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DISSERTATION

The Role of Herpes Simplex Virus in Chemotherapy-induced Mucositis in Pediatric Patients

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I dedicate this dissertation to my parents and family

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Abstracts

In patients undergoing chemotherapy, mucositis is a common and serious complication that can significantly affect the treatment outcomes and the patients' survival rate. The pathogenesis of mucositis is divided into five biological phases: initiation, upregulation and message generation, amplification and signaling, ulceration, healing. Some factors are considered to increase the risk of oral mucositis in patients receiving chemotherapy, such as age, gender, oral health and hygiene, salivary secretory function, renal/liver function as well as chemotherapy agents and dosage. Several studies suggest a correlation of oral mucositis with leucopenia (neutropenia) and isolation of microorganisms, particularly herpes simplex virus (HSV).

Our study evaluated retrospectively the development of oral mucositis in 83 children with hematologic malignancies and solid tumours during intensive chemotherapy. The chemotherapy-induced mucositis was found in 76 patients (91.6%). Of 33 patients with mucositis examined for HSV-PCR, 15 patients (45.5%) had HSV in oral swabs or throat washings; 7 of them had repeatedly HSV-PCR positive in their oral lesions, which was detected during different mucositis episodes. 24 of 50 mucositis episodes (48%) were found positive for HSV. In the severe mucositis episodes, HSV was isolated more frequently (50% in grades 3 and 4 mucositis according to the mucositis scale of National Cancer Institute) than in mild to moderate mucositis episodes (46.4% in grades 1 and 2 mucositis). Although HSV was frequently found in children with chemotherapy-induced mucositis, there was no significant relationship between HSV presence and the severity of the chemotherapy-induced mucositis in pediatric population (*p=0.222, Chi-Square-Test or Fisher's exact test)*.

The mucositis episodes occurred mostly during leucopenia (71.7%). Furthermore, HSV was more frequently isolated in mucositis episodes associated with leucopenia (54%) and less frequent without leucopenia (30.8%).

Based on these results and considering the retrospective nature of the study, we propose to have a prospective intervention study using prophylactic acyclovir therapy in high-risk children with leucopenia and who are HSV seropositive, who develop mucositis under high intensity chemotherapy, to determine the role of acyclovir in

preventing the HSV infection that often occurs during the mucositis episode and not only complicates the course of antineoplastic treatment but could also cause a widespread systemic disease and mortality in patients with malignancy due to immunosuppression.

Zusammenfassung

Mukositis ist eine häufige und schwerwiegende Komplikation bei Patienten mit malignen Erkrankungen, die durch Chemotherapie ausgelöst wird. Sie kann die Behandlungsergebnisse und die Überlebensrate der Patienten beeinträchtigen. Die Pathogenese von Mukositis wird in fünf biologischen Phasen eingeteilt: Initiierung, Hochregulation und Meldungsgenerierung, Amplifikation und Signalisierung, Ulzeration, Heilung. Faktoren, die das Risiko der oralen Mukositis bei Patienten unter Chemotherapie erhöhen können, sind Alter, Geschlecht, orale Hygiene, Speicheldrüsenfunktion, Nieren- und Leberfunktion sowie Art und Dosierung der Chemotherapeutika. Einige Studien zeigen eine Korrelation zwischen oraler Mukositis, Leukopenie (Neutropenie) und einer Isolierung von Mikroorganismen, insbesondere Herpes Simplex Virus (HSV) auf.

In unserer Arbeit wurden 83 Kinder mit hämatologischer Malignität und soliden Tumoren während der intensiven Chemotherapie retrospektive für die Entwicklung der oralen Mukositis evaluiert. Die Chemotherapie-induzierte Mukositis wurde bei 76 Patienten (91,6%) gefunden. 15 von 33 Patienten (45,5%) mit Mukositis, die nach HSV-PCR untersucht wurden, hatten einen positiven Nachweis von HSV in oralen Abstrichen oder Rachenspülwasser. 7 von diesen 15 Patienten hatten in der HSV-PCR wiederholte positive Ergebnisse in den oralen Läsionen, die während unterschiedlicher Mukositis Episoden nachgewiesen wurden. 24 von 50 Mukositis Episoden (48%) waren HSV positive. In schweren Mukositis Episoden, war HSV etwas häufiger nachzuweisen (50% in Mukositis Grad 3 und 4 nach dem Mukositis Grad von National Cancer Institute) im Vergleich zu milden bis moderaten Mukositis Episoden (46,4% in Mukositis Grad 1 und 2). Obwohl HSV bei Kindern mit Chemotherapie-induzierter Mukositis häufig nachgewiesen wurde, gab es keine statistisch signifikante Beziehung zwischen HSV und dem Schweregrad der Chemotherapie-induzierten Mukositis bei pädiatrischen Patienten (*p=0,222, Chi-Square-Test or Fisher's exact test*).

Die meisten Mukositis Episoden traten während Leukopenie auf (71,1%). Weiterhin wurde HSV häufiger bei Mukositis Episoden während Leukopenie nachgewiesen (54%) im Gegensatz zu Phasen ohne Leukopenie (30,8%). Hinsichtlich dieses Ergebnisses

dieser retrospektiven Bewertung, wäre eine prospektive Interventionsstudie mit Acyclovir-Prophylaxe für Kinder mit hohem Risiko (HSV seropositive Kinder mit Leukopenie), die unter intensiver Chemotherapie eine orale Mukositis entwickeln, durchzuführen, um die Rolle von Acyclovir bei der Prävention der HSV Infektion zu erfassen, die während der Mukositis Episode häufig auftritt und nicht nur den antineoplastischen Therapieverlauf kompliziert sondern auch wegen Immunsuppression bei Patienten mit Malignität eine verbreitende systemische Erkrankung und Mortalität verursachen kann.

The Role of Herpes Simplex Virus in Chemotherapy-induced Mucositis in Pediatric Patients

1. Introduction

Mucositis is one of the most common complications in patients treated for malignant disease, which occurs during cytotoxic chemotherapy, radiation therapy, or bone marrow transplantation. It is characterized by inflammation and ulceration in the oro-oesophageal and gastrointestinal mucosa that results in pain, dysphagia, diarrhoea, difficulties in chewing and speaking, and dysfunction depending on the tissue affected.[1-3] Severe mucositis can greatly complicate the management of malignant disease and the survival rate of the patients, because it often leads to treatment interruption, secondary infection and sepsis.

The incidence of chemotherapy-induced mucositis ranges from 40% to 76% for patients treated with standard and high-dose chemotherapy.[4] Several studies indicated that children are more susceptible to develop oral mucositis during chemotherapy than adults.[5-7] The frequency of chemotherapy-induced oral mucositis in pediatric patients is reported at around 65% compared to adult patients at approximately 40%.[8] Mucositis occurs in almost all patients receiving radiation therapy involving the head and neck region, with the severity depending on the radiation fractionation, radiation field size and the use of combined chemoradiation. About 30-50% of patients receiving bone marrow transplantation are reported to develop mucositis during the treatment.[9]

The etiology of mucositis in patients undergoing therapy for malignant diseases is still only partly understood. Several risk factors have been suggested to contribute to the incidence of mucositis such as age, gender, oral health and hygiene, salivary secretory function, renal and liver function, type of malignancy.

It is assumed that oral mucositis is associated with self-toxic effects of the chemotherapeutic agents[10,11], non-specific direct-effect of radiation[9], therapy-induced neutropenia[12-15], Enterobacteriaceae[16] and Candida spp..[17]

Interestingly, several studies both in adult and pediatric population revealed that oral mucositis is also associated with a herpes simplex virus infection.[17-22] Based on these observations, we aimed to retrospectively determine the prevalence and intensity of mucositis in relation to the presence of herpes simplex virus (HSV) in pediatric patients treated with high intensity chemotherapy protocols. This investigation should evaluate the need to further establish a role for an intervention study using prophylactic acyclovir therapy.

The following chapters review important information on mucositis and herpes simplex virus infections with reference to risk factors, pathogenesis and clinical scoring systems since this relates to the retrospective analysis presented.

1.1. Mucositis

Mucositis is a painful inflammation and ulceration of oro-oesophageal and gastrointestinal mucosa. It is a common complication in many patients receiving treatment for malignancy (chemotherapy, radiation therapy, bone marrow transplantation). Many studies were performed to understand more about the risk factors and pathogenesis of mucositis in patients with malignancy in order to give a better treatment and prevention against mucositis.

1.1.1. Risk factors

The risk factors of mucositis development in patients treated for malignancy are still poorly understood and sometimes conflicting. Not much is known about the risk factors of mucositis in pediatric population since the available studies were performed mostly with adult patients. A number of patient- and treatment-related factors have been suggested to associate with the incidence of oral mucositis.[23]

a.) Patient-related factors

Several patient-related factors associated with the frequency, duration, and severity of mucositis during treatment for malignancy are described in *Table 1*.[23] In terms of age, children and the elderly have an increased risk of developing mucositis compared with adults.[3,8,24-27] This is probably related to the higher proliferation rate of basal cells of the mucosa in children, causing the loss of ability of the tissue to renew itself. Moreover, the higher incidence of hematologic malignancies in children accompanied by a high degree of immunosuppression facilitates the colonization of microorganisms to destroy further the epithelial cells of the mucosa.[3,8] The risk of severe mucositis in the elderly population is assumed to be associated with the increased toxicity of antineoplastic drugs due to the decreased renal function, which alters the pharmacokinetics and pharmacodynamics of the drugs. This condition is enhanced by poor recovery of tissue losses caused by the decline in stem cell reserve due to aging.[26]

Gender is also considered a risk factor of mucositis during chemotherapy in adults. Several studies in adult patients receiving 5-fluorouracil-based chemotherapy have shown that women experienced more significant oral mucositis compared with men.[28-30] However, studies in the pediatric population with hematologic malignancies and/or solid tumours under chemotherapy found no correlation between gender and incidence or severity of oral mucositis.[12, 31-33]

Salivary secretory function and oral health and hygiene play an important role in the occurrence of oral mucositis during antineoplastic therapy. A role for saliva in protecting the oral mucosa with its washing function and bactericidal proteins was reported. A study from McCarthy et al. in adult patients receiving 5-fluorouracil showed that reduced salivary flow significantly increased the susceptibility to oral mucositis.[25] The increased saliva production related to gum use in children receiving chemotherapy was associated with a decreased incidence of WHO grade 1-2 oral mucositis. [13] Intensive oral hygiene significantly reduces the frequency of mucositis among patients with malignancy receiving bone marrow transplant.[34]

In children receiving chemotherapy, Cruz et al. [35] found a significant negative correlation between the number of tooth brushing sessions and the prevalence of mucositis on day 8, suggesting that good oral hygiene with daily brushing help to reduce pathogens in the oral cavity and consequently reduce the prevalence of oral mucositis.

Genetic factors have also been suggested to influence the risk of mucositis through modulation of the inflammatory response. Patients who express high levels of pro-inflammatory cytokines were reported to be at higher risk of mucositis.[3] This contention was indirectly supported by a study on pediatric patients undergoing chemotherapy. In this study, a correlation between high anxiety level and a greater risk of developing oral mucositis was reported and related to higher levels of pro-inflammatory cytokines in patients with increased anxiety level.[15]

A low body mass index (<20 for males, <19 for females) in adults was reported to correlate with the development of oral mucositis[14] as a result of poor nutritional status affecting mucosal regeneration due to decreased cellular renewal. However, this was not confirmed in pediatric patients, since in this study, a lower body weight prior to chemotherapy was associated with a greater risk of developing oral mucositis.[12]
Adult and pediatric patients with decreased renal function or elevated serum creatinine level were reported most likely to develop severe oral mucositis due to increased chemotherapy toxicity through the compromised excretion of the cytotoxic drugs and accumulation of metabolites within cells.[12, 36]

Smokers or patients with previous malignancy treatment have a higher risk for developing mucositis, because smoking can affect the healing capacity of the oral tissue and prior antineoplastic therapy can make the mucosa more vulnerable to cell damage. [37, 38]

Neutropenia is also considered as a risk factor of oral mucositis in pediatric and adult patients receiving chemotherapy.[12,14,15,18] It has been suggested that the oral mucosal defense mechanism and the proliferation response of oral epithelial cells to the cytotoxic effects of chemotherapy in neutropenic patients are impaired. Furthermore, neutropenic patients are more susceptible to microbial oral infection, which may aggravate the oral mucositis.

Altered liver function characterized by elevated transaminase level has also been suggested to correlate with the incidence of oral mucositis in children undergoing chemotherapy.[12] The exact mechanism is unclear, but it is assumed that there is an increased risk of drug accumulation within cells caused by impaired drug metabolism in the liver due to liver dysfunction.

