# Immunological Aspects of Carbohydrate and Lipid Metabolism in Patients with Periprosthetic Infection and Osteomyelitis

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# Immunological Aspects of Carbohydrate and Lipid Metabolism in Patients with Periprosthetic Infection and Osteomyelitis

## Immunologische Aspekte des Kohlenhydrat- und Lipidstoffwechsels bei Patienten mit periprothetischer Infektion und Osteomyelitis

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glucose, cholesterol, infection, sensitization, antibodies

**Bibliography**

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### ZUSAMMENFASSUNG

**Einleitung** Die Behandlung von Patienten mit periprothetischer Infektion und Osteomyelitis ist recht kompliziert. Das Ziel dieser Studie war es, den Kohlenhydrat- und Lipidstoffwechsel sowie das immunologische Profil bei Patienten mit periprothetischer Infektion und Osteomyelitis zu untersuchen.

**Material und Methoden** Eine Studie zum Kohlenhydrat- und Lipidstoffwechsel und zur immunologischen Untersuchung von Patienten mit periprothetischer Infektion nach Knie- und Hüftgelenkendoprothetik (n = 16) und Osteomyelitis (n = 20) wurde durchgeführt.

**Ergebnisse** Patienten mit einer periprothetischen Infektion neigten zu Anämie, gestörter Glukosetoleranz, einer Veranlagung zu verstärkter Atherogenese, Autoimmun-Granulozytotoxizität und einer Sensibilisierung gegen die Synovialmembran und Staphylococcus aureus. Patienten mit Osteomyelitis zeigten eine Abnahme der Produktion des Leukozyten-Migrationshemmungsfaktors (LIF), sowohl unspezifisch als auch spezifisch gegenüber den Antigenen des Knorpelgewebes sowie eine Sensibilisierung gegenüber Streptokokkus und E. coli. Bei den untersuchten Patienten wurden die Störungen des Glukosestoffwechsels mit dem Vorhandensein von autoimmunen lymphozytotoxischen Antikörpern, einer verminderten Produktion von LIF, sowohl unspezifisch als auch spezifisch gegen die Antigene der Synovialmembran in Verbindung gebracht. Die Atherogenese war mit einer erhöhten Anzahl zirkulierender Immunkomplexe, autoimmunen lymphozytotoxischen und granulozytotoxischen Antikörpern, einem verminderten LIF und einer Sensibilisierung gegenüber Antigenen des Bindegewebes, Streptokokkus und Proteus assoziiert. Eine Anämie war mit einem Anstieg der autoimmunen lymphozytotoxischen Antikörpern, einer verzögerten Sensibilisierung gegen Knochen- und Knorpelgewebe, gegen Staphylokokken und einer beschleunigten Sensibilisierung gegen E. coli und Proteus assoziiert.

**Schlussfolgerungen** Ärzte sollten bei der Behandlung und Überwachung von Patienten mit Bindegewebesinfektion und einem gestörten Glukose- und Cholesterinstoffwechsel auch immunologische Aspekte analysieren.

### ABSTRACT

**Introduction** Treatment of patients with periprosthetic infection and osteomyelitis is rather challenging. The objective of the research was to evaluate the metabolism of carbohydrates and lipids and immunological profile in patients with periprosthetic infection and osteomyelitis.

**Material and methods** A study on carbohydrate and lipid metabolism and immunological examination of patients with periprosthetic infection after knee and hip joint endoprosthetics (n = 16) and osteomyelitis (n = 20) was conducted.

**Results** The patients with periprosthetic infection were characterized by anemia, impaired glucose tolerance, predisposition to enhanced atherogenesis, autoimmune granulocytotoxic antibodies and sensitization to synovial mem-

brane and Staphylococcus aureus. The patients with osteomyelitis showed decrease of production of the leukocyte migration inhibition factor (LIF), both nonspecific and to the antigens of cartilage tissue, as well as sensitization to Streptococcus and E. coli. In the patients studied glucose metabolism disturbances have been associated with the presence of autoimmune lymphocytotoxic antibodies, reduced production of LIF, both nonspecific and to the antigens of synovial membrane. Atherogenesis was associated with increased circulating immune complexes, autoimmune lymphocytotoxic and granulocytotoxic antibodies, decreased LIF and sensitization to the antigens of connective tissue, Streptococcus and Proteus. Anemia was associated with increased autoimmune lymphocytotoxic antibodies, delayed type sensitization to bone and cartilage tissue, to Staphylococcus and sensitization by accelerated type to E. coli and Proteus.

**Conclusions** Physicians should analyze immunological data while treating and monitoring the patients with connective tissue infections and disturbances of glucose and cholesterine metabolism.

