Number and dynamics of micronuclei and near-tetraploidy predict prognosis in childhood acute leukaemia

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Objectives. This study aims to identify factors possibly contributing to complications in children with acute leukaemia. Despite diverse etiological causes, similar processes trigger the process of cell malignancy. Genomic instability has received considerable attention in this context.
Method. We conducted chromosomal analysis of bone marrow cells and measured the micronuclei (Mn) level in buccal cells over time. Statistical reliability assessment was performed using Analysis of variance (ANOVA), and the data were analyzed and visualized using the SPSS 12 statistical analysis software package.
Results. On the 15th day of treatment, our findings confirmed a statistically significant correlation (χ^2=3.88, *P*=0.04) between the number of blasts in the bone marrow and unfavourable outcome in patients with a near-tetraploid chromosome clone. Additionally, on the 33rd day of treatment, we observed a correlation between an elevated number of Mn and relapses.
Discussion. While it is commonly believed that a hyperdiploid clone with >50 chromosomes in childhood acute lymphoblastic leukaemia confers favorable outcome, our study revealed partially heterogeneous results and poor prognosis in patients with a near-tetraploid clone. We have also identified a correlation between the Mn level on the 33rd day of treatment and the development of complications. It is possible that the increased Mn values and the occurrence of relapses were influenced by the individual patient's sensitivity to the genotoxic effect of the medication.

Key words: buccal micronuclei (Mn), children leukaemia, predictors

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INTRODUCTION

Leukaemia is the most common childhood malignant disease. Based on the 2022 data from the National Center for Disease Control and Public Health of Georgia and the Haematology Oncology Department at Iashvili Paediatric Tertiary Referral Hospital, 35–40 children are diagnosed acute leukaemia yearly, which corresponds approximately to 4 per 100,000 of the child population of Georgia. According to the Haematology Oncology Center data, 70–75% of the patients fully recover[1].

Childhood acute leukaemias are divided into two primary forms (lymphoblastic and non-lymphoblastic, i.e. myeloblastic) with their subvariants. Acute lymphoblastic leukaemia (ALL) is more common (70–85%). Over 80% of ALL develop from B-cell precursors and approximately 15% are T-ALL (ref.[2]).

In recent decades, significant achievements have been made in treating childhood leukaemia, however, late complications are often noted. There is an effort to identify specific biomarkers to predict the course of leukaemia. Regardless of the different etiological factors causing malignancy, the process occurring during the period of tumorigenesis are mostly identical. Damage to the DNA structure can be triggered by various mutagens[3-5].

Genomic instability leads to changes in DNA sequences and can be associated with different chromosomal aberrations[6-8]. Recently, special attention has been paid to the testing of genomic instability and its correlation with the course of leukaemia and possible complications. Micronucleus assay, the study of Mn in different tissues, is considered to be a relatively new direction to assess genetic instability and genotoxicity. Mn is defined as an entire chromosome or its fragment, dropped from the nucleus during cell division. The recent literature describes of the levels of Mn in lymphocytes related to the course and outcome of cancer[9,10].

Neverthless, more research is needed, to establish correlation between genomic instability, the risk of late complications and the underlying mechanisms.

We settled on Mn in buccal cells since this method is non-invasive and easily accessible. We aimed to develop tests for genetic instability and the degree of genotoxicity of leukaemia treatment, considering the individual characteristics of patients to identify the most informative predictors of subsequent complications.

MATERIALS AND METHODS

The material for the study was the peripheral blood and bone marrow of 86 children with acute leukaemia admitted to the Haematology Oncology Department at Georgian Iashvili Paediatric Tertiary Referral Hospital.

Diagnosis and treatment were performed according to the BFM (Berlin, Frankfurt, Münster) – type protocol (ALL-BFM-2017). Cytomorphology, immunophenotyping, cytogenetic, molecular genetic investigation and CNS status assessment were performed at diagnosis. Treatment included three stages: remission induction, consolidation and intensification. On the 15th day of the treatment, minimal residual disease (MRD) was assessed to assign a risk group. According to MRD status, patients were grouped into high, medium, and low-risk groups. After intensive chemotherapy, the patients were given maintenance therapy.

