ORIGINAL PAPERS

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An Assessment of the Number of Cariogenic Bacteria in the Saliva of Children with Chemotherapy-Induced Neutropenia

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;
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Abstract

Background. Anticancer therapy entails qualitative and quantitative changes in the physiological bacterial flora of the organism, including the oral microflora.

Objectives. The aim of the study was to assess the number of cariogenic bacteria – *Streptococcus mutans* and *Lactobacillus* spp. – in the saliva of children with chemotherapy-induced neutropenia, and the relationship between the dynamics of neutrophils in the blood and the number of cariogenic bacteria in the saliva.

Material and Methods. The study included 52 children aged 3–17.5 years, diagnosed with cancer and undergoing antineoplastic treatment. The control group comprised 52 generally healthy children matched for age and gender. Both groups underwent microbiological analysis of the saliva. The Dentocult SM Strip Mutans test (Orion Diagnostica, Espoo, Finland) was used to evaluate the number of *Streptococcus mutans* colonies in the saliva, while the Dentocult LB test (Orion Diagnostica) was used to assess the number of *Lactobacillus* spp. bacteria. The statistical analysis was carried out using STATISTICA 10 software (StatSoft Inc., Tulsa, USA).

Results. The statistical analysis using Kendall's tau test showed a significant inverse correlation between the number of neutrophils in the blood and the number of the *Streptococcus mutans* and *Lactobacillus* spp. colonies in the saliva of the children undergoing anticancer therapy. The highest titres of cariogenic bacteria in the saliva were observed during severe neutropenia, which was frequently observed between day 7 and day 21 of the chemotherapy course.

Conclusions. A significant increase in the number of cariogenic bacteria in the saliva during episodes of chemotherapy-induced neutropenia suggests that the activity of dental caries increases in children undergoing antineoplastic treatment (**Adv Clin Exp Med 2016, 25, 1, 11–19**).

Key words: chemotherapy, neutropenia, saliva, cariogenic bacteria.

Among children over one year of age cancers are the fourth most common cause of death, after poisoning, injuries and accidents [1]. In most countries, 70 to 160 new cases of cancer are reported annually per 1 million children aged up to 15 years. In Poland, in the population of children aged up to 17 years, approximately 1100–1200 cases of cancer occur every year [2–5]. The primary goal of antineoplastic treatment in a child is obtaining permanent remission and recovery. Currently, a significant number of childhood cancers are treatable in more than 80% of the cases [3]. Huge progress in the treatment of cancer has been made thanks to advances in basic tests, diagnostics,

epidemiology and therapeutic methods. Modern antineoplastic therapy is complex, involving surgery, chemotherapy (frequently both pre- and post-operative, usually lasting for several months), radiotherapy and megatherapy with transplantation of hematopoietic stem cells (HSCT). In almost all pediatric cancers, recovery – meaning total, permanent removal of all cancer cells from the organism – became possible thanks to the introduction of chemotherapy. Due to the anti-proliferative properties of cytostatic drugs, tissues and organs with the highest number of cell divisions (bone marrow, airway mucosa, the gastrointestinal tract, skin, hair and gonads) are the most sensitive

to the toxicity of chemotherapy. Therefore, one of the most common side effects of cytostatic treatment is neutropenia, defined as a reduction in the number of neutrophils in the peripheral blood to the level of < 1500/μL. The maximum reduction in the number of peripheral blood granulocytes (the nadir) is generally observed between day 7 and day 14 after the administration of the drugs; this period varies quite considerably depending on the type of chemotherapy [6, 7]. Neutropenia below 0.5×10^3/μL and especially neutropenia below 0.1×10^3/μL favors infections, especially those caused by Gram-negative rods, Gram-positive cocci, Candida species and other pathogenic fungi. Nowadays, infections caused by enterococci in neutropenic patients are becoming an increasingly significant problem [6–9]. The origin of infections during neutropenia may be either exogenous or endogenous. The prevention of exogenous infections is limited to providing patients with appropriate conditions during hospitalization. The prevention of endogenous infection includes, among other things, suppression of the intestinal flora and preservation of the integrity of the gastrointestinal tract epithelium. In neutropenic patients, translocation of bacteria and fungi from the gastrointestinal tract can lead to bacteremia, candidemia and multiorgan infections [10, 11]. Anticancer therapy entails qualitative and quantitative changes in the physiological bacterial flora of the organism, including the oral microflora. Both physiological and opportunistic floras are often the primary source of oral infection, constituting the etiological agent in 30% of the bacteremia cases in cancer patients [12]. Translocation of *Streptococcus viridans* into the blood leads to minor transient bacteremia in healthy people, while in neutropenic patients it can induce severe sepsis [13].