## Introduction

Treatment of patients with periprosthetic infection and osteomyelitis presents many difficulties for clinicians and requires complete information not only on the type of the pathogen and its sensitivity to medication, but also on the activity of immune processes, proinflammatory and antiinflammatory status of the patient, biochemical and immunological background. The use of antimicrobial therapy is known not to be always effective. Balanced cooperation of carbohydrates, lipids, enzymes, hormones, cytokines and immune cells is rather important for the successful healing of connective tissue. Disturbances of glucose and lipid metabolism contribute to the development of infection in these patients. On the other hand, there is a limited number of studies on specific immunological parameters in disturbances of glucose metabolism, such as leukocyte migration inhibition factor (LIF) and leukocyte migration activity induced by specific antigens. LIF is known to inhibit the migration of polymorphonuclear leukocytes in vitro in response to antigens or mitogens. LIF is involved in the host defense against bacteria as well as in the tissue-specific mechanisms of damage [1, 2]. LIF has emerged as a key factor of inflammatory diseases, nevertheless its associations with glucose intolerance and involvement into autoimmune processes have not been reported.

Disturbances in carbohydrate and lipid metabolism are usually considered as a distinct disorder without taking into account their possible associations with overgrowth of the certain pathogen microflora. Nevertheless recent studies have shown an important interrelationships of glucose intolerance and obesity with metabolism of certain bacteria [3]. Most of the studies have been performed on animals and human data are still lacking.

The contribution of intestinal bacterial strains (gut microbiota) in human metabolism and obesity is being increasingly recognized. This “external endocrine organ” has started to gain acclaim as a regulator of obesity, low-grade inflammation, insulin resistance, and diabetes mellitus. Various links have been proposed between the intestinal microbiome, obesity, and altered glucose and lipid metabolism [4]. Thus, obese patients and patients with diabetes mellitus type 2 (T2DM) have a moderate decrease in intestinal microbiota diversity with changes of the 2 dominant bacterial divisions, the Bacteroidetes and the Firmicutes. Moreover, metagenome-wide association studies showed that patients with insulin resistance as well as T2DM had a decrease in important butyrate producing bacteria (Faecalibacterium prausnitzii and Roseburia species) and an increase in Gram-negative, lipopolysaccharide (LPS)-containing pathogens such as Escherichia coli. Because LPS has been shown to promote the accumulation of macrophages, the studies suggest that an altered (trained) innate immune response of macrophages might be crucial in the development of bacterial translocation and subsequent chronic inflammation [5]. In this regard, it is noteworthy that the DNA of intestinal bacteria has been found in mesenteric visceral adipose tissue of mice fed a high-fat diet. Indeed, bacterial DNA load in plasma was shown to be an independent marker of development of T2DM. The authors suggest a causal connection between malfunctioning of the innate immune system and development of metabolic syndrome.

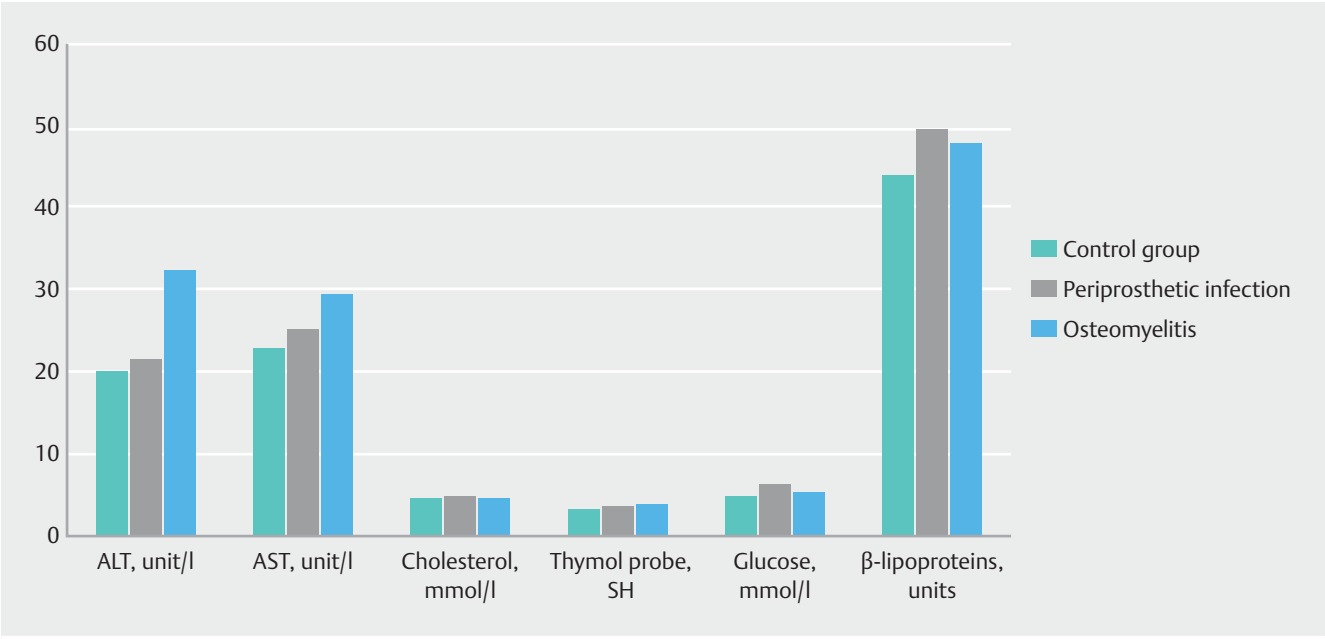
The conducted studies revealed difficulties in the treatment of the patients with bone and joint pathology [6, 7] and the patients with impaired carbohydrate and lipid metabolism are highly predisposed to the development of infection.

