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Corresponding Author: Anna Moniuszko

Corresponding Author's Institution:

First Author: Wojciech Łuczaj

Order of Authors: Wojciech Łuczaj;Anna Moniuszko;Magdalena Rusak;Sławomir Pancewicz;Joanna Zajkowska;Elżbieta Skrzydlewska

Abstract: Aim: Lipid peroxidation products - malondialdehyde, 4-hydroxynonenal and 8-isoPGF2 α were determined in the plasma and urine of patients with Lyme arthritis and healthy people. Methods: The group consisted of 19 patients with Lyme arthritis (mean age - 47 years). The control group consisted of 16 healthy individuals (mean age -38 years). Diagnosis of Lyme disease was confirmed by epidemiological anamnesis, clinical manifestation of arthritis and serological examinations. Lipid peroxidation was estimated by the measurement of aldehydes (malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) determined by HPLC) and prostaglandin derivatives (8-isoPGF2 α - determined by LCMS).

Results: Malondialdehyde and 4-hydroxynonenal levels were increased about 2-4-fold in the plasma, while in the urine about 2-fold. More significant increase, which referred to the 8-isoPGF2 α total plasma level, was enhanced over 4-fold, while the urine 8-isoPGF2 α level was increased over 8-fold. The 8-isoPGF2 α total plasma level consists of free and esterified form. During infection the ratio of free to esterified form is significantly smaller compared to healthy people.

Conclusions: The ratio of free to esterified form of 8-isoPGF2 α may be a useful indicator of Lyme arthritis. Moreover the complementarities of three lipid peroxidation products levels may be helpful in the diagnosis of Lyme arthritis.

Response to Reviewers: Dear Reviewers,
Thank You very much for all comments on our paper. Below are answers for Your suggestions.

Answers for comments of Reviewer 1:

1.The correlation tables have been omitted. The results section contains only one sentence (last sentence) refers to the correlations:

"Moreover the analysis of correlation showed that in the group with Lyme arthritis the correlations between all analyzed lipid peroxidation products are significantly weaker than in the control."

2.The discussion has been significantly shortened.

3.The problem with control we tried to solve as follows;

*In the Materials and methods section the following information was added

"In the history of the control group no other diseases, which could be responsible for increased lipid peroxidation (e.g. arthritis of any etiology) were observed."

*In the Discussion after statement

"Therefore 8-isoPGF2 α can be implicated as mediators of Lyme arthritis development. " the following sentences have been added [with citation of Basu et al paper]

"However it has been shown that an increase in 8-isoPGF2 α and its major metabolite in the course of other type of arthritis including rheumatoid arthritis, reactive arthritis, psoriatic arthritis and osteoarthritis may be observed, which in consequence may cause diagnostic problems [32]. In case of Lyme disease, both immunological data and lipid peroxidation products levels should allow to establish the diagnosis."

Answers for comments of Reviewer 2:

1.In order not to stress only one lipid peroxidation product as the most important and to indicate that the used technique for MDA and HNE determination is HPLC and for 8-isoPGF2 α is LCMS the following changes have been done in the abstract and discussion:

in abstract:

"Lipid peroxidation was estimated by the measurement of aldehydes (malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) determined by HPLC) and prostaglandin derivatives (8-isoPGF2 α - determined by LCMS) in the plasma and urine."

"Moreover the complementarities of three lipid peroxidation products (MDA, 4-HNE and 8-isoPGF2 α levels may be very helpful in the diagnosis of Lyme arthritis."

in discussion:

In conclusion, it should be emphasized that during Lyme arthritis lipid peroxidation is enhanced. In the course of infection the ratio of free to esterified form of 8-isoPGF2 α is significantly smaller compared to the level in healthy people. This finding indicates that the ratio of free to esterified form of 8-isoPGF2 α may be a useful indicator of Lyme arthritis. Moreover the complementarities of the three lipid peroxidation products (MDA, 4-HNE and 8-isoPGF2 α levels may be very helpful in the diagnosis of Lyme arthritis.

2. Information that HNE level reflects only free aldehydes level has been added however the comparison of advantages of ELISA and HPLC technique has not been done, because this paper is not methodological paper. We want to prepare the methodological paper showing 8-isoPGF2 determinations and there we will include information about comparison of the LC and ELISA techniques.

3. The discussion has been significantly shortened and significance of MDA and HNE has been emphasized.

4.Conclusion indicates for complementarities of three lipid peroxidation products as above (point 1).

5.Errors have been corrected.

