

**Research Article****Biological Age in Young People with Biliary Pathology as a Criterion for the Integrated Assessment of the Functional State of the Human Body****L.V. Volevach, L.V. Gabbasova, N.A. Demidova,  
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**Abstract.**

The relevance of the conducted study lies in the emergence of the premature aging phenomena in young people. In this regard, the presented paper is aimed at studying the biological age in young people with biliary pathology. The leading methods for the study of this problem are the methods of counting in the outpatient and inpatient conditions, allowing a comprehensive review of the issues of aging. The presented research reviews the dependence of the pace of aging on nosology and its duration, frequency of relapses, disorders of the intestinal microbiocenosis and blood lipid spectrum and their interdependencies; the study also revealed, that the information ability of the biological age counting models is sufficient to assess the degree of aging in the groups examined. The main group was represented by the following subgroups: 52 patients with biliary tract dysfunction, 76 patients with chronic non-calculous cholecystitis, and 54 patients with chronic calculous cholecystitis. The control group consisted of 46 participants. The aging by the criteria of biological age was most pronounced in the group with diseases of the biliary system, where the past medical history (PMH) lasted more than 10 years and consisted of averaged  $11.19 \pm 1.04$  years. 130 patients with chronic cholecystitis were selected; the biological age in all of them was determined by the 'outpatient' method (stage 1 - group 1), then all 130 patients were examined by the 'inpatient method' (stage 2 - group 2). The results were compared with the control group. Excess biological age was shown in patients with chronic cholecystitis compared with the control group in a screening study ('outpatient' method). The results of the study of biological age by 'inpatient' method in patients with chronic cholecystitis were thoroughly analyzed. The value of the biological age in patients was higher than in the control group by  $4.62 \pm 0.7$  U ( $p < 0.001$ ); both techniques showed sufficient information ability. The study also has shown the relationship between the history of the disease and the number of relapses with the biological age. A high level of the correlation coefficient between biological age and calendar age ( $r = 0.796$ ,  $p < 0.001$ ) indicated the presence of a strong interdependence between these parameters. The materials of the paper are of practical value for general practitioners, physicians, and gastroenterologists of both outpatient clinics and hospitals.

**Keywords:** biological age, young age, chronic cholecystitis, biliary tract dysfunction, counting method, correlation, disease relapse, risk factors.

**Introduction.**

Biliary obstruction commonly refers to blockage of the bile duct system leading to impaired bile flow from the liver into the intestinal tract. Bile is

a substance that contains bile salts, bilirubin, and cholesterol and is continuously synthesized in the liver hepatocytes. Bile is then transported via the

bile ducts into the second portion of the duodenum to assist with the metabolism of fats.[30] Many researchers believed that biological age (BA) is a measure of the systemic disintegration of the human body during aging. They considered it as a degree of vitality, and also as a measure of the biological capabilities of an organism over time [18, 23, 25, 27-29].

It is important to note that in determining the biological age two tasks are being solved: 1) theoretical - analyzing the contribution of various factors associated with the age-related decline in the viability of the body and 2) practical - identifying contingent risks at an accelerated aging rate [24, 26].

The scientists usually considered the determination of biological age in the background of the general problems of medical diagnostics. They reviewed it as a peculiar method of setting a clinical and functional diagnosis, which is fundamentally focused on a quantitative assessment of the state of the body through a quantitative assessment of the degree of its aging, namely, 'wear and tear aging'. According to WHO experts, the BA determination is an important diagnostic technique, the use of which allows estimating the degree of aging of an individual, in particular, depending on the nature of the activity and lifestyle [17]. The experts have concluded that, whatever the possible shortcomings of individual methods for BA determination and health assessments, these methods are still of tremendous practical value, because they allow making quantitative estimates in preventive and clinical medicine [1, 3, 4, 16]. Therefore, the BA problem interlocks with the most important issues of medical diagnostics.

The importance of studying not only the current state, but also the biological capabilities of the body (and its reliability) allows concluding that a set of criteria is necessary to determine BA, including, on the one hand, health indicators that are gradually and naturally decreasing with age, and on the other - functional tests and loads, assessing the adaptive capabilities of a man.

