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Original scientific paper

WILMS TUMOR (WT)1 GENE EXPRESSION IN CHILDREN WITH ACUTE LEUKEMIA IN SERBIA

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Acute leukemias constitute the most common malignancy in childhood, accounting for 25-35% of all cancer in children. They are divided into acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML). Genetic susceptibility is known to play a major role in childhood leukemias. Wilms tumor (WT)1 is a zinc finger transcription factor involved in regulating the process of cell differentiation; it has been implicated in a wide range of human neoplasms. WT1 overexpression in the bone marrow at diagnosis is reported to be an independent negative prognostic factor in adults and children with AML. The aim of the present investigation was to determine the expression of WT1 in the bone marrow of children with AML and ALL in Serbia and its possible impact on patient survival. We determined bone marrow WT1 expression levels by reverse-transcription polymerase chain reaction (RT-PCR) at diagnosis in 20 children with AML and 20 children with ALL (16 B-ALL and 4 T-ALL), as well as 15 age- and sex-matched controls who were evaluated for immune thrombocytopenic purpura (ITP). For children with AML, follow-up samples were also analyzed one month after treatment initiation and at variable later timepoints of control punctures. The results were normalized based

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on WT1 expression in controls. We found that children with AML had significantly higher WT1 expression at diagnosis (median ± SD: 139.42 ± 244.03) than those with ALL (1.18 ± 54.37; Mann-Whitney U=82; p<0.01) and ITP (0.76 ± 1.01; U=32; p<0.01). Patients with T-ALL had higher WT1 expression than those with B-ALL, though significance was not reached due to subgroup size; differences between AML subgroups according to the French-American-British (FAB) classification were also below the level of significance, though a tendency toward higher values in M3 and M4 leukemias was notable. There was also a tendency toward higher values in 14 children with AML who were still alive after a median follow-up of 1.5 years (181.42 ± 192.52) than in 6 who succumbed to the disease (104.29 ± 354.87). All children with AML who had WT1 expression 1 month after diagnosis below the fourth quartile (10 of 10) were still alive, while only 2 of 5 with 1-month WT1 expression in the fourth quartile survived (Fisher's exact test: p=0.0952). Taken together, our results support a role for WT1 in the diagnostic workup in children with acute leukemia, although it needs to be considered in view of a complex and indvidualized context.

Key words: children, leukemia, Wilms tumor (WT)1

INTRODUCTION

Acute leukemias, as a group, constitute the most common malignancy in childhood, accounting for 25-35% of all cancer in children (RADHI *et al.*, 2015). Genetic susceptibility is known to play one of the major roles in the pathogenesis of this complex category of neoplastic disorders (BRISSON *et al.*, 2015). They are also highly curable by modern chemotherapy protocols and, in some cases, hematopoetic stem cell transplantation. Although it is accepted that all leukemias arise from neoplastic transformation of early hematopoietic progenitor cells (PUI, 2012), acute lymphoblasic leukemias (ALL) and acute myeloid leukemias (AML) are essentialy different diseases. The former is further divided into B- or T-cell leukemia, while the latter is still routinely subclassified according to the morphological French-American-British (FAB) classification into types M0-M7 (RUBNITZ and INABA, 2012). Risk stratification relies on patient age, number of peripheral blood leukemic cells at diagnosis, early (day 8) treatment response, presence or absence of CNS affection, immunophenotype, karyotype and a number of aberrations detected by molecular genetic analyses. Minimal residual disease (MRD) at day 15, determined by multiparameter flow cytometry, also dictates risk stratification in ALL (LAZI and JANKOVI , 2012). B-cell leukemias account for ~80% of all childhood leukemias and can be divided by immunophenotyping according to the expression of cytoplasmic immunoglobulin heavy chains and CD10 into pro-B, common-B and pre-B leukemia, together constituting B-cell precursor leukemias; mature B cell leukemia is usually considered apart, because its treatment is quite different. T-cell leukemias are also divided according to phenotypical maturity attained by the neoplastic cell into early, middle and late form, though there is significant overlap (PUI, 2012). Among many genetic aberrations with potential impact on the course and outcome of childhood leukemia, routine testing for genetic rearrangements TEL/AML1 (most common), E2A/PBX, MLL/AF4 and BCR/ABL for B-cell precursor ALL (MULLIGHAN, 2012); SIL/TAL1 for T-ALL; and PML/RAR , AML1-ETO, CBFB/MYH11, as well as FLT3 internal tandem duplication (ITD) for AML are routinely performed in Serbia (KRSTIC *et al.*, 2010; KRSTOVSKI *et al.,* 2010). There is a tendency toward refinement of genetic analysis-based risk stratification within the larger scope of highly desirable personalization of treatment (HUNGER and MULLIGHAN, 2015). Next-generation

