Oral mucositis and saliva IgA, IgG and IgM concentration during anti-tumor treatment in children suffering from acute lymphoblastic leukemia

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
D – writing the article; E – critical revision of the article; F – final approval of the article

Abstract

Background. Oral mucositis is a problem occurring within the oral cavity, which is the most difficult to deal with during anti-tumor treatment. The first symptom reported by the patient is discomfort. Salivary immunoglobulins play an important role in pathological processes occurring in the oral cavity.

Objectives. The study objective was to assess the occurrence of oral mucositis and to assess changes in the saliva IgA, IgG, and IgM concentration in children with acute lymphoblastic leukemia during anti-tumor treatment.

Material and methods. The study included 78 children with acute lymphoblastic leukemia (ALL) and a control group of healthy children. All the participants underwent 3 examinations.

Results. Mucosal opacity followed by redness usually occurred within 2–4 days after the methotrexate infusion. The most severe lesions of the oral mucosa were observed after the 1st month of chemotherapy. Correlations were found between hard-to-heal wounds and ulcers and blood morphology parameters. The mean saliva IgA concentration in children with ALL during chemotherapy was significantly lower than in children in the control group. A comparison of the mean saliva IgG in a given patient in subsequent examinations revealed a significant saliva IgG decrease occurring between the 1st and 3rd examinations.

Conclusions. Wounds and ulcers that were difficult to heal were related to blood morphology parameters. A low salivary IgA concentration in children with ALL may result in the development and potentiation of oral lesions typical of mucositis during anti-tumor treatment. A significant decrease in salivary IgG and IgM concentrations in children with ALL during chemotherapy may cause potentiation of pathological lesions in the oral mucosa.

Key words: children with oncological diseases, oral mucositis, saliva immunoglobulins, acute lymphoblastic leukemia

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E. Pels. Saliva immunoglobulin in children with ALL

Oral mucositis is a problem occurring within the oral cavity that is extremely difficult to deal with during anti-tumor treatment. Generally, the first symptom reported by the patient is discomfort. This is followed by burning of mucosa, dry mouth, mucosal erosion, and ulceration, which causes strong pain. Pain caused by oral mucositis may hinder proper nutrition, which in turn leads to weight loss, cachexia, and dehydration of the body, which is already weakened by disease.[1–3] Pain caused by mucositis also affects drug administration, speech, and breathing.[4–6] Oral mucosa may become deteriorated to such an extent that the patients require changes in the anti-tumor treatment and/or administration of parenteral analgesia. Observations reveal that oral mucositis occurs more often in children than in adults with a similar tumor disease. Oral inflammation is also more often observed in patients after bone marrow transplantation.[7,8] Early diagnosis and prompt treatment of oral mucositis that leads to patient deterioration are of crucial importance in the multidisciplinary treatment of patients. Although the symptoms of mucositis have been known for a long time and a 5-grade pathomechanism of these changes has been developed, there is no effective method to treat and eliminate the pain related to oral mucosa lesions.[8–10] Both the disease and treatment dramatically change the oral environment. Insufficient defense mechanisms are an important factor in the pathogenesis of numerous diseases. Pathological lesions of the oral mucosa observed in children with acute lymphoblastic leukemia are often caused by impaired functioning of the immune system.[11,12] Salivary immunoglobulins play an important role in pathological processes occurring in the oral cavity.[13] It is also believed that a tendency to develop mucositis symptoms is related to poor oral hygiene prior to treatment.[14,15]

The aim of the study was to assess the occurrence of oral mucositis and to evaluate changes in the level of immunoglobulins A (IgA), G (IgG) and M (IgM) in the saliva of children with acute lymphoblastic leukemia during anti-tumor treatment.

