Original Paper

Involvement of Oxidative Stress in Resistance to Tyrosine-Kinase Inhibitors Therapy in Chronic Myeloid Leukemia

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ABSTRACT: Oxidative stress involves disruption of the cellular redox status through excessive production of reactive oxygen species or through deficiency in the cellular antioxidant capacity. It is involved in the pathogeny of multiple entities (hematological diseases, metabolic disorders, cardiovascular and renal pathology etc.), as well as in the pharmacokinetics of specific treatments for these pathologies. Chronic myeloid leukemia is a chronic myeloproliferative disease for which current standard treatment is BCR-ABL tyrosine kinase inhibitors. The innovation of this therapy has significantly improved life expectancy for patients with chronic myeloid leukemia, but in some cases, this treatment becomes ineffective, installing the resistance to tyrosine kinase inhibitors therapy. There were described two types of tyrosin kinase inhibitors resistance: primary and secondary resistance. In the present paper we proposed to evaluate the involvement of oxidative in the resistance to tyrosine kinase inhibitors therapy, in the clonal instability in chronic myeloid leukemia and in the progression of the disease to an advanced stage. We concluded that oxidative stress can play a dual role in the evolution of chronic myeloid leukemia: on the one hand it can promote genomic instability and accelerate the progression of the disease to advanced stages associated with tyrosin kinase inhibitors resistance and, on the other hand, it can contribute to leukemic cell apoptosis. It seems to be outlined a fragile balance between the pro- and anti-apoptotic effects of the reactive oxygen species, closely related to their level in the leukemic cells.

KEYWORDS: *Oxidative stress, Reactive oxygen species, Tyrosin-kinase inhibitors, Therapeutic resistance, Chronic myeloid leukemia.*

Introduction

Chronic myeloid leukemia (CML) is a chronic myeloproliferative neoplasia featured by presence of the balanced reciprocal translocation t (9; 22) (q34; q11) and the BCR-ABL fusion gene responsible for p210 bcr-abl protein synthesis with intense tyrosine kinase activity [1,2].

This abnormal protein modulates multiple signaling pathways involved in cell growth and differentiation, in cellular interaction with the medullary microenvironment, in apoptosis and genomic instability: phosphatidyl-inositol-3 kinase pathway (PI3K), NOTCH, mitogen-activated protein kinase pathway (MAPK), HEDGEHOG, Signal Transducer and Activator of Transcription (STAT) 1/3 pathway[3].

Currently, standard CML treatment targets the activity of the bcr-abl oncoprotein, being represented by first generation tyrosine kinase inhibitors BCR-ABL (TKIs) (imatinib), second generation TKIs (dasatinib, nilotinib, bosutinib) and third generation TKIs (ponatinib) [4].

Patients under the age of 65, without therapeutical response to TKIs therapy, benefit from stem cell allotransplantation.

Imatinib is a 2-phenyl-amino-pyrimidine [5] that binds to the aminoacids at the ATP binding site of BCR-ABL tyrosine kinase and stabilizes the inactive form, thus preventing tyrosine autophosphorylation and phosphorylation of its substrates [4,6].

The onset of imatinib resistance or suboptimal response required the development of second and third generation TKIs.

Dasatinib is a thiazolylamino-pyrimidine, 300 times more potent than imatinib, which inhibits the family of Src kinases and ABL1, c-KIT, PDGFRβ, EphA2, HER1 and p38 MAP kinases [7,8].

Nilotinib is a phenylamino-pyrimidine derivative, structurally similar to imatinib [9], but more selective for BCR-ABL1 and 20 to 30 times more potent than imatinib. Bosutinib inhibits BCR-ABL1 with approximately 1 log higher potency comparing to imatinib [10].

Several randomized clinical trials have shown that second generation TKIs induce faster and deeper molecular responses and reduce the disease progression to the blast phase, comparing to imatinib [11,12].

Instead, the side effects are more severe. Ponatinib is a third generation TKI used in CML cases with T315I mutation, against which all the other TKIs are ineffective [13].

The innovation of TKIs has significantly improved life expectancy for patients with

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CML. The choice of TKI type is made according to the mutational status (Y253H, Y253F, E255V -resistance to nilotinib, V299L, T315A-resistance to dasatinib, T315I-resistance to imatinib, nilotinib, dasatinib and bosutinib, sensitivity only to ponatinib) and to the associated comorbidities. Treatment is continued or switched according to molecular response and tolerability [14,15].

Most CML patients following TKIs therapy achieve major molecular responses (MMR) (BCR-ABL<=0.1%) or early MMR (in less than 3 months of treatment). Patients with early MMR have a low risk of disease progression and an increased long-term survival rate [16].

Recently, several clinical studies have shown that in 40-60% of patients who achieve a deep MMR (BCR-ABL<=0.00032) after treatment with TKIs (at least 3 years of treatment), and maintain it for at least one year, TKIs therapy can be safely discontinued, keeping the disease remission and reducing the side effects of TKIs. In the conditions of losing the MMR, upon resumption of TKIs therapy, recovery of deep MMR was observed in almost all cases [17-19].

