Oral and dental status of immunocompromised children
Prevention and treatment options

Dr. Márta Alberth

Prof. Dr. Ildikó Márton
Prof. Dr. Csongor Kiss

University of Debrecen, Medical and Health Science Center,
Faculty of Dentistry

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1. Introduction

Although malignancies are rare, they represent the most common cause of death in childhood. Children differ greatly from adults not only in their incidence of cancer, but also in the type of cancer that they develop. The major part of pediatric malignances are hematologic (leukemias, lymphomas), sarcomas or embryonal tumors, whereas the majority of adult cancers are carcinomas. Advances in treatment of malignancy in childhood have resulted in an increasing number of long-term survivors. The complex treatment includes multiagent chemotherapy alone or in combination with radiotherapy. In certain cases the bone marrow transplantation has become a part of the therapeutic modality. Each of the treatment components has negative side effects, and the components of the modality used in combination increase the negative impact of the treatment.

The impressive advances made in the treatment of childhood cancer have now directed increasing attention to these acute and late side effects and to the quality of life of the survivors. Acute manifestations of cancer therapy include mucositis and ulcerations, different infections and dental changes. Late complications are a particular concern in children, because cranial growth and dental development may be altered. Considering these facts, it seemed appropriate to conduct a study designed to investigate the incidence and nature of oral problems occurring in child cancer patients. Children with cancer should be followed and given an early treatment besides the prophylactic measurements to reduce the consequences of the baseline diseases and of the anticancer therapy given. Management of pediatric oncology patients requires team approach that should include pediatric dentist as well, this way the majority of late sequels of chemo-irradiation can be prevented or at least decreased. Idiopathic thrombocytopenic purpura is not a malignant life threatening disease, but the thrombocytopenic bleeding, the immunosuppressant state caused by the treatment, and the often

occurring infections make the oral and dental status the same to be found in children with malignant diseases.
The beneficial effect of steroids are well known and widely used in medicine and dentistry, but the use of steroids has a negative impact on the overall health of patients. The “soft” or “novel” steroids are designed that way that after acting on the target cell the molecule brakes into inactive metabolites without having any side effects.
In my study I summarize all the results I gained with investigating the oral health of children earlier treated with malignant diseases and with ITP compared them to healthy controls, the results of studying the physicochemical properties one of the locally used “soft” steroids, the loteprednol etabonate.

2. The aim of the present study was to investigate*:*

- the microbiological changes during anticancer treatment of newly diagnosed children with malignant diseases,
- the oral health and disturbances in dental development in long-term survivors after antineoplastic therapy and ITP and compare them to healthy subjects,
- to find the most effective way of dental treatment and prevention of diseased children,
- to study the lypophilicity, solubility and in vitro permeability of loteprednol etabonate.

3. Material and methods

3. 1. Microbiological changes during anticancer treatment

3. 1. 1. Patient group

The study group consisted of 30 children and adolescents (15 girls and 15 boys, mean age 8.9 years, range from 2 to 24 years) with newly diagnosed cancer, admitted consecutively to the Hematology/Oncology Ward of the Department of Pediatrics of the Medical and Health Science Center of the University of Debrecen

(MHSCUD). The underlying diseases were acute lymphoblastic leukemia (ALL) in 16 cases and solid tumors in 14 cases. Patients were treated according to standard protocols as accepted by the Hungarian Pediatric Oncology Group (HPOG). In febrile patients, empiric antibiotic therapy (ceftazidime, w/o amikacin) was started immediately and modified if necessary according to the result of microbiological cultures or to the condition of the patients. Systemic antifungal therapy (fluconazole 6 mg/kgbw/die) was introduced if fungal infection was diagnozed by clinical sings or microbiological samples, or empirically, if febrile neutropenia lasted over 7 days despite of antibiotic treatment. Fluconazole was changed for amphotericin B (starting dose 0.5 mg/kgbw/die to be escalated up to 1.0 mg/kgbw/die in 5 to 7 days) if culture results proved fluconazole-resistant strains.