Lipid peroxidation products as potential bioindicators of Lyme arthritis

Wojciech Łuczaj¹, Anna Moniuszko², Magdalena Rusak¹, Sławomir Pancewicz², Joanna Zajkowska²,

Elżbieta Skrzydlewska¹

¹Department of Analytical Chemistry, Medical University of Białystok

²Department of Infectious Diseases and Neuroinfections, Medical University of Białystok

Correspondent Footnote:

Anna Moniuszko

Department of Infectious Diseases and Neuroinfections,

Medical University in Białystok, Żurawia Street 14, 15-540 Białystok, Poland

Tel.: 00 48 85 740 95 14

fax: 00 48 85 740 95 15

E-mail: annamoniuszko@op.pl, apuntamento@wp.pl

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4 **Abstract**

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6 **Aim:** Lipid peroxidation products - malondialdehyde, 4-hydroxynonenal and 8-isoPGF_{2α} were
7 determined in the plasma and urine of patients with Lyme arthritis and healthy people.
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9
10 **Methods:** The group consisted of 19 patients with Lyme arthritis (mean age - 47 years). The control
11 group consisted of 16 healthy individuals (mean age -38 years). Diagnosis of Lyme disease was confirmed by
12 epidemiological anamnesis, clinical manifestation of arthritis and serological examinations. Lipid peroxidation
13 was estimated by the measurement of aldehydes (malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE)
14 determined by HPLC) and prostaglandin derivatives (8-isoPGF_{2α} - determined by LCMS).
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20 **Results:** Malondialdehyde and 4-hydroxynonenal levels were increased about 2-4-fold in the plasma,
21 while in the urine about 2-fold. More significant increase, which referred to the 8-isoPGF_{2α} total plasma level,
22 was enhanced over 4-fold, while the urine 8-isoPGF_{2α} level was increased over 8-fold. The 8-isoPGF_{2α} total
23 plasma level consists of free and esterified form. During infection the ratio of free to esterified form is
24 significantly smaller compared to healthy people.
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31 **Conclusions:** The ratio of free to esterified form of 8-isoPGF_{2α} may be a useful indicator of Lyme
32 arthritis. Moreover the complementarities of three lipid peroxidation products levels may be helpful in the
33 diagnosis of Lyme arthritis.
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39 **Key words:** Lyme disease, malondialdehyde, 4-hydroxynonenal, 8-isoPGF_{2α}
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Introduction

The *Ixodes ricinus* ticks belonging to the arachnids are responsible for transferring many bacteriae, viruses and rickettsiae pathogenic to humans and animals. In Central Europe TBEV (Tick-Borne Encephalitis Virus) and spirochete *Borrelia burgdorferi* cause the most wide spread zoonoses, namely Lyme disease and TBE [1]. In the last two decades a dramatic increase of incidence of both diseases was reported. 100 000 new cases in Europe and 15.000-20.000 in North America are diagnosed annually. Considering all the tick-borne diseases, several European countries such as Poland, Estonia, Sweden, Austria, Czech Republic or Slovenia are characterized by the highest incidence in the northern hemisphere.

In the pathomechanism of Lyme disease, a significant role is played by the inflammatory and immunological mechanisms. *B. burgdorferi* causes damage of various organs and systems including skin, nervous system, osteoarticular system, heart, or eyes. Clinical outcome of Lyme disease depends on the spirocheates genotype and the immunological system of patients. This infection may lead to various pathologies, most commonly inflammatory arthritis characterized by cellular infiltration in the joints, predominantly with neutrophils and macrophages [2]. It predisposes to the generation of reactive oxygen species, which cause changes in antioxidant abilities of the organism [3,4]. In consequence, cellular components such as lipids, DNA and proteins are oxidatively modified, which leads to changes in their structure and functions, and significantly contributes to the pathophysiology of oxidative stress.

Lipids are readily attacked by reactive oxygen species, which results in the formation of numerous peroxidation products. One group of oxidation products that may exert toxic effects on the cells is very reactive carbonyls, such as malondialdehyde and 4-hydroxynonenal [5]. Unlike reactive oxygen species, aldehydes are rather long-lived and can therefore diffuse from the site of their origin reaching and attacking the intracellular and extracellular targets [5]. They disrupt various important structural and protective functions associated with biomembranes in various pathological events in vivo [6]. Moreover, the generated aldehydes are highly reactive and may act as “secondary toxic messengers” of the primary free radical event. These biogenic aldehydes possess strong electrophilic property which is reflected in their ability to form adducts with nucleophilic proteins amino acids residues as well as with DNA bases accompanied by alterations in their structure and functions.