The most appropriate method seems to be the use of a multiple regression model of biological age [9]. It characterizes the age and nosological changes of mental and physical performance, allowing not only to identify and evaluate the dependence of premature aging of a human body

at a young stage on the degree of influence of a complex of behavioral factors in the youth environment, but also to diagnose pre-nosological and non-nosological conditions at the stages of therapeutic and preventive effects.

### **Screening methods and materials.**

Currently, there is no single generally accepted method for determining BA. The opinion on how the aging of the individual corresponds to one's chronological age is usually being formed based on the use of various mathematical models and tests.

The indicators used to determine BA should meet the following requirements:

- objectively reflect the functional and (or) natural state of an organ, system, metabolic processes, and regulatory features of the body;
- change significantly with age;
- differ as much as possible from other indicators, without being linked to the same processes;
- be technically feasible for people of any age;
- be easy to quantify;
- be reliable and easily reproducible with repeated research, yielding stable and comparable results;
- do not cause immediate death.

Test Batteries that were used in determining BA were arranged as follows:

1. Systolic blood pressure (sBP) and diastolic blood pressure (dBP) were measured according to the generally accepted method using the Riva-Rocci apparatus on the right arm in a sitting position, three times with 5 minutes confidence intervals. The results of the measurements at which the blood pressure had the lowest value were taken into account along with the difference between systolic and diastolic blood pressure - Pulse blood pressure (PBP).
2. The velocity of pulse wave propagation through the arterial vessels was measured by direct arterioplethysmography using P-2A sensors on the 6 channel EK-6T electrocardiograph and Cardiolux-300 on elastic vessels (EV: carotid artery – femoral artery) and vascular smooth muscle (SM: carotid artery - radial artery) according to rheovasography.
3. The breath holding when inhaling breath (BHB) and forced expiratory time (FET) were measured three times with an interval of 5 minutes using a

stopwatch. The highest values of BHB and FET were recorded.

4. The mean acuteness of vision (MAV) was determined for the leading eye by finding the nearest clear point of view when reading font No. 2 from the Sivtsev Table under the conditions of ametropia and presbyopia.

5. The hearing threshold or auditory acuity (AA) was measured at an acoustic frequency of 4000 TC using the audiometer.

6. The static balancing (SBL) was determined when the test person was standing on the left leg, with no shoes on and eyes closed, arms lowered along the body and pressed to the hips (without prior training). The duration of the SBL was measured using a stopwatch three times with an interval of 5 minutes; the best result was placed on record.

7. The body weight (BW) was recorded using medical weights while the test person was wearing light clothing with no shoes on. Body height was determined using a height meter in cm.

8. Subjective health assessment (SHA) was conducted using a questionnaire comprising 29 questions. After filling in the questionnaire, the total number of adverse responses was calculated (which can range from 0 to 29), and this value was included in the formula for determining BA.

9. The Wechsler Adult Intelligence Scale test (WAIS) was performed using a standard protocol form. The test person was assigned the task of entering the characters corresponding to the digits located in each cell for 90 seconds as fast as possible. The number of correctly filled cells within 90 seconds timeframe was counted. This number was also a part of the formula for determining BA.

When calculating BA, the values of certain indicators were expressed in the following units: sBP, dBP and PBP - in mmHg, EV and SM - in m/s, BHB, FET and SBL - in sec., MAV - in diopters, AA - in decibels, BW - in kg, SHA - in arbitrary units (the number of correctly filled cells), and WAIS - in conventional units.

Two versions of the BA determination proposed by the Institute of Gerontology of the Academy of Medical Sciences of Ukraine (developed by V.P. Voitenko, I.I. Tokar and A.M. Polyukhov) were used in the course of the study.

The first option allowed evaluating BA using 4 fairly informative, but technically simple tests. Authors are entitled to the opinion that this variant of BA assessment should be used when examining large contingents on an outpatient basis.

