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FULL PAPER

Clinical features and outcome of hospitalized patients with HSV-1 DNA in the lower respiratory tract

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SUMMARY

The aim of this study was to define the clinical impact of Herpes simplex virus-1 (HSV-1) DNA detection in the low respiratory tract of hospitalized patients. Forty-nine patients admitted to the University Hospital Tor Vergata, Rome, Italy, from May 2013 to June 2014, were analysed. Inclusion criteria were the presence or absence of HSV-1 DNA in clinical routine bronchoalveolar lavage (BAL) fluid specimens. Nineteen individuals were positive (cases) and 30 negative (controls) for the presence of HSV-1 DNA. The two groups were matched for age, gender and month of BAL collection. Cases and controls differed significantly according to length of stay in hospital (p=0.027), ICU transfer (p=0.02), disease severity (p=0.003), death (p=0.009), haematological and blood chemistry tests. Among cases, survivors and deceased patients differed significantly regarding ICU transfer (p=0.0001), mechanical ventilation (p=0.0048), disease severity (p=0.028) and risk of death (p=0.013). A trend towards higher HSV-1 loads was observed in the cases who died. These results suggest that detection of HSV-1 DNA in BAL fluid specimens is a marker of disease severity and poor outcome. Further prospective studies are necessary to deepen the clinical significance of HSV-1 DNA detection in the lower respiratory tract of hospitalized patients.

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INTRODUCTION

Herpes simplex virus 1 and 2 (HSV-1 and HSV-2) are members of the double-stranded DNA family named *Herpesviridae,* ubiquitous and highly prevalent worldwide. HSV infections account for a wide array of human diseases ranging from mild and localized clinical manifestations in immunocompetent people to severe life-threatening infections in immunocompromised individuals (Whitley and Roizman, 2001).

HSV-1 infection is generally acquired during childhood and adolescence and when symptomatic is characterized by oral or facial lesions (Whitley and Roizman, 2001). The majority of adults are HSV-1 seropositive, while the percentage of adults seropositive for HSV-2, i.e. the main etiologic agent of *herpes genitalis*, is lower albeit with variations depending on geographical and social settings (Smith and Robinson, 2002; Pica and Volpi, 2012).

HSV are neurotropic and after primary infection establish latency in neuronal tissues. Different stimuli such as sunlight, fever, hormonal changes, trauma or physical stress may trigger HSV reactivation, which may be symptomatic, with the presence of characteristic vesicular lesions or ulcers, or asymptomatic. Common sites of HSV shedding or disease are the respiratory tract, eye, oral or genital mucosa and the central nervous system. Clinical manifestations of HSV-1 reactivation include cold sores, gingivostomatitis and keratitis but also tracheobronchitis and lower respiratory tract (LRT) infections (Saugel *et al.,* 2016). Cell-mediated immunity plays a key role in the control of HSV reactivation (Nash AA, 2000).

Previous studies have implicated HSV-1 as a causative agent of pneumonitis/pneumonia in immunocompromised or immunocompetent patients (Nash and Foley, 1970; Ramsey *et al.,* 1982; Prellner *et al.,* 1992; Schuller *et al.,* 1993; Shimokawa *et al.,* 2001; Witt *et al.,* 2009). The increasing use of molecular biology techniques together with a more frequent use of fiberoptic bronchoscopy and bronchoalveolar lavage (BAL) in recent decades has led to a higher frequency of HSV detection in the LTR of hospitalized patients, raising questions about the clinic relevance of this finding (Simoons-Smi *et al.,* 2006; Van den Brink *et al.,* 2004). In particular, HSV-1 has been isolated in BAL fluid specimens from patients of the Intensive Care Units (ICU) (Cook *et al.,* 1998; Bruynseels *et al.,* 2003; Ong *et al.,* 2004; Luyt *et al.,* 2007; De Vos *et al.,* 2007; Linssen *et al.,* 2008). Critically ill patients present several risk factors for HSV reactivation: systemic stress related to the critical illness, increased stress hormones activity, immune impairment linked to multiorgan failure or treatments, and local airway trauma (Linssen *et al.,* 2008). Considering that HSV-1 can reach the LRT by aspiration of viral shedding from the upper respiratory tract (Van den Brink *et al.,* 2004), HSV-1 isolation or detection by PCR in BAL fluid specimens does not allow differentiation of true disease from asymptomatic shedding (Simoons-Smit, *et al.,* 2006; Van den Brink *et al.,* 2004).