Material and methods

The study included 78 children (34 girls and 44 boys) aged from 2 to 18 years with acute lymphoblastic leukemia (ALL) and a control group of 78 age- and gender-matched healthy children. The mean age of the subjects was 8.14 ±4.61 years. The study was carried out among patients who were diagnosed and undergoing anti-neoplastic treatment according to the protocols/blocks for risk groups in the ALL-IC BFM 2002 trial. The study was conducted at the Department of Paediatric Haematology and Oncology and Transplantation at the Medical University of Lublin (Poland), which belongs to the multicenter Polish Pediatric Group for Leukemia and Lymphoma and the Polish Pediatric Group for the Treatment of Solid Tumors (PPGLBC). This center coordinates a nationwide program of treatment for acute lymphoblastic leukemia. Under this program, children are stratified into 3 risk groups: standard risk (SR), intermediate risk (IR), and high risk (HR). Children from the SR group accounted for 25.4% of the study group; children from the IR group accounted for 50.79%; and children from the HR group accounted for 23.81%. In the study group, 5 children had cerebrospinal ALL recurrence; 2 had bone marrow recurrence; 7 had their CNS affected; and 3 had Down's syndrome. The children with ALL were examined in 3 stages: examination 1 was conducted prior to chemotherapy, examination 2 was conducted between 2 days and 5 months after the onset of chemotherapy, and examination 3 was conducted between 6 and 18 months of anti-tumor treatment. The 2nd and 3rd stages of saliva sample collection were always carried out with the consent of the pediatric hematologists who were treating the patients. Saliva collections were carried on the most secure day, ensuring no impact on the sick children. In the vast majority of the children, the 2nd saliva sample was taken in between the 6th and 8th week of chemotherapy, while in the case of 4 children, because of the general condition of the patients, the 2nd saliva sample was collected after 4.5 months of therapy. The stomatological clinical study was conducted by a dentist with the use of basic diagnostic tools in artificial lighting. The condition of the oral mucosa of the children in the study group was assessed with the use of a 5-grade WHO classification of oral mucositis.[2] Changes in oral mucosa were monitored every day.

The control group consisted of 78 generally healthy children, matched for age and gender. These children were patients at the Department of Paedodontics at the Medical University of Lublin. The children were undergoing check-ups or were being treated for dental caries. The control group consisted of 34 girls and 44 boys, and their ages ranged from 3 to 18 years, with a mean age of 8.51 ±2.38 years.

Unstimulated saliva was collected from the children in the study in the morning, 2 h after eating, in order to determine immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM) concentration. The collected saliva was centrifuged for 15 min at 5000 rpm. The centrifuged saliva was frozen to a temperature of -80°C until the laboratory test was conducted. The concentrations of the selected parameters were determined with commercially available ELISA tests for determining immunological parameters in biological fluids (DRG Diagnostics GmbH, Marburg, Germany). IgA concentrations were determined by means of the Salivary IgA ELISA; IgM concentrations by the CIC IgM ELISA; and IgG concentrations by the IgG CIC ELISA.

The findings were analyzed statistically using STATISTICA v. 10.0 software (StatSoft Inc., Tulsa, USA). The characteristics of the measurable parameters were presented as mean, median, minimum and maximum

values, and standard deviation. The Mann-Whitney U test was used to compare 2 independent groups. The Wilcoxon signed-rank test was used to compare dependent groups. A logistic regression analysis was also performed. The level of statistical significance was set at p < 0.05, and statistically significant differences are marked hereafter with asterisks (*).

Results

Lesions of the mucositis type were observed in the ALL children in the period from 48 h to 6 months after the initiation of chemotherapy. The lesions were of various intensities; and there were periods without pathological lesions, which were related to the intensity of the chemotherapy. Mucosal opacity followed by redness usually occurred within 2−4 days after a methotrexate infusion. The most severe lesions of the oral mucosa were observed after the 1st month of chemotherapy. Changes of varying severity were observed in the oral mucosa: localized erythema of the mucosa (grade I) were noted in 35% of the children; pseudomembranous mucosa (grade II) in 18%; ulcers with extensive erythema (grade III) in 40%; massive mucosal ulcers and tissue necrosis (grade IV) in 4%.

Hard-to-heal wounds and ulcers were related to the blood parameters. It was observed that healing was faster, especially with regard to oral mucosa ulceration, when blood morphological parameters were improved. Lesions of the mucositis type were also dependent on the level of neutropenia. Neutropenia was recognized when the number of neutrophils was <1500/µL. Data on blood cell counts was obtained from case records. Each child with neutropenia also had fungal complications in the oral mucosa.

