INTRODUCTION
Numerous studies in recent years have proved the pathogenetic relationship of the intestinal microbiota with such diseases as hyperlipidemia, atherosclerosis, arterial hypertension, steatohepatitis, diabetes mellitus [1]. It is known that on the background of the syndrome of excessive bacterial growth in the intestine, proatherogenic changes in lipid spectrum are possible [2].

Microorganisms which colonize the intestine are capable to impact on cholesterol metabolism by affecting the key stages of its synthesis. Excessive microbial growth of anaerobes in the jejunum, which is typical for syndrome of bacterial overgrowth (SIBO), leads to its damage, with the development of system inflammation, that causes early deconjugation of bile acids with the formation of their toxic salts and impaired enterohepatic circulation [3,4]. Deconjugated bile acids can also damage the epithelium of the small intestinal mucosa by its detergent properties.

As a result, synthesis and sorption of enzymes on its surface are reduced, which leads to disruption of membrane digestion and absorption of fats and fat-soluble vitamins A, D, E, K, amino acids and carbohydrates [5,6]. Moreover, there was a data, linking SIBO with subclinical atherosclerosis, through the vitamin K-dependent activity of the matrix Gla-protein (MGP), that maintains arterial structure and function through prevention of calcification of vessel walls and the regulation of the extracellular matrix [7].

Bile acids induce impaired sodium absorption, increase the secretion of chlorides and water into the intestinal lumen, accelerate peristalsis of the small intestine, which aggravates diarrhea syndrome [8]. It should also be noted that deconjugated bile acids are rapidly absorbed, which prematurely turns them off from digestion processes [9,10]. In this case, induction of hypercholesterolemia is possible, especially in individuals with hereditary predisposition [11].

The only class of lipids with anti-atherogenic activity is high-density lipoproteins (HDL), that are synthesized in the liver and small intestine. An increasing amount of
endotoxins that is produced by gram-negative microflora of intestine, leads to declining the anti-atherogenic HDL [12,13].

Permanent inflammation also causes the changes, that impact on cholesterol catabolism reducing and its excretion in the liver by decreasing the expression of matrix ribonucleic acids and the activity of bile acid synthesis key enzymes - CYP7A1, CYP27A1 and CYP7B1 [14,15].

Taking into account these facts, there is a big interest in searching the way of hyperlipidemia development, where the SIBO is one of the essential factors. The main attention is dedicated to enterohepatic circulation disturbance which evolves on the background of the early bile acids deconjugation with further endotoxin production and oxidative stress in the liver with hyperproduction of cholesterol and atherogenic lipoproteins [16,17].

THE AIM
The aim of this study therefore was to determine the prevalence and features of SIBO in a series of patients with hyperlipidemia and in control subjects.

MATERIALS AND METHODS

Nineteen patients with hyperlipidemia (9 men and 10 women) aged from 24 to 50 years (average age 33,69±1.73 years) with average BMI 24,4±1.54 were examined in “Medicover Ukraine” (Lviv, Ukraine). The diagnostic criteria, except the hyperlipidemia, which were used for patient including into the program of examination were: BMI not more that 25, waist circumference <94 cm for male, <80 cm for female, no significant alcohol consumption, defined as no greater than 20 g of alcohol per day. Ten control subjects (4 men and 6 women) aged from 24 to 34 years (an average 29,9±0.68 years) and average BMI 24,2±1.21 were matched with main group patients by age and metabolic characteristics. All control subjects had normal lipid range and no history of coronary disease. None of both groups subjects was taking drugs known to affect lipid profile or microbiota composition, including antibacterial medicines 1 month before and during the data.

Both groups of patients underwent biochemical evaluation of serum that included blood cell count and lipid profile. For the evaluation of the inflammation, as one of the pathogenetically ways for hyperlipidemia formation due the SIBO activity, C-reactive protein (CRP) was measured in serum, obtained on the day of SIBO testing. Another biochemical tests included alaninaminotransferase (ALT), aspartaminotransferase AST, gamma glutamyl transpeptidase (GGTP), bilirubin (total, direct, indirect), apolipoprotein B (apo B). Biochemical tests were carried out using commercially available test kits.

Additionally, the data plan involved the determination of calprotectin in feces, which positive result was the reason of excluding the patient from the data. Values >50 μg/g were considered as increased.

Ultrasound examination was proved to all patients of both groups. with aim to exclude the patients with fatty liver disease as one of the reasons of increased cholesterol level and aggravation factor for SIBO presence. The ultrasound criteria for fatty infiltration existence was a diffuse increase in the echogenicity of the liver parenchyma, decreased attenuation on the liver and ratio between the brightness level of the liver and the right kidney that was calculated for the hepato-renal index (HRI) determination [18].