Patients developing neutropenic episodes while on cytostatic chemotherapy were examined daily at least twice with respect of their physical condition by a pediatrician and at least once with respect of their oral status by one of the dentists participating in the study. Body temperature was taken at least three times, complete blood count and differential count were determined at least once, daily. Additional imaging and laboratory investigation, including chest X-ray and other imaging techniques, ESR, CRP, electrolyte and blood gas analysis, liver enzyme and renal function tests were performed according to the patients' needs.

3. 1. 2. Microbiological screening

Oral cultures were obtained at least 3 times in each patient: before initiating intensive cytostatic therapy (30 cultures) to determine the patient characteristic microflora, during treatment-related neutropenic episodes (30 cultures) to investigate the microbiological changes and identify the dominant pathogen if existed, and after recovery from neutropenia (30 cultures) to examine the effectiveness of treatment. In cases of prolonged neutropenia (> 7 days) sampling was repeated at

least weakly (15 additional cultures). Altogether 105 oral cultures were conducted. In 23 patients, developing fever in association with moderate-to-severe neutropenia, simultaneous blood, throat and urine culture samples were obtained. Oral candidiasis was diagnosed, and mucositis was scored according to the "National Cancer Institute Common Toxicity Criteria v. 2.0.

Oral cultures were obtained by swabbing the palatal and buccal mucosa using Transystem Standard Tube (Copan Italia, Brescia, Italy). The specimens were inoculated on blood agar, chocolate agar, eosin-methylen blue agar and Sabouraud-dextrose agar (SD) plates (Scharlau Chemie, S.A, Barcelona, Spain).Yeasts, growing on Sabouraud agar, were identified using CHROMagar Candida (Becton Dickinson, Franklin Lakes, NJ, USA) and API ID32C (BioMerieux, Hazelwood, MO, USA). The determination of antifungal susceptibility was performed using Etest (AB Biodisk, Solna, Sweden).

Throat samples were cultured similarly as oral samples. Blood samples were incubated using BACT/Alert (Organon Teknika, Cambridge, UK) automatic device. Urine samples were cultured on Uricult Plus Plates (Orion Diagnostica, Espoo, Finland).

3**. 2. Compare the oral health status and dental development disturbances of long-lasting survivors and healthy controls.**

3. 2. 1. Patient group

Fourty-five children in long-term remission, previously treated for neoplastic diseases at the Division of Hematology–Oncology, Department of Pediatrics, UDMHSC were included into the study. The survey was performed in the Institute of Dental Sciences of the UDMHSC. Of the 45 patients 25 boys and 20 girls, aged 4 to 25 yrs (mean 12.9 yrs), were examined. At the time of diagnosis the age ranged from 1 to 22 yrs (mean 6.9 yrs). Mean survival time was 1 to 14 yrs (mean 5.9 yrs) at the time of the survey. The underlying diseases were 23/45 acute lymphoblastic leukaemia, 5/45 acute

myeloblastic leukaemia and 17/45 solid tumors. Patients were treated according to standard protocols as accepted by the Hungarian Pediatric Oncology Group (HPOG). In course of the combined cytotoxic treatment 28 patients (ALL, AML) 13 received irradiation (12 Gy and 18 Gy, respectively) in addition to chemotherapy, while 17 patients (solid tumors) 11 received irradiation (18-40 Gy).
For each patient an age and sex-matched control was chosen randomly from kindergarden and school children of similar socio-economic background attending at the Department of Pediatric Dentistry, MHSCUD. Oral and dental status, including X-ray imaging, was assessed with informed consent.

3. 2. 2. Oral and dental survey

The DMF numeric scores are generally accepted in the stomatologic literature to quantify the extent of dental disorders. The scores represent the total number of decayed (D), missing (M) or filled (F) permanent teeth (DMF-T) and, allowing a more precise evaluation of dental status, the tooth surfaces (DMF-S). Of these indices we used the combined DM scores (i.e. the number of decayed+missing teeth/surfaces within the denture of a proband) and F scores characteristic for dental pathology and treatment, respectively. In addition to the numeric score system, we also recorded congenital and aquired oral and dental malformations.