Key words:
HSV-1, Viral load, BAL fluids, Clinical outcome, Hospitalized patients.

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Herein we compared the clinical characteristics and outcome of patients with or without HSV-1 DNA in their BAL fluids to define the impact of HSV-1 detection on the clinical outcome of hospitalized patients in our Institution.

METHODS

Patients and study design

The study included 49 patients admitted to the ICU or the Respiratory Diseases Division of the University Hospital Tor Vergata, Rome, Italy, from May 2013 and June 2014. Inclusion criteria were the documented presence or absence of HSV-1 DNA, alone or in combination with other respiratory pathogens of LRT, in clinical routine BAL fluid specimens.

The study was performed according to the Declaration of Helsinki and in accordance with the International Conference on Harmonization Good Clinical Practice Guidelines [ICH-GCP E6 (R2)]. The Ethical Committee of the University of Rome Tor Vergata approved the study protocol. All patients provided written informed consent before participating in any study-related activities.

Clinical and laboratory data of the patients at the time of BAL fluids collection were obtained from the respective medical records and were gathered anonymously for this research purpose. A list of pre-defined variables, such as demographic data, admission and discharge departments, underlying diseases or co-morbidities, reasons for admission and surgical interventions were analysed. The APR-DRGs (All Patient Refined-Diagnosis Related Groups), which assign to each case a subclass of illness severity or of risk of mortality, were calculated. Among the haematological and blood chemistry tests, white and red blood cell counts, haemoglobin and erythrocyte sedimentation rate (ESR) were analysed.

BAL fluid sampling and laboratory assessment

Bronchoscopy and BAL fluid sampling were performed in the course of disease, in case of persistent or new fever (i.e. body temperature above 38°C), or persistent or new radiographic alveolar consolidation or interstitial abnormalities of unknown origin or both.

In addition to HSV-1 DNA, BAL fluid samples were tested for the presence of nucleic acids of other viruses, including HSV-2, Human Cytomegalovirus (CMV), Epstein-Barr virus (EBV), Varicella-Zoster virus (VZV), Herpesvirus-6 (HHV-6), Herpesvirus-8 (HHV-8), Adenovirus (ADV), Enterovirus and Human immunodeficiency virus type-1 (HIV-1).

DNA/RNA were extracted from BAL fluids using the QIAsymphony DSP Virus/Pathogen kit V.1 kit and the QIASymphony^{SP} automated extractor (Qiagen, Hilden, Germany). Briefly, 1.2 ml of BAL sample was loaded on the extractor and nucleic acid eluted in 110 µl TE buffer. The obtained nucleic acid was amplified by ELITe MGB® Real-Time PCR kits targeting HSV 1 & 2, CMV, EBV, HHV6, VZV, ADV, (Elitech 100 Nanogen Group, Turin, Italy) on the 7300 ABI PRISM instrument (Applied Biosystem, Foster City, CA, USA), adding 20 µl of extracted DNA to 20 µl of amplification mixture. External standards at known concentrations (10^5, 10^4, 10^3, 10^2 genome equivalents) were added in each PCR to construct the reference curve to determine the viral titre in the samples examined. The assay detects about 10 genome equivalents (limit of detection, LOD) in the 20 µl of DNA added to the PCR mix; the linear range of quantification of the assay is between 10 to 10^6 genomes in the same volume (Turriziani *et al.,* 2014). After amplification, dedicated software allows the conversion of the viral titre determined in each sample in copies/ml taking into account the sample extracted volume, the final elution volume, the DNA volume utilized in the amplification reaction and the conversion factor. So, the limit of quantification (LOQ) of our assay is 56 viral copies/ml for HSV 1 & 2, EBV, HHV6, VZV, ADV and 72 copies/ml for CMV, while the upper limit of quantification is $5.55x10^6$ copies/ml for all the viral targets reported above. Below the lower limit of quantification, the assay reports a qualitative result as detected on not detected depending on the presence or absence of the viral target. The Enterovirus Real Time kit (Biomerieux, Florence, Italy) and Cobas Taqman HIV-1 v2.0 (Roche Diagnostics, Branchburg, NJ, USA) were also used. *Pneumocystis jiroveci* (*Pneumocystis carinii*) was amplified by conventional PCR as reported previously (Dimonte *et al.,* 2013). Cultures for aerobic, facultative anaerobic bacteria and fungi were performed using standard media.