In the periods between protocols of chemotherapy, there were usually no lesions.

After 6 months of chemotherapy, lesions in the oral mucosa were less intense and were observed in 3.17% of the study children. The lesions were usually observed as redness and erosion. No ulcers in the oral cavity were observed.

For prophylactic purposes, the children were directed to regularly rinse the oral cavity with preparations of chlorhexidine (Corsodyl, GlaxoSmithKline PLC, London, UK) or benzydamine hydrochloride (Tantum verde, Angelini S.P.A., Ancona, Italy), as well as with a herbal mixture (Dentosept, Phytopharm Klęka SA, Nowe Miasto nad Wartą, Poland). When lesions appeared in the oral mucosa, children were administered a mixture for oral swabbing containing bicarbonate, gentamicin, colimycin, and nystatin. When massive ulceration in the oral cavity occurred, the children received Solcoseryl ampules intravenously and Solcoseryl adhesive paste (Meda Pharma GmbH & Co. KG, Bad Homburg, Deutschland) on the oral mucosa. In cases of massive milky white opacities, the treatment included antifungal preparations of the azole group, e.g., fluconazole (10 mg/kg/day).

The findings concerning saliva IgA, IgG, and IgM levels in the study participants are presented in Table 1. The mean saliva IgA concentration in children with ALL during chemotherapy was significantly lower than in the control group (Table 1). Saliva IgA measured in subsequent examinations of a given ALL patient did not reveal significant differences between the study stages being compared (Wilcoxon signed-rank analysis) (Fig. 1).

In examination 1, the mean saliva IgG concentration in the children with ALL was significantly higher than in the healthy children, which was also confirmed by a logistic regression analysis (logistic regression: $\chi^2(1) = 13.5820$; p = 0.0002*). During the anti-tumor therapy, however, the mean saliva IgG in the study children gradually decreased; and in examination 3 it was lower than in the control group (Table 1). A comparison of mean saliva IgG in subsequent examinations of any given patient revealed a significant saliva IgG decrease between examinations 1 and 3 (Wilcoxon signed-rank test: Z = 2.303654;

Table 1. Salivary immunoglobulin A (IgA), immunoglobulin G (IgG), and immunoglobulin M (IgM) concentrations (µg/mL) in the children with ALL and in the healthy controls

Saliva Ig	Studied group of children		Mean value	Me	Min	Max	SD	Mann-Whitney U Test	
								Z	p-value
IgA µg/mL	all	exam 1	218.1	245.8	8.89	458.2	153.9	3.631	0.0003*
		exam 2	225.8	233.7	5.68	450.8	118.4	4.099	0.00004*
		exam 3	188.5	171.0	8.14	480.0	147.2	4.937	0.000001*
	healthy		353.1	337.2	15.27	804.7	171.2	the above values refer to healthy children	
IgG µg/mL	all	exam 1	4.8	5.2	0.00	12	4.6	-2.83	0.0046*
		exam 2	2.3	1.3	0.05	13	3.3	-0.8019	0.423
		exam 3	1.5	0.8	0.0	6	1.9	1.8192	0.0689
	healthy		2.1	0.8	0.16	12	2.7	the above values refer to healthy children	
IgM µg/mL	all	exam 1	31.5	28.1	15.15	62	12.6	-8.2676	0.00001*
		exam 2	22.3	22.7	7.57	66	13.4	-4.8644	0.000001*
		exam 3	20.0	13.0	6.49	66	13.6	-2.3299	0.019811*
	healthy		11.7	11.9	0.0	31	6.1	the above values refer to healthy children	

exam – examination; Me – median; Min – minimum; Max – maximum; SD – standard deviation; p – significance level; * statistically significant value.

p = 0.0212*). These findings were also confirmed by a logistic regression analysis (logistic regression: $\chi^2(3) = 20.592$; p = 0.00013*) (Fig. 2).

