Introduction: Chemotherapy, neoplasms, and their complications linked to malabsorption, malnutrition, and metabolic disorders may lead to improper tooth development and frequent severe caries in patients during/after antineoplastic treatment and to a more frequent improper tooth development in patients undergoing chemotherapy during odontogenesis. However, the causes of these abnormalities remain unknown; there are no studies on the impact of antineoplastic treatment and its complications on the chemical composition of mineralised teeth.

Aim of the study: To compare the chemical composition of mineralised teeth extracted due to complicated caries in children after chemotherapy, and of teeth extracted due to orthodontic treatment in generally healthy children.

Material and methods: The treatment group included five teeth extracted due to complicated caries in children after antineoplastic treatment. The control group included five teeth extracted due to orthodontic treatment in generally healthy children. The chemical composition of enamel, dentine, cementum, interior of the canal, and enamel abnormalities in teeth extracted from patients after chemotherapy and in generally healthy patients were assessed with energy-dispersive X-ray spectroscopy. Results were analysed statistically.

Results: The magnesium (Mg) and zinc (Zn) mass contents in the enamel of patients after chemotherapy increased and so did the calcium (Ca) to phosphorus (P) ratio when compared to controls. Areas with abnormal enamel in patients after chemotherapy and in generally healthy patients were assessed with energy-dispersive X-ray spectroscopy. Results were analysed statistically.

Key words: children, chemotherapy, chemical composition of mineralised teeth.
Patients received different chemotherapy regimens, including vincristine, etoposide, cisplatin, 5-fluorouracil, cyclophosphamide, and doxorubicin. Three patients started antineoplastic treatment after removing fully formed teeth, including two patients with fully formed crowns.

The extracted teeth were rinsed under running water, mechanically cleaned from soft tissues, cut lengthwise, and kept in ethanol. Dental cross-sections were visualised with a stereo microscope (SZX16, Olympus) and, after applying carbon dust with an ionic duster (JEC 530 Auto Carbon Coater, Jeol), with a scanning electron microscope (SEM; JSM-7600F Jeol). Chemical elements in the different parts of the enamel, dentine, cementum, and interior of the canal were analysed with energy-dispersive X-ray spectroscopy (EDS; Oxford X-Max) together with an electron microscope.

The analysis was performed with a magnification of 250×, at 15kV at fixed voltage measurement points (n = 6 for each area), as shown in Fig. 1.

The chemical composition was presented as mean element mass contents (arithmetic mean ± standard deviation) of elements prevailing in the assessed tooth areas in children after chemotherapy (group 1) and in generally healthy children (group 2). The calcium-to-phosphorus ratio for subsequent dental tissues was also calculated for both groups. The results were statistically analysed with the \( U \) Mann-Whitney test. Significance was set at \( p \leq 0.05 \).

## Results

The microanalysis of the chemical composition of enamel within the opaque areas in children after chemotherapy showed that levels of phosphorus were statistically significantly lower than in healthy enamel. The calcium-to-phosphorus ratio in these areas was 1.851 ±0.524. It was higher in the teeth of patients after chemotherapy (1.760 ±0.232) than in those of controls (1.694 ±0.011) (Fig. 2).

The mass contents of trace elements (Cl, Mg, and Na) within the opaque areas were higher in patients treat-
Changes in the chemical composition of mineralised teeth in children after antineoplastic treatment

ed with chemotherapeutics than in macroscopically unchanged enamel after chemotherapy or in healthy enamel (Table 1).

The enamel mass content of Mg was also higher in patients after chemotherapy than in controls, while that of Cl was lower. Na levels were also assessed in both groups (Table 2).

Furthermore, the enamel of two teeth after chemotherapy tested positive for zinc (Zn) (Fig. 3), whereas healthy enamel did not.

Chlorine and a lower magnesium level were detected in the dentine of patients after chemotherapy when comparing to controls (Table 3).

Chlorine was detected in the cementum of patients after chemotherapy and sulphur was also detected in two teeth. Sulphur was not detected in any of the teeth in the controls (Table 4, Fig. 4).