3. 2. 3. Statistical analysis

DM and F data points did not follow normal distribution as checked with Kolmogorov-Smirnov test therefore, we used nonparametric tests for the further statistical analysis of our data. Since there was a strong and statistically significant correlation between the age and the DM scores within the patients' group (Sperman's correlation), we created three age groups: 5-11, 12-15 and 16-25 years to control for age. DM and F scores of patients and controls of these age groups were

compared with the Wilcoxon probe. Differences were considered significant if p values were below 0.05.

3. 3. Children with thrombocytopenia

The data of six children with ITP was taken and analysed with the above mentioned methods (3. 2. 2.; 3. 2. 3). The six boys age ranged from 5-12 years (mean age: 8.7 years).

Through a case history of a 9-year- old male patient with amegacariocytic thrombocytopenic purpura I wanted to demonstrate the importance of the regular dental screening, early treatment and prevention in case of children with systemic diseases.

3. 4. Physicochemical properties of a "soft" steroid, the loteprednol etabonate

In this study, the physicochemical properties solubility, lipophilicity and permeability of a soft steroid, the loteprednol etabonate compared to other steroid commonly used in medicine were investigated.

A high performance liquid chromatography (HPLC) method was used. A Water NOVA PAK phenyl column (4 μm, 3.9 mm x 7.5 cm) was connected to a component system from Spectra Physics, which consisted of SP 8810 precision isocratic pump, Rheodyne 7125 injector with a 20 μl loop, SP 8450 UV/VIS variable wave length detector operated at 254 nm and an SP 4290 integrator. The HPLC system was operated at ambient temperature. The mobile phase consisted of acetonitrile, acetic acid and water (45:1:54). At a flow rate of 1.5 ml/min, the retention time of loteprednol etabonate was 4.2 min and that of hydrocortisone-17-valerate was 2.0 min.

3. 4. 1. Solubility determination

Solubility of the compound in water and solutions of various solubilizing agents was determined by adding excess amounts of the compound to the aqueous solutions and sonicating for one hour until equilibration was attained. An aliquot was centrifuged and the supernatant was filtered (0.45 μm membrane filter, Nihon Millipore

Kigyo, KK Yonesava, Japan). The filtrate was diluted and analysed by HPLC

3. 4. 2. Lipophilicity determination

The lipophilicity index, log K, was obtained from the relationship log $k' = \log [(t_r - t_o) / t_o]$. The HPLC method was used to determine t_r and t_o where t_r represents the tested compound and t_o is the formaldehyide. The mobile phases used were various concentrations of acetonitrile in aqueous solution. Log K value was calculated by extrapolating log K' values to 0% acetonitrile.

3. 3. 3. In vitro permeability experiment

3. 3. 3. 1. Preparation of the hamster cheek pouch as biological membrane

Male Golden Syrian Hamsters age 6-8 weeks and weighing 50-80 g were used. The animals were sacrificed by i.v. lethal injection of sodium pentobarbital, and both cheek pouches were rinsed several times with normal saline to remove food debris. Incision was made between mucous and skin; fat and tissue were removed carefully from the undersurface. After rinsing with normal saline solution, the excised pouches were immediately mounted on the diffusion cells providing a 0.71cm^2 surface; the mucosal side of the membrane was exposed to the donor phase. The same area of the pouch was used for all the experiments.

3. 3. 3. 2. Determination of the penetration

The diffusion cells (KRESCO Engineering Consultants, Palo Alto,Ca) consisted of the parts.

The lower part of the diffusion cell was then filled with 4.5 ml of degassed isotonic phosphate buffer saline solution containing 0.3% Brij 58 as the receptor phase and the dermal side was bathed by the receptor phase. The drug to be tested was saturated and suspended in the vehicle of choice (20% or 50% propylene glycole or 50% 2 hydroxypropyl-β-cyclodextrin containing 2% myristoyl choline bromide) and filled in the upper part of the diffusion cell (1 ml) as the

donor phase. The diffusion cells were placed on a temperature controlled magnetic stirrer (36.8 ^{o}C±0.2^{o}C; 400 rpm, PIERCE Recti-therm, Pierce Chemical Co, Rockford, NM). At various time intervals, 0.2 ml samples were removed from the receptor phase and replaced with fresh receptor phase solution. Flux of the drug through the mucous membrane was calculated by comparing the peak area of the drug to a standard calibration curve. The amount of drug penetrating the buccal mucous at each time interval was calculated by he sample concentration multiplied with the volume of the receptor phase. The steady state flux was obtained from the slope of the linear portion of individual cumulative amount versus time plot divided by the area of the mucous membrane (0.71cm^2). The permeability coefficient was calculated by dividing the flux with the initial concentration in the donor phase.