An association between level of nausea/vomiting and oral mucositis was also hypothesized. This study established a higher frequency of WHO grade ≥2 nausea/vomiting (transient vomiting 1-5 emetic episodes/day) in children with oral mucositis under high-dose Methotrexate (MTX) therapy.[39] The exact mechanism is unclear, but it is assumed that severe nausea/vomiting reduces glomerular filtration and thus resulting in decreased renal clearance and increased MTX toxicity, as it was observed in this study that children with prolonged clearance of MTX (plasma level of MTX at 66 h ≥0.2 µmol/l) had higher incidence of mucositis.

Underlying disease of the patients is also associated with the incidence of mucositis. Otmani et al.[33] has suggested that pediatric patients with hematologic malignancies experienced more oral mucositis than those with solid tumours (59.8% against 48.6%). They also found that patients with acute leukemia, non-Hodgkin lymphoma and undifferentiated carcinoma of nasopharyngeal type were at greater risk of severe oral mucositis. Figliolia et al.[32] found 46% incidence of oral mucositis in children with acute lymphoblastic leukemia during treatment.

Table 1. Major patient-related risk factors for mucositis

1. Age Children and the elderly are at greater risk of mucositis.
2. Gender [a] Women are at greater risk for severe (grade ≥3) oral mucositis.
3. Oral health and hygiene Poor oral health and hygiene increase the risk of mucositis.
4. Salivary secretory function Reduced salivary flow increases susceptibility to oral mucositis.
5. Genetic factors Patients who express high levels of cytokines may be at higher risk of mucositis.
6. Body mass index [b] Low body mass (BMI <20 for male and <19 for female) increase the risk of mucositis.

7. Body weight
   Children with lower body weight prior to chemotherapy are at greater risk of developing mucositis.
8. Renal function
   Decreased renal function increases the risk of mucositis.
9. Smoking
   Patients who smoke may be at higher risk of mucositis.
10. Previous cancer treatment
    Patients who received previous cancer treatment may be at higher risk of mucositis.
11. Neutropenia
    Patients with neutrophil count ≤ $1x10^3$/µl are at greater risk of developing oral mucositis.
12. Liver function
    Patients with elevated transaminase level may have increased risk of mucositis.
13. Level of nausea/vomiting
    High frequency of WHO grade ≥ 2 nausea/vomiting increases the risk of mucositis.
14. Methotrexate (MTX) clearance
    High-risk plasma MTX concentration at 66 h would increase the risk of mucositis.
15. Type of cancer
    Patients with hematologic malignancies may be more prone to developing mucositis than those with solid tumours.

[a] In pediatric patients' gender has not been suggested as a risk factor of oral mucositis
[b] BMI is not associated with the incidence of oral mucositis in pediatric patients

b.) Treatment-related factors

Treatment-related factors associated with an increased risk for mucositis in patients treated for malignancy are summarized in *Table 2*.[23] The incidence for chemotherapy-induced mucositis ranges from 40% to 76%, in which the type and dose of chemotherapeutic agents used mainly determine the frequency and severity of mucositis. *Table 3* provides several chemotherapeutic agents responsible for oral mucositis.[40]

Pediatric patients who more often receive methotrexate or adriamycin-based chemotherapy are more susceptible to develop oral mucositis (24.6% and 29.8%).[15] In one study, the incidence of oral mucositis in children receiving high-dose methotrexate therapy was found to be as high as 52%.[41] The high methotrexate plasma concentration at 66 h (≥ 0.2 µmol/l) would increase the risk of oral mucositis in children.[39] Children treated with Busulfan before autologous stem cell transplantation were also described to have a higher prevalence of oral mucositis (63.5%).[31]

Chemotherapy regimens combining an alkylating agent, anthracycline, a vinca-alkaloid, and methotrexate or etoposide such as the one used to treat Burkitt lymphoma and high-risk acute lymphoblastic leukemia (COPADM = cyclophosphamide, vincristine, prednisone, methotrexate, doxorubicin) or the one used to treat Ewing tumor (VIDE = vincristine, ifosfamid, doxorubicin, etoposide) were reported to be associated with a very high rate of severe oral mucositis in children.[13]
Etoposide and methotrexate have a direct mucotoxic potential and are also secreted in the saliva, which might explain their marked oral mucotoxicity.[46]

In adult patients being treated with fluorouracil (5-FU) and cisplatin, 90% develop mucositis.[42] The degree and severity of fluorouracil-induced mucositis seem to be influenced by the administration schedule of 5-FU. A continuous infusion of 5-FU increased the incidence of mucositis compared to intermittent bolus treatment.[43] However, in a meta-analysis of randomized trials comparing continuous infusion versus intermittent bolus of 5-FU in 1219 patients, no significant difference in mucositis frequency or severity was found between these two schedules.[44]

Mucositis is also common in patients treated with doxorubicin, vinblastine, and taxanes.[45] Irinotecan (CPT-11) is associated with a high risk of severe gastrointestinal toxicity in the form of a secretory, delayed diarrhea.[47] Mucositis is uncommon with asparaginase and carmustine.[45] High-dose chemotherapy regimens used in order to obtain a better therapy response have unfortunately resulted in such patients increasingly developing mucositis and sometimes due to mucositis the further treatment intensification could also be limited.

Allogeneic marrow transplantation recipients experience higher risk and severity of mucositis than autologous marrow transplantation recipients. The increased mucotoxicity in allograft recipients is believed to result from differences in conditioning regimens and the use of methotrexate for the prevention of graft-versus-host disease.
The study from Sonis et al.[48] in 92 patients receiving stem cell transplant has shown that 36% of the allograft recipients developed grade 3 or greater mucositis (on a 0 to 5 scale) compared with only 21.4% of the autograft patients.

Radiation therapy can also cause oral and gastrointestinal mucositis, depending on the radiation site and radiation fractionation. Radiation therapy directed at parts of the body containing epithelial cells of mucosa, such as the head and neck, thorax, abdomen and anal-rectal region, might produce higher rates of mucositis. Altered radiation fractionation, such as hyperfractionation and acceleration also increase the risk of mucositis. Moreover, when the chemotherapy is combined with radiation therapy (chemoradiation), the mucotoxicity caused by this kind of treatment is more aggravated.[49]

Zerbe et al.[50] reported that in bone marrow transplant patients given chemoradiation regimens involving total body irradiation, mucositis was more severe and occurred at earlier time points than in patients given busulfan-based chemotherapy alone for bone marrow ablation.

Table 2. Major therapy-related risk factors for mucositis *(From Avritscher EBC, Cooksley CD, Elting LS (2004): Scope and epidemiology of cancer therapy-induced oral and gastrointestinal mucositis.* Seminars in Oncology Nursing ***20:*** *3-10)*

1. Chemotherapy agent 5-FU, methotrexate, and etoposide produce high rates of mucositis.
2. Chemotherapy dosage High-dose chemotherapy regimens are associated with greater risk and severity of mucositis.
3. Type of bone marrow transplantation Allogeneic bone marrow transplantation recipients experience higher rates of mucositis than autologous bone marrow transplantation patients.
4. Radiation site Radiation administered directly to the head and neck, thorax, abdomen, and anal-rectal region produce high rates of mucositis.
5. Radiation fractionation Altered fractionation schemes (hyperfractionation and acceleration) increase the risk of mucositis.
6. Combined modality The use of chemotherapy in conjunction with radiation therapy is associated with increased risk and severity of mucositis

Table 3. Main chemotherapeutic agents responsible for oral mucositis *(From Scully C, Epstein J, Sonis S (2003: Oral mucositis: A challenging complication of radiotherapy, chemotherapy, and radiochemotherapy: Part 1, pathogenesis and prophylaxis of mucositis.* Head and Neck 1057-1070*)*

Alkylating agents	Anthracyclines	Antibiotics	Antimetabolites	Taxanes	Vinca-alkaloids
Busulfan	Daunorubicin	Actinomycin D	Cytosine arabinoside	Docetaxel	Vinblastin
Cyclophosphamide	Doxorubicin	Amsacrine	5-Fluorouracil	Paclitaxel	Vincristin
Mechlorethamine	Epirubicin	Belomycin	Hydroxyurea		Vinorelbine
Procarbazine		Mithromycin	Methotrexate		
Thiotepa			6-Mercaptopurine		
			6-Thioguanine		

1.1.2. Pathogenesis

In the past, mucositis was thought to be the result of damage to the epithelium because of the non-specific direct effects of radiation or chemotherapy on the rapidly dividing mucosal basal cells. As a result, the basal epithelial cells are injured and die. The mucosa becomes thin and atrophic, causing a higher susceptibility to trauma from normal oral function like eating.

Mucositis studies in animal models suggest that the pathogenesis of mucositis is more complex. Sonis et al. identified five biological phases involved in the development and resolution of mucositis. They are: (1) initiation, (2) upregulation and message generation, (3) amplification and signaling, (4) ulceration, (5) healing.[51, 52]

The ***initiation phase*** occurs immediately after exposure of the oral mucosa to radiation or chemotherapy. Both irradiation and chemotherapy cause direct damage to the DNA of epithelial basal cells and also generate reactive oxygen species (ROS). ROS are free radicals that appear to have a significant role in mucosal injury induction. The activation of ROS directly damages cells, tissues and blood vessels, and can initiate a cascade of biological events.

Because the impairment of mucosal defence structures occurs already in this phase, we could assume that herpes simplex virus (HSV), which in several studies was frequently found in the oral lesions of patients treated for malignancy, might already pose a risk to patients starting from this phase. Invading the damaged epithelial cells of mucosa is the way HSV spreads by replicating in the cells. The virus replication will aggravate the mucosal injury. *(Figure 1)*

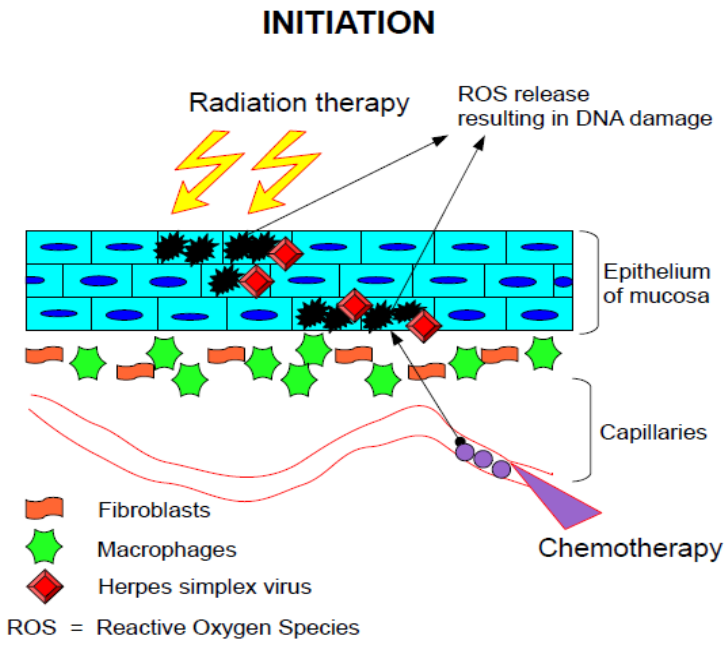


Figure 1. Initiation Phase

Radiation therapy and chemotherapy cause DNA damage of the epithelial cells and generate reactive oxygen species (ROS). The release of ROS causes not only tissue injury, but initiates also a cascade of biological events leading to mucositis. At this phase, herpes simplex virus can invade the damaged mucosa easily and replicate in the epithelial cells of the mucosa, causing further the mucosal damage.

In the ***up-regulation and message generating phase*****,** transcription factors are activated by radiation, chemotherapy and ROS. Of the transcription factors, nuclear factor-kappa B (NF-κB) is considered to have the principal role in the mucositis induction. Once activated, NF-κB upregulates genes that control the synthesis of the pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6), leading to tissue injury and apoptosis.

Upregulation of other genes causes the expression of adhesion molecules, activation of the cyclooxygenase-2 pathway subsequently and then angiogenesis.

