Pediatric Hematology and Oncology, 32:322–330, 2015

ISSN: 0888-0018 print / 1521-0669 online
DOI: 10.3109/08880018.2015.1034819

informa
healthcare

Oral Mucositis in Pediatric Acute Lymphoblastic Leukemia Patients: Evaluation of Microbiological and Hematological Factors

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Objective: to investigate the associations of oral microbiota, leucocytes count, neutrophil count, platelet counts and hemoglobin level, and the severity of oral mucositis in pediatric patients with acute lymphoblastic leukemia (ALL) receiving chemotherapy. *Materials and Methods*: 71 prospective patients were included. Analyses of oral microbiota and blood sample were conducted on days 14 (D14) and 56 (D56) of the Brazilian GBTLI-99 treatment protocol. Herpes simplex virus (*HSV*) identification was performed by PCR followed by DNA sequencing analysis. Bacteria and fungi identification was obtained by standard microbiological culture tests. *Results*: 103 episodes of mucositis occurred, being 65 at D14 and 38 at D56. Most cases positive for herpes viral DNA sequences were identified as *HSV-1*. At D14, we found a significant association between the severity of mucositis and presence of *HSV-1* ($p = 0.0347$), *Candida spp.* ($p = 0.0078$), and low platelet count ($p = 0.0064$). At D56, we found a significant association between the severity of mucositis and the presence of *HSV-1* ($p = 0.0317$), previous *HSV-1* presence on D14 ($p < 0.0001$) and neutrophil count ($p = 0.0211$). *Clinical relevance*: the identification of risk factors for mucositis in children and adolescents may contribute to the development of new strategies for prevention and/or treatment, reducing the complications associated with this condition. *Conclusions*: the presence of *HSV*, platelet count, and *Candida spp.* presence at D14 of ALL induction treatment is associated with increased severity of mucositis in children and adolescents. At D56 of ALL treatment, mucositis severity was associated with neutrophil count, *HSV* presence, and previous presence of *HSV* (at D14).

Keywords acute lymphoblastic leukemia, candida spp, hematological parameters, herpes virus, oral bacteria, oral mucositis

INTRODUCTION

One of the often overlooked and under researched complications of cancer treatment is oral mucositis (OM), which is defined as inflammation of oral mucosa resulting from cancer therapy typically manifesting as atrophy, swelling, erythema, and ulceration [1].

Received 3 March 2015; accepted 24 March 2015.
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Mouth pain and difficulties in swallowing, eating, drinking, and talking are the most prevalent and debilitating symptoms, have been shown to affect many patients throughout the course of OM, and can cause profound psychological distress and impairment of patients' quality of life and functional status [2, 3]. Hospitalization also poses economic constrains on the health care system in addition to its clinical implications [4].

Oral mucositis begins 3 to 10 days after chemotherapy is initiated and can persist for 3 weeks. It has been shown to peak at around 7 to 14 days, at which time it slowly resolves unless complicated by infection [5, 6]. This condition can range from mild to severe and represents a common cause of dose reduction and treatment delays. Development of OM can also increase mortality by nearly 40% in severe cases [7]. Up to 80% of children undergoing chemotherapy will experience some degree of mucositis, although the incidence of OM differs according to the type of cancer and treatment regimen. Children with hematologic malignancies experience mucositis more frequently than those with solid tumors. Furthermore, this group of patients is also more likely to have severe mucositis compared with patients suffering other malignancies [8, 9].

Significant progress has been made in understanding the pathobiology of mucositis. What was once thought to be a simple reflection of direct epithelial damage induced by cytotoxic therapy is now considered to be a complex phenomenon that also affects the connective tissue. The model includes events that have been described in five overlapping stages: initiation, upregulation, message generation, ulceration, and healing. Initiation involves the generation of reactive oxygen species, direct damage to cells, tissues and blood vessels, and the start of other biological events that create a cascade of reactions contributing to tissue damage. Activation of transcription factors such as nuclear factor-kappa B leads to a local increase in proinflammatory cytokines including interleukin IL-6, and tumor necrosis factor (TNF). Positive feedback mechanisms result in amplification and acceleration of the process, which finally leads to ulceration. Oral bacteria colonize denuded connective tissues and their cell wall components activate macrophages to produce additional inflammatory cytokines. All these events lead to pain and in neutropenic patients, bacteria may invade into the systemic circulation causing bacteraemia and sepsis. Following cessation of the injurious therapy, healing occurs and the epithelium appears normal again. However, ongoing alterations may occur predisposing for future complications [4, 7].