gene sequencing approach is already showing considerable promise in this regard (GLUMAC *et al.*, 2015).

Wilms tumor (WT)1, encoded by an eponymous gene located at chromosome 11p13, is a zinc finger transcription factor deeply involved in regulating the process of cell differentiation, both under physiological conditions and in the settings of malignant alteration. Initially disocovered in connection with the Wilms' tumor, it has been implicated as a pathogenetic factor in a wide range of human neoplasms and appears to play both the role of an oncogene and that of a tumor-suppressor gene (SUGIYAMA, 2001). It is also a known regulator of apoptosis (YANG *et al.*, 2007) and extensively involved both in normal and malignant hematopoiesis (ARIYARATANA and LOEB, 2007). WT1 is hyperexpressed in the bone marrow of acute myeloid leukemia (AML) patients (BERGMANN *et al.*, 1997). WT1 overexpression is reported to be an independent negative prognostic factor in adults and children with AML (TRKA *et al.*, 2002; GARG *et al.*, 2003; RODRIGUEZ *et al.*, 2007; LYU *et al.*, 2014); However, some authors have found no correlation between WT1 expression at the time of diagnosis and general prognosis (BARRAGAN *et al.*, 2004; NORONHA *et al.*, 2009), and in one study in adults WT1 overexpression was found to be associated with good prognosis in non-M3 AML (MIGLINO *et al.*, 2011). A study by the Japanese Childhood Leukemia Cooperative Study Group found WT1 expression levels at diagnosis not to be related with outcome, after correction for FLT3-ITD status; however, WT1 levels in the bone marrow after induction treatment did correlate with the likelihood of treatment success (SHIMADA *et al.*, 2012). In contrast, WT1 expression levels in acute lymphoblastic leukemia (ALL) tend to be close to those of healthy controls; however, some patients do show a moderately high expression (ZHANG *et al.*, 2015).

Given that there is currently no data on WT1 expression in children suffering from acute leukemia in Serbia, the aim of this study was to determine the status of this important genetic parameter in our patient population and its potential impact on survival.

PATIENTS AND METHODS

Patients

Twenty children with AML, 12 male and 8 female, median age 9½ years (range 3 months – 16 years); 20 children with ALL, 11 male and 9 female, median age 5 years (range 6 months – 17 years); and 15 age- and sex-matched controls (children who underwent diagnostic bone marrow puncture because of immune thrombocytopenic purpura [ITP]) were enlisted in the study after obtaining informed consent from their parents. Children with AML were diagnosed in the period April 2011-December 2015 and treated according to the BFM-AML-2004 Protocol, while children with ALL were treated according to the BFM-ALL-IC-2009 Protocol. Karyotype was determined as part of routine diagnostic workup. Gene rearrangements PML/RAR , AML1/ETO, CBHB/MYH11 and FLT3-ITD for AML patients, and TEL/AML1, MLL/AF4, BCR/ABL and PBX2/E2A were also routinely determined by standard reverse-transcription polymerase chain reaction. Patient characteristics are presented in Table 1.