Therefore, the aim of the present work was to evaluate the associations of metabolism of carbohydrates and lipids with immunological profile in patients with periprosthetic infection and osteomyelitis.

## Materials and methods

The biochemical and immunological examination of the patients with periprosthetic infection after knee and hip joint endoprosthetics (n = 16) and osteomyelitis (n = 20) was performed. The patients were divided into 2 groups. The first group consisted of 16 patients (10 females and 6 males) and the second group comprised 20 patients (11 females and 9 males). The control group consisted of 20 healthy donors of the corresponding sex and age.

Periprosthetic infection developed within 1 year after arthroplasty and presented in discomfort, pain and loss of proper function of the joint. The patients needed repeated surgery and bacterial investigation of synovial fluid was performed. The major bacteriological findings were Staphylococcus aureus, St. epidermidis, Streptococcus pyogenes and Candida Lusitaniae.




▶ **Fig. 1** Biochemical parameters of the studied patients. Note: ALT = alaninaminotransferase, AST = aspartataminotransferase.

Patients were aged from 41 to 68 years, on average 61.4 ± 2.10 (group 1) and 57.3 ± 1.8 years old (group 2).

To obtain the sera, the samples of blood were kept during 30 minutes at 20 °C and then centrifuged at 1500 rpm for 15 minutes. The leukocytes were obtained after adding heparin (in dilution – heparin:whole blood = 1:100).

Complement of the guinea pig (Pharmstandard-Biolek, Ukraine) was used in dilution with the serum in proportion 1:1.

The content of cholesterine, glucose, glycoproteins, calcium, chondroitine sulfates, alkaline phosphatase, total protein, ALT, AST and urea were measured in the serum by biochemical methods [8]. Determination of circulating immune complexes in serum was performed with polyethyleneglycol 6000 by precipitation method [9]. The levels of IgA, IgM and IgG in serum were measured by ELISA kits (Human IgG total ELISA, Human IgA total ELISA, Human IgM total ELISA, eBioscience, Austria).

LIF production was estimated in leukocyte migration inhibition (LMI) assay by a capillary migration technique [1, 10]. To reveal the bacteria induced leukocyte migration activity antigens of Staphylococcus aureus (St. aureus), Streptococcus pyogenes (Str. pyogenes) and Pseudomonas aeruginosa (Ps. aeruginosa) were used. The strains of bacterial cultures were killed by heating. In order to estimate LMI to the heart tissue antigens we used tissue homogenates on 0,9 % NaCl solution with a final protein content of 100 μg/ml. Leukocytes migration area was determined using ocular micrometer. To assess LMI we calculated leukocyte migration index (MI), which corresponded to the ratio of leukocyte migration area induced by specific antigens to the leukocyte migration area in the control sample.

Complement independent serum autocytotoxicity against lymphocytes and granulocytes was assessed as a percentage of stained cells under microscope after cell culture incubation with serum without adding a complement in the assay and subsequent staining for determination live/dead cells according to the method described by Isaeva AD et al. [11].

The study protocol was approved by Kharkiv National Medical University ethics committee. The study is within clinical trial № 0118U000929. No funding sources were used in the study.

Statistical analysis was performed using a package of software «Statistica 10.0». For the comparison of the data Mann-Whitney criterion was used. The data were considered as significant at $p < 0.05$.

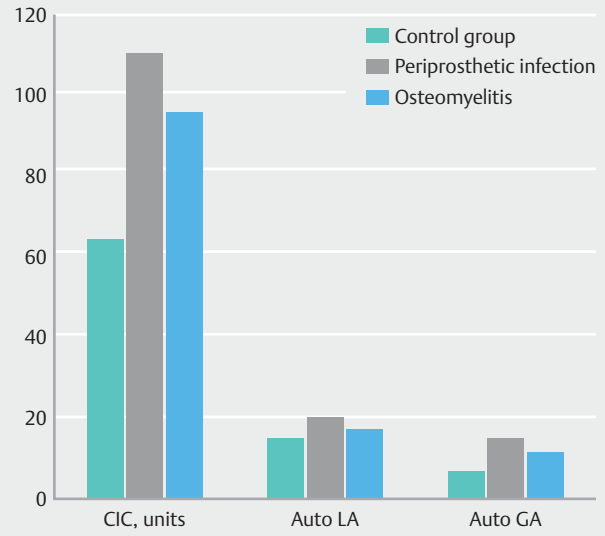
## Results

The patients with periprosthetic infection as compared with controls showed increased glucose (6.32 ± 1.0 mmol/l), B-lipoproteins (49.57 ± 10.1 units), alkaline phosphatase 328.3 ± 24.4 IU/l, glycoproteins (0.83 ± 0.18 g/l), haptoglobin (1.52 ± 0.14 g/l), and increased prothrombin time (15.42 ± 2.17 mm/sec) ($p < 0.05$) (▶ **Fig. 1**).