Saliva IgM concentration in the ALL patients was at all stages significantly lower than in the control group (Table 1). A comparison of the mean saliva IgM in subsequent examinations of any given patient revealed significant differences between examinations 1 and 2 (Wilcoxon signed-rank test: Z = 3.4098; p = 0.0006*) and between examinations 1 and 3 (Wilcoxon signed-rank test: Z = 3.0544; p = 0.0022*). These findings were confirmed by a logistic regression analysis (logistic regression: $\chi^2(3) = 74.952$; p < 0,0001*) (Fig. 3).

Discussion

In the present study, mucositis symptoms were correlated with blood parameters. Similar correlations have also been observed by other authors.[16–20] Mucositis is most frequently induced by a reduced number of white blood cells, the use of cytotoxic antibiotics and by alkylating factors.[16,17] A significant decrease in salivary S-IgA following chemother-apy in children with leukemia was reported by Karolewska et al., although the authors did not report differences in the S-IgA concentration in comparison with the control group prior to anti-tumor treatment. According to those authors, a statistically significant low salivary S-IgA level was maintained after chemotherapy.[18] A statistically significant lower level of saliva IgA in patients with leukemia (2.9–1.9 µg/mL) as compared to controls (5.4 µg/mL) was reported by Thomaz et al.[19] Månsson-Rahemtulla et al. also reported that the value of saliva IgA in patients with ALL undergoing chemotherapy was significantly reduced. The authors revealed that at the time of diagnosis, salivary IgA in patients with ALL was 0.183 ±0.193 mg/mL, and that it was higher than in the control group. After 3 and 5 weeks of chemotherapy, salivary IgA levels were statistically significantly reduced in those patients, both as compared to patients with acute myeloid leukemia and to healthy patients.[20] A low level of saliva IgA and IgG has been reported to result from the effects of chemotherapy on salivary gland secretion, which in turn leads to impairment of the local immune system. Local disturbances of the immune system may affect systemic immunity of patients undergoing chemotherapy.[18]
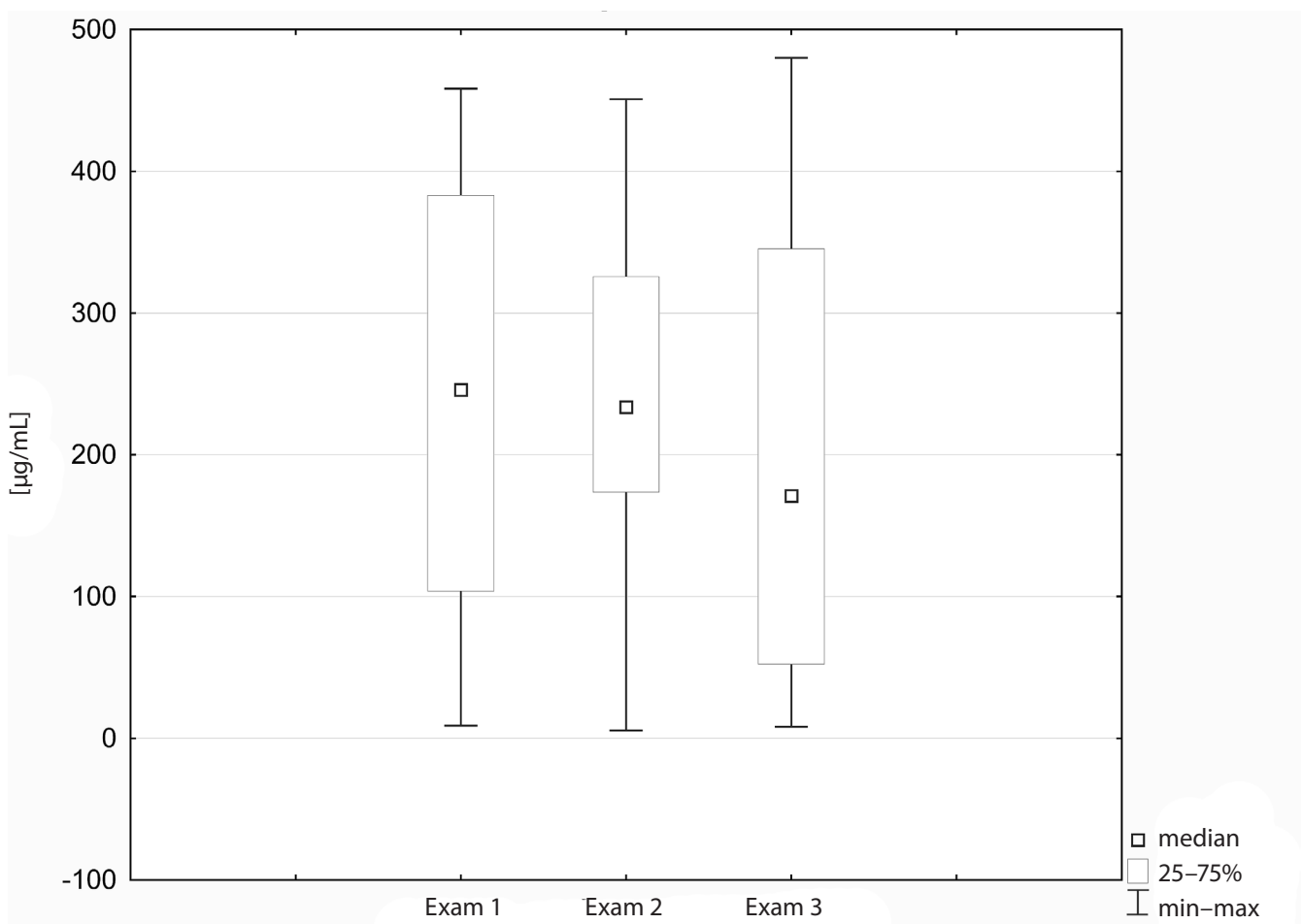