Discussion

The analysis of the chemical composition of various tooth areas in children and adolescents after antineoplastic treatment indicated a lower calcium level (Ca) and an increased calcium to phosphorus ratio in enamel, when compared to controls. The level of magnesium (Mg) decreased; chlorine (Cl) was detected in dentine; trace amounts of sulphur (S) and important amounts of chlorine (CL) were detected in the cementum of certain teeth, when compared to controls.

Table 2. Mass content of elements in macroscopically unchanged enamel

| Chemical element | Patients after chemotherapy (group 1) | Controls (group 2) | p   |
|------------------|---------------------------------------|-------------------|-----|
| Ca   | 34.676 ±0.744 | 35.560 ±0.283 | 0.0601 |
| P    | 19.926 ±1.898 | 20.990 ±0.166 | 0.2101 |
| Na   | 0.790 ±0.261 | 0.770 ±0.125 | 0.5309 |
| Cl   | 0.634 ±0.127 | 0.758 ±0.073 | 0.0937 |
| Mg   | 0.302 ±0.215 | 0.224 ±0.127 | 0.5284 |
| O    | 41.498 ±0.486 | 41.470 ±0.265 | 0.8345 |

Table 3. Mass percent of elements in dentine

| Chemical element | Patients after chemotherapy (group 1) | Controls (group 2) | p   |
|------------------|---------------------------------------|-------------------|-----|
| Ca   | 36.288 ±1.574 | 36.104 ±0.877 | 0.8345 |
| P    | 20.062 ±0.547 | 20.304 ±0.266 | 0.5309 |
| Na   | 0.908 ±0.350 | 0.838 ±0.294 | 0.6761 |
| Cl   | 0.138 ±0.171 | - | 0.1797 |
| Mg   | 0.842 ±0.453 | 1.048 ±0.281 | 0.4034 |
| O    | 41.424 ±0.355 | 41.640 ±0.226 | 0.4633 |

Table 4. Mass percent of elements on cementum surface

| Chemical element | Patients after chemotherapy (group 1) | Controls (group 2) | p   |
|------------------|---------------------------------------|-------------------|-----|
| Ca   | 32.936 ±14.143 | 34.242 ±2.437 | 0.6974 |
| P    | 14.472 ±6.211 | 19.482 ±1.002 | 0.2963 |
| Na   | 1.016 ±0.643 | 2.058 ±1.143 | 0.2963 |
| Cl   | 0.386 ±0.355 | - | 0.0720 |
| Mg   | 0.700 ±0.368 | 1.428 ±0.463 | 0.0947 |
| O    | 45.228 ±10.646 | 41.852 ±0.444 | 0.6761 |

Fig. 3. Enamel spectrogram of teeth in patients after chemotherapy, indicating the prevalence of trace elements: F, Zn (A), and Si (B)

Fig. 4. Cementum spectrogram of tooth in patient after chemotherapy, indicating the prevalence of sulphur (S)
Table 5. Mass percent of elements on the interior surface of the canal

| Chemical element | Patients after chemotherapy (group 1) | Controls (group 2) | p     |
|------------------|--------------------------------------|-------------------|-------|
| Ca               | 26.870 ±9.839                       | 33.420 ±2.789     | 0.4034|
| P                | 17.472 ±6.247                       | 19.528 ±0.698     | 0.8345|
| Na               | 1.326 ±1.192                        | 1.594 ±0.873      | 0.4034|
| Cl               | 0.234 ±0.265                        | -                 | 0.1797|
| Mg               | 1.444 ±0.897                        | 5.320 ±7.106      | 0.2492|
| O                | 46.106 ±8.916                       | 42.236 ±0.755     | 0.8345|

Hydroxyapatite, a crucial enamel component, is mainly composed of calcium and phosphorus. The normal Ca level in healthy enamel is between 36.5 and 40.0 mass percent, depending on age, and the normal phosphorus (P) level is between 17.25 and 18.25 mass percent [12]. A study reported the levels at 32.68 mass percent for Ca and 17.48 for P [12]. However, the Ca level increased with age [13].

