Chapter 5

Clinical correlates of herpes simplex virus type 1 loads in the lower respiratory tract of critically ill patients

Evelien Assink- de Jong, AB Johan Groeneveld, Annika Pettersson , Alex Koek, Christina MJE Vandenbroucke-Grauls, Albertus Beishuizen and Alberdina M Simoons-Smit

J ClinVirol. 2013 Sep;58(1):79-83

Abstract

The significance of isolation of herpes simplex virus (HSV) type 1 from the lower respiratory tract in critically ill patients on mechanical ventilation is still unclear. In the current study, we used polymerase chain reaction techniques to quantify HSV-1 to further evaluate its role. The hypothesis was that high loads reflect invasive pulmonary disease with prolonged mechanical ventilation and increased mortality, as opposed to shedding from the upper respiratory tract, which leads to lower viral loads. We prospectively studied 77 consecutive patients admitted to the intensive care unit and analyzed 136 tracheal aspirates or bronchoalveolar lavage fluids, taken when clinically indicated in the diagnostic workup of fever, radiologic pulmonary infiltrates, progressive respiratory insufficiency or combinations. Samples were cultured for bacteria and yeasts according to routine microbiological methods and HSV-1 loads were determined by real time quantitative PCR. Viral loads were expressed per number of cells recovered. HSV-1 load directly related to the simplified acute physiology score II (r_s=0.47, P=0.04) when the first specimen taken proved positive for HSV-1. HSV-1 positivity concurred with *Candida* spp. colonization. Patients with and without a HSV-1 load did not differ with respect to pulmonary and systemic courses and vital outcomes. In conclusion, the data suggest that HSV-1 in the lower respiratory tract originates from shedding in the upper respiratory tract in about 30% of critically ill patients, following immune suppression and reactivation, without invasively infecting the lung. No attributable mortality was observed.

Introduction

Critical illness, immune suppression and mechanical ventilation predispose to nosocomial infections, which in turn increase morbidity and mortality. Upper respiratory tract shedding of reactivated latent herpes virus simplex (HSV) type 1 is increasingly recognized in critically ill patients, even when not prior immune-compromised, but the clinical significance and therefore need for antiviral treatment remains largely unclear, unless associated with visible mucosal lesions positive for HSV-1 in the oropharynx and lips.[1-7]

HSV-1 positivity of lower respiratory tract samples in the critically ill varies from 6 to 70%, depending on inclusion criteria and study design, whereas risk factors, such as immunodeficiency, vary among studies.[3,8-16] It is now thought that immune suppression, for instance following prior bacterial infection or sepsis, and resultant upper respiratory tract shedding is the major cause of lower respiratory tract HSV-1 positivity,[3,10] but tracheobronchial and alveolar pathogenicity remain difficult to establish.[2,7,9,17-19] Alternatively, hematogenous spread (detection of HSV in circulating lymphocytes or buffy coat) or reactivation of latent infection within vagal nerve ganglia with spread along the nerve may lead to viral pneumonia in susceptible patients, as documented by pathologic studies, while cross-contamination can be the origin of outbreaks in the intensive care unit (ICU).[2,4,7,8,18,20] In any case, direct demonstration of HSV-1-induced tissue damage, by airway disease on bronchoscopy (herpetic tracheobronchitis), typical findings on chest radiography or computer tomography scanning and characteristic cytologic or histologic abnormalities to truly diagnose HSV-1 related pulmonary disease, is often lacking, even in studies where HSV-1 is considered pathogenic for the lungs.[2,3,7-9,19,21,22,30] HSV-1 in the lower respiratory tract may also predispose to superimposed bacterial ventilator-associated pneumonia (VAP)[9,13,14,24], so that bacterial causes may have been overlooked in some reports suggesting VAP caused by HSV-1 in about 20% of critically patients on prolonged mechanical ventilation [8,14,22,24,25] Lower respiratory tract HSV-1 positivity is regarded as mostly harmless by other investigators.[4,11,17,21,22,25] Mortality may be increased, but not independently of age and severity of underlying disease, in patients with HSV-1 in some [3,8,10,13,21,26] but not in other studies.[9,12,15]

