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Relationship between redox status in blood of patients with chronic myeloid leukemia (CML) and values of selected inflammatory markers – preliminary studies

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ABSTRACT

The results of several experimental and clinical studies confirm the relationship between chronic inflammation and the concurrent oxidative stress as well as the development of many cancers.

The aim of this study was to assess the relationship between the redox status in the blood serum of the patients with chronic myeloid leukemia (CML) and the hs-CRP concentration as well as the values of the calculated markers: neutrophil-lymphocyte ratio (NLR), lymphocyte-monocyte ratio (LMR) and platelet-lymphocyte ratio (PLR).

Blood serum, obtained from 35 subjects (16 women and 19 men, aged 27-86) the CML, the patients of the Clinic of Hematology, Blood Neoplasms and Bone Marrow Transplantation of Medical University in Wroclaw, was used in this investigation. All the patients suffered from a chronic phase of the disease.

In the patients' serum the malondialdehyde (MDA) concentration as the end product of lipid peroxidation was determined using the TBARS method. A degree of DPPH radical reduction was measured in order to estimate the total antioxidative capacity of the deproteinized serum (TAC). Additionally, the serum hs-CRP concentration was also determined. Based on the results of the patients' blood count tests, the markers for NLR, LMR and PLR were calculated.

The serum MDA concentration in women was 7.72 μM/L and in men 6.92 μM/L. The TAC value of women was 251.55 mM Trolox/L whereas of men it was 244.67 mM Trolox eqvivalents/L. Statistically significant correlations were observed between the markers: PLR, NLR, LMR, including the negative correlations between the PLR and LMR as well as NLR and LMR, and the positive correlation between the NLR and PLR. However, no statistically significant correlation was found between MDA, TAC and hs-CRP levels and PLR, NLR, LMR values.

INTRODUCTION

CML is the disease entity that is caused by a clonal proliferation of multipotent bone-marrow stem cells resulting from the occurrence of the BCR/ABL gene mutation localized in Philadelphia chromosome (Ph) (Żołnierowicz, 2010; Jabbour, 2018). Chromosome Ph is an aberration consisting in the translocation between chromosomes 9 and 22, which results in mutant BCR/ABL proteins with tyrosine kinase activity (Druker, 2006).

Exposure to ionizing radiation has been proved to be an etiological factor of CML. It has been suggested that reactive oxygen species (ROS) take part in CLM patho-physiology (Ahmad, 2008 a).

At the moment tyrosine kinase inhibitors (TKI) are used in a standard CML therapy as they make it possible to get a complete cytogenetic response in a significant percentage of patients. However, increasingly frequent cases of resistance to imatinib treatment are observed, which is attributed to the significant role of ROS (Koptyra, 2006; Ammar, 2020). It has been established that BCR/ABL kinase induces the ROS growth due to the activation of the PI-3K/mTOR, kinase pathway, which in consequence results in the increase of proliferative activity of cancerous cell and the inhibition of apoptosis. (Kim, 2005; Sattler, 2000).

ROS are natural products of cellular oxygen metabolism (Ścibior-Bentkowska, 2009).

Oxidative stress is an imbalance resulting from overproduction or accumulation of ROS due to the insufficiency of antioxidant systems or dysfunction of antioxidant enzymes. The role of oxidative stress has been the subject of numerous studies for the last decades (Reuter, 2010; Sosa, 2013; Toyokuni, 2016; Valko, 2007; Udensi, 2014).

When the ROS level increases significantly it may result in the damage of nucleic acids, proteins and other cell parts, multisystem dysfunctions as well as mutations (Reuter, 2010). Apart from ROS, the concentration of endogenic antioxidants, that is the antioxidant capacity, has a significant impact of the oxidative stress induction. When a person is healthy, endogenic antioxidants play a protective role against the increase of ROS level and the cells with the balanced redox status are less susceptible to the genetic material damage (Sosa, 2013).

Inflammatory states favour formation of a large number of ROS in cells; therefore, they increase the oxidative stress intensity and stress – induced DNA mutations. ROS may contribute to abnormal gene expressions and dysfunction of signaling pathways and as a result of the progressive cell damage may cause cancerous transformation. (Hole, 2011). On the other hand the state of oxidative stress in the early stages of tumour growth may be the consequence of the inflammatory state which accompanies cancerous disease (Reuter 2010).