The present analysis of enamel established a Ca level at 35 mass percent in children not treated with chemotherapeutics, and at 34.67 mass percent in children treated with chemotherapeutics; therefore considerably higher than in adult teeth. This did not confirm the generally suggested tendency when comparing the teeth of children/adolescents and young adults [13].

Enamel hardness depends on calcium and phosphorous levels, i.e. the calcium-to-phosphorus ratio. The higher the ratio, the lower the enamel mineralisation. Normal ratio should fluctuate between 1.8 and 2.3 [12]. The present results established the calcium to phosphorus ratio at 1.76 in healthy enamel and at 1.851 in carious enamel, both being within the normal limits. Studies varyingly described the calcium-to-phosphorus ratio in demineralised enamel; Pieciak-Pańczyszyn et al. reported it to be decreased in carious enamel vs. healthy enamel (1.5 vs. 1.9) [12]; so did Jalevik et al. (1.8 vs. 1.4) [9]; however Knychalska-Karwan (2.34 vs. 3.38) et al. and Fagrell et al. reported it to be increased, similarly to the present study [8, 14]. Divergences could result from unidentified defects during tooth mineralisation.

Furthermore, the present study detected such trace elements as chlorine, sodium, and magnesium in the enamel of both groups, i.e. in patients after chemotherapy and in controls, with slightly higher results in the former. These elements could promote caries [10, 11, 15, 16]. Magnesium (Mg) is known to increase the susceptibility of mineralised dental tissues to acids and therefore also to caries. In the present study, the level of Mg in dental enamel was higher in the group after chemotherapy than in controls, which could be responsible for more severe caries in that group. Amr et al. determined that the level of magnesium in carious enamel was higher than in healthy enamel of teeth removed due to orthodontic treatment [17]. The level of Mg in enamel could also be related to environmental factors because, in industrialised regions, levels of magnesium contained in animal and plant-based foods have been decreasing. Opalko et al. observed a lower Mg level in the enamel of children living in the region of the city of Szczecin than in that of children living in the region of the city of Białystok [18]. At the meantime, it resulted in less severe caries in children from the Szczecin region than in those from the Białystok region [18]. Jalevik et al. also confirmed higher levels of Mg in demineralised enamel than in the healthy one [9]. The correlation between Cl prevalence in enamel and enamel mineralisation remained unclear, Jalevik et al. did not confirm that the prevalence of Cl affected enamel demineralisation and promoted caries [9]. In the present study, the amount of Cl in the enamel of patients after chemotherapy was lower than the amount of Cl in controls, although the difference was not statistically significant. Furthermore, Zn was detected in one tooth from group 1 and was not detected in any of the teeth from group 2. Mazurek-Machol et al. and Gomes et al. established that Zn was necessary for proper enamel formation and its deficiency meant teeth were more prone to developing caries [15, 19]. They showed that zinc helped strengthen enamel and prevented caries. Therefore, the prevalence of Zn detected in the enamel of patients after chemotherapy and not detected in controls remained unclear.

Many studies have emphasised that even a low level of trace elements could impact the size of the formed enamel prisms, determining their hardness, and therefore their resistance to acids [10, 11]. Such trace elements as lead (Pb), titanium (Ti), manganese (Mn), selenium (Se), chromium (Cr), and nickel (Ni) affected the crystal structure of hydroxyapatites [10].

Such trace elements as fluorine (F), aluminium (Al), iron (Fe), selenium (Se), and strontium (Sr) were detected in teeth at a low risk of caries; manganese (Mn), copper (Cu), and cadmium (Cd) were detected in teeth at a high risk of caries. In the mean time, the levels of fluorine (F), strontium (Sr), potassium (K), and aluminium (Al) were higher in healthy than in carious enamel, and the level of silicon (Si) was higher in carious enamel [11]. In the present study, fluorine was detected in one patient after chemotherapy and in one control, and silicon only in one patient after chemotherapy. No other trace elements were detected in the enamel in both groups.