Radiation, chemotherapy and ROS will also stimulate sphingomyelinase and ceramide synthase that activate the ceramide pathway leading to apoptosis. Fibronectin break-up also occurs during this phase and it activates macrophages subsequently, leading to stimulation of matrix metalloproteinases, which cause tissue injury or increased production of TNF-α. As a result, the mucosa starts to thin, becomes erythematous and begins to be painful. *(Figure 2)*

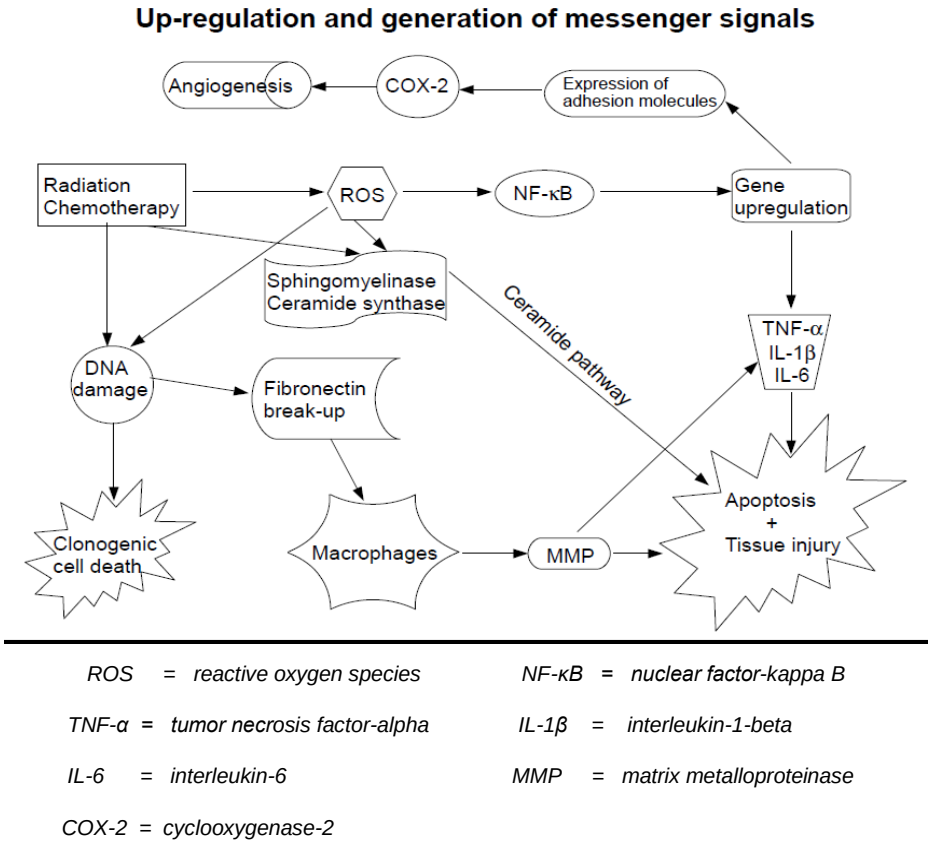


Figure 2. Up-regulation and message generating phase

Radiation therapy, chemotherapy and ROS activate NF-κB that upregulate genes, which lead to production of proinflammatory cytokines TNF-α, IL-1β and IL-6 that results in tissue injury and apoptosis. Radiation, chemotherapy and ROS stimulate also sphingomyelinase and ceramide synthase that activate the ceramide pathway leading to apoptosis. Activation of macrophages through fibronectin break-up during this phase stimulates matrix metalloproteinases (MMP), which cause tissue injury or increased production of TNF-α. Upregulation of other genes causes expression of adhesion molecules and activation of the cyclooxygenase-2 pathway resulting subsequently in angiogenesis.

Besides damaging the mucosal tissue, the proinflammatory cytokines also make simultaneously a "feedback loop" that ***amplifies and signals*** the destructive process further. TNF-α activates the ceramide and caspase pathways leading to tissue damage and apoptosis, and activates the transcription pathway mediated NF-κB resulting in further production of the pro-inflammatory cytokines TNF-α, IL-1β and IL-6. Consequently, many of the injuries continue to occur, even after radiation or chemotherapy has been completed. *(Figure 3)*

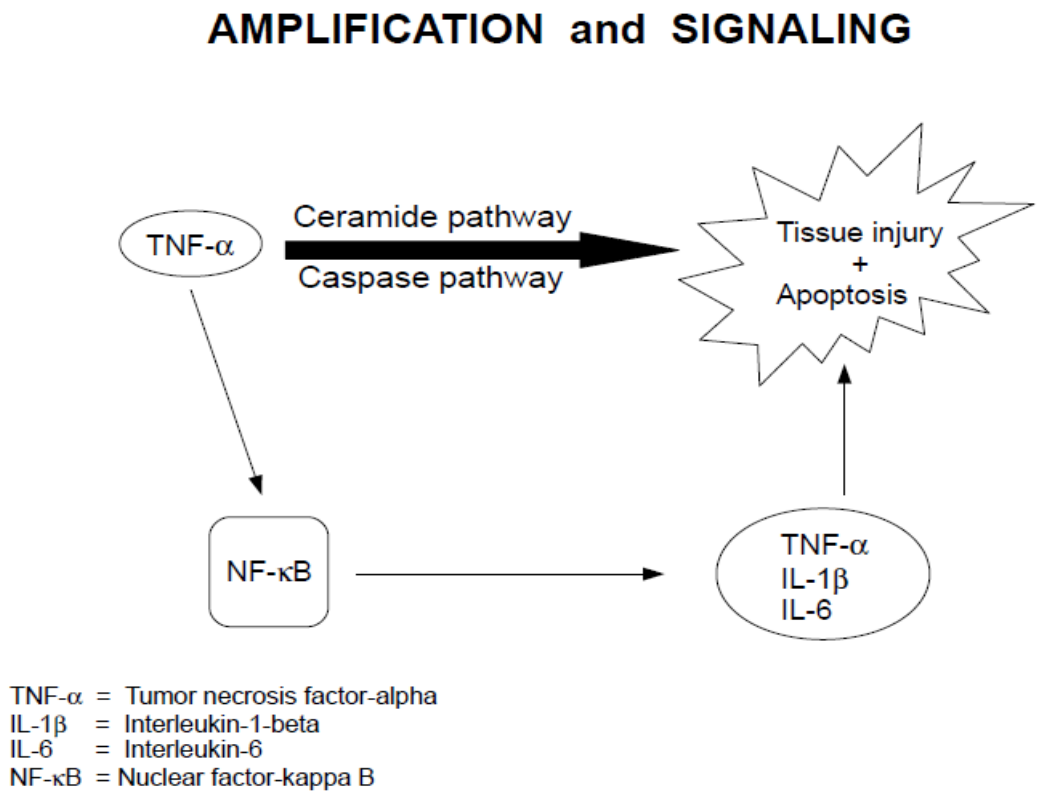


Figure 3. Amplification and Signaling Phase

The proinflammatory cytokines amplify and signal the destructive process further ("feedback loop"). TNF-α activates ceramide and caspase pathways leading to tissue damage and apoptosis. It also activates the transcription pathway mediated NF-κB, that produces further the proinflammatory cytokines TNF-α, IL-1β and IL-6, which lead to tissue injury.

The culmination of the mucosal injury is ***ulceration*****.** At this point, the ulcerated surface will be colonized by the oral microbial flora that also release toxins and stimulate inflammatory cells, which in turn lead to more production of the proinflammatory cytokines TNF-α, IL-1β, and IL-6. This will cause further inflammation and pain. Indeed, the neutropenic patients may experience bacteremia and sepsis. Furthermore, the replication of herpes simplex virus will also increase and aggravate the mucosal injury. *(Figure 4)*

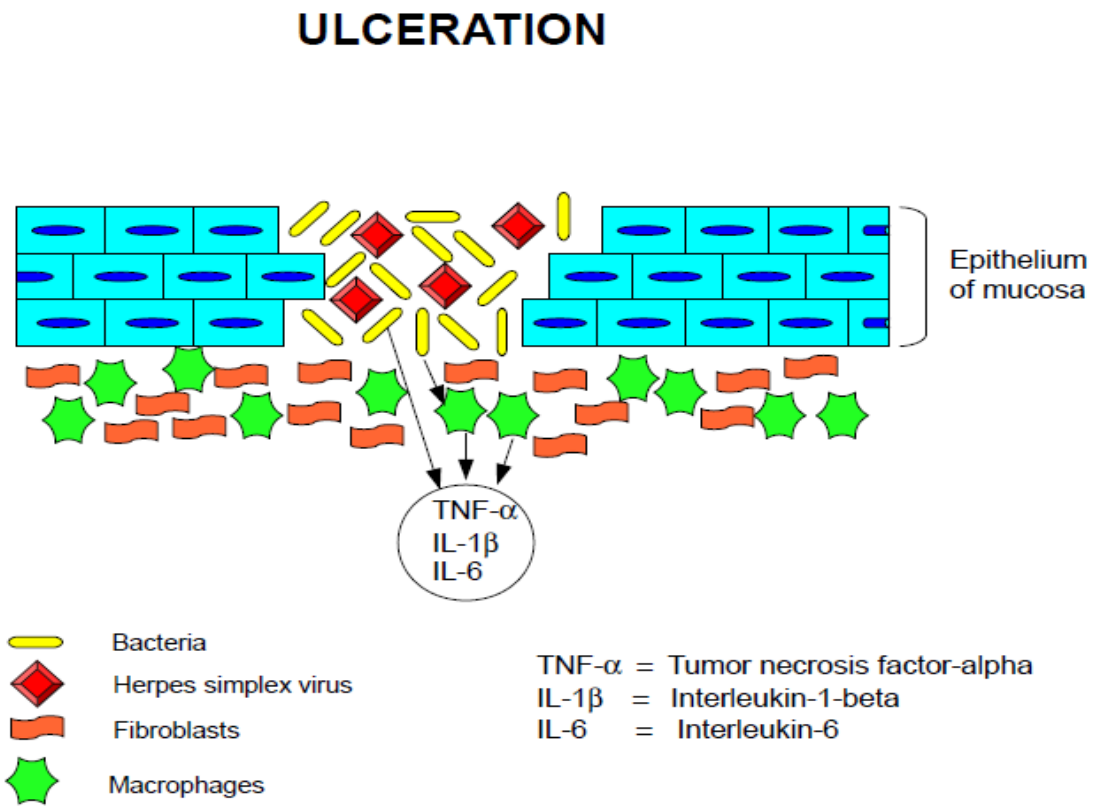


Figure 4. Ulceration Phase

A continuous process of tissue damage and apoptosis causes loss of epithelial integrity, which results subsequently in ulceration. The ulcerated surface will be colonized by the oral microbial flora that invade into the submucosa, release toxins and activate macrophages, leading to production of more proinflammatory cytokines TNF-α, IL-1β, IL-6. In neutropenic patients, bacteremia and sepsis can occur. Moreover, the replication of herpes simplex virus will increase and cause further destruction of mucosal tissue.

Finally the ***healing phase*** occurs. The extracellular matrix releases new messenger molecules that cause the epithelium adjacent to the ulcer to divide, migrate and differentiate into new healthy mucosa. At the same time other messages are also released to downregulate the process so that the hyperplasia does not occur. *(Figure 5)*

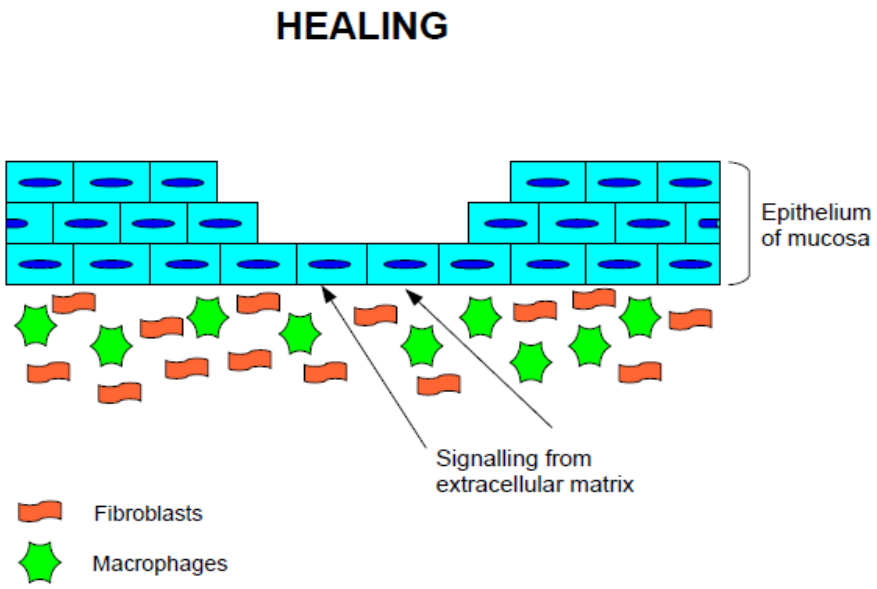


Figure 5. Healing Phase

In the healing phase, the extracellular matrix releases a signal that leads to a renewal of epithelial proliferation and differentiation and re-establishment of the local microbial flora. Although the oral mucosa appears normal after the healing phase, the mucosal environment has been altered significantly (e.g. residual angiogenesis) and the patient has now an increased risk of oral mucositis following the next cancer therapy.