There are some widely known methods used to determine the oxidative stress level (Nielsen, 1997; Singh, 2009,). These methods are based on the measurement of ROS and antioxidant concentration in blood (Al-Gayyar, 2007). Malondialdehyde (MDA), the stable end product of lipid oxidation is adopted to be the marker of unfavourable effects of ROS activity (Ahmad, 2010). Looking at different methods determining antioxidant concentration in blood serum, DPPH assay turned out to be useful, cheap and simple to perform. Thanks to this method, it is possible to measure the total antioxidant capacity of the deproteinised blood serum (TAC) (Chrzczanowicz, 2008; Kedare, 2011).

Markers such as C-reactive protein is used to assess the inflammatory state in the organism (Cavicchia, 2009). The determination of hs-CPR value in the blood serum using an ultrasensitive method finds its application in diagnostics and monitoring of the inflammatory states (Bassuk, 2004). The C-reactive protein level may indicate the activity of carcinoma cells as well as the host's response to the presence of tumour (Stasik, 2008).

The inflammatory state markers: NLR (neutrophil-to-lymphocyte ratio), LMR (lymphocyte-to-monocyte ratio) or PLR (platelet-to-lymphocyte ratio) are widely inve-stigated with regard to their prognostic suitability for cancerous disease. (Wójcik, 2016; Forget, 2017; Yang, 2018) These markers reflect the systemic inflammatory response (SIR) which is appropriate in the prognostic assessment of the patients with numerous tumours (Ying, 2014). The advantages of this solution are the low cost of research and the fact that the marker levels can be easily determined only on the basis of the complete blood count (Sylman 2018).

Taking into consideration the fact that the oxidative stress, the chronic inflammatory state and the incidence of cancerous diseases are closely linked, it can be assumed that the all-in observation of the markers of the oxidative stress and the inflammatory state during the therapy may be helpful in the assessment of the disease advancement. The observation could also provide valuable information leading to a more targeted patient care (Reuter 2010).

The aim of this study was to assess the relationship between the redox status in the blood serum of the patients with chronic myeloid leukemia (CML) and the hs-CRP concentration as well as the values of the calculated markers: NLR, LMR and PLR.

MATERIALS AND METHODS

PATIENTS

The study was conducted in a group of 35 subjects (16 women and 19 men) with chronic myeloid leukemia (CML), at the median age of 57.5 years (in the age range: 27-86). The patients were from the Clinic of Hematology, Blood Neoplasms and Bone Marrow Transplantation of Medical University in Wroclaw. The diagnosis was made based on the standard clinic-hematological and cytogenetic criteria. The studies involved patients in the chronic phase of the disease treated with tyrosine kinase inhibitors.

All detailed information about the CML patients (the clinical stage of disease, the body mass index – BMI, smoking status, and the level of education) is presented in table 1. The research was approved by the Ethics Board of Wroclaw Medical University (No KB 172/2018) and all the patients provided their written consent for volunteering in the research.

BLOOD SAMPLE COLLECTION AND PREPARATION

The blood samples were taken from the patients' antecubital vein under fasting conditions. Hematological parameters including white blood cell count (WBCs) and the types (neutrophil, lymphocyte and monocyte) and platelets were determined by a hematological analyser (Sysmex XN2000, TOA Medical Electronics, Japan). NLR (the neutrophil-to-lymphocyte ratio) was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count. The same calculation method was applied for other markers, that is, PLR (platelet-lymphocyte ratio) and LMR (lymphocyte-monocyte ratio).

The sample tubes were centrifuged for ten minutes at 3500 rpm to obtain a clear serum. The serum was separated and then stored at -80°C prior to the analysis.

DETERMINATION OF HS-CRP CONCENTRATION

The hs-CRP protein concentration was determined in the patients' blood serum using the immunoturbidimetric method, consisting in measurement of the changes in light dispersion and transmission of the reaction product of CRP protein molecules with anti-human CRP antibodies. The hs-CRP concentration measurement was carried out in the Kornelab 7.2.1 (Thermo Fisher Scientific, USA) automatic analyser.

DETERMINATION OF MALONDIALDEHYDE (MDA) CONCENTRATION

Serum malondialdehyde concentration (MDA) was evaluated with the spectro-photometric method using a Spectronic GENESYS 6 UV-visible spectrophotometer (Thermo Electron Corporation, USA); the measurement was based on the reaction between MDA and thiobarbituric acid (TBA) and the extraction of the product: 1-butanol. The absorbance of the pink supernatant was measured at λ=535 nm and the results were calculated using the molar coefficient, and expressed in μM of MDA/L of serum (Ahmad, 2008 b).

