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ABSTRACT

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LIPID PEROXIDATION MARKER LEVELS AND BASIC LABORATORY HEALTH INDICATORS OF BLOOD DONORS DURING WARTIME: POSSIBLE CONSEQUENCES FOR PRESERVED PACKED RED BLOOD CELLS QUALITY

Introduction. During storage at a moderately low-temperature, a preserved packed red blood cells (PRBCs) undergo metabolic and morphological changes commonly known as "storage lesion" or in vitro aging. Such changes inevitably increase during the permitted storage period, which is usually 21-42 days. But the degree and speed of their development in each individual preserved PRBCs unit is largely related to the special donor characteristics. The initial level of pro-oxidant process activities in the donor's body at the blood donation time can be an important starting point for the further kinetics of pathological changes since oxidative reaction activations are considered one of the main pathophysiological erythrocyte aging pathways. In particular, intense peroxidation of lipids as the main structural components of cell membranes causes significant changes with a usually negative impact on the dynamics and quality of cell physiological processes, and the induction of apoptosis and necrosis.

Numerous oxidative stress causes with adverse health consequences, such as acute and chronic psychological stress, significant physical exertion, work in adverse environmental conditions (air temperature, smog, altitude), etc., are known today. So, the danger of the spread of the conditions described above, which are frequent during the war period, both among the military and the civilian population, can significantly affect public health in Ukraine as a whole with an indirect negative impact on blood donation. Therefore, the activity levels of lipoperoxidation processes in the donor's body, along with other blood indicators that characterize the functional state of the main organs and systems, are critically important for the preservation of the blood components provided by this donor, in particular PRBCs.

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The work aimed to study individual laboratory indicators of the donors' health during wartime. A number of tasks to be performed were set, namely: to investigate the lipoperoxidation activity in venous blood, as well as the liver functional state, iron metabolism, indicators of a general blood analysis; to compare the data obtained in the studied group of wartime donors with the corresponding indicators obtained from archival data in the group of peacetime donors.

Materials and Methods. General blood analysis, protein metabolism, liver functional state, iron metabolism, and venous blood lipid peroxidation activity level were studied in donors. The research group included wartime donors of the Kharkiv region (2023 donations), whose activity types were military, civilian, and critical infrastructure. Archival data on donations from the pre-war period 2007 (I) were considered a comparison group (II). Statistical processing and data analysis were performed using STATISTICA 10 (StatSoft, USA). Since the distribution of the trait was not normal, the non-parametric Mann-Whitney U test was used to assess the differences between the two independent groups. Differences between the results were considered significant at p-value <0.05.

Results. It was established that the pro-oxidant activity indicators in the wartime blood donors significantly exceeded the control group indicators. This was evidenced by the data on the content of the entire range of lipid peroxidation molecular products, where the data excess of the experimental group compared to the control group ranged from 1.7 to 17.7 times. Thus, the levels of substrates and molecular peroxidation products of lipids extracted to the heptane phase (neutral lipids) were, in according to the groups and the investigated indicators: for substrates (isolated double bonds (IDB)) - Me (I) = 2.40 (2.07; 3.35) U/ml vs Me (II) = 0.47 (0.19; 1.41) U/ml, p = 0.000001; for intermediate products such as dienic (DC), trienic (TC) and oxodienic conjugates (ODC) - Me (I) = 1.84 (2.07; 2.78) U/ml vs Me (II) = 0.10 (0.29; 0.91) U/ml, p = 0.000001; Me (I) = 0.56 (0.46; 0.82) U/ml vs Me (II) = 0.16 (0.13; 0.26) U/ml, p = 0.000001; Me (I) = 0.55 (0.44; 0.82) U/ml vs Me (II) = 0.15 (0.11; 0.25) U/ml, p = 0.000001; and for the Schiff bases type end products (ShB) - Me (I) = 0.15 (0.10; 0.28) U/ml vs Me (II) = 0.02 (0.02; 0.04) U/ml, p = 0.000001. Phospholipid peroxidation products, determined in the lipid extract isopropanol phase, also had significant intergroup differences, namely: according to the IDB concentration - Me (I) = 4.39 (3.89; 4.87) U/ml vs Me (II) = 1.63 (1.21; 1.92) U/ml, p = 0.000001; for the DC, TC, and ODC concentrations, respectively, - Me (I) = 2.07 (1.72; 2.62) U/ml vs Me (II) = 0.91 (0.65; 1.09) U/ ml, p = 0.000001; Me (I) = 1.09 (0.91; 1.36) U/ml vs Me (II) = 0.65 (0.48; 0.77) U/ml, p = 0.000001; Me (I) = 1.05 (0.86; 1.45) U/ml vs Me (II) = 0.50 (0.42; 0.61) U/ml, p = 0.000001; and for the ShB concentration - Me (I) = 0.26 (0.14; 0.43) U/ml vs Me (II) = 0.13 (0.08; 0.16) U/ml, p = 0.000001. The data of the general blood analysis, protein metabolism, functional state of the liver, and iron metabolism were within the reference values.

The significant role of oxidative stress in PRBC aging during cold storage, accompanied by a substantial deterioration of their transfusion efficiency, confirmed by many studies, confirms the importance of the demonstrated results and the continuation of work in the chosen direction.

Conclusions. The revealed features of the pro-oxidant activity of blood donors can influence the stability of their erythrocytes to standard long-term storage conditions at a temperature of 4-6 °C. Further research in the direction of analyzing the relationships between oxidative stress markers,

in particular the lipoperoxidation activity, as special parameters of the donor, as well as substantiating the feasibility of considering these and other additional donor factors of the rate of erythrocyte aging development during storage are promising from the point of view of finding ways to improve the blood component quality.

Keywords: blood donors, lipid peroxidation, public health, packed red blood cells, quality, oxidative stress markers.

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РІВНІ МАРКЕРІВ ЛІПОПЕРОКСИДАЦІЇ ТА ОСНОВНІ ЛАБОРАТОРНІ ПОКАЗНИКИ ЗДОРОВ'Я У ДОНОРІВ КРОВІ ПІД ЧАС ВІЙНИ: МОЖЛИВІ НАСЛІДКИ ДЛЯ ЯКОСТІ КОНСЕРВОВАНИХ ЕРИТРОЦИТІВ

Вступ. Під час зберігання в умовах пониженої температури (4–6 °С) консервовані еритроцити (КЕ) зазнають метаболічних та морфологічних змін, що мають загальні ознаки псування, або так званого «старіння» *in vitro*. Такі зміни невідворотно зростають упродовж усього терміну зберігання, що стандартно складає від 21 до 42 діб. При цьому ступінь та швидкість їх нарощування у кожній окремій збереженій одиниці КЕ значною мірою залежить від особливих характеристик донора. Оскільки одним з основних патофізіологічних шляхів старіння еритроцитів вважають активацію окислювальних реакцій, вихідний рівень активності прооксидантних процесів у донора на момент кроводачі може бути важливою відправною точкою для подальшої кінетики розвитку патологічних змін. Зокрема, інтенсивна пероксидація ліпідів, що є основними структурними складовими клітинних мембран, спричинює вагомі зміни із зазвичай негативним впливом на динаміку та якість клітинних фізіологічних процесів, а також індукцією апоптозу й некрозу.