HSV-1-associated tracheobronchitis or pneumonia in the critically ill are thus controversial entities, with differing etiology, epidemiology, criteria for diagnosis and impact on the clinical courses of these patients among studies.[2,7,21,26] Polymerase chain reaction (PCR) techniques to quantify viral DNA load may help to solve the controversy on the marker or mediator role of HSV-1. Indeed, high viral loads are more likely to reflect viral replication and thus tissue damage than low loads.[9,11,21] Prior prospective studies on HSV-1 loads in the critically ill suggest that high loads are associated with viral pneumonia and a downhill clinical course, although results vary widely among these studies.[8-12,14,15,21,26] Therapy with acyclovir, an antiviral drug active against HSV-1, prevented expression of the virus in upper or lower respiratory tract in mechanically ventilated patients with acute respiratory

distress syndrome (ARDS), but outcome of the patients did not improve, possibly because of lack of statistical power of these studies.[16]

We hypothesized that a high HSV-1 load in the lower respiratory tract in critically ill patients relates to severe underlying disease, clinical pulmonary infection and a downhill clinical course, thereby necessitating antiviral treatment. We therefore prospectively studied the role of HSV-1 in the lower respiratory tract of consecutive patients admitted into the ICU and analysed tracheal aspirates and bronchoalveolar fluids obtained for clinical reasons by quantitative real-time PCR assay. Clinical and infectious pulmonary and overall courses were studied according to (serial changes in) HSV-1 loads and risk factors for the latter were examined.

Patients and methods

Patients and data collection.

From June until August 2006, 136 consecutive tracheal aspirates (n=130) or bronchoalveolar lavage fluids (n=6) were obtained from 77 adult (>18 years) patients admitted to the ICU of the VU University medical center in this prospective observational study. Informed consent was waived. The specimens were obtained when pulmonary infection was suspected on clinical grounds in the presence of fever, pulmonary infiltrates on chest radiography, progressive respiratory insufficiency or combinations. Patient characteristics, including age, sex, body mass index, comorbidities, source and reason for admission and the Simplified Acute Physiology Score II (SAPS II) upon ICU admission were noted. Furthermore, data collection was performed on pulmonary, systemic and treatment parameters on the day of tracheal aspiration or bronchoalveolar lavage. Systemic inflammatory response syndrome criteria (SIRS), sequential organ failure assessment (SOFA) score,[27] the lung injury score (LIS) and the clinical pulmonary infection score (CPIS) were also assessed. Patients meeting 2 or more SIRS criteria were considered to suffer from the syndrome. To calculate the LIS,[28] we used routine chest radiographs, blood gas analyses and ventilator settings, when appropriate. The LIS varies between 0 (no lung injury) and 4. Consolidations on chest radiography are expressed as number of affected quadrants (0-4). Variables required to calculate the CPIS score,[29] were recorded, including body temperature, routinely obtained leukocyte counts (and band neutrophils), the arterial PO_2 to inspiratory O_2 fraction, P_aO_2/F_iO_2 ratio, the quantity of tracheal secretions, the result of bacterial cultures and chest radiography. The maximum CPIS is 12, with values above 6 in the presence of mechanical ventilation for 48 h or more and recovery of a (non-viral) pathogen from tracheal aspirate or bronchoalveolar lavage fluid regarded as evidence for ventilator-associated pneumonia. Clinical evidence for herpes labialis and antiviral (acyclovir) and antibiotic therapy within 1 week around sampling for HSV-1 were recorded. Treatment with acyclovir was divided into prophylactic, empiric or therapeutic treatment. Patients were followed up until discharge or death in the ICU. Durations of stay and mechanical ventilation were recorded.