MEASUREMENT OF TOTAL ANTIOXIDANT STATUS (TAC)

The total antioxidant capacity (TAC) in serum was measured making use of the DPPH assay described by Chrzczanowicz (Chrzczanowicz, 2008). The serum samples were deproteinized with the use of the equal volume of acetonitryle. DPPH radical has an unpaired valence electron in its structure, therefore, when it reacts with antioxidants, which are electron donors or hydrogen radicals, 1.1-diphenyl-2-picrylhydrazine with a pale purple colour is formed. TAC was measured spectrophotometrically at λ = 517 nm using a Spectronic GENESYS 6 UV-visible spectrophotometer (Thermo Electron Corporation, USA). The results were expressed in mM Trolox equivalents/L serum.

STATISTICAL ANALYSIS

The data were analysed using Statistica Stat Soft 13.1. The nonparametric Mann–Whitney U test was used for group comparisons, and the Spearman correlation analysis was performed for the measurements of the relationship between MDA concentration and TAC value in female and male blood serum as well as NLR, LMR PLR levels and TAC value, hs-CRP value and MDA concentration.

For all statistical procedures, the significance level was set at $p < 0.05$.

RESULTS

Table 1 presents the characteristics of the examined women and men with CML.

Table 1. Characteristics of CLM patients with respect to gender

Parameter	Women (n=16)	Men (n=19)
Age, median (range) [years]	57 (28-86)	58 (27-79)
BMI, n (%) [kg/m^2]		
18,5-24.9	5 (31.2)	6 (31.6)
≥25	11 (68.8)	13 (68.4)
Clinical stage of disease (%)	chronic phase (100)	chronic phase (100)
Treatment, n (%):		
Imatinib	11 (68)	14 (73.7)
Nilotinib	3 (18.7)	3 (15.8)
Dasatinib	2 (12.5)	2 (10.5)
Smoking status, n (%):		
never smoker	15 (93.7)	13 (68.4)
current smoker	0	4 (21.1)
previous smoker	1 (6.3)	2 (10.5)
Educational level [%]: primary		
secondary	0	5.3
vocational	68.8	36.8
university	12.5	26.3
	18.7	31.6

BMI – body mass index

The median age of women and men was similar, in both groups the patients over 60 years of age constituted approximately 45%. The BMI was over 25 in approximately 68% of the examined group, which indicates obesity of a large number of the individuals. All the examined patients suffered from the chronic phase of CML. 68% women and 73% men were treated with imatinib. A small number of patients in the tested groups (5 females and 5 males) were treated with the second-generation kinase inhibitor (nilotinib and dasatinib). Majority of the patients were non-smokers – only 3 individuals of the examined groups admitted to smoking. Taking into account education there were twice as many females having secondary education whereas twice as many males had higher education.

Table 2 shows the results of the oxidative stress parameters (MDA, TAC), hs-CRP concentration as well as inflammatory state markers calculated from the results of the hematological parameters of the white blood cell system.

Table 2. Results of the redox state parameters, hs-CRP concentration in CLM patients' blood serum and calculated NLR, LMR and PLR markers

Parameter	Women (n=16)	Men (n=19)	p value
MDA [μM/L]			NS*
median	7.72	6.92	
(range)	5.28-10.41	5.62-10.33	
TAC			NS
[mM Trolox equiv./L]			
median	251.55	244.67	
(range)	28.0-493.43	31.33-426.29	
hs-CRP [mg/L]			NS
median	2.18	2.14	
(range)	(0.23-10.69	(0,12-9.28)	
NLR			NS
median	2.26	1.54	
(range)	(0.90-5.97)	(0.09-4.97)	
LMR			NS
median	2.96	3.28	
(range)	(1.29-9.50)	(1.25-23.32)	
PLR			NS
median	125.81	106.49	
(range)	(67.29-244.53)	(3.02-188.00)	

NS* – not statistically significant; NLR – neutrophil-lymphocyte ratio; LMR – lymphocyte-monocyte ratio; PLR – platelet-lymphocyte ratio; MDA – malondialdehyde concentration; TAC – total antioxidant capacity

During the examination, it was observed that MDA and TAC concentrations were slightly higher in the blood serum of the female CLM patients if compared to the male. However, the differences were not statistically significant. The median of hs-CRP concentration in blood serum of both female and male patients did not exceed the reference values set up for this marker (hs-CRP < 3 mg/L). No significant differences were observed between the two gender groups.