All subjects were examined by a lactulose breath test what is one of the most diagnostically valuable methods for determining excessive bacterial growth under clinical conditions [19]. The test allows to determine the concentration of hydrogen (H2) in exhaled air, what is growing up when there are a lot of hydrogen-producing bacteria in the small intestine [20,21]. The patient was given from 10 g of lactulose. A change in the level of exhaled hydrogen gas above 20 parts per million (ppm) within 120 minutes from a basal value was the basis for SIBO diagnosis [22,23]. Furthermore, after the recording SIBO by enormous H2 growing (more than 30 points), the test could be stopped before 120 minute [24,25]. Before the test, subjects were asked to brush their teeth and rinse mouth with antiseptic mouth wash and tap water, to eliminate an early hydrogen peak due to action of oral bacteria on test sugars [26,27]. Patients were required to comply with a low residue diet the day before the test and not to smoke within two hours of the test to prevent high basal levels of H2 [28,29].

Lactulose breath test was carried out on the “Gastro+Gastrolyzer” (Bedfont® Scientific Ltd) device in the laboratory of “Medicover Ukraine”. Except the standart interpretation, we have analyzed the difference between the basal and the highest range of exhaled H2 during the 120 minutes of test in subjects of both groups. An estimate of small intestinal transit time was calculated, where possible, by observing the time taken from ingestion of lactulose to the appearance of the H2 peak, indicating colonic catabolism of lactulose.

Statistical analysis was carried out using Statistica 5.0 for Windows software (Statsoft Inc, Tulsa, USA). Comparisons between groups for parametric data were performed using the Student’s t test. Differences were considered statistically significant for p<0,05. The correlation between the values was measured by Person correlation coefficient.

RESULTS

Patient characteristics are shown in Table I. Due to the results, the statistically significant difference was matched between the cholesterol level, low density lipoproteins, very low density lipoproteins of main and control groups. The level of CRP was more than in 1,4 significantly higher in group with hyperlipidemia in contrast to the controls.

The measurement of SIBO by lactulose test showed the equal result of the basal dose of hydrogen in both groups. In contrast, the maximal dose was particularly higher in
patients with hyperlipidemia in comparison with control group (94.7±13.69 vs. 36.13±5.4) (Table II).

According to the fact, that SIBO existence is based on the hydrogen level increasing more that 20 ppt, not depending the amount of H2, we have analyzed the prevalence of intestinal bacterial overgrowth and the small intestinal transit time in both groups. On the other hand, the fact of difference in result of H2 between group of patients with hyperlipidemia and without was essential.

The prevalence of SIBO in hyperlipidemia group was 78.9%. Small intestinal transit time amounted 100 minutes. Meanwhile, the SIBO occurrence in controls was 40% with average time of small intestine transit 140 minutes.

We have analyzed the data, where different methods of small intestine transit time were compared and found the substantial remark, that lactulose is non-physiologic for small intestine time transit measurement since it accelerates small bowel transit, presumably due its osmotic activity. Based on this evidence, we did not accent on the difference of transit time between both groups, because in both groups it was shorter than normal range. On the other hand, we could not ignore the fact of meaningful difference in SIBO existence between main and control groups.

In accordance to this result, we have calculated the correlation coefficient between different biochemical markers and lactulose test results with the purpose to find the influencing factors in each group that could be the reason of SIBO occurrence.

The results showed that there is a strong correlation between AST level and SIBO existence in both groups (r=1). Moreover, the correlative connection was marked between AST/ALT ratio and bacterial overgrowth in main group (r=0.59). An interesting detail was found during the correlation analysis – CRP, that is strongly connected with SIBO in different data, did not interrelate with bacterial overgrowth in both groups. On the other hand, the relationship between cholesterol, LDL and CRP in patients with hyperlipidemia has been found (r=0.58, r=0.59 respectively). Regarding to lipid profile – there was remarkable positive connection between LDL, TG, VLDL and the dose of exhaled hydrogen on 120 minute (r=0.6, r= 0.62, r=0.7 respectively) and strong negative correlation between HDL and 120 minutes dose (r=-0.74) in main group.

