Original article

Connective tissue dysplasia as a predictor of premature skin aging

Nikolai N. Potekaev [1,2], Olga B. Borzykh [3], Elena I. Karpova [1], Marina M. Petrova [3], Natalia A. Shnayder [3,4], Diana V. Dmitrenko [3], Maria A. Zatolokina [5], Olga M. Demina [1]

[1] Pirogov Russian National Research Medical University, Moscow, Russia
[2] Moscow Research and Practical Center for Dermatology and Cosmetology, Moscow, Russia
[3] Voino-Yasenetsky Krasnoyarsk State Medical University, Krasnoyarsk, Russia
[4] Bekhterev National Medical Research Center for Psychiatry and Neurology, St. Petersburg, Russia
[5] Kursk State Medical University, Kursk, Russia

Abstract: *Background* — One of the manifestations of connective tissue dysplasia (CTD) is premature skin aging. The latter can have different etiological factors. *The objective* of our study was to investigate the effect of predictors of premature skin aging, especially CTD, on the severity of involutional changes in the skin, as well as to develop the approach for the management of patients with premature aging.

Material and Methods — We included in our study 78 women 35-45 years of age with no substantial anti-aging treatment procedures in their anamneses. We considered genealogical and cosmetological anamneses, and life history (including somatic pathology), physical examination; determined prevailing type of facial skin aging; identified predictors of CTD, assessed psychoemotional state of the patient, and performed sonography of the skin and biochemical examination of patient serum. At a clinical stage, we conducted a randomized comparative study of biorevitalizant efficacy in patients with normal and premature patterns of skin aging.

Results — Our study established the role of CTD in assessing the risk of premature skin aging. In addition to the presence of CTD, the importance of identifying isolated phenotypic manifestations of CTD (such as arachnodactyly, hypermobility syndrome, low relative weight of the patient, and skin manifestations of CTD) was shown. Other predictors were also analyzed; their identification can help assessing the risk of premature skin aging. Among them, somatic pathology (varicose veins, herniated discs, visceroptosis, autonomic vascular dystonia) and physical examination data (such as pallor of the facial skin, swelling, skin hyperelasticity) were noted. When assessing the clinical efficacy of biorevitalization, the greatest satisfaction of patients with premature skin aging regarding its results was characteristic for the group of patients after 7% collagen treatment. The clinical efficacy according to sonography and histological examination in patients with premature skin aging was significantly higher after the use of collagen or a complex hyaluronic acid (HA) preparation, compared with native HA. Patients with premature skin aging exhibited high level of anxiety and/or depression, which in turn increased the risk of underestimating the outcome of procedures.

Conclusion — The presented study confirmed the role of CTD in assessing the risk of premature skin aging. Predictors were revealed (somatic pathology, type of facial aging, physical examination data of the patient, etc.) that could be used to assess the risk of premature skin aging. In patients with premature aging, preparation with native HA (as a biorevitalizant) had a low clinical efficacy comparable to using placebo. A collagen-based preparation and a complex HA-based preparation exhibited high clinical efficacy. Also, patients with premature aging of the skin had a higher score of anxiety and depression, while patients with high levels of anxiety and depression were more likely to underestimate the satisfaction with the results of their aesthetic treatment.

Keywords: involutional changes in the skin, aging, CTD, biorevitalization.

Introduction

Interest in CTD, which is not a hereditary (monogenic) disease, has appeared relatively recently. Published studies showed the role of CTD in the risk of developing various somatic pathologies [1]. Also, some authors emphasized the role of CTD in the development of certain skin pathologies, early onset of involutional changes in the skin, and premature aging [2].

It is known that aging is a natural and inevitable programmed process that begins after birth [3, 4]. However, premature aging occurs when the severity of age-related loss of organ system functions exceeds the calendar age. An extremely rare variant of repeatedly accelerated aging is genetic pathology (progeria) [5]. The latter is based on various mutations, because of which early loss of functions of all organ systems occurs in patients [6]. However, this disease is not considered in the context of aesthetic

medicine since the primary task for such patients is to maintain the functioning of vital organ systems.