Fig. 1. Saliva IgA (µg/mL) concentration in children with ALL in subsequent tests

According to a study by Pajari et al., neither children with the remission of ALL, nor children in the acute phase of the disease showed a significant difference in saliva IgA, IgG and IgM as compared to the control group, but patients with other hematological neoplasms had significantly higher levels of salivary IgG and IgM than the healthy controls.[21] These authors stated that the significant reduction in salivary IgG and IgM observed in the children cured of neoplastic diseases indicates a probability that the treatment and/or disease may impair the immune response in these patients. Other authors studying children with ALL observed normal serum IgG, IgA and IgM levels at the time of diagnosis, whereas during chemotherapy, they observed a decrease in the concentration of 1 or more immunoglobulins; the greatest decreases were observed for IgG and IgM. In those studies, the permanent IgM deficit was related to a higher risk of disease recurrence and death in the ALL children. In patients with good prospects for remission and recovery, the immunoglobulins normalized within a year following the completion of treatment.[22–24]

Human IgA occurring in serum induces a significant increase in interleukin-1 receptor antagonist (IL-1RA) excretion by mononuclear cells of the peripheral blood, as well as increased adherence of monocytes. By inducing IL-1RA and decreasing excretion of pro-inflammatory cytokines such as IL-1β, TNF-α and IL-6, IgA contributes to regulating the inflammatory response. According to a pathomechanism outlined by Sonis et al., a low S-IgA concentration in patients undergoing anti-tumor treatment could be partially responsible for a higher risk of mucositis development.[8,18] In my own previous studies, decreases in salivary TNF-α and IL-2 was observed in patients with ALL as compared to healthy children.[25,26]