The patients with periprosthetic infection as compared to the controls were characterized by decreased hemoglobin (108.22 ± 5.33 g/l), erythrocytes (4.00 ± 0.37 * $10^{12}$/l), leukocytes (6.7 ± 2.11 * $10^{9}$/l) and higher values of ESR (38.11 ± 6.7 mm/h), neutrophiles (66.11 ± 4.55 %) and eosinophiles (3.16 ± 0.5 %) ($p < 0.05$).

Increased CIC (110.22 ± 14.4 units) and autoimmune granulocytotoxic antibodies (15.44 ± 2.71 %) ($p < 0.05$) were also determined in the patients with periprosthetic infection (▶ **Fig. 2**).

In the patients with periprosthetic infection, a decreased leukocyte migration to the antigens of synovial membrane was observed: the index of migration (MI) = 0.89 ± 0.01 when compared with controls and comparison group ($p < 0.05$) (▶ **Fig. 3**, ▶ **Table 1**).




▶ **Fig. 2** Immunological parameters of the studied patients. Note: CIC: circulating immune complexes, Auto LA = autoimmune lymphocytotoxic antibodies, Auto GA = autoimmune granulocytotoxic antibodies.

The patients with periprosthetic infection showed decreased MI to Staphylococcus aureus antigens (0.86 ± 0.03), increased IgG (14.8 ± 2.4 g/l), IgM (2.0 ± 0.30 g/l) and IgA (2.75 ± 0.38 g/l) ($p < 0.05$).

The patients with osteomyelitis were characterized by increased leukocytes ($7.54 \pm 2.4 \times 10^9$/l), stab neutrophiles (4.05 ± 0.41 %), ALT, and chondroitinesulfates ($p < 0.05$). The patients with osteomyelitis were also characterized by reduced production of LIF (1.22 ± 0.03), increased MI to cartilage (1.1 ± 0.02) and decreased – to Streptococcus (0.86 ± 0.03) ($p < 0.05$).

There were not statistically significant difference in the values of CRP and urea between the studied groups. Nevertheless, the level of CRP was higher in both groups as compared to controls.

Thus, among the studied groups of the patients with connective tissue infection only the patients with periprosthetic infection developed disturbances of glucose and lipid metabolism.

Therefore, it was interesting to perform an analysis of glucose and lipid associations with the studied parameters in order to reveal the cause of impaired glucose and lipid metabolism in the patients with periprosthetic infection.

Thus, the glucose level correlated with autoimmune lymphocytotoxic antibodies ($r = 0.38$), LIF ($r = 0.49$) ($p < 0.01$), MI to synovial membrane ($r = 0.78$), Pseudomonas aeruginosa ($r = 0.23$), E. coli ($r = 0.13$), Proteus vulgaris ($r = 0.13$), IgA ($r = 0.13$), fibrinogen and fibrinolytic activity of the blood ($r = -0.72$, $r = -0.57$) ($p < 0.01$).

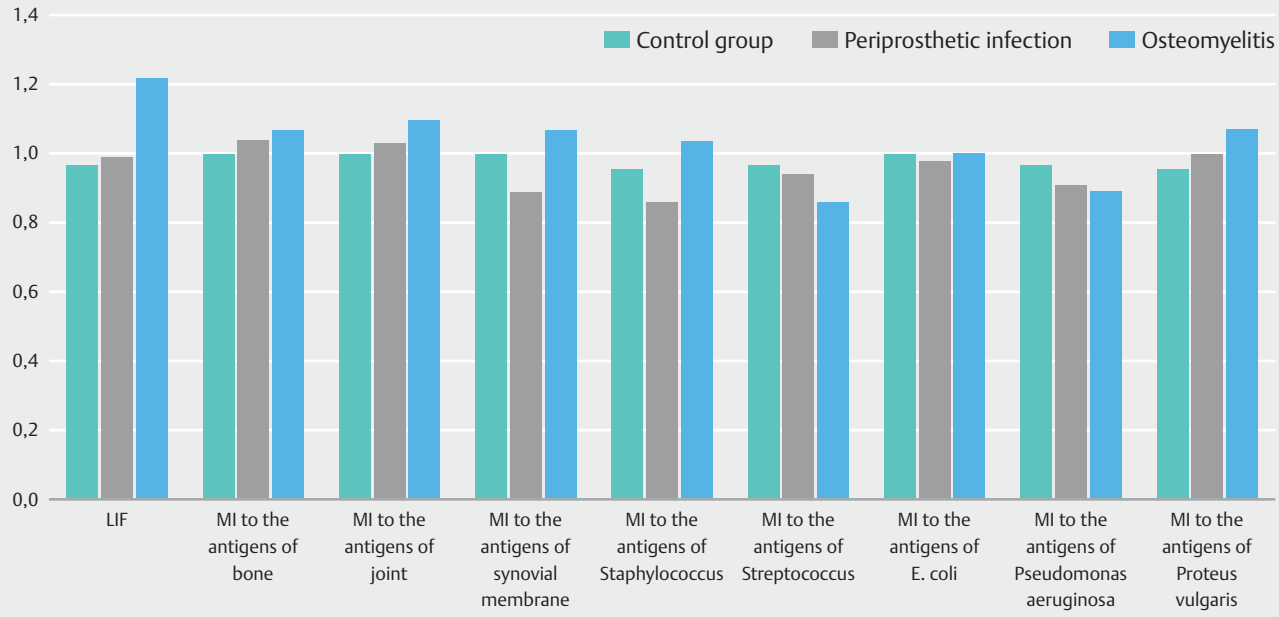
β-lipoproteins correlated with autoimmune granulocytotoxic antibodies ($r = 0.27$), MI to joint ($r = -0.39$), synovial membrane ($r = -0.6$), E. coli ($r = -0.15$). Cholesterol showed associations with autoimmune lymphocytotoxic ($r = 0.3$) and granulocytotoxic antibodies ($r = 0.27$), LIF ($r = 0.32$), MI to joint ($r = -0.29$), synovial membrane ($r = -0.42$), Staphylococcus ($r = -0.33$), Streptococcus ($r = -0.45$), E. coli ($r = -0.6$), MI to Proteus vulgaris ($r = -0.32$).