Нині відомі численні причини розвитку окислювального стресу з несприятливими наслідками для здоров'я, такі як гострий та хронічний психологічний стрес, значні фізичні навантаження, робота в несприятливих умовах навколишнього середовища (температура повітря, задимленість, висотність) тощо. Небезпека розповсюдженості охарактеризованих вище станів, що є частими у воєнний період, як серед військових, так і серед цивільного населення, значним чином може відображатись на громадському здоров'ї в цілому в Україні з опосередкованим негативним впливом на донорство крові. Отже, рівні активності процесів ліпопероксидації в організмі донора, поряд з іншими показниками крові, що характеризують функціональний стан основних органів та систем, потребують ретельного вивчення, оскільки є важливими для подальшої збереженості наданих цим донором компонентів крові, зокрема КЕ.

Метою роботи було дослідження окремих лабораторних показників здоров'я донорів крові у воєнний час. Для її досягнення були поставлені наступні завдання: -дослідити активність процесів ліпопероксидації у венозній крові, а також функціональний стан

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печінки, метаболізм заліза, показники загального аналізу крові; - порівняти отримані дані у досліджуваній групі донорів воєнного періоду з відповідними показниками, отриманими за архівними даними у групі донорів мирного часу.

Матеріали і методи. У донорів досліджено загальний аналіз крові, білковий обмін, функціональний стан печінки, а також рівень активності процесів ліпопероксидації в венозній крові. До дослідної групи увійшли донори харківського регіону воєнного часу (I -донації 2023 року), що за родом діяльності відносились до військових, цивільних та робітників критичної інфраструктури. Групою порівняння слугували архівні дані довоєнного періоду (II - донації 2007 року). Статистичну обробку та аналіз даних проводили за допомогою програмного забезпечення STATISTICA 10 (StatSoft, США). Оскільки розподіл ознаки не був нормальним, для оцінки відмінностей між двома незалежними групами використовували непараметричний U-критерій Манна-Уітні. Відмінності між результатами вважалися достовірними при p-значенні <0,05.

Результати. Встановлено, що показники прооксидантної активності крові донорів воєнного часу значно перевищують показники контрольної групи. Про це свідчили дані щодо вмісту усього спектра молекулярних продуктів пероксидації ліпідів, де перевищення даних дослідної групи відносно групи контролю складало від 1,7 до 17,7 раза. Так, рівні субстратів та молекулярних продуктів пероксидації екстрагованих до гептанової фази ліпідів (нейтральні ліпіди), відповідно до груп та досліджених показників, складали: для субстратів (ізольвані подвійні зв'язки - $ІПЗ_н$) - Me (I) = 2,40 (2,07; 3,35) Од/мл та Me (II) = 0,47 (0,19; 1,41) Од/мл, p = 0,000001; для проміжних продуктів, таких як дієнові ($ДК_н$), триєнові ($ТК_н$) та оксодієнові кон'югати (ОДКн) – Me (I) = 1,84 (2,07; 2,78) Од/мл та Me (II) = 0,10 (0,29; 0,91) Од/мл, p = 0,000001; Me (I) = 0,56 (0,46; 0,82) Од/мл та Me (II) = 0,16 (0,13; 0,26) Од/мл, p = 0,000001; Me (I) = 0,55 (0,44; 0,82) Од/мл та Me (II) = 0,15 (0,11; 0,25) Од/мл, p = 0,000001; для кінцевих продуктів типу основ Шиффа ($ШО_н$) – Me (I) = 0,15 (0,10; 0,28) Од/мл та Me (II) = 0,02 (0,02; 0,04) Од/мл, p = 0,000001. Продукти пероксидації фосфоліпідів, визначені в ізопропанольній фазі ліпідного екстракту, також мали значні міжгрупові відмінності, а саме: за концентрацією $ІПЗ_ф$ – Me (I) = 4,39 (3,89; 4,87) Од/мл та Me (II) = 1,63 (1,21; 1,92) Од/мл, p = 0,000001; для $ДК_ф$, $ТК_ф$ та $ОДК_ф$, відповідно, – Me (I) = 2,07 (1,72; 2,62) Од/мл та Me (II) = 0,91 (0,65; 1,09) Од/мл, p = 0,000001; Me (I) = 1,09 (0,91; 1,36) Од/мл та Me (II) = 0,65 (0,48; 0,77) Од/мл, p = 0,000001; Me (I) = 1,05 (0,86; 1,45) Од/мл та Me (II) = 0,50 (0,42; 0,61) Од/мл, p = 0,000001; для $ШО_ф$ – Me (I) = 0,26 (0,14; 0,43) Од/мл та Me (II) = 0,13 (0,08; 0,16) Од/мл, p = 0,000001. Дані загального аналізу крові, білкового обміну, функціонального стану печінки та метаболізму заліза не виходили за межі референтних значень.

Продемонстрована багатьма дослідженнями значима роль окислювального стресу у старінні еритроцитів під час холодового зберігання, що супроводжується суттєвим погіршенням їх трансфузійної ефективності, підтверджує важливість продемонстрованих результатів та продовження роботи у обраному напрямку.

Висновки. Виявлені особливості прооксидантної активності крові донорів можуть впливати на стійкість їх еритроцитів до

стандартних умов тривалого зберігання при помірно низькій температурі 4–6 °С. Подальші дослідження в напрямку аналізу зв'язків між маркерами окисного стресу, зокрема активністю ліпопероксидації, як особливими параметрами донора, а також обґрунтування доцільності розгляду цих та інших додаткових донорських факторів швидкості розвитку старіння еритроцитів в процесі зберігання є перспективними з точки зору пошуку шляхів покращення якості компонентів крові.

Ключові слова: донори крові, перекисне окислення ліпідів, громадське здоров'я, консервовані еритроцити, якість, маркери окисного стресу.

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INTRODUCTION

Preserved packed red blood cells (PRBC) are the most necessary component of transfusion therapy in critical and severe conditions caused by deficiencies of these cells and hemoglobin. The acquisition of high efficiency and safety of these agents depends on the biochemical, biophysical, and morphological properties of cells, which are the main quality indicators in PRBC production and storage [1]. The history of the development of biomedical technologies of such manufacturing shows that many of them, which are used in the blood service operations, are aimed at stabilizing such components as cell membranes, their intracellular medium, as well as functional activity. Preservative solutions modernization due to new compositions of chemical compounds is practically exhausted today [2, 3]. Traditionally, they contain salts (NaCl, $NaHCO_3$, Na_2HPO_4, sodium citrate), citric acid, adenine, glucose and other components. Unfortunately, cell protection with such solutions is not complete. Gradual but significant cell damage with their acquiring signs of "in vitro aging" during the permitted period (from 21 to 42 days) under standard production conditions of hypothermic PRBC storage always occurs, and is collectively scientifically referred to as the "storage lesion" [4]. At the same time, the oxidative processes activation accompanied by RBC membrane damage is a general characteristic of such damage. As a result, the risk of post-transfusion immune and inflammatory complications in recipients increases.

In addition to these and other production parameters, such as fractionation methods, the presence of leukocytes, as well as oxygen access, there are also a number of the donor characteristics (gender, age, metabolic features, genetic factors, etc.) that are somehow related to quality and preservation of PRBC in the blood banks conditions [5–10]. In view of these individual characteristics, the storage lesion progression rates vary from donor to donor [6]. Recently, there has been a significant revival of interest in the study of such correlations, but the specified donor characteristics are not yet taken into account during the realization of the donor suitability assessing procedure for blood donation under standard conditions. In particular, the initial parameters of the pro-oxidant processes activity, which should play a very important role in the RBC aging induction intensity during blood bank storage, are not taken into account [11].