4. Results

4. 1. Microbiological changes in patients treated with malignancies

From the 30 patients 7 developed mild, 12 moderate and 11 severe neutropenia during the treatment periods. In patients with **mild neutropenia** absolute neutrophil count (ANC) varied from 1.1 to 1.9 G/L (mean 1.5 G/L). Neutropenic episodes lasted from 5 to 7 days (mean 6 days). No patients developed fever (axillary temperature >38.0 Cº) and no significant oral lesions were noticed. In patients with **moderate neutropenia** ANC was between 0.6 to 1.0 G/L (mean 0.8 G/L). Neutropenic episodes lasted from 5 to 26 days (mean 16 days). Fever was registered in all 12 children. Ulcerative mucosal lesions (grade 2 and 3) were found in 3 children. Clinical signs of oral candidiasis were observed in 7 patients. In patients with **severe neutropenia** ANC varied from 0.2 to 0.5 G/L (mean 0.4 G/L). Neutropenic episodes lasted from 19 to 40 days (mean 29 days). Each patient was febrile, 8/11 patient had severe ulcerative mucositis (grade 3) and 9 had clinical sign of candidiasis.

Six patients (1 with moderate and 5 with severe neutropenia) developed signs of fungal esophagitis, otherwise no severe organ manifestation was observed. Fungemia (*C. albicans*) was identified in 1 sample and bacteremia was identified in 13 samples obtained from patients with moderate-to-severe neutropenia. Eight patients (2 with moderate and 6 with severe neutropenia) developed clinical signs of septicemia associated with hemoculture-proven fungal and bacterial infections. Every patient survived and recovered from the infectious complications.

No dominant pathogens were found in cultures taken before and after neutropenic episodes. From the analyzed 45 oral cultures taken during the neutropenic episode, 38 (84.4 %) contained dominant pathogens. No changes to the initial oral microflora were observed in patients with mild neutropenia (7 cultures). All 38 positive cultures were obtained from patients having developed moderate-to-severe neutropenia. All 23 patients with moderate-to-severe neutropenia, i.e. 76.7% of study patients, were noted with at least one positive oral culture during the time of observation.

The most frequently isolated pathogens were yeasts (33/38). Bacteria were found in 6/38 cultures. There was no association between the type of cancer and the occurrence of the positive cultures.

Out of the 38 positive oral cultures, 12 were obtained from children with moderate neutropenia. The distribution of these positive samples was as follows: 10/12 yeast, 2/12 bacteria. *C. albicans,* was detected only in 1/12 sample obtained from the pharynx and none from the urine or blood samples in addition to the positive oral samples. Pathogenic bacteria were found in 2/12 samples taken from the pharynx, 1/12 from the urine and 5/12 from the blood.

Twenty-six out of the 38 positive oral cultures were obtained from children with severe neutropenia. The distribution of these samples was as follows: 23/26 yeast, 4/26 bacteria. In addition to positive oral samples, yeasts were found in 8/11 of the pharyngeal swabs, 6/11

from the urine and 1/11 from blood culture samples. Bacteria were detected in 3/12 samples obtained from the pharynx, 5/12 from the urine and 8/12 from the blood.

Initially, *C. albicans* was detected in each of the 33 positive oral cultures. Non-albicans *Candida* species (2 *Candida kefyr*, 1 *Candida lusitaniae*, 1 *Candida sake* and 1 *Candida tropicalis*) were observed exclusively in patients with severe neutropenia and were always preceeded by *C. albicans* infection, 4 to 6 days before the identification of the non-albicans strains.

As expected, all *C. albicans* strains were susceptible to fluconazole and itraconazole, in addition to amphotericin B. Among the non-albicans *Candida* strains we found 1 fluconazole- and 3 itraconazole-resistant strains (MIC values were 64 and 1 mg/L, respectively). All non-albicans *Candida* species were susceptible to amphotericin B.