Thus, presence of autoimmune reactivity to the Pseudomonas aeruginosa, E. coli, Proteus vulgaris and synovial membrane found in patients with periprosthetic infection contributed to the development of glucose metabolism disturbances. Whereas sensitization to E. coli, Proteus vulgaris, Staphylococcus, Streptococcus, joint and synovial membrane were associated with impaired lipid metabolism.

▶ **Table 1** Biochemical parameters of the studied patients.

| Parameters | Periprosthetic infection, n = 16 | Osteomyelitis, n = 20 |
|---|---|---|
| ALT, unit/l | 21.4 ± 4.1* | 32.3 ± 5.4 |
| AST, unit/l | 25.11 ± 4.2 | 29.3 ± 4.4 |
| Cholesterol, mmol/l | 4.7 ± 0.82 | 4.6 ± 0.8 |
| Thymol probe, SH | 3.44 ± 0.72* | 3.87 ± 0.75 |
| Glucose, mmol/l | 6.32 ± 1.0* | 5.26 ± 0.7 |
| β-lipoproteins, units | 49.57 ± 10.1 | 47.6 ± 8.7 |
| Alkaline phosphatase, unit/l | 328.3 ± 24.4* | 255.63 ± 22.0 |
| Ca, mmol/l | 2.33 ± 0.17 | 2.29 ± 0.13 |
| Haptoglobin, g/l | 1.52 ± 0.14* | 1.32 ± 0.11 |
| CHS, g/l | 0,107 ± 0.003* | 0.169 ± 0.004 |
| CIC, units | 110.22 ± 14.4* | 96.0 ± 11.4 |
| Auto LA | 19.77 ± 3.65 | 17.15 ± 3.2 |
| Auto GA | 15.44 ± 2.71* | 11.5 ± 2.1 |
| LIF | 0.99 ± 0.02* | 1.22 ± 0.03 |
| MI to the antigens of | | |
| – bone | 1.04 ± 0.01 | 1.07 ± 0.01 |
| – joint | 1.03 ± 0.01* | 1.10 ± 0.02 |
| – synovial membrane | 0.89 ± 0.01* | 1.07 ± 0.02 |
| – Staphylococcus | 0.86 ± 0.03* | 1.04 ± 0.02 |
| – Streptococcus | 0.94 ± 0.01* | 0.86 ± 0.01 |
| – E. coli | 0.98 ± 0.01 | 1.0 ± 0.01 |
| – Pseudomonas aeruginosa | 0.91 ± 0.01 | 0.89 ± 0.01 |
| – Proteus vulgaris | 1.0 ± 0.02 | 1.07 ± 0.02 |
| IgG, g/l | 14.8 ± 2.4* | 11.8 ± 2.1 |
| IgA, g/l | 2.75 ± 0.38* | 2.26 ± 0.34 |
| IgM, g/l | 2.08 ± 0.30* | 1.5 ± 0.24 |

Note: ALT = alaninaminotransferase, AST = aspartataminotransferase, CHS = chondroitinsulfates, CIC = circulating immune complexes, Auto LA = autoimmune lymphocytotoxic antibodies, Auto GA = autoimmune granulocytotoxic antibodies, LIF = leukocyte migration inhibition factor, MI = migration index
* $p < 0.05$ when compared with a group of osteomyelitis




▶ **Fig. 3** Specific immunological parameters of the studied groups. Note: LIF = leukocyte migration inhibition factor, MI = migration index.