It is well known that the development of various pathological conditions, as well as the aging acceleration, occurs under conditions when the oxidative reaction degree exceeds the antioxidant system ability to self-renew. The generation of free radicals or reactive oxygen species (ROS) is caused by many factors. Infectious agents, ionizing and ultraviolet radiation, tobacco smoke, environmental toxins, exposure to herbicides/ insecticides, and many other exogenous influences are the main ones [12]. In case of predominance of pro-oxidant processes, oxidative molecular damage of cells develops with the induction of apoptosis or necrosis due to peroxidation of lipids, proteins, DNA and RNA [13].

Thus, lipids are targets of peroxidation modification. At the same time, free radicals formed from oxygen, such as hydroxyl and hydroperoxyl species, mainly oxidize membrane lipids containing polyunsaturated fatty acids. In particular, phospholipids in the composition of cell membranes are mainly prone to peroxidation [14].

Since membrane lipids are responsible for maintaining cellular membrane integrity, their intense peroxidation changes the assemblage, composition, structure, and dynamics of these cellular structures. As highly reactive compounds, lipid peroxides are able to spread the further formation of ROS or decompose into reactive particles [15].

In view of the above, the lipid peroxidation (LPO) processes activity levels in the donor's body are critically important for the initial quality characteristics of cellular components, in particular RBCs intended for transfusion therapy. The LPO activation predictably affects their quality during hypothermic storage of the reserve accumulation in the blood bank.

The work aimed to study individual laboratory indicators of the donors' health during wartime. A number of tasks to be performed were set, namely: to investigate the lipoperoxidation activity in venous blood, as well as the liver functional state, iron metabolism, indicators of a general blood analysis; to compare the data obtained in the studied group of wartime donors with the corresponding indicators obtained from archival data in the group of peacetime donors.

MATERIALS AND METHODS

The research group included 28 donors from the Kharkiv region (group I, n = 28). All of them were in the combat zone, and by type of activity, they were military, critical infrastructure workers, and civilians. Donors were examined for hematological and biochemical indicators. Archival data from pre-war (2007, group II – the controls, n = 50) served as a comparison group. All donors were primary and were allowed to donate in accordance with national eligibility criteria for blood donation after signing the appropriate questionnaires and informed consent forms (Order of the Ministry of Health of Ukraine dated August 1, 2005 No. 385 "Procedure for medical examination of blood and blood component donors" in the edition of February 8, 2021 No. 207). Among such criteria are compliance with age restrictions, weight from 50–55 kg, absence of chronic diseases, restrictions on taking medications and denial of risky behavior. Accordingly, the age of the donors in the study was from 18 to 51 years. By gender, 21 and 23% of the total number of experimental and comparative groups were women. When studying the dependence of LPO indicators on age, donors were divided according to two categorical variables, namely: age from 18 to 40 years and from 41 to 51 years, which made up 50% of the total sample.

The study was carried out according to the plan of research and development of the National Academy of Sciences of Ukraine, and passed the examination of the medical ethics committee of the State Institution "Institute of Hematology and Transfusiology of the NAMS of Ukraine".

In whole blood, taken in a vacuum tube with an EDTA filler, indicators of a general blood analysis with the leukocyte formula were determined [16]. Biochemical studies were carried out in serum from the vacuum tube without a filler. We used photometric methods of determination for such indicators as total protein (g/L) [17], total and direct bilirubin according to Endrashik (μmol/L) [18], thymol test by Shank–Hoagland turbidity units (S-H U) [19], alkaline phosphatase (ALP (ncat/L)) [20], iron and total iron-binding capacity (TIBC) of donor blood serum (μmol/L) [21]. Manual photometric calibration methods were also used to determine the activities of alanine aminotransferase (ALT (μmol/(ml·h)) and aspartate aminotransferase (AST (μmol/(ml·h)) according to Reitman–Frenkel [22, 23], and gamma-glutamyl transpeptidase (γ-GGT (μcat/L)) [24].

The activity of lipid peroxidation (LPO, U/ml) processes was investigated using the Volchegorsky IA, et al. method in the Anoshyna MYu modification [25]. The method modification allows separate determination of the relative content of the intermediate LPO products, which clarifies the character of the LPO processes. In general, the method is characterized by differentiated determination of acyl peroxidation in the structure of the phospholipids ((PhLs) extracted to the isopropanol phase) and the unesterified intermediates of the neutralized lipids (NLs) under fatty acid peroxidation (extracted to the heptane phase) according to the concentration of dienic conjugates (DC), trienic conjugates (TC), oxodienic conjugates (ODC) and final products by type Schiff basics (ShB) and substrates of the lipid peroxidation (the content of isolated double bonds (IDB). The optical density of each phase of the lipid extract against the corresponding control was measured on a spectrophotometer (Helios α, Thermo Electron Co, UK). The wavelengths correspond to the absorption of the indicated range of LPO molecular products and their substrates. The concentration of IDB was measured by absorption at 220 nm wavelength, DC – at 232 nm; TC – at 268 nm, ODC – at 278 nm, and ShB – at 400 nm. There were no differences in the conducting research conditions using harmonized techniques in the comparison groups.

Statistical processing and data analysis were performed using STATISTICA 10 (StatSoft, USA). Since the distribution of the traits was not normal, the non-parametric Mann–Whitney U test was used to assess the differences between two independent groups. Differences between the results were considered significant at p-value <0.05 [26]. The data are presented as median (Me) and interquartile range (25 %; 75 %).

RESULTS

As evidenced by the data in Fig. 1 and Fig. 2, the blood pro-oxidant activity of wartime donors (group I) significantly exceeds the indicators of prewar donors (group II – the controls).

The absolute content of the LPO molecular products (both in the case of neutral lipids peroxidation (fig. 1) and in the case of phospholipids peroxidation (fig. 2)) significantly exceeded the group II data. The levels of

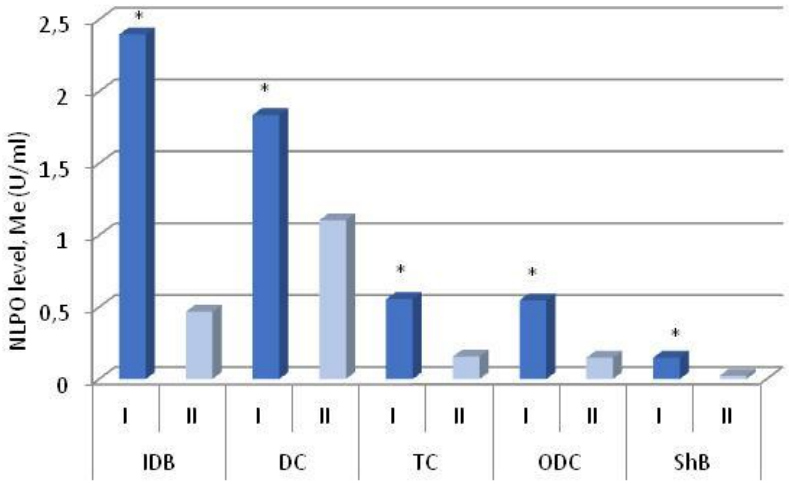

Figure 1 – Levels of neutral lipid peroxidation products (NLPO) in the blood of wartime donors (I) and the control group (II): Me – median; IDB – isolated double bonds; DC, TC, ODC – diene, triene, and oxodienic conjugates. Statistical differences are indicated with * – P < 0.000001