WT1 expression analysis

Patient bone marrow mononuclear cells (BMMCs) were purified on Ficoll-Paque^{TM} Plus (GE Healthcare) density gradients, suspended in TRI Reagent (Ambion) and total RNA was extracted. One microgram of total RNA was used for the cDNA synthesis in 20 µL reactions using RevertAid Reverse Transcriptase (Thermo Scientific). RT PCR was performed using 7900

HT Fast Real-Time PCR System (Applied Biosystems). We have performed PCR in a 20 µl reaction volume using 1 µl of cDNA with TaqMan® Universal Master Mix II (Applied Biosystems), TaqMan® Gene Expression Assay for WT1 (Hs01103751_m1) and primers and probe for ABL gene as endogenous control. All samples were run in duplicates. Relative quantification analysis was performed using comparative ddCt method, using healthy controls as calibrator in order to define the normal range of WT1 expression in healthy subjects.

Statistical analysis

WT1 expression in each group was presented by median ± standard deviation. Where feasible, differences between groups were statistically analyzed using Mann-Whitney U test. This choice of test was dictated by cytogenetic and molecular genetic heterogeneity of patients in all groups, precluding the use of tests dependent on Gaussian distribution.

Table 1A. Characteristics of patients with AML

Patient No.	Sex	Age at dg.	FAB	Immunophenotype	Karyotype	Molecular genetics
1	m	14 y	M3	APL	46, XY	PML/RAR
2	f	8 y	M5	AMxLL (Mo/B)	N.d	Neg
3	m	10 y	M5	AMxLL (Mo/B)	46,XY/47,XY+C	Neg
4	m	8 y	M3	APL	46,XY/46,XY, +17q-	PML/RAR
5	m	10 y	M2	AMxLL (M/T)	N.d.	Neg
6	f	9 y	M4	AMMoL	46,XX	Inv(16)
7	m	19 mo	M5	AMoL	48,XY, +8, +21/46,XY	Neg
8	m	9 y	M2	AML	46, XY	AML1/ETO
9	m	12 y	M2	AML	46, XY	Neg
10	f	5 y	M5	AMoL	46, XY	Neg
11	f	8 y	M5	AMoL	46, XX	FLT3-ITD
12	f	15 y	M3	APL	t (15:17)	PML/RAR
13	m	15 y	M4	AMMoL	46, XY	Neg
14	m	9 mo	M5	AMoL	46, XY	Neg
15	m	13 y	M3	APL	t (15:17)	PML/RAR , FLT3-ITD
16	f	16 y	M5	AMoL	46, XX	Neg; Moz/CBB*
17	m	9 y	M1	AML	t (7;8)	AML1/ETO
18	f	16 y	M3	APL	t (15:17)	PML/RAR
19	m	15 y	M1	AML	46, XY	Neg
20	f	3 mo	M3	APL	t (15:17)	PML/RAR

APL, Acute promyelocytic leukemia; AMxLL, acute mixed-lineage leukemia; AMMoL, acute myelomonocytic leukemia; AMoL, acute monocytic leukemia; AML; acute myeloid leukemia (sensu stricto); Patients not carrying any of the routinely investigated genetic aberrations are marked “Neg”.

* This rearrangement, although not part of routine workup, was found by extensive testing in another center.

Table 1B. Characteristics of patients with ALL

Patient No.	Sex	Age	Immunophenotype	Karyotype	Molecular Genetics
1	f	2 y	Pre-B	t(9;22)	BCR/ABL
2	m	3 y	Pre-B	N.d.	Neg
3	m	2 y	Common B	46, XY	Neg
4	m	6 mo	Common B	46, XY	Neg
5	f	7 y	Pre-B	46, XX	PBX1/E2A
6	f	16 y	Pro-B	46, XX	Neg
7	f	3 y	Pre-B	46, XX + 21	Neg
8	f	5 y	Common B	46, XX	Neg
9	f	5 y	Pre-B	t(12;21)	TEL/AML1
10	m	5 y	Common B	46, XY	Neg
11	m	4 y	Common B	46, XY	Neg
12	m	22 mo	Common B	45, XY -7	Neg
13	f	3 y	Common B	N.d.	Neg
14	m	23 mo	Pre-B	46XY	Neg
15	f	10 y	Common B	46XX	Neg
16	f	15 y	Pre-B	t(9;22)	BCR/ABL
17	m	17 y	T cell	46, XY	Neg
18	m	14 y	T cell	47,XY,+mar/66~68,XXY/46,XY	Neg
19	m	5 y	T cell	46, XY	Neg
20	m	5 y	T cell	46, XY	Neg

Patients not carrying any of the routinely investigated genetic aberrations are marked “Neg”.