Objectives

The aim of the study was to assess the number of *Streptococcus mutans* and *Lactobacillus* spp. bacteria in the saliva of children during chemotherapy-induced neutropenia, and the relationship between the dynamics of neutrophils in the blood and the number of cariogenic bacteria in the saliva.

Material and Methods

The study included 52 children aged 3–17.5 years diagnosed with cancer and undergoing antineoplastic treatment at the Department of Pediatric Hematology, Oncology and Transplantation at the Medical University in Lublin, Poland.

The study group consisted of 22 girls and 30 boys. The mean age of the subjects was 7.83 ± 3.86 years. There were 37 patients with hematologic malignancies and 15 patients with solid tumors. All the patients were treated according to the recommendations of the Polish Pediatric Group for Leukemia and Lymphoma and the Polish Pediatric Group for the Treatment of Solid Tumors [3]. The control group consisted of 52 generally healthy children, matched for age and gender, who were being treated at the Department of Pediatric Dentistry at the Medical University of Lublin. The control group also consisted of 22 girls and 30 boys, and their age ranged from 3 to 18 years, with a mean age of 8.19 ± 4.04 years. The study was approved by the Bioethics Committee of the Medical University in Lublin.

The children undergoing anti-cancer treatment were tested three times during their hospitalization. The first examination was carried out within the first seven days of the chemotherapy cycle (T1), the second examination was 7–21 days after the start of the treatment (T2), and the third evaluation was 21–30 days from the start of chemotherapy (T3). The children from the control group were tested once.

Information on the diagnosis, duration and course of cancer, concomitant diseases, the protocol of treatment, medications and laboratory blood test results was obtained from the children's medical records. Their blood cell count was monitored during antineoplastic treatment. The current number of neutrophils in the blood (NEU) was part of the material for this study.

Neutropenia was recognized when the number of neutrophils was < 1500/μL, and it was classified as follows: mild (granulocyte count of $1–1.5 \times 10^3$/μL), moderate (granulocyte count of $0.5–1 \times 10^3$/μL), severe (granulocyte count of $< 0.5 \times 10^3$/μL), and life-threatening (granulocyte count of $< 0.1 \times 10^3$/μL).

The saliva of both groups was subjected to microbiological analysis. The saliva was collected in the morning after stimulation by chewing a paraffin cube. In most cases, the patients were fasting; they had not brushed their teeth or rinsed their mouth with an antibacterial oral rinse for 1–3 h before the test. The saliva was placed on an agar surface. The number of bacterial colonies of *Lactobacillus* spp. was assessed using Dentocult LB tests (Orion Diagnostica, Espoo, Finland), while the number of *Streptococcus mutans* was determined using Dentocult SM Strips Mutans tests (Orion Diagnostica). The material was incubated at 37°C, for 48 h in case of the *Streptococcus mutans* cultures and for 96 h for *Lactobacillus* spp. After that specified time, the result

was read in accordance with the kit manufacturers' instructions. The following evaluation criteria of the number of colonies were accepted for *Streptococcus mutans*: class 0: a colony count < 10,000 CFU/mL; class 1: a colony count < 100,000 CFU/ /mL; class 2: colonies in 100,000 – 1,000,000 CFU/ /mL range; class 3: colonies > 1,000,000 CFU/mL. For *Lactobacillus* spp., over 10,000 CFU/mL was defined as a high number, while < 1000 CFU/mL was a low number. The results of the microbiological testing of the saliva were then analyzed in relation to the current number of neutrophils in the peripheral blood.