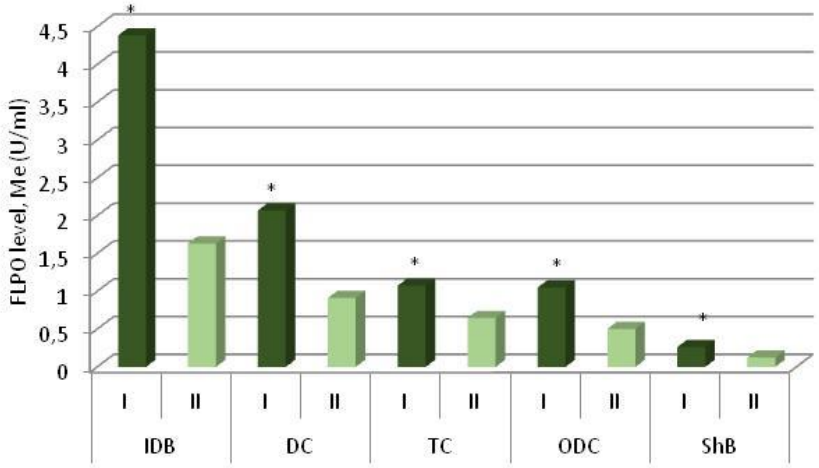

Figure 2 – Levels of phospholipids peroxidation products (FLPO) in the blood of wartime donors (I) and the control group (II): Me – median; IDB – isolated double bonds; DC, TC, ODC – diene, triene, and oxodienic conjugates. Statistical differences are indicated with * – P < 0.000001

substrates and molecular peroxidation products of lipids extracted to the heptane phase (neutral lipids) were, in according to the groups and the investigated indicators: for substrates (IDB) – Me (I) = 2.40 (2.07; 3.35) U/ml vs Me (II) = 0.47 (0.19; 1.41) U/ml, p = 0.000001; for intermediate products such as DC, TC, and ODC – Me (I) = 1.84 (2.07; 2.78) U/ml vs Me (II) = 0.10 (0.29; 0.91) U/ml, p = 0.000001; Me (I) = 0.56 (0.46; 0.82) U/ml vs Me (II) = 0.16 (0.13; 0.26) U/ml, p = 0.000001; Me (I) = 0.55 (0.44; 0.82) U/ml vs Me (II) = 0.15 (0.11; 0.25) U/ml, p = 0.000001; and for the end products (ShB) – Me (I) = 0.15 (0.10; 0.28) U/ml vs Me (II) = 0.02 (0.02; 0.04) U/ml, p = 0.000001. Phospholipid peroxidation products, determined in the lipid extract isopropanol phase, also had significant intergroup differences, namely: according to the IDB concentration – Me (I) = 4.39 (3.89; 4.87) U/ml vs Me (II) = 1.63 (1.21; 1.92) U/ml, p = 0.000001; for the DC, TC, and ODC concentrations, respectively, – Me (I) = 2.07 (1.72; 2.62) U/ml vs Me (II) = 0.91 (0.65; 1.09) U/ ml, p = 0.000001; Me (I) = 1.09 (0.91; 1.36) U/ml vs Me (II) = 0.65 (0.48; 0.77) U/ml, p = 0.000001; Me (I) = 1.05 (0.86; 1.45) U/ml vs Me (II) = 0.50 (0.42; 0.61) U/ml, p = 0.000001; and for the ShB concentration – Me (I) = 0.26 (0.14; 0.43) U/ml vs Me (II) = 0.13 (0.08; 0.16) U/ml, p = 0.000001.

Thus, the content of lipid substrates, which are characterized by the IDB concentration, in the case of neutral lipids peroxidation at the wartime donor group exceeded the control data of the second group by as much

as 5 times. This indicator exceeded the data of the control donor group by almost 3 times in the phospholipids peroxidation case. The increase for primary LPO products (NLs DC and PhLs DC) was even more pronounced and amounted to 17.7 and 2.3 times, respectively. The excess concentration of lipoperoxidation intermediates (NLs TC, NLs ODC, PhLs TC, PhLs ODC) in the blood of wartime donors was 3.6, respectively; 3.7; 2,3; 1.7 times. A significant difference was also found for the end products of the type of Schiff bases, both NLs and PhLs, which reached an excess of 6.2 and 2.1 times, respectively, in the experimental donor group.

The Mann–Whitney test for independent groups was used to assess the relationships between LPO activity in the venous blood of donors and their gender (fig. 3, 4) and age (fig. 5, 6).