1.1.3. Mucositis scoring system

There are many methods or scoring systems to measure the level of mucositis. The most commonly used system is the mucositis scale from World Health Organization (WHO) and the National Cancer Institute (NCI)[53] *(Table 4)*, which are commonly applied in the pediatric population. WHO divides mucositis into grades 0-4 by evaluating the presence of erythema – ulceration and the capacity to eat.

NCI differentiates mucositis due to radiation, chemotherapy and bone marrow transplantation into grades 0-4.

Table 4. Mucositis Scale WHO und NCI *(From López-Castaño F, Oñate-Sánchez RE, Roldán-Chicano R, Cabrerizo-Merino MC (2005). Measurement of secondary mucositis to oncohematologic treatment by means of different scale.* Med Oral Patol Oral Cir Bucal ***10:*** *412-21)*

Scale	Antineoplastic Therapy	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
WHO	-	Normality	Generalized erythema (painless pink mucosa with abundant saliva and normal voice function)	Erythema involving small ulcerations and preserved solid swallowing capacity	Extensive ulcers with edematous gingival tissue and thick saliva, preserved liquid swallowing capacity, pain and speech difficulties	Very extensive ulcers with bleeding gums, infections, the absence of saliva, incapacity to swallow, and intense pain
NCI	Chemotherapy	None	Painless ulcers, erythema, or mild soreness in the absence of lesions	Painful erythema, edema, or ulcers, but can eat or swallow	Painful erythema, edema, or ulcers requiring intravenous hydration	Severe ulceration or requires parenteral or enteral nutritional support or prophylactic intubation
NCI	Radiation	None	Erythema of the mucosa	Patchy pseudomembranous reaction (patches generally ≤ 1,5 cm in diameter and non-contiguous	Confluent pseudomembranous reaction (contiguous patches generally > 1,5 cm in diameter)	Necrosis or deep ulceration; may include bleeding not induced by minor trauma or abrasion
NCI	Bone marrow transplantation	None	Painless ulcers, erythema, or mild soreness in the absence of lesions	Painful erythema, edema or ulcers but can swallow	Painful erythema, edema, or ulcers preventing swallowing or requiring hydration or parenteral (or enteral) nutritional support	Severe ulceration requiring prophylactic intubation or resulting in documented aspiration pneumonia

WHO : World Health Organization

NCI : National Cancer Institute

1.2. Herpes simplex Virus

Herpes simplex virus (HSV) belongs to the subfamily of alphaherpesvirus. It is a double-stranded DNA-containing enveloped virus with a 150-200 nm diameter. The virus consists of an icosahedral protein core that is surrounded by a lipid envelope in which a number of glycoproteins responsible for the viral-target cell interaction and infection are embedded. Two strains of HSV are identified: HSV-1 commonly infects skin and mucous membranes above the waist, and HSV-2 commonly infects the genitals and the neonate.[54, 55]

HSV infection is a common infection among humans with a variety of clinical manifestations determined by the immune competence of the host. The infection can involve the skin, mucous membranes, eye, central nervous system (CNS), the genital tract and can also result in widespread systemic disease, especially in immunocompromised patients such as patients with AIDS, primary immunodeficiencies, or malignancy.

Patients treated for malignancy develop commonly immunosuppression due to the intensive treatment, therefore the risk of HSV infection in these patients is elevated. Not only can it complicate the therapy for malignancy, but it can also result in a fatal disease. A 4-year-old patient with relapsed ependymoma and immunosuppression was reported to develop HSV pneumonia (HSV type 1) resulting in death.[56] Another fatal case of a 22-year-old patient with ALL having generalized HSV infection (also HSV type 1) has also been reported.[57] In immunosuppressive patients, the viral shedding is usually greater; approximately 38%.[58] The high level of viral replication and spreading in these patients are due to impaired local and systemic defense mechanisms, particularly the cell-mediated immunity which is responsible for clearing HSV from host. Therefore, it is important for physicians to focus more on the risk and impact of HSV infection in patients treated for malignancy, especially in those treated for infection caused by HSV type 1.

1.2.1. Epidemiology

Herpes virus infection is a disease affecting humans worldwide. The incubation period is 2-12 days (average 6 days). The spread of infection appears to be determined by two factors: close body contact and mucosal/skin trauma such as teething or a break in the skin. HSV can also be transmitted by organ transplantation. Concerning this way of spread, it is believed that patients treated for malignancy are at high risk of HSV infection because they commonly develop oral mucosal damage as a complication of the intensive therapy (chemotherapy, radiation therapy). Moreover, due to this treatment the patients are greatly immunosuppressive, causing the infection to be more severe.

The infection in neonates (85%) occurs in birth canal through an infected maternal genital tract (perinatal infection). The incidence of neonatal HSV infection is estimated to range from 1 in 3000 to 1 in 20000 live births. Infants born to mothers with primary genital HSV infection have a much greater risk of developing neonatal HSV infection (25-60%) than those who are born to mothers with recurrent genital herpes (2%).[59] About 10% of infants acquire the HSV infection postnatally, not necessarily from the mother, but also from persons (family member, caregiver) shedding HSV (often HSV type 1). In approximately 5% rare cases, the HSV infection occurs within the uterus.[55]

In children, HSV spreads from body fluid such as saliva and close body contact. The seroprevalence of HSV-1 antibodies is increased in children aged 1 to 4 years.[55] In the United States, 33% of children from the lower socioeconomic populations are infected by HSV-1 by the age of 5 years and 70% to 80% by late puberty.[60] HSV-1 antibodies are found in more than 80% of adults[61] and HSV-2 antibodies are present in up to 60% of adults in lower socioeconomic groups, 10-30% of adults in higher socioeconomic groups, and 3% in nuns.[55] The higher incidence of HSV antibodies in lower socioeconomic groups correlates with crowded living conditions.

1.2.2. Pathogenesis

There are three types of HSV-infection: primary infection; first infection, nonprimary; and recurrent.[55] ***Primary infection*** means infection in HSV-seronegative persons; the host's first experience with the virus, which is commonly subclinical infection or limited to the skin and mucous membrane lesions accompanied by varying degrees of systemic reaction. ***First infection, nonprimary,*** is infection in a person with immunity to one type of HSV but infection by another type. These infections are usually less severe than primary infection. ***Recurrent infection*** means reactivation of a latent infection in an immune host with circulating antibodies.

Damaged mucosa is the port of entry for HSV to invade hosts. In immunocompetent patients, once HSV invades the mucosa, the immune system will limit the viral replication and spreading, and eliminate the virus optimally, causing a subclinical infection. But in immunocompromised patients this process will not be optimal.

HSV replicates in oral and genital mucosal tissue, and in immunocompromised patients it also replicates in the respiratory and gastrointestinal tracts. After the primary infection, the virus will be in a latent state in ganglia of the dorsal spinal cord or in cerebral nerves. HSV-1 migrates mostly to the ganglion of trigeminus nerve and HSV-2 migrates frequently to the sacral ganglion. Reactivation of this latent infection can occur after these stimuli:[54]

- ultraviolet light, burn, skin lesions or skin laser therapy
- inflammation of the neuronal ganglia
- fever, menstruation, emotional stress, high temperature

After reactivation, the virus leaves the ganglia to the mucosal surface of the correlated dermatom through the peripheral sensory nerves and starts to develop vesicles with active virus replication.[62]

1.2.3. Clinical manifestations [55,59,61,62]

The characteristic of HSV-infection is a development of vesicular skin lesions. A viremia that results in disseminated disease or neurogenic transmission that leads to meningoencephalitis is rarely described, and if it does occur then usually in neonates and immunocompromised patients. HSV infections in children mostly are either asymptomatic or so mild that the illness is not noticed.

a.) Children and adults

- ***Skin and oral mucous membranes***

Skin lesions consist of aggregates of thin-walled vesicles on an erythematous base, which heal within 7-10 days without leaving scars except after repeated episodes or secondary bacterial infections. Mild irritation or burning at the local site or severe neuralgic pain in the region may precede the lesions. In immunocompromised persons the primary infection may uncommonly result in a generalized vesicular eruption, which may continue to appear over a period of 2-3 weeks.

Traumatic lesions of the skin or burns can be infected by HSV, which leads to different degrees of skin infection that usually disappear within one week. **Eczema herpeticatum (Kaposi varicelliform eruption)** is the most serious manifestation of a widespread HSV infection in patients with eczema. Large numbers of vesicles develop over the area of eczematous skin and persist for 7-9 days. If the clinical situation worsens, death may result from physiologic disturbances because of loss of fluid, electrolytes and protein through the skin; from invasion of the virus to the brain or other organs; from secondary bacterial infection usually with Staphylococcus or group A Streptococcus.

Acute herpetic gingivostomatitis is the common HSV infection in children marked by mucocutaneous vesicular eruptions on gingivae, buccal mucosa, tongue, lips, hard and soft palates. The vesicles are 1-2 mm in diameter and rapidly rupture; forming shallow and painful ulcers covered with a yellow-gray membrane and surrounded by erythematous base. Fever, pain in mouth, salivation, fetor oris, and refusal to eat or drink are the accompanying symptoms. The acute phase lasts about 4-9 days and is

self-limited. HSV infection recurs commonly as **stomatitis and herpes labialis**, that are usually accompanied by local pain, tingling, or itching and last 3-7 days. Because of the overlapping mucosal damage, these typical herpetic lesions are difficult to recognize in patients developing mucositis during malignancy therapy if they are concurrently infected by HSV. The vesicular lesions are rarely seen; instead of that ulcers are dominant, particularly in higher grade mucositis.

❖ ***Ocular infections***

HSV can also invade the eyes and manifest as **conjunctivitis and keratoconjunctivitis.** Recurrent herpetic corneal infection may result in scarring of the cornea and vision impairment.

❖ ***Genital herpes***

Genital infections are usually caused by HSV-2 and occur most commonly in adults and adolescents through sexual activity. In females, the cervix is the primary site of infection, but the vulva and vagina may be involved with vesicles and ulcers. In males, the infection is seen on the glans penis, prepuce, or shaft of the penis. An autoinoculation of HSV-1 from orolabial lesions can cause genital herpes in children.

❖ ***Central nervous system infections***

HSV-1 and HSV-2 can cause a meningoencephalitis, which if untreated has a mortality rate of 75%. The clinical signs and symptoms include fever, altered consciousness, headache, personality changes, seizures, dysphasia, and focal neurologic signs. The cerebrospinal fluid reveals a lymphocytic pleocytosis and protein elevation. In typical cases, EEG and neuroimaging studies show unilateral or bilateral changes in the temporal lobe. HSV is also considered to be the cause of the most cases of recurrent aseptic meningitis (Mollaret meningitis).

b.) Immunocompromised persons

A severe HSV infection can occur in persons with severe malnourishment, malignancies, immunsuppresive therapy, AIDS, burns or primary immunodeficiency

diseases that impair cell-mediated immunity. The disease is widely disseminated involving gastrointestinal tract, liver, lungs, adrenal gland, kidneys, spleen, central nervous system, and has a high mortality rate even with therapy.

c.) Neonates

HSV infections in neonates are always symptomatic and can occur within the uterus, perinatally (peripartum) and postnatally (postpartum). The most frequent one is the perinatal infection due to maternal infection during delivery and it is caused in approximately 75% of the cases by HSV-2. Mothers with primary or first episode of genital herpes are 10 to 30 times more likely to transmit the virus to the neonates compared to mothers with recurrent disease. Postnatal infection is acquired not only from the mother but also from other family members shedding HSV (often HSV-1), from fever blisters, finger infections, or lesions at other sites. Intrauterine infection is very rare, accompanied by vesicular skin lesions at birth, microcephaly, chorioretinitis and microophthalmia.

There are 3 major categories of neonatal herpetic infections: localized skin, eye, and mouth infection; central nervous system (CNS) infection; and disseminated infection. The vesicular, ulcerative skin lesions of neonatal HSV infection are present in 40-45% of cases; one third manifest as CNS infection and 20% of cases as disseminated infection. The risk of mortality of HSV infection with CNS involvement is about 6% and around 31% in disseminated disease.

1.2.4. Diagnosis[55,62]

A mucocutaneous HSV infection can be clinically diagnosed in most cases by the characteristic vesicle lesions. With a microscopic examination using Tzank stain we can identify multinuclear giant cells and intranuclear inclusions from the lesions. The disadvantages of Tzank stain are: low sensitivity and it cannot differentiate between HSV 1, HSV 2 and Varicella-zoster virus. HSV can be detected in the lesions, cerebrospinal fluid, and blood using polymerase chain reaction (PCR-analysis). Serologic test to detect HSV antibody in serum or cerebrospinal fluid is not a useful tool

for rapid diagnosis, because the HSV serologic changes (fourfold rise or seroconversion from negative to positive) usually occur after the critical period for diagnosis and therapy.