In an effort to minimize the side effects of radiation therapy and chemotherapy used in cancer treatment, there have been studies aimed at developing therapeutic strategies to regenerate oral mucosal tissues affected by mucositis.[3,5] Certain agents have been examined for prevention or treatment of oral mucositis caused by chemotherapy or radiotherapy, but none of them has been confirmed as fully effective.[27] A study by Pereira Pinto et al. revealed that in a group of children with ALL who used 0.12% chlorhexidine gluconate to rinse the mouth, 26% developed mucositis in comparison with 80% who did not use the oral rinse.[28] Initially, the lesions were
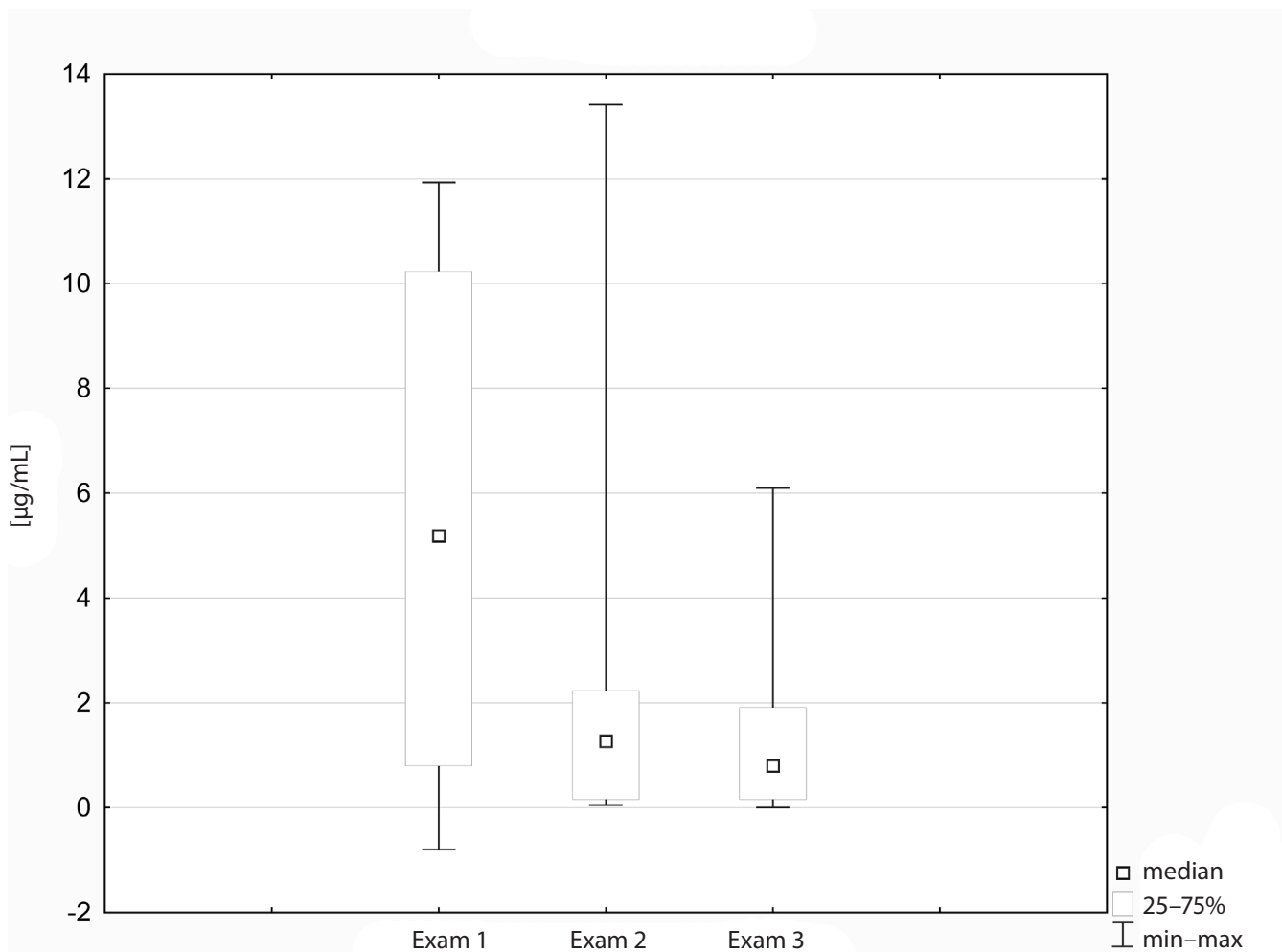