The data was analyzed using STATISTICA 10 for Windows Software (StatSoft Inc., Tulsa, USA). Due to the nature of the variables, the following statistics were applied: descriptive statistics, i.e. the number (N) and percent (%), arithmetic mean (M), standard deviation (SD), the minimum and maximum (min, max); and statistical tests. The χ^2 test was used to assess the significance of qualitative data; Kendall's tau test was used to determine the relationship between two measured quantities; the Friedman ANOVA test was used to compare the results of the three measurements (T1, T2, T3) [14]. A p-value of < 0.05 was considered significant.

Results

The number of blood neutrophils was monitored in children during antineoplastic treatment. In Test 1, within the first seven days of chemotherapy, half the patients (50%) did not manifest neutropenia. Mild and moderate neutropenia were diagnosed in equal proportions (21.15%). Severe neutropenia was observed in 7.69% of the patients. There were no cases of life-threatening neutropenia. In Test 2, 7–21 days after the start of anticancer treatment, all the patients in the study group had neutropenia. In most cases it was severe neutropenia (34.61%), followed by mild neutropenia (32.69%) and moderate neutropenia (25%). Life-threatening neutropenia was observed in 7.69% of the patients. In Test 3, 21–30 days after the start of chemotherapy, the patients were mostly diagnosed with mild (40.38%) and moderate neutropenia (30.77%). Severe neutropenia was observed in 5.77% of the patients. There were no cases of life-threatening neutropenia; and 23.08% of the subjects did not manifest neutropenia. The statistical analysis revealed statistically significant differences in the incidence of neutropenia in Tests 1, 2 and 3 (p < 0.05, Table 1).

The number of *Streptococcus mutans* colonies in the saliva in the study group and the control group are shown in Table 2. In Test 2 Pearson's

Table 1. The incidence of neutropenia in the study group in Test 1 (T1), Test 2 (T2) and Test 3 (T3)

Test	Neutropenia									
	none		mild		moderate		severe		life-threatening	
	n	%	n	%	n	%	n	%	n	%
T1	26	50	11	21.15	11	21.15	4	7.69	0	0
T2	0	0	17	32.69	13	25	18	34.61	4	7.69
T3	12	23.08	21	40.38	16	30.77	3	5.77	0	0
	Friedman test: ANOVA = 21.9467; df = 2; p = 0.0001*									

Table 2. The number of *Streptococcus mutans* colonies in the saliva in the study group and the control group

Group	Test	*Streptococcus mutans* class								Pearson χ^2 test
		0		1		2		3		
		n	%	n	%	n	%	n	%	
Study group	T1	11	21.2	28	53.8	12	23.1	1	1.9	χ^2 = 3.7482; df = 3; p = 0.29
	T2	7	13.5	11	21.2	19	36.5	15	28.8	χ^2 = 10.55426; df = 3; p = 0.0144*
	T3	8	15.4	16	30.8	25	48.1	3	5.8	χ^2 = 3.3629; df = 3; p = 0.3389
Control group		12	23.1	20	38.5	16	30.8	4	7.7	

