

ORIGINAL ARTICLE / ОРИГИНАЛНИ РАД

The reasons for unusable lipemic blood plasma in transfusion treatment

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Introduction/Objective The increased presence of lipid particles in blood is one of most common reasons that transfusion units are unusable. The risk factors for lipemic plasma in donated blood are not completely known. The aim of this study is to identify the factors that influence plasma to be fatty so that we can prevent further storage costs and eliminate unusable transfusion units.

Methods This case-control study was conducted in 2017, and 1552 respondents were included in the study. The control group included 1502 subjects whose blood was not lipemic, while 50 patients with lipemic blood were selected for the case group. The presence of lipemic blood was assessed by inspection, while data were collected by clinical laboratory tests and a questionnaire.

Results Our findings show that multiple blood donors with lipemic blood were significantly older ($p < 0.0005$) and have higher systolic and diastolic pressure ($p < 0.0005$), high triglyceride levels ($p < 0.0005$), and lower levels of hemoglobin ($p < 0.0005$). Additionally, the presence of lipemic plasma was associated with female sex ($p = 0.002$), blood type ($p = 0.016$), heart disease ($p < 0.0005$), smoking ($p < 0.005$), diabetes ($p = 0.001$), lipid intake prior to blood donation ($p < 0.005$) and venipuncture therapy ($p < 0.0005$). Systolic pressure is a reliable predictor of lipemic blood (AUROC = 0.901, $p < 0.0005$).

Conclusion Our study provided a rational explanation and identified some of the risk factors that may help identify potential donors with lipemic blood.

Keywords: blood transfusion; blood donors; plasma; preventive measures; cholesterol; triglyceride

INTRODUCTION

The availability of safe blood and blood products has always been a key strategy for addressing health-related challenges [1, 2, 3]. In transfusion practice, blood and blood components with changed plasma color are often present [4]. Color changes occur due to factors such as hemolysis, bacterial contamination, bubble formation, the presence of clots and fibrin strands and bright yellow to brown lipemic plasma with a specific “strawberry milkshake” appearance [4]. The increased presence of lipid particles in the blood is called lipemia and is one of most common reasons why transfusion units are unusable [4]. The causes of occurrence are different, but lipemia is usually associated with excessive nutrition [2, 5]. Donors who eat a fatty meal before donating blood are known to have an increased plasma triglyceride concentration for several hours. [6]. Other causes of lipemia include conditions such as obesity, diabetes mellitus, Cushing disease, acromegaly, nephritic syndrome, hypothyroidism, pregnancy and the use of various medications [4].

Increased blood fat values are associated with atherosclerotic diseases and heart disease

and greatly increase the possibility of the occurrence of blood pressure and stroke [7]. Low-calorie nutrition and some foods, such as vitamins, minerals, and unsaturated fatty acids (omega 3), reduce the level of fat in blood [7].

There are no up-to-date international transfusion guidelines [4]. Therefore, some transfusion centers use visual inspection to detect the turbid appearance of plasma [8]. Further, the effects of lipemic plasma or platelet donations on recipients are not known [4]. Similarly, some countries consider lipemic units acceptable for transfusion [4]. However, excessive lipemia obstructs testing of all blood components and is a rational reason to discard such blood units [4]. This has been applied in our practice in compliance with the Blood Safety Strategy implemented by the Government of the Republic of Montenegro and Institute for Blood Transfusion of Montenegro since 2006 and in accordance with EU Directives 2002/98 EC and the WHO resolution on the availability, safety and quality of blood products.

We tried to discover the collaboration and the origin of the factors that affect the occurrence of increased fat content in donated blood. Knowledge about risk factors and what

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is causing the risk may provide options for the prevention of lipemic plasma donation. The presence of large numbers of such transfusion units in the future could lead to an even greater increase in costs related to the process and length of this type of treatment [1, 9].

METHODS

Ethical approvals

The study was conducted at the Blood Transfusion Institute of Montenegro, Bar Organizational Unit, Bar, Montenegro. Patients included in the study gave their informed consent, and the research project was approved by the ethics committees of the University Medical Center Kragujevac, Serbia. Additionally, the study adhered to the Principle of Good Clinical Practice and the Declaration of Helsinki at all times.