Statistics

Statistical analysis and data processing were performed using SPSS software - version 20 for Windows™. All two-sided statistical tests were performed with a 5% significance level. Quantitative variables were analysed by descriptive statistics including mean values, standard deviation, median, minimum and maximum. Clinical and demographic characteristics were analysed by Mann-Whitney *U* test, Chi-square test and Analysis of variance (ANOVA).

RESULTS

Demographic and clinical characteristics of the patients included in this study are summarised in *Table 1*. Overall, the group consisted of 49 Caucasian individuals (15 females and 34 males), whose median age was 63 years (range 16-81 years). Nineteen patients were positive (cases) and 30 negative (controls) for the presence of HSV-1 DNA in BAL fluid specimens. The two groups of patients were matched for age, gender and month of BAL fluid sampling. There was a prevalence of males in both groups (*Table 1*).

The main diagnostic categories (MDC) were diseases or disorders of respiratory and cardiovascular system, while a minority of patients showed haematological, oncological or psychiatric disorders or infectious diseases. *Table 1* also shows diagnoses on discharge, comorbidities, mechanical ventilation and transfer from the ICU. There was a high prevalence of pulmonary diseases in the total group of patients as well as among controls, but respiratory failure occurred only among cases (*Table 1*). The median copy number of HSV-1 DNA detected in BAL fluids from cases was $71.1x10^3$/ml (range: 56/ml - $5.5x10^6$/ml). HSV-1 was the only virus detected in BAL fluids from nine out of the 19 cases (47.4%), while in the remaining ten it was co-detected with one or more other viruses, including CMV (nine patients), EBV (six patients), HSV-2 (three patients), VZV (one patient), HHV-6 (one patient), ADV (one patient), Enterovirus (one patient) or HIV-1 (one patient). No prophylaxis with acyclovir was performed. All the patients with HSV-DNA in their BAL

fluids were prescribed acyclovir (10 mg/kg i.v. three times daily). Microbiological cultures of BAL fluid specimens or PCR amplification revealed the presence of *Pseudomonas aeruginosa* (two patients), *Acinetobacter baumannii* (three patients), *Klebsiella pneumonia*e (two patients), *Pneumocystis jiroveci* (seven patients), *Enterobacter cloacae subsp cloacae* plus *Stenotrophomonas maltophili* and *Candida albicans* (one patient), and *Aspergillus niger* (one patient). There was no significant relationship between the number or type of co-infections and clinical outcome (data not shown).

As shown in Table 1, HSV-1 DNA positivity of BAL fluids was significantly associated with length of stay in hospital (p=0.027), ICU transfer (p=0.02), disease severity (p=0.003) and death (p=0.009). In addition, among cases 9 individuals died (47.4%) and 10 survived (52.6%), whereas among controls only 3 patients died (10%) and 27 survived (90%) (*Tables 1* and *2*). Higher leukocyte (p=0.011) and lower lymphocyte (p=0.0001) counts, lower haemoglobin (p=0.002) and higher ESR (p=0.026) were significantly associated with HSV-positivity in BAL fluid samples (*Table 1*).

Between cases who survived and those who died there was a statistical significant difference in relation to the ICU transfer (p=0.0001), mechanical ventilation (p=0.0048), disease severity (p=0.028) and risk of death (p=0.013), but not to the results of the blood tests (*Table 2*). The median number of HSV-1 DNA copies detected in BAL fluids from the cases who survived was lower than in those who