Class 0 – colony count < 10 000 CFU/mL; class 1 – colony count < 100 000 CFU/mL; class 2 – the number of colonies in the range of 100 000–1 000 000 CFU/mL; class 3 – the number of colonies > 1 000 000 CFU/mL.
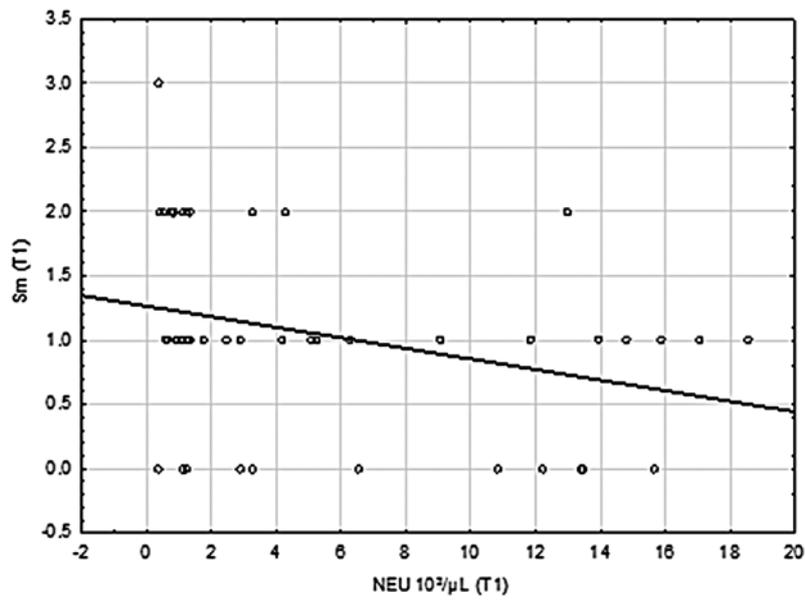
Fig. 1. Correlation between blood neutrophil count (NEU) and the number of *Streptococcus mutans* (Sm) colonies in the saliva in the study group in Test 1 (T1)
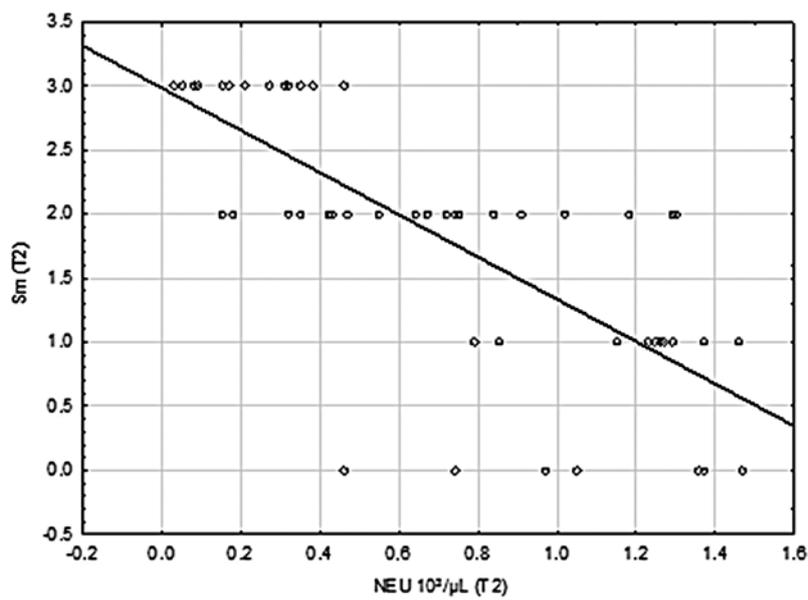

Fig. 2. Correlation between blood neutrophil count (NEU) and the number of *Streptococcus mutans* (Sm) colonies in the saliva in the study group in Test 2 (T2)
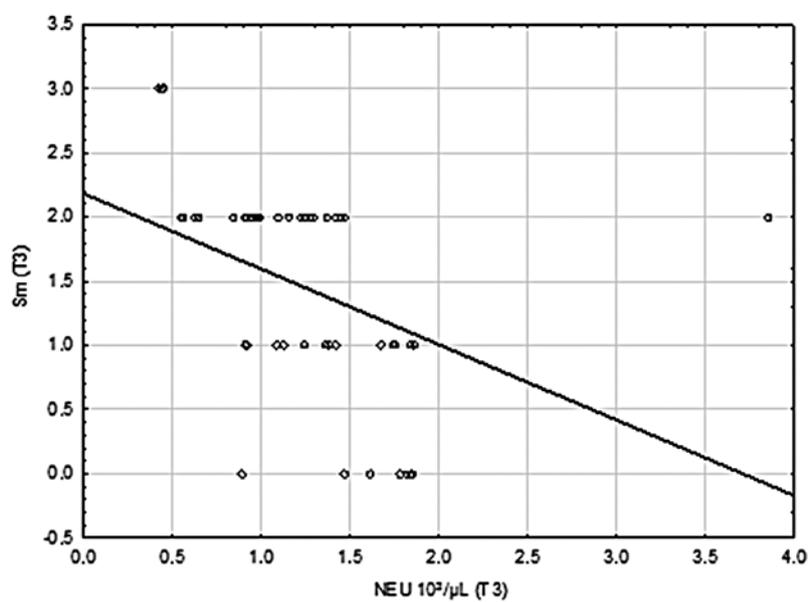