1.2.5. Therapy

The therapy of HSV infection is aimed to inhibit the replication of the virus, reduce the duration of pain and avoid systemic complications. Acyclovir (9-[-2-hydroxyethoxymethyl] guanine) is still the drug of choice to treat HSV infection. It is a purine nucleoside analog, which is pharmacologically an inactive substance. After acyclovir penetrates the herpes-infected cells, the viral thymidine kinase, an enzyme that the herpes virus needs to replicate, phosphorylates acyclovir into acyclovir-monophosphate. By cellular kinases acyclovir monophosphate is then triphosphorylated into acyclovir-triphosphate that acts as an HSV-DNA polymerase inhibitor and DNA chain terminator.[55]

Other oral anti herpetic drugs with an excellent oral bioavailability of about 55-80% are valacyclovir and famciclovir. Valacyclovir is the valine ester prodrug of acyclovir, which is rapidly and completely converted into acyclovir in the liver and intestine. Its oral bioavailability is 3-5 times greater than acyclovir.[60] Famciclovir is a diacetyl ester prodrug of penciclovir and is also a synthetic acyclic guanine derivative. It is rapidly absorbed and converted into penciclovir, which is then phosphorylated to penciclovir monophosphate by HSV thymidine kinase. By cellular kinases, penciclovir monophosphate is converted into penciclovir triphosphate, which is a competitive inhibitor of viral DNA polymerase.[63] However, valacyclovir and famciclovir are not recommended for HSV treatment in pediatric population since no studies have been performed with these drugs in children or adolescents.[59, 63]

All neonates with HSV infection should be treated with acyclovir, regardless of clinical findings. Antiviral therapy is not routinely recommended in immunocompetent children or adults with mucocutaneous HSV infection (gingivostomatitis, herpes labialis), but in patients with severe gingivostomatitis or eczema herpeticatum, acyclovir should be given intravenously or orally for 7 days.[55, 62] Topical therapy with acyclovir or penciclovir is not effective for orolabial lesions.

Topical therapy with idoxuridine, trifluridine, vidarabine and acyclovir has proven to be effective for herpes keratoconjunctivitis.[62] The treatment of choice for herpes encephalitis is intravenous acyclovir 3 x 10 mg/kg daily for 14-21 days. Primary genital herpes in children is treated with intravenous or oral acyclovir for 10 days.
In older adolescents or adults, the recommended treatment regimen for initial genital herpes is oral acyclovir, valacyclovir or famciclovir for 7-10 days.[55]

The emergence of acyclovir-resistant HSV is rare and when it occurs, the drug of choice is intravenous foscarnet (40 mg/kg/dose 3 times daily), that inhibits the HSV replication by preventing cleavage of pyrophosphate from deoxynucleotide triphosphate. Foscarnet has not been studied in pediatric population either.[54, 63]

1.3. Association of Herpes Simplex Virus and Chemotherapy-induced Mucositis

In patients with malignancy, mucositis is the most common complication that develops during or after chemotherapy. Many authors have assumed that self-toxic effect of the chemotherapeutic agents, a therapy-induced neutropenia, Enterobacteriaceae, and Candida spp. are related to this complication. Several studies also suggested an association between the chemotherapy-induced mucositis and the presence of herpes simplex virus (HSV) in the oral lesions.

A study from Rand et al. (1982)[19] in adults with malignancy found that HSV was recovered significantly more often from throat washings in HSV seropositive patients who were receiving chemotherapy (8 / 114 patients ~ 7%) compared to those who were not receiving chemotherapy (1 / 91 patients ~ 1.1%). This study also demonstrated that HSV was found more frequently in HSV seropositive patients with oral lesions during chemotherapy (12 / 14 ~ 85.7%) compared to those without oral lesions (4 / 14 ~ 28.6%). In all HSV seronegative patients receiving chemotherapy, there was no HSV recovered in throat washings.

Bergmann et al. (1990)[20] performed a prospective study in 46 adult patients with hematologic malignancies undergoing antineoplastic treatment. Among those patients, 18 developed intraoral ulcers during the study, and concomitant signs of herpes labialis were detected in 2 patients with intraoral ulcers. HSV-1 was detected in cultures from the surfaces of the ulcers in 61% of the cases (11 of 18 patients).
Excluding patients with concomitant herpes labialis, HSV-1 cultures were positive in 9 of 16 patients with intraoral ulcers (56%). Based on these findings Bergman et al. concluded that some intraoral ulcers are likely to be caused by HSV. They also assumed that an association between leucopenia and HSV infection is plausible, because the patients in the study were generally leukopenic when the ulcers were detected.

Djuric et al (2007)[22] investigated the presence of HSV-1 and HSV-2 on the oral mucosa in 40 adult patients with different malignancies receiving chemotherapy by means of polymerase chain reaction (PCR) and identified HSV-1 in 35 patients (87.5%), but HSV-2 was not detected in any of the patients. Of those 35 patients, 23 patients (65.7%) presented oral mucosa damage.

A study from Carrega et al. (1994)[21] in 20 children with oral mucositis following chemotherapy showed that HSV was isolated from the surface of the oral lesions in 50% of patients. Two patients had HSV-2 and 8 patients had HSV-1 in the oral cultures. Because HSV was only isolated from the HSV seropositive patients and none from the HSV seronegative patients, it was thought that the HSV isolation was probably due to reactivation of a latent infection rather than to a primary infection. This study also indicated that neutropenia (<1000/mm^3) seemed to be an important cofactor for the development of mucositis, since 18 of 20 patients (90%) were neutropenic at the development of severe mucositis.

Sepúlveda et al. have also suggested a strong association between chemotherapy-induced mucositis in children and the presence of HSV-1. One study from them in 2003 examining 30 oral lesions in 19 pediatric patients under oncologic therapy found the presence of HSV-1 in 33.3% of the samples.[64] Another study from Sepúlveda et al. two years later revealed that 12 of 20 ulcers occurred in 15 children undergoing

chemotherapy were HSV-1 positive (60%). They also found that the chemotherapy-induced mucositis in the leukemic patients was observed more frequently in neutropenia (absolute neutrophil count <500/mm^3).[18]

A recent study from Mendonca et al. (2011)[17] concluded that the presence of HSV (mainly HSV-1) and Candida spp. on days 14 and 56 of treatment in children and adolescents with acute lymphoblastic leukemia was associated with mucositis severity. Therefore, it should be considered to screen the children under chemotherapy for pre-clinical HSV positivity to determine a prophylactic antiviral therapy.

1.4. Objective

Based on the limited information about the relation between herpes virus and oral mucositis in children treated with high intensity chemotherapy protocols, we aimed to retrospectively analyze prevalence and intensity of mucositis in relation to the presence of herpes simplex virus (HSV).

The objectives of this study are:

- to describe the prevalence of HSV in children with chemotherapy-induced mucositis
- to establish a relationship between the presence of HSV and the severity of the chemotherapy-induced mucositis
- to evaluate a relationship between leucopenia and HSV infection

2. Patients and Methods

2.1. Patients

In this retrospective study, the medical records of children with acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), relapsed ALL, relapsed AML, non-Hodgkin lymphoma, Ewing's sarcoma and osteosarcoma, who underwent an intensive

chemotherapy at the Department of Pediatric Hematology and Oncology at the Charité Universitätsmedizin Berlin from January 2008 until July 2010, were evaluated.
There were 83 patients analyzed in this study. These patients were receiving intensive chemotherapy according to the following protocols:

a.) Berlin-Frankfurt-Münster study group (BFM)
- ALL-BFM 2000 (acute lymphoblastic leukemia)
- ALL-Rezidiv BFM 2002 (relapsed ALL)
- AML-BFM 2004 (acute myeloid leukemia)
- AML-Rezidiv 2001 (relapsed AML)
- B-NHL-BFM 90 and 2004 (B-non Hodgkin lymphoma)

b.) European intergroup study on post-induction treatment of Philadelphia positive ALL (EsPhALL)

c.) International Registry Relapsed AML 2009

d.) EURO-EWING 99 and 2008: European Ewing Tumour Working Initiative of National Groups

e.) CWS 2002 P (Cooperative Weichteil Sarkomstudie)

f.) EURAMOS-1 (The European and American Osteosarcoma Studygroup)

During this intensive chemotherapy, the children were examined for the development of oral mucositis and the presence of HSV on the oral lesions. We used these chemotherapy protocols as one criterion in our study because children with these protocols seemed to have a tendency of developing oral mucositis due to the high treatment intensity with many mucotoxic chemotherapeutic agents (e.g. methotrexate) applied in these protocols compared to others.

2.2. Methods

2.2.1. Data Collection from Patients' Medical Records

At the beginning of our retrospective study, we first compiled a list of the patients who underwent chemotherapy at the Department of Pediatric Hematology and Oncology at the Charité Universitätsmedizin Berlin from January 2008 until July 2010. From that list,

we chose the patients who had an intensive chemotherapy for ALL, AML, relapsed ALL, relapsed AML, non-Hodgkin lymphoma, Ewing's sarcoma and osteosarcoma. After getting the patients' names, we went regularly to the archive department to collect their medical records. All medical records of the patients were further evaluated to obtain information about the occurrence and frequency of oral mucositis episode developed during the intensive chemotherapy. Here we found out that the children admitted to the pediatric hematology/oncology ward as well as those seen in the daycare clinic had been examined daily on the ward or during each appointment at the daycare clinic by the physicians on duty. When the children experienced a mucositis episode, the clinical signs of mucositis were systematically documented in the patient's chart. Based on the documentation results recorded by the physicians, we classified the mucositis episodes that had occurred in patients into grades 1 to 4 according to the mucositis scale of the National Cancer Institute (NCI) *(see Table 4).* The reason why we used the NCI mucositis scale rather than the one from WHO was because the NCI mucositis scale describes the symptoms more specifically based on the different types of antineoplastic therapy given (chemotherapy, radiation therapy or bone marrow transplantation). For this study, we took the NCI mucositis scale for patients treated with chemotherapy.

It was mentioned previously that neutropenia is considered to be one of the risk factors of oral mucositis in patients receiving chemotherapy. In this study we analyzed the laboratory findings documented in the medical records, particularly the leucocyte counts during mucositis episode. By evaluating the leucocyte counts within a period of 7 days since the beginning of the mucositis episode in patients who were examined for HSV-PCR, we tried to determine the role of the leucocyte counts in HSV mucositis in patients receiving chemotherapy. During these 7 observation days, the lowest leucocyte count (leucocyte nadir) was taken as the representative leucocyte count for that mucositis episode experienced by the patient. The leucocyte count < 1500/µl is considered as leucopenia.

2.2.2. HSV Examination using Polymerase Chain Reaction (PCR)

Materials: Plasmid DNA (ca. 125 copies/PCR), reagent LightCycler FastStart DNA Master ^{plus} HybProbe from Roche, Nuclease-Free Water from Promega,

QIAamplification DNA Mini Kit from Qiagen, real-time PCR-unit LightCycler 2.0 from Roche

In the medical records we noticed a virology examination for HSV, which was carried out on patients who had suffered from oral mucositis during the chemotherapy. However, since this was a retrospective study, neither every mucositis episode nor all patients suffering from mucositis were examined for HSV. The virology test itself was carried out at the Institute of Virology in Campus Charité Mitte in Berlin. The method used is based on a PCR.

Briefly, the presence of HSV in the mucositis episode was determined using the PCR technique from throat washings or oral swab materials, which were examined representatively on the sites where the mucosa was affected. Initially, each sample was added with an internal control (IC), which is plasmid DNA (ca. 125 copies/PCR) containing 247 bp-fragments with a lambda DNA-sequence with binding site for the IC-probe and the HSV-primer sequences. Oral swabs were rinsed in 1 ml NaCl 0.9%. Then a 200µl solution of sample plus IC was processed using QIAamplification DNA Mini Kit from Qiagen to get a 100µl eluate of HSV-DNA plus IC. A reaction mixture comprised of 5µl eluate (HSV-DNA+IC), reagent LightCycler FastStart DNA Master ^{plus} HybProbe from Roche, and Nuclease-Free water from Promega was amplified using real-time PCR-unit LightCycler 2.0 from Roche. The PCR consisted of an initial denaturation of 10 minutes at 95°C followed by 55 cycles (denaturation at 95°C for 10 seconds, annealing at 60°C for 30 seconds) and a final annealing of 30 seconds at 40°C. The sample was determined positive if it exceeded the detection limit of 1500 copies/ml.

2.2.3. Statistical Analysis

In this study we also wanted to analyze a relationship between the presence of HSV and the severity or degree of the chemotherapy-induced mucositis. Therefore, we performed a statistical analysis using the computer program SPSS statistics 17.0. We used Chi-Square test or Fisher's exact test. A p-value less than 0.05 (two-sided) was considered significant.

3. Results

3.1. Description of the patient population

Of the 83 patients evaluated with an age range from 1.33 to 19.83 years old (mean age 8.29 ± 5.28 years old), 52 were male (62.7%) and 31 were female (37.3%). The underlying diseases were acute lymphoblastic leukemia, acute myeloid leukemia, relapsed acute lymphoblastic leukemia, relapsed acute myeloid leukemia, non-Hodgkin lymphoma, Ewing's sarcoma, and osteosarcoma *(Table 5)*.

Table 5. Classification of the patient population based on the underlying disease and therapy

Diagnosis	Therapy	Male patients	Female patients	Total
Acute lymphoblastic leukemia (ALL) [(1)]	ALL-BFM 2000 / EsPhALL	27	23	50
Relapsed acute lymphoblastic leukemia (relapsed ALL) [(2)]	ALL-Rez. BFM-2002	7	1	8
Acute myeloblastic leukemia (AML) [(3)]	AML-BFM 2004	5	2	7
Relapsed acute myeloblastic leukemia (relapsed AML) [(4)]	AML-Rez. 2001 / Relapsed AML 2009	3	0	3
Non-Hodgkin Lymphoma [(5)]	B-NHL-BFM 2004 / NHL-BFM 90	6	0	6
Ewing's sarcoma [(6)]	EURO-EWING 99 / EWING 2008 / CWS 2002 P	1	5	6
Osteosarcoma	EURAMOS-1	3	0	3
TOTAL		52	31	83

[(1)] three patients have trisomy 21 (2 males, 1 female); one female patient died during the intensive chemotherapy; only one female patient was treated with EsPhALL protocol; two male patients had preventive radiation therapy at the end of the intensive chemotherapy

[(2)] one male patient has trisomy 21; four male patients had radiation therapy at the end of the intensive chemotherapy; three other male patients and one female patient underwent bone-marrow transplantation

[(3)] one male patient has trisomy 21; one female patient had radiation therapy; two male patients and one female patient underwent bone-marrow transplantation (1 male patient died after bone-marrow transplantation)

[(4)] one patient was treated with relapsed AML 2009; two patients underwent bone-marrow transplantation

[(5)] four patients were treated with B-NHL-BFM 2004; two patients were treated with NHL-BFM 90

(6) four female patients were treated with EURO-EWING 99; one male patient was treated with EWING 2008; one female patient was treated with CWS 2002; three female patients and one male patient had radiation therapy; one female patient died during the intensive chemotherapy

3.2. Incidence and degree of mucositis

In this study, we observed that during the intensive chemotherapy 76 patients (91.6%) developed at least one episode of oral mucositis and only 7 patients (8.4%) did not develop any mucositis; they were 5 patients with ALL, 1 patient with relapsed AML and 1 patient with Ewing's sarcoma. *(Figure 6)*

Furthermore, we also found many patients who experienced several mucositis episodes. *(Figure 7)*

Figure 6. Number of patients related to mucositis during chemotherapy

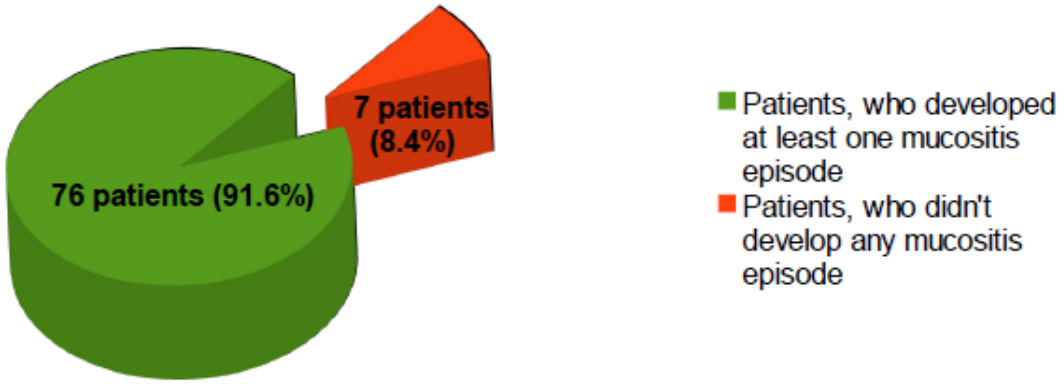
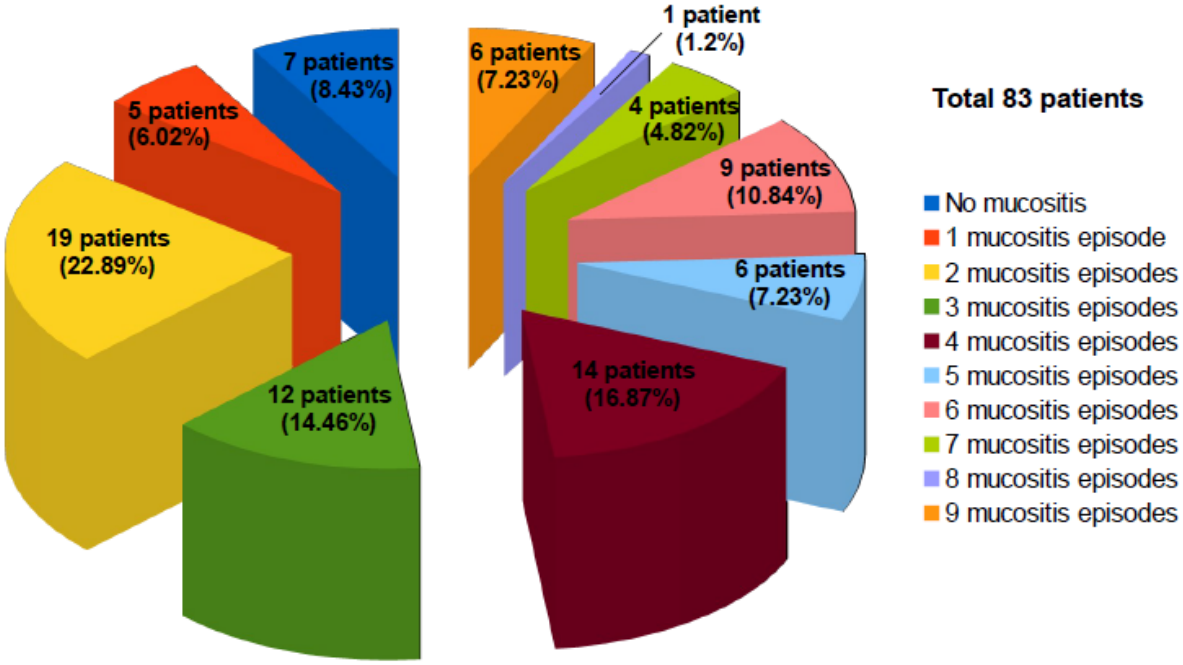


Figure 7. Distribution of patients based on the number of mucositis episodes experienced during chemotherapy

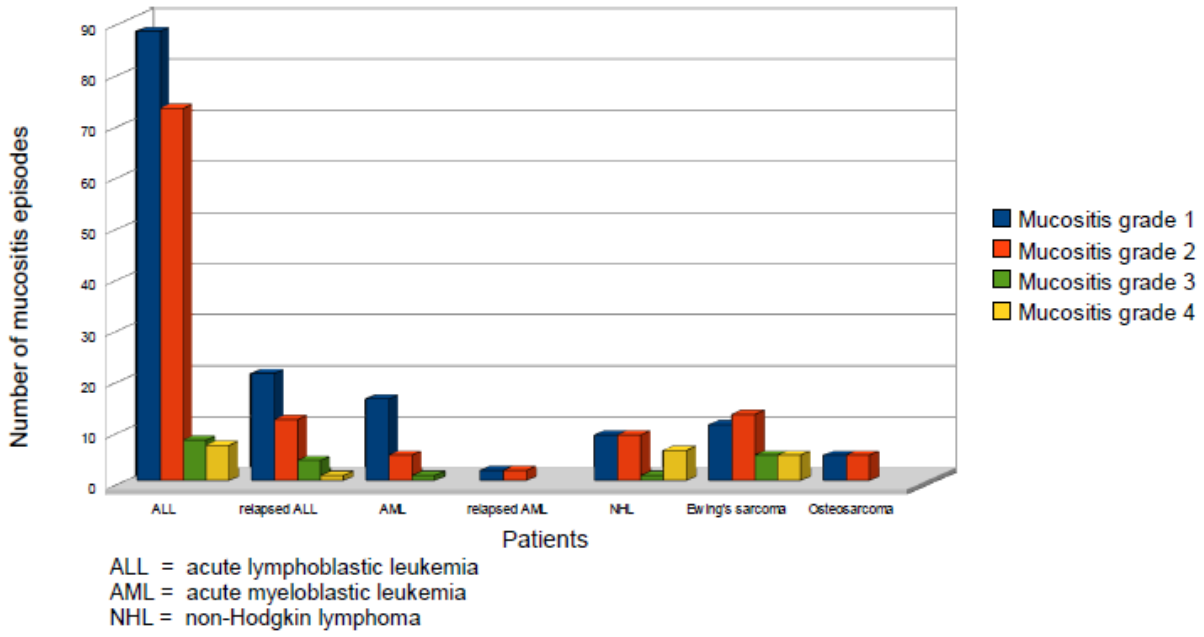


There was a total of 309 mucositis episodes which were classified according to the mucositis scale from the National Cancer Institute. 152 episodes of mucositis were considered as grade 1 (49.2%), 119 episodes as grade 2 (38.5%), 19 episodes as grade 3 (6.15%), and 19 episodes as grade 4 (6.15%). Of the total number of evaluated patients, we discovered that the group of patients with ALL experienced 176 mucositis episodes, the group of patients with relapsed ALL had 38 mucositis episodes, the group of patients with AML had 22 mucositis episodes, the group of patients with relapsed AML experienced 4 mucositis episodes, the group of patients with non-Hodgkin lymphoma had 25 mucositis episodes, the group of patients with Ewing's sarcoma had 34 mucositis episodes, and the group of patients with osteosarcoma experienced 10 mucositis episodes. *(Table 6 and Figure 8)*

Table 6. Number of mucositis episodes experienced by each group of patients

Mucositis scale from NCI	Number of mucositis episodes in group of patients with							Total number of mucositis episodes
	ALL	relapsed ALL	AML	relapsed AML	non-Hodgkin lymphoma	Ewing's sarcoma	Osteo-sarcoma	
Grade 1	88	21	16	2	9	11	5	152 *(49.2%)*
Grade 2	73	12	5	2	9	13	5	119 *(38.5%)*
Grade 3	8	4	1	0	1	5	0	19 *(6.15%)*
Grade 4	7	1	0	0	6	5	0	19 *(6.15%)*
TOTAL	176 *(56.96%)*	38 *(12.3%)*	22 *(7.12%)*	4 *(1.29%)*	25 *(8.09%)*	34 *(11%)*	10 *(3.24%)*	309 *(100%)*

Figure 8. Number of mucositis episodes based on the severity of mucositis and the patients' diseases



ALL = acute lymphoblastic leukemia
AML = acute myeloblastic leukemia
NHL = non-Hodgkin lymphoma