HSV-1 real-time PCR

Real-time PCR to determine the HSV-1 load was performed on all samples of trachea aspirates or bronchoalveolar lavage fluids. Hundred µl of specimen was incubated with 12 units of proteinase K for 1h at 56 °C. Thereafter the specimens were diluted 1:100 before isolation to avoid overloading of the isolation robot. From PCR negative and weakly positive samples DNA was isolated again from undiluted specimens. One µl of diluted Phocine Herpesvirus (PhHV) was added prior to extraction to control DNA isolation and PCR inhibition. DNA extraction was performed using the NucliSens EasyMAG platform with the specific A stool protocol, as described by the manufacturer (BioMérieux, Lyon, France). Purified nucleic acids were eluted in 100 µl of elution buffer and stored at -20 °C until further analysis. The HSV-1 load was determined by real-time PCR using a standard consisting of a quantified plasmid (pGEM T-easy) containing the PCR target. The plasmid was quantified using a quality control sample from QCMD (http://www.qcmd.org/) as a standard. DNA isolation and PCR Inhibition were controlled by a separate PhHV PCR.[11] The number of cells in the specimens was determined by measuring leukocyte DNA with a quantitative human leukocyte antigen PCR (HLA DQA1) an comparing this to a standard of DNA isolated from blood containing human leukocytes of a known concentration. Primers and probes for the HSV-1 and HLA DQA1 PCR are described in Table 1. The PCR's were performed in a 30 µl reaction mix containing TaqMan Universal PCR Master Mix (Applied Biosystems), primers and probe, and 10 µl of DNA template. Cycling conditions were 2 min at 50 °C, followed by 10 min at 95 °C and 45 cycles of 15 s at 95 °C and 1 min at 60 °C. Amplification, detection and data analysis was performed with an ABI 7500 real-time PCR and sequence detection system (Applied Biosystems). All samples containing HSV-1 were tested four times, from DNA isolated twice at different dates. The lower detection limit was 44 copies/mL. The average HSV-1 load per cell was calculated. Finally, tracheal aspirates and bronchoalveolar lavage fluids were examined according to routine methods for non-viral pathogens and bacterial and fungal cultures were recorded. HSV-1 cultures were done on clinical grounds only.

Table 1 Primers and probes used in this study.

Oligonucleotide	Sequence 5'-3'	Concentration in PCR, nM
HSV-F	ATGACCAAGTGGCAGGARG	300
HSV-R	GGTCAGGTTGGTGGTGAAG	300
HSV1-P	FAM-TGCGCTCCGAGTACGGCG-TAMRA	150
HLA-F	TTGTACCAGTTTTACGGTCCC	830
HLA-R	TGGTAGCAGCGGTAGAGTTG	830
HLA-P	FAM-TTCTACGTGGACCTGGAGAGGAAGGAG-TAMRA	200

Statistical analysis

Many data were distributed non-normally (Kolmogorov-Smirnov test $P<0.05$). Data are therefore expressed as median (range) or as number of patients (percentage) where appropriate. Mann-Whitney U or Fisher exact tests were used, where appropriate. We used generalized estimating equations (GEE), after logarithmic transformation to normalise distributions to determine differences according to HSV-1 loads, taking repeated measurements in patients into account. This also allowed to assess the potential contribution of (changes in) viral load to disease severity measures on mortality prediction. The Spearman correlation coefficient was used, where appropriate. All tests were two-sided and a P-value <0.05 was considered statistically significant. Exact P values are given, unless <0.001.

Results

One-hundred-thirty-six specimens (130 tracheal aspirates and 6 bronchoalveolar lavage fluids) were obtained from 77 patients, at day 1-56 after admission in the ICU. Of these, 39 (28%) were positive and 97 (71%) were negative by HSV-1 PCR. Twenty-six patients (34%) were HSV-1 positive at any time of ICU admission and 51 were HSV-1 negative (66%). Repeated samples (2-8) were obtained from 59 patients; in 15 of them the HSV-1 load was positive in at least one specimen.

Patient characteristics

Patient characteristics did not differ between HSV-1-positive (at least one specimen) or HSV-1-negative patients (Table 2). Five HSV-1-negative patients were immunosuppressed, because of corticosteroid administration for systemic lupus erythematosus or lung carcinoma, or because of chemotherapy for non-seminoma testis, acute myeloid leukaemia or chronic myelomonocytic leukaemia. Lengths of stay in the ICU tended to be longer in HSV-1 positive than negative patients, particularly prior to first sampling for HSV-1.