Another class of lipid oxidation products formed in abundance in vitro and in vivo is the prostaglandin F₂-like compounds group – isoprostanes, mainly 8-isoPGF_{2α} [7]. 8-isoPGF_{2α} are produced in vivo independently of cyclooxygenases, primarily by free radical induced peroxidation of arachidonic acid [8]. It has been shown that levels of 8-isoPGF_{2α} are increased in a number of human diseases. Therefore, the measurement of these

1 molecules is currently recognised as the most accurate analytical method to assess the oxidative injury in vivo.
2 Moreover they are more stable than aldehydes generated during lipid peroxidation. In addition, products of the 8-
3 isoPGF_{2α} pathway have been found to exert potent biological actions and thus may be pathophysiologic
4 mediators of diseases [7,9].
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8 The clinical symptoms caused by *B. burgdorferi*, especially in the early stage of infection, may not be
9 characteristic and may not allow to base a diagnosis on them. Specific IgM and IgG antibodies titer in the serum
10 of the Lyme disease patients enable etiological diagnosis. The results of serological examinations do not
11 correlate with the severity of the disease and are not useful in the treatment monitoring and potential
12 complications prediction.
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18 Therefore, the objective of our study was the measurement of the serum lipids peroxidation products
19 concentration and potential designation of their role in the pathomechanism of the Lyme disease.
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22 **Material and methods**