RESULTS

WT1 expression across main groups

As a group, AML patients displayed a significantly higher WT1 expression (139.42 ± 244.03) than those with ALL (1.18 ± 54.37; U=82; p<0.01) and ITP (0.76 ± 1.01; U=32; p<0.01). WT1 expression in ALL patients was not significantly different from ITP controls (U=105.5; p>0.05) (Figure 1).

WT1 Expression and Remission/Survival of AML patients

WT1 expression in 16 children with AML who achieved complete remission was 139.42 ± 186.51, while four children who died without entering remission had WT1 expression of 300.80 ± 407.01. At the time of analysis (median follow-up 1½ years), 14 children were still alive and their WT1 expression was 181.42 ± 192.52, while that of the six children who succumbed to their disease (four children who died without entering remission, one who suffered a relapse after having achieved full remission and one who died of an infectious complication) was 104.29 ± 354.87. This difference was not statistically significant (U=41, p>0.05). Among survivors, one also suffered a relapse (WT1 expression 305.91) and was being administered appropriate treatment. In the group of children who had WT1 expression at diagnosis in the 4th quartile

(>350.10), five of seven (71.4%) were still alive, as compared to nine of 13 children (69.2%) with WT1 expression at diagnosis below this cutoff. This difference is not statistically significant by Fischer's exact probability test.

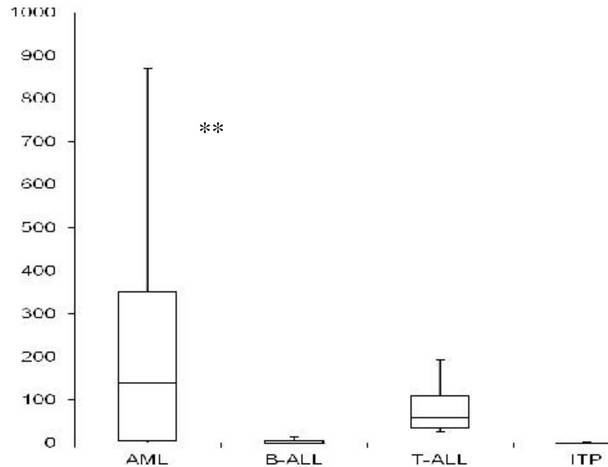

Figure 1. A box plot showing bone marrow WT1 expression at diagnosis in major types of leukemia vs. controls (ITP); ** p<0.01

WT1 Expression in AML Subgroups

A group of six patients with acute promyelocytic leukemia, all of whom were positive for t(15;17) (as well as confirmed PML/RAR rearrangement), had WT1 expression of 379.53 ± 183.52. If one patient who had FLT3-ITD in addition to PML/RAR is excluded, WT1 expression for the remaining five was 320.68 ± 196.53. Due to small sample size, Mann-Whitney U test did not yield significance between WT1 expression in children with APL and those with other FAB types of AML (U=18, p>0.05) (Figure 2). Five children with FAB M1 or M2 subtype had WT1 expression of 5.20 ± 100.7; U=22, p>0.05, while the M5 group (N=7) showed WT1 expression of 51.02 ± 186.66; U=24, p>0.05. One of the two children diagnosed with M4 leukemia had the highest of all values for WT1 expression in the series, amounting to 874.92, while the other child's expression was 305.91. Of note, only the latter child was found to carry inv(16) (i.e., CBFB/MYH11 rearrangement).