There was no difference in disease severity or mortality between HSV-1 positive and negative patients. In multivariate analysis (GEE), mortality in the ICU was solely predicted by SOFA score at the day of testing ($P=0.003$) and not by (changes in) HSV-1 load (per cell, data not shown). The 4 patients who died had HSV-1 <44 copies/mL. In only 2 patients, serial HSV-1 loads became negative during their stay in the ICU, none of them died on the ICU.

Table 2 Patient characteristics according to herpes simplex virus type 1 (HSV-1) status			
	HSV-1+	HSV-1 -	P
	n=26	n=51	
Age, year	66 (40-89)	63 (15-94)	0.33
Gender , male	17 (65)	34 (67)	1.00
BMI, kg/cm2	25 (20-33)	26 (15-37)	0.81
SAPS II	48 (24-98)	43 (11-78)	0.42
Days from admission to first specimen	3 (1-24)	2 (1-57)	0.01
Duration mechanical ventilation, days	16 (1-69)	9 (1-53)	0.19
ICU length of stay, days	17 (3-87)	10 (1-59)	0.20
ICU mortality	4 (15)	13 (25)	0.39
Underlying diseases			
Heart disease	15 (58)	20 (39)	0.15
Lung disease	5 (19)	9 (18)	1.00
Renal disease	3 (12)	2 (4)	0.33
Neurological disease	3 (12)	7 (14)	1.00
Malignancy	3 (12)	10 (20)	0.52
Immune deficiency	2 (8)	5 (10)	1.00
Source of admission			0.32
Home	11 (42)	18 (35)	
Other hospital	7 (18)	9 (27)	
Internal medicine	1 (4)	13 (26)	
Surgery	5 (19)	9 (18)	
Other	2 (4)	2 (4)	
Reason of admission			0.24
Major surgery	7 (27)	12 (24)	
Respiratory	6 (23)	21 (41)	
Pneumonia	2 (8)	-	
Renal	1 (4)	-	
Neurological	5 (19)	5 (10)	
Sepsis	2 (8)	3 (6)	
Herpes labialis	1 (4)	-	0.26
Culture positive for HSV-1	2 (8)	0	0.11

Median (range), or number (percentage), where appropriate.

Table 3 Herpes simplex virus type 1 (HSV-1) load and concomitant pulmonary and systemic clinical status and treatment

	HSV-1+	HSV-1-	P(GEE)
	N=39	N=97	
HSV-1, copies/mL	462 (<44-108)	0	-
Cells, x106/mL	1.8 (0.01-66)	-	-
HSV-1, copies/cell	0.001 (0-127)	-	-
Pulmonary variables			
Lung injury score	2.2 (0.7-3.7)	2.0(0.5-3.5)	0.66
Chest radiograph (quadrants)	1 (0-4)	1 (0-4)	0.06
CPIS	6 (2-10)	5 (1-10)	0.81
Bacterial VAP	8 (21)	19(20)	0.73
Mechanical ventilation	38(97)	92(95)	0.51
Pulmonary variables without acyclovir			
Lung injury score	2.0 (0.7-3.7)	2.0 (0.5-3.5)	0.90
Chest radiograph (quadrants)	1 (0-4)	1 (0-4)	0.21
CPIS	6 (3-10)	5 (1-10)	0.29
Bacterial VAP	8 (24)	18 (23)	0.89
Mechanical ventilation	32(97)	73(94)	0.24
Systemic variables			
SIRS	38(97)	93(96)	0.95
SOFA	7 (3-15)	8 (1-17)	0.24
Renal replacement therapy	4 (10)	13 (13)	0.64
Inotropic/vasopressor drug support	20 (51)	63 (65)	0.24
Antibiotics	18 (46)	77 (79)	<0.001
Acyclovir, prophylaxis	0	1 (1)	0.65
empiric or therapeutic treatment	6 (15)	18(19)	
Steroids	20 (51)	51 (53)	0.49
SDD	22 (56)	32 (33)	0.22

Median (range) or number (percentage), where appropriate. Abbreviations: GEE, generalized estimating equations; CPIS, clinical pulmonary infection score; VAP, ventilator-associated pneumonia; SIRS, systemic inflammatory response syndrome; SOFA, sequential organ failure assessment; SDD, selective decontamination of the digestive tract.