At present, there is a scarcity in the literature investigating OM in children and the exact risk factors of the disease in this population are still poorly understood, despite their increased risk of developing this complication when compared to the adult population [9, 10].

Development of mucositis depends on a number of factors, related to both therapy and patient characteristics. Treatment variables that may affect the prevalence and the severity of mucositis include the type, dose, and schedule of systemic cytotoxic medications, radiation dose and field, and concomitant use of chemotherapy and radiation. Among patient associated factors, age, body mass index, gender, alterations in salivary production, poor oral health and mucosal trauma have been reported to influence mucositis [11]. It has become increasingly clear that genetic factors play a role in toxicity risk [12]. Genetic determinants of mucositis risk include genes that regulate the availability of active chemotherapy drug metabolites. Oral microflora is considered to play a secondary role in the pathogenesis of mucositis. Bacteria colonizing ulcerations may contribute to increased severity and delayed healing. The duration of mucositis in myeloablative hematopoietic progenitor cell transplantation (HPCT) was reported to be associated with neutrophil engraftment [13], but the relative contribution of innate and adaptive immunity in mucositis remains to be defined.

Clinical improvement of mucositis seems to correlate with neutrophil recovery [1, 11]. For patients who have been made neutropenic by their chemotherapy, coincident mucositis offers a ready portal of entry for oral bacteria. It is not surprising, therefore, that the presence of mucositis in granulocytopenic cancer patients is strongly associated with bacteremia and sepsis [7].

Patients with impaired local or systemic immunity, in particular low baseline neutrophil levels, are at high risk of infection and mucositis [14]. Furthermore, the development of neutropenia during chemotherapy or radiotherapy greatly increases the incidence of oral mucositis. In addition, the time-scale of mucosal healing depends, at least in part, on that of the recovery of immune function [15, 16].

The objective of this study was to prospectively investigate the associations of oral microbiota, leucocytes count, neutrophil count, platelet counts and hemoglobin level, and the severity of oral mucositis in pediatric patients with acute lymphoblastic leukemia (ALL) receiving chemotherapy.

MATERIALS AND METHODS

Methodology is similar to our recently published study [17]. The study cohort includes a total of 71 consecutive pediatric ALL patients, enrolled on the Brazilian Childhood Cooperative Group-protocol ALL-99 (GBTLI ALL-99) [18] and treated at the Boldrini Children's Center, from August 2005 to October 2006. All patients received the same drugs on similar schedules and dosages. Patients were aged 3 to 276 months (mean, 93.8 ± 65.1); 61.1% were male. Parents, legal guardians, or patients, as appropriate, agreed to participate in the study. Research was conducted with the approval of the Brazilian National Ethical Committee. The collection of mucosal material and blood samples was carried out on days 14 and 56 after the beginning of treatment. All patients treated according to the GBTLI ALL-99 protocol, regardless of risk group, received a 4 weeks induction regimen including prednisone, vincristine, doxorubicin, L-asparaginase, and triple intrathecal therapy (TIT): methotrexate, ara-C and dexamethasone. TIT was performed at D0, D14, D28, and, in addition, at days 7 and 21 if the patient was positive for central nervous system leukemia.

The severity of mucositis was evaluated according to the criteria established by the NCI (2003) [19]. The highest mucositis severity reached along the 10-day period of observation was used for the statistical analysis. All patients received the same orientation regarding oral hygiene and mouth rinsing with physiological saline at 1%. All patients received routine dental management for oral hygiene, cavity repair, and necessary restorations or extractions. Patients did not receive any prophylactic medicine for fungus or virus.

On D14, 71 patients were evaluated. On D56, only 67 of the 71 patients were evaluated. Although oral samples were collected on D14 and D56, the assessment of oral mucosa was carried out on a daily basis for up to 10 days. All patients were examined daily by the same investigator (RMHM).