Study design and study population

This case–control study was conducted in 2017. In the observation period, 1639 respondents self-reported as potential blood donors, of whom 127 were rejected as ineligible. The survey included the remaining 1552 respondents (1512 seemingly healthy service providers and 40 patients treated with venipuncture). Of the 1512 healthy donors, 10 gave lipemic blood (Table 1).

Table 1. Study participants

Respondents	n	Normal plasma (control group)	Turbid plasma (case group)
Potential blood donors	1639	-	-
Rejected as ineligible	127	-	-
Seemingly healthy service providers	1512	1502	10
Patients treated with venipuncture	40	0	40
Total	1552	1502	50

The respondents were divided into two groups. The control group consisted of 1502 subjects whose blood was not lipemic and consisted exclusively of healthy donors. For the group of cases, 50 subjects with lipemic blood were selected, which consisted of the healthy donors who gave lipemic blood – 10 previously mentioned persons – as well as patients treated with therapeutic venipuncture – 40 sick persons (Table 1).

The basic criteria for assessing donor eligibility were 18–65 years of age, body weight over 55 kg, hemoglobin values (in males, higher than 135 g/l; in females, higher than 125 g/l), blood pressure values (systolic 120–150 mmHg; diastolic 80–100 mmHg) and the presence of elevated glycemia (greater than 6.1 mmol/l). To examine the factors whose elevated values affect the appearance of lipemic blood, we included 40 patients undergoing venipuncture (hypertension, other cardiovascular disease, obesity, and blood with an elevated erythrocyte count) in the study. According to the clinical and laboratory findings,

these subjects are not eligible as donors and based on the presence or absence of blood fat, were grouped into a control or a case group. Subjects who were undergoing therapeutic venipuncture included persons 65 years and older who weighed significantly more than 55 kg and had elevated values of the parameters tested (blood pressure, hemoglobin levels, and glucose levels) and/or the presence of chronic diseases (diabetes and cardiovascular disease).

Data collection

Before giving blood, all subjects routinely had their blood type and Rh factor determined and laboratory glucose and hemoglobin values, body weight, and blood pressure values measured. Respondents completed a questionnaire compiled according to European blood donation directives, providing data on sex, age, habits (alcohol and smoking), the presence and type of chronic diseases, previous injuries and surgeries, and the possible presence of infectious and blood-transmissible diseases. Based on the survey questionnaire and clinical laboratory analyses, part of the analyzed data was collected, and donor eligibility was assessed.

Blood collection and laboratory analyses

A total volume of 350–450 ml of blood was collected into standardized blood bags of foreign production (Terumo Corporation, Tokyo, Japan). Citrate-phosphate-dextrose-adenine (CPD) and CPD A1 were used as anticoagulants in the blood bags, each with a duration of 28–42 days. The majority of blood was collected at the Blood Transfusion Department in Bar (80%), while the rest of the blood was collected as voluntary blood donations in the field (20%). The blood units taken were stored at 4°C. After 3–5 hours, by inspection, we detected and recorded the presence of turbid and greasy contents in the bags. Milky and fatty units were removed, while unchanged units were sent for centrifugation and then divided into fractions. All blood units were processed according to the Standard Operational Procedures for treatment with blood and converted into two fractions: fresh frozen plasma and concentrated erythrocytes [1, 10]. Plasma units in which milky color and lipemic content were detected were recorded and eliminated from further use, as were erythrocytes obtained by this procedure. The processing was performed by adequately trained staff on the appropriate standardized equipment.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 20.0. (IBM Corp., Armonk, NY, USA). Continuous variables were presented as the mean \pm standard deviation. Comparisons between two groups were analyzed by t-test or the Mann–Whitney U-test. Comparisons of categorical data between the groups were performed by the χ^2 test or the Fisher's exact test. Univariate and multivariate binary logistic regression analyses, including odds ratios, were performed to determine the effects of each factor on the dependent variable