Fig. 3. Correlation between blood neutrophil count (NEU) and the number of *Streptococcus mutans* (Sm) colonies in the saliva in the study group in Test 3 (T3)
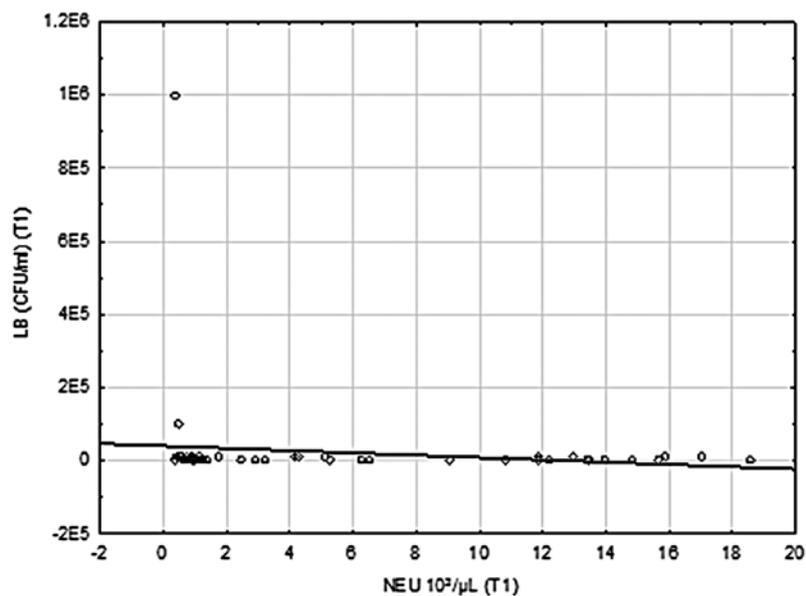
Fig. 4. Correlation between blood neutrophil count (NEU) and the number of *Lactobacillus* spp. (LB) in the saliva in the study group in Test 1 (T1)

Fig. 5. Correlation between blood neutrophil count (NEU) and the number of *Lactobacillus* spp. (LB) in the saliva in the study group in Test 2 (T2)
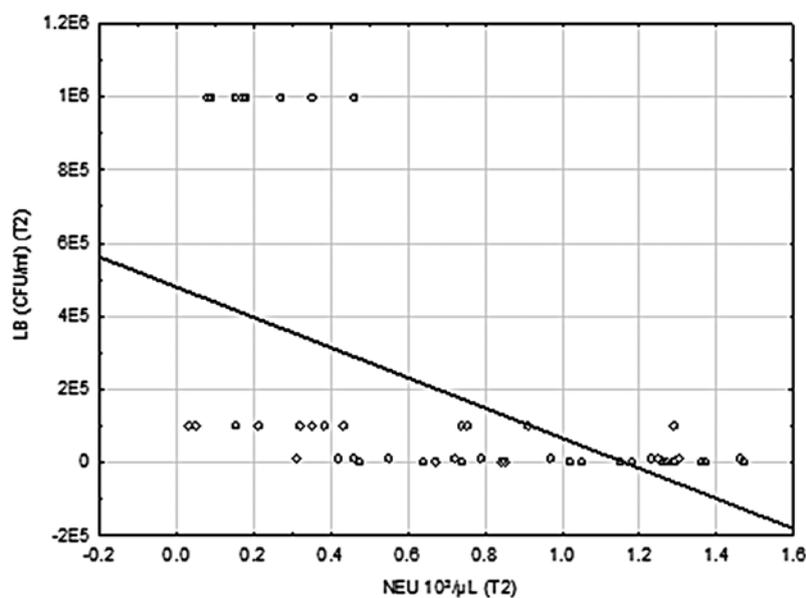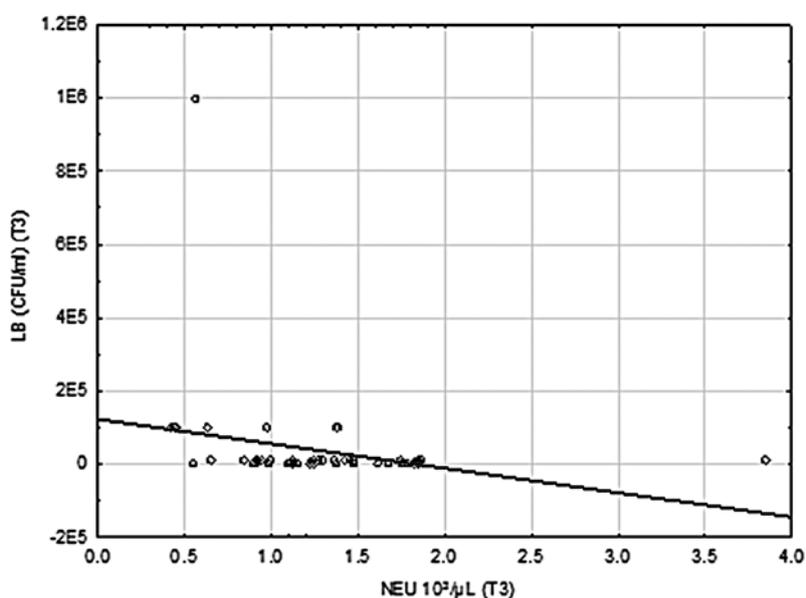