Consequently, the formula for determining BA in men resulted in:

$$BA = 26.985 + 0.215 * sBP - 0.149 * 3FET - 0.151 * SBL + 0.723 * SHA$$

The formula for determining BA in women was:

$$BA = 1.463 + 0.415 * PBP - 0.140 * SBL + 0.248 * BW + 0.694 * SHA$$

In accordance with the recommendations by V.P. Voitenko, A.V. Tokar, and A.M. Polyukhov and using the above formulas it was possible to calculate the BA values for each test person. The pronouncement of 'aging' was assessed by comparing the BA either directly with the calendar age (CA), or with the due biological age – DBA. According to authors, DBA is a population average for the rate of aging.

DBA value was calculated by the following formulas:

$$\text{Men: } DBA = 0.837 * CA + 8.13;$$

$$\text{Women: } DBA = 0.64 * CA + 14.8$$

Further, the determination of the 'rate of aging' was carried out for the groups surveyed according to the formula:

$$BA = BA - DBA$$

By calculating the index (BVA/DBA), one could say what time the BA of the patients being examined is greater or less than their peers' DBA. If the degree of aging of the subject was less than the average degree of aging of persons of equal calendar age (CA): then  $BA/DBA < 1$ , and  $BA - DBA < 0$ . If the degree of aging of the subject was greater than the average degree of aging of persons of equal CA: then  $BA/DBA > 1$ , and  $BA - DBA > 0$ . Finally, if the degree of aging of the subject corresponds to the average degree of aging of persons of equal CA: then  $BA/DBA$  approaches 1, and  $BA - DBA$  approaches 0. Authors used a visual graphic comparison of the individual BA with DBA and CA in the process of dynamic observation of the patients.

The biological age was studied in young people with various disorders of the biliary system. Depending on the particular disease all the examined individuals were divided into 3 groups; the paired comparison (AHP) method was used.

The first subgroup included patients with biliary tract dysfunction (52 participants), the second - patients with chronic acalculous cholecystitis (AAC, 76 participants), and the third - patients with chronic calculous cholecystitis (CC, 54 participants). These three subgroups constituted the main group of the study.

The control group consisted of 49 volunteers who had no disorders in the biliary system; they attended 'Healthy Lifestyle' School and exercised high physical activity. It is also essential to make a pointed reference to the fact that, first of all, the normal healthy control subjects represented a group of volunteers of approximately the same calendar age; secondly, they all had approximately the same initially good health condition; thirdly, they all embraced a healthy lifestyle (balanced diet, physical activity, etc.). The gender and age composition of the examined participants of young age is presented in Table 1. The BA-DBA index calculation in patients with various disorders of the biliary system was conducted with respect to the duration of the history of the disease using the 'outpatient method' (Table 2). This study (in terms of the examination of research projects) was approved by the Ethics Committee of the Bashkir State Medical University in the city of Ufa.

### Results and discussion.

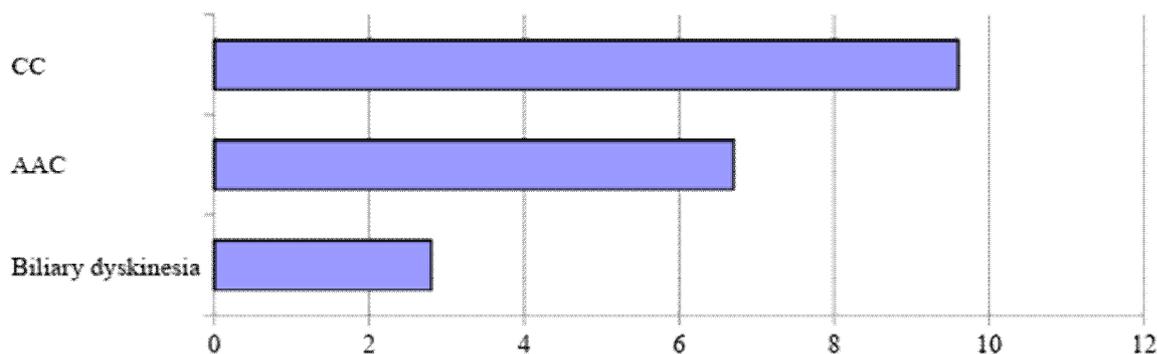
As follows out of Table 2, the BA-DBA rate varied in the main group depending on the