Pulmonary and systemic status

Table 3 shows that HSV-1 positive and negative-specimens were not associated with differences in pulmonary or systemic variables, with or without acyclovir treatment: the load did not relate to LIS, CPIS, SIRS or SOFA. *Candida* spp. co-isolation was more frequent in HSV-1 positive than negative specimens (Table 4). Fig. 1 shows the direct relation between SAPS II score and HSV-1 load in the first specimen when tested positive. The r_s for SAPS II score and HSV-1 load for all 39 positive specimens was 0.41, P=0.009, and for all 139 positive and negative specimens r_s=0.21, P=0.02. In multivariate analysis (GEE), HSV-1 load related to SAPS II score only (P=0.041).

Table 4 Coisolations in lower respiratory tract specimens of herpes simplex virus type 1 (HSV-1)

	HSV- 1+ n=39	HSV-1- n=97	P (GEE)
Enterobacter spp.	3 (8)	8 (8)	0.81
Klebsiella oxytoca	2 (5)	6 (6)	0.84
Stenotrophomonas maltophilia	5 (13)	5 (5)	0.36
Pseudomonas aeruginosa	1 (3)	5 (5)	0.63
Haemophilus influenza	1 (3)	5 (5)	0.60
Escherichia coli	3 (8)	5 (5)	0.36
Staphylococcus aureus	6 (15)	8 (8)	0.32
Streptococcus pneumonia	0	1 (1)	1.00
Candida spp.	14 (36)	9 (9)	0.001
Other	3 (8)	7 (7)	0.31

Number (percentage); GEE, generalized estimating equations.
Six Candida spp. isolates were non-albicans.

Discussion

Our results suggest that the presence of HSV-1 in the lower respiratory tract in critically ill, mostly mechanically ventilated patients does not correlate with indicators of pulmonary injury but only with acuity and severity of underlying disease. The latter may have led to immune suppression, viral reactivation and shedding, concomitantly with airway colonisation by *Candida* spp., in about 30% of critically ill patients. There was no evidence for pulmonary pathogenicity nor mortality attributable to HSV-1. The observation that the respiratory HSV-1 load primarily relates to severity of underlying

disease is in agreement with other studies, suggesting low pathogenicity and a marker rather than a mediator role of HSV-1 in the respiratory tract.[4,13,17,21,25]