Table 2. Lipid levels in the study

Types of analyses mmol/l	Turbid plasma SD	Turbid plasma %	Normal plasma SD	Normal plasma %	SD 95% CI	p X ²
Total cholesterol	0.98	1.96	1.88	0.12	-0.10 (-0.63–0.54)	
Desirable (< 5.2)	36.97	73.94	1360.5	90.57		
Borderline high (5.2–6.1)	9.24	18.48	124.6	8.25		
Low (> 6.2)	2.52	5.04	15.02	1		
HDL	0.11	0.22	4.95	0.33	4.84 (4.43–5.29)	< 0.01
High (> 1.55)	0.25	0.5	688.96	45.87		
Medium (1.03–1.54)	4.29	8.58	711.94	47.4		
Low (< 1.03)	45.35	90.7	96.12	6.4		
LDL	0.86	1.72	21.02	1.4	20.17 (18.46–22.054)	< 0.01
Low (< 1.55)	0.315	0.3	132326	88.1		
Medium (1.55–4.52)	3.94	6.08	139.68	9.3		
High (> 4.53)	45.9	91.8	18.02	1.2		
Triglycerides	0.87	1.75	24.7	1.6	23.83 (21.82–26.057)	< 0.01
Normal (< 1.70)	1.6	3.2	1108.73	73.8		
Borderline high (1.7–2.25)	0.75	1.5	184.30	12.3		
High (2.26–5.64)	25.6	51.2	181.27	12.1		
Very high (> 5.65)	21.1	42.35	3	0.2		
Total	50	100%	1502	100%		

HDL – high-density lipoprotein; LDL – low-density lipoprotein

Table 3. Numerical variables in relation to lipemic blood

Variable	Not lipemic blood	Lipemic blood	p
	Arithmetic mean +SD	Arithmetic mean + SD	
Age	40.74 ± 11.72	57.1 ± 12.47	< 0.0005
Systolic pressure (mmHg)	129.77 ± 6.62	156.1 ± 15.98	< 0.0005
Diastolic pressure (mmHg)	86.29 ± 5.58	102.8 ± 10.46	< 0.0005
Hemoglobin (g/l)	143.88 ± 5.58	138.08 ± 4.66	< 0.0005
Weight (kg)	84.68 ± 5.84	87.84 ± 6.8	0.002

Table 4. Lipemic blood compared to categorical variables

Variable	Lipemic blood	Non-lipemic blood	p
Sex	Male	40 (2.8%)	0.002
	Female	10 (8.5%)	
Blood type	A	11 (1.8%)	0.041
	B	13 (5.6%)	
	AB	4 (3.8%)	
	O	22 (3.6)	
Professional qualifications	Basic and less	16 (15.8%)	< 0.0005
	Medium	33 (2.3%)	
	High and higher	1 (2.7%)	
Retiree	No	47 (3%)	0.001
	Yes	3 (60%)	
Heart disease	No	42 (2.7%)	< 0.0005
	Yes	8 (57.1%)	
High blood glucose	No	48 (3.1%)	0.001
	Yes	2 (100%)	
Venipuncture therapy	No	28 (1.9%)	< 0.0005
	Yes	22 (55%)	

(fatty blood). A receiver operating characteristic (ROC) curve was generated, and the area under the curve (AROC) was calculated. Sensitivity and specificity for the optimal cut-off value were calculated. Differences were considered significant at $p < 0.05$.

RESULTS

During the study, out of the 1639 people who volunteered for donation, a total of 127 were rejected (7.8%) for various reasons. Seemingly healthy service providers (1512) and patients treated with venipuncture (40) constituted a group of 1552 respondents. Of this number (1552), 50 individuals donated lipemic blood that was then discarded (3.2%), of which 10 units were from seemingly healthy donors and 40 units were from sick persons treated with venipuncture (Table 1). In developed countries of Europe, this percentage ranges 0.05–0.15%.

The total cholesterol level was 0.1 (-0.63 to -0.54) higher in turbid/lipemic plasma cases than in the control group. Additionally, high-density lipoprotein cholesterol levels were higher in donors with turbid plasma than in the control group 84 (4.43–5.29), and low-density lipoprotein cholesterol was 20.17 (18.46–22.05). The value of triglyceride was also higher at 23.83 (21.82–26.05) in donors from the case group compared to those from the control group who gave normal plasma (difference, 95% CI) (Table 2).