Currently, premature biological aging is well described in Russia. Indicators of biological age reflect the functional state of internal organs [7, 8]. Skin aging can also go at a different pace. The term premature aging of the skin is widely used; however, in the available literature, we did not find an accurate diagnostic algorithm and patient management approach [9]. Most often, the concept of premature aging describes photoaging, when aggressive environmental factors (mainly UV radiation) are superimposed on the natural rate of biological aging [10, 11]. Thus, the objective of our study was to examine the effect of predictors of premature skin aging, especially CTD, on involutional changes in the skin, as well as to develop approaches for managing patients with premature skin aging and to compare the effectiveness of drugs used to correct involutional changes in the skin in patients with normal and premature aging patterns.

Material and Methods

Our study was conducted in 2021–2022 on the basis of the Department of Skin Diseases and Cosmetology at Pirogov Russian National Research Medical University (Moscow), the Molecular and Cellular Technology Center for Collective Use at Voino-Yasenetsky Krasnoyarsk State Medical University (Krasnoyarsk), and Dr. Albrecht Clinic of Plastic Surgery and Cosmetology (Voronezh).

General characteristics of patients

We Included 78 patients in the study based on the following inclusion criteria: apparently healthy women 35–45 years of age; without a history of dermal filler injections, thread lifts, plastic surgery and other cosmetic procedures at least a year before their inclusion in the study; without endocrine disorders and pronounced photoaging; not taking medications that affect skin condition. The total sample of patients was subsequently divided into two groups: the main group (52 patients with premature skin aging) and the comparison group (26 patients with normal skin aging).

Study design

The study was conducted in three stages: screening, laboratory examination, and clinical stage (*Figure* 1). At the screening stage, we analyzed genealogical and cosmetological anamneses, and life histories (including somatic pathology); carried out physical examination; determined the prevailing type of facial skin aging; identified the predictors of CTD; assessed psychoemotional state of each patient; performed skin sonography and photographed patients. At the laboratory stage, we carried out a biochemical study of the patient serum before biorevitalization. At the third stage, we conducted a comparative clinical study of biorevitalization efficacy in patients with normal and premature types of skin aging. The results were documented in the official patient record approved by the Ethics Committee of the Pirogov Russian National Research Medical University (Protocol # 206 of 22 March 2021). All patients signed an informed consent to participate in the study.
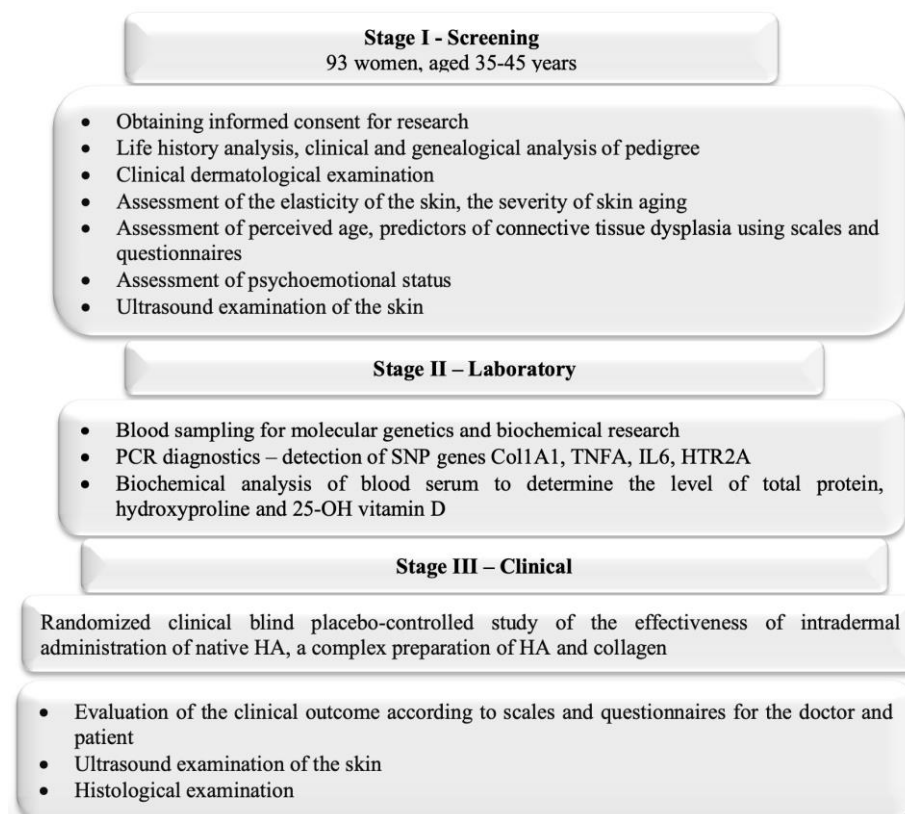