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24 The group consisted of 19 patients with Lyme arthritis (7 females and 12 males) aged 25 to 67 years
25 (mean age- 47 years). The control group consisted of 16 healthy individuals (12 females and 4 males) aged 23 to
26 62 years (mean age-38 years).
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31 Diagnosis of Lyme disease was confirmed by epidemiological anamnesis, clinical manifestation –
32 arthritis caused by *B. burgdorferi* and serological examinations results - detection of anti/ *B. burgdorferi* IgM
33 and IgG antibodies in ELISA method (Borrelia recombinant IgG and IgM High sensitivity of Biomedica
34 Company, Austria) and at least 4- time increase of its concentration in IgG class. The mean IgM anti/*B.*
35 *burgdorferi* antibodies titer was 18 BBU/ml (min-11 BBU/ml, max-88 BBU/ml) and the mean IgG anti/*B.*
36 *burgdorferi* antibodies titer was 38 BBU/ml (min-11 BBU/ml, max-87 BBU/ml). In all cases results of ELISA
37 test were confirmed by Western blot testing. In the history of control group no other diseases, which could be
38 responsible for increased lipid peroxidation (e.g. arthritis of any etiology) were observed.
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47 In epidemiological anamnesis 90% of patients reported previous tick bites. In all cases pain limiting
48 movement of big joints (especially knee, elbow, shoulder) and muscles, especially of thighs and shoulders was
49 observed.
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53 From patients with diagnosis of Lyme disease as well as from healthy people the blood was taken into
54 EDTA tubes. The blood was centrifuged at 3 000 x g (4°C) to obtain the plasma. From the same people urine
55 was collected.
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1 Lipid peroxidation was estimated by measuring malondialdehyde (MDA), 4-hydroxynonenal (4-HNE)
2 and 8-isoPGF_{2α} [10, 11,12] in the plasma as well as in the urine. Malondialdehyde was determined as a
3 malondialdehyde-tiobarbituric acid adducts separated by HPLC with spectrofluorometric quantification at 532
4 nm excitation and 553 nm emission. 4-hydroxynonenal was determined as a one of the fluorescent
5 decahydroacridine derivatives formed in reaction with 1,3-cyclohexanodione. It was determined by HPLC with
6 fluorescence detector with excitation at 380 nm and emission at 445 nm.
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11 The plasma 8-isoPGF_{2α} were determined as free 8-isoPGF_{2α} (hydrolyzed in the organism), not-
12 esterified in phospholipids and as total 8-isoPGF_{2α} contained free and esterified 8-isoPGF_{2α}.
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16 To determine total 8-isoPGF_{2α} 1.0 ml of plasma was thawed and homogenised with 30μl internal
17 standard (300 pg 8-isoPGF_{2α}), 80μl of a solution containing BHT (10mM) and triphenylphosphine (1 mM) in
18 ethanol, and the mixture was vortexed thoroughly. Then 400 μl of a solution containing 25% (w/v) KOH was
19 added and the mixture was heated on a water bath for 45 minutes at 40°C in order to hydrolyse esterified
20 isoprostanes. After hydrolysis the sample was diluted with 3ml water (in order to prevent protein precipitation)
21 followed by vortexing. Acetic acid was added to pH 3 to prevent dissociation of the isoprostanes. A solid phase
22 column (tC18 Sep-pak Vac, 3ml, 200mg; Waters Chromatography) was conditioned with 3ml methanol,
23 followed by 3ml water that was adjusted to pH 3 with glacial acetic acid. The sample was vortexed again and
24 loaded onto the SPE column. The test-tube was washed with 3mL water (pH 3, corrected with glacial acetic
25 acid) and the washing solution was also loaded onto the column and cartridge was dried. The cartridge was then
26 washed with 3ml heptane to remove lipids. The cartridges were dried again under vacuum after which the
27 sample was eluted with 7ml of eluent consisting of ethyl acetate/heptane/methanol (50/40/10%). The resulting
28 solution was evaporated to dryness under a gentle stream of nitrogen. The residue was dissolved in 60 μl
29 acetonitrile/water (60/40% with 0.05% (v/v) acetic acid).
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45 To determine free 8-isoPGF_{2α} 1.0 ml of plasma was thawed and homogenised with 30μl internal
46 standard (300 pg 8-isoPGF_{2α}), 80μl of a solution containing BHT (10mM) and triphenylphosphine (1 mM) in
47 ethanol, and the mixture was vortexed thoroughly. Then 3400 μl of water was added. Acetic acid was added to
48 pH 3 to prevent dissociation of the isoprostanes. A solid phase column (tC18 Sep-pak Vac, 3ml, 200mg; Waters
49 Chromatography) was conditioned with 3ml methanol, followed by 3ml water that was adjusted to pH 3 with
50 glacial acetic acid. The sample was vortexed again and loaded onto the SPE column. The test-tube was washed
51 with 3mL water (pH 3, corrected with glacial acetic acid) and the washing solution was also loaded onto the
52 column and cartridge was dried. The cartridge was then washed with 3ml heptane to remove lipids. The
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1 cartridges were dried again under vacuum after which the sample was eluted with 7ml of eluent consisting of
2 ethyl acetate/heptane/methanol (50/40/10%). The resulting solution was evaporated to dryness under a gentle
3 stream of nitrogen. The residue was dissolved in 60 µl acetonitrile/water (60/40% with 0.05% (v/v) acetic acid).
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6 To urine 8-isoPGF_{2α} determination samples (1 ml) were added to 0.5 ml of 15% KOH and mixed and
7 then allowed to stand for 30 min. The pH was then adjusted with 6 M HCl to ~2.5. The sample was then
8 subjected to solid phase extraction (SPE). BondElut LMS polymer 100-mg SPE cartridges were used (purchased
9 from Varian). The SPE cartridge was conditioned with 1 ml of ethanol and 1 ml of Sorensen buffer. The sample
10 was applied to the cartridge, which was then sequentially washed with 1 ml each of Sorensen buffer and 5%
11 ethanol in water. The analyte and internal standards were eluted from the cartridge by using 1 ml of ethanol. The
12 eluate was collected and dried under a gentle stream of nitrogen. The resulting residue was then reconstituted
13 with 100 µl of 20% acetonitrile.
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22 For chromatographic analysis Phenomenex RP C18 column (150x2 mm, 3µm) was used. A gradient
23 separation was performed starting with a solvent that consisted of 60% water (pH 3.85) and 40% acetonitrile
24 running for 4 minutes. From 4 to 5 minutes a gradient was run to 100% acetonitrile which ran till 8 minutes.
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26 Between 8th and 9th minute a gradient was used to return to the original eluent composition and the system was
27 equilibrated till 15 minutes. The flow rate was 200 µl/min, the temperature column was set at 30°C and the
28 injection volume was 10 µl. The following parameters were employed throughout all MS experiments for ESI
29 with negative ion polarity the capillary voltage was set to 3.5 kV, the drying temperature to 350°C, the nebulizer
30 pressure to 45 psi, and drying gas flow to 10 l/min. The maximum accumulation time was 400 msec, the scan
31 speed was ultra scan mode and the fragmentation time was 40 ms. The target ions m/z 353, EIC (extracted ion
32 chromatogram): m/z 193 and 255, the fragments detected over scan range of m/z 150-400.
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43 *Statistical analysis*

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45 Data obtained in the current study are expressed as mean ± SD. These data were analyzed by standard statistical
46 analyses, one-way analysis of variance (ANOVA) with Tukey's test, to determine significant differences
47 between different groups. A p value of < 0.05 was considered statistically significant.
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52 **Results**

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54 In the pathogenesis of Lyme arthritis lipid peroxidation is significantly enhanced. The levels of lipid
55 peroxidation products are enhanced in the plasma as well as in the urine of patients. In the plasma the MDA level
56 is enhanced by about 2 fold, while the HNE level is increased by about 4 fold (p<0.001) (Table 1). In the urine
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2 of the Lyme arthritis patients, the level of MDA as well as HNE is about 2 fold higher than in the control group
3 (p<0.01) (Table 1). Significantly important differences are observed in prostanoids derivatives - 8-isoPGF_{2α} -
4 nonenzymatic lipid oxidation products level. In patients plasma the total 8-isoPGF_{2α} level is about 3 fold higher
5 than in the control while in the urine it increased tenfold (p<0.001) (Table 1). However the level of free 8-
6 isoPGF_{2α} in the control patients plasma gives 32 percent of the total level while the level of free 8-isoPGF_{2α} in
7 the Lyme arthritis patients plasma gives 4 percent of the total level (p<0.001) (Table 1, Fig 1, Fig 2).