The survival of CML leukemic cells has been shown to be based on oxidative metabolism [20].

The involvement of oxidative stress (OS) in the pathogenesis of hematological diseases has been demonstrated, as well as in their response to specialized treatments (chronic myeloid leukemia [2], essential thrombocythemia [21-23], chronic lymphoproliferation [24].

OS is also involved in dysfunctions of carbohydrate metabolism, lipid metabolism, in renal pathology, its level varying depending on specialized treatments and the association of comorbidities [24,25].

Resistance to TKIs therapy is established in some patients with CML, either by dependent BCR-ABL mechanisms [26] or by independent BCR-ABL mechanisms [27] and possibly by defective transport of TKIs [28].

BCR-ABL1 kinase mutations are the most intensively studied mechanism of TKIs resistance [26], but independent BCR-ABL1 mechanisms have also been reported in patients with imatinib resistance (activation of compensatory survival pathways/antiapoptotic pathways-overexpression of members of the SRC kinase family (LYN, HCK) [29], molecules such as FOXO1 [30], β-catenin [31], STAT3 [32], nucleocytoplasmic transport molecules RAN and XPO1, Cobll1 and NF-κB signaling, AXL tyrosine kinase [33].

Primary resistance was related to altered expression and/or function of imatinib transport molecules (Pgp, MDR1 ABCB1, hOCT1) [34,35].

Genomic instability in hematopoietic stem cells associated with bcr-abl oncoprotein, DNA alteration and defective DNA repair, antiapoptotic effect of p210 protein, dominance of one or more malignant clones or subclones are just a few aspects of CML resistance to TKIs therapy in which the involvement of OS has been proven [26,31,32].

OS involves disruption of the cellular redox status through excessive production of reactive oxygen species (ROS) or through deficiency in the cellular antioxidant capacity [36-38].

There were described a type of primary resistance (absence of MMR) and a type of secondary resistance to TKI therapy (loss of the initially achieved MMR and disease progression) [14,39].

It was demonstrated the involvement of OS in the BCR-ABL-dependent resistance mechanisms to TKIs [40,41], but also in BCR-ABL-independent resistance mechanisms [42].

Purpose and objectives of the study

The aim of the study is to evaluate the involvement of OS in the resistance to TKIs therapy, in the clonal instability and disease progression in CML.

The main objectives of the study are to evaluate the level of ROS and total antioxidant capacity (TAC) in patients with CML treated with first or second generation TKIs, correlated with BCR-ABL transcript level and to evaluate the involvement of OS in clonal instability in CML by determining 8OH-2deoxyguanosine (8OH-2dG) level as an indirect marker of the DNA oxidative damage.

Patients and infrastructure.

Materials and Methods

The study group contains 75 CML patients (diagnosed according to ELN/WHO criteria), registered in the Hematology Clinic of the Filantropia City Hospital Craiova (patients agreed the study enrolment by signing an informed consent).

The control group contains 20 healthy subjects. Our practice unfolds with the acknowledgment of the University and Scientific Ethics and Deontology Commission within the University of Medicine and Pharmacy of Craiova, according to the approval no.

74/23.02.2017 and following the patients' rights established by WHO and fixed in the Patients' Rights Law no. 46/2003, the Helsinki Declaration revised in 2002 and the General Data Protection Regulation (EU) 2016/679.

The group of CML patients was divided according to the type of treatment (first generation TKI-imatinib, second generation TKIs-dasatinib, nilotinib) and to the evolution under the treatment with a certain type of TKI (MMR-BCR-ABL transcript ≤0.1% obtained during treatment, primary resistance-BCR-ABL transcript >1% after 12 months of treatment with a certain type of TKI or secondary resistance-losing the initially obtained MMR by increasing the BCR-ABL transcript level during treatment with a certain type of TKI).

Both CML patients and subjects in the control group were determined ROS levels, respectively TAC levels. Also, CML patients with disease progression to advanced stages during monitoring, as well as subjects in control group, were determined 8OH-2dG levels, as an indirect marker of OS on cellular DNA.

The determinations were performed in the Oxidative Stress Assessment Laboratory within the University of Medicine and Pharmacy of Craiova.

The evolution of patients following TKIs therapy was monitored by BCR-ABL transcript level performed by RQ-PCR technique in specialized laboratories (Ritus Biotec, Personal Genetics, Genetic Center).

A FLUOstar Omega microplate reader (BMG LABTECH GmbH, Ortenberg, Germany) and a kit for determining the antioxidant capacity from Sigma-Aldrich (CS0790) were used to determine TAC. A flowcytometer CyFlow SPACE Sysmex (Sysmex Partec GmbH, Görlitz, Germany) and a kit for quantitative determination of cellular ROS from Abcam (ab113851) were used for determining ROS levels, while for the 8OH-2dG determination the FLU Ostar Omega microplate reader and a 8OH-2dG kit from Abcam (ab201734) were useful.