Stage I - Screening
93 women, aged 35-45 years

- Obtaining informed consent for research
- Life history analysis, clinical and genealogical analysis of pedigree
- Clinical dermatological examination
- Assessment of the elasticity of the skin, the severity of skin aging
- Assessment of perceived age, predictors of connective tissue dysplasia using scales and questionnaires
- Assessment of psychoemotional status
- Ultrasound examination of the skin

Stage II – Laboratory

- Blood sampling for molecular genetics and biochemical research
- PCR diagnostics – detection of SNP genes Col1A1, TNFA, IL6, HTR2A
- Biochemical analysis of blood serum to determine the level of total protein, hydroxyproline and 25-OH vitamin D

Stage III – Clinical

Randomized clinical blind placebo-controlled study of the effectiveness of intradermal administration of native HA, a complex preparation of HA and collagen

- Evaluation of the clinical outcome according to scales and questionnaires for the doctor and patient
- Ultrasound examination of the skin
- Histological examination

Figure 1. Study design.

Table 1. Phenotypic signs in patients with connective tissue dysplasia according to the phenotypic map of M.J. Glesby 1989 and the evaluation table of L.N. Abbakumova 2008

The phenotypic map of M.J. Glesby 1989	The evaluation table of L.N. Abbakumova 2008
Asthenic constitution	Asthenic constitution
High-arched palate	High-arched palate
Joint hypermobility syndrome	Joint hypermobility syndrome
Scoliosis	Scoliosis
Flatfeet	Flatfeet
Myopia	Myopia
Pectus excavatum	Pectus excavatum
Pectus carinatum	Pectus carinatum
Skin hyperelasticity	Skin hyperelasticity
Widespread hyperpigmentation	Widespread hyperpigmentation
Loss of normal posture	Deviated nasal septum
Arachnodactyly	Skin with visible veins
Positive wrist sign	Presence of transverse striation on the foot skin
Positive thumb sign	Corns
Straight back	Hallux valgus
Ectopia lentis	Heavily trampled foot
	Presence of scars on the skin

Table 2. Distribution of patients by groups and subgroups depending on their treatment (M±SD)

Subgroup	Group 1	Group 2
Subgroup A, Σn=40	n=20, mean age 38.4±2.96*	n=20, mean age 39.9±3.03*
Subgroup B, Σn=46	n=21, mean age 39.8±2.58*	n=25, mean age 40.5±2.96*
Subgroup C, Σn=20	n=20, mean age 38.7±3.29*	
Subgroup D, Σn=21	n=21, mean age 39.3±3.51*	

Comparison between groups; *, $p>0.05$; statistical significance of differences between independent quantitative indicator samples was assessed using the Mann-Whitney test.
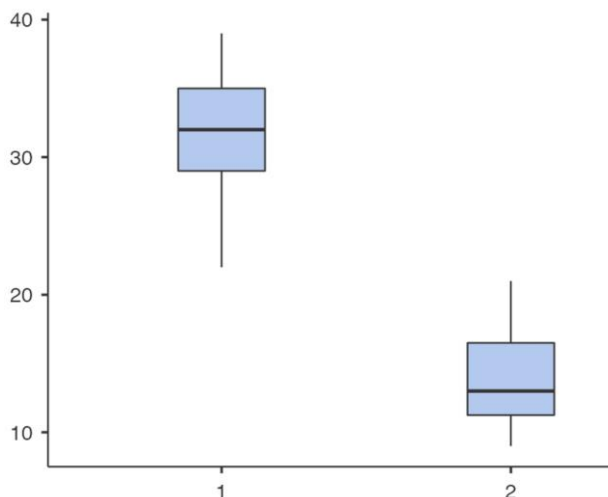

Figure 2. The index of involutional changes in the main group (1) vs. the comparison group (2). The sum of points is shown on the vertical axis ($p<0.001$).