When considering the impact of trace elements on enamel mineralisation, it is important to note that because of fluctuations in the levels of other elements, which ranged from very low to very high, averaging the results could lead to interpretation errors [20, 12]. Divergences between the chemical composition of carious and healthy teeth could also result from environmental factors (diet and pollution), the impact of other diseases, different patient ages in subsequent studies, and the use of different measurement tools. The main dentine components – calcium, inorganic phosphorous, and fluorine – play a key role in tooth demineralisation and remineralisation [21, 22].

Pawlícki et al. assessed the chemical composition of dentine [13] and found lower mass percentage of calcium (21.76–25.82 depending on age group) and phosphorous (7.91–15.20) than in the present study. The present calcium and phosphorous mass percentages in outer dentine was higher than in the interior of the canal. These results differed from those of Magnus et al. [22], who compared Ca and P mass percentages in both outer dentine and in the interior of the canal. Chlorine was detected in the dentine of teeth after chemotherapy but not in that of controls. How-
ever, according to Jalevik et al., chlorine did not affect tooth mineralisation [9].

Brodzikowska assessed the mass percentage of these elements in healthy and carious cementum [23]. She established that the calcium mass percentage (38.1) was higher than in the present study [23]. Furthermore, carious cementum contained less Ca (33.1) than healthy cementum. Brodzikowska also showed that the phosphorus mass percentage was higher in both healthy (18.08) and carious (17.02) cementum than in the present group 1 (14.47), but higher than in the present group 2 (19.48). In the present study, the calcium to phosphorus ratio in the cementum of children after chemotherapy was higher (2.92) than Brodzikowska’s ratio in healthy cementum (2.12). For controls, the calcium to phosphorus ratio (1.76) was lower than Brodzikowska’s. It is difficult to compare the present study to the existing ones because there are no publications reporting on the element composition in teeth extracted after antineoplastic treatment. Höltta and Macleod analysed teeth extracted from patients after chemotherapy with a microscope, however they only determined the morphology of mineralised tissues [5, 24].

The present X-ray analysis of the chemical composition of mineralised dental tissues showed that multidrug antineoplastic treatments in childhood, together with the complications they cause, including malnutrition, malabsorption, and metabolic disorders, could considerably impact developmental anomalies, caries, and their severity. It could be caused by abnormal calcium and phosphorus levels and the prevalence of trace elements in these tissues. Since antineoplastic treatments are complex and may impact oral health in numerous ways, studies on this impact should be continued.

In conclusion, antineoplastic treatment, and the complications it causes, in childhood may lead to a decrease in the calcium-to-phosphorus ratio and also modify the levels of these elements in mineralised teeth. Antineoplastic treatments and concurrent disorders could also have an impact on the levels of trace elements. A higher predisposition to caries in children after oncological treatment could result from modifications in the chemical composition of teeth.

The authors declare no conflict of interest.