Processing of blood samples

There were performed repeated sequences of centrifugation-washing with blood samples obtained by collecting in EDTA vacutainers in order to obtain the necessary plasma for TAC and 8OH2dG determinations and the necessary leukocytes for the cellular ROS measurements.

Determination of TAC in plasma

This test is based on detecting ABTS +, a green soluble chromogen which can be read spectrophotometrically at a wavelength of 405 nm using a microplate reader. ABTS+forms by oxidation of ABTS (2,2'-azino-bis (3-ethylbenzothiazolin-6-sulfonic acid) under the action of ferryl-myoglobin radical (resulting from metmioglobin and hydrogen peroxide); for measurements there were used colorless, flat-bottomed microplates.

The antioxidants in the samples suppress the production of the ferryl-myoglobin radical in a concentration-dependent manner. Consequently, the color intensity (spectrophotometrically detected) decreases proportional to the plasma TAC concentration. The kit includes Trolox, a soluble vitamin E analogue, useful for tracing the standard curve.

Determination of ROS in leukocytes

The kit for quantitative determination of cellular ROS uses the reagent 2 ', 7' - dichlorofluorescein diacetate (DCFDA), a fluorogenic dye, in order to quantify intracellular ROS compounds.

The principle of the test is that after diffusion into the cell, DCFDA is deacetylated by cellular esterases to give a non-fluorescent compound that will be oxidized by ROS to form 2 ', 7' - dichlorofluorescein (DCF). DCF can be detected by fluorescence spectroscopy at 495nm and 529nm spectra, respectively, being a highly fluorescent compound.

Determination of 8OH-2dG in plasma

It was used an ELISA kit for the competitive and quantitative measurement of 8OH-2dG in plasma.

A 8OH-2dG precoated microplate and a HRP conjugated antibody are used for detection. 8OH-2dG is produced as a consequence of the DNA damage caused by reactive oxygen and nitrogen species.

Statistical interpretation

Statistical analysis was performed in SPSS, after plotting data points in Microsoft Excel.

Statistical differences between the groups of continuous data have been sought utilizing a wan way analysis of variance (ANOVA) testing, with Fisher's Least Significant Difference (LSD) post-hoc analysis.

Consequently, there were evidenced the pairs exhibiting the actual differences. Statistical significance was considered for p values<0.05.

All data have been reported as average±standard error of the means (SEM).

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Results

Patients with CML in the study group underwent treatment with first generation TKIs (imatinib) or second generation TKIs (dasatinib, nilotinib), some of them requiring the switch from first generation TKIs to second generation TKIs or even between second generation TKIs. In Table 1 are presented the numerical distribution of CML patients according to the type/types of TKIs and, also, TAC and ROS values in patients with CML treated with 1/2/3 types of TKIs (Table 1).

Table 1. Mean values of TAC and ROS in CML patients following treatment with TKIs.

ITK therapy	Number		TAC (mM)	ROS (FI)
Imatinib only	35	**1-TKI**	0.232±0.029	10.587±0.191
Dasatinib only	7			
Nilotinib only	11			
Imatinib →Dasatinib	11	**2-TKIs**	0.255±0.056	10.234±0.386
Dasatinib→Nilotinib	4			
Imatinib→Nilotinib	5			
Imatinib→Dasatinib→Nilotinib	2	**3-TKIs**	0	11.545±0.775
		CML	0.23±0.025	10.537±0.168
		Control	0.363±0.037	10.065±0.278

It was found that there is a statistical difference regarding TAC between the patients who received treatment with one, two, respectively three types of TKIs (p=0.03).

Post-hoc LSD analysis also revealed a statistically significant difference between TAC value for 3-TKI and C (p=0.02), between 1-TKI and C (p=0.01).

No statistically significant differences were obtained regarding ROS values between 1-TKI, 2-TKIs, 3-TKIs categories, but it should be noted that the maximum ROS value was recorded in 3-TKI category.

The mean values of TAC and ROS in CML patients in the study group, depending on the undergoing treatment, as well as the mean values of TAC and ROS in CML patients depending on the type of response to TKIs therapy are found in Table 2.

Table 2. Mean TAC and ROS values in CML patients depending on the response to TKIs treatment.

	Number	TAC (mM)	ROS (FI)	TAC (mM)	ROS (FI)
PR-I	26	0.26±0.045	10.377±0.281	0.248±0.032	10.328±0.187
SR-I	3	0.236±0.12	10.746±1.631		
MMR-I	21	0.236±0.05	10.208±0.209		
PR-D	6	0.153±0.098	10.79±0.721	0.165±0.055	10.71±0.348
SR-D	0	-	-		
MMR-D	11	0.171±0.07	10.666±0.396		
PR-N	10	0.28±0.078	10.58±0.546	0.246±0.062	10.644±0.414
SR-N	1	0	9.22±0		
MMR-N	5	0.18±0.11	10.772±0.675		

TAC and ROS values were evaluated in CML patients with TKIs primary resistance. Although there was no statistically significant difference between the groups of patients PR-I, PR-D, PR-N (p>0.05), TAC recorded lower mean values for all categories of CML patients comparing to the control group. Regarding ROS, mean values tend to increase in the categories of CML patients with second generation TKIs primary resistance (Figure 1, Table 3).