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12 Moreover the analysis of correlation showed that in group with Lyme arthritis the correlations between
13 all analyzed lipid peroxidation products are significantly weaker than in the control group.
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16 17 18 **Discussion**

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20 In this study, a significant increase in the level of membrane phospholipids peroxidation products in the
21 course of Lyme arthritis has been revealed. The enhanced lipid peroxidation results from ROS generated by
22 stimulated phagocytes located near the joints during *B.burgdorferi* infection [3]. The lipid peroxidation process
23 is additionally intensified by a decrease in the antioxidant abilities observed during inflammation especially by
24 the decrease in the level of vitamin E, which acts as an efficient antioxidant in the lipid phase of the
25 biomembrane [13]. ROS reacting with phospholipids polyunsaturated fatty acids produce mainly superoxides
26 and low molecular aldehydes [5]. The augmentation of the malondialdehyde (MDA) and 4-hydroxynonenal
27 (HNE) level in the plasma as well as in urine of patients with *B.burgdorferi* infection has been presented in this
28 paper. These aldehydes have longer lifetime than ROS and can diffuse from the formation site and cause greater
29 cell damages. In addition, aldehydes may be considered as a secondary transmitter of lipid peroxidation, because
30 they are very reactive electrophilic compounds which react e.g. with antioxidants participating in diminishing
31 lipid peroxidation, such as GSH and glutathione peroxidase [14]. Aldehydes also form adducts with nucleophilic
32 sulfhydryl, amino and histidyl groups of proteins, which causes changes in the membrane proteins structure and
33 functions [15]. High reactivity of aldehydes generated during lipid peroxidation contributes to the fact that
34 estimation of their total level is difficult. In consequence, the level of their free form in the plasma as well as in
35 urine during Lyme arthritis is only about 2 fold higher in comparison to the control group. However, the level of
36 another, more stable, lipid peroxidation product - 8-isoPGF_{2α} that was determined in the plasma and urine of
37 patients with Lyme arthritis was significantly higher than the level of aldehydes and its level in plasma was over
38 3 fold higher, while in urine was over 8 fold higher than in healthy people.
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8-isoPGF_{2α} belongs to the eicosanoids family that comprise a diverse class of over one hundred bioactive lipid mediators derived from the metabolism of polyunsaturated fatty acids by enzymatic pathways connected with the action of cyclooxygenase, lipoxygenase and cytochrom P450 as well as non-enzymatic pathways [16]. The non-enzymatic mechanism generation of eicosanoids derivatives results from enhanced ROS generation and their reactions with nonsaturated fatty acids from phospholipids. However, very little is known about the role of eicosanoids in infectious arthritis. Analysis of joints from Lyme arthritis-resistant and – susceptible mouse strains revealed e.g. production of PGE₂ and PGD₂ [17]. In such a situation, the nonenzymatic mechanisms of arachidonic acid oxidation may play important role. This acid can be autooxidized in vivo by free radicals to yield a series of PGF₂-like compounds termed 8-isoPGF_{2α}. The formation of 8-isoPGF_{2α} results in the generation of four regioisomers that contain 16 stereoisomers within each regioisomeric group [18]. It is important to note that unlike COX, which generates stereoisomerally pure eicosanoids, 8-isoPGF_{2α} are formed as racemic diastereomers [19]. These compounds possess potent bioactivity and their quantification represents the most reliable index of oxidative stress in vivo [8].

A novel aspect of the 8-isoPGF_{2α} formation is that they are generated in situ esterified to phospholipids and realized by phospholipases, mainly by phospholipase A₂ – enzyme hydrolyzing bond in sn-2 position of the glycerin skeleton of phospholipids, the place of the location of unsaturated fatty acids including arachidonic acid [20,21]. It was shown that *B.burgdorferi* induced cytosolic phospholipase A₂ mRNA level in the joints of infected mice [22]. Phospholipase A₂ that separates arachidonic acid, indirectly contributes to increased ROS generation because the further arachidonic acid metabolism is partially connected with COX and LOX action in which ROS generation is observed [23].