**Table 1:** Distribution of patients with diseases of the biliary system and participants of the control group by gender, age and duration of a disease (%)

Nosological entity	Abs. / relative quantity %	By gender		By age		Duration of a disease (Years)		
		Men	Women	17-29	30 and older	Up to 5	5-10	10 and over
1. Biliary dyskinesia <i>abs.</i> %	$\frac{52}{22.5}$	$\frac{19}{8.2}$	$\frac{33}{14.3}$	$\frac{38}{16.5}$	$\frac{14}{6.1}$	$\frac{19}{26.0}$	$\frac{12}{21.0}$	$\frac{21}{40.4}$
2. Chronic acalculous cholecystitis <i>abs.</i> %	$\frac{76}{32.9}$	$\frac{29}{12.6}$	$\frac{47}{20.3}$	$\frac{39}{16.7}$	$\frac{37}{16.0}$	$\frac{20}{27.4}$	$\frac{31}{54.4}$	$\frac{25}{48.1}$
3. Chronic calculous cholecystitis <i>abs.</i> %	$\frac{54}{23.4}$	$\frac{21}{9.1}$	$\frac{33}{14.3}$	$\frac{17}{7.4}$	$\frac{37}{16.0}$	$\frac{34}{46.6}$	$\frac{14}{24.6}$	$\frac{6}{11.5}$
4. Control group <i>abs.</i> %	$\frac{49}{21.2}$	$\frac{25}{10.6}$	$\frac{24}{10.4}$	$\frac{24}{12.6}$	$\frac{20}{8.7}$	—	—	—
Total	$\frac{231}{100}$	$\frac{94}{40.7}$	$\frac{137}{59.3}$	$\frac{123}{53.2}$	$\frac{108}{46.8}$	$\frac{73}{40.1}$	$\frac{57}{31.3}$	$\frac{52}{28.6}$

duration of the biliary system disease. The aging according to the BA criteria was most pronounced in the group with diseases of the biliary system, where the PMH was over 10 years ( $11.19 \pm 1.04$  years). The BA-DBA index accounted for  $1.16 \pm 0.46$  years in young people with the history of up to 3 years; however, there is a sharp spike in the aging of people with diseases of the biliary system with the disease history of 3-6 years. So, the difference between BA and DBA in the groups of participants reached  $5.07 \pm 1.42$  years, stabilizing with a history of 6-10 years at the level of  $5.78 \pm 1.39$  years with a subsequent increase to  $11.19 \pm 1.04$ . The BA indicators improved with an increase in the duration of observations, which also indicated an increase in the viability of individuals of the 'healthy lifestyle' group, which was observed in dynamics. The BA-DBA index in the control group had significant differences from the main group (Table 1).

As can be seen from Figure 1, the rate of aging of participants with diseases of the biliary system according to the BA criteria composed  $9.59 \pm 1.0$  for patients with CC;  $6.68 \pm 1.24$  for AAC patients; and  $2.77 \pm 1.19$  years for patients with biliary dyskinesia. The duration of the disease of the biliary tract in all studied groups was approximately the same and ranged from 8.61 to 14 years.



**Fig. 1.** ‘The pace of aging’ (BA-DBA) of human body of the young age with various disorders of the biliary system according to the BA criteria (M±m)

Left, top to bottom: CC, AAC, Biliary dyskinesia

Noteworthy is the magnitude difference analysis of the BA-DBA index (which characterizes the ‘aging rate’) in the studied groups, assessed depending on the medical history (Table 2, Figure 1).

So, in the analyzed groups of patients with CC, the BA-DBA index increased from  $8.4 \pm 1.42$  years (with 3-6 years duration of the disease) and up to  $12.9 \pm 1.48$  with 10-year disease history. In patients with AAC, the difference in these groups was different, being the most significant with a disease history of 3-6 years:  $5.24 \pm 2.17$ ; it ranged in subsequent groups from  $5.09 \pm 1.88$  to  $11.28 \pm 2.24$  years. In the group of patients with biliary dyskinesia, the BA-DBA indexes with disease duration of 3-6 years were  $1.21 \pm 2.36$  years, with 7-10 years -  $2.46 \pm 4.57$ , and over 10 years -  $3.77 \pm 1.32$  years. The BA-DBA index of the control group was  $0.88 \pm 0.97$ , which is significantly different from the patients with diseases of the biliary system.

Thus, the results of the BA (BA-DBA) study in young people with biliary pathology depending on the duration of the disease confirmed that the presence of a lesion in the biliary system affects the aging processes of the body. The most pronounced negative effect was observed in patients with CC with a history of the disease over 10 years.

**Table 2:** BA-DBA distribution in the examined groups with diseases of the biliary system depending on the duration of the history of the disease (M±m)

Nosological entity	BA-DBA	Duration of a disease (Years)							
		Up to 3		3-6		7-10		Over 10	
		Qty	BA-DBA	Qty	BA-DBA	Qty	BA-DBA	Qty	BA-DBA
1. Biliary dyskinesia n=52	2.77±1.19	4	0.98±0.53	15	1.21±2.36	12	2.46±4.57	21	3.77±1.32
2. Chronic acalculous cholecystitis n=76	6.68±1.24	7	1.30±0.72	13	5.24±2.17	31	5.34±1.88	25	11.28±2.24
3. Chronic calculous cholecystitis n=54	9.59±1.00	22	1.23±0.63	12	8.38±1.42	14	8.8±2.37	6	12.9±1.48
4. Control group n=49	0.88±0.97	–	–	–	–	–	–	–	–

It should be noted that there is equalization in terms of BA-DBA with a history of biliary system disease over 10 years; the data are almost the same for the groups with chronic cholecystitis. This gives reason to believe that the stabilization process starts with the beginning of active collaboration of the doctor and patient. The results obtained suggest that not only the state of health predetermines changes in the BA-DBA parameters

but also the typical behavioral features of the young generation.

In the course of the further study the interdependence of BA and clinical-functional and behavioral features in patients with chronic cholecystitis was studied. The BA determination was performed in 130 patients with chronic cholecystitis by an ‘outpatient’ method (1 stage - 1 group), then all 130 patients were examined by the

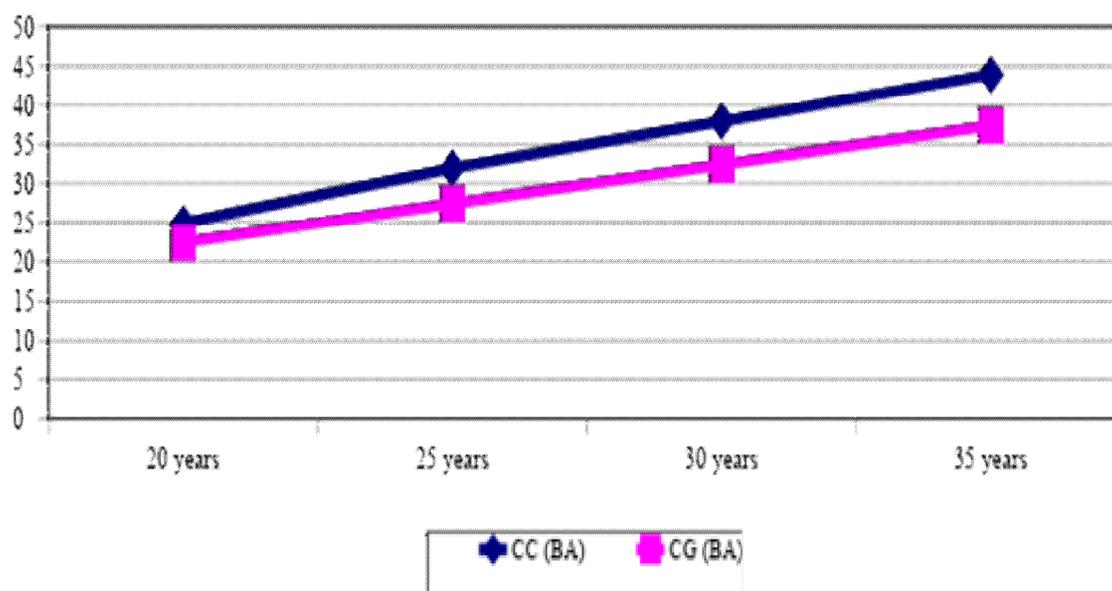
'inpatient method' (stage 2 - group 2). The obtained results were compared with the control group of 49 individuals with no pronounced issues related to the biliary system. This group was chosen as a model for assessing BA in individuals influenced by behavioral factors and with various changes in the biliary system. In order to objectify the duration of exposure to behavioral factors, the duration of the presence of this factor for at least 1 year for the patients with diseases of the biliary system was adopted as a measurement unit of such an impact.

The results of the study of biological age, obtained by the 'outpatient method' were as follows:

The BA values in the first group suffering from chronic cholecystitis were generally higher when compared with the control group by  $4.76 \pm 0.5$  U of biological age ( $p < 0.001$ ).

The results of the dependence of certain values of BA on CA in patients with chronic cholecystitis and in the control group obtained by the 'outpatient' method are presented in Figure 2.

It was revealed during the analysis of the equations obtained by the method of dispersive analysis, that in both cases the information capacity of the models was quite sufficient because the calculated F-test for both cases was  $F = 54.4726$ ,  $F = 83.9618$ , respectively; the dependence level of both models was  $< 0.001$ .



**Fig. 2.** Linear biological values dependence on the calendar age in patients with chronic cholecystitis (CC) and in the control group (CG) ('outpatient' method)

*Left to right/ top: 20, 25, 30, 35 years*

*Bottom: CC (BA); CG(BA)*

Thus, the BA excess in the intent-to-treat population suffering from chronic cholecystitis has been established in comparison with the control group during the screening study ('outpatient' method).

To examine the associations of BA indicator and diseases of the biliary system in more detail, a correlation analysis of BA parameter with the history of the disease was conducted; the analysis also included the number of disease relapses (typified by outpatient treatment for acute exacerbation of chronic cholecystitis with a medical disability certificate issued 2 times or more per year), the presence of dysbiotic intestinal disorders (second and third degrees), and dyslipidemia in the group examined; all interdependencies are presented in Table 3.

The study revealed that BA had a statistically significant direct correlation with the duration of the history of the disease and the number of relapses, but there was no such connection with dyslipidemia and dysbiotic intestinal disorders. The canonical correlation coefficient between the four characteristics of the test batteries (sBP, BHB, SBL, SHA) and BA of  $r = 0.504$  was highly significant ( $\rho < 0.05$ ).

**Table 3:** Pair correlation coefficients between the calendar and biological age for a number of clinical characteristics in patients with chronic cholecystitis

Characteristics n=130	Pair correlation coefficients			
	BA	Level of significance	CA	Level of significance
Duration of a disease	0.447	0.0001	0.602	0.0001
Number of relapses	0.355	0.002	0.327	0.005
Presence of dysbiotic intestinal disorders	0.198	0.5	0.092	0.5
Presence of dyslipidemia	0.116	0.5	-0.189	0.05

Therefore, there is a statistically significant correlation between the duration and the nature of the disease progression on the one hand, and indicators of biological age on the other. Correlation coefficients between BA parameters and the indicated characteristics are displayed in Table 4.

**Table 4:** Paired correlation coefficients of BA parameters and some clinical characteristics of chronic cholecystitis

Characteristics	BA parameters			
	sBP	FET	SBL	SHA
Duration of a disease	0.136	-0.397	-0.182	0.436
Number of relapses	0.055	-0.342	-0.108	0.446
Presence of dysbiotic intestinal disorders	0.052	-0.238	0.047	0.241
Dyslipidemia	0.106	-0.107	-0.056	0.216

In addition, a significant correlation was noted between the characteristics of the BHB and SHA test batteries with the past medical history duration and the number of relapses of the disease in young people.

Also, the results of the regression analysis showed that the information ability of the models was quite significant. The reliability of the fact of exceeding the biological age in patients with diseases of the biliary system was positively high.

The data for building a regression model on the parameters of the own sample obtained from young volunteers coincided with the results obtained by the authors of the V.P. Voitenko method. The information significance of the model according to F-test  $F = 7986.5$  ( $p < 0.0001$ ) and the coefficient of BA determination was rather large:  $R\text{-squared} = 0.9747$ .

The following calculated degree of influence of the indicators on the BA parameter was manifested in the course of the study: sBP = 29.8%, FET = 31.4%, SBL = 24.1%, SHA = 14.7%.

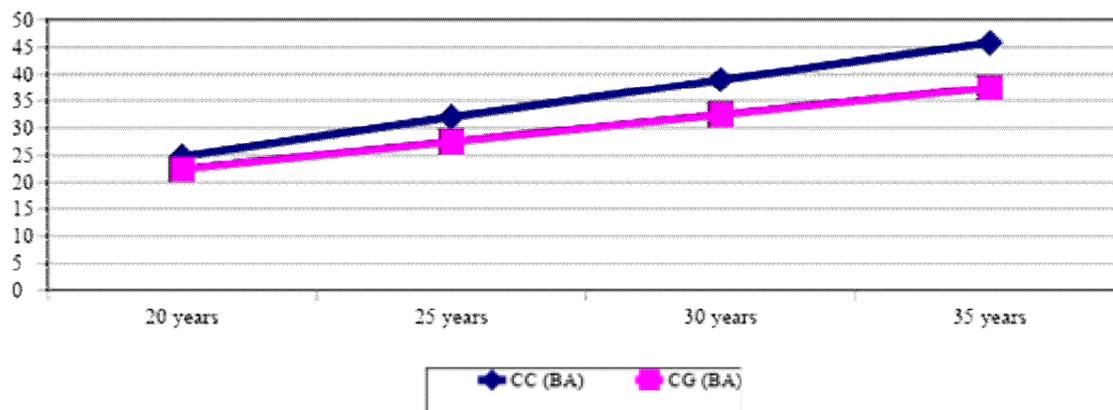
The coincidence between the data obtained in the course of own research and the data of the authors of the method indicated sufficient reliability and consistency of the method and the appropriateness of its application to the specified contingents, that is, to young people with biliary pathology.

Thus, the results of the analysis proved that one of the clinical signs of the disease such as the duration of the history of the disease is quite closely related to the indicators that determine BA in this group.

As a part of the research, the results of the study of biological age by another ('inpatient') method in patients with chronic cholecystitis were analyzed.

When assessing BA in the group of individuals with chronic cholecystitis (130 test persons), the BA group value as a whole was higher than in the control group by 4.62-0.7 U ( $p < 0.001$ ). The results of BA evaluation in the control group are presented below.

A simple linear dependence between the CA and its corresponding BA value is depicted in Figure 3.



**Fig. 3.** Linear biological values dependence on the calendar age in patients with chronic non-calculous cholecystitis and in the control group ('inpatient' method)

Left to right: 20, 25, 30, 35 years

Bottom: CC (BA); CG (BA)

The variance analysis revealed that in both cases the information capacity of the models was sufficient ( $F = 126.7$ ;  $F = 149.7$ , respectively), as well as the level of significance of the models ( $p < 0.0001$ ). In both cases, the resulting model accounted for 63-69% of the total variance of BA indicators.

A number of correlation patterns were found while conducting a correlation analysis of such clinical characteristics of the disease as the duration of the history, the number of relapses, dyslipidemia, and dysbiotic disorders (Table 5, Figure 4).