Assessment of the severity of skin aging and perceived age

Assessment of the severity of involutional changes in the skin was performed by photographic scales [12, 13] and during physical examination (via assessment of the skin elasticity and extensibility). The assessment of the severity of aging was scored in points ranging from 0 (no changes) to 4 (extremely pronounced changes).

To calculate the perceived age, a focus group of respondents was created, who were asked to estimate age of patients from photographs taken under similar conditions. The focus group included 21 respondents (11 women and 10 men) aged from 24 to 52 years. The respondents included 7 cosmetologists, 7 plastic surgeons and 7 individuals without medical education.

Diagnosing CTD

Diagnosis of CTD was carried out by questioning and physical examination of patients. It was established on the basis of the presence of three or more phenotypic signs *sensu* the Glesby phenotypic map and Abbakumova evaluation table criteria (*Table 1*) [14, 15]. Skin manifestations of CTD were noted, such as skin hyperelasticity, thin skin, increased skin venosity, easily wounded skin, presence of pathological scarring and telangiectasia. The presence of minor developmental anomalies characteristic of patients with CTD, along with the presence of joint hypermobility syndrome and arachnodactyly, were also noted.

Biochemical study

To conduct a biochemical analysis, blood was sampled from the cubital vein into test tubes with a coagulation activator. Further on, centrifugation was carried out, followed by the separation of serum. The serum levels of vitamin D and total protein were determined.

Assessment of psychoemotional state

The assessment of the psychoemotional state was carried out by the method of questioning patients on the Hospital Anxiety and Depression Scale (HADS) [16]. Patients filled out a questionnaire in the presence of a doctor before and after the procedures.

Comparative randomized placebo-controlled study of biorevitalization efficacy

To compare the efficacy of intradermal injection of preparations used to correct involutional changes in the skin, all patients were randomly divided into six subgroups of comparable ages (*Table 2*):

• Subgroup 1A: 20 patients of the main group with intradermal placebo injection;

• Subgroup 1B: 21 patients of the main group with intradermal injection of native 1.8% HA (Hyon 1.8%, Infarm, Russia, RU No. 2018/7614);

• Subgroup 1C: 20 patients of the main group with intradermal injection of a complex preparation based on 1.5% HA, amino acids, vitamins, antioxidants and minerals (Teosyal Redensity 1, Teoxane, Switzerland, RU No. 2011/09821);

• Subgroup 1D: 21 patients of the main group with intradermal injection of collagen (Collost 7%, BioPHARMAHOLDING, Russia, RU No. 2008/02112);

• Subgroup 2A: 20 patients of the comparison group with intradermal injection of placebo;

• Subgroup 2B: 25 patients of the comparison group with intradermal injection of native 1.8% HA (Hyon 1.8%, Infarm, Russia, RU No. 2018/7614).

Table 3. Evaluation of phenotypic signs of connective tissue dysplasia in patients of the main group vs. the comparison group, scores (Me [LQ; UQ])

Criteria for connective tissue dysplasia	Main group	Comparison group	P (Mann-Whitney test)
The phenotypic map of M.J. Glesby 1989	4 [1.5; 5]	1 [0; 2]	p<0.001
The evaluation table of L.N. Abbakumova 2008	4 [3; 5.5]	1 [1; 2]	p<0.001

Statistical significance of differences between independent quantitative indicator samples was assessed using the Mann-Whitney test.