It was proved that over 60% of 8-isoPGF_{2α} present in the plasma was carried as lipid esters, with the remainder circulating as free 8-isoPGF_{2α} [24], what is confirmed in this study. Measurement of free 8-isoPGF_{2α} in the plasma provides an index of production of 8-isoPGF_{2α} representing the equilibrium between the formation of esterified 8-isoprostanes and further released as the free acid, which indicates for metabolism. The concentration of free 8-isoPGF_{2α} seems to change more dynamically during the course of the disease. Free 8-isoPGF_{2α} are formed by oxidation of the tissues or plasma lipids and then released as free acids by the action of phospholipases. Because it was proved that *B.burgdorferi* induce the cytosolic phospholipase A₂ mRNA level in the joints of infected mice [17], the level of the free 8-isoPGF_{2α} should be increased. However significant decrease in the level of free 8-isoPGF_{2α} was observed in the plasma of patients with Lyme arthritis. It may be connected with the fact that oxidatively modified phospholipids, such as esterified 8-isoPGF_{2α} are good

1 substrates for plasma platelet-activating factor acetylhydrolase (PAF-AH) [25], which also hydrolyzes the long
2 chain polyunsaturated fatty acyl groups attached to the sn-2 position of the membrane phospholipids [26]. It was
3 also shown that during some diseases such as asthma, atopy or stroke [27,28], the plasma PAF-AH was
4 decreased [29]. Because 8-isoprostanes are initially formed in situ from esterified arachidonic acids in
5 phospholipids, we estimated the content of esterified 8-isoPGF_{2α} in phospholipids by subtracting the amount of
6 free 8-isoPGF_{2α} from the total amount of 8-isoPGF_{2α}. The present study shows that the total 8-isoPGF_{2α} levels in
7 the plasma from Lyme arthritis patients were significantly higher than those of the control group, whereas the
8 free 8-isoPGF_{2α} levels stayed at the similar level as in controls. These results indicate that the esterified 8-
9 isoPGF_{2α} levels were increased in patients with Lyme arthritis and also imply that accumulation of oxidized
10 phospholipids was more pronounced in the plasma of patients with Lyme arthritis than that of the control
11 probably because of the impaired oxidized phospholipids-hydrolyzing activity. Because most isoprostanes,
12 which are produced under oxidative stress, are in the esterified form, it is possible that their release from
13 phospholipids is a key step for the biological action.

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Independently of the significant increase in the plasma level of 8-isoPGF_{2α}, a higher than 8-fold increase in the urine 8-isoPGF_{2α} from patients with Lyme arthritis in comparison with the control group was observed. Measurement of the urinary 8-isoPGF_{2α} does not allow the COX to influence on the level of 8-isoPGF_{2α} existing only as a free form [30]. It was proven that the majority of 8-isoprostanes present in the human urine is derived from the free radical-oxidation of arachidonic acid rather than via COX [31], because COX inhibitors do not suppress the urinary levels of 8-isoPGF_{2α} and the major urinary metabolites of eicosanoids such as PGE₂ are significantly reduced [30]. The source of the urinary PGF is presumed to be from the tissue phospholipids-esterified PGF that is hydrolyzed by the action of phospholipase A₂ and excreted into the urine [29]. The evidence that the levels of 8-isoPGF_{2α} are significantly increased in human disorders associated with oxidative stress provides additional support for the mechanism of formation of 8-isoPGF_{2α} via free-radicals mechanism. A number of important physiological and pathophysiological issues have emerged from this study. This is related with the fact that formation of bioactive 8-isoPGF_{2α} occurs in vivo to a significant extent via free-radical mechanisms at the baseline and is markedly increased in oxidative stress e.g. by *B.burgdorferi* infection. Therefore 8-isoPGF_{2α} can be implicated as mediators of Lyme arthritis development. However it has been shown that increased in 8-isoPGF_{2α} and its major metabolite in the course of other type of arthritis including rheumatoid arthritis, reactive arthritis, psoriatic arthritis and osteoarthritis may be observed, which in consequence may cause diagnostic problems [32]. Therefore the comparison of the level of different

lipid peroxidation products (MDA, HNE and 8-isoPGF_{2α}) may be helpful in diagnosis. Additionally in case of Lyme disease, using both immunological data and lipid peroxidation products levels should allow to establish the correct diagnosis.

In conclusion, it should be emphasized that during Lyme arthritis lipid peroxidation is enhanced. During infection the ratio of free to esterified form of 8-isoPGF_{2α} is significantly smaller compared to the level in healthy people. This finding indicates that the ratio of free to esterified form of 8-isoPGF_{2α} may be a useful indicator of Lyme arthritis. Moreover the complementarities of three lipid peroxidation products (MDA, 4-HNE and 8-isoPGF_{2α}) levels may be very helpful in the diagnosis of Lyme arthritis.

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Fig. 1. The level of total (a), esterified (b) and free 8-isoPGF_{2α} (c) in the plasma and the level of 8-isoPGF_{2α} in the urine (d) of patients with Lyme arthritis (N=19) and control subjects (N=16)

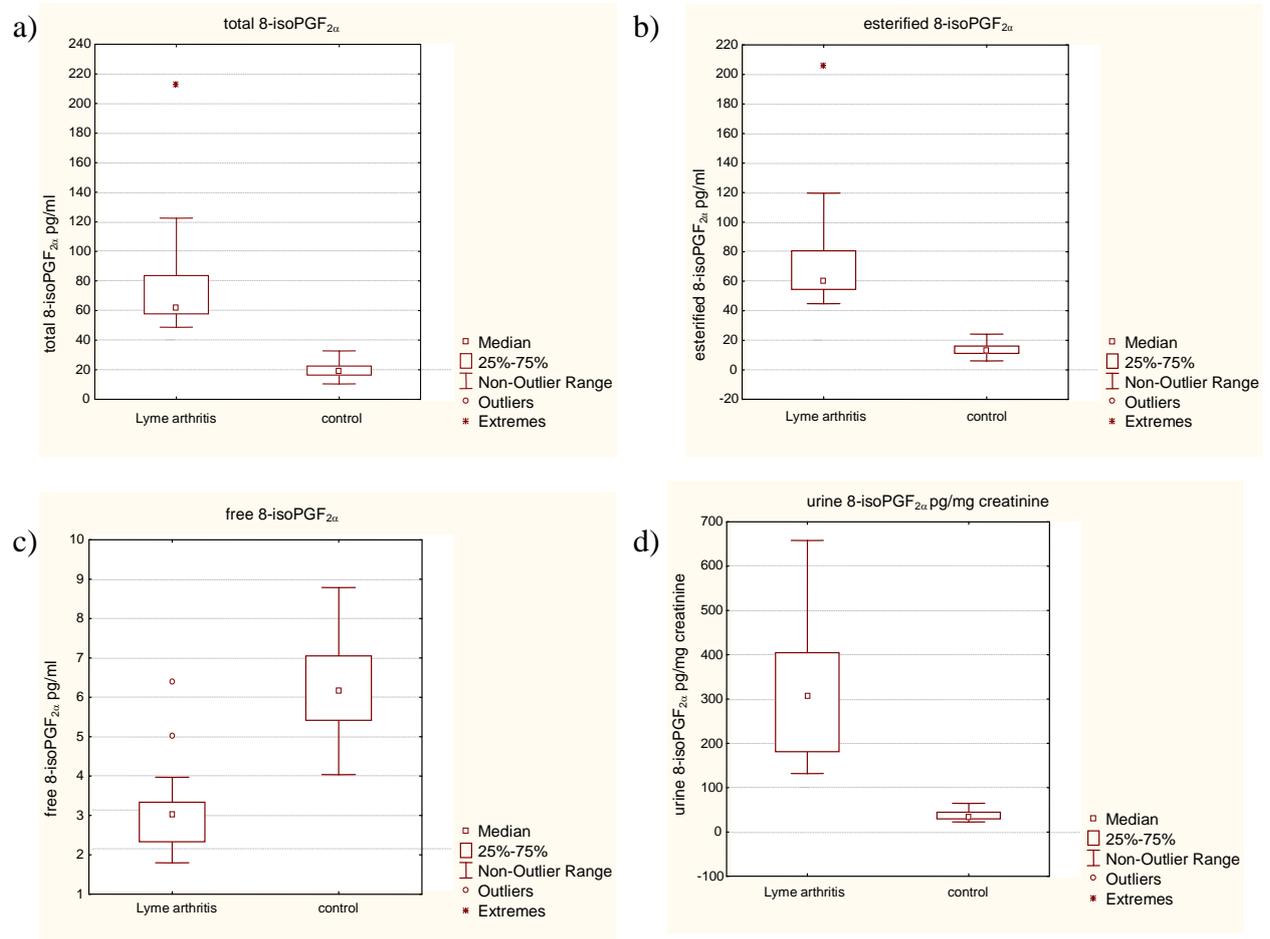


Fig. 2. The plasma MDA (a) and HNE (b) level and the urine MDA (c) and HNE (d) level of patients with Lyme arthritis (19) and control subjects (16)