The donors whose blood was lipemic were significantly older ($p < 0.0005$) and had higher systolic and diastolic pressure ($p < 0.0005$), a higher body mass index ($p = 0.022$), and a lower level of hemoglobin ($p < 0.0005$) (Table 3). Women ($p = 0.002$), diabetic patients ($p = 0.001$), and donors with heart disease who were treated with venipuncture ($p < 0.0005$) had a higher percentage of lipemic blood (Table 4). Interestingly, the occurrence of lipemic blood depended on the blood group ($p = 0.041$), professional qualification ($p < 0.0005$) and occupation ($p < 0.0005$) (Table 4). Fear, Rh factors, injuries, alcoholism, psychoactive substances, suspicion of the presence of transmissible diseases, places of blood collection, other diseases and seasons were not statistically significantly related to the donation of unusable lipemic units.

The univariate binary logistic regression showed that the appearance of fatty blood was influenced by age ($p < 0.0005$), systolic pressure ($p < 0.0005$), diastolic pressure ($p < 0.0005$), hemoglobin level ($p < 0.0005$), female sex ($p = 0.001$), blood group A ($p = 0.016$), primary schooling ($p < 0.0005$), working status retiree ($p < 0.0005$), and venipuncture therapy ($p < 0.0005$) (Table 5). Multivariate binary logistic regression showed

Table 5. Influence of examined variables on the occurrence of lipemic blood

Variable	Univariate binary regression		Multivariate binary regression	
	Risk quantity	p	Risk quantity	p
Age	1.132 (1.099–1.165)	< 0.0005		
Systolic pressure (mmHg)	1.168 (1.139–1.197)	< 0.0005	1.166 (1.138–1.196)	< 0.0005
Diastolic pressure (mmHg)	1.246 (1.201–1.293)	< 0.0005		
Hemoglobin (g/l)	0.766 (0.716–0.818)	< 0.0005		
Female	3.259 (1.586–66.98)	0.001	3.218 (1.185–8.738)	0.022
Blood group A	0.435 (0.221–0.856)	0.016		
Primary school	7.845 (4.165–14.777)	< 0.0005		
Pensioner	47.872 (7.814–298.282)	< 0.0005		
Therapeutic venipuncture	64.778 (31.326–133.95)	< 0.0005		

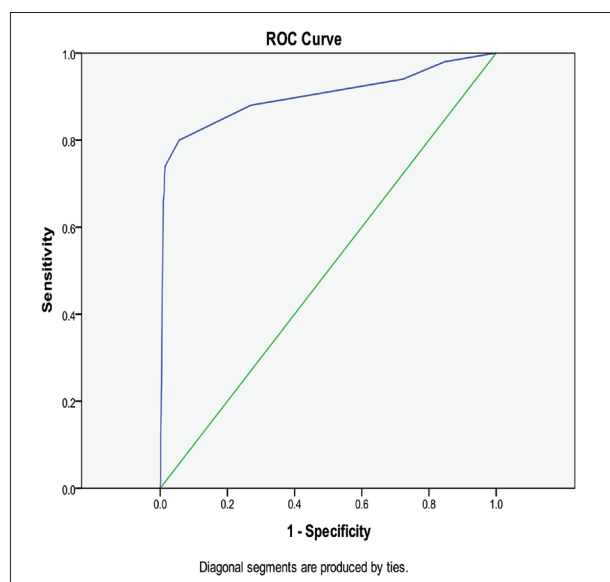


Figure 1. Receiver operating characteristic (ROC) curve for systolic pressure; systolic pressure may be a marker indicating to lipemic blood (the area under the ROC = 0.901, $p < 0.0005$) if we use systolic pressure cut-off value of 140 mmHg (sensitivity = 0.740, specificity = 0.985; negative predictive value = 0.991, and positive predictive value = 0.627); 94.3% of blood donors with systolic pressure above 150 mmHg had lipemic blood

that the appearance of fatty blood affected systolic pressure ($p < 0.0005$) and female sex ($p = 0.022$) (Table 5). The risk quantity for systolic pressure was 1.166 (1.138–1.196), and for females it was 3.218 (1.185–8.738) (Table 5).

The ROC curve shows that systolic pressure may be a marker indicating lipemic blood (AUROC = 0.901, $p < 0.0005$) (Figure 1).