Table 4. Predictors of premature aging in patients of the main group vs. the comparison group (n; %)

Predictors	Main group	Comparison group	Fisher's exact test
CTD	38; 73.1%	5; 19.2%	p<0.001
Skin hyperelasticity (extension >2 cm)	40; 76.92%	1; 3.85%	p<0.001
Thin skin with visible veins	37; 71.15%	3; 11.54%	p<0.001
Easily wounded skin	35; 67.31%	2; 7.69%	p<0.001
Telangiectasia	33; 63.46%	5; 19.23%	p<0.001
Easy bruising	35; 67.3%	2; 7.7%	p<0.001
Reduced skin regeneration	20; 38.5%	4; 15.4%	p=0.042
Abnormal scarring	10; 19.2%	0	p=0.017
Joint hypermobility syndrome	26; 50%	2; 7.69%	p<0.001
Arachnodactyly	20; 38%	0	p<0.001
Autonomic vascular dystonia	16; 30.77%	0	p<0.001
Frequent colds (more than 4 times a year)	10; 19.23%	0	p=0.013
Varicose veins	17; 32.69%	3; 11.54	p=0.037
Visceroptosis/spinal hernia	14; 26.9%	2;7.7%	p=0.047
Pale, grayish complexion without a healthy glow	44; 84.61%	5; 19.23%	p<0.001
Dark circles under eyes	49; 94.2%	3; 11.5%	p<0.001
Swelling of the face	27; 51.9%	6; 23.1%	p=0.015
Varga body build index less than 1.7	25; 48.1%	1; 3.8%	p<0.001

CTD, connective tissue dysplasia; statistical significance of differences between independent quantitative indicator samples was assessed using Fisher's exact test.

Patients could participate in different subgroups with an interval of 12 months (for preparations) and 6 months (for placebo).

Three intradermal injections of preparations in the facial area were carried out with an interval of 2-3 weeks; the efficacy was monitored for 30 days after the last procedure.

Assessing clinical efficacy of procedures

Clinical evaluation of the procedures' efficacy was carried out based on validated and non-validated (developed by the authors) scales. The Global Aesthetic Improvement Scale (GAIS) was used as a validated scale, with an assessment of the treatment outcome by the patient and the doctor. The result was recorded on a following scale: 1, marked improvement; 2, very good improvement; 3, some improvement; 4, no change; 5, deterioration of the skin condition [17]. A questionnaire for patients on changes in their skin parameters after procedures was used as a non-validated scale. This questionnaire asked patients to evaluate the change in the qualitative indicators of their skin (color, glow, moisture, pleasant texture, enlarged pores, skin tone, fine wrinkles) in points as follows: 0, no changes; 1, some changes; 2, pronounced improvement. Results obtained in subgroups after placebo were used as an efficacy control. denotes marked improvement, 2 denotes very good improvement, 3 stands for some improvement, and 4 means no change.

Patients were photographed before and after the end of the course of procedures. All photos were taken in identical conditions (illumination, head position).

Sonography

For ultrasound examination of skin thickness (before and after the course of procedures), we used the Mindray DC-70 ultrasound scanner. The measurement of skin thickness by a high-density linear sensor L12-3E was carried out at six points on the patient facial skin: in the projection of the middle of the zygomatic arch (right and left), in the projection of the lower jaw body (right and left) and in the submental region (right and left). The skin thickness was noted from the entrance echo of the epidermis to the border of the dermis (the place of transition of the hyperechoic layer of the dermis to the hypoechoic layer of subcutaneous fat). Results obtained in subgroups after placebo, as well as those obtained in the submental space (where injections were not performed) were used as a control for efficacy estimates.

Histological examination

For histological examination, patients underwent skin biopsy in the parotid fold before and after the course of procedures (before the biopsy, intradermal injections were carried out, including in this particular area). The skin biopsies were fixed in a 10% formalin solution, followed by hematoxylin and eosin staining, Van Gieson and Malory staining, and Sirius Red staining with a subsequent polarization microscopy.

Statistical data processing

Statistical data processing was carried out using the Jamovi and SSPS software. The statistical significance of differences between the groups was examined Using the Mann–Whitney U test for independent samples, the Wilcoxon signed-rank test for paired data, and the Fisher's exact test for nonparametric data. All indicators were assessed for normality based on Shapiro–Wilk test. At p>0.05, the results were presented as mean (M) ± standard deviation (SD). At p<0.05, the data were presented as median and interquartile range, Me [LQ; UQ]. ANOVA was employed for multivariate analysis. The results were assumed statistically significant at p<0.05.