Alkaline phosphatase correlated with CIC (r = 0.39), autoimmune lymphocytotoxic antibodies (r = 0.24), MI to Staphylococcus (r = – 0.24), Streptococcus (r = – 0.32), Proteus (r = – 0.31), immunoglobulins (IgG [r = 0.29], IgA [r = 0.26], IgM [r = 0.17]), decreased hemoglobin (r = – 0.49), red blood cells (r = – 0.26), erythrocyte sedimentation rate (r = 0.77) and haptoglobin (r = 0.59).

Increased glycoproteins were associated with decreased red blood cells (r = – 0.37) and hemoglobin (r = – 0.36) and increased erythrocyte sedimentation rate (r = 0.47), monocytes (r = 0.37), eosinophils (r = 0.27), increased migration of leukocytes to the antigens of Streptococcus (r = 0.36), Proteus vulgaris (r = 0.23) (▶ **Table 2**, ▶ **Table 3**) ($p < 0.05$).

Low hemoglobin level in the patients was associated with increased CIC (r = – 0.23), autoimmune lymphocytotoxic antibodies (r = – 0.28), sensitization by the delayed type to the antigens of bone (r = 0.32), synovial membrane (r = 0.29) and Staphylococcus aureus (r = 0.23). Red blood cells count was associated with increased CIC (r = – 0.18), autoimmune lymphocytotoxic antibodies (r = – 0.21), sensitization to the antigens of bone tissue, synovial membrane (r = 0.43) and enhanced migration of leukocytes to Proteus vulgaris (r = – 0.21) and E. coli (r = – 0.24). Increased ESR was associated with increased CIC, autoimmune lymphocytotoxic and granulocytotoxic antibodies, sensitization by delayed type to the antigens of bone tissue, synovial membrane and Staphylococcus aureus. Increased stab neutrophiles were associated with high LIF (r = – 0.21) and enhanced migration of leukocytes to the joint tissue (r = 0.16), synovial membrane (r = 0.26), pathogenic antigens, especially to E. coli (r = 0.41). Neutrophiles were associated with increased CIC (r = 0.12), autoimmune granulocytotoxic antibodies (r = 0.23) and sensitization by delayed type to bone tissue (r = – 0.24), synovial membrane (r = – 0.26) and enhanced migration of leukocytes to E. coli (r = 0.22). Lymphocytosis was associated with sensitization by delayed type to E. coli (r = – 0.24) and increased immunoglobulins. Monocytosis correlated with enhanced migration of leukocytes to the antigens of synovial membrane (r = 0.27), Streptococcus (r = 0.25), Proteus vulgaris (r = 0.34). Eosinophils count correlated with elevated CIC (r = 0.19), autoimmune granulocytotoxic antibodies (r = 0.27), delayed sensitization to the joint tissue (r = – 0.32), high LIF (r = 0.43) and increased IgM (r = 0.32).

The level of CRP was associated with increased migration of leukocytes to synovial membrane antigens (r = 0.40) ($p < 0.01$) and sensitization to E. coli (r = – 0.25) and Proteus vulgaris (r = – 0.38).The level of aminotransferases showed direct associations with LIF (ALT and LIF: r = – 0.3, AST and LIF: r = – 0.3), ALT and AST with MI to E. coli (r = – 0.35, r = – 0.31) and CRP (r = – 0.47 with ALT, r = – 0.55 with AST). The level of calcium was associated with lymphocytotoxic antibodies (r = – 0.21), migration of leukocytes to the joint tissue (r = 0.36) and E. coli (r = – 0.44), IgG (r = 0.24), IgM (r = – 0.22), chondroitinsulfates (r = – 0.55). The level of haptoglobin was associated with migration of leukocytes to the bone (r = – 0.47) ($p < 0.01$) and synovial membrane (r = – 0.64), Staphylococcus aureus (r = – 0.34), Pseudomonas aeruginosa (r = – 0.46) ($p < 0.01$), Proteus vulgaris (r = – 0.24). The level of urea was associated with LIF (r = 0.25), MI to E. coli (r = 0.26) and Proteus vulgaris (r = 0.69).

Level of chondroitinsulfates correlated with granulocytotoxic antibodies (r = 0.19), leukocyte migration to joint tissue (r = 0.36), Streptococcus (r = 0.27), Pseudomonas aeruginosa (r = 0.22), E. coli (r = – 0.44). Prothrombin time correlated with migration of leukocytes to the joint (r = 0.33), synovial membrane (r = 0.37), E. coli (r = 0.33) and Proteus vulgaris (r = 0.42),

▶ **Table 2** Correlations of immunological and biochemical parameters in the studied patients.