The presence of different herpes viral species (*HSV-1* to *HSV*−8) was investigated using the consensus primer polymerase chain reaction (PCR) technique developed by VanDevanter et al. [20]. The evaluation of fungi and bacteria was determined by methods of microbiological culture (Sabouraud, Chocolate, Sanguis, and MacConkey agar). The tests used to identify fungi and bacteria were performed according to the norms of the American Society of Microbiology [21].

The Fisher *t*-test was used to analyze the associations between the grade of oral mucositis and the presence of *HSV*, number of bacteria/mm^3, presence of *Candida spp.*, leucocytes count, neutrophil count, platelet counts, and hemoglobin level, using a 5%

significance level. The median value was used as a cutoff point in analyzing data of bacteria colony numbers. The SAS software was used throughout [22].

RESULTS

We evaluated 44 males (61.9%) and 27 females (38.1%). The mean age ± SD was 93,1 ± 63,4 months (range, 3–228 months) at the time of diagnosis. One hundred and three episodes of mucositis occurred, being 65 at D14 and 38 at D56. Typically, patients developed mucosal ulcers, roof of the mouth, lips, and hard palate. The lesions were followed by pain, hyperemia, and fibrin deposition. The mean time to onset of oral mucositis was 6.8 ± 3.9 days. Mucositis started to ameliorate at an average time of 9 days, with neutrophils recovered and. Most cases positive for herpes viral DNA sequences were identified as having *HSV-1*. One patient was positive for *HSV-4* (EBV) at D14. More than 95% of patients were positive for *Streptococcus viridans* and *Neisseria sp* on both time points (days 14 and 56).

At D14, we found a significant association between the severity of mucositis and the presence of *HSV-1* ($p = 0.0347$), *Candida spp.* ($p = 0.0078$) and low platelet count ($p = 0.0064$ (Table 1).

At D56, we found a significant association between the severity of mucositis and the presence of *HSV-1* ($p = 0.0317$), previous *HSV-1* presence on D14 ($p < 0.0001$), and neutrophil count ($p = 0.0211$) (Table 2).

DISCUSSION

Most researches on the risk factors for OM have been focused on adult patients. Pediatric and adolescent patients are greatly under represented. Further, the majority of the studies on pediatric and adolescent populations were cross sectional, retrospective studies or secondary analysis of clinical trials [23]. In this study, the association of mucositis severity and oral microflora and hematological parameters, in a cohort of consecutive ALL pediatric patients were prospectively evaluated.

Our findings are consistent with previous studies in which the incidence of OM in pediatric and adolescent patients receiving chemotherapy was moderately high [24]. A recent retrospective medical record found a 46% incidence of OM in ALL pediatric patients undergoing chemotherapy. Patient age ($p = 0.33$) and gender ($p = 0.08$) showed no correlation with the oral mucositis occurrence [25]. Cheng et al. [6, 26], Fadda et al. [27] and Otmani et al. [9] also reported that no association was found between OM grades and age or gender in pediatric patients. These results are in agreement with our data. However, Vokurka et al. [28] suggested that females appear to be more susceptible to mucositis and that gender may play an important role as an independent risk factor and as a predictor for OM in high-dose chemotherapy settings.

Chemotherapeutic agents inhibit other rapidly dividing cells, especially those in the bone marrow. Thus maximum stomatotoxicity is most frequently observed in the nadir of white blood cell count since oral mucosal cells and leukocytes have essentially the same renewal rate. In the present study we found a significant association between mucositis and neutrophil count at D14 of induction of therapy. McCarthy et al. [29] conducted a prospective cohort study in adult patients to investigate factors associated with mucositis in adult patients receiving 5-FU for cancer of the digestive tract. Multiple logistic regression analysis indicated that xerostomia at baseline or baseline neutrophil level less than 4000 cells/mm^3 were significant predictors of mucositis. Their results are similar to ours; however the study was conducted in adult patients, making it difficult to compare results. Cheng et al.[26] conducted a study to determine the risk factors associated with chemotherapy-induced OM in children and evaluated age,

TABLE 1 Mucositis Severity, *HSV* Presence, *Candida spp*. Presence, Total Bacteria Count, Leucocytes Count, Neutrophil Count, Platelet Count, and Hemoglobin Level in Pediatric Patients with ALL at day 14 of Induction Therapy