Results

The selection of groups of patients with premature and normal skin aging was performed based on the calculation of the perceived age according to the results of a survey of a focus group of respondents. The age difference was determined between an average perceived age and actual age. Considering that the perceived age is affected by overweight, patients with a BMI of up to 24.9 kg/m2 were included in the initial assessment. The age difference in the group of patients with premature skin aging (the main group) was 3.82±1.90 years (i.e., patients perceived to be older than their actual age). In the group with normal skin aging (comparison group), the age difference was (-)3.62±2.40 years (i.e., patients perceived to be younger than their actual age).

Furthermore, in the main group and the comparison group, a comparative analysis of the severity of signs of age-related changes in the face was carried out. The signs by which the groups differed statistically significantly were summarized and used to calculate the index of involutional changes. To do so, we summed up scores assessing skin tone and elasticity, enlarged pores,

severity of infraorbital and mid-buccal furrows, nasolabial folds, marionette lines, accordion lines, wrinkles in front of the ears, lower eyelid hernias, severity of brow ptosis and face oval ptosis, excess skin of the upper eyelid, and age-related changes in the neck [18].

In the main group, the index of involutional changes was from 22 to 39 points, median of 32 [29; 35] points; in the comparison group, it was from 9 to 21 points, median of 13 [11; 15] (*Figure* 2).

For further study, we added patients with a BMI of 25 kg/m2 to the groups. Thus, in the main group there were 52 patients with premature skin aging (mean age 38.9±3.13 years), while in the comparison group, there were 26 patients with normal skin aging (mean age 40.5±2.92 years).

CTD was diagnosed in 38 patients (73.1%) of the main group and 5 patients (19.2%) of the comparison group based on the presence of signs *sensu* the phenotypic map of M.J. Glesby and the evaluation table of L.N. Abbakumova. The mean number of phenotypic traits in the two groups was statistically significantly different (*Table* 3).

CTD is a multiorgan pathology of connective tissue with varying penetrance of symptoms. For dermatologists and cosmetologists, skin manifestations of CTD are of greater importance. The most characteristic skin manifestations of CTD include thin flaccid skin with visible veins, petechiae and hyperelasticity, as well as with reduced regeneration and pathological scars. Skin manifestations in the two groups differed statistically significantly (*Table* 4). Therefore, not only CTD, but also the presence of isolated skin manifestations, can be considered predictors of premature skin aging. The study also noted impaired regeneration and scarring in patients of the main group. Atrophic scars of unknown origin in the form of striae were not identified in patients of both groups, and the prevalence

values of striae caused by fluctuations in body weight or pregnancy did not differ statistically significantly.

Moreover, we detected patients with hypermobility syndrome, albeit without a full diagnosis of CTD, and with arachnodactyly in the group of premature skin aging.

In the course of the genealogical anamnesis, we identified three patients with premature skin aging on the maternal side in the main group, while no relatives with premature skin aging were detected in the comparison group.

Among somatic pathology, autonomic vascular dystonia, frequent colds (more than 4 times a year), varicose veins, visceroptosis and/or spinal hernias were statistically significantly more common in the main group of patients.

According to the examination data, the patients of the main group were more likely to have a tired aging morphotype (39 patients or 75.01%), pale facial skin with no healthy glow (44; 84.61%), and dark circles under eyes (49; 94.23%). Also, patients of the main group more often complained of swelling of the face and in the area under the eyes (14; 26.92%). There was no statistically significant difference in the prevalence of hyperpigmentation and skin types (p>0.05).

In addition, one of the diagnostic criteria for CTD is a decrease in the Varga body build index of less than 1.7 (reflecting the patient's BMI-to-age ratio). In our study, a smaller Varga body build index was statistically significantly found in patients of the main group (with premature skin aging).