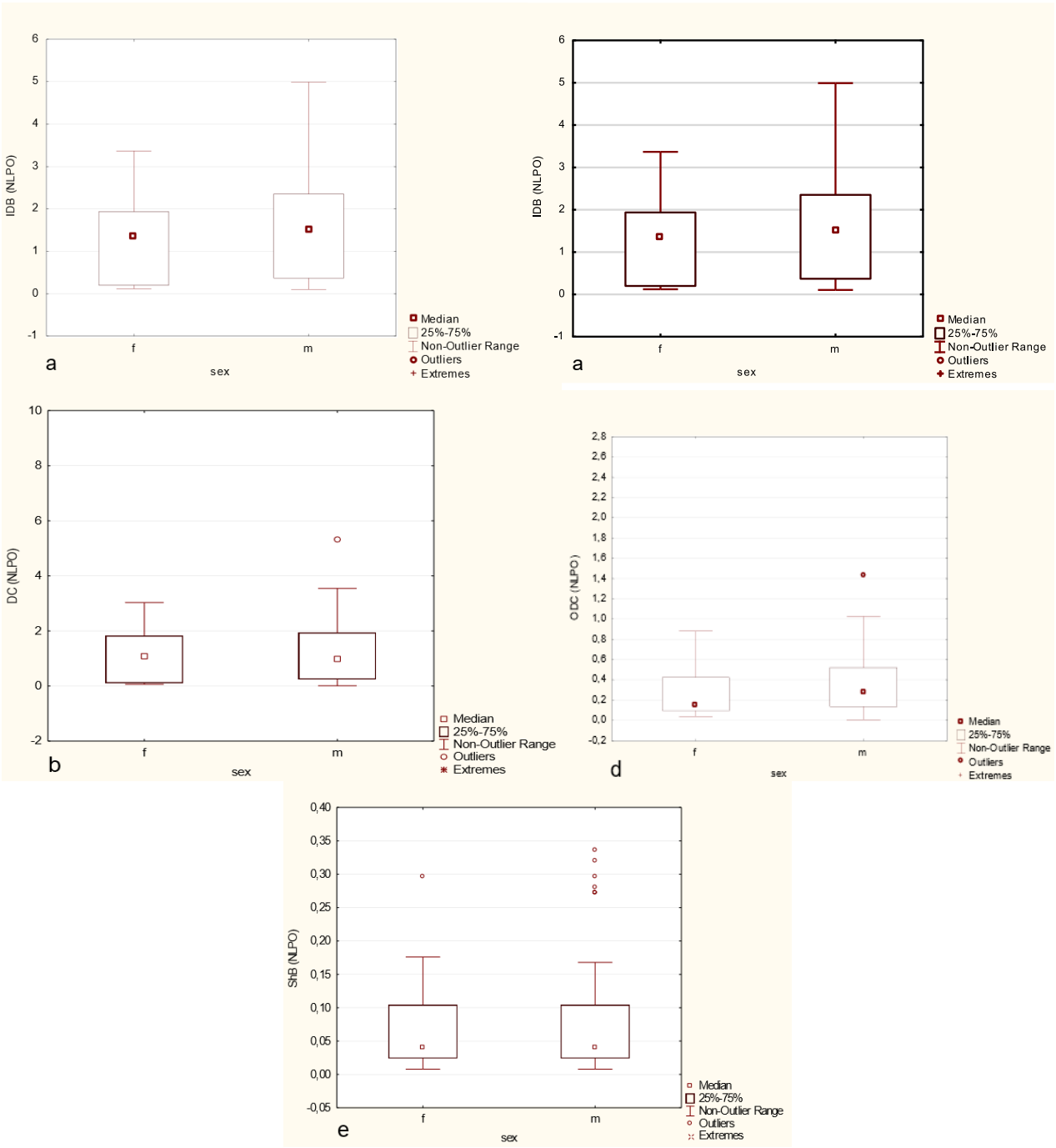

Figure 3 – The intergroup difference of median for neutral lipid peroxidation products (NLPO, U/ml) in the donor peripheral blood (distribution by gender: f – female, m – male) according to Mann–Whitney U test: a – the concentration of isolated double bonds (IDB), p-value = 0.4280; b – the concentration of dienic conjugates (DC), p-value = 0.7036; c – the concentration of trienic conjugates (TC), p-value = 0.2021; d – the concentration of oxodienic conjugates (ODC), p-value = 0.2457; and final products by type Schiff basics (ShB), p-value = 0.6102

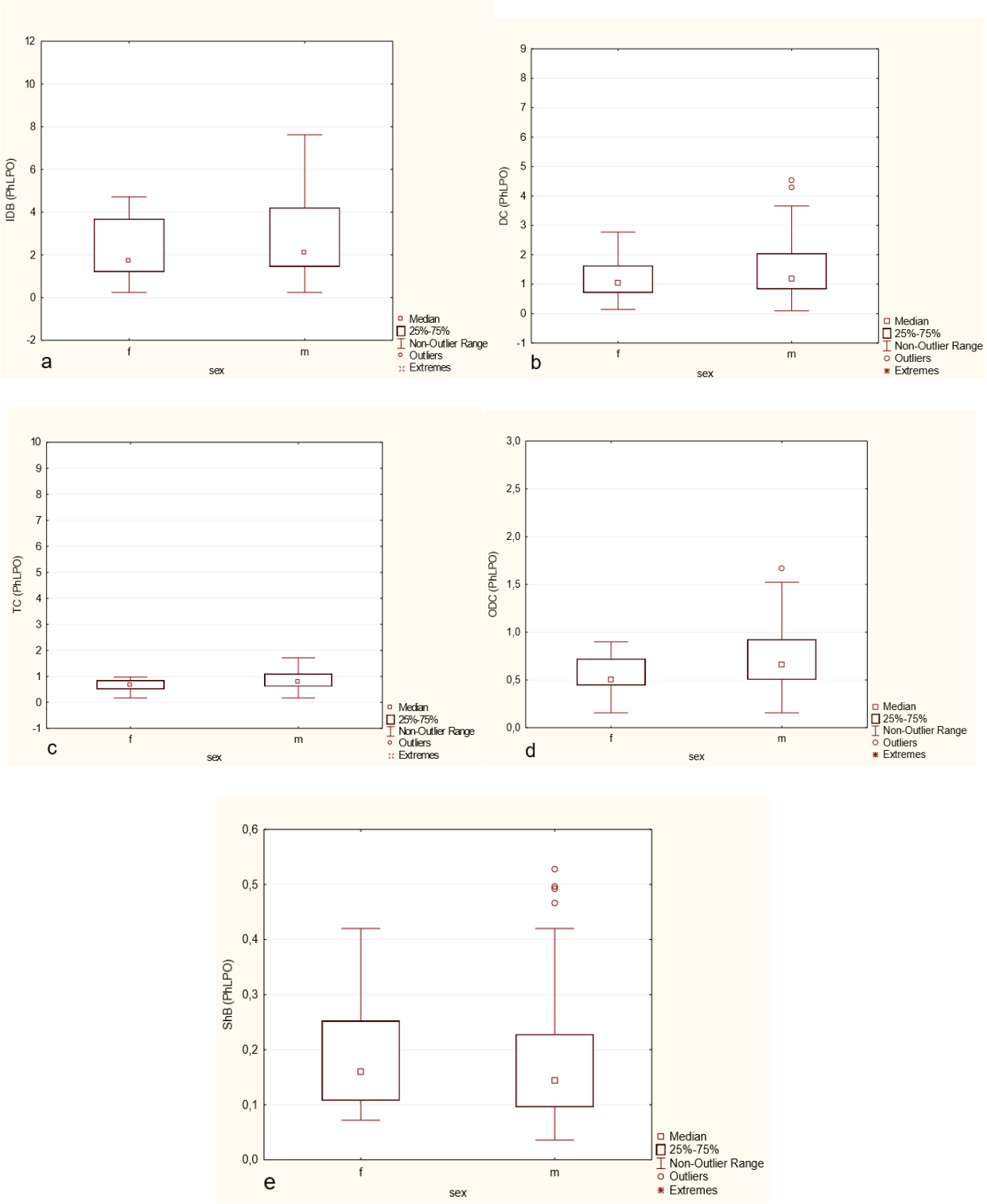

Figure 4 – The intergroup difference of median for phospholipids' peroxidation products (PhLPO, U/ml) in the donor peripheral blood (distribution by gender: f – female, m – male) according to Mann–Whitney U test: a – the concentration of isolated double bonds (IDB), p-value = 0.7567; b – the concentration of dienic conjugates (DC), p-value = 0.7643; c – the concentration of trienic conjugates (TC), p-value = 0.6383; d – the concentration of oxodienic conjugates (ODC), p-value = 0.9840; and final products by type Schiff basics (ShB), p-value = 0.7643