The results of white blood cell parameters – neutrophils, lymphocytes and monocytes as well as platelet count measured during the patient's blood count were used to determine NLR, PLR and LMR markers. The differences between the groups of females and males with CML were not statistically significant in the values of NLR, PLR and MLR markers.

Table 3 presents statistically significant correlations between NLR, PLR and LMR markers.

Table 3. Statistically significant correlations between NLR, PLR and LMR markers of CLM patients with respect to gender

Variables	R	p-value
Women n=16		
PLR vs LMR	-0,618	0,011
NLR vs PLR	0,624	0,010
NLR vs LMR	-0,568	0,022
Men n=19		
PLR vs LMR	-0,411	0,081
NLR vs PLR	0,711	0,0007
NLR vs LMR	-0,491	0,033

n-number of subjects; R-correlation coefficient

High correlations between the values of PLR and LMR (negative), NLR and PLR (positive) as well as NLR and LMR (negative) were found in the groups of the examined women. On the other hand, in the group of the male patients the relationships between PLR a LMR and NLR a LMR (negative) were average whereas between NLR and PLR (positive) they were very high (R = 0.711).

Table 4 shows correlations between MDA, TAC, hs-CRP levels and PLR, NLR, LMR values.

Table 4. Correlations between NLR, PLR and LMR markers and MDA, TAC and hs-CRP concentration of CLM patients with respect to gender

Variables	PLR R p-value	NLR R p-value	LMR R p-value	MDA R p-value	TAC R p-value
Women n=16					
hs-CRP	-0,150 0,579	0,415 0,110	0,106 0,696	0,221 0,411	0,041 0,880
MDA	0,116 0,668	0,249 0,353	0,091 0,737	-	0,121 0,656
TAC	0,259 0,333	0,106 0,696	-0,124 0,649	0,121 0,656	-
Men n=19					
hs-CRP	0,063 0,797	-0,007 0,977	0,153 0,533	0,088 0,721	0,161 0,509
MDA	-0,049 0,842	-0,125 0,611	0,086 0,726	-	0,151 0,538
TAC	0,049 0,842	-0,002 0,994	0,200 0,412	0,151 0,538	-

n – number of subjects; R – correlation coefficient

There was no statistically significant correlation between the PLR, NLR, LMR markers and the concentration of MDA, TAC and hs-CRP in both the female and male groups.

DISCUSSION

There is a lot of evidence of ROS participation in modifications of cellular reactions and there are implications that oxidative stress may be a significant pathophysiological factor of various types of leukemia, due to the initiation of lipid peroxidation and DNA damage (Devi, 2000, Oltra, 2001, Al-Gayyar 2007, Ciarcia, 2010). It is suggested that patients with leukemia may experience oxidative stress due to a greater number of mature and immature myeloid cells and the cell-related unknown factors (Rajeshwari, 2013).

BCR-ABL kinase supposedly stimulates ROS, which causes oxidative DNA damage and genome instability, which in consequence makes an intracellular environment more susceptible to mutations and may lead to disease progression. This fact suggests that ROS may play a significant role in resistance to treatment, which in turn may cause CLM progression (Ahmad, 2010).

In the available literature, few studies deal with the redox status in blood of patients suffering from different types of leukemia.

In this study the concentration of the lipid peroxidation products was measured and antioxidant ability to neutralise ROS was determined in order to assess fully redox status in CML patient's blood serum.

The mechanism of the formation of a great number of lipid peroxidation products may be based on the increased ROS production by the mature or immature myeloid cells, which leads to the oxidative stress (Gutowicz, 2011). Formed lipoperoxyl radicals can be regrouped in the cyclisation reaction to endoperoxides with the stable end peroxidation product – MDA with mutagenic and carcinogenic activity (Valko, 2007).

The examinations conducted so far, have shown that high oxidative stress occurs in CML and other types of leukemia (Petrola, 2012, Ahmad, 2010). According to Petrola (Petrola, 2012), MDA concentration reflects the range of lipid peroxidation and modulates gene expression connected with tumour promotion. The studies conducted by Ahmad (Ahmad, 2008 a, Ahmad, 2008 b, Ahmad, 2010) show that the application of drugs from different groups (imatinib, hydroxycarbamite) and different derivatives

within a certain group (I and Il generation kinase inhibitors) had a great impact on the MDA level in the CLM patients' blood. The CML phase was of importance as well, the considerably higher MDA concentration was found in the acceleration phase if compared with the chronic one. Nielsen et al. (Nielsen, 1997), based on determining the level of MDA in the blood of healthy Danes of both sexes aged 20-79, proposed reference values of this indicator in the range 0.36-1.24 μMm/L. In this study, both women and men had MDA serum levels approximately 6 times higher, which may reflect the degree of oxidative stress associated with the disease (Tab.2).

The available literature lacks information about determination of the total oxidation capacity (TAC) with the use of method of DPPH radical reduction in CML patients' blood serum. Mazor et al. (Mazor, 2008) observed that DPPH concentration in blood serum of children suffering from ALL was significantly lower if compared with the control group. It clearly indicated that the capacity of low-molecular-weight anti-oxidants to scavenger ROS was depleted.

The DPPH assay is based on the measurement of the scavenging capacity of ROS in different biological material. In this study the method, adapted by Chrzczanowicz et al. (Chrzczanowicz, 2002), was applied to determine the complete oxidation capacity (TAC) of deproteinised blood serum. The DPPH measurement method used in the serum was only reflecting the content of low molecular-weight-antioxidants. Hence, there could be no significant correlation between MDA and DPPH levels in patients' blood (Tab.4). Various factors, such as the structure of the molecules of these low-molecular-weight antioxidants, as well as the impact of the disease itself and therapy, could have an effect on the changes in the concentration of these components and their antioxidant activity.

CRP as an acute phase protein is a very sensitive marker of the inflammatory state (Bassuk, 2004). It is believed that the hs-CRP test, which is cheap and easy to use due to a low diagnostic sensitivity of many tumour markers, may provide useful infor-mation to assess the prognosis in patients and the choice of a therapy (Stasik, 2008).

The median value of in this investigation did not exceed the reference values both in female and male groups, which similarly as for other markers can be the consequence of the fact that all the patients were in the chronic phase of the disease and revealed a positive response to the performed therapy (Tab.2).

NLR, PLR or LMR markers can provide valuable prognostic information about different tumors. Studies on the prognostic role of these markers in various cancer diseases have been published (Li, 2018, Sun, 2018, Zhou, 2014, Yang, 2018, Li, 2017, Ying, 2018, Feng, 2018).

In literature there are no unequivocal reference ranges for the values of NLR, PLR and LMR markers that could be used to compare the obtained results. Lee et al. (Lee, 2018) conducted a retrospective study in which the values of NLR, PLR and LMR markers were calculated in 12000 healthy adults from the South Korean population in the age range 19-70 years. The conducted investigation showed that the mean values of NLR for all age groups was 1.65, and the values for males and females were 1.63 and 1.66, respectively. The average LMR value was 5.31 (for males and females – 5.05 and 5.60, respectively) whereas the mean PLR value was 132.40 (for males and females 122.73 and 142.60, respectively). The values of the NLR, LMR and PLR markers in the CML patients in this investigation were similar to these dates. It is difficult to compare these values with the cut off points put forward by the South Korean researchers due to inter-individual variability, racial and population differences. Statistically significant correlations between PLR, NLR and LMR markers in both women and men, found in this study, may result from the specificity of the disease, as well as from the inter-dependence of individual components of the white blood cell system (tab. 3).

This study revealed no statistically significant differences in MDA, DPPH, hs-CRP concentrations and the values of NLR, PLR and MLR markers between the groups of females and males with CML (tab. 4). The lack of correlation between the above markers could be caused by the applied therapy, which stabilized the patients' condition.

LIMITATIONS OF THE STUDY

There are limitations to this study. These were preliminary and pilot studies. The number of patients was not too high with a wide age range. Moreover, the studies were carried out only with the participation of patients in the chronic phase of the disease, treated with drugs from the group of tyrosine kinase inhibitors, which could have influenced the obtained results. Another limitation of the study was the determination of a few selected markers of inflammation and redox status. In further studies, the range of the determined redox status indicators should be increased.

SHORT CONCLUSION

The study found that statistically significant correlations occur between individual markers of the inflammatory state: PLR, NLR, LMR, including negative correlations between the PLR and LMR as well as NLR and LMR markers and the positive correlation between NLR and PLR markers. It speaks for the interdependence of the components of the white blood cell system and may suggest the inflammatory state in progress. No correlation was found between the mentioned markers and the MDA, TAC and hs-CRP concentration. Further investigations are necessary to determine relationships between the oxidative stress level and the concomitant inflammatory state and the response of the CLM patients' antioxidative system. The investigation should involve a bigger number of participants and should focus on the determination of values of a wider marker range for the redox status and the inflammatory state.

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