4. 2. Oral health of long-lasting survivors compare it to healty controls.

We found strong and statistically significant, positive correlation between age and the DM difference (Sperman correlation, Corr=0.463, P< 0.003 for teeth, Corr= 0.523, P<0.001 for surface). To control for this effect of age, we created three age groups 4-11, 12-15, and 16-25 years. To check the relevance of these age intervals, we compared the mentioned DM differences in the three groups (P< 0. 023) for teeth, P<0.007 for surface). Both the differences between the mean values and the level of significance became more expressed with increasing age. In parallel with the higher DM scores representing a more severe tooth decay, periodontal status of patients (GI and PII) was more frequently and more severely affected than the controls (P< 0.001). In contrast, there were no significant differences between the F scores of patients and controls, representing dental treatment activity.

We did not observe any sex- or therapy related differences between the patient and control groups.

Regarding developmental anomalies, we found 17/45 (36%) patients having short V-shaped roots, mostly of the lower incisors. There were 12/45 patients who had enamel hypoplasia in 48 teeth. Only one child in the control group had this problem. Microdontia involving the lower frontal and premolar regions was found in one case in the patient group. Furthermore, one patient had one extra tooth in the premolar region of each quadrant. Dental agenesis was found in three children in the patient group. This anomaly affected the upper lateral incisors and premolars, six teeth were affected. In the control group, one child had two lower premolars missing. The prevalence of dental anomalies in the patient and control group were highly significant ($P<0.001$).

4. 3. Oral health of children with ITP

As it was expected, because of the low number of patients in this group, we found no significant differences ($p<0.2$) in the DM/dm and F/f indicis in children with ITP compare it to healthy controls, though the tendency was the same that we found in long- lasting survivor group. From the six patients two had only primary dentition, and the eruption of the permanent was not expected (late eruption), while others had only permanent dentition. The observed malformations in the patient's group were significantly more than it was in the control group ($p<0.001$). From the screened six children one had mild and one had very serious enamel disturbances. PII and GI values were also significantly higher in the patient's group ($p<0.03$) than in the control group.

4. 3. Physicochemical properties of loteprednol etabonate

Lipophilicity is one of the main factors in controlling the penetration of drugs through biological membranes. The log K value of loteprednol etabonate was higher than that of other steroids, such as hydrocortisone, dexamethasone and hydrocortisone-17-valerate. The

greater lipophilicity of loteprednol etabonate indicates the possibility of better affinity or permeability through a biological membrane. Penetration of the seroids was not observed when using 20% or 50% PG or 50% 2 hyroxypropyl-β-cyclodextrin, so we added 2% myristoyl-cholin-bromid to the vehicle sytem. In the presence of the myristoyl cholin bromide as a penetration enhancer, the total amount of the compounds penetrating through the membrane from the donor phase to receptor phase.

The results indicate that in the presence of the myristoyl cholin bromide, the penetration of loteprednol etabonate was increased by increasing the concentration of PG, however, the opposite was observed in the case of hydrocortisone-17-valerate; the higher the concentration of PG, the lower the amount of drug diffusion. The reason for this observation remains unknown. The results also show that in the twelve hour period, the penetration amount of both drugs was significantly higher if cyclodextrin instead of PG was used as vehicle ($P<0.001$).

In the presence of the 2% myristoyl cholin bromide, the steady state flux of drug permeation was considered to be obtained between 6 and 12 hours or between 4 and 12 hours when 20 or 50% PG was used The lag time for lteprednol eatbonate and hydrocortisone valerate was 4.83 and 3.13 hours (20%) or 1.32 and 2.3 hours (50%) respectively. In the case of 50% cyclodextrin the diffusion pattern of both drugs was different from a standard plot. The initial flux was relatively fast, therefore a convex parabolic curve was obtained, and the lag time was considered to be relatively short. These unusual results are probably due to the addition of penetration enhancer which could adhere to the cyclodextrin instead of the steroid.