	Mucositis				
Clinical and laboratory data	Grade 0	Grade I	Grade II	Grade III	*p-value*
Age (years)					
≤ 5	3 (4.22)	23 (22.29)	3 (4.22)	3(4.22)	
> 5 ≤ 10	2 (2.82)	12 (16.90)	2 (2.82)	3 (4.23)	
> 10 ≤ 14	0 (0.00)	9 (12.68)	1(1.41)	1(1.41)	
≥ 14	1 (1.41)	3 (4.23)	4 (5.63)	1 (1.41)	
					$p = 0.3624$
Gender					
Female	3 (4.23)	19 (26.76)	2 (2.82)	3 (4.23)	
Male	3 (4.23)	28 (39.44)	8 (11.27)	5 (7.04)	
					$p = 0.6161$
HSV presence					
Negative	6 (8.57)	44 (62.86)	9 (12.86)	1 (1.43)	
Positive	0 (0.00)	2 (2.86)	1 (1.43)	7 (10.00)	
					$p < 0.0001$
Candida spp. Presence					
No	5 (7.04)	39 (54.93)	7 (9.86)	2 (2.82)	
Yes	1 (1.41)	8 (11.27)	3 (4.23)	6 (8.45)	
					$p = 0.0078$
Leucocytes count (/mm^3)					
≥3000	0 (0.00)	8 (11.27)	0 (0.00)	0 (0.00)	
2000 ≥ <3000	2 (2.82)	12 (16.90)	3 (4.23)	1 (1.41)	
1000 ≥ <2000	2 (2.82)	20 (28.17)	3 (4.23)	5 (7.04)	
<1000	2 (2.82)	7 (9.86)	4 (5.63)	2 (2.82)	
					$p = 0.5224$
Neutrophil count (/mm^3)					
≥ 1500	0 (0.00)	3 (4.23)	0 (0.00)	0 (0.00)	
1000 ≥ < 1500	2 (2.82)	3 (4.23)	0 (0.00)	0 (0.00)	
500 ≥ <1000	1 (1.41)	13 (18.31)	0 (0.00)	1 (1.41)	
< 500	3 (4.23)	28 (39.44)	10 (14.08)	7 (9.86)	
					$p = 0.1801$
Platelet count (/mm^3)					
≥ 75.000	2 (2.82)	35 (49.30)	4 (5.63)	2 (2.82)	
50.000 ≥ <75.000	1 (1.41)	4 (5.63)	0 (0.00)	3 (4.23)	
25.000 ≥ <50.000	2 (2.82)	5 (7.04)	3 (4.23)	1 (1.41)	
< 25.000	1 (1.41)	3 (4.23)	3 (4.23)	2 (2.82)	$p = 0.0064$
Hemoglobin level (g/dL)					
≥ 10.0	0 (0.00)	9 (12.68)	1 (1.41)	1 (1.41)	
8.0 ≥ <10.0	3 (4.23)	17 (23.94)	3 (4.23)	3 (4.23)	
6.5 ≥ <8.0	2 (2.82)	11 (15.49)	2 (2.82)	1 (1.41)	
< 6.5	1 (1.41)	10 (14.08)	4 (5.63)	3 (4.23)	$p = 0.9366$
Total CFU[b] of bacteria/mm^3					
≤380.000	3 (4.23)	26 (36.62)	4 (5.63)	4 (5.63)	
> 380.000	3 (4.23)	21 (29.58)	6 (8.45)	4 (5.63)	$p = 0.892$

[a]Because of poor DNA quality, 1 patients' sample was excluded from PCR analysis at D14.
[b]CFU = colony-forming units

type of cancer and chemotherapy regimen. These authors suggest that neutropenia may be one important risk factor. Their finding is consistent with current understanding of indirect cytotoxicity in the pathogenesis of OM [4]. It has been suggested that a decrease in the neutrophil count may result in an impaired ability to promote an adequate inflammatory response to the chemotherapy cytotoxic effects on the oral mucosa. In addition, in the absence of adequate neutrophil counts or function, relatively minor mucosal toxicity may progress to overt ulceration, because of an impaired basic

