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

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ABSTRACT

Background: 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.
Objectives: The hypothesis was that high loads reflect invasive pulmonary disease related to prolonged mechanical ventilation and increased mortality, as opposed to shedding from the upper respiratory tract, which leads to lower viral loads.
Study design: 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.
Results: HSV-1 load was 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.
Conclusions: 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.

1. Background

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 in the intensive care unit (ICU), 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,[3,16] but tracheobronchial and alveolar pathogenicity remain difficult to establish.[5,7,11,17–19] 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 pathology studies, while cross-contamination can be the origin of outbreaks in the ICU.[4–6,9,17,20] While isolation of HSV-1 is of unknown significance in the critical care setting, isolation in severely immunocompromised, bone marrow or organ transplant patients would be more likely to represent potentially

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fatal HSV-1 pneumonia.[17] 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.[3,5,6,9,11,18,21–23] HSV-1 in the lower respiratory tract may also predispose to superimposed bacterial ventilator-associated pneumonia (VAP),[11,12,15,24] 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[9,12,21,24,25] Lower respiratory tract HSV-1 positivity is regarded as mostly harmless by other investigators.[4,10,19,21,23,25] Mortality may be increased, but not independently of age and severity of underlying disease, in patients with HSV-1 in some[3,9,15,16,23,26] but not in other studies.[11,13,14]

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.[5,6,23,26] 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.[10,11,23] 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.[9–14,16,23,26] 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 underpowering.[8]

2. Objectives

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 analyzed 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.

3. Study design

3.1. 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 Amsterdam 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 or empiric treatment treatment. Patients were followed up until discharge or death in the ICU. Durations of stay and mechanical ventilation were recorded.

3.2. 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 1 h 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, Zaltbommel, The Netherlands). 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, Promega Corporation, Leiden, The Netherlands) 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.[12] 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, Bleiswijk, The Netherlands), 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

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

PCR, polymerase chain reaction.