In case of the hamster pouch, the flux of the loteprednol eatbonate relative to hydrocortisone-17-valerate varied with the vehicle. When 20% PG was used, the hydrocortisone-17-valerate flux was

approximately twice that of loteprednol eatbonate whereas when 50% cyclodextrin was used the flux of both drugs was about the same.
The results shows that both compounds are marginally soluble in water (0.5 μg/ml and 39 μg/ml for loteprednol eatbonate and hydrocortisone-17-valerate, respectively) and the solubility of loteprednol etabonate in all types of vehicle system used was one order of magnitude lower than the solubility of hydrocortisone-17-valerate.
In PG water system, the solubility of both compounds was increased more than ten folds by raising the concentration of PG from 20% to 50%; however, it could be much more remarkably increased by using 50% of 2 hydroxypropyl-β-cyclodextrin instead of PG. The solubilities of loteprenol eatbonate and hydrocortisone-17-valerate in the 50% cyclodextrin system were more than 33 and 25 times higher compared to the 50% PG system. The penetration enhancer, myristoyl cholin bromide, significantly increased the solubility of both drugs in water. In cyclodextrin system, the presence of myristoyl cholin bromide slightly decreased the solubility of the drugs, this could be explained if myristoyl cholin bromide, instead of the steroids, occupies the cyclodextrin cavity.
The permeability coefficients were calculated by deviding the flux by the saturated concentration of the drug in the donor phase. In this study, in the presence of myristoyl choline bromide, the permeability coefficient of loteprednol etabonate in all system was at least 10 times higher than that of hydrocortisone-17-valerate. This result is consistent with the lipophilicity of the compound, which here is a strong function of the partition coefficient, as is a usual for penetration phenomena. Therefore, one can conclude that although loteprednol etabonate is less soluble than hydrocortisone-17-valerate in various vehicle systems, the permeation activity of loteprednol etabonate is significantly higher, as the total amount of penetration and steady state flux of loteprednol etabonate were greater than that of hydrocortisone-17-valerate in some cases.

5. Discussion

Cancer and cancer treatment profoundly impairs oral health. Oral complications, presenting as mucositis, xerostomia, bleeding and infections, are three times more common in children than in adults. The clinical diagnosis of oral infections may be difficult due to reduced inflammatory responses in the immunocompromized host.

In this study, we have assessed 105 oral swabs of 30 consecutive children with newly diagnosed cancer. Of the 38 positive cultures 33 (86.8%) were positive for fungi and 6 (15.8%) revealed pathogenic bacteria, indicating that fungal pathogens are about five times as common as bacteria in the oral cavity of children with cancer. Altogether 23 of 30 patients (76.7%) were diagnosed at least on one occasion with oral colonization and/or infection. Studies suggest that oropharingeal colonization of Candida species may increase the risk of systemic infection especially when oral ulcers develop during the neutropenic episodes. Risk factors for systemic fungal infection also include the use of broad-spectrum antibiotics, steroids. Well-designed, large studies, utilizing reliable microbiological or histopathological methods estimated the frequency of fungal colonization and infection in pediatric oncologic patients. In the classical study from the St. Jude Children's Research Hospital, Hughes analyzed 109 fatal cases of systemic candidiasis by complete autopsy. He identified fungal lesions in the oral cavity of 26% of the deceased children. *Antemortem* cultures identified *Candida* strains in 69% of the throat and 23% of the nasopharyngeal samples within 2 months to the fatal outcome. In addition to case reports and small-scale studies, 3 groups of investigators assessed oropharyngeal fungal colonization and infections of 42, 26 and 36 children, respectively, with newly diagnosed leukemia and lymphoma. One study analyzed yeast colonization and infections in 64 pediatric hematopoietic stem cell transplant recipients. Similar to our results, reported rates of fungal colonization and infections varied from 35% to 69%.

Regarding microbiological speciation, Gozdasoglu et al. identified exclusively *C. albicans* from 36 surveillance cultures and Stinnett et al. identified 2 *C. tropicalis*- and 1 *Rhodotorula rubra*-positive samples in addition to 37/40 *C. albicans*. Even in the St. Jude study investigating fatal cases of advanced pediatric cancer patients, *C. albicans* was the predominant pathogen. These observations are in contrast with our results identifying 5/33 (15.2%) non-albicans *Candida* strains of the positive fungal cultures. Four of the 5 non-albicans *Candida* strains identified in this study were resistant to azole-type antifungal agents. A similar impact of non-albicans *Candida* strains in children with cancer has only been reported in hematopoietin stem cell transplant recipients. In adults with advanced neoplastic diseases an increasing importance of azole-resistant oral fungal pathogens has already been acknowledged. Interestingly, in our cases, colonization with *C. albicans* always preceded that of with non-albicans *Candida* strains and occurred only in patients with severe, long-lasting neutropenia.

