower respiratory tract and specific herpetic inclusions in BALF or bronchial biopsies. Using the HSV-1 viral load in BALF to predict the presence of HSV-1 bronchopneumonitis led to a sensitivity of 81% and a specificity of 83% at a cut-off value of 10^4 copies/10^6 cells present in BALF. The fact that they found a lower cut-off value may be due to the fact that they normalised the HSV-1 load in BALF by the number of cells in BALF, whilst we used the dilution factor of BALF compared to ELF. Since the number of cells present in BALF increase due inflammation[30] and the total cell count of BALF depends upon technical variables[31], the number of copies HSV per cells present in BALF may not be the most objective way of representing the HSV load in BALF.

In a recent study, it was concluded that HSV-1 has a low pulmonary pathogenicity[32]. This notion was based on a normal capillary permeability measured in the lungs of only four critical ill patients without quantifying HSV-1 in the respiratory tract. Although the study was not designed to prove HSV-1 to be the absolute cause of mortality, in at least two of the four patients with a HSV-1 in BALF of $>10^5$ ge/ml HSV-1 pneumonia was determined as the cause of death at post-mortem examination. These results indicate that HSV-1 could play a causative role in the development of respiratory pathology, as was previously described for immunocompromised patients[6,33,34]. Apart from the more general causes of immunosuppression, such as immunosuppressive therapy, it has been shown that major surgery, trauma and excessive blood loss may also cause a dramatic decrease in cell-mediated immunity and hence induce the reactivation of HSV-1.

Besides the presence of HSV-1 in BALF, we also determined the presence of HSV-2. In only two out of 521 BALF samples HSV-2 was recovered, with relatively low loads of 253 and 104 ge/ml. This finding corresponds with the notion that HSV-1 infections in our population are generally restricted to the oropharynx, whereas HSV-2 infections are usually limited to the genital mucosa.

Using HSV-1 immunofluorescent staining of the BALF cytospin-preparations we were able to identify 7/11 (64%) of samples with quantities of HSV-1 exceeding 10^7 ge/ml including the two patients that died from a HSV-1 pneumonia. Since this technique could only detect 7 out of 36 (19%) of the HSV-1 loads exceeding 10^5 ge/ml, and only approximately half of the samples exceeding 10^7 ge/ml, it was of no additional value in the diagnostic workup of BALF.

Although this study shows an increase in mortality in patients with a HSV-1 load in BALF exceeding 10^5 ge/ml, the design of this study was insufficient to prove HSV-1 to be the cause of death in all but 2 patients. Due to the retrospective nature of the study it was not possible to correlate HSV-1 load in BALF with adequate therapy (aciclovir) and clinical outcome. A large prospective, randomized medical intervention study in a wide range of critically ill patients is needed. Using the proposed cut-off level of 10^5 ge/ml HSV-1 in BALF patients this may provide the opportunity to ultimately unravel the role of HSV-1 in pulmonary disease.

In conclusion, HSV-1 was present in approximately one third of all BALF samples acquired from ICU patients. Furthermore, our results indicated that a HSV-1 load in BALF of $>10^5$ ge/ml is an independent predictor for a poor outcome in critically ill patients, with an increase in mortality rate of 21%. It remains to be determined whether HSV-1 is causally linked with a low survival or whether it is a marker of a severely disturbed immune system. Until additional data of a large prospective, randomised intervention study are

available, the administration of aciclovir in ICU patients with a HSV-1 load in BALF exceeding 10^5 ge/ml should be considered.

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