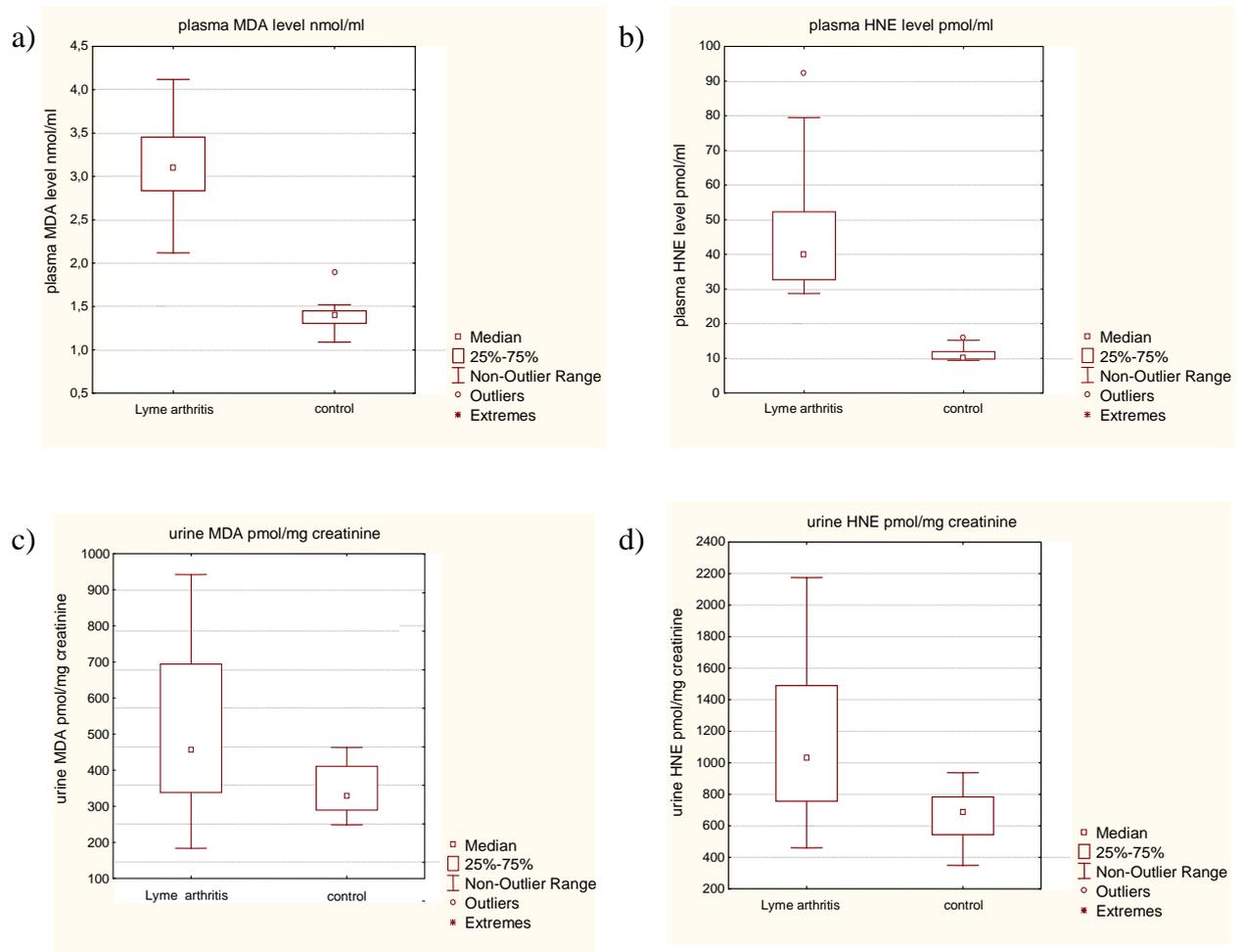


Table 1. Comparison of means and standard deviations from lipid peroxidation products level in plasma and urine of patients with Lyme arthritis (19) and control subjects (16)

Parameter	Group	N	Mean	SD	p<
plasma total 8-isoPGF _{2α} level [pg/ml]	Lyme arthritis	19	76.55	37.38	0.001
	control	16	19.94	5.53	
plasma free 8-isoPGF _{2α} level [pg/ml]	Lyme arthritis	19	3.13	1.10	0.001
	control	16	6.30	1.38	
plasma esterified 8-isoPGF _{2α} level [pg/ml]	Lyme arthritis	19	73.42	36.73	0.001
	control	16	13.58	4.42	
plasma MDA level [nmol/ml]	Lyme arthritis	19	3.13	0.48	0.001
	control	16	1.39	0.17	
plasma HNE level [pmol/ml]	Lyme arthritis	19	45.31	16.86	0.001
	control	16	11.24	2.19	
urine 8-isoPGF _{2α} level [pg/mg creatinine]	Lyme arthritis	19	328.21	154.76	0.001
	control	16	36.88	12.15	
urine MDA level [pmol/mg creatinine]	Lyme arthritis	19	528.47	222.51	0.008
	control	16	344.69	72.96	
urine HNE level [pmol/mg creatinine]	Lyme arthritis	19	1 124.16	482.52	0.002
	control	16	675.13	169.16	