When analyzing external factors affecting aging (poor sleep, sedentary lifestyle, excessive consumption of fast digesting carbohydrates, frequent exposure to stress and to sunlight without SPF protection, smoking), we found no statistically significant differences between the two groups. As for laboratory parameters, there were no statistically significant differences in the levels of serum total protein and vitamin D (p>0.05).
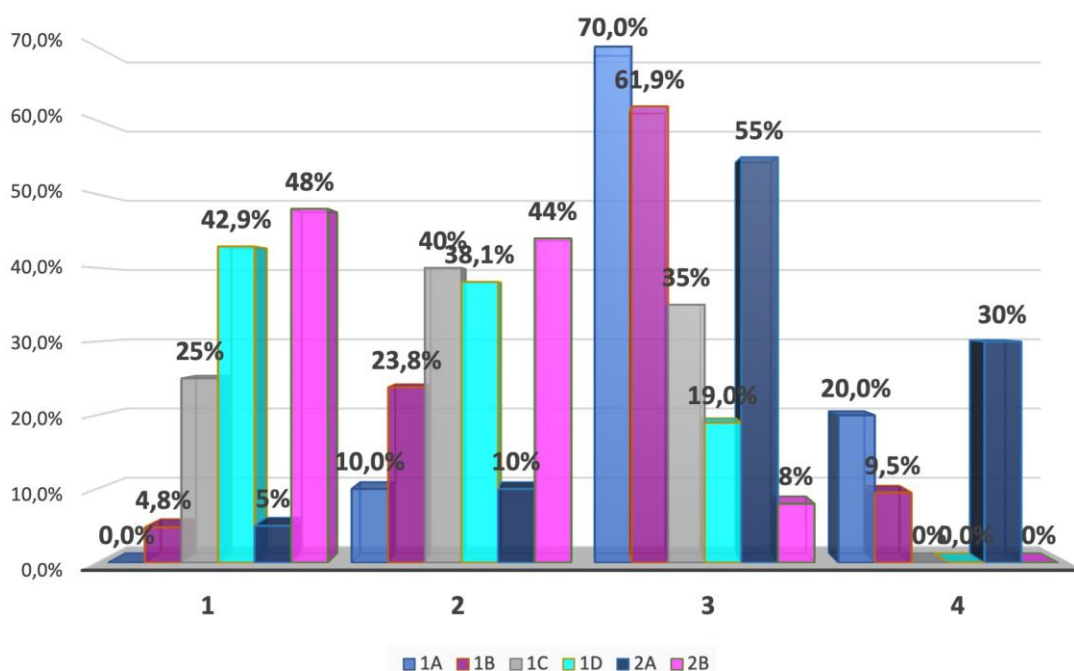

Figure 3. Comparison of clinical efficacy (*sensu* GAIS) of biorevitalizant in the main group vs. the comparison group. 1 denotes marked improvement, 2 denotes very good improvement, 3 stands for some improvement, and 4 means no change.
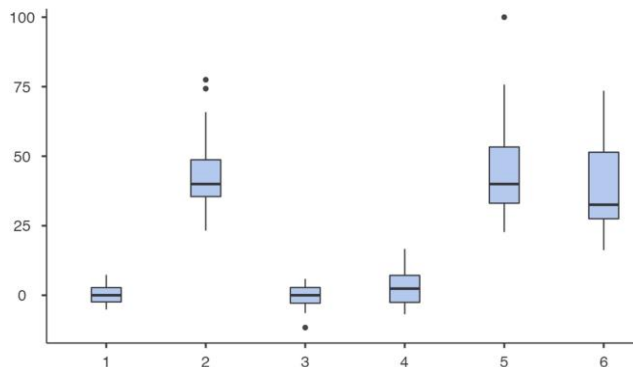
Figure 4. Change in the skin thickness of the middle third of the face in patients of the main group vs. the comparison group after aesthetic treatment. 1 denotes patients of subgroup 2A, 2 denotes patients of subgroup 2B, 3 denotes patients of subgroup 1A, 4 denotes patients of subgroup 1B, 5 denotes patients of subgroup 1C, 6 denotes patients of subgroup 1D; % change in skin thickness is shown on the vertical axis.
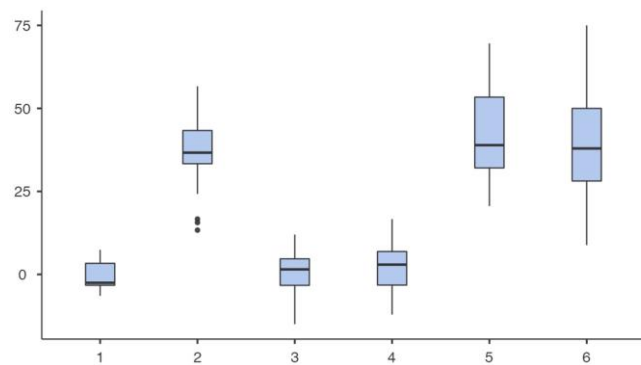