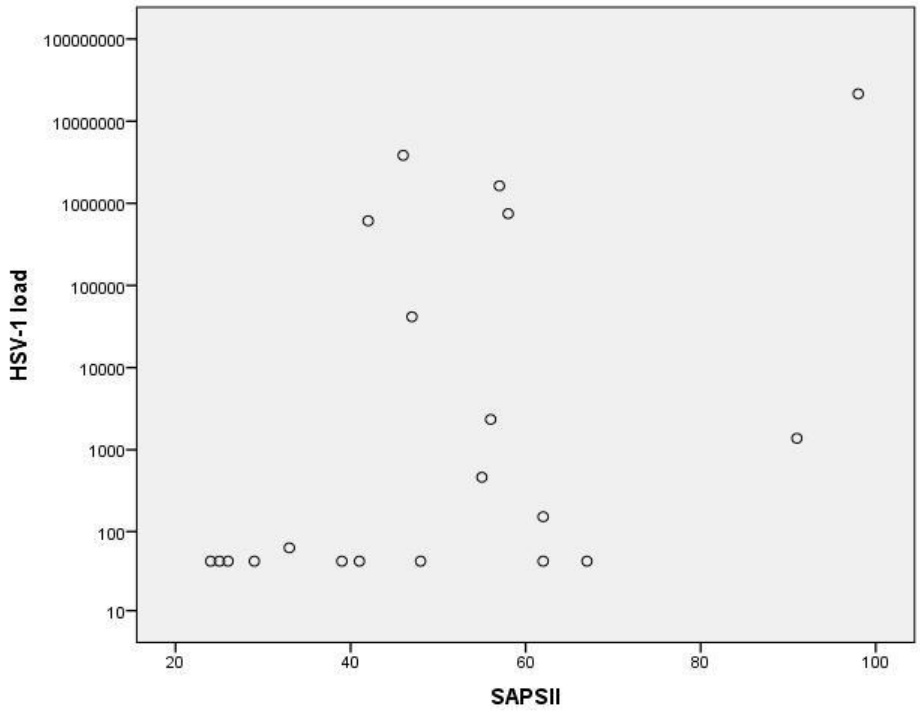

Fig 1: Simplified acute physiology score (SAPS II) versus herpes simplex virus (HSV) type-1 load (in copies/mL, semilogarithmic scale) in first specimen when positive: rs=0.47, P=0.04 (n=20 patients).

Risk factor for HSV-1 positive cultures, according to other studies include, in non prior immune compromised patients, sepsis, prolonged mechanical ventilation and prolonged durations of ICU stay and extensive organ failures.[3,8-10,22] Our study only marks underlying disease acuity and severity, as expressed by the SAPS II score, as the predominant risk factor for high HSV-1 loads.[4] In the prior studies on HSV-1 PCR-determined loads in the critically ill, risk factors included advanced age, duration of stay in the ICU, use of steroids, but not always acuity and severity of disease, [11,12,14,15,26] as in our study. HSV-1 positivity was mostly not documented as an independent risk factor for mortality [3,8,10,13,21,26], whereas the observed unchanged mortality with HSV-1 positivity in our study is supported by others.[12,15] We were also unable to establish a relation between changes in HSV-1 load in time with clinical courses in those patients with serial load observations,[12,14] again suggesting relatively harmless shedding rather than harmful tissue invasiveness. Shedding and tracheal spread apparently occurred in about 30% of patients already 3 days after ICU admission, in agreement with previous studies in the critically ill [4,12.21,24,26] but more often or earlier than in other studies.[3,4,11,13,14] Our data indicate colonisation with *Candida* spp in the lower respiratory tract, along with the HSV-1 shedding,

suggesting a common origin such as the oropharyngeal cavity and upper respiratory tract.[3,6] Indeed, *Candida* spp isolated in the lower respiratory tract may also stem from the oral cavity with subsequent downstream spreading, even in intubated patients.[30] Conversely, the association between *Candida* spp. colonisation and HSV-1 presence suggests immune suppression in the course of severe disease, in agreement with studies on septic shock.[10]

We were unable to demonstrate a relationship between HSV-1 load, even when expressed per cell, and pulmonary injury as measured by lung injury scores. This suggests lack of pulmonary pathogenicity, in agreement with previous studies,[4,11,17,25] but not with others, showing that about 30% of respiratory HSV-1-positive critically ill patients may have some evidence of viral pneumonia.[9] In the latter study, however, the criteria for pneumonia caused by the virus were relatively loose and not systematically pursued. We also demonstrated that HSV-1 can coexist with bacterial VAP, as noted before,[8,9,14,22,24,25] but our study does not suggest that HSV-1 predisposes to the latter (or vice versa) as suggested before.[9,13] Antiviral drug treatment was equally often given in HSV-positive and negative episodes, whereas a prior prospective study showed that antiviral drug therapy prevents HSV-1 shedding in patients with ARDS, without altering mortality.[16] Conversely, the observation that HSV-1 shedding in our patients was not associated with attributable mortality may also disfavour the need of routine treatment of HSV-1 shedding by antiviral drugs in the critically ill.

Limitations of the study include absence of histologic confirmation of pulmonary disease by HSV-1, which would constitute the reference standard.[18,19] Obviously, obtaining a lung biopsy routinely is not feasible in the critically ill.

In conclusion, our prospectively collected data suggest that immunosuppression results in HSV-1 reactivation, shedding and spread in the lower respiratory tract in about 30% of critically ill patients. This may not invade the lungs nor increase mortality and may therefore not require treatment.

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