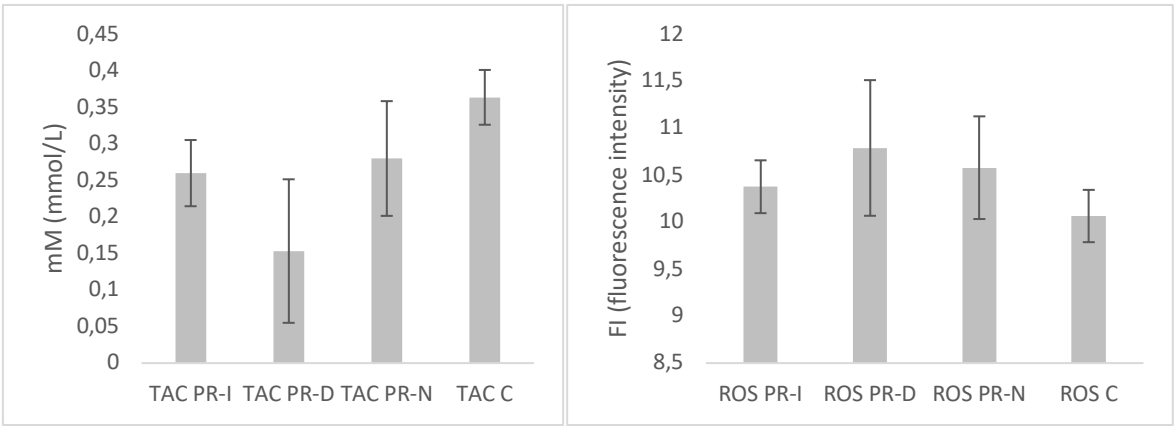

Figure 1. TAC and ROS in CML patients with TKI primary resistance.

Table 3. TAC and ROS in CML patients with TKI primary resistance.

	TAC (mM)	ROS (FI)
I	0.26±0.045	10.377±0.281
D	0.153±0.098	10.79±0.721
N	0.28±0.078	10.58±0.546
C	0.363±0.037	10.065±0.278

Analyzing separately the mean TAC values for the MMR-I, MMR-D, MMR-N categories, we observed that the mean values in all categories are lower than the control group one.

Post-hoc LSD analysis revealed a statistically significant difference between MMR-D and the control group (p=0.02).

Regarding ROS, mean values tend to increase in the categories of CML patients with MMR undergoing second generation TKIs therapy and all CML patients categories registered higher values than the control group (Figure 2, Table 4).

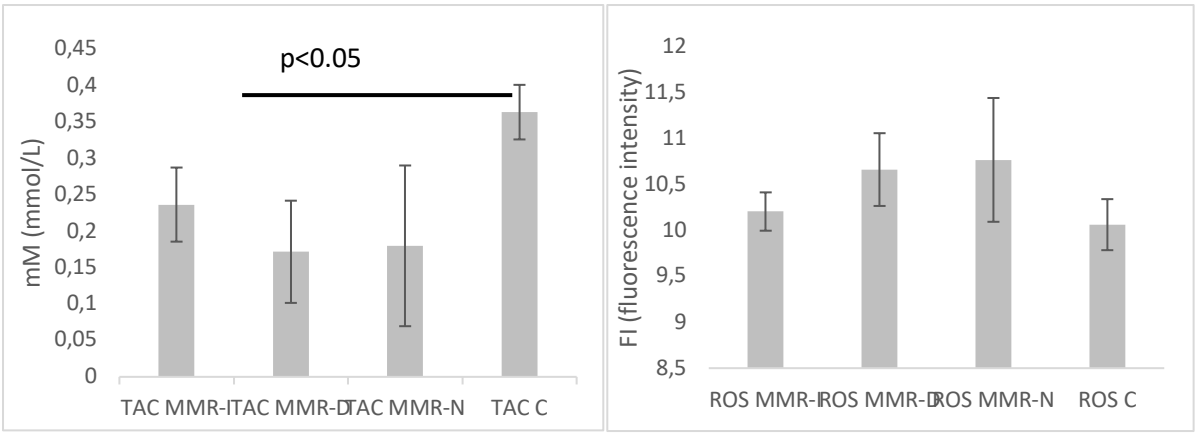

Figure 2. TAC and ROS in CML patients who obtained MMR following TKI therapy.

Table 4. TAC and ROS in CML patients who obtained MMR following TKI therapy.

	TAC (mM)	ROS (FI)
I	0.236±0.05	10.208±0.209
D	0.171±0.07	10.666±0.396
N	0.18±0.11	10.772±0.675
C	0.363±0.037	10.065±0.278

Regarding secondary resistance in the study group, this type of evolution was registered only for imatinib and nilotinib.