| | GP | Total protein | ALT | AST | Cholest. | Thymol probe | Gluc. | β-lipo prot. | APh | Ca | Hapto-globin | Urea | CRP | CHS |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CIC | 0.10 | | | −0.19 | 0.20 | 0.25 | 0.17 | | 0.39 | | | 0.19 | −0.26 | |
| Auto LA | | 0.20 | | | 0.30 | 0.20 | 0.38 | | 0.24 | −0.21 | 0.23 | | | |
| Auto GA | | | −0.13 | −0.10 | 0.27 | | 0.14 | 0.27 | | | | | | 0.19 |
| LIF | | 0.22 | −0.30 | −0.30 | 0.32 | | 0.49* | | 0.12 | | | 0.25 | | |
| MI to the antigens of | | | | | | | | | | | | | | |
| – bone | 0.10 | 0.24 | 0.12 | | | | | | −0.21 | | −0.47* | | | |
| – joint | | | −0.20 | −0.22 | −0.29 | | −0.21 | −0.39 | | 0.36 | | | | 0.36 |
| – synovial membrane | | | −0.12 | −0.36 | −0.42 | 0.40 | 0.78* | −0.6 | | | −0.64 | | 0.40 | |
| – Staph. | | 0.32 | | | −0.33 | | | | −0.24 | | −0.34 | | | |
| – Strept. | 0.36* | 0.27 | −0.18 | −0.15 | −0.45* | | | | −0.32 | | | | | 0.27 |
| – Ps. aerug. | | 0.25 | −0.23 | | −0.20 | | 0.23 | | | | −0.46* | | | 0.22 |
| –E. coli | | 0.37 | −0.35 | −0.31 | −0.6* | −0.25 | 0.13 | −0.15 | −0.16 | −0.44 | | 0.26 | −0.25 | −0.44 |
| – Proteus vulgaris | 0.23 | 0.32 | −0.47* | −0.55* | −0.32 | −0.37 | 0.13 | | −0.31 | | −0.24 | 0.69* | −0.38 | |
| IgG | | | 0.20 | 0.10 | | | | | 0.29 | 0.24 | | 0.33 | −0.34 | |
| IgA | | | 0.20 | 0.10 | | | 0.13 | | 0.26 | | | | | |
| IgM | | | 0.10 | 0.10 | | | | | 0.17 | −0.20 | | | | |

Note: given data at $p < 0.05$, APh-alkaline phosphatase, Cholest. = cholesterol, CHS = chondroitinesulfates, GP = glycoproteins, CRP = C reactive protein, ALT = alaninaminotransferase, AST = aspartataminotransferase, Ca = calcium, Gluc. = glucose, β-lipoprot. = β-lipoproteins, CIC = circulating immune complexes, Auto LA = autoimmune lymphocytotoxic antibodies, Auto GA = autoimmune granulocytotoxic antibodies, LIF = leukocyte migration inhibition factor, MI = migration index, Staph. = Staphylococcus, Strept. = Streptococcus, Ps. aerug. = Pseudomonas aeruginosa, E. coli = Escherichia coli.
* $p < 0.01$

▶ **Table 3** Correlation analysis of immunological data and parameters of peripheral blood.

| | CIC | Auto LA | Auto GA | LIF | MI to the antigens of | | | | | | | | IgG | IgA | IgM |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | bone | joint | synovial membrane | Staph. | Strept. | E. coli | Ps. aeruginosa | Proteus vulgaris | | | |
| Hb | −0.23 | −0.28 | | | 0.32 | | 0.29 | 0.23 | | | | −0.22 | 0.25 | | |
| Erythr. | −0.18 | −0.21 | | | | | 0.43 | | | | −0.24 | −0.21 | | | |
| Leuc. | 0.16 | | 0.12 | | | | 0.14 | | | | | | | | |
| ESR | 0.27 | 0.22 | | | −0.24 | | −0.31 | −0.31 | | | 0.13 | | | | |
| Stab neutr. | 0.16 | | | −0.21 | | 0.16 | 0.26 | 0.20 | 0.18 | 0.16 | 0.41 | 0.18 | | | |
| Segm. neutr. | 0.12 | | 0.23 | | −0.24 | | −0.26 | | −0.12 | | 0.22 | 0.13 | −0.26 | −0.30 | −0.13 |
| Lymph. | −0.27 | 0.19 | | | 0.13 | | | | | | −0.24 | | 0.19 | 0.24 | 0.15 |
| Monoc. | 0.27 | | | | 0.15 | | 0.27 | | 0.25 | | | 0.34 | | | |
| Eosin. | 0.19 | 0.17 | 0.27 | 0.43 | | −0.32 | | −0.19 | 0.13 | −0.15 | | | | | 0.32 |

Note: given data at $p < 0.05$, Cholest. = cholesterol, CHS = chondroitinesulfates, GP = glycoproteins, CRP = C reactive protein, ALT = alaninaminotransferase, AST = aspartataminotransferase, Ca = calcium, Gluc. = glucose, β-lipoprot. = β-lipoproteins, CIC = circulating immune complexes, Auto LA = autoimmune lymphocytotoxic antibodies, Auto GA = autoimmune granulocytotoxic antibodies, LIF = leukocyte migration inhibition factor, MI = migration index, Staph. = Staphylococcus, Strept. = Streptococcus, Ps. aerug. = Pseudomonas aeruginosa, E. coli = Escherichia coli

IgG (r = −0.53), IgA (r = −0.67), IgM (r = −0.55). Activated thromboplastin time correlated with autoimmune lymphocytotoxic antibodies (r = 0.4), MI to bone (r = −0.31), Proteus vulgaris (r = −0.5), IgG (r = −0.67) ($p < 0.01$), IgA (r = −0.69), IgM (r = −0.55). The patients demonstrated associations of fibrinogen and CIC (r = 0.29), LIF (r = −0.4), MI to synovial membrane (r = −0.69), Streptococcus (r = 0.5), E. coli (r = 0.4), Proteus vulgaris (r = 0.49). Fibrinolytic activity correlated with granulocytotoxic antibodies (r = −0.4), LIF (r = −0.22), MI to synovial membrane (r = 0.26), Streptococcus (r = 0.32), Pseudomonas aeruginosa (r = −0.69).

## Discussion

The major end products of bacterial fermentation in colon are organic acids such as acetate, propionate, butyrate, lactate and succinate, together with hydrogen, methane and $CO_2$. Mounting evidences suggest that fatty acids (FA) have a high impact on host physiology since they can act as energy source for epithelial cells, signalling molecules and gene expression modulator [12]. The amount and relative abundance of each FA depend on diet's indigestible fraction, but also on a cross-feeding mechanisms established in the bacterial community. The most abundant FA are acetic acid (C2), propionic acid (C3) and butyric acid (C4), which together represent nearly 90–95 % of the FA present in the colon. Acetate is a net product of carbohydrate fermentation of most anaerobe bacteria, while propionic and butyric acid are generated from carbohydrate or protein fermentation by a distinct subset of bacteria. The researchers revealed that the faecal proportion of acetic acid is indicative of a more unfavourable lipid profile, while propionic acid – of more favourable one. It was also revealed that individuals with hypercholesterolemia possess a particular faecal bacterial signature [13], characterized by lower prevalence of the genera *Anaeroplasma* and *Haemophilus* and higher prevalence of *Odoribacter*, which seems to be associated with a wide range of blood lipid biomarkers, including those ones commonly linked to a higher risk of cardiovascular diseases and others considered as novel promising biomarkers related to both amount and size of lipoproteins. It was found that intestinal bacteria can influence blood glucose and lipid concentrations [14].

The obtained data from the present study showed that increased glucose and B-lipoproteins are associated with lack of LIF. Increased cholesterine and β-lipoproteins were associated with inhibition of migration of leukocytes to the joint. Importantly, atherogenesis was also associated with the presence of the sensitization to the pathogens (Staphylococcus, Streptococcus, Pseudomonas aeruginosa, Proteus vulgaris and especially to E. coli). Moreover, sensitization to E. coli and Proteus vulgaris contributes to the distruction of the cell membrane, since there was association with increased aminotransferases.

The association of the intensive LIF production with elevated transferases corresponds to the existing data on the inflammatory properties of LIF [1, 10, 15], nevertheless there has not been any information concerning LIF production in patients with increased glucose and lipids.

It is important that the increase of autoimmune lymphocytotoxic antibodies was associated with the impaired metabolism of carbohydrates and lipids, as well as with cholestasis. Thus, in atherosclerosis therapeutic measures aimed to decrease autoimmune lymphocytotoxic antibodies might be of benefit by the usage of reosorbilact, neohemodez and reopoliglucinum. In the studied patients with infectious complications reosorbilact showed the highest potential. The enhanced migration of leukocytes to the synovial membrane is associated with increased glucose and CRP, and decreased migration of leukocytes – with increased cholesterine, β-lipoproteins and AST.

Interestingly, inhibition of leukocyte migration to the bone was associated with alkaline phosphatase and haptoglobin.

The obtained associations between specific cell reactivity to joint tissue and the level of calcium allow to use the value of leukocyte migration index to joint tissue for the monitoring of calcium metabolism. There are data on participation of E. coli in the process of absorption of calcium in the gut, thus the revealed association of the enhanced leukocyte migration to the antigens of E. coli and calcium level might allow to use leukocyte migration index to E. coli as a parameter to control the calcium level in serum.

The association of increased urea with enhanced migration of leukocytes to E. coli and, especially, to Proteus vulgaris indicate that these microorganisms are involved in the metabolism of the nitrogen.

## Conclusions

The increased serum glucose in patients with periprosthetic infection and osteomyelitis is associated with the presence of autoimmune lymphocytotoxic antibodies, reduced LIF production and autoimmune reactivity to the antigens of synovial membrane, E. coli, Proteus vulgaris and Pseudomonas aeruginosa. Increased atherogenesis has also autoimmune pathogenetic mechanism, being associated with increased circulating immune complexes, autoimmune lymphocytotoxic and granulocytotoxic antibodies, decreased non-specific LIF production and sensitization to connective tissue antigens and pathogenic microorganisms, especially, to Streptococcus and E. coli. Anemia is associated with increased autoimmune lymphocytotoxic antibodies, sensitization to the bone and joint tissue and Staphylococcus, as well as with autoimmune reactivity to E. coli and Proteus vulgaris.

Thus, using targeted vaccination to boost the immune system against pathogenic microbiota may be a novel therapeutic option for the regulation of metabolism and the prevention of metabolic diseases.

## Competing interests

The authors declare that no conflicts of interest exist.

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