WT1 Expression in ALL Patients

The small number of T-ALL patients in our study (N=4) precluded statistical comparison of this subgroup with other subgroups. However, all four had WT1 expression exceeding the median for ITP controls by more than 3 SD. The same is true for four of the 16 B-ALL patients (25%). Of these four, three had common immunophenotype, while one was classified as having pro-B leukemia (there was no expression of CD10). All four had a normal karyotype and routinely investigated gene rearrangements were absent. Conversely, two children in our series that turned

out to have BCR/ABL rearrangement, as well as one child with PBX/2A and TEL/AML1 rearrangements, respectively, did not show WT1 hyperexpression. At the time of analysis, two children were lost to follow-up, while all of the remaining 14 children with B ALL were alive and well (two have undergone HSCT). Three of the four T-ALL patients were also alive and free of disease. Median follow-up time for all children with ALL was 2.0 years.

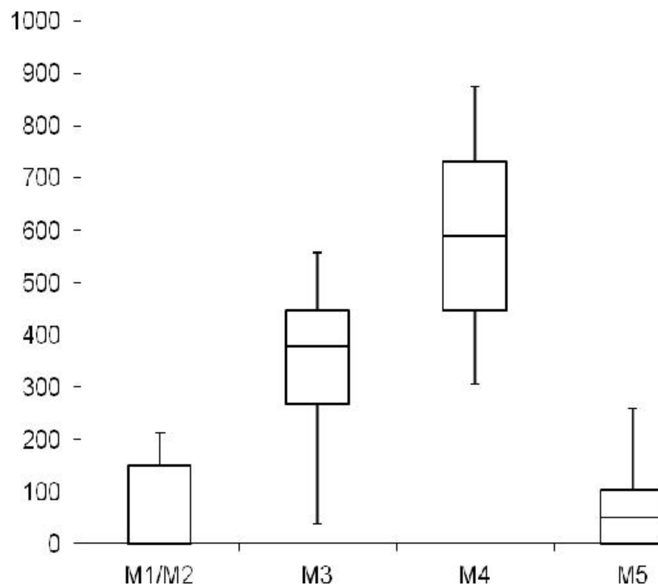

Figure 2. A box plot showing bone marrow WT1 expression at diagnosis in FAB subtypes of children with AML

WT1 Expression at Follow-Up Bone Marrow Investigations

Follow-up measurements of bone marrow WT1 expression in children with AML, where available, are presented in Table 2. For most patients, initial control examination was performed one month after presentation; further control examinations were performed at variable time points. The data for the one-month time point are available for 15 children. Only two of five children whose WT1 expression at one month after diagnosis was in the 4th quartile were alive at the time of analysis, while 10 out of 10 children with WT1 expression below the 4th quartile were alive. However, due to insufficient number of patients, this difference was not found to be significant by Fisher's exact probability test (p=0.0952).

Table 2. Follow-up bone marrow WT1 expression values for individual patients

Patient No.	Sex	Age at dg.	FAB	Immunophenotype	Karyotype	Molecular Genetics
1	m	14 y	M3	APL	46,XY	PML/RAR
Presentation	11 mo	1 y 5 mo	2 y 8 m		Relapse	Outcome
556.41	0	0	0.43		No	Alive at 4 y 8 m

Patient No.	Sex	Age at dg.	FAB	Immunophenotype	Karyotype	Molecular Genetics
2	f	8 y	M5	ALML (Mo/B)	N.d	Neg
Presentation	1 mo				Relapse	Outcome
520.59	47.05				No	Exitus leth. at 1 1/2 mo

Patient No.	Sex	Age at dg.	FAB	Immunophenotype	Karyotype	Molecular Genetics
4	m	8 y	M3	APL	46,XY/46,XY, +17q-	PML/RAR
Presentation	1 mo				Relapse	Outcome
37.71	0				No	Alive at 2 y 9 mo

Patient No.	Sex	Age at dg.	FAB	Immunophenotype	Karyotype	Molecular Genetics
6	f	9 y	M4	AMMoL	46,XX	Inv(16)
Presentation	1 mo	2 mo	3 mo	4 mo	1 y 10 mo (relapse)	1 y 11 mo
305.91	0	0.32	0	1.34	180.39	330.38
					Relapse	Outcome
					Yes	Alive at 2 y