Lack of the use of appropriate microbiological culture techniques may result in an underestimation of fungal colonization. Among the 30 patients enrolled in this study, 11 (36.7%) had ulcerative mucositis and 16 (53.5%) had clinical sign of candidiasis. Stinnett et al. identified only 15% of the leukemic children presenting with clinical signs of oral fungal infections, whereas 46% of their patients proved colonized with fungi when investigated by culture methods. Similarly, in an early study of a pediatric leukemia population, the reported incidence of symptomatic oral fungal lesions was 21%.

Neutropenia has been recognized as one of the major risk factors of developing nosocomial fungal infections both in the oral cavity and in other organs, in particular deep-sited lesions. We detected oral yeasts only in association with moderate-to-severe neutropenic episodes of children with cancer. In turn, oral *Candida* colonization and infections occurred in 100% of these patients. In addition to oral

candidiasis, 1 patient with moderate and 5 with severe neutropenia (20.2%) developed signs of fungal esophagitis. One patient (3.3%) with severe neutropenia was found with fungemia accompanied by clinical signs of septicemia. Prevalence rates of fungemia were reported to occur in 0-6% in children with cancer, representing a rare but severe complication in pediatric oncology.
In conclusion, oral fungal infections develop frequently in children with cancer, in particular in patients with prolonged, severe neutropenic episodes. The pathogenic agents colonizing the oral cavity may induce symptomatic infections, often systemic ones and may promote the development of infections with drug resistant strains. Regular dental check-up examinations, oral microbial surveillance and application of professional oral hygienic measures in children with cancer may decrease the incidence, duration and severity of infectious complications. Early introduction of systemic antifungal treatment in pediatric cancer patients with microbiologically proven oral yeast colonization and infections seems to be a prudent approach.

As survival rate of children with cancer improved considerably during the past decades, late effects of antineoplastic therapy gained an increasing importance. Oral and dental care seem to be neglected areas resulting in considerable impairment of quality of life in adolescents and young adults cured from cancer. Even literature data surveying oral and dental status of children with preceeding neoplastic and hematologic disease are sparse. As far as we know, DMF-T and DMF-S scores were rarely investigated in children with neoplastic disorders. The reported results are controversary. British and Scandinavian investigators found no significant differences in dental caries frequency of long-term childhood cancer survivors. However, those patients were on a regular stomatologic prevention, inducling fluor-prophylaxis while receiving anti-cancer therapy. Our results suggesting that previous antineoplastic therapy exerted a negative

impact on the dental status of patients confirm the observations of Prudell-Lewis et al. and Pajari et al. who reported on increased DMF-S and dmf-s values the latter ones indicative of primary denture status, in children antineoplastic therapy compared to controls.

The negative stomatologic impact of cytostatic drugs and irradiation as well as poor oral hygiene during chemotherapy has been acknowledged. Animal studies showed that chemotherapeutic agents and irradiation induced changes in dental tissues affecting enamel and dentin formation as well as odontogenesis. Reduced salivary flow induced changes in the spectrum of bacteria colonizing the oral cavity favouring caries related microflora. Another consequence of hyposalivation is, that children on chemotherapy or irradiation often require mouth moistening, usually with sugar containing soft drinks, a further risk factor of caries development. Swallowing may become difficult in periods of cancer- or treatment-related mucositis and oral bleeding and cariogenic soft, sweet food are more frequently chosen. Moreover, stomatologic and dental problems are often underestimated by the patients and their parents because of the severity of the underlying neoplastic disease. Therefore, regular dental check-ups and necessary treatment remain often neglected. Even some dentists may be reluctant to provide appropriate treatment for children with cancer because of unjustified fear of serious infections or bleeding.