Figure 5. Change in the skin thickness of the lower third of the face in patients of the main group vs. the comparison group after aesthetic treatment. 1 denotes patients of subgroup 2A, 2 denotes patients of subgroup 2B, 3 denotes patients of subgroup 1A, 4 denotes patients of subgroup 1B, 5 denotes patients of subgroup 1C, 6 denotes patients of subgroup 1D; % change in skin thickness is shown on the vertical axis.

According to the results of analyzing the cosmetic treatment anamnesis, 30 patients of the main group and 15 patients of the comparison group had previously undergone cosmetic procedures that did not affect their perception of age (mesotherapy, biorevitalization, peelings, resurfacing, plasma lift, botulinum toxin therapy). At the same time, in 7 patients of the main group, the presence of undesirable effects after cosmetic procedures was noted, such as persistent swelling after botulinum toxin therapy, an autoimmune response to plasma lift, or thin skin. There were no patients with adverse events after aesthetic procedures in the comparison group.

Based on the above, predictors of premature skin aging can be identified that can be applied to all patients, including patients with a history of aesthetic procedures affecting the severity of involutional changes in the skin (*Table* 4).

In a comparative assessment of the clinical efficacy of biorevitalization (based on GAIS), we obtained the following results (*Figure* 3):

- In patients with premature skin aging, the clinical efficacy of native HA was comparable to the clinical efficacy of placebo;

- In patients with normal skin aging, the clinical efficacy of native HA was statistically significantly higher than the clinical efficacy of placebo and native HA in patients with premature skin aging;

- In patients with premature skin aging, the clinical efficacy of the collagen preparation and the complex preparation based on HA was statistically significantly higher than the efficacy of placebo or native HA in patients with premature skin aging, and it was comparable to the clinical efficacy of native HA in patients with normal skin aging.

According to skin sonography, results comparable with clinical efficacy were obtained (*Figures* 4 and 5). However, despite the positive assessment by patients based on the GAIS scale, there were no statistically significant changes in skin thickness after placebo according to sonography data.

Despite similar clinical efficacy of the complex HA preparation and collagen preparation, the overall level of patient satisfaction after the course of intradermal administration of collagen in the main group of patients was higher. Based on the GAIS, pronounced improvement was stated by 42.9% of patients after the collagen preparation and only 25% of patients after the complex HA preparation. Also, higher satisfaction in the main group after the collagen was due to such parameters as:

- Improvement of skin color (95% vs. 75% after the complex preparation);

- Increased skin hydration (100% vs. 95% after the complex preparation);

- Increased skin tone (95.2% vs. 90% after the complex preparation).

Table 5. Results of the patient psychoemotional state study in the main group vs. the comparison group (M±SD).

Indicators of psychoemotional state	Main group	Comparison group	p (Mann-Whitney test)
Anxiety (HADS)	6.57±3.13	5.50±2.69	p=0.071
Depression (HADS)	4.51±2.91	3.92±2.61	p=0.213

Statistical significance of differences between independent quantitative indicator samples was assessed using the Mann-Whitney test; HADS, Hospital Anxiety and Depression Scale.

Table 6. Results of the patient psychoemotional state study in patients with and without connective tissue dysplasia (CTD), M±SD.

Indicators of psychoemotional state	Patients with CTD	Patients without CTD	p (Mann-Whitney test)
Anxiety (HADS)	6.88±2.98*	5.35±2.87*	p=0.011
Depression (HADS)	4.58±2.98	3.97±2.59	p=0.193

Statistical significance of differences between independent quantitative indicator samples was assessed using the Mann-Whitney test; HADS, Hospital Anxiety and Depression Scale.

Table 7. Assