SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PhD)

THE PROGNOSTICAL ROLE AND THERAPICAL USE OF WILMS TUMOR ANTIGEN IN HEMATOLOGICAL MALIGNANCIES

BY ZSÓFIA JÓNA-UJJ MD

SUPERVISOR: LÁSZLÓ REJTŐ MD, PhD

UNIVERISTY OF DEBRECEN
DOCTORAL SCOOL OF KÁLMÁN LAKI
DEBRECEN, 2017

The prognostical role and therapical use of Wilms tumor antigen in hematological malignancies

By Zsófia Ágnes Jóna-Ujj, MD

Supervisor: László Rejtő, MD, PhD

Doctoral School of Kálmán Laki, University of Debrecen

Head of the **Examination Committee**:
György Balla, MD, PhD, DSc, member of HAS
Members of the Examination Committee:
Miklós Egyed, MD, PhD
Imelda Marton, MD, PhD

The Examination takes place at the Library of the Department of Pediatrics, Faculty of Medicine, University of Debrecen, June 30, 2017, 11:00 AM

Head of the **Defense Committee**:
György Balla, MD, PhD, DSc, member of HAS
Reviewers: Csongor Kiss, MD, PhD, DSc
László Szerafin, MD, PhD
Members of the Defense Committee:
Miklós Egyed, MD, PhD
Imelda Marton, MD, PhD

The PhD Defense takes place at the Lecture Hall of Bldg. A, Department of Internal Medicine, Faculty of Medicine, University of Debrecen, June 30, 2017, 1 PM

Introduction

The WT1 gene was originally isolated from Wilms tumor, a childhood kidney malignancy. It encodes a zinc finger protein which acts as a transcription factor that regulates gene expression. Several types of embryonic cells initially express WT1, in adults however, the gene is only expressed in select tissue types, if it is expressed at all. Originally it had been known as a tumor suppressor gene until its role was revealed as an oncogenic factor in malignancies such as breast, lung, colon, and pancreas cancer. Soon after its discovery, studies also revealed its expression in acute leukemia, pertaining to worse prognosis. These views are considered out of date, since the gene's expression is not necessarily related to shorter survival. However, the assessment of WT1 expression is a useful tool in the monitoring of minimal residual disease. There is little knoen data on the presence of WT1 gene expression in malignant lymphomas due to a lack of sufficient trials. Broadening our knowledge about WT1 could help improve the assessment for prognosis, follow-up and treatment of adult and childhood leukemia.

Overview

The WT1 gene

After the discovery of Wilms tumor, the WT1 gene was described as a tumor suppressor gene, although later its role as an oncogene also became obvious. Its dual role depends on the co-factors present, the functions of related genes, cell types and the degree of differentiation.

WT1 is often expressed in several tissue types throughout the embryonal development. It is essential for kidney growth, since a disturbance in its expression leads to developmental abnormalities, even Wilms tumor. It partakes in gonadal sex differentiation, helps regulate the proliferation of cardiomyocytes, it is also present in the development of coronary arteries and the mesothelium which constitutes the pleura and the peritoneum. It has an important role in liver development, additionally, heavy WT1 expression was observed in skeletal muscle, brain and spinal ependymal tissue at embryonic age.

In healthy adults, few organs and tissues express WT1. About 1% expression rate was observed in adult kidney podocytes, gonadal Sertoli and granulosa cells, mesothelial cells and cells of the bone marrow – the hemopoietic stem cells.

Although WT1 was discovered first in Wilms tumor, its role in the evolution of other solid tumors was also confirmed in the past decade. It has shown increased expression in mesothelial carcinoma, rhabdomyosarcoma, breast cancer, colorectal carcinoma, pancreas carcinoma and non-small cell lung cancer. An early discovery was its increased expression in hematological malignancies such as AML, ALL, and MDS. There is little known data about WT1's relation to lymphomas. Expression has been observed in lymph nodes affected by Hodgkin's lymphoma, Burkitt's Lymphoma, DLBCL and ALCL (anaplastic large cell lymphoma), the prognostic value of this phenomenon has not yet been assessed.

Acute Myeloid Leukemia

In cases of acute myeloid leukemia (AML), the ratio of myeloblasts in the bone marrow is equal to or above 20%. Assessment of chromosome abnormalities are crucial for proper evaluation of patients' prognosis. Aside from that, patient age, clinical condition, primary or secondary disease, response to induction and the depth of remission achieved, along with the presence of specific markers or lack thereof play an important part in it. The optimal treatment for adult acute myeloid leukemia has not yet been made clear. There is obvious difference between younger (<60 years of age) and older patients concerning treatment, since older patients require dose reduction depending on their biological state, furthermore, treatment could shift into palliative and supportive care. Chemotherapeutic treatment for AML patients may be divided into induction and consolidation phases. Treatment success can be measured by evaluating remission completion, depth, disease-free survival and overall survival. Rescue protocols have been used to deal with recurrence. In case of recurrence, stem cell transplantation should be the goal kept in mind. There are numerous promising new drugs being studied that might aid patients with resistance or recurrence.

Diffuse Large B-Cell Lymphoma

Diffuse large B-cell lymphoma (DLBCL) is the most common adult lymphoid malignancy. Diagnosis is based on lymph node biopsy and the Ann Arbor system is used for staging. The choice for treatment also depends on patient risk assessment with IPI (International Prognostic Index). Aside from

the usual prognostic markers, cases with poor therapeutic reaction can be detected using interim PET-CT. Survival for non-disseminated disease is between 80-85%, for disseminated disease however, the rate is around 50%. The treatment for DLBCL usually implies intensive chemotherapy (such as RCHOP). After the achievement of CR by agressive chemotherapy, autologous stem cell transplantation is recommended in case of recurrence or an individually bad prognosis. The treatment arsenal for DLBCL has also grown in the last few years.

Goals

Working in cooperation with the Department of Human Genetics, this study seeks to answer the following

1. Can WT1 expression be assessed from peripheral blood samples, using our method?
2. Can a quantifyable range for pathological WT1 expression be established?
3. What is the prognostic value of WT1 expression evaluation in AML at the time of diagnosis and after the first induction therapy?
4. Can WT1 expression trials be used for monitoring MRD in AML?
5. Can WT1 expression be measured reliably using peripheral blood in DLBCL?
6. Does WT1 expression have any prognostic value in DLBCL?

Materials and Methods

60 AML patients and 25 DLBCL patients were included between October 2006 and October 2014.

The peripheral blood samples were analyzed in the Department of Human genetics, University of Debrecen. Samples were collected into PAXgene Blood RNA Tubes for mRNA isolation. RNA isolation was performed using PAXgene Blood RNA Kit. Real Time qRT-PCR was used for detecting WT1 gene expression (Applied Biosystems 7500), evaluation was presented by TaqMan reaction (Applied Biosystems Hs00240913-ml

Assay). To quantify WT1 expression, a reference gene was needed that shows constant and high levels of expression, we applied the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene. Instead of using the 'Ct' cycle threshold for the calculations, we decided to use the amplification rate of a DNA solution of known concentration for both genes. We applied the Ct values on a calibration curve, allowing the evaluation of the mRNA concentration in the samples. A standardized numerical value (arbitrary unit) was set up, namely the number of WT1 mRNA molecules per 10^4 GAPDH mRNA molecules. For further calculation, we used the aforementioned values.

To mark WT1 expression as positive, a cut-off value had to be determined. A control group of 35 of healthy volunteers of different age and sex was introduced. The WT1 expression measurements from blood samples were normalized to GAPDH expression levels, ranging between 0.002-0.109. All control peripheral blood samples expressed WT1 to some degree. The data gathered has shown a normal distribution which we fitted on a χ^2 function ($p=0.9923$). When using a normal distribution variable, the upper 2.5 percentage is usually considered significant, therefore we rounded up the 97.5 percentile value (0.108) and set the WT1 positivity threshold to 0.1. Since the WT1 expression varied greatly among patients (0.0077-239.0), another threshold value had to be added for high WT1 expression levels (10.0). Categorizing patients as either WT1 positive or WT1 negative became possible using these threshold values.

Results

WT1 expression in AML patients

WT1 expression at the time of diagnosis

53 out of 60 patients (88.33%) showed WT1 positivity at the time of discovery. No statistic correlation was shown between the level of WT1 expression at the time of diagnosis with neither the white blood cell count, medullary blast ratio, the primary or secondary nature of the disease, patient age and cytogenetic alterations.

All seven non-expressing patients showed better survival than the 52 WT1 positive patients, the difference however was insignificant ($p=0.0812$).

22 out of the 53 positive WT1 cases (41%) achieved complete remission with induction treatment. Three of the 7 (43%) initially WT1

negative patients achieved CR so the disease-free survival (remission time) could only be assessed in these cases. The difference in overall survival was also insignificant (p=0.1232).

Similar results were observed when comparing the initially WT1 negative and WT1 positive patients' overall survival (41 cases of de novo AML) (p=0.134). WT1 positive patients' overall survival seemed better, yet the difference was not significant.

Changes in WT1 expression after the first induction treatment

Another set of WT1 expression tests were conducted after the first chemotherapeutic treatment in 49 cases. All 49 patients underwent induction therapy. Aside from the 7 initially WT1 negative cases, another additional 11 patients became WT1 negative, making up a total of 18 sub-threshold WT1 values and 31 supra-threshold values.

The overall survival of the now 18 WT1 negative patients and the 31 WT1 expressing patients showed significant differences ($p<0.0001$). The WT1 negative patients estimated 2-year survival was 61%, with a median survival of 206 weeks, while the WT1 positive patients 2-year survival estimate was 24%, with a median survival of 46 weeks.

There was also a significant difference in disease-free survival (p=0.018) between the 14 WT1 negative and 11 WT1 positive patients who achieved CR.

Changes in WT1 expression and the survival of the three patient groups after induction treatment

After the induction therapy, the surviving patients were divided into three groups to better evaluate the difference in survival related to WT1 negativity. The first group involved 7 subjects who were WT1 negative both before and after induction treatment, only 3 of them were in complete remission, therefore they were the only ones available for determining DFS. The second group involved patients who were WT1 positive all along. This included 31 subjects, 11 of which achieved CR, DFS calculation thus became available. The third group included patients who were initially WT1 positive and became WT1 negative after induction treatment. WT1 expression levels were below threshold in 11 patients in total, all of them achieved CR.

OS was significantly different among the three groups (p=0.0015). The best OS result (median survival 222 weeks, 50-324 weeks) was seen with

those 11 patients who initially expressed WT1 and became WT1 negative after induction, in such case, the 2-year survival vas 63%. OS was somewhat worse (median 180 weeks, 34-324 weeks) with patients who did not express WT1 at all (7 patients), their 2-year survival rate was 57%. The worst OS (median 43 weeks, 5-209 weeks) was seen with patients expressing WT1 all along, these 31 patients had a 2-year survival rate of a mere 7,8%.

Significant difference was seen in disease-free survival as well (p=0.0471). Disease-free survival was 100% in the WT1 negative group, since all three patients achieved CR stayed in remission (median 167 weeks, 71-167 weeks). The initially WT1 positive and later WT1 negative patients' (11 patients) DFS was 65% at two years (median 192 weeks, 27-282 weeks). The worst two-year DFS (27%) was seen in the only WT1 positive group (median 73 weeks, 7-205 weeks)

Therapeutic results after the first induction and during follow-up, divided by patient groups

- After the first chemotherapeutic treatment, the 25 (51%) out of the 49 patients achieved CR. The remission rate among all 60 patients was 42%.

- Out of the 7 patients all along being WT1 negative, 3 patients achieved CR and 4 patients developed PR (42% and 58%, respectively). The three CR patients remained alive even after follow-up and kept their remission. The 4 PR patients did not survive by the end of follow-up.

- Concerning the 31 patients expressing WT1 during the entire study, 11 patients (35%) achieved CR after the first induction, 10 (32%) patients developed PR and 10 patients (32%) showed resistance. The 11 CR patients' WT1 expression levels did not reach sub-threshold. By the end of the follow-up, only 4 out of these 11 CR patients survived, 2 out of 4 (25%) were still in remission. None of the other 20 patients survived.

- The initially WT1 expressing then WT1 negative group of 11 patients showed CR in all cases. 7 patients survived by the end of the follow-up, 6 of which were still in CR.

- By the end of the follow-up, 11 out of the 14 surviving patients were in CR. 3 patients (27%) belonged in the completely WT1 negative group, with a median survival of 136 weeks. Six patients (55%) belonged in the initially WT1 expressing then WT1 negative group, median survival was 247.5 weeks, and 2 patients (18%) belonged in the steadily WT1 positive group, their survival was 209 weeks and 156 weeks. The three non-CR patients were still being treated due to their active state of disease.

- In general terms, patients who showed WT1 positivity during the entire study had the worst survival rates and CR values, only 2 out of the 11 patients who achieved remission managed to stay in remission by the end of the follow-up. The remission rate was lower in patients who had WT1 negativity during the entire study when compared to those who developed WT1 negativity during this period, not many of them could achieve remission (42%), although those who did remained so for the rest of the follow-up. In contrast, the patients who acquired WT1 negativity during the study achieved a 100% CR, yet 45% of the patients showed recurrence and 36% of them eventually died by the end of the follow-up.

- Upon examination of the patients who later became WT1 negative, we have decided that four non-APL and three APL patients should be mentioned in detail. One of them relapsed after the second induction, two patients relapsed during consolidation and one patient relapsed after chemotherapy. All four relapses expressed WT1 positivity, none of the WT1 negative patients relapsed. All three APL patients achieved CR.

WT1 gene expression levels

We wished to evaluate whether the amount of WT1 expression affects prognosis. To do so, we have divided the 53 initially WT1 positive patients into two groups based on WT1 expression levels. 19 patients were put in the first group with their WT1 expression levels varying between 0.1 and 9.99, they were determined as slightly positive, while the highly positive group included 34 patients (expression level above 10.0). No significant difference

was observed in overall survival (p=0.5286). 8 slightly positive and 14 highly positive patients achieved CR, their DFS curves did not differ significantly (p=0.4719).

We observed no difference when observing those 31 patients who remained WT1 positive after treatment. We compered the overall survival between 14 slightly and 17 highly expressing patients (p=0.6773). No significant difference was seen in DFS, either (11 patients in total: 2 slightly WT1 positive, 9 highly WT1 positive) (p=0.401).

There was no significant difference in OS (p=0.3112) even when comparing the overall survival among those 11 patients who became WT1 negative after first induction treatment (5 slightly WT1 positive, 6 highly WT1 positive patients). The results were similar with all 11 patients when comparing DFS (p=0.3674).

Observing those 31 patients whose WT1 positivity was above cut-off value even after the first induction treatment, it appears a singe logarithmic decrease in expression by value (7 patients) does not affect overall survival, when compared to those patients whose WT1 expression levels did not decrease by a logarithmic value (24 patients) (p=0.9228). There was no significant difference in DFS, either (p=0.2254). Similarly, decrease in WT1 expression by 2 logarithmic values had no influence on overall survival (p=0.5186) or disease-free survival (p=0.0648) in patients who remained WT1 positive after the first induction treatment (3 patients expressed decreased values, 4 did not). In broad terms, instead of the decrease in expression, the WT1 negativity (i.e. achievement of sub-threshold level expression) developing after treatment proves to be the determining factor for improvement in OS and DFS.

WT1 expression in DLBCL

No correlation was found between initial WT1 positivity and the disease's other features such as clinical stage, IPI score and the presence of B symptoms.

OS and DFS results of WT1 positive and WT1 negative patients were compared. The 17, initially WT1 negative patients' overall survival (median 131 weeks; range 5-141 weeks) was significantly better (p=0.0475) than the 8 WT1 positive patients' (median 103 weeks; range 40-224 weeks). The WT1 negative patients' 2-year survival probability was 68.8%, while the WT1 positive group achieved only 37.5%.

After treatment, 17 (68%) of all DLBCL patients achieved CR, 6 achieved PR and two patients showed resistance. In terms of WT1 expression levels, 5 out of 8 (63%) WT1 positive patients achieved CR, two (25%) achieved PR and one (12%) developed resistance. 12 out of the 17 (70%) WT1 negative patients achieved CR, 4 (24%) achieved PR and one (6%) remained resistant to treatment. Among the 14 patients who died during the study, 7 belonged in the WT1 positive group and 7 belonged in the WT1 negative group.

There was a significant difference in disease-free survival related to WT1 expression (p=0.0004). The remission's duration among WT1 positive patients were shorter (median 22 weeks; range 11-204 weeks) than WT1 negative patients (median 113 weeks; range 66-152 weeks).

Aside from assessing the initial WT1 values, post-treatment changes were also measured. We have separated the steadily WT1 negative patients, the initially non-WT1 expressive then WT1 positive patients, and the 2 patients who developed WT1 expression later, and compared the overall survival rates. Although the small number of patients does not allow us to establish solid conclusions, we could say that those who became WT1 positive during the study period and those who were WT1 positive all along had significantly poorer survival rates than WT1 negative patients (p=0.006), therefore this phenomenon could predict especially bad prognosis.

There was a crucial difference between those who achieved remission if we compare the three group's DFS periods. Patients who were WT1 negative during treatment showed significantly better survival than entirely WT1 positive patients or those who became WT1 positive during the study period (p=0.0002).

Discussion

The relationship between Wilms tumor gene and AML

The gene's oncogenic function was proven merely two years after its discovery, in contrast to this, its role in the development of leukemia remains uncertain. Previously, it was believed that high WT1 expression levels at the time of discovery meant poor prognosis. The latest data are controversial as the initially WT1 negative patients' prognosis was not significantly different. Our results were coherent to these findings. Our studies suggest that the presence of gene expression after chemotherapy correlates with worse DFS

and OS. We believe the WT1 gene expression's value lies in MRD assessment. WT1 positivity post-treatment indicates MRD positivity and a higher chance for relapse.

Wilms tumor gene expression in AML patients

There is a rich literary background on WT1 assessment from bone marrow samples in leukemia patients, still there is little data supporting peripheral blood samples as an adequate source for gene expression studies, even though there is less 'background noise' from the 1-2% percent of pluripotent stem cells that physiologically express this gene and the sample can be taken non-invasively from both patient and healthy control groups. We analyzed peripheral blood samples, which is rarer in this scenario. We have done concurrent bone marrow and peripheral blood analysis initially; the results were in accord with data found in literature. It should be noted that we have detected (sub-threshold) WT1 expression in healthy volunteers as well, this was in accordance with the literary finding which states about 30-40% healthy people's peripheral blood samples may express WT1 activity.

Determining gene expression levels

Logarithmic decrease (or change) in WT1 mRNA levels relating to patients' response to therapy was used in studies available in literature. Our study was the first to use GAPDH gene as a reference for normalization to determine WT1 expression threshold. Based on the WT1 expression found in healthy volunteers, we set the WT1 positivity threshold to 0.1. This number has no denomination and we used it to quantify WT1 expression, using this threshold allowed us to mark patients as either WT1 negative or WT1 positive. Since patients' observed WT1 expression levels varied on a large scale, a second threshold value had to be added to separate strongly positive, high rate of WT1 expression (10.0).

Clinical Features of Leukemia

We confirmed WT1 expression in 88.33% of leukemia patients at the time of discovery. This rate is in accordance with literary results. There was no correlation between the presence and rate of WT1 expression and the FAB subtype, medullary blast invasion, initial peripheral cell count, karyotype,

FLT3 positivity or NPM1 negativity. Similar results were observed in international studies involving larger patient groups.

WT1 Expression at the Time of Diagnosis

The 2-year survival, overall survival and disease-free survival were all more favorable in WT1 negative cases when compared to WT1 positive cases, however this was not statistically significant. Therefore, we did not find any prognostic value of higher WT1 expression levels at AML's discovery, this is consistent with findings in the literature.

Changes in WT1 expression after induction treatment

All patients entered CR who initially showed WT1 positivity and then achieved sub-threshold WT1 expression levels. Those who were WT1 negative at the time of discovery or achieved this later had significantly better OS and DFS.

To assess the importance of WT1 negativity after the first induction treatment, we divided our patients into three groups. Aside from a group containing WT1 positive patients since discovery and a group of WT1 negative patients since discovery, we also included a group that initially was WT1 positive but then became WT1 negative after treatment. According to our data, the initially WT1 expressing then WT1 negative group achieved significantly better OS.

The three groups responded to induction therapy in different ways. 42% of the entirely WT1 negative group achieved CR and kept it (DFS 100%). In contrast, all the initially WT1 positive then WT1 negative patients achieved CR after first induction therapy, but 45% of them relapsed. The entirely WT1 positive patients' response to therapy and time of remission was the most unfavorable.

Our results were in accordance with the model that proposes WT1 gene's dual role in leukemogenesis, stating that in case of WT1 negativity achieved by chemotherapy (if there is lasting remission) WT1's oncogenic effect is supposedly eliminated and its tumor suppressor activity may reach normal values. Based on the observation that even patients with sub-threshold WT1 levels may respond poorly to treatment (not achieving CR) it is possible that there is a loss off tumor suppressor function which is not always corrected

by the induction treatment. Patients expressing the supposedly all along active (i.e. oncogenic) WT1 gene responded poorly at best.

Several studies claim that high WT1 values in patients achieving CR is unfavorable, suggesting that increased WT1 levels after induction treatment may be of prognostic value. Our results could support this, since those who were all along WT1 positive (WT1 expression above the 0.1 threshold) had unfavorable results.

Decrease in one or two logarithmic values in WT1 expression showed no prognostic value concerning survival if WT1 expression levels were in the pathologic range, i.e. sub-threshold value, as our data suggests.

Out of the 11 patients who became WT1 negative after the first induction treatment, 4 patients WT1 expression values rose above the threshold. According to previous studies an increase in WT1 expression may predict clinical relapse by weeks. The lack of WT1 level elevation in our APL patient helped us exclude recurrence.

Our work also supports that the determination and follow-up of WT1 gene expression may be an effective tool to observe MRD in patients with acute leukemia. This is in accordance with other, international research committee's results. An increase in gene expression could be an early sign of relapse.

Relations between WT1 Gene and DLBCL

One of the first questions during our work was sampling method. Evaluation of WT1 gene expression by immunohistochemistry from histological samples is well known, however, subsequent sampling could be difficult. In our study, we tried to determine whether WT1 gene expression can be safely assessed by qRT-PCR from peripheral blood samples. While writing this essay, we encountered no data in the literature explaining increased WT1 expression in the peripheral blood of patients with NHL. Presumably, the increase in number of WT1 expressing pathological cells could be an explanation to the issue.

WT1 Expression in DLBCL Patients

32% of the patients were found WT1 positive. Despite the small number of patients, the rate of WT1 positivity is in accordance with the data from studies that used lymph node biopsy in NHL. The similar results in WT1

expression level with different sampling methods lead to suggest that it may not necessary to measure WT1 expression from lymph node biopsy for follow-up, peripheral blood sampling may suffice.

WT1 expression levels did not correlate with initial tumor mass, clinical stage, patient age, IPI score or the presence of B symptoms.

There was however, a significant difference in overall survival between WT1 expressing and non-expressing patients. Presumably, a greater number of malignant cells enter the bloodstream in WT1 positivity, suggesting a more aggressive disease which comes with poor prognosis.

Disease-free survival of WT1 negative patients achieving CR was proven to be better than with patients who initially achieved CR but remained WT1 positive. Although a larger number of WT1 positive patients achieved CR, the time of remission (and overall survival) was considerably shorter, the leading cause of death being lymphoma progression. This might suggest that a more intensive induction treatment, an early autologous stem cell transplant or a treatment targeting a different area of effect is required with initially WT1 positive patients.

Two, initially WT1 negative patients showed WT1 expression during treatment, their disease made quick progression and they soon relapsed. Naturally, we cannot make any conclusions from merely two cases but their remarkably bad survival calls for further evaluation, since standard immunochemotherapeutic treatment obviously needs addition or revision in such cases.

In general, our data suggests that WT1 gene may also have a role in the pathogenesis of DLBCL, aside from acute leukemia, which may be related to the oncogenic function of WT1. Our results point out that determining WT1 gene expression may be useful in elucidating a better prognosis, monitoring treatment and in the early prediction of relapse in DLBCL patients, this may change therapeutic approach. If consecutive MRD tests show disease progression, even initial WT1 positivity may suggest the need to intensify the treatment which may very well be stem cell transplant during first CR. In a wider scope, it might become an immunotherapeutic tool. Studies with greater number of patients are required for further exploration.

New findings and considerations

1. We were first to measure WT1 expression threshold from peripheral blood and healthy controls, using GAPDH as an endogenous reference gene, results were proven pathological above said threshold. WT1 positivity begins above 0.1, the threshold for strong WT1 positivity was set at 10.0

2. We were first to prove in a Hungarian patient population that the initially WT1 negative group's survival seemed more favorable, yet the difference was not statistically significant

3. We found that AML patients who became WT1 negative due to treatment (i.e. achievement of molecular remission) showed significantly better survival when compared to the altogether WT1 positive and altogether WT1 negative patients

4. The rate of therapeutic response was lower in constantly WT1 negative AML patients, however, after having achieved CR, no recurrence was seen

5. Decrease in supra-threshold WT1 expression by 1 log value or 2 log values did not affect survival in AML patients

6. We were first to measure WT1 expression from peripheral blood samples in DLBCL. We observed the same occurrencerates than in lymph node biopsy as the literary background suggests

7. The initial increase in WT1 expression in DLBCL did not correlate to any characteristic parameters used for prognosis (age, clinical stage, IPI score, B symptoms)

8. DLBCL patients' overall survival and disease-free survival was proven to be significantly better if they did not show WT1 expression

9. As with AML, pathological increase in WT1 during treatment may suggest relapse in patients with DLBCL, therefore the evaluation of WT1 expression is a useful tool for establishing MRD and for disease monitoring

Acknowledgements

First of all, I would like to give thanks to my mentor, László Rejtő, MD, for his irreplaceable guidance, his continuous support over the years and his motivation which allowed the making of this paper

I am grateful to Professor Sándor Bíró, MD from the Department of Human Genetics and to my co-author, Gergely Buglyó, MD, since the results of this study were born by our conjoint effort, and I would like to extend my special thanks to Katalin Kosztolányi for her help in processing the samples.

I would like to thank my colleagues who took a great part in sample collection – Márta Hevér, Ágnes Gyáni, Zsuzsanna Kiss, Mrs. Ferenc Kovács, Éva Szikora, I am grateful for their ongoing help.

I would like to thank Professor Miklós Udvardy, MD and Professor Árpád Illés, MD for their help throughout the entire research and their aid in writing this paper.

And last but not least, this dissertation could not come to be without the unconditional support, cheering and help from my loving family. I wish to thank my husband Ádám Jóna, MD, my daughter Panni and my Mother. I would like to give my special thanks to my Father for his professional support and constant guidance.

Registry number: DEENK/29/2017.PL
Subject: PhD Publikációs Lista

Candidate: Zsófia Ujj
Neptun ID: D1MQO4
Doctoral School: Kálmán Laki Doctoral School

List of publications related to the dissertation

1. **Ujj, Z.**, Buglyó, G., Udvardy, M., Beyer, D., Vargha, G., Biró, S., Rejtő, L.: WT1 Expression in Adult Acute Myeloid Leukemia: assessing its Presence, Magnitude and Temporal Changes as Prognostic Factors.
   Pathol. Oncol. Res. 22 (1), 217-221, 2016.
   DOI: http://dx.doi.org/10.1007/s12253-015-0002-0
   IF: 1.94 (2015)
2. **Ujj, Z.**, Buglyó, G., Udvardy, M., Vargha, G., Biró, S., Rejtő, L.: WT1 Overexpression Affecting Clinical Outcome in Non-Hodgkin Lymphomas and Adult Acute Lymphoblastic Leukemia.
   Pathol. Oncol. Res. 20 (3), 565-570, 2014.
   DOI: http://dx.doi.org/10.1007/s12253-013-9729-7
   IF: 1.855

List of other publications

3. Telek, B., Rejtő, L., Batár, P., Miltényi, Z., Reményi, G., Simon, Z., **Ujj, Z.**, Mezei, G., Szász, R., Kiss, A., Udvardy, M., Illés, Á.: Az akut myeloid leukaemia gyógyszeres kezelése: Jelenlegi lehetőségek, jövőbeli kilátások.
   Orvosi Hetilap. 157 (22), 843-848, 2016.
   DOI: http://dx.doi.org/10.1556/650.2016.30433
   IF: 0.291 (2015)
4. **Ujj, Z.**, Jóna, Á., Udvardy, M., Tornai, I., Kiss, A., Telek, B., Szász, R., Reményi, G., Ilonczai, P., Illés, Á., Rejtő, L.: Hepatitis B virus reactivation in a patient with follicular lymphoma treated with fludarabine and rituximab containing immuno-chemotherapy.
   Research. 1, 796-801, 2014.
   DOI: http://dx.doi.org/10.13070/rs.en.1.796

5. Selmeczi, A., Udvardy, M., Illés, Á., Telek, B., Kiss, A., Batár, P., Reményi, G., Szász, R., **Ujj, Z.**, Márton, A., Ujfalusi, A., Hevessy, Z., Pinczés, L., Bedekovics, J., Rejtő, L.: Heveny myeloid leukaemiás betegeink kezelésével szerzett tapasztalataink (2007-2013).
Orv. Hetil. 155 (17), 653-658, 2014.
DOI: http://dx.doi.org/10.1556/OH.2014.29884

6. Jóna, Á., Miltényi, Z., **Ujj, Z.**, Garai, I., Szilasi, M., Illés, Á.: Late pulmonary complications of treating Hodgkin lymphoma: bleomycin-induced toxicity.
Expert Opin. Drug Saf. 13 (10), 1291-1297, 2014.
DOI: http://dx.doi.org/10.1517/14740338.2014.946901
IF: 2.911

7. Telek, B., Rejtő, L., Kiss, A., Batár, P., Reményi, G., Szász, R., **Ujj, Z.**, Udvardy, M.: A felnőttkori heveny myeloid leukaemia korszerű kezelése.
Orv. Hetil. 153 (7), 243-249, 2012.
DOI: http://dx.doi.org/10.1556/OH.2012.29304

8. Rejtő, L., Méhes, L., Kiss, A., Telek, B., Batár, P., Reményi, G., Szász, R., **Ujj, Z.**, Udvardy, M.: Fenntartó kezelés myeloma multiplexben autológ perifériás őssejtátültetés után - eredményeink.
Hematol. Transzfuziol. 44 (1), 30-33, 2011.

Total IF of journals (all publications): 6,997
Total IF of journals (publications related to the dissertation): 3,795

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

17 February, 2017