Fig. 6. Correlation between blood neutrophil count (NEU) and the number of *Lactobacillus* spp. (LB) in the saliva in the study group in Test 3 (T3)

χ^2 test demonstrated a significantly higher number of *Streptococcus mutans* bacteria in the saliva in the study group than in the control group (p < 0.05). It should be noted that during the T2 period (7–21 days after the start of chemotherapy) the largest decreases were observed in blood neutrophil count. The comparison of the number of *Streptococcus mutans* colonies in the saliva between the study group in Tests 1 and 3 and the control group showed no statistically significant differences. The number of *Lactobacillus* spp. in the saliva in the study group and the control group are shown in Table 3. As in the case of *Streptococcus mutans*, the highest number of *Lactobacillus* spp. colonies was observed in the saliva in the study group in Test 2, where the most severe neutropenia was also reported. The number of *Lactobacillus* spp. colonies in the study group in Test 2 was significantly higher than in the control group (p < 0.05). The comparison of the number of *Lactobacillus* spp. bacteria in the saliva between the study group in Tests 1 and 3 and the control group showed no statistically significant differences. The Friedman ANOVA test showed significant differences in the number of *Streptococcus mutans* and *Lactobacillus* spp. bacteria in the study group in the three measurements. The highest number of cariogenic bacteria in the study group was found in Test 2, and it was significantly higher than the number found in Tests 1 and 3.

Kendall's tau test showed a significant inverse correlation between the number of neutrophils in the blood and the number of *Streptococcus mutans* colonies in the saliva of children from the study group in all three measurements (Test 1: p < 0.05; Test 2: p < 0.05; Test 3: p < 0.05). In patients with neutropenia along with a decrease in the number of neutrophils in the blood, an increase in the number of *Streptococcus mutans* colonies in the saliva was observed (Table 4). Moreover, a significant inverse correlation was noted between the number of neutrophils in the blood and the number of *Lactobacillus* spp. colonies in the saliva of children from the study group in all three measurements (Test 1: p < 0.05; Test 2: p < 0.05; Test 3 p < 0.05). Along with the decrease in the number of neutrophils in the blood, an increase in the number of *Lactobacillus* spp. colonies in the saliva was observed (Table 5).