DISCUSSION

Blood treatment is an expensive process, and blood collection has always been extremely demanding and difficult,

especially in countries where the standard of living is quite low. The leading cause of unusable blood units in our research is the presence of fat content in fresh frozen plasma produced immediately after collecting blood. We showed that the percentage of unused lipemic blood units is 22.1%, which is quite high. The reasons for unusable plasma units are also found in some insufficiently educated blood donors in terms of diet before giving blood. As all the blood collected in our blood transfusion department is processed into fresh frozen plasma, it is only then that it is possible to notice the fat content in the bag with the plasma. Such a plasma bag, due to the unauthorized presence of fat inside, automatically becomes unusable [11, 12]. In line with this, milky plasma due to the presence of fat constitutes the strictest ban on its use in transfusion treatment [13]. Vassallo and

Stearns [3] reported a detailed study of the occurrence of lipemic plasma, its composition – particularly the level of triglycerides that is significant for the onset of many diseases and draws attention to the habits of donors, risk factors and the possibility of removing these phenomena. de Kort et al. [14] observed the reasons for the refusal of blood donors following the relationship between the Dutch and the general population, as well as the possibility of disease occurrence with the popularization of a healthy lifestyle. Some authors try to introduce new methods for treating various metabolic diseases, especially fatty liver and diabetes [15]. This is possible by the identification of bioactive lipids and their mechanisms of action [16].

The problem of the presence of fat in donor blood and the consequent association with the onset of cardiovascular diseases has long attracted the attention of many authors [1, 2, 17, 18, 19]. Wittcock et al. [20] observed the demographic characteristics of donors across Europe, bringing them into the relationship between the presence of fat in the blood and the onset of cardiovascular diseases. One of the leading causes of these diseases is the presence of fat in donor blood [20]. On the appearance of fat in donor plasma, Peffer et al. [2] pointed out in his study that it is linked to risk factors and the occurrence of many heart and blood vessel diseases. The aim of this study was to objectively determine clouding and to identify risk factors for cloudy plasma [2]. Donors that have given cloudy plasma have a less favorable cardiovascular profile than other donors [2]. The most common independent risks are consuming dinner, high levels of triglycerides and smoking [2]. Adverse health behaviors (smoking, poor diet, higher body mass index, and unhealthy lifestyle) combined with risk factors (hyperlipidemia, hypertension, and diabetes) lead to the development of cardiovascular diseases (85–95%) [17]. Elimination of unhealthy habits and using aspirin can help in the treatment [17]. O'Neill et al. [21] found several factors associated with what we assessed to be “appropriate” documentation of risk factors sufficient for cardiovascular risk assessment, such as an

increasing number of clinical encounters, male sex, and increasing BMI and age. The focus of the research was the relationship between the composition of lipid plasma and the appearance of cardiovascular diseases, as well as elevated serum lipid levels and the appearance of coronary heart disease [18, 19]. Many risk factors that lead to the onset of this disease have been described [19]. Goel et al. [1] showed the risks of cardiovascular disease due to the presence of fat in donor blood and the manner of their care and treatment. Otherwise, cardiovascular diseases prolong the length of treatment for sick donors, shorten their length and quality of life and permanently eliminate them from the blood donation process [2, 12].

Some of the authors have also observed sociological moments that influence donor motivation for giving blood [1, 22, 23]. Their goal is to increase the number of healthy blood donors. In two independent studies, the authors showed a change in the behavior of blood donors, according to the diverse social circumstances in which they are found. They emphasize the importance of sociological institutions in increasing the number of fully healthy blood donors [22, 23]. Unequivocally, it is easier to motivate a satisfied donor to give blood [24]. In our community, quite a large number of blood donors are unemployed, and they have a major problem supporting themselves; therefore, it is difficult to motivate them to give blood. In addition, they have poor and irregular nutrition, which certainly affects the presence of fat in their blood [25].

Most authors advocate mandatory testing of the level of fat in blood donors. In this way, it would be possible to profile the production of lipids in blood donors, which would contribute to more effective treatment of persons with an increased content of fat in their blood [26]. The presence of fatty blood from donors suggested the standardization of detection through testing, as well as documentation that contributes to the optimal resolution [27]. In a case-control study, Si et al. [28] analyzed 61 lipidomic markers in baseline plasma using targeted nuclear magnetic resonance spectroscopy. Pechlaner et al. [10] showed patents for cardiovascular diseases biomarkers that refer to the standardization of blood lipidemia measurements. Beginning in 2016, in our practice, all donors with a recorded finding of increased fat in plasma bags, present in two consecutive cases after giving blood, are personally informed of the findings and are obliged to undergo a medical examination.