The finding of significantly elevated DM-T and DM-S scores within two age groups of patients (12-15 and 16-25 years) when comparing with controls suggests that patients have aquired a considerably more severe damage of their permanent dentures in course of the previous neoplastic disease and the required aggressive cytotoxic treatment despite of a similar level of dental treatment as indicated by the similar F-T and F-S scores. The negative impact of cytotoxic agents on patients' dentures is underscored by the observed grossly disturbed amelogenesis.

Similarly to our observations, Alpaspan et.al. documented various pathologic oral and dental malformations in patients treated for malignant diseases. V shaped roots, early apical closure, microdontia and agenesis were the most common findings in addition to enamel hypoplasia .

In conclusion, children with cancer require special attention and dental care in parallel with antineoplastic treatment. Proper oral hygiene and oral health can be maintained and restored before development of irreversible dental damage with a close cooperation of the pediatric oncohematology staff, pediatric dental surgeons and dental hygienists. The British and Scandinavian experiences suggest, that although side effects of antineoplastic therapy affecting the oral cavity are unavoidable, their detrimental impact on oral health can and should be avoided contributing to a better quality of life of children who become cured from cancer.

Loteprednol etabonate is an active "soft" anti-inflammatory corticosteroid. The log K values of loteprednol etabonate and other steroids were used as indices of lipophilicity and by this criterion loteprednol eatbonate was more lipophilic than the other steroids examined. The solubility of loteprednos etabonate in aqueous solution was low, but could be increased by the addition of solubilizing agents such as propylene glycol, myristoyl cholin bromide or 2 hydroxypropyl-β-cyclodextrin. Using hamster cheek pouch as a mucous membrane the loteprednol etabonate exhibited higher penetration activity than did hydrocortisone valerate. The good anti-inflammatory effect and the high penetration activity might make the loteprednole etabonate a very useful medicament in dentistry for curing mucous membrane inflammation. Though the time I spent in the USA was not enough to complete the drug for stomatological use, the co-workers finishing the study made a new "soft" steroid eye drops.

6. Summary

Changes in oral health and microbiological conditions were investigated in children during anticancer treatment. The study consisted of thirty consecutive patients with malignant disease who were assessed for infections, especially Candida and bacteria during myeloimmuno- suppressive therapy. Cultures were taken at baseline, at hematological nadir and at hematological recovery. Patients with moderate to severe neutropenic episodes had a greater frequency of oral ulcerations, as well as occurrence of Candidosis. In addition, there was an increased incidence of fungemia and bacteremia with septic signs and symptoms. Candida albicans was by far the predominant yeast isolated. Non albicans species developed in only the severely neutropenic patients. Neutropenia, the use of broad-spectrum antimicrobial agents and steroids were implicated as risk factors in the development of infections.

Oral health and disturbances in dental development were studied in 45 long-term survivors after antineoplastic therapy compared that to healthy individuals. The results showed that children with malignant diseases previously treated with chemoirradiation exhibited a wider range of disorders in the oral cavity, an increased caries experience, and a significantly higher number of extracted teeth. Teeth affected by disturbances in enamel mineralization and root development could be found in a significant amount of cases of children cured from cancer as compared to the healthy controls.

Loteprednol eatbonate is an active “soft” steroid preparation with an anti-inflammatory potency similar to that of other conventional steroids, but associated with fewer side effects. The investigated compound proved more lipohilic than other steroids examined. The solubility of loteprednol etabonate in aqueous solution was low, but could be increased by the addition of solubilizing agents such as propylene glycol and 2-hydroxypropyl-β-cyclodextrin together with myristoyl cholin bromide. The permeability studies were carried out in

vitro using hamster cheek pouch and diffusion cells as a model to compare the total diffusion. The steady state flux and the permeability coefficient of loteprednol etabonate and hydrocortisone-17-valerate were determined in various vechicle systems mentioned above. The results indicated that in the presence of 2% myristoyl cholin bromide, as a permeation enhancer, when 50% propylene glycol or 50% 2-hydroxypropyl-β-cyclodextrin were used as vehicles, loteprednol etabonate exhibited a higher penetration activity through hamster cheek pouch than hydrocortisone-17-valerate.

Publications related to the thesis:

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