Additionally, on the 15th and 33rd days of treatment, we examined the level of Mn in buccal cells in 35 patients. We studied its correlation with clinical and laboratory data according to the treatment protocol. Of these, 20 patients were also examined after three years. In brief, scrape cytology of the oral mucosa was used. The smear was placed on the glass slide and fixed with Carnoy's fixative, stained with azur eosin and Lichtgreen solution. One thousand cells were analyzed under a light microscope, and the number of Mn was counted. We used ANOVA for statistical assessment and the SPSS 12 statistical software package for the calculations and the visualization of the data. The number of Mn was determined at the beginning of treatment and on the 15th and 33rd day, because these time points are generally accepted the most relevant for prognosis of leukaemia. The level of Mn in buccal cells up to 5 per 1000 was considered normal.

RESULTS

Based on the fact that one of the indicators of genetic instability is structural and numerical chromosomal aberration, we began our research with the analysis of data from 86 children (37 girls and 49 boys, 0–18 years of age) with acute leukaemia admitted to the Haematology Oncology Department at Iashvili Paediatric Tertiary Referral Hospital. In most patients (88.6%), ALL was diagnosed. In all cases, a cytogenetic analysis of the bone marrow was performed upon admission and on days 15 and 33. On admission, the number of blast cells was 63–89%.

We noted the correlation between the number of blast cells and the presence of polyploidy, which is frequent in this dynamic variant. In 18 patients (20%), a hyperdiploid clone with a chromosome number >50 was identified, in some cases as singleton and sometimes coexisting with other abnormal clones.

The number of chromosomes was 50–54 in 8 patients, 55–59 in 8 patients and 83–90 near tetraploid number in 2 cases. On the 15th day of treatment, the number of blasts in 14 patients with polyploid clones decreased and was < 5%, which indicated a good effect. In 4 patients, a decrease in the number of blasts was also observed, but only to the level between 5% and 20%. These patients later relapsed. We confirmed statistically significant correlation (χ^2=3.88, *P*=0.04) between the number of blasts in the bone marrow on the 15th day after the start of treatment and the further course of the diseases (33th day and after 2–3 years).

Fig. 1. shows a causal relationship between the number of blasts on the 15th day of treatment and the likelihood of relapse.

We also noted an unfavourable outcome in patients with a near-tetraploid clone and additional chromosomal aberrations.

Hyperdiploid number of chromosomes can be considered as having causal relationship with the likelihood of relapse. Detection of Mn in buccal cells was conducted in 35 patients, 17 girls and 18 boys.

At diagnosis, the Mn number in 1000 analyzed cells was between 1 to 5 in 10 patients and between 6 and 32 in 25 patients.

During the treatment, all the patients showed an increase in the level of Mn, but using cluster analysis, two groups of patients were identified, differing in the intensity of the rise in the number of Mn, and factorial analysis of variance revealed a statistically significant relationship between the rate of growth in the number of Mn and their initial level.

On the 15th day of treatment, the count of Mn was normal only in 6 patients; in 29 patients, it exceeded 5 in 1000 and the maximum number was 56 per 1000. An increased level of Mn at diagnosis was also noted in the latter group. On day 33, the level of Mn did not exceed 5 per 1000 in 10 patients, and in 25, it reached 32. Even

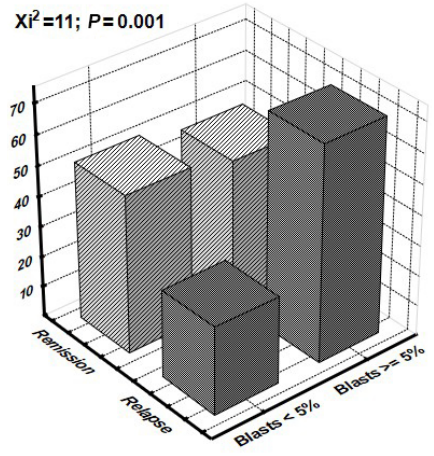

Fig. 1. Contingency histogram between the percentage of blasts in bone marrow on the 15th day after the start of treatment and outcome (relapse or remission).
X axis: Outcome category
Y axis: Groups of patients with blast cell content in bone marrow on the 15th day of treatment, <5% and >=5%, respectively
Z axis – percentage of remission and relapse in Groups of patients with blast cell content in bone marrow <5% and >=5%, respectively

though by day 33, 31 out of 35 patients were in clinical and haematological remission, 23 of them developed various complications by the end of the first year such as cardiological and neurological complications, aneurysm, drug-induced diabetes, venous thrombosis, etc.

After 2–3 years, 6 (14.7%) of the 35 patients developed a relapse and 3 (8.8%) patients died.

The correlation between the number of Mn on admission and- on day 15 with the state of patients after 2–3 years was not confirmed. However, in patients who were in remission, the level of Mn on the 33rd day of treatment was significantly lower than in the patients with relapses. These data are shown in Fig. 3.

DISCUSSION

The present study demonstrated that karyotypic instability affects the development of malignant disease. Although the presence of a hyperdiploid clone with a chromosome number of more than 50 in children with acute lymphoblastic leukaemia is considered prognostically supperior[11-14], we observed partially discordant results.

Like other authors[11,12], we have also detected an unfavourable outcome in patients with a near-tetraploid clone and additional chromosomal aberrations.

A high hyperdiploid number of chromosomes can be considered as one of the factors determing the course of

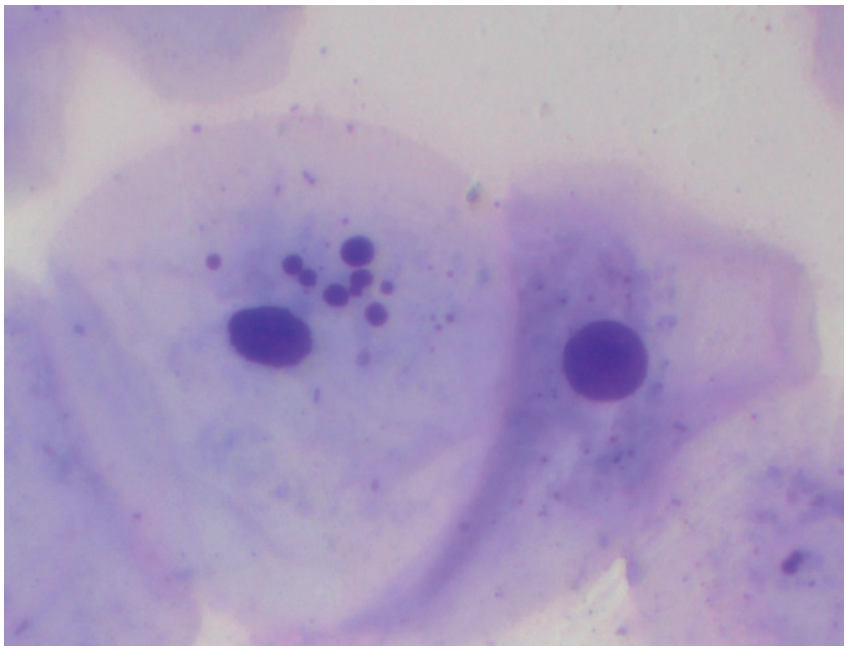

Fig. 2. Buccal cells of a patient with numerous Mn (relapse after 2 years).

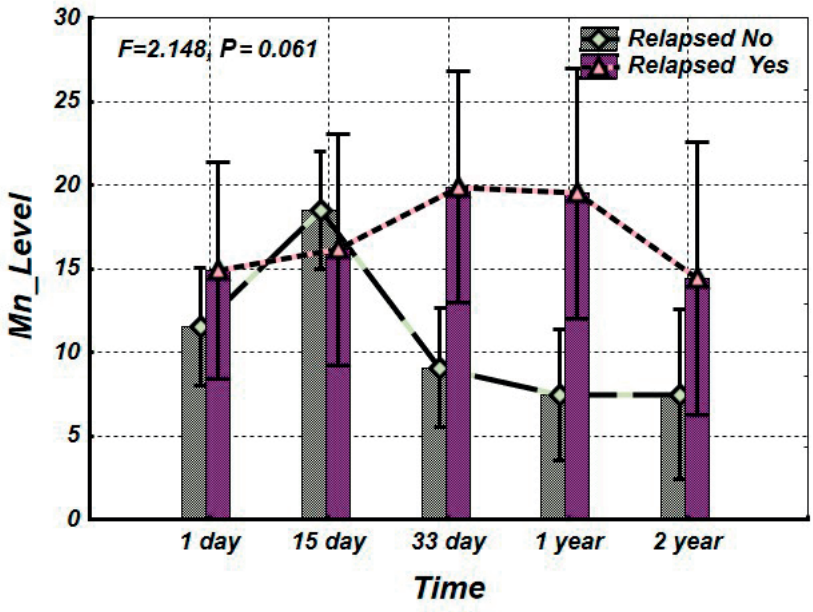

Fig. 3. Dynamics of the distribution of buccal micronucleus level in relapsed (purple) and non-relapsed (grey) patients.

the disease. The interaction of prognostic markers should always be considered, together with an individual characteristics of the patient[13].

In addition to commonly used indicators for assessing the condition of a patient, such as the number of blasts, cellular composition of the bone marrow, and widely employed genetic methods such as cytogenetic and PCR, we have conducted a novel study regarding a readily accessible genetic process, known as the buccal micronucleus testing, to predict immediate and long-term complications in paediatric leukaemia.

In the available literature, some data on the significance of Mn exist, but include different conditions. A significant part of the studies was performed on peripheral blood lymphocytes and other tissues and tumours of various localizations, such as skin, digestive tract, and prostate[15.] Many authors have noted the necessity of continuing these studies. Very few works address the prognostic value of Mn in buccal cells[11], despite the fact that the method of sdudying buccal Mn is much more accessible, non-invasive and unlike micronuclei in lymphocytes, does not require cell culturing.

Some authors note Mn and frequencies in leukemic patients were significantly higher than those in exposed workers and control groups (P<0.05). To conclude, with the knowledge of the risk status, various interventions can be initiated, and the Mn test can be performed to assess the cellular improvements and prognosis after the completion of treatment/chemoprevention.

It can be assumed that the increased Mn values on the 33rd day of treatment, depends on the individual sensitivity of the patients to the genotoxicity of the drugs, which can explain their subsequent disease course and help understand the role of genomic instability in predicting the prognosis and possible complications of childhood leukaemia.

Our data emphasize the importance of evaluating the number of blasts, polyploidy and level of Mn in both the diagnosis of acute leukaemia in children and the identification of individual patient sensitivity to the genotoxicity of therapy. Additionally, these factors can help predict the development of the disease. This underscores the limitless potential of laboratory research in medicine and suggests the need to adjust the treatment regimens based on these indicators accordingly.

CONCLUSION

Our work showed that prognosis of childhood acute leukaemia with polyploid clone was associated with modal number of chromosomes in the polyploid clone. There was a distinct correlation with the dynamics of blasts during the treatment. On the 33rd day of the treatment, patients with subsequent relapse had significantly higher number of Mn than the patients in long-term remission. These data emphasize the importance of evaluating the number of blasts, polyploidy and level of Mn in acute leukaemia in children. The identification of individual patient's susceptibility to genotoxic therapy can help predict the development of the disease.

Author contributions: SJ: wrote the original article, studied the level of micronuclei in buccal cells; AZ: collected patient for cytogenetic study and performed cytogenetic study; GO: performed data analysis and designed the study; AS: collected children with leukemia on which the study was performed.

Conflict of interest statement: The authors state that there are no conflicts of interest regarding the publication of this article.

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