3.3. Frequency of HSV detection

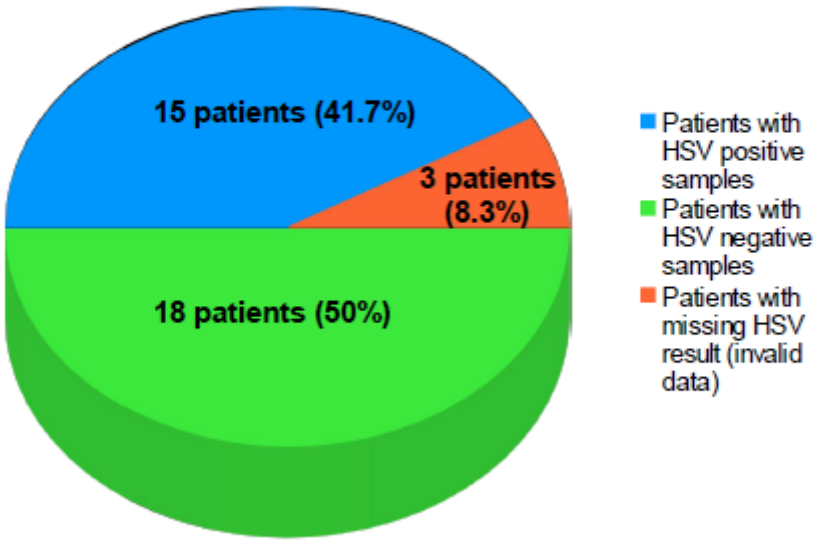
Of the 83 patients, only 36 patients were tested for HSV-PCR using oral swab or throat washing. HSV was then isolated in 15 patients (41.7%), not detected in 18 patients (50%) and in 3 patients (8.3%) the result of HSV-PCR examination was technically not evaluable (invalid data). Excluding these 3 invalid patients, HSV was detected in 15/33 patients (45.5%) and not found in 18/33 patients (54.5%). All positive samples showed HSV type 1 and none of them had HSV type 2. *(Table 7 and Figure 9)*

Table 7. HSV-PCR results in examined patients

Diagnosis	Number of patients with			Total patients examined for HSV-PCR
	HSV-PCR positive[1]	HSV-PCR negative[2]	unknown HSV-PCR result[3]	
ALL	8	8	1	17
Relapsed-ALL	3	4	1	8
AML	-	3	1	4
Relapsed-AML	-	-	-	-
NHL	2	1	-	3
Ewing's sarcoma	2	1	-	3
Osteosarcoma	-	1	-	1
Total	15	18	3	36

[1] HSV-PCR positive at least in one of the examinations
[2] HSV-PCR negative in all examinations
[3] No result found in any examination *(invalid patients)*

Figure 9. Number of patients based on HSV examination result



Of 36 patients tested for HSV-PCR, there was a total of 53 examinations; 11 patients were tested more than once at different mucositis episode. Three of 53 examinations were excluded because of technically not evaluable HSV-PCR results (not enough DNA, poor quality of the swab); therefore later only 50 mucositis episodes were included. HSV was isolated from the mucosal lesions in 24 episodes (48%) and not isolated in 26 episodes (52%). *(Table 8)*

Table 8. Total episodes of mucositis analysed for HSV-PCR

Mucositis episode with	Diagnosis							TOTAL
	ALL	Relapsed ALL	AML	Relapsed AML	NHL	Ewing's sarcoma	Osteo-sarcoma	
HSV-PCR positive	13	5	-	-	2	4	-	24
HSV-PCR negative	12	4	3	-	3	3	1	26
No result (invalid data)	1	1	1	-	-	-	-	3
TOTAL	26	10	4	0	5	7	1	53

Of 53 mucositis episodes, we classified 12 episodes as grade 1, 19 episodes as grade 2, 10 episodes as grade 3, and 12 severe episodes as grade 4. *(Figure 10)*

Figure 10. Frequency of mucositis episodes according to the severity grade

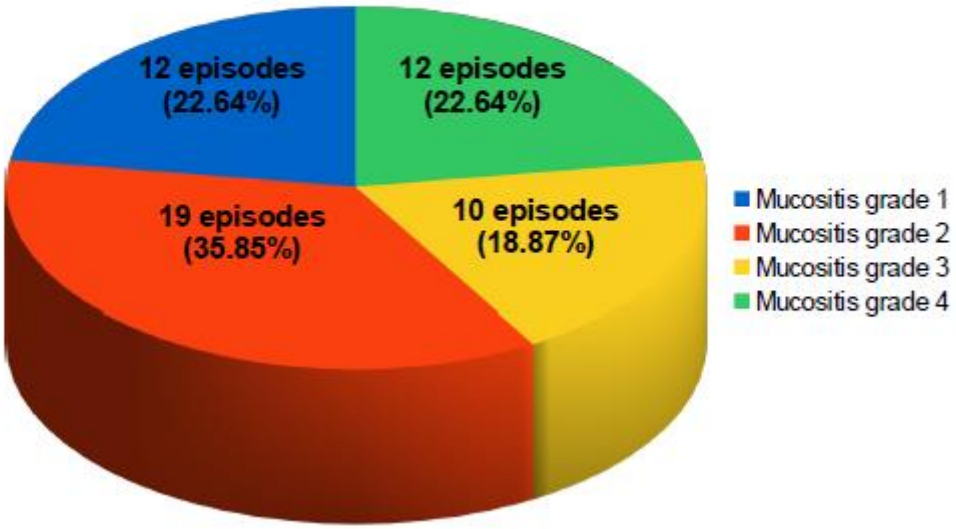


Table 9 below describes the grade of mucositis episodes and HSV-PCR results of those 36 patients who were examined for HSV-PCR from their oral lesions. Furthermore, the lowest leucocyte count (leucocyte nadir), which was observed within a period of 7 days from the beginning of each mucositis episode in every examined patient, is also documented in *Table 9*.

Table 9. Mucositis grade, HSV-PCR result and leucocyte nadir of the examined patients

Patient	Mucositis grade	HSV-PCR	Leucocyte count (cells/mm^3)
Acute Lymphoblastic Leukemia (ALL)			
LL	2	negative	1800
	1	negative	1500
	1	negative	600
AB	2	negative	1100
PS	2	**positive**	1400
	2	**positive**	2400
DO	2	**positive**	790
	1	negative	5700
	2	**positive**	1700
HC	1	**positive**	1100
RR	1	*unknown*	1500
RD	3	negative	500
	1	negative	2200
MJ	3	negative	1200
SB	1	**positive**	2700
ME	2	negative	1900
FL	4	**positive**	500
	4	**positive**	100
NN	2	negative	1700
EM	2	negative	3800
SV	3	negative	800
VJ	4	**positive**	100
	2	**positive**	3300
	4	**positive**	0
SM	3	**positive**	1100
AB	3	**positive**	300
Relapsed ALL			
KO	3	negative	400
PP	2	negative	500
RL	2	negative	1500
WA	2	*unknown*	600
AK	4	**positive**	800
	2	**positive**	400

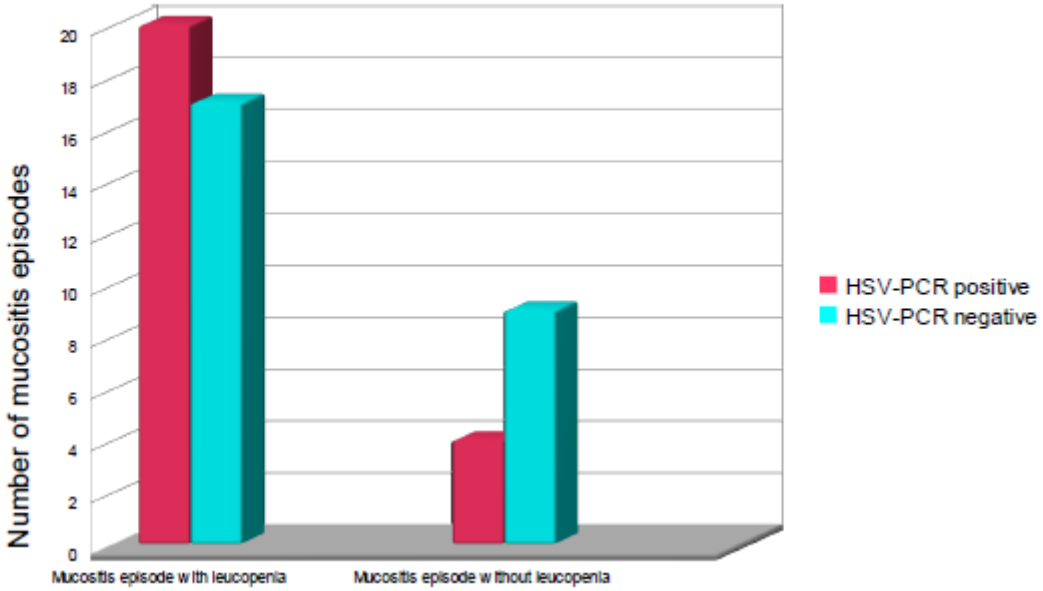
TT	1	**positive**	200
SA	3	negative	1100
WE	1	**positive**	960
	2	**positive**	100
Acute Myeloblastic Leukemia (AML)			
BM	1	*unknown*	1540
BR	1	negative	100
GE	1	negative	100
KY	2	negative	400
Non-Hodgkin Lymphoma			
JA	4	negative	110
HV	4	negative	0
	4	negative	3700
	4	**positive**	110
NM	3	**positive**	300
Ewing's sarcoma			
US	2	**positive**	300
JL	4	**positive**	110
	4	**positive**	200
	3	negative	180
	2	**positive**	100
MJ	3	negative	400
	4	negative	170
Osteosarcoma			
AM	2	negative	1200

As shown in *Table 9*, 8 of 15 patients positive for HSV were examined more than once and at different mucositis episode. Interestingly, in 7 of 8 patients (87.5%) HSV was detected repeatedly (more than once) on their oral lesions at different mucositis episode. We also observed that 38 of 53 (71.7%) mucositis episodes occurred during leucopenia (leucocyte count < 1500/mm^3). Interestingly, of 50 mucositis episodes with their available results of HSV-PCR, we found that HSV was more frequently isolated in mucositis episodes associated with leucopenia (20 of 37 episodes, 54%) and less frequent in mucositis episodes without leucopenia (4 of 13 episodes, 30.8%). *(Table 10 and Figure 11)*

Table 10. Number of mucositis episodes related to leucopenia and HSV-PCR result

Mucositis episodes	HSV-PCR positive	HSV-PCR negative	Total
with leucopenia	20 *(54%)*	17 *(46%)*	37 *(100%)*
without leucopenia	4 *(30.8%)*	9 *(69.2%)*	13 *(100%)*
Total	24 *(48%)*	26 *(52%)*	50 *(100%)*

Figure 11. Mucositis episodes based on leucocyte count and HSV-PCR result



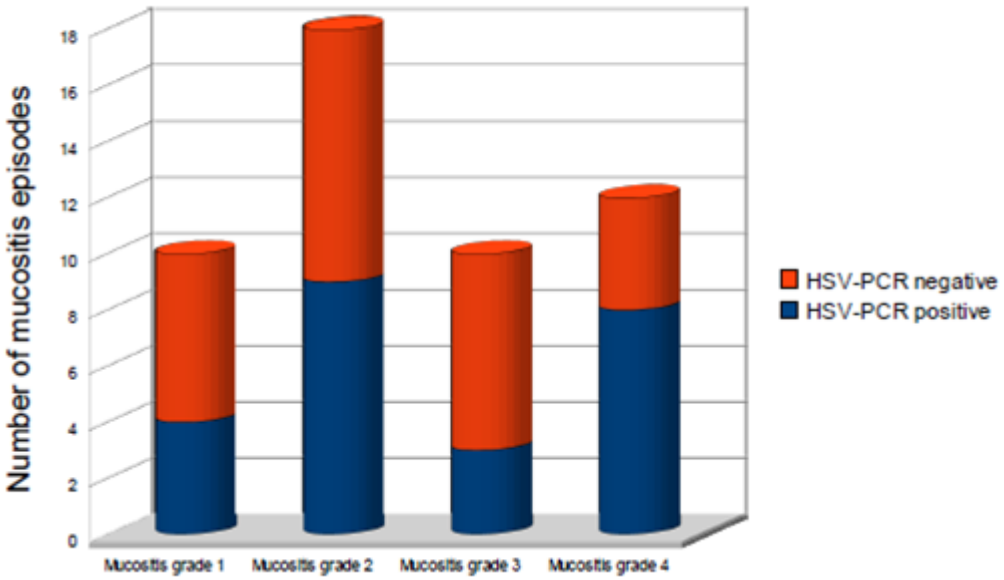
3.4. Correlation of the degree of mucositis with the presence of HSV

The distribution of HSV presence in the mucositis episodes is shown in *Table 11* and *Figure 12*. Excluding those 3 invalid mucositis episodes, we found that of 50 valid mucositis episodes, HSV was isolated in about 40% of the cases in mucositis grade 1, 50% in mucositis grade 2, 30% in mucositis grade 3, and 66.7% in mucositis grade 4. If we divide the mucositis into severe episode (grade 3 and grade 4) and mild to moderate episode (grade 1 and grade 2), we find that HSV was slightly more frequently isolated in the severe episodes (50%) than in the mild to moderate episodes (46.4%).