Discussion

A few authors have pointed out that in the course of antineoplastic treatment some quantitative and qualitative changes take place in the

Table 3. The number of *Lactobacillus* spp. in the saliva in the study group in Test 1 (T1), Test 2 (T2) and Test 3 (T3) and in the control group

Group	Test	The number of *Lactobacillus* spp.										Pearson's χ^2 test
		none		10^3 CFU/mL		10^4 CFU/mL		10^5 CFU/mL		10^6 CFU/mL		
		n	%	n	%	n	%	n	%	n	%	
Study group	T1	14	26.9	21	40.4	15	28.8	1	1.9	1	1.9	χ^2 = 4.6653; df = 4; p = 0.3234
	T2	9	17.3	8	15.4	12	23.1	15	28.8	8	15.4	χ^2 = 14.0495; df = 4; p = 0.0071*
	T3	7	13.5	15	28.8	21	40.4	8	15.4	1	1.9	χ^2 = 7.0305; df = 4; p = 0.1280
Control group		11	21.2	22	42.3	11	21.2	5	9.6	3	5.8	

Table 4. Correlation between blood neutrophil count (NEU) and the number of *Streptococcus mutans* (Sm) colonies in the saliva in the study group in Test 1 (T1), Test 2 (T2) and Test 3 (T3)

Variables	Kendall's tau correlation			
	N	Kendall's tau correlation coefficient	Z	p
NEU 10^3/μL (T1) & Sm (T1)	52	−0.2793	−2.9221	0.0035*
NEU 10^3/μL (T2) & Sm (T2)	52	−0.6373	−6.6686	0.0001*
NEU 10^3/μL (T3) & Sm (T3)	52	−0.4192	−4.3865	0.0001*

Table 5. Correlation between the blood neutrophil count (NEU) and the number of *Lactobacillus* spp. (LB) colonies in the saliva in the study group in Test 1 (T1), Test 2 (T2) and Test 3 (T3)

Variables	Kendall's tau correlation			
	N	Kendall's tau correlation coefficient	Z	p
NEU $10^3/\mu L$ (T1) & LB (CFU/mL) (T1)	52	−0.2448	−2.5617	0.0104*
NEU $10^3/\mu L$ (T2) & LB (CFU/mL) (T2)	52	−0.5829	−6.1001	0.0001*
NEU $10^3/\mu L$ (T3) & LB (CFU/mL) (T3)	52	−0.26179	−2.7393	0.0061*

physiological bacterial flora of the body, including the oral microflora. According to O'Sullivan et al., the quantitative changes in the microorganisms in the oral cavity may reflect systemic changes in the biocenosis, and may precede the development of sepsis in the course of leukemia. Therefore, non-invasive microbiological tests of the oral cavity provide important information for the prevention of systemic infections in patients undergoing anticancer therapy [15]. Peterson et al. studied the number of bacteria in the subgingival and supragingival dental plaque in patients with acute leukemia accompanied by gingivitis or periodontitis [16]. In that study, microbiological tests of the dental plaque were performed before chemotherapy and on the 14th day of myelosuppressive anticancer therapy, when the majority of patients had severe neutropenia (< 100/mm³). The results showed that the presence of potentially pathogenic bacteria such as *Veillonella* spp., *Neisseria* spp., *Pseudomonas aeruginosa* and *Staphylococcus* spp. in the dental plaque is highly correlated with the degree of chemotherapy-induced neutropenia [16]. Evaluating quantitative changes in the bacterial flora of the oral cavity and pharynx in children with acute lymphoblastic leukemia (ALL) and in healthy children, Krzeminski and Majda found a general tendency for the population of bacteria to increase in children with ALL, although the growth was not statistically significant in every case [17]. The rise in the number of bacteria, which was undoubtedly the major cause of morbidity and of the increased intensity of dental and periodontal diseases, was more pronounced in the oral cavity (it involved all bacteria capable of being cultured: aerobic, streptococci, enterococci and cariogenic lactobacilli) than in the pharynx, where it was restricted to only staphylococci and the emergence of *Enterobacteriaceae* [17]. Microbiological tests conducted by Konopka et al. investigating the condition of the oral cavity in children with leukemia revealed that the most frequently isolated bacteria were Gram-positive cocci constantly colonizing the oral cavity. The presence of known pathogenic bacteria, such as *S. aureus* and *Pseudomonas aeruginosa,* was also confirmed [18].