There were no statistically significant differences between mean TAC values of SR-I and SR-N (=0.09).

For SR-I, it was obtained a mean ROS value higher than the control group (Figure 3, Table 5).

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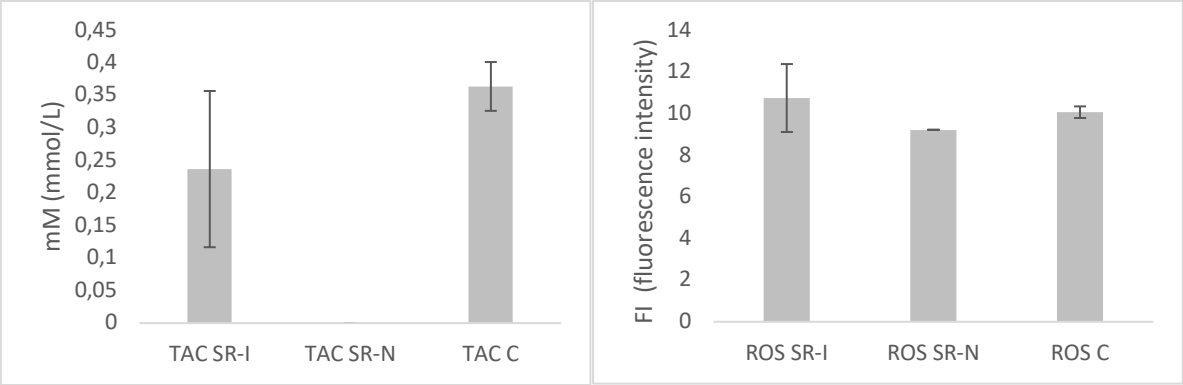

Figure 3. TAC and ROS in CML patients with TKIs secondary resistance.

Table 5. TAC and ROS in CML patients with TKIs secondary resistance.

	TAC (mM)	ROS (FI)
I	0.236±0.12	10.746±1.631
N	0	9.22
C	0.363±0.037	10.065±0.278

Analyzing the TAC and ROS values of the patients who received imatinib treatment depending on the type of response, no statistically significant differences were obtained ($p>0.05$ for both TAC and ROS in PR-I, SR-I, MMR-I), but there were observed approximately equal mean TAC values for SR-I and MMR-I, with an increasing tendency to PR-I, all values being lower than the control value.

Regarding ROS, the values tend to increase in the following order: MMR-I, PR-I, SR-I (Figure 4, Table 6).

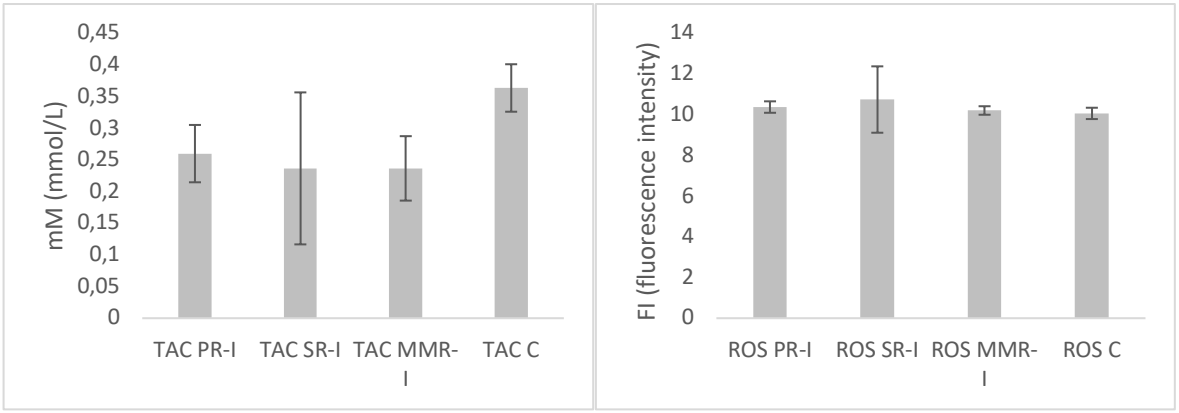

Figure 4. TAC and ROS in CML patients following imatinib therapy.

Table 6. TAC and ROS in CML patients following imatinib therapy.

	TAC (mM)	ROS (FI)
PR	0.26±0.045	10.377±0.281
SR	0.236±0.12	10.746±1.631
MMR	0.236±0.05	10.208±0.209
C	0.363±0.037	10.065±0.278

Analyzing OS in patients treated with dasatinib, a statistically significant difference was obtained between mean TAC values for PR-D, MMR-D, C ($p=0.01$). Post-hoc LSD analysis also showed a statistically significant difference between MMR-D and C ($p=0.01$), between PR-D and C ($p=0.03$); according to Tukey HSD and Bonferroni there is a statistically significant difference between MMR-D and C ($p=0.04$).

Although statistically insignificant ($p>0.05$), it was observed that ROS value in PR-D tends to be higher than ROS value in MMR-D, respectively C (Figure 5, Table 7).