Table 1 - *Demographic and clinical characteristics of patients.*

	All patients (n=49)	*Cases (n=19)*	*Controls (n=30)*	p-*value**
Age in years, mean (SD)	59.7 (15.4)	59.8 (16.6)	59.6 (14.9)	
median (range)	63 (16-81)	62 (21-80)	65 (16-81)	0.88
Gender, male/female	34/15	12/7	22/8	0.45
Diagnosis on discharge				
Pulmonary disease	29	5	24	0.0001
Cardiac disease	8	4	4	0.7
Respiratory failure	3	3	0	0.053
Circulatory failure	2	2	0	0.14
Other	7	5	2	0.09
Comorbidity				
COPD	3	2	1	0.3
Cardiovascular disease	11	0	11	0.003
Diabetes	5	1	4	0.36
Lenght of stay in days, median (range)	12 (1-137)	15 (6-112)	10.5 (1-137)	0.027
Mechanical ventilation	9	7	2	0.071
Death	12	9	3	0.009
Transferred from ICU	14	9	5	0.02
Surgery	18	7	11	0.99
APR-DRG (disease severity), median	2	3	2	0.003
1	10	1	9	
2	16	5	11	
3	16	7	9	
4	7	6	1	
APR-DRG (risk of death), median	2	2	2	0.09
1	12	3	9	
2	22	8	14	
3	10	4	6	
4	5	4	1	
Laboratory Tests, median				
WBC x1000/ml	9.7	12.83	8.22	0.011
LYMPH x1000/ml	1.43	0.87	1.87	0.0001
Hemoglobin (g/dl)	12.55	10.7	12.9	0.002
ESR (mm/h)	40	92	36	0.026
HSV-1 (copy number x1000/ml)	-	71.12	-	-

COPD = Chronic obstructive pulmonary disease; ICU = Intensive Care Unit; APR-DRG = All Patient Refined - Diagnosis Related Groups; WBC = White Blood Cells; LYMPH = Lymphocytes; ESR = Erythrocyte Sedimentation Rate; *by Mann-Whitney U test or Chi-square test, where appropriate.

died (i.e. 28.1x10^3 copies/ml [range: 56-472.3x10^3] *versus* 114.1x10^3 copies/ml [range: 111-5.5x10^6], respectively), albeit this datum did not reach the statistical significance (p=0.90; *Table 2*). Moreover, 4 of the 9 cases who died, but only one of the 10 cases who survived, had more than one million HSV-1 copies *per* ml in their BAL fluid samples, even though the difference was not statistically significant (p=0.22).

DISCUSSION

In recent decades, increased attention has been devoted to the frequency and clinical relevance of herpes virus reactivations in hospitalized patients. In particular, HSV-1 invasion of the lower respiratory tract of critically ill patients has been extensively investigated (Cook *et al.,* 1998; Bruynseels *et al.,* 2003; Ong *et al.,* 2004; Luyt *et al.,* 2007;

Table 2 - *Characteristics of patients with HSV-1 DNA in their BAL fluids in relation to the clinical outcome.*

	Survivors (n=10)	*Dead (n=9)*	p-value*
Age in years, mean (SD)	56.7 (19.1)	63.3 (13.5)	
median (range)	61 (21-79)	62 (39-80)	0.50
Gender, male/female	7/3	5/4	0.51
Diagnosis on discharge			
Pulmonary disease	3	2	1.0
Cardiac disease	2	2	1.0
Respiratory failure	1	2	0.58
Circulatory failure	0	2	0.21
Other	4	1	0.30
Comorbidity			
COPD	0	2	0.21
Cardiovascular disease	0	0	-
Diabetes	1	0	1
Lenght of stay in days, median (range)	27.5 (6-103)	11 (7-112)	0.21
Mechanical ventilation	0	7	0.0048
Death	0	9	-
Transferred from ICU	3	6	0.0001
Surgery	3	4	0.65
APR-DRG (disease severity), median	2.5	4	0.028
1	1	0	
2	4	1	
3	4	3	
4	1	5	
APR-DRG (risk of death), median	2	3	0.013
1	2	1	
2	7	1	
3	1	3	
4	0	4	
Laboratory Tests, median			
WBC x1000/ml	12.85	11.66	0.78
LYMPH x1000/ml	1.07	0.84	0.16
Hemoglobin (g/dl)	12.4	8.4	0.11
ESR (mm/h)	70	92	0.78
HSV1 (copy number x1000/ml)	28.13	114.11	0.90

COPD = Chronic obstructive pulmonary disease; ICU = Intensive Care Unit; APR-DRG = All Patient Refined - Diagnosis Related Groups; WBC = White Blood Cells; LYMPH = Lymphocytes; ESR = Erythrocyte Sedimentation Rate; *by Mann-Whitney U test or Chi-square test, where appropriate.