Few authors have studied the behavior of the cariogenic flora in children during anticancer treatment. The present study found a significantly higher number of *Streptococcus mutans* and *Lactobacillus* spp. colonies in the saliva of children during anticancer therapy than in the healthy controls. Pajari et al. observed that in children undergoing intensive chemotherapy, non-stimulated saliva contained increased number of *Streptococcus mutans* and *Lactobacillus* spp., elevated activity of lysozyme and decreased pH of the saliva [19]. Krzemiński and Majda-Stanisławska investigated the prevalence and the number of cariogenic bacteria in children with acute lymphoblastic leukemia undergoing intensive chemotherapy and in healthy children. The cariogenic bacteria *Streptococcus mutans* was noted in 77.3% of the healthy children and in 75.0% of the unhealthy children; *Lactobacillus* spp. in 79.6% of the healthy children and 83.3% of the unhealthy children; *Actinomyces* spp. in 38.7% of the healthy controls and 81.3% of the unhealthy subjects. The percentage of children with high titres increased in children with ALL for each tested organism. The authors reported that in children undergoing intensive chemotherapy for acute lymphoblastic leukemia, the risk of dental caries increased because of the substantial rise in the number of cariogenic bacteria in the oral cavity [20].

In a study on the condition of the oral cavity and saliva in children with ALL, Pels observed a statistically significant decrease in the number of *Streptococcus mutans* and *Lactobacillus* spp. colonies in the saliva and dental plaque in the course of antineoplastic therapy [21]. That author also found a significantly lower number of *Streptococcus mutans* colonies in the saliva and dental plaque in children with ALL than in healthy children, and no statistically significant differences in the number of *Lactobacillus* spp. colonies in the saliva and dental plaque in a group of children with ALL and in healthy children [21]. The difference between Pels's results and those in the current study is probably due to the fact that in Pels's study microbiological tests were not performed in the period of chemotherapy-induced neutropenia.

The present study revealed a strong correlation between the number of neutrophils in the blood and the number of cariogenic bacteria *Streptococcus mutans* and *Lactobacillus* spp. in the saliva of children undergoing antineoplastic treatment. The highest titres of cariogenic bacteria in the saliva were observed during severe neutropenia, which was most frequently observed between days 7 and 21 of the chemotherapy course.

Examining the qualitative and quantitative changes in the bacterial flora of the mouth in children being treated for acute lymphoblastic leukaemia and in healthy children, Majda-Stanisławska and Krzeminski found that there was a negative correlation between those numbers and neutrophil counts in the peripheral blood [22]. Examining the number of neutrophils present in the oral cavity and comparing it with the number of bacteria that reside there, Zarzycka et al. showed that the number of certain types of bacteria – including the cariogenic bacteria *Streptococcus mutans* and *Lactobacillus* spp. – depends on the number of granulocytes. This indicates that oral neutrophils, through their phagocytic activity and the secretion of various antimicrobial peptides, play a more important role in controlling the population of cariogenic bacteria and thus, indirectly, the development of dental caries than was previously supposed [23]. The same authors phagocytized *Streptococcus mutans* cells and lactic acid bacteria in the granulocytes isolated directly from the oral cavity, demonstrating that neutrophils of the oral cavity are among the factors that undoubtedly reduce the population of cariogenic bacteria in this environment [24]. In a further study, the authors demonstrated that the number of bacteria phagocytized in the oral cavity depends, among other things, on the population of certain types of bacteria in this environment and the phagocytic activity of neutrophils in an individual [25].

Based on the study results, it can be concluded that the significant increase in the number of cariogenic bacteria in the saliva during episodes of chemotherapy-induced neutropenia indicates increased activity of dental caries in children undergoing antineoplastic treatment. Due to the frequent oral complications of cancer therapy, children with cancer should be provided with specialist dental care for the entire period of antineoplastic therapy, with a strong emphasis on dental caries prophylaxis. Close cooperation between dental professionals and the team of pediatric oncologists and hematologists is highly recommended.

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