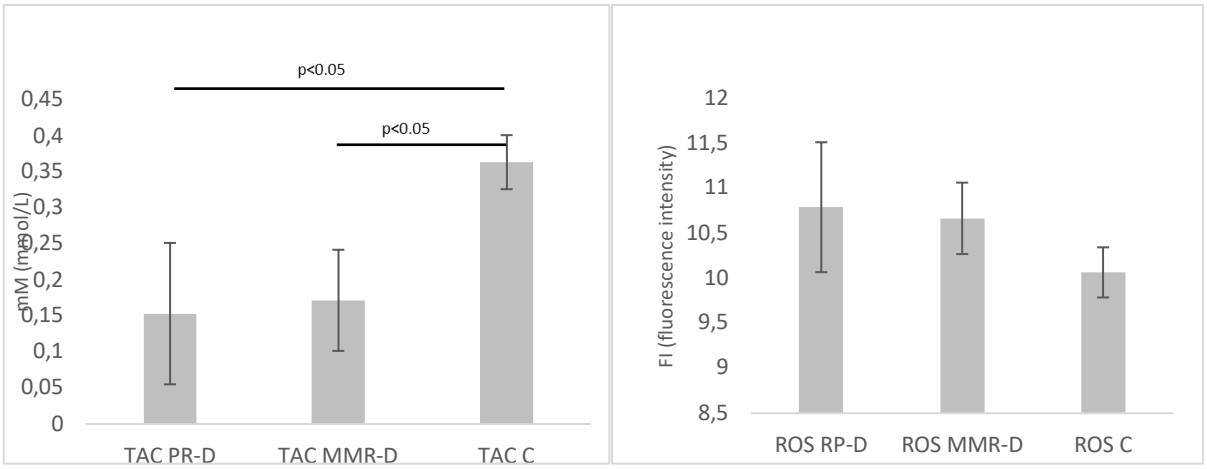

Figure 5. TAC and ROS in CML patients following dasatinib therapy.

Table 7. TAC and ROS in CML patients following dasatinib therapy.

	TAC (mM)	ROS (FI)
PR	0.153±0.098	10.79±0.721
MMR	0.171±0.070	10.666±0.396
C	0.363±0.037	10.065±0.278

No statistically significant differences were found in patients treated with nilotinib for TAC and ROS values, but a ROS mean value exceeding C was found in the MMR-N category, contradictory to SR-N (Figure 6, Table 8).

Figure 6. TAC and ROS in CML patients following nilotinib therapy.

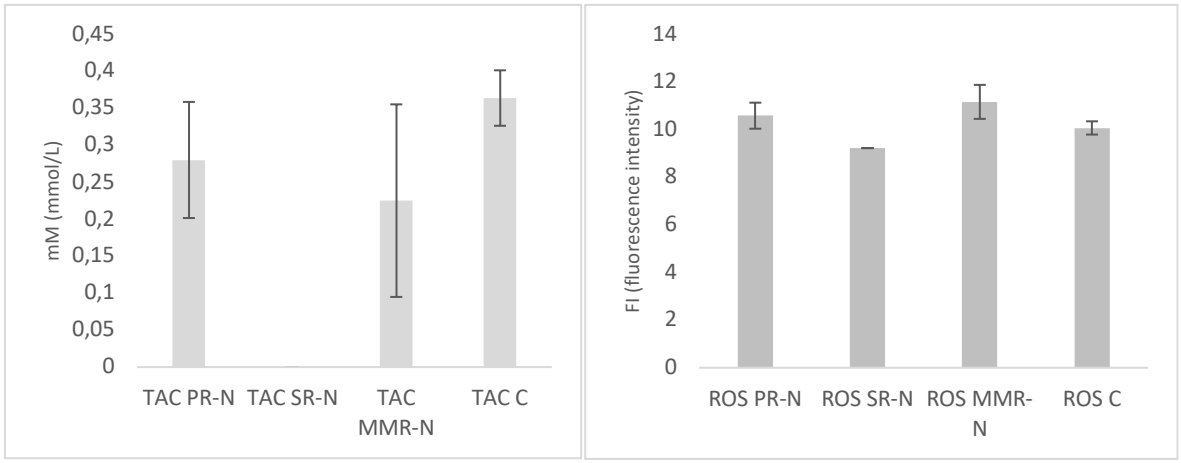

Table 8. TAC and ROS in CML patients following nilotinib therapy.

	TAC (mM)	ROS (FI)
PR	0.28±0.078	10.58±0.546
SR	0	9.22
MMR	0.225±0.130	11.16±0.713
C	0.363±0.037	10.065±0.278

Regarding the 8OH-2dG concentration determined in CML patients with unfavorable evolution under TKIs treatment (progression to accelerated/blastic phase), no statistically significant value was obtained (p=0.07).

It was found that there is a statistically significant association between the age of the patients and the concentration of 8OH-2dG (Fischer test, F=0.03), although no association was found between age and TAC, respectively ROS (Table 9).