Patient No.	Sex	Age at dg.	FAB	Immunophenotype	Karyotype	Molecular Genetics
8	m	9 y	M2	AML	46, XY	AML1/ETO
Presentation	1 mo	2 mo	3 mo	1 y 9 mo	Relapse	Outcome
151.27	2.01	0.09	0.36	1.55	No	Alive at 1 y 4 mo

Patient No.	Sex	Age at dg.	FAB	Immunophenotype	Karyotype	Molecular Genetics
9	m	12 y	M2	AML	46, XY	Neg
Presentation	1 mo				Relapse	Outcome
5.2	0.45				No	Alive at 1 y 11 mo

Patient No.	Sex	Age at dg.	FAB	Immunophenotype	Karyotype	Molecular Genetics
10	f	5 y	M5	AMoL	46, XY	Neg
Presentation	1 mo				Relapse	Outcome
51.02	0.22				No	Alive at 1 y 10 mo

Patient No.	Sex	Age at dg.	FAB	Immunophenotype	Karyotype	Molecular Genetics
11	f	8 y	M5	AMoL	46, XX	Neg, FLT3-ITD
Presentation	4 mo	6 mo (rel.)			Relapse	Outcome
127.56	8.75	260.47			Yes (6 mo)	Exitus lethalis 6 1/2 mo

Patient No.	Sex	Age at dg.	FAB	Immunophenotype	Karyotype	Molecular Genetics
12	f	15 y	M3	APL	t (15:17)	PML/RAR
Presentation	1 mo	2 mo	5 mo		Relapse	Outcome
320.68	37.48	11.24	39.89		No	Alive at 1 y 9 mo

Patient No.	Sex	Age at dg.	FAB	Immunophenotype	Karyotype	Molecular Genetics
13	m	15 y	M4	AMMoL	46, XY	Neg
Presentation	1 mo				Relapse	Outcome
874.92	465.29				No	Exitus lethalis 1 1/2 mo

Patient No.	Sex	Age at dg.	FAB	Immunophenotype	Karyotype	Molecular Genetics
15	m	13 y	M3	APL	t (15:17)	PML/RAR , FLT3-ITD
Presentation	1 mo	2 mo			Relapse	Outcome
449.76	4.2	0.5			No	Alive at 9 mo

Patient No.	Sex	Age at dg.	FAB	Immunophenotype	Karyotype	Molecular Genetics
16	f	16 y	M5	AMoL	46, XX	Neg; Moz/CBB (t16;18)
Presentation	1 mo				Relapse	Outcome
0.05	3.28				No	Alive (post HSCT) at 9 mo

Patient No.	Sex	Age at dg.	FAB	Immunophenotype	Karyotype	Molecular Genetics
17	m	9 y	M1	AML	t (7;8)	AML1/ETO

Presentation	1 mo	4 1/2 mo			Relapse	Outcome
211.57	12.07	0.22			No	Alive at 8 mo

Patient No.	Sex	Age at dg.	FAB	Immunophenotype	Karyotype	Molecular Genetics
18	f	16 y	M3	APL	t (15:17)	PML/RAR
Presentation	1 mo				Relapse	Outcome
438.37	74.85				No	Alive at 5 mo

Patient No.	Sex	Age at dg.	FAB	Immunophenotype	Karyotype	Molecular Genetics
19	m	15 y	M1	AML	46, XY	Neg
Presentation	1 mo	1 mo			Relapse	Outcome
2.17	4.37	4.22			No	Alive at 3 mo

Although high WT1 expression at first follow-up (1 month after presentation) did confer a poor prognosis (although not reaching statistical significance) and appeared to herald a relapse in our patients (patients 6 and 11), this was not invariably so – namely, patients 12 and 18 have, so far, not suffered a relapse in spite of significant WT1 minimal residual disease (MRD) levels. The use of WT1 as an MRD marker has been extensively studied (MIYAMURA *et al*., 2004; LAPILLONNE *et al*., 2006). This study was not powered to assess the possible use of WT1 as a marker of MRD and further investigations are warranted. Some attempts in this direction have already been made (CILLONI *et al*., 2009). It would be of particular interest to identify potential genetic subpopulations for whom this marker might display a stronger predictive value compared to others. Some steps in this direction have already been taken (PARK *et al*., 2015; STEINBACH *et al*., 2015). It is also possible that defining an appropriate cutoff value for WT1 overexpression may actually be more helpful than continuous scale of WT1 expression values (UJJ *et al*., 2016).