Table 11. Mucositis Grade * HSV-PCR Crosstabulation

Mucositis	HSV-PCR positive	HSV-PCR negative	Total
Grade 1	4	6	10
% of total mucositis grade 1	*40%*	*60%*	*100%*
% of total HSV-PCR	*16.7%*	*23.1%*	*20%*
Grade 2	9	9	18
% of total mucositis grade 2	*50%*	*50%*	*100%*
% of total HSV-PCR	*37.5%*	*34.6%*	*36%*
Grade 3	3	7	10
% of total mucositis grade 3	*30%*	*70%*	*100%*
% of total HSV-PCR	*12.5%*	*26.9%*	*20%*
Grade 4	8	4	12
% of total mucositis grade 4	*66.7%*	*33.3%*	*100%*
% of total HSV-PCR	*33.3%*	*15.4%*	*24%*
Total	**24**	**26**	**50**
% of total mucositis	***48%***	***52%***	***100%***

Figure 12. Frequency distribution of HSV in different degree of mucositis



Our findings that HSV was frequently isolated in the mucositis episodes of children who had undergone intensive chemotherapy enabled us to find out how HSV could affect the chemotherapy-induced mucositis. Therefore, we tried to statistically correlate the presence of HSV with the degree of the chemotherapy-induced mucositis. For this purpose, we evaluated here the presence of HSV only in one mucositis episode of each patient of the 33 patients with valid HSV-PCR results.

In 11 of 33 patients, HSV was examined in more than one episode of mucositis. Therefore, the mucositis episode taken for the sample group from these patients was the first examined episode *(Table 12)*. In order to analyze a correlation between the categorical HSV data and mucositis scale, Chi-Square-Test (Fisher's exact test) was applied. Statistically, the result indicates a non-significant correlation between the frequency of positive HSV samples and the degree of chemotherapy-induced mucositis in children (*p-value= 0.222, Fisher's exact test)*.

Table 12. HSV-PCR * Mucositis Grade Crosstabulation

			Mucositis grade				Total
			1	2	3	4	
HSV-PCR	**negative**	**Count**	2	9	6	2	19
		% of total HSV-PCR	10.5%	47.4%	31.6%	10.5%	100%
	positive	**Count**	4	3	3	4	14
		% of total HSV-PCR	28.6%	21.4%	21.4%	28.6%	100%
Total		**Count**	6	12	9	6	33
		% of total mucositis	18.2%	36.4%	27.3%	18.2%	100%

4. Discussion

In patients receiving cytotoxic chemotherapy, mucositis is a frequent complication associated with a high degree of morbidity and mortality. Mucositis will negatively impact on the treatment density and has therefore a documented adverse effect on the survival. Furthermore treatment-related toxic deaths occur as a result of mucositis and secondary infections followed by sepsis. The mucositis in patients receiving chemotherapy can occur as a result of the cytotoxic effect of the chemotherapeutic agents themselves on the oral tissue and also as a result of underlying immunodeficiency. This immune dysfunction can enhance opportunistic infections of bacteria, fungi or viruses to develop and cause pathological changes in the oral mucosa.

A number of studies, mostly performed in adult patients, assumed that herpes simplex virus (HSV) was particularly associated with the oral mucositis in patients receiving chemotherapy.[19,20,22] There are only limited studies available in pediatric populations concerning the relationship between HSV and chemotherapy-induced mucositis.[18,21,64]

In our retrospective study evaluating 83 children with hematologic malignancies and solid tumours during their intensive chemotherapy, we found that 76 patients (91.6%) developed oral mucositis. The incidence of mucositis in our study is higher than the results of other studies done in pediatric populations, such as a study from Cheng et al.[15] that found only a 41% incidence of mucositis in pediatric patients under chemotherapy also with hematologic malignancies and solid tumours. The different mucositis scale used (Chinese version of the Mouth and Throat Soreness-Related Questions of the Oral Mucositis Daily Questionnaire) and the mucositis assessment done by the patients themselves or their parents applied in the study from Cheng et al. might be the factors that explain the difference in our results. Figliolia et al.[32] revealed 46% incidence of mucositis but only in children treated for ALL. Cruz et al.[35] suggested approximately 40% incidence of mucositis in children undergoing chemotherapy, but the assessment of mucositis was only done on day 1 (before the chemotherapy) and days 8 and 15. In our study, the oral assessment was done daily when the children were hospitalized or during their visits in the daycare clinic. Furthermore, the selection of our patient population also contributed to the high incidence of mucositis, since only patients receiving chemotherapy with intensive treatment protocols were included in our study. It is believed that, due to the mucotoxic effect of the chemotherapeutic agents, patients undergoing high intensity treatment are at greater risk for developing oral mucositis compared to the patients with moderate or low intensity treatment. This high incidence of mucositis shows how children with chemotherapy are at such great risk of developing mucositis and it is important to reduce the risk or severity of mucositis by applying supportive efforts such as maintaining good oral hygiene, avoiding oral irritants (rough or spicy foods), or applying topical analgesics.

Of 76 patients with oral mucositis, only 36 patients were analyzed for HSV-PCR. Three patients were excluded because their HSV-PCR results were not available (invalid patients). Thus, only 33 patients were eligible for the next evaluation. HSV-1 was isolated in 15 of 33 patients (45.5%) and none of the patients was positive for HSV-2. This finding is in accordance to the characteristics of HSV itself, which is that HSV-1 commonly infects skin and mucous membranes above the waist. Our result is similar to the study from Carrega et al.[21], who found HSV from mucosal lesions in 10 of 20 children (50%) under chemotherapy. Of 33 patients there was a total of 50 mucositis episodes, which were examined for HSV. 24 of 50 mucositis episodes (48%) were found positive for HSV. This result doesn't differ much from the one from Sepúlveda et al.[64], who found HSV in 10 of 30 oral lesions (33.3%) from 19 pediatric patients.

Furthermore, we also found HSV in different mucositis episodes experienced by the same patient. In this study, 8 of 15 patients positive for HSV were examined more than once and at different mucositis episodes. The result is interesting, because HSV was detected repeatedly (more than once) in 7 of 8 patients (87.5%) from their oral lesions at different mucositis episode. These findings are in accordance with the suggestion that HSV isolation in mucositis developing after antineoplastic therapy reflects a reactivation of a latent infection rather than a primary infection.[21]

Several studies in adult patients showed a higher incidence of HSV presence in mucositis under chemotherapy. Bergmann et al.[20] found HSV in 61% of the ulcerated lesions from 11 of 18 adult patients with hematologic malignancies. A high incidence of HSV presence of about 87.5% in adults with chemotherapy-induced mucositis (35 of 40 patients) was also discovered by Djuric et al.[22] The higher incidence of oral HSV in adult patients undergoing chemotherapy than in the pediatric patients might be explained by the previous assumption that the HSV-isolation from the oral lesions is probably due to reactivation of a latent infection rather than a primary infection; as it is recognized that HSV-seropositive patients present a higher risk for developing HSV mucositis during chemotherapy[19,21], and that the prevalence of HSV antibodies increases corresponding with age, where HSV-antibodies are found in 37.5% of children less than 10 years of age and in 88.9% of adults above 30 years of age.[65]

In this study, we also found that 38 of 53 mucositis episodes (71.7%) occurred during leucopenia (leucocyte count < 1500/mm^3). This result confirms the finding from Bergmann et al.[20], which has revealed that patients, who were treated for hematologic malignancies, were generally leucopenic when the ulcers were detected. Some authors have even suggested an association between chemotherapy-induced mucositis and neutropenia.[12,14,15,18,21] The leucopenia occurs as a result of the high toxicity of the chemotherapy and might relate to the dose or intensity of the chemotherapy regimen used. It is usually more severe in patients treated for hematologic malignancies. Patients who are leucopenic have an impaired protection against oral mucosal damage, and the proliferation of oral epithelial cells is also compromised.

We further evaluated 50 mucositis episodes with their available results of HSV-PCR and found that HSV was more frequently isolated in mucositis episodes associated with leucopenia (20 of 37 episodes, 54%) and less frequent in mucositis episodes without leucopenia (4 of 13 episodes, 30.8%). Leucopenic patients have indirectly low lymphocyte counts, which might explain the higher frequency of positive HSV samples in mucositis episode with leucopenia, because as we know that the optimal clearance of HSV depends upon the generation of a T helper cell-associated immune response particularly CD4+ and CD8+ T cells. Epstein et al.[66] even showed a statistically significant relationship between lymphocyte counts and monocyte counts and the occurrence of and recovery from HSV infection in adult patients treated for leukemia.

The mucositis episodes in our study were classified into grades 1, 2, 3 and 4 according to the mucositis scale from the National Cancer Institute. Of 50 mucositis episodes examined for HSV, we found that HSV was isolated in 40% of mucositis grade 1, 50% of mucositis grade 2, 30% of mucositis grade 3, and 66.7% of mucositis grade 4. HSV was slightly more frequently detected in severe episodes of mucositis (in 50% of mucositis grades 3 and 4) compared to mild to moderate episodes of mucositis (in 46.4% of mucositis grades 1 and 2). These findings enabled us to find out how HSV could influence the chemotherapy-induced mucositis in children. Using Chi-Square-Test or Fisher's exact test we found that there was no significant association between HSV presence and the severity of chemotherapy-induced mucositis (p-value= 0.222).

There are several arguments that could be considered why the result of our study showed no statistical significance between HSV presence and the severity of chemotherapy-induced mucositis. If we consider the study of Mendonca et al., which suggested an association between HSV presence (mainly HSV-1) and mucositis severity, then the procedure on how the clinical assessment of mucositis is done and how the mucositis episode is classified might play an important role in affecting the end result. In the study from Mendonca et al., the examination of patients and the classification of the mucositis were carried out by the same investigator. Meanwhile in our study, the clinical assessment of mucositis was done by different investigators and the mucositis episode itself was later classified or graded by us based on the examination results from the investigators documented in the patients' medical records. Thus, there might be different opinions or biases in establishing the degree of mucositis and this factor could influence the result of the study. Another factor to be considered is that in our study, since it is not a prospective study, not all patients with mucositis were examined for HSV-PCR. Therefore, in the end we might get less accurate results of HSV-PCR due to "omitted patients"; those are patients who were not analyzed but might have positive result of HSV-PCR during their oral mucositis episode. In this case, the results of HSV-PCR in our study could be higher than they are now. Considering these factors, it would be reasonable to do a prospective study in the future to get a more accurate result about how significant the association is between HSV and mucositis severity.

Viewing the results of our study, we conclude that pediatric patients, particularly those who underwent high intensity chemotherapy protocol, had high incidence of oral mucositis and the positive HSV samples were frequently discovered in the mucositis episodes of these patients. But statistically, there is no relationship between the presence of HSV and the severity/degree of the chemotherapy-induced mucositis in pediatric patients. However, we should consider again some factors mentioned above, which could influence the statistical result. Furthermore, leucopenia seems to be an important cofactor for the presence of HSV in the chemotherapy-induced mucositis, since pediatric patients, who had leucopenia during the mucositis episode, experienced HSV oral infection more frequently.

Not only that, we also found that pediatric patients, who had already had once HSV infection (HSV seropositive patients), have a higher risk of experiencing a reactivation of HSV infection during the chemotherapy.

Based on this conclusion that high-risk pediatric patients (children with leucopenia, HSV seropositive children) had frequently HSV infection during their mucositis episode while undergoing high-intensity chemotherapy raises the question of, whether it is necessary to give an acyclovir-prophylaxis to these patients to prevent the HSV infection, so that the management of antineoplastic therapy will not be complicated and the patient's chances of survival rate can be improved. As is well known, HSV infection in immunosuppressive patients can develop into a widespread disseminated disease and also not rarely cause a fatal disease. In adult population, several studies have already been performed. Bergmann et al.[67] has demonstrated a significant benefit of acyclovir in preventing HSV oral infection in HSV-seropositive adult patients with AML during chemotherapy by giving 400 mg acyclovir orally twice daily. In this study, HSV was isolated from 15 placebo recipients and from only 1 acyclovir recipient. Study from Warkentin et al.[68] has established similar clinical efficacy and safety of valacyclovir to acyclovir in the prevention of mucocutaneous HSV infections in adolescent and adult patients with hematologic malignancies.

Regarding this matter, we suggest that a prospective study with large samples of children receiving high-intensity chemotherapy protocol should be performed, in which those children with risk factors (leucopenia, HSV seropositive) will receive placebo or acyclovir during the mucositis episodes. Not to forget, the factors mentioned before, namely the procedure on how to assess the mucositis clinically and to classify the mucositis itself (by the same investigator); also the HSV-PCR examination of all patients with mucositis should be considered thoroughly in this prospective study, so that an accurate result about the necessity of acyclovir prophylaxis for children receiving chemotherapy can be obtained, with the aim of minimizing mucositis complications due to HSV infection during chemotherapy.

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Lebenslauf

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

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