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Table 9.TAC, ROS and 8OH in CML patients with unfavorable evolution under TKIs treatment, depending on age.

Case no.	Age (years)	8OH (ng/mL)	TAC (mM)	ROS (FI)
1	38	60.36	0	10.77
2	72	2.81	0.46	10.75
3	65	0.60	0	9.86
4	58	0.60	0	13.5
5	81	0.63	0.66	11.31
6	61	0.59	0	9.22
7	61	0.64	0.52	9.64
Control (mean values)	58	0.624±0.014	0.363±0.037	10.065±0.278

Discussions

CML can be considered a typical model for the molecular pathogenesis of malignancy, the involvement of OS in the pathogenesis of this entity being not completely understood, while the available data in the literature provide contradictory information. On the one hand, it has been shown that the tyrosine kinase activity of the BCR-ABL oncogene, as well as the implicit activation of other signaling pathways, provide the production of oxygen free radicals [43,44].

By the fact that in our study we obtained in CML patients following TKIs therapy lower mean values for TAC and higher mean values for ROS than in the control group, we claim the theory that the genetic and biomolecular substrate of CML generates the production of OS [43].

On the other hand, it has been shown that treatment with TKIs in CML acts by inducing OS and, depending on its level, signaling pathways responsible for either apoptosis or survival can be activated in leukemic cells [45].

Regarding the relationship between OS and TKIs therapy, it is known that dasatinib treatment reduces antioxidant status and increases ROS in various tissues [46] while low-dose imatinib treatment stimulates antioxidant defense [47].

In CML patients treated with second generation TKIs a higher level of OS was found comparing to those treated with first generation TKIs. It was considered that, in the presence of a high level of OS, patients express resistance to first generation TKIs and need to be initiated second generation TKIs therapy [48].

Regarding these data, we observed higher ROS values and lower TAC values in patients who received treatment with dasatinib, comparing to those treated with imatinib and nilotinib, respectively.

Also, in patients with PR in our study group, it was found that ROS values were higher in patients with second generation TKIs therapy than in those treated with imatinib. Patients treated with dasatinib recorded the lowest TAC value, these data being supported by pre-existing information in the literature [46,47].

There are some studies that incriminate the OS in producing genetic mutations generating resistance to TKIs [49], this being only one of the possible factors involved in TKIs therapy resistance.

Ammar demonstrated that in imatinib resistant patients, concentrations of oxidative markers are significantly higher than in patients without imatinib resistance[50].

Nieborowska-Skorska demonstrates that ROS overproduction is associated with the existence of imatinib resistant cellular clones [51].

In this sense, similar data were obtained in our study: ROS value is higher in SR-I than in PR-I, while PR-I have higher ROS values than MMR-I. We did not obtain statistically significant differences regarding TAC in PR-I, SR-I and MMR-I, but all these values were lower than TAC value for the control group, aspect supported by official data that evidenced the decrease of antioxidant capacity in CML as well as in CML patients following TKIs treatment [53].

Regarding SR to TKIs therapy, we observed that all cases of SR, except for one case of SR-N, occurred in patients treated with imatinib. It has been shown that in these patients the main cause of SR is the occurrence of BCR-ABL gene mutations [54], while in patients with PR to imatinib, changes in the absorption and metabolism of treatment are rather incriminated [55]. Related to this aspect,

we observed in our study a higher ROS value in SR-I, in contrast to those with PR-I or MMR-I. Most likely, under conditions of an oxidative status dominated by the production of ROS, it was set a favorable environment for the occurrence of BCR-ABL mutations which provided treatment resistance.

Analyzing the oxidative status of CML patients who obtained MMR, we observed that MMR-I had higher mean TAC values and lower mean ROS values comparing to patients who obtained MMR following second generation TKIs treatment. This aspect suggests that a favorable oxidative status (quantitatively reduced ROS and efficient antioxidant systems) creates the optimal conditions for the therapeutic effect of first generation TKIs, while an oxidative status with high concentrations of ROS and deficient antioxidant defense requires the use of second generation TKIs in order to obtain MMR. There are several studies in the literature that claim the effectiveness of dasatinib and nilotinib in cases of CML with various BCR-ABL mutations (possibly some of them produced under the action of OS) that generate resistance to imatinib therapy [56-58].

On the other hand, there are studies showing that the apoptotic activity of dasatinib and nilotinib generates ROS and oxidative compounds [59].

In this regard, Bazi demonstrated that TKIs therapy in CML acts by inducing OS, its level being able to induce either a favorable response or an unfavorable response correlated with the dual possibility of triggering both apoptotic and survival processes inside leukemic cells [45].

Analyzing OS in patients treated with dasatinib, statistically significant differences were obtained between mean TAC values for PR-D, MMR-D and C. The fact that TAC value is significantly lower in patients with resistance to dasatinib than in those who obtained MMR, to which is added the observation that ROS value in PR-D tends to be higher than in RMM-D, strengthens the opinion of other researchers that a redox status balanced by reducing the mitochondrial ROS production would promote the action of antineoplastic treatments [60,61].

It is interesting that, although patients treated with dasatinib had higher mean ROS and lower TAC values than those treated with imatinib or nilotinib, none of them developed SR to dasatinib, although it has been reported in the literature that CML patients treated with either imatinib, dasatinib or nilotinib have the same susceptibility to develop secondary resistance to the therapy [62]. On the other hand, Cortes demonstrated that patients treated with dasatinib have the lowest susceptibility to develop genetic mutations responsible for second generation TKIs resistance, after the failure of imatinib therapy [63].

The fact that, in our study, none of the patients undergoing dasatinib treatment developed SR, although they had higher ROS and lower TAC concentration comparing to other treatment categories, may suggest that there are several mechanisms responsible for genetic mutations that generate resistance to treatment and ROS overproduction is just one of them.

The same aspect emerges from the only case of SR-N, whom ROS value was lower than the mean ROS value of the control group. Related to this situation and adding that in this case the detected TAC value was 0, it is not excluded that the genetic mutation generating secondary resistance to nilotinib would be T315I (impossible to certify in the absence of a mutational analysis). It was demonstrated that leukemic cells presenting the T315I mutation show a low proliferation rate, as well as a reduction in the synthesis of lactate, fatty acids, ROS and antioxidant products such as SOD2, catalase, GPx1 [64].

We observed that, in nilotinib treated patients, ROS values tend to be higher in cases with a favorable response to this TKI (ROS MMR-N higher than ROS PR-N respectively ROS SR-N, although statistically insignificant).

This suggests either that nilotinib resistance installs by mechanisms independent of the redox, or that nilotinib, as known from the literature, being approximately 30 times more effective than imatinib, is useful even in the presence of some genetic mutations [65] that could have occurred under the influence of OS.

We reservedly report this observation, considering the reduced number of patients who received treatment with nilotinib in our study group.

We observed that TAC values vary statistically significant in the groups of patients who received during the monitoring period treatment with one/two/three types of TKIs. All these values are significantly lower than the TAC value for the control group. While TAC value in the 3-TKIs was 0, the maximum TAC value was obtained for 2-TKIs.

Regarding the two cases that required two therapeutic switches during the evolution of the

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disease, it should be noted that one of them, with unfavorable evolution-blastic transformation and death, presented SR-I, PR-D and PR-N, recording a ROS value of 10.77 FI, while the other one presented PR-I, PR-D, requiring the switch on nilotinib (currently in hematological response) and recorded a ROS value of 12.32 FI.

The suspicion in these cases was the presence of T315I mutation (no mutational analysis was performed to certify this suspicion), thus explaining the 0 value of TAC, according to data from literature [64]; however, the increased value of ROS, which in the conditions of the T315I mutation would have been a low one [64], it is not explained.

Regarding the concentration of 8OH-2dG we found a higher value in patients with unfavorable evolution of the disease, comparing to the control group. This aspect supports once again the theory according to which pathophysiological changes in CML are based on genetic changes, and OS is involved in both their production and in clonal instability and progression to an advanced stage of the disease [43,44].

It was found that there is a statistically significant association between the age of the patients and the concentration of 8OH-2dG. The maximum value of 8OH-2dG was recorded in a 38-years old patient (minimum age in the study group), whose TAC value was 0 mM and ROS value was 10.77 FI. The evolution was towards death, this patient presenting during the disease evolution SR-I, PR-D and PR-N. It is thus supported the hypothesis that the resistance to different types of TKIs is based on the changes of the genetic material induced by ROS [66].

Interestingly, no correlation was found between ROS concentration and 8OH-2dG, suggesting that not only ROS overproduction is responsible for DNA oxidative changes, but also the susceptibility of the genetic material to mutagenesis, the intrinsic DNA repair ability and the characteristics of the medullary microenvironment [67], as well as the intervention of the antioxidant systems [68].

This study sediments the previous results of our research group confirming the involvement of OS in the appearance and evolution of chronic myeloproliferative and lymphoproliferative disorders [69-71].

Conclusions

1. OS can play a dual role in the evolution of CML: on the one hand it can promote genomic instability and accelerate the progression of CML to advanced stages of disease associated with acquiring TKIs resistance (primary/secondary) and, on the other hand, it can contribute to leukemic cell apoptosis.

A fragile balance between the pro-and anti-apoptotic effects of ROS is outlined, closely related to their level in the cells of the leukemic clone.

2. It seems that TKIs therapy induces the optimal level of ROS in order to determine apoptosis in the leukemic clone by their prooxidant effect.

3. Research in this field remains an open subject, the association of OS modulators with TKIs therapy being an attractive idea.

A personalized therapy and, in selected cases, mutational analysis are needed to achieve therapeutic success.

4. The results of our research were reservedly presented because of the numerically limited study group, CML being a relatively rare pathology.

This research topic can be developed through a future multicenter collaboration, with the inclusion in the study group of a larger number of patients with CML.

Conflict of Interest

None to declare

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