De Vos *et al.,* 2007; Costa *et al.,* 2012; Assink-de Jong *et al.,* 2013). Indeed these patients show altered immune and inflammatory responses, which in turn may favour viral reactivation. Whether HSV reactivation represents a cause or a consequence of the poor outcome of critically ill patients is currently debated (Van den Brink *et al.,* 2004; Simoons-Smit *et al.,* 2006). Some authors hypothesize that viral reactivation is caused by the immune impairment observed in the course of sepsis or multi-organ failure, thus representing a marker of illness severity (Hotchkiss *et al.,* 2009; Walton *et al.,* 2014). Other investigators report the association of HSV-1 detection in LRT with clinical episodes of ventilator-associated pneumonia (VAP) supporting a pathogenic role of the agent in the specific disease (Scheithauer *et al.,* 2010; Tuxen *et al.,* 1987; Coisel *et al.,* 2012; Traen *et al.,* 2014).

Besides HSV-1, there is also increasing evidence that reactivation of CMV, EBV or HHV-6 is frequent and often associated with mortality or morbidity in ICU patients (Connolly *et al.,* 1994; Kalil *et al.,* 2009; Razonable *et al.,* 2002; Desachy *et al.,* 2001). Indeed, because of their elevated seroprevalence and their tendency to establish latent infection and to reactivate in association with host defences breakdown, herpesviruses detection in clinical specimens might represent a useful marker of disease severity. In addition, a relationship between CMV and EBV reactivations, which results in an increased mutual pathogenicity in critically ill patients, has been reported very recently (Libert *et al.,* 2015).

To gain insight into this issue, we compared the clinical characteristics and outcome of patients with or without HSV-1 DNA in their BAL fluids aiming to define the clinical impact of HSV-1 reactivation in patients admitted to the ICU or the Respiratory Division of our Institution.

Our results show that HSV-1 DNA positivity of BAL fluids is significantly associated with the length of stay in hospital, ICU transfer, disease severity and death. Indeed, almost a half of the cases but only ten percent of the controls died. Our cases showed significant leucocytosis and lymphopenia, lower haemoglobin and higher ESR than controls, indicating a poorer health status, which may favour endogenous reactivation of the virus, as prospected previously (Simoons-Smit *et al.,* 2006). Several cases showed the co-presence of CMV, EBV and other viral, bacterial or fungal opportunistic pathogens in BAL fluid specimens, but we did not find a significant relationship between the number or type of co-infections and clinical outcome. To date, there is no general agreement on the impact of multiple viral reactivations and other co-infections in this clinical context (Libert *et al.,* 2015). The exact nature of their link with different conditions of immune impairment also needs further investigation. Reasonably, lymphopenia and a general dysfunction of innate and adaptive cell immunity may be determinant, but the effects might not be univocal (Nash and Foley, 1970; Nash AA, 2000).

Among cases, we again found a significant difference between survivors and deceased patients according to selected clinical variables such as the ICU transfer, disease severity, risk of death and mechanical ventilation. Consistently, several studies outline the impact of mechanical ventilation on the frequency of HSV detection in the LRT of critically ill patients and on their outcome (De Vos *et al.,* 2009; Luyt *et al.,* 2007; Coisel *et al.,* 2012). The results of blood tests of the cases who survived did not significantly differ from those who died, albeit clearly indicating the worse health status of the deceased. Similarly, the median copy number of HSV-1 DNA found in BAL fluids from survivors was lower than in those who died, but the difference was not statistically significant. Thus, probably, the lack of statistical significance between the two groups is attributable to the low number of the patients studied, which also represents a limitation of this study. There is also a problem of viral quantitation, due to the lack of normalization of BAL fluid dilution, which could explain the lack of significance of differences of viral load.

In conclusion, in agreement with part of the international literature, our results indicate that the presence of HSV-1 DNA in BAL fluid specimens is a marker of a clinically poor condition and more severe outcome in hospitalized patients. Further prospective longitudinal studies are necessary to improve our knowledge on the pathogenic role of the HSV-1 DNA presence in the lower respiratory tract of hospitalized patients.