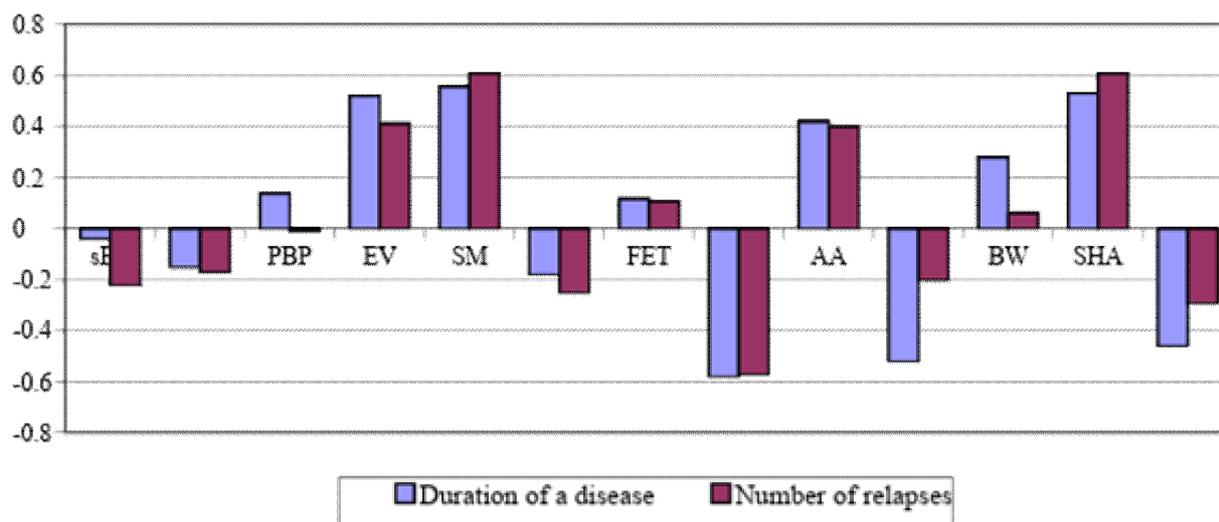
It ought to be remarked that a number of BA indicators had a significant correlation with the

history of anamnesis: from the test batteries of SM, MAV, EV, SHA, and AA.

Moreover, the same five indicators out of thirteen had a significant correlation with the number of relapses. In addition, the coefficients of the pair correlation of the calendar ( $r = 0.618$ ;  $p < 0.001$ ) and biological ( $r = 0.523$ ;  $p < 0.001$ ) age with the history of the biliary system diseases had approximately the same indicators. These coefficients with the number of relapses of the disease corresponded to  $r = 0.534$ ;  $p < 0.001$  and  $r = 0.528$ ;  $p < 0.001$ .

**Table 5:** Pair correlation coefficients between biological age, the history of chronic cholecystitis, the number of relapses and dyslipidemia

Indicators	Duration of a disease	Number of relapses	Dyslipidemia
1. CA	0.592	0.506	
2. BA	0.502	0.526	
3. sBP	-0.036	-0.216	-0.204
4. dBP	-0.106	-0.167	-0.102
5. PBP	0.144	-0.112	-0.167
6. EV	0.517	0.407	-0.106
7. SM	0.562	0.608	-0.016
8. BHB	-0.176	-0.254	-0.136
9. FET	0.124	0.112	-0.064
10. MAV	-0.582	-0.566	0.116
11. AA	0.422	0.398	0.224
12. SBL	-0.523*	-0.213	-0.208
13. BW	0.284	0.056	-0.219
14. SHA	0.529	0.608	-0.164
15. WAIS	-0.462	-0.295	-0.188



**Fig. 4.** Pair correlation coefficients between clinical and physiological indicators of biological age, duration of the history of chronic cholecystitis and the number of relapses of the disease

*O-Scale, Left to Right: sBP, dBP, PBP, EV, SM, BHB, FET, MAV, AA, SBL, BW, SHA, WAIS*

*Below: Duration of a disease; Number of relapses*

Consequently, the BA rate in young people was associated with the presence of glandulae endocrinae diseases in the examined group of patients, as indicated by the excess of BA indicator in comparison with the control group of healthy individuals, as well as by the relationship between the duration of a disease and the number of relapses. The high level of the correlation coefficient between BA and CA ( $r = 0.796$ ,  $p < 0.001$ ) indicates the presence of a strong interdependence between these parameters.

### Conclusion.

Thus, the BA indicator includes a significant 'chronic' component. However, it also has a 'pathological' constituent, since it simultaneously reflects the effect of the disease on the patient's body. A significant correlation coefficient between BA and anamnesis indicates the existence of relationships between them due to the number of relapses of the disease, which allows a comprehensive assessment of the presented data that influenced the aging process.

### Availability of data and materials

The data supporting the findings of this study are available from the corresponding author upon reasonable request. Taken consent from patients.

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**Conflict of interest:** The authors declare no conflict of interest.

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