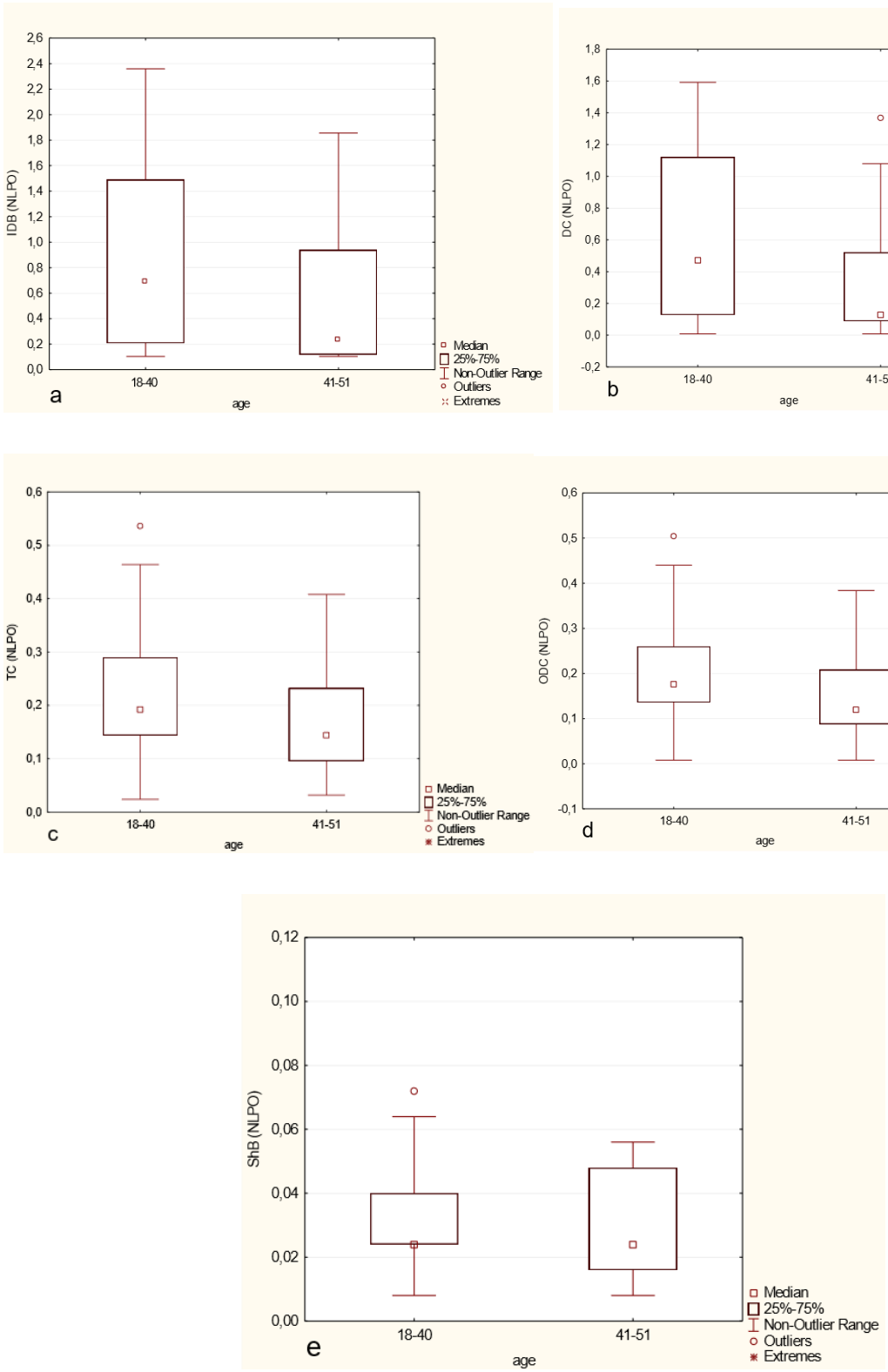

Figure 5– The intergroup difference of median for neutral lipid peroxidation products (NLPO, U/ml) in the donor peripheral blood (distribution by age: from 18 to 40, and from 41 to 51 years old) according to Mann–Whitney U test: a – the concentration of isolated double bonds (IDB), p-value = 0.0786; b – the concentration of dienic conjugates (DC), p-value = 0.0563; c – the concentration of trienic conjugates (TC), p-value = 0.0467; d – the concentration of oxodienic conjugates (ODC), p-value = 0.0912; and final products by type Schiff basics (ShB), p-value = 0.8338

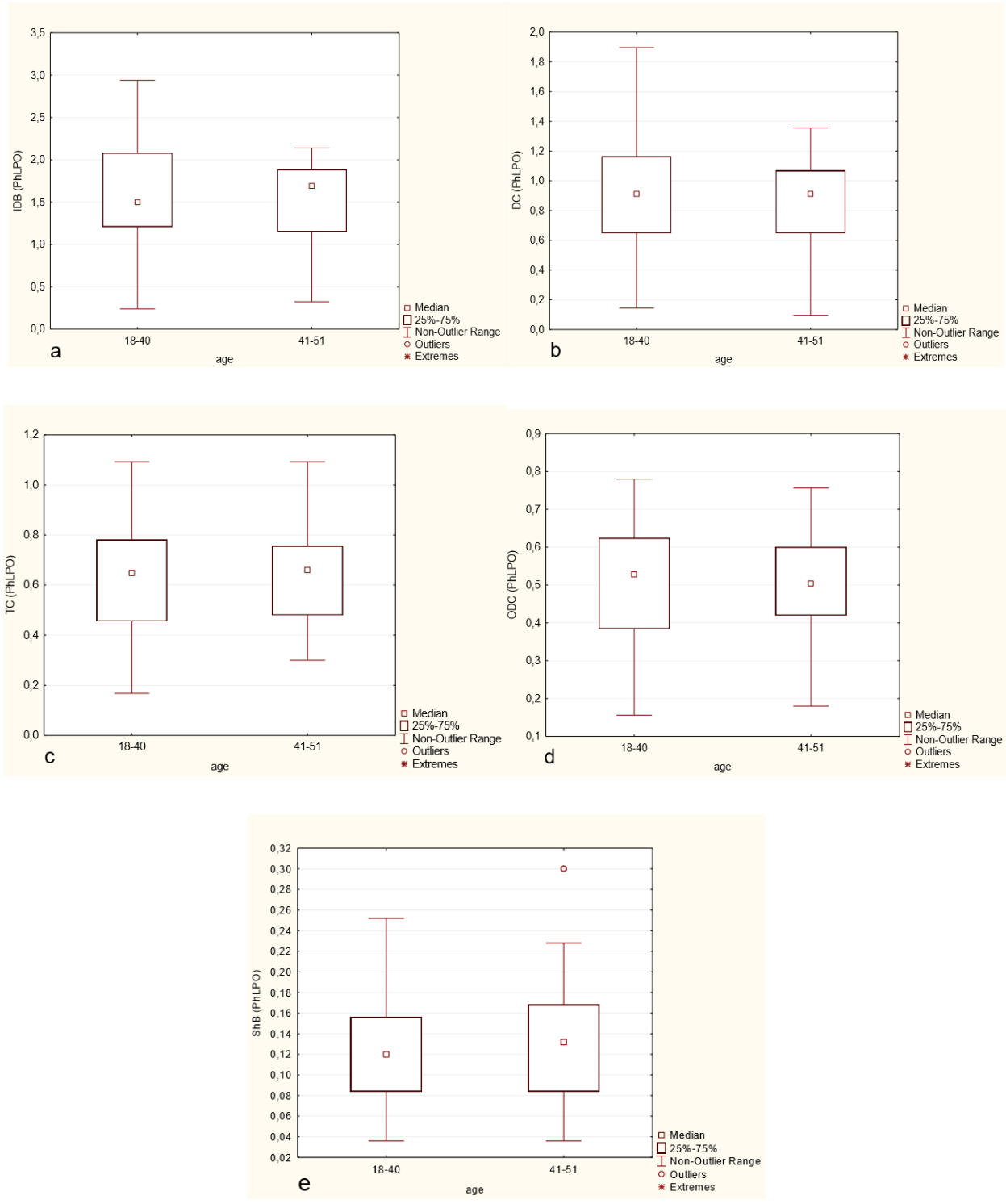

Figure 6 – The intergroup difference of median for phospholipids' peroxidation products (PhLPO, U/ml) in the donor peripheral blood (distribution by age: from 18 to 40, and from 41 to 51 years old) according to Mann–Whitney U test: a – the concentration of isolated double bonds (IDB), p-value = 0.7566; b – the concentration of dienic conjugates (DC), p-value = 0.7643; c – the concentration of trienic conjugates (TC), p-value = 0.6386; d – the concentration of oxodienic conjugates (ODC), p-value = 0.9841; and final products by type Schiff basics (ShB), p-value = 0.7643

As it turned out when comparing the data of donors, divided according to the above-mentioned grouping features, the activity of lipoperoxidation processes did not differ in the blood of female and male donors (fig. 3, 4). There was also no difference in groups divided by age (fig. 5, 6). This is evidenced by the significance levels of differences with respect to the absolute majority of lipo peroxidation products (p>0.05).

Some intergroup differences in biochemical indicators of venous donor blood, distributed in groups by the terms of donation, were also revealed.

A summary comparison of indicators of functional liver condition and iron metabolism selected for statistical significance is presented in Table 1. Other investigated indicators did not have statistically significant differences between the experimental and control groups (data not presented).

Table 1 – *Intergroup differences in indicators of functional liver status and iron metabolism in blood samples of wartime (group I) and peacetime (II) donors, which are statistically significant according to the Mann–Whitney test*

Indicators	Group	Reference values	Me	25 %	75 %	$P_{I–II}$
Thymol test, S-H U	I	0.00–4.00	1.50	1.24	1.91	0.001843
	II		2.30	1.80	2.90	
Direct bilirubin, μmol/l	I	2.20–5.13	2.03	1.23	2.30	0.000001
	II		0.86	0.83	0.90	
Indirect bilirubin, μmol/l	I	6.30–15.40	8.28	7.86	11.89	0.002437
	II		10.82	10.11	10.82	
ALT, μmol/(ml·h)	I	0.10–0.69	0.41	0.33	0.51	0.010873
	II		0.56	0.41	0.69	
ALP, ncat/L	I	820.00–2190.00	1658.00	1458.00	1773.00	0.000008
	II		1388.00	1223.00	1503.00	
γ-GGT, μcat/L	I	0.21–1.44	0.38	0.34	0.50	0.003310
	II		0.47	0.42	0.55	
Serum iron, μmol/L	I	9.15–28.45	20.00	15.65	22.65	0.006154
	II		15.00	12.20	18.90	
TIBC, μmol/L	I	44.80–76.10	69.80	67.15	72.00	0.021631
	II		73.25	67.90	76.40	
LIBC, μmol/L	I	25.00–48.00	49.75	46.50	54.85	0.027227
	II		56.00	47.10	63.00	
TSC, %	I	15.00–50.00	28.95	22.45	32.55	0.010395
	II		21.35	16.20	28.10	
Transferrin, g/L	I	2.00–2.60	2.68	2.58	2.77	0.012423
	II		2.82	2.62	2.95	

Note: ALT – alanine aminotransferase, ALP – alkaline phosphatase; γ-GGT – gamma-glutamyl transpeptidase; TIBC – total iron-binding capacity, LIBC – latent serum iron-binding capacity, TSC – transferrin saturation coefficient; Me – Median

The data obtained during non-parametric statistical analysis show that the indicators of the liver functional state in donors of both groups do not cross the reference values limits, although they have certain statistically significant differences (Table 1). Thus, direct bilirubin in the blood of wartime donors exceeds the control group data ($p = 0.000001$). All other data either do not have a significant statistical difference (data not presented) or are lower compared to the indicators of the II donor group. The latter include such dates as indirect bilirubin, ALT, ALP, thymol test, and γ-GGT.

Indicators of iron metabolism in both groups also do not cross the limits of reference values (Table 1). At the same time, the iron content of serum was 25% higher in donors of the 1st group, which had an effect on other calculated indicators of its exchange. So, Me TSC by 7.6% in the donors of the experimental group exceeds the control data. It should be noted that normally the percentage of transferrin saturation with iron is about 30%. This indicator decreases under conditions of insufficient intake of iron in the body. As for transferrin, the concentration reference indicators are slightly different for women and men (for women, they are higher), but in general, the content of transferrin should be in the range of 1.3 to 3.8 g/l. We observed exactly such normal values in both donor groups. At the same time,

the level of transferrin in the group of wartime donors was higher by 5.2%.

The calculated indicators of total and unsaturated serum iron-binding capacity (TIBC and LIBC) also, without going beyond the reference values, had statistical differences according to the I and II groups: the difference for Me TIBC was 4.9%; for Me LIBC – 12.6%.

In addition, indicators of general clinical blood analysis were investigated in the donor groups. According to the non-parametric Mann–Whitney test, statistically significant differences were found in eight indicators in the general blood analysis of donors of the I and II groups, namely: absolute content of erythrocytes, absolute content of leukocytes, and their differentiated indicators: absolute content of band neutrophils, segmented neutrophils, and basophilic white blood cells (WBCs), as well as between the relative indicators of the content of eosinophils, monocytes, and basophils.

A detailed analysis of the identified discrepancies in the indicators of the general blood analysis in the two donor groups is presented in Table 2. The data that did not have statistically significant discrepancies are not presented.

Table 2 – *The intergroup difference in the parameters of the general blood analysis of wartime (group I) and peacetime (group II) donors, which is statistically significant according to the Mann–Whitney test*

Indicators	Group	Reference values (for donors)	Me	25%	75%	$P_{I–II}$
RBCs, $\cdot10^{12}$/L	I	4.30–5.75	5.35	5.21	6.46	0.000118
	II		5.00	4.69	5.20	
WBCs, $\cdot10^{9}$/L	I	3.60–10.50	4.32	4.00	5.40	0.000063
	II		5.80	5.00	6.90	
Band neutrophils, $\cdot10^{9}$/L	I	0.00–0.40	0.07	0.04	0.12	0.001072
	II		0.13	0.08	0.20	
Segmented neutrophils, $\cdot10^{9}$/L	I	1.70–7.20	2.63	2.37	2.91	0.049369
	II		2.92	2.65	3.84	
Eosinophils, %	I	0.5–5.5	3.50	1.50	5.50	0.026311
	II		2.00	1.00	3.00	
Monocytes, %	I	2.0–9.5	0.12	0.06	0.22	0.000001
	II		0.45	0.34	0.57	
Basophils, %	I	0.0–1.8	1.00	0.75	2.00	0.000069
	II		0.00	0.00	0.00	
Basophils, $\cdot10^{9}$/L	I	0.00–0.20	0.05	0.03	0.09	0.000194
	II		0.00	0.00	0.00	

Note: RBCs – red blood cells, WBCs – white blood cells; Me – Median

As shown in Table 2, all eight indicators that have differences between groups of wartime and peacetime donors do not go beyond the reference limits. At the same time, such data as the content of erythrocytes, the relative content of eosinophils, basophils, as well as the absolute content of basophilic WBCs in the 1st group statistically reliably exceed the data obtained in the 2nd group with a high degree of statistical reliability.

Discussion

Metabolic reactions occurring inside the bags during PRBC storage lead to cell damage [27]. However, the initial quality of the harvested material is of critical importance for the dynamics of these processes. Above all, it depends on the state of the donor's health. The main goal of the today transfusiology practice is to reduce the risk of infectious and non-infectious complications during the transfusion of blood components. Obtaining medical and behavioral anamnesis from potential blood donors is one of the first steps in this process. However, PRBC metabolism is closely related to the donor systemic metabolism [8]. Therefore, in this study, we turned to the study of donor individual health indicators, which may have changes in wartime conditions.

We paid special attention to studying the activity of lipid peroxidation processes in the red blood cells of the donor. The important role of membrane LPO in cell biology and human health is well understood. At the same time, the value of such methods for the presented

study is due to the need to characterize the red blood cell initial parameters, as a cellular object of storage in the blood bank, as well as the leading role of the lipid bilayer, as the main component of the cell membrane, in maintaining its structural integrity. Here, the study of the wide range of LPO molecular products (substrates, primary, secondary, final) is used as a marker of pro-oxidant activity, which in the circumstances of significant activation disrupts the membrane structure with an inevitable effect on the functional ability of the cells. In particular, in this case, there is a loss of fluidity, and regulatory mechanisms are disturbed, including lipid-lipid and lipid-protein interaction, permeability, transport of ions and nutrients, functioning of signaling pathways, and metabolic processes. It should be noted that the molecular products of lipid peroxide breakdown studied here are considered the most useful tools for the detection and quantification of LPO in biological samples [14]. Therefore, in our study, among the important characteristics of donors' health indicators, particular attention was paid to the content of LPO products in venous blood. On the one hand, they are used as critical mediators of the development of pathological conditions in the donor's body; on the other hand, they are used as indicators of the packed red blood cell initial quality.

The research group consisted of donors from the Kharkiv region who had donated blood in 2023 under special conditions of extreme acute and chronic stress caused by hostilities, what, among other reasons, could also have an impact on red blood cell donation. In particular, the harsh experiences of war and man-made disasters are known to often cause disorders of varying severity degrees, including post-traumatic stress disorder (PTSD), depression, and deep-seated anxiety states. Research in the psychiatry field indicates the considerable duration of such consequences, especially in war trauma cases. Many people do not recover for three years.

From the point of view of public health in general and the health of donors in particular, the latest results of numerous studies demonstrating general changes in the functioning of various organs and systems in persons with the consequences manifestations of psychological trauma are extremely important. A number of epidemiologic studies have found associations with newly discovered metabolic disorders such as diabetes and abnormal lipid profiles [28, 29], which are risk factors for cardiovascular dysfunction and failure [30, 31]. Staying in extreme stressful conditions affects human hemodynamic, oxygen transport, microcirculation, and erythropoietin production [32]. Psychological stress disrupts iron metabolism and influences erythropoiesis [33]. Evidence from the past decade has confirmed the close relationship between the brain and the body as a whole in the context of exposure to stress/trauma. The key role of impaired immune regulation with a blood reaction in the extreme form of the manifestation of suffered psychological traumas, which is PTSD, has been shown recently [34–36]. The mass of such phenomena in the combat zone has a significant impact on public health and can indirectly have a negative impact on blood donation in view of the quality of its components, in particular PRBC.

The symptoms of such disorders reflect a distinct inability to restore homeostasis primarily through the peripheral blood, which serves as a conduit for neuroendocrine and immune signaling [37]. Dysregulation leads to inflammation, changes in the adaptive activities of the hypothalamic-pituitary-adrenal system, as well as the sympathetic nervous system sensitivity [38]. This affects both peripheral blood immune cells and neuroimmune dynamics in the brain.

In our study, we did not find changes that can accompany stress disorders of varying degrees. On the contrary, some of the obtained results, in particular, data on iron metabolism, in the donors of the experimental group looked slightly better compared to the control group. However, over time, under the influence of chronic and acute stress, regulation failure of all three main lines of hematopoiesis can be manifested. Unfortunately, biological dysregulation of markers of the immune and endocrine systems may become more apparent with increasing time after injury [35]. Such clinically valuable shifts with evidence of a significant correlation for the development of inflammatory responses have been demonstrated by several research groups recently [39–41].

At the same time, we found important data on indicators of pro-oxidant activity of wartime blood donors. According to our observations, there was a significant excess of LPO indicators in the experimental group, compared to the control. In this study, the method of measuring oxidative damage by the LPO product levels in biological samples of blood donors was used, since direct quantitative analysis of reactive oxygen species is considered a difficult task due to their high activity and short half-life [11]. At the same time, the reaction of oxygen with unsaturated lipids gives the oxidation product range (from substrates (IDB), primary, secondary (DK, ODK, TK), and to the final products (ShB) of LPO, which were investigated in this work. So, we found that the levels of the entire range of the molecular LPO products with a high statistical probability exceeded the data of the control group (from 1.7 to 17.7 times, depending on the studied indicators, $P_{I-II} = 0.000001$). This is important and can have a significant impact on the main quality parameters of both a freshly prepared dose of PRBC and one that is stored for a long time under normal conditions of a blood bank, as it is known that under such conditions the risks of

irreversible processes in the vital activity of the cell increase significantly. [11, 42, 43]. Such a direct relationship between the content of LPO products in the cellular components of umbilical cord blood prepared for cryopreservation and the cryosensitivity levels of its RBC, as well as granulocyte-macrophage hematopoietic cells, was already demonstrated by us earlier [44, 45].

In general, predicting the RBC response to different storage conditions is burdened by the complexity of relationships between biochemistry, cytoskeleton structure, and cell membrane properties. Heterogeneity between donors is manifested in variable susceptibility to hemolysis and subsequent post-transfusion increase in hemoglobin [46]. Therefore, such donor-related additional factors, not previously studied, should be considered extremely important for the biotechnological practices of manufacturing and long-term storage of PRBCs in blood banks.

CONCLUSIONS

It was shown that the data of the general blood analysis, protein metabolism, functional state of the liver, and iron metabolism in the blood samples of wartime and peacetime donors did not cross the limits of reference values. Still, that have certain statistically significant differences in some indicators, namely: the content of direct and indirect bilirubin, alanine aminotransferase, alkaline phosphatase, thymol test, and gamma-glutamyl transpeptidase. Indicators of iron exchange in donors of the research group exceeded the data of the control group in terms of such parameters as serum iron (by 25%), transferrin saturation coefficient (by 7.6%), and transferrin level (by 5.2%). The concentration of RBCs, the percentage of eosinophils, and basophils, as well as the absolute concentration of basophilic WBCs in the blood of donors belonging to the experimental group statistically significantly exceeded the data obtained in the control group.

At the same time, it was found that the indicators of blood pro-oxidant activity of wartime donors significantly exceed the indicators of peacetime donors. This was evidenced by concentration data on the entire spectrum of lipid peroxidation molecular products with an excess of the data for the control group from 1.7 to 17.7 times, depending on the type of such product. Such features may have significant implications for cell biology in the context of the PRBC transfusion medium stability, and therefore for the biotechnological practices of its manufacture and storage.

PROSPECTS FOR FUTURE RESEARCH

Since this is important from the point of view of understanding the factors affecting the quality of PRBCs, as well as finding ways to improve it, further studies should be devoted to the analysis of the relationship between established special parameters of the donor and the individual sensitivity of the harvested RBCs to standard conditions of long-term hypothermic storage.

AUTHOR CONTRIBUTIONS

All authors substantively contributed to the drafting of this paper. They take full responsibility for the integrity of all aspects of the work.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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