Fig. 2. Saliva IgG (µg/mL) concentration in children with ALL in subsequent tests

manifested as a characteristic erythema on the buccal and labial mucosa, followed by edema and ulceration. Lesions of the mucositis type developed within 2–4 days after administration of methotrexate, and made normal nutrition impossible. In a study by de Oliveira Lula et al., patients with acute lymphoblastic leukemia treated according to the GBTLI-93 and the BFM protocols developed oral problems irrespective of the chemotherapy protocol. Pathological lesions were mainly observed shortly after the initiation of anti-tumor therapy. Mucosal pallor occurred in 20% of the subjects treated according to the GBTLI-93 protocol and in 23.3% of patients treated according to the BFM protocol. Ulceration was observed at the same level in both groups of patients. Bleeding gums, candidiasis, and xerostomia occurred mainly in patients treated according to the GBTLI-93 protocol.[29] Some scientists suggest applying chlorhexidine when used in a 0.1% or 0.12% solution to prevent and mitigate pain during chemotherapy as a way to avoid grade II and III oral mucositis.[28,30] According to some researchers, both chlorhexidine and benzydamine contribute to the reduction of oral mucositis during chemotherapy, but only in children over 6 years of age.[2,18,31–35] Currently, opioids seem to be the most effective in the treatment of mucositis-induced pain. However, patients develop tolerance to opioids relatively quickly, and responses to their analgesic effect vary.[5] Despite the use of opioids, pain related to oral lesions of the mucositis type in patients subject to hematopoietic stem cell transplantation was significantly correlated with increased expression of the TNF-α gene in buccal cells on the 9th day of therapy in comparison with the baseline.[33]

Randomized trials do not allow recommendations, but may suggest possible preparations for use in the treatment of mucositis. Only palifermin is recommended by both the US Food and Drug Administration and the European Medicines Agency for the treatment of oral mucositis.[9] Prevention of mucositis in patients with hematological neoplasms who have undergone stem cell transplantation is based on palifermin, which contains the recombinant human keratinocyte growth factor (KGF). Following the use of palifermin in these patients, grade III and grade IV oral mucositis was significantly reduced, and the average duration of its occurrence was shorter than in the control group.[36] Palifermin appears to reduce the incidence of oral mucositis in patients treated for head
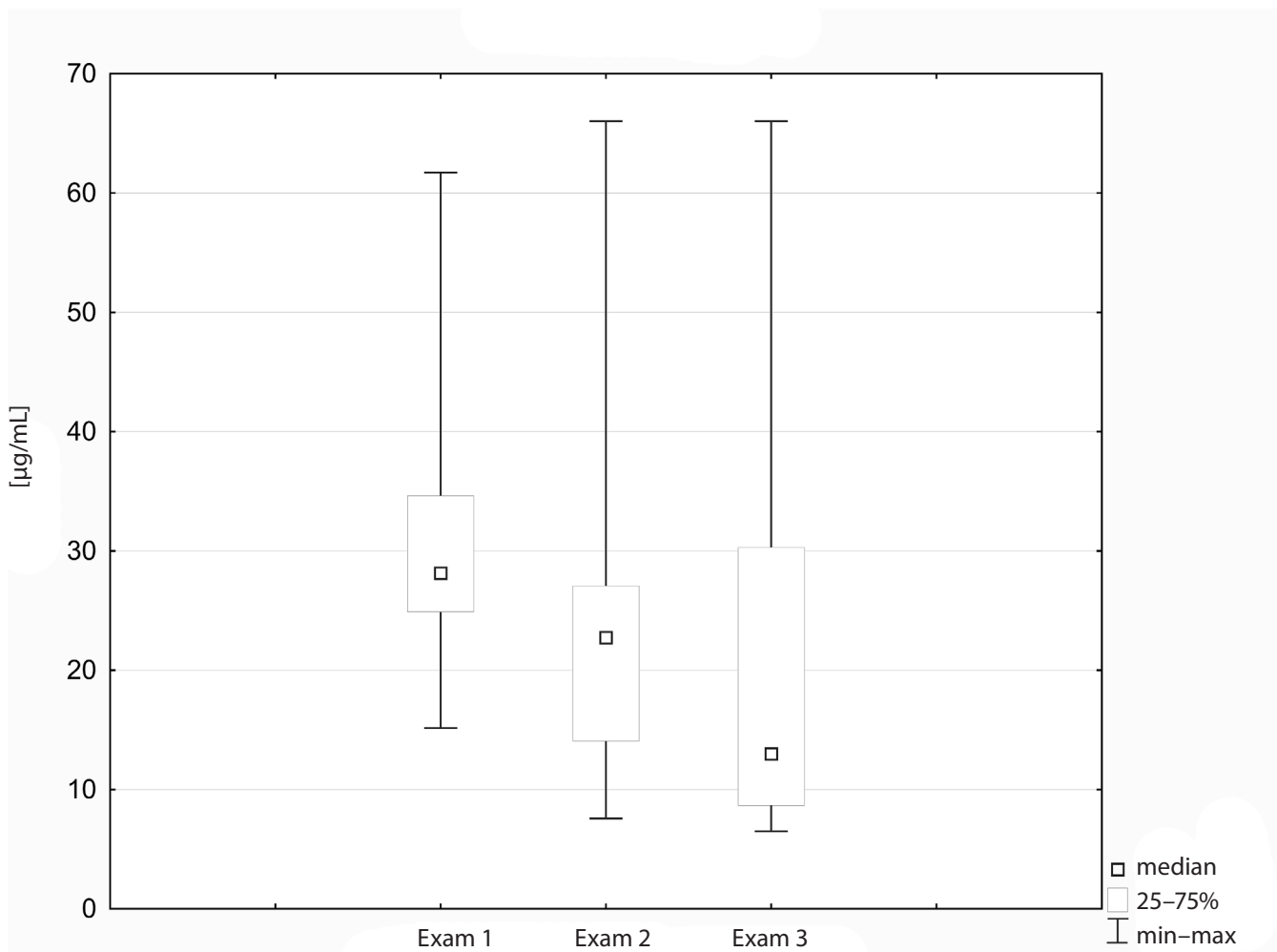