Our study indicates that older donors, higher blood pressure, lower levels of hemoglobin, increased body weight, profession pensioner, venipuncture therapy, and some blood groups significantly increase the risk of fat presence in blood or plasma (Tables 3 and 4). The reason is irregular diet, low social status of donors, and the more frequent occurrence of disease among elderly persons. We showed that the value of systolic pressure is a reliable predictor of lipemic blood (Figure 1). Previous research has shown the association between the presence of fat in the blood and older age and the time of day when the blood

was taken [29]. Female sex and lower education levels (primary school and less) also lead to a higher risk of blood being fatty (Table 4). It has been proven that women are more inclined to increase body mass than men. People with lower levels of education consume higher amounts of fat in their diets compared to people with higher education levels [14]. The blood of donors with blood group A has a lower risk of being fatty than the blood of donors in other blood groups. This data has to be regarded with certain amount of reserve due to the fact that there was a relatively small number of tested donors with blood type A (11 donors). Our findings could be verified in a more extensive study in the future. Blood group B donors have a higher risk of fat presence in their blood. In our community, the highest percentage of individuals in blood group B are members of the Roma population, and their diet can explain the increased presence of fat in the blood of donors with this blood group (Table 5).

We have also introduced continuous education of blood donors by blood transfusion teams in our service, which, through lectures and written flyers for donors, point out the danger of the presence of an elevated level of fat in the blood, resulting in severe cardiovascular disease. A healthy lifestyle is proposed, as well as proper nutrition with reduced intake of fat through meals.

CONCLUSION

Our study provided a rational explanation and identified some of the risk factors that may help in the identification of potential donors with lipemic blood.

Determining the presence of these risk factors in donors and excluding them from the blood donation process can reduce the cost of storage and elimination of lipemic blood units. Additionally, such testing can assist in the early diagnosis and timely treatment of some chronic diseases of potential "healthy" donors. The detection of such blood donors and the speed of their care and treatment must be priorities in the future. Further, larger studies are necessary to confirm the effects of lipemic blood units and the safety of their recipients.

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NOTE

Authors Dragan Radonjić and Saša Raičević have contributed equally to this work.

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Разлози неупотребљивости липемичне крвне плазме у трансфузиолошком лечењу

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САЖЕТАК

Увод/Циљ Повишено присуство липидних честица у крви је један од најчешћих разлога неупотребљивости трансфузиолошких јединица. Фактори ризика за липемичну плазму код давалаца нису потпуно познати.

Циљ рада је био утврђивање фактора који утичу на настајак липемичне плазме, чиме би се спречили, последично, даљи трошкови складиштења и уклањања неупотребљивих трансфузиолошких јединица.

Методе Студија случаја и контрола спроведена је током 2017. године и укључена су 1552 испитаника. Контролна група је обухватала 1502 испитаника чија крв није била липемична, док је у испитивану групу одабрано 50 болесника са липемичном крвљу. Присуство липемичне крви утврђивано је инспекцијским прегледом, док су подаци прикупљени лабораторијским тестовима и анкетним упитником.

Резултати Наши налази показују да вишеструки даваоци крви, чија је крв липемична, припадају следећим категоријама: значајно старији ($p < 0,0005$), имају виши систолни и дијастолни притисак ($p < 0,0005$), висок ниво триглицерида ($p < 0,0005$) и нижи ниво хемоглобина ($p < 0,0005$). Такође, присуство липемичне плазме повезано је са женским полом ($p = 0,002$), крвном групом ($p = 0,016$), болестима срца ($p < 0,0005$), пушењем ($p < 0,005$), дијабетесом ($p = 0,001$), као и са уносом масне хране пре давања крви ($p < 0,005$) и терапије венепунктуром ($p < 0,0005$). Систолни притисак је позудан предиктор липемичне крви ($AUROC = 0,901$, $p < 0,0005$).

Закључак Наша студија је пружила рационално објашњење и идентификовала је неке од фактора ризика који могу помоћи у идентификацији могућих давалаца липемичне крви.

Кључне речи: трансфузија крви; даваоци крви; плазма; мере превенције; холестерол; триглицериди