In conclusion, the results obtained in our limited patient series support the inclusion of bone marrow WT1 expression measurements as a potentially useful addition to the routine diagnostic protocol for childhood AML, and possibly certain instances of ALL; however, this does need to be interpreted in view of a complex and highly individualized context.

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EKSPRESIJA GENA ZA VILMSOV TUMOR (WT)1 KOD DECE SA AKUTNOM LEUKEMIJOM

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Izvod

Akutne leukemije predstavljaju naj eš i malignitet kod dece, obuhvataju i 25-35% svih slu ajeva. Dele se na akutne limfoblastne (ALL) i akutne mijeloidne leukemije (AML). Poznato je da genska predispozicija igra jednu od glavnih uloga u nastanku de jih leukemijâ. Vilmsov tumor (WT)1 je transkripcijski inilac koji u estvuje u regulaciji elijske diferencijacije, povezan sa mnoštvom neoplazmi kod oveka. Nagovešteno je da je hiperekspresija WT1 u koštanoj srži pri dijagnozi inilac loše prognoze kod dece i odraslih sa AML. Cilj ovog istraživanja bilo je utvr ivanje ekspresije WT1 u koštanoj srži dece obolele od leukemije u Srbiji i mogu eg uticaja na prognozu bolesti. Uz pomo lan ane reakcije polimeraze sa reverznom transkripcijom (RT-PCR) ispitivali smo ekspresiju WT1 kod 20 dece sa L i 20 dece sa ALL (16 B-ALL 4 T-ALL), kao i 15 kontrolnih osoba, ujedna enog uzrasta i pola, ispitivanih zbog imunske trombocitopenijske purpure (ITP). Kod dece sa AML, ispitivani su i uzorci koštane srži uzeti mesec dana po dijagnozi i u kontrolnim punkcijama u razli ito vreme. Rezultati su normalizovani na osnovu ekspresije WT1 u kontrolnoj grupi. Utvrdili smo da je kod dece sa AML ekspresija WT1 pri dijagnozi (medijana ± standardna devijacija: 139,42 ± 244,03) bila zna ajno viša nego kod ALL (1,18 ± 54,37; Man-Vitnijev test U=82; p<0,01) i ITP (0,76 ± 1,01; U=32; p<0,01). Deca sa T-ALL imala su višu ekspresiju WT1 od dece sa B-ALL, mada bez statisti ke zna ajnosti usled malih podgrupa; razlike izme u podgrupa AML na osnovu francusko-ameri ko-britanske (FAB) klasifikacije tako e nisu dostigle zna ajnost, mada je uo ena tendencija viših vrednosti u podgrupama 3 i 4. Kod 14 dece sa AML koja su bila živa nakon perioda pra enja (medijana 1½ godina), ekspresija WT1 je bila nešto viša (181,42 ± 192,52) nego kod šestoro dece koja su podlegla bolesti (104.29 ± 354.87). Sva deca (10/10) sa AML ija je ekspresija WT1 mesec dana po dijagnozi bila ispod etvrtog kvartila bila su živa nakon perioda pra enja, dok je od onih sa ekspresijom WT1 u etvrtom kvartilu preživelo samo 2/5 (Fišerov test ta ne verovatno e: p=0,0952). U celini, naši rezultati potkrepljuju potencijalnu ulogu WT1 u dijagnosti koj obradi dece sa akutnom leukemijom, ukoliko se posmatra kao deo složenog i individualizovanog konteksta.

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