**DISCUSSION**

One of the essential findings of this study was a significantly higher prevalence of SIBO in patients with

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<tr>
<th>Table I. Clinical and biochemical variables for patients with hyperlipidemia (n=19) and controls (n=10)</th>
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<td>Variable (normal range)</td>
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<tr>
<td>Age (years)</td>
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<td>BMI, kg/m²</td>
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<tr>
<td>Apo B, g/l (normal range 0.66-1.33-men, 0.6-1.17 women)</td>
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<td>Bilirubin total, mmol/l (normal range &lt;21)</td>
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<td>Direct bilirubin, mmol/l (normal range &lt;5)</td>
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<tr>
<td>Indirect bilirubin, mmol/l (normal range &lt;75% of bilirubin total)</td>
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<td>AST, IU/L (normal range &lt;40)</td>
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<td>ALT, IU/L (normal range &lt;41)</td>
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<td>AST/ALT ratio (normal range 0.91-1.75)</td>
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<tr>
<td>GGTP, IU/L (normal range &lt;55 men, &lt;38 women)</td>
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<td>CRP, mg/l (normal range £5)</td>
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<tr>
<td>Cholesterol, mmol/l (normal range £5.2)</td>
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<tr>
<td>Triglycerides, mmol/l (normal range ≤1.7)</td>
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<td>LDL, mmol/l (normal range £2.59)</td>
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<td>VLDL, mmol/l (normal range =0.26-1.0)</td>
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<td>HDL, mmol/l (normal range =³1.56)</td>
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<th>Table II. H2 level during the lactulose breath test in patients of main and control groups</th>
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<td>Main group (19)</td>
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<td>Basal dose, ppm</td>
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<td>Maximal dose, ppm</td>
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hyperlipidemia compared with controls (78.9% vs. 40%). Furthermore, an interesting point was found during the analysis of main and control group results – the highest range of exhaled hydrogen during the lactulose test in patients with hyperlipidemia was in above 2.5 times higher than in controls (94.7±13.69 compared to 36.13±5.4 ppm), in while the basal level was equal in both groups (10.7±0.93 and 11.1±1.2 ppm).

Apparently, the way of hyperlipidemia development on the SIBO background could be realized by next steps. Under the influence of SIBO, the protective mechanisms of small intestine mucous membrane are injured, which causes both local and systemic pathological processes, complemented by inflammation, that are closely interrelated. The bacterial pool of colonic flora, which has, in case of SIBO, the properties of conditionally pathogenic flora, by causing the violation of the small intestine barrier function on the background of inflammation, induces the bacterial hydrolysis of proteins with the formation of ammonia and ketone acids, the oxidation of fatty acids, deconjugation of bile acids and the formation of short-chain fatty acids from carbohydrates.

However, CRP, as one of the main markers of system inflammation, that could impact on SIBO occurrence, did not exceed the upper limit of norm, in both groups, not depending the significant difference between it in patients of main and control groups. Thus, the way of SIBO development could be connected not only with injured intestine, but with another way of pathological process that is associated with liver. Due the results of this study, the singular sign that had strong correlation with SIBO presence or absence was AST, which is always associated not only with cardiac muscle, but with liver parenchyma injuring. This indicator was connected with bacterial overgrowth in both groups, but there was more specific strong correlation between de Ritis ratio and SIBO existing in patients of main group, that could be the second point of definitely including liver and cardiovascular system in SIBO manifestation in patients with hyperlipidemia.

Finally, the analysis of correlation between the lipids, CRP and SIBO demonstrated, that increasing of LDL, TG and VLDL is interlinked with higher dose of exhaled hydrogen in main group and with CRP increasing. In contrast, there was no connection in both groups between CRP and H2 growth on any minute of lactulose test. It could be the explanation of considerably higher rate of H2 in patients with hyperlipidemia – there are strong connection between the lipoproteins and SIBO manifestation. Between this, maybe CRP increasing is not before SIBO occurrence, but after its development and is the result of SIBO, not the reason.

That could be the answer for the relationship presence between LDL, VLDL, TG with H2 and CRP, with absence of correlation between CRP and SIBO. High hydrogen rate leads to LDL, VLDL and TG increasing, and HDL decreasing, that provokes the inflammation with next CRP growing. In that way, the main target organ becomes the liver. Thus, it could be the next “vicious circle”: disruption of intestinal microecology * SIBO occurrence * accumulation of endotoxins in the intestine * violation of enterohepatic circulation of bile acids * impairing of liver function * impairing of lipid metabolism * impairing of structure (fatty infiltration, fibrosis) * impairing of lipid metabolism * maintaining (aggravating) disturbed intestinal dysbiosis.

**CONCLUSIONS**

1. The prevalence of SIBO in patients with hyperlipidemia is predominantly higher than in patients without lipid metabolism disturbance.
2. The hydrogen level is significantly higher in patients with hyperlipidemia in comparison with controls.
3. There is an axis between high LDL, VLDL and TG level and hydrogen rate in patients with hyperlipidemia.
4. CRP is not interrelated with SIBO, but is strongly connected with LDL and cholesterol level in patients with hyperlipidemia.

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Authors' contributions:
According to the order of the Authorship.

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