Fig. 3. Saliva IgM (µg/mL) concentration in children with ALL in subsequent tests

and neck cancer, but its position in therapy has not yet been established.[37] The efficacy of the preparation has been confirmed for intravenous administration, whereas local administration seems to be less effective.[5,10,15,34,38,39] Palifermin is an expensive preparation, whose use is difficult to justify when the risk of severe oral mucositis is low. However, if physicians could predict which patients would suffer from oral mucositis after standard doses of chemotherapy and/or radiotherapy, then, from a pharmaco-economic point of view, the use of new and more expensive preparations could be well justified.[35] In certain cases, oral mucositis makes it necessary to change or stop anti-tumor therapy. Moreover, the additional costs of treating oral mucositis has economic consequences that can be difficult for national health funds to bear.[5,9,10]

One factor reducing the risk of oral complications that is especially important, and simple at the same time, is regular oral hygiene. Motivating the patient to properly clean all tooth surfaces and oral soft tissues, to brush the teeth at least twice a day, and to rinse the oral cavity may contribute to reducing the incidence of oral mucositis or mitigate its symptoms.[9,10,35] The participation of dentists in multidisciplinary teams treating children with ALL can greatly contribute to better health protection for these patients.[16] At the same time, it must be emphasized that the immunoregulatory mechanisms of cytokines and immunoglobulins involved in oral inflammations play a special role in protecting the host and maintaining oral homeostasis. Saliva may become a good source for detecting pro-inflammatory markers. In the future, salivary cytokines and immunoglobulins might play an important role as replacement biomarkers in assessing the efficacy of chemotherapy.[40]

In conclusion, the findings of this study demonstrate that the condition of the oral mucosa in children with acute lymphoblastic leukemia was not satisfactory during chemotherapy. Low salivary IgA concentrations in children with ALL may result in the development and potentiation of oral lesions of the mucositis type during anti-tumor treatment, and significant decreases in salivary immunoglobulin G and immunoglobulin M concentrations in children with ALL during chemotherapy may cause a potentiation of pathological lesions in the oral mucosa.

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