TABLE 2 Mucositis Severity, *HSV* Presence, *Candida spp*. Presence, Total Bacteria Count, Leucocytes Count, Neutrophil Count, Platelet Count, and Hemoglobin Level in Pediatric Patients with ALL at day 56 of Induction Therapy

	Mucositis				
Clinical and laboratory data	Grade 0	Grade I	Grade II	Grade III	*p-value*
Age (years)					
≤ 5	15 (22.39)	10 (14.93)	1 (1.49)	4 (5.97)	
$> 5 \leq 10$	6 (8.96)	8 (11.94)	5 (7.46)	0 (0.00)	
$> 10 \leq 14$	5 (7.46)	3 (4.48)	1 (1.49)	2 (2.99)	
≥ 14	3 (4.48)	4 (5.97)	0 (0.00)	0 (0.00)	
					$p = 0.2057$
Gender					
Female	11 (16.42)	6 (8.96)	5 (7.46)	3 (4.48)	
Male	18 (26.87)	19 (28.36)	2 (2.99)	3 (4.48)	
					$p = 0.1238$
HSV presence at D56[a]					
Negative	28 (43.08)	24 (36.92)	6 (89.23)	3 (4.62)	
Positive	0 (0.00)	0 (0.00)	1 (1.54)	3 (4.62)	
					$p < 0.0001$
Previous HSV presence at D14[a]					
Negative	27 (41.54)	23 (35.38)	7 (10.77)	1 (1.54)	
Positive	2 (3.08)	1 (1.54)	0 (0.00)	4 (6.15)	
					$p < 0.0001$
Previous mucositis at D14					
No	1 (1.49)	2 (2.99)	1 (1.49)	0 (0.00)	
Yes	28 (41.79)	23 (34.33)	6 (8.96)	6 (8.96)	
					$p = 0.5422$
Leucocytes count (/mm^3)					
$\geq$3000	12 (17.91)	9 (13.43)	2 (2.99)	2 (2.99)	
$2000 \geq$ <3000	13 (19.40)	12 (17.91)	1 (1.49)	2 (2.99)	
$1000 \geq$ <2000	3 (4.48)	3 (4.48)	2 (2.99)	2 (2.99)	
<1000	1 (1.49)	1 (1.49)	2 (2.99)	0 (0.00)	
					$p = 0.3366$
Neutrophil count (/mm^3)					
≥ 1500	14 (20.90)	8 (11.94)	2 (2.99)	2 (2.99)	
$1000 \geq < 1500$	10 (14.93)	9 (13.43)	0 (0.00)	3 (4.48)	
$500 \geq$ <1000	4 (5.97)	4 (5.97)	0 (0.00)	1 (1.49)	
< 500	1 (1.49)	4 (5.97)	5 (7.46)	0 (0.00)	
					$p = 0.0211$
Platelet count (/mm^3)					
≥ 75.000	26 (38.81)	22 (32.84)	6 (8.96)	6 (8.96)	
$50.000 \geq$ <75.000	2 (2.99)	0 (0.00)	1 (1.49)	0 (0.00)	
$25.000 \geq$ <50.000	0 (0.00)	3 (4.48)	0 (0.00)	0 (0.00)	
< 25.000	1 (1.49)	0 (0.00)	0 (0.00)	0 (0.00)	
					$p = 1.000$
Hemoglobin level (g/dL)					
≥ 10.0	10 (14.93)	11 (16.42)	0 (0.00)	3 (4.48)	
$8.0 \geq$ <10.0	9 (13.43)	8 (11.94)	2 (2.99)	3 (4.48)	
$6.5 \geq$ <8.0	9 (13.43)	3 (4.48)	3 (4.48)	0 (0.00)	
< 6.5	1 (1.49)	3 (4.48)	2 (2.99)	0 (0.00)	
					$p = 0.1097$
Total CFU[b] of bacteria/mm^3					
≤ 300.000	13 (19.40) 16	16 (23.88)	3 (4.48)	5 (7.46)	
> 300.000	(23.88)	9 (13.43)	4 (5.97)	1 (1.49)	
					$p = 0.2314$
Candida spp. presence					
No	24 (35.82)	22 (32.84)	6 (8.96)	5 (7.46)	
Yes	5 (7.46)	3 (4.48)	1 (1.49)	1 (1.49)	
					$p = 0.9491$

[a]Because of poor DNA quality, 2 patients' samples were excluded from PCR analysis at D56.
[b]CFU = colony-forming units

repair response [30]. An inverse relationship between neutrophil counts and the severity of OM has been reported by several investigators [6, 31, 32]. In the present study was observed an inverse association between platelets count and mucositis grade ($p = 0.0064$). These results are in agreement with the results of previous study [32]. Probably the bleeding related to lower platelet counts difficult the ulcers healing and increase the severity of mucositis.

We found no association between hemoglobin levels and mucositis intensity in the studied patients. Ye et al. [32] showed that before chemotherapy a more heterogeneous bacterial community was found in the patients who later developed OM compared with no mucositis group. In addition, they found a more pronounced shift of the bacterial composition after the initiation of chemotherapy in patients who later developed OM compared with those who did not, indicating that oral microbial 'stability' might be beneficial. We do not found a direct association between the count of CFU bacteria of the oral cavity and mucositis severity ($p = 0.8921$ and $p = 0.2314$, at D14 and D56 respectively). As described in a previous publication, one possible explanation for the lack of this relationship is that patients under chemo treatment used trimethoprim–sulfamethoxazole prophylactically, which caused a reduction in the oral bacterial microbiota, or the dental preventive treatment received by patients, which may have led to a significant decrease in the bacterial population [17]. Anirudhan et al. [33] studied 70 children and found no significant difference in oral bacteria prevalence between groups of patients with mild and severe mucositis.

In this study, it was shown that the presence of *Candida spp.* is associated with increased severity of mucositis but these results are in conflict with Anirudhan et al. [8] who found no significant difference in fungi prevalence between groups of patients with mild and severe mucositis.

According to Sonis [34] speculation that *HSV-1* might be associated with the development of mucositis has been mentioned in the literature since at least the 1980s. However, Aggarwal et al.[35] has confirmed a high (66%) prevalence of *HSV-1* in oral mucositis in children receiving chemotherapy for cancer, but found no association of *HSV-1* with mucositis severity. These authors believe that a relatively small sample size with a reasonably high prevalence of *HSV* could be the confounding factors for identifying such association. Herpes simplex (mainly *HSV-1*) stands out among the viral agents related to infections in pediatric leukemia patients, in whom the virus is found most frequently in lesions located in the oral mucosa [36]. Figliolia et al. [25] retrospectively analyzed records from 169 children with ALL and herpes was recorded in 13 (16.9%) patients. Santos de Faria et al.[37] analyzed the data of 92 patients diagnosed with ALL and considered the *HSV-1* virus as a risk factor for the mucositis severity. These findings are in agreement with the results of the present research, which demonstrated that infection by *HSV* was a risk factor for aggravation of the severity of mucositis.

CONCLUSION

We conclude that the presence of *HSV*, platelet count, and *Candida spp.* presence at D14 of ALL induction treatment is associated with increased severity of mucositis in children and adolescents with this disease. At D56 of ALL treatment, mucositis severity was associated with neutrophil count, *HSV* presence, and previous presence of *HSV* (at D14).

Acknowledgments

We thank Dr. Marcio J. da Silva from the State University of Campinas (Campinas, SP, Brazil) for sequencing analysis. We also thank Dr. José Dirceu Ribeiro and Dr. Antônio

Fernando Ribeiro, from the State University of Campinas (Campinas, SP, Brazil) for the critical reviewing of this work.

Declaration of Interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

This work was supported by the State of São Paulo Research Foundation (FAPESP) grant number 04/11274-5 to SRB.

REFERENCES

[1] Miller MM, Donaldm DV, Hageman TM. Prevention and treatment of oral mucositis in children with cancer. *J Pediatr Pharmacol Ther* 2012;17:340–350.
[2] Tomlinson D, Judd P, Hendershot E, Maloney AM, Sung L. Establishing literature-based items for an oral mucositis assessment tool in children. *J Pediatr Oncol Nurs* 2008;25:139–147.
[3] Cheng KKF, Lee V, Li CH, Yuen HL, Epstein JB. Oral mucositis in pediatric and adolescent patients undergoing chemotherapy: the impact of symptoms on quality of life. *Support Care Cancer* 2012;20:2335–2342.
[4] Sonis ST, Elting LS, Keefe D, Peterson DE, Schubert M, Hauer-Jensen M, Bekele BN, Raber-Durlacher J, Donnelly JP, Rubenstein EB. Perspectives on cancer therapy-induced mucosal injury: pathogenesis, measurement, epidemiology, and consequences for patients. *Cancer* 2004; 100:1995–2025.
[5] Medina PJ, Shord SS. Cancer treatment and chemotherapy. In: Talbert RL, DiPiro JT, Matzke GR, et al., eds. *Pharmacotherapy: A Pathophysiologic Approach*. 8th ed. McGraw-Hill, New York, 2011: 2191–2228.
[6] Cheng KK, Molassiotis A, Chang AM, Wal WC, Cheunq SS. Evaluation of an oral care protocol intervention in the prevention of chemotherapy induced oral mucositis in paediatric cancer patients. *Eur J Cancer* 2001;37:2056–2063.
[7] Sonis ST. Oral mucositis in cancer therapy. *J Support Oncol* 2004;2:3–8.
[8] Cheng KK, Chang AM, Yuen MP. Prevention of oral mucositis in paediatric patients treated with chemotherapy; a randomized crossover trial comparing two protocols of oral care. *Eur J Cancer* 2004;40:1208–1216.
[9] Otmani N, Alami R, Hessissen L, Mokhtari A, Soulaymani A, Khattab M. Determinants of severe oral mucositis in paediatric cancer patients: a prospective study. *Int J Paediatr Dent* 2011;21:210–216.
[10] Tomlinson D, Judd P, Hendershot E, Maloney AM, Sung L. Measurement of oral mucositis in children: a review of the literature. *Support Care Cancer* 2007;15:1251–1258.
[11] Raber-Durlacher JE, Elad S, Barasch A. Oral mucositis. *Oral Oncology* 2010;46:452–456.
[12] Sonis ST. Regimen-related gastrointestinal toxicities in cancer patients. *Curr Opin Support Palliative Care* 2010;4:26–30.
[13] Blijlevens N, Schwenkglenks M, Bacon P, D'Addio A, Einsele H, Maertens J, et al. Prospective oral mucositis audit: oral mucositis in patients receiving high-dose melphalan or BEAM conditioning chemotherapy – European Blood and Marrow Transplantation Mucositis Advisory Group. *J Clin Oncol* 2008;26:1519–1525.
[14] Duncan M, Grant G. Oral and intestinal mucositis—causes and possible treatments. *Aliment Pharmacol Ther* 2003;18:853–874.
[15] Filicko J, Lazarus HM, Flomenberg N. Mucosal injury in patients undergoing hematopoietic progenitor cell transplantation: new approaches to prophylaxis and treatment. *Bone Marrow Transplant* 2003;31:1–10.
[16] Sonis ST, Fey EG. Oral complications of cancer therapy. *Oncology (Huntington)* 2002;16:680–686.
[17] Mendonça RM, de Araújo M, Levy CE, Morari J, Silva RA, Yunes JA, Brandalise SR. Prospective evaluation of HSV, Candida spp., and oral bacteria on the severity of oral mucositis in pediatric acute lymphoblastic leukemia. *Support Care Cancer* 2012;20:1101–1107.
[18] Scrideli CA, Assumpção JG, Ganazza MA, Araújo M, Toledo SR, Lee ML, Delbuono E, Petrilli AS, Queiróz RP, Biondi A, Viana MB, Yunes JA, Brandalise SR, Tone LG. A simplified minimal residual disease polymerase chain reaction method at early treatment points can stratify children with acute lymphoblastic leukemia into good and poor outcome groups. *Haematologica* 2009;94:781–789.
[19] Cancer Therapy Evaluation Program (2003) Common terminology criteria for adverse events v.3. Available from http://ctep.cancer.gov. Accessed 12 December 2006
[20] VanDevanter DR, Warrener P, Bennett L, Schultz ER, Coulter S, Garber RL, Rose TM. Detection and analysis of diverse herpesviral species by consensus primer PCR. *J Clin Microbiol* 1996;34:1666–1671.

[21] Murray PR, Baron EJ, Jorgenson JH, Pfaller MA, Yolken RH. *Manual of clinical microbiology*. Washington, DC: ASM Press; 2003.
[22] SAS Institute Inc. *SAS/STAT software changes and enhancements though release 8.2*. Cary, NC: SAS Institute, Inc. 1999–2001
[23] Cheng KKF, Lee V, Li CH, Goggins W, Thompson DR, Yuen HR, Epstein JB. Incidence and risk factors of oral mucositis in paediatric and adolescent patients undergoing chemotherapy. *Oral Oncology* 2011;47:153–162.
[24] Cruz LB, Ribeiro AS, Rech A, Rosa LG, Castro CG Jr, Brunetto AL. Influence of low-energy laser in the prevention of oral mucositis in children with cancer receiving chemotherapy. *Pediatr Blood Cancer* 2007;48:435–440.
[25] Figliolia SLC, Oliveira DT, Pereira MC, Lauris JR, Maurício AR, Oliveira DT, Mello de Andrea ML. Oral mucositis in acute lymphoblastic leukaemia: analysis of 169 paediatric patients. *Oral Dis* 2008;14:761–766.
[26] Cheng KKF, Goggins WB, Lee VWS, Thompson DR. Risk factors for oral mucositis in children undergoing chemotherapy: a matched case–control study. *Oral Oncol* 2008;44:1019–1025.
[27] Fadda G, Campus G, Luglie P. Risk factors for oral mucositis in paediatric oncology patients receiving alkylant chemotherapy. *BMC Oral Health* 2006;18(6):13.
[28] Vokurka S, Bystrická E, Koza V, Scudlová J, Pavlicová V, Valentová D, Visokaiová M, Misaniová L. Higher incidence of chemotherapy induced oral mucositis in females: a supplement of multivariate analysis to a randomized multicentre study. *Support Care Cancer* 2006;14:974–976.
[29] McCarthy GM, Awde JD, Ghandi H, Vincent M, Kocha WI. Risk factors associated with mucositis in cancer patients receiving 5-fluorouracil. *Oral Oncol* 1998;34:484–490.
[30] Witko-Sarsat V, Rieu P, Descamps-Latscha B, Lesavre P, Halbwachs-Mecarelli L. Neutrophils: molecules, functions and pathophysiological aspects. *Lab Invest* 2000;80:617–53.
[31] Lockhart P, Sonis S. Relationship of oral complications to peripheral blood leukocyte and platelet count in patients receiving cancer chemotherapy. *Oral Surg Oral Med Oral Pathol* 1997;48:4821–4828.
[32] Ye Y, Carlsson G, Agholme MB, Wilson JA, Roos A, Henriques-Normark B, Engstrand L, Modéer T, Pütsep K. Oral bacterial community dynamics in paediatric patients with malignancies in relation to chemotherapy-related oral mucositis: a prospective study. *Clin Microbiol Infect.* 2013;19:E559–67.
[33] Anirudhan D, Bakhshi S, Xess I, Broor S, Arya LS. Etiology and outcome of oral mucosal lesions in children on chemotherapy for acute lymphoblastic leukemia. *Indian Pediatr* 2008;45:47–51.
[34] Sonis ST. Mucositis: the impact, biology and therapeutic opportunities of oral mucositis. *Oral Oncol* 2009;45:1015–20.
[35] Aggarwal R, Bansal D, Naru J, Salaria M, Rana A, Minz RW, Trehan A, Marwaha RK. HSV-1 as well as HSV-2 is frequent in oral mucosal lesions of children on chemotherapy. *Support Care Cancer* 2014;22:1773–1779.
[36] Alexander SW, Walsh TJ, Freifeld AG, Pizzo PA Infections complications in the pediatric cancer patients. In: Pizzo PA, Poplack P (eds) *Principles and practice of pediatric oncology*, 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2002:1239–1283.
[37] Santos de Faria AB, Silva IH, de Godoy Almeida R, Silva SP, Carvalho AT, Leão JC. Seroprevalence of herpes virus associated with the presence and severity of oral mucositis in children diagnosed with acute lymphoid leukemia. *J Oral Pathol Med* 2014;43:298–303.