Conflict of interest

Authors declare no conflict of interest.

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References

Assink-de Jong E., Groeneveld A.B., Pettersson A.M., Koek A., Vandenbroucke-Grauls C.M., et al. (2013). Clinical correlates of herpes simplex virus type 1 loads in the lower respiratory tract of critically ill patients. *J Clin Virol.* **58**, 79-83.

Bruynseels P., Jorens P.G., Demey H.E., Goossens H., Pattyn S.R. et al. (2003). Herpes simplex virus in the respiratory tract of critical care patients: a prospective study. *Lancet.* **362**, 1536-1541.

Coisel Y., Bousbia S., Forel J-M., Hraiech S., Lascola B. et al. (2012). Cytomegalovirus and Herpes Simplex Virus Effect on the Prognosis of Mechanically Ventilated Patients Suspected to Have Ventilator-Associated Pneumonia. *PLoS One.* **7**, e51340.

Connolly M.G. Jr, Baughman R.P, Dohn M.N., Linnemann C.C. Jr. (1994). Recovery of viruses other than cytomegalovirus from bronchoalveolar lavage fluid. *Chest.* **105**, 1775-1781.

Cook C.H., Yenchar J.K., Kraner T.O., Davies E.A., Ferguson R.M. (1998). Occult herpes family viruses may increase mortality in critically ill surgical patients. *Am. J. Surg.* **176**, 357-360.

Costa C., Sidoti F., Saldan A., Sinesi F., Balloco C., et al. (2012) Clinical impact of HSV-1 detection in the lower respiratory tract from hospitalized adult patients. *Clin Microbiol Infect*. **18**, E305-307.

De Vos N., Van Hoovels L., Vankeerberghen A., Van Vaerenbergh K., Boel A., et al. (2007). Monitoring of herpes simplex virus in the lower respiratory tract of critically ill patients using real-time PCR: a prospective study. *Clin. Microbiol. Infect*. **15**, 358-363.

Desachy A., Ranger-Rogez S., Francois B., Venot C., Traccard I., et al. (2001). Reactivation of human herpesvirus type 6 in multiple organ failure syndrome. *Clin. Infect. Dis.* **32**, 197-203.

Dimonte S., Berrilli F., D'Orazi C., D'Alfonso R., Placco F., et al. (2013). Molecular analysis based on mtLSU-rRNA and DHPS sequences of Pneumocystis jirovecii from immunocompromised and immunocompetent patients in Italy. *Infect. Genet. Evol.* **14**, 68-72.

Hotchkiss R.S., Coopersmith C.M., McDunn J.E., Ferguson T.A. (2009) The sepsis seesaw: tilting toward immunosuppression. *Nat Med.* **15**, 496-497.

Kalil A.C., Florescu D.F. (2009). Prevalence and mortality associated with cytomegalovirus infection in non-immunosuppressed patients in the intensive care unit. *Crit. Care. Med.* **37**, 350-358.

Libert N., Bigaillon C., Chargari C., Bensalah M., Muller V., et al. (2015). Epstein-Barr virus reactivation in critically ill immunocompetent patients. *Biomed. J.* **38**, 70-76.

Linssen C.F., Jacobs J.A., Stelma F.F., van Mook W.N., Terporten P. et al. (2008). Herpes simplex virus load in bronchoalveolar lavage fluid is related to poor outcome in critically ill patients. *Intensive Care Med.* **34**, 2202-2209.

Luyt C.E., Combes A., Deback C., Aubriot-Lorton M.H., Nieszkowska A., et al. (2007). Herpes simplex virus lung infection in patients undergoing

prolonged mechanical ventilation. *Am. J. Respir. Crit. Care. Med.* **175**, 935-942.
Nash A.A. (2000). T cells and the regulation of herpes simplex virus latency and reactivation. *J. Exp. Med.* **191**, 1455-1458.
Nash G., Foley F.D. (1970). Herpetic infection of the middle and lower respiratory tract. *Am. J. Clin. Pathol.* **54**, 857-863.
Ong G.M., Lowry K., Mahajan S., Wyatt D.E., Simpson C., et al. (2004). Herpes simplex type 1 shedding is associated with reduced hospital survival in patients receiving assisted ventilation in a tertiary referral intensive care unit. *J. Med. Virol.* **72**, 121-125.
Pica F., Volpi A. (2012). Public awareness and knowledge of herpes labialis. *J. Med. Virol.* **84**, 132-137.
Prellner T., Flamholc L., Haidl S., Lindholm K., Widell A. (1992). Herpes simplex virus: the most frequently isolated pathogen in the lungs of patients with severe respiratory distress. *Scand. J. Infect.Dis.* **24**, 283-892.
Ramsey P.G, Fife K.H., Hackman R.C., Meyers J.D., Corey L. (1982). Herpes simplex virus pneumonia: clinical, virologic, and pathologic features in 20 patients. *Ann. Intern. Med.* **97**, 813-820.
Razonable R.R., Fanning C., Brown R.A., Espy M.J., Rivero A. et al. (2002). Selective reactivation of human herpesvirus 6 variant a occurs in critically ill immunocompetent hosts *J. Infect. Dis.* **185**, 110-113.
Saugel B., Jakobus J., Huber W., Hoffmann D., Holzapfel K., et al. (2016) Herpes simplex virus in bronchoalveolar lavage fluid of medical intensive care unit patients: Association with lung injury and outcome. *J. Crit. Care.* **32**, 138-144.
Scheithauer S., Manemann A.K., Kruger S., Hausler M., Kruttgen A., et al. (2010). Impact of herpes simplex virus detection in respiratory specimens of patients with suspected viral pneumonia. *Infection.* **38**, 401-405.
Schuller D., Spessert C., Fraser V.J., Goodenberger D.M. (1993). Herpes simplex virus from respiratory tract secretions: epidemiology, clinical characteristics, and outcome in immunocompromised and nonimmunocompromised hosts. *Am. J. Med.* **94**, 29-33.
Shimokawa S., Watanabe S., Taira A., Eizuru Y. (2001). Herpes simplex virus pneumonia after cardiac surgery: report of a case. *Surg. Today.* **31**, 814-816.
Simoons-Smit A.M., Kraan E.M., Beishuizen A., Strack van Schijndel R.J., Vandenbroucke-Grauls C. (2006). Herpes simplex virus type 1 and respiratory disease in critically-ill patients: Real pathogen or innocent bystander? *Clin. Microbiol. Infect.* **12**, 1050-1059.
Smith J.S., Robinson N.J. (2002). Age-specific prevalence of infection with herpes simplex virus types 2 and 1: A global review. *J. Infect. Dis*. **186**, S3-S28.
Traen S., Bochanen N., Ieven M., Schepens T., Bruynseels P., et al. (2014). Is acyclovir effective among critically ill patients with herpes simplex in the respiratory tract? *J. Clin. Virol.* **60**, 215-221.
Turriziani O., Falasca F., Maida P., Gaeta A., De Vito C., et al. (2014) Early collection of saliva specimens from Bell's palsy patients: quantitative analysis of HHV-6, HSV-1, and VZV. *J Med Virol.* **86**, 1752-1758.
Tuxen D.V., Wilson J.W., Cade J.F. (1987). Prevention of lower respiratory herpes simplex virus infection with acyclovir in patients with the adult respiratory distress syndrome. *Am. Rev. Respir. Dis.* **136**, 402-405.
Van den Brink J.W., Simoons-Smit A.M., Beishuizen A., Girbes A.R., Strack van Schijndel R.J. (2004). Respiratory herpes simplex virus type 1 infection/colonisation in the critically ill: marker or mediator? *J. Clin. Virol.* **30**, 68-72.
Walton A.H., Muenzer J.T., Rasche D., Boomer J.S., Sato B., et al. (2014) Reactivation of multiple viruses in patients with sepsis. *PLoS One*. **9**: e98819.
Whitley R.J., Roizman B. (2001). Herpes simplex virus infections. *Lancet*. **357**, 1513-1518.
Witt M.N., Braun G.S., Ihrler S., Schmid H. (2009). Occurrence of HSV-1-induced pneumonitis in patients under standard immunosuppressive therapy for rheumatic, vasculitic, and connective tissue disease. *BMC. Pulm. Med.* **9**, 22.