References
1. Olczak-Kowalczyk D, Daszkiewicz M, Adamowicz-Klepalska B, Mielnik-Błaszczyk M, Dombowska-Bąginska B, Perek D. The status of dentition and oral hygiene in children after anticancer treatment. Ann Acad Med 2004; 34: 237-55.
2. Aşvar A, Ellı M, Darka O, Pınarlı G. Long-term of chemotheraphy on caries formation, dental development, and salivary factors in childhood cancer survivors. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007; 104: 781-9.
3. Maciel Cordova JC, de Castrojr CG, Brunatto Al Di Leone LP, da Silva HE. Oral Health and dental abnormalities in patients treated for leukemia in childhood and adolescence. Pediatr Blood Cancer 2009; 53: 361-5.
4. Höltta P, Alaluusua S, Saarinen-Pihkala UM, Wolf J, Nyström M, Hovi L. Long-term adverse effects on dentition in children with poor-risk neuroblastoma treated with high-dose chemotherapy and autologous stem cell transplantation with or without total body irradiation. Bone Marrow Transpl 2002; 29: 121-7.
5. Höltta P. Developmental aberrations of permanent teeth after high-dose anticancer therapy in childhood. Dissertation, Helsinki 2005.
6. Cubucku CE, Sevinir B, Erkan I. Disturbed dental development of permanent teeth in children with solid tumors and lymphomas. Pediatr Blood Cancer 2012; 58: 80-4.
7. Nemeth O, Hermann P, Kivicsics P, Garami M. Long-term effects of chemotherapy on dental status of children cancer survivors. Pediatr Hematol Oncol 2013; 30: 208-15.
8. Fagrell TG, Dietz W, Jalevik B, Norén JG. Chemical, mechanical and morphological properties of hypomineraled enamel of permanent first molars. Acta Odontol Scand 2010; 68: 215-22.
9. Jalevik B, Odellius H, Dietz W, Norén J. Secondary ion mass spectrometry and X-ray microanalysis of hypomineralized enamel in human permanent molars. Arch Oral Biol 2001; 46: 239-47.
10. Ghadimi E, Eimar H, Marelli B, Nazhat SN, Asgharian M, Vali H, Tamimi F. Trace elements can influence the physical properties of tooth enamel. Springerplus 2013; 2: 499-53.
11. Shashikiran ND, Sultha CV, Reddy Himath MC. Estimation of trace elements in sound and carious enamel of primary and permanent teeth by atomic absorption spectrophotometry: an in vitro study. Indian J Dent Res 2007; 18: 157-62.
12. Piesiak-Panczyzsyn D, Czajczytysz-Waszkiewicz A, Kaczmarek U. Comparative ultrastructure analysis of image and chemical components of early caries lesion and sound hard tissue of the teeth. Dent Med Probl 2005; 42: 443-8.
13. Pawlicki R, Knysz-Pisalka-Karwan. Tooth hard-tissues of adults of different ages. Examinations in the scanning electron microscope and X-ray microanalysis. Czas Stomat 1994; 47: 672-80.
14. Knysz-Pisalka-Karwan Z, Pawlicki R. Caries lesions of tooth smooth surfaces. SEM and rentgen microanalysator study. Magaz Stomat 1997; 8: 9-12.
15. Mazurek-Machol M, Machoy-Mokrzyńska A. The level of zinc in the blood, urine, bones and teeth of rats following intra-oral application of this element. Czas Stomat 2005; 58: 195-200.
16. Schalk-van der Weide Y, Steen WH, Bosman F. Taurodontizm and length of teeth with patients with oogondontia. J Oral Rehabil 1999; 20: 401-12.
17. Amr M, Fattah A, Helal I. Analysis of trace elements in teeth by ICP-MS implications for caries. J Phys Sci 2010; 21: 1-12.
18. Opalko K, Lagocka R, Marczuk-Kolada G, Stokowska W. Magnesium content in permanent dental enamel in children from Białystok and Szczecin (in vitro studies). Nowa Stomat 1999; 4: 15-17.
19. Gomes VE, Wada RS, Cury AJ, Rosario de Sousa ML. Lead level, enamel defects and dental caries in deciduous teeth. Rev Saude Publica 2004; 38: 1-6.
20. Mattthers-Brzozowska T, Surdacka A, Kobyłasza M, Józwiak K, Stachecki B. Renermalisation of induced enamel defects in vivo study. Czas Stomat 1991; 44: 251-7.
21. Dowker SE, Anderson P, Elliott JC, Gao XL. Crystal chemistry and dissolution of calcium phosphate in dental enamel. Mineralog Magaz 1999; 63: 791-800.
22. Magnus L, Maltz M, Baveresco C, Bastos LF, Hashizume L. Biochemical composition of carious dentin and different layers of sound dentin. J Oral Sci 2013; 55: 133-7.
23. Brodzikowska A. X-ray microanalysis of carious lesion in the root cement following application of Fluor Protector fluoride varnish and Cervitec chlorhexidine varnish. Czas Stomat 2005; 58: 167-74.
24. Macleod RI, Welbury RR, Soames J. Effects of cytotoxic chemotherapy on dental development. J Royal Soc Med 1987; 80: 207-9.

Address for correspondence
Ewa Krasuska-Slawańska
The Children’s Memorial Health Institute
Al. Dzieci Polskich 20
04-730 Warsaw, Poland
e-mail: e.krasuska@czd.pl

Submitted: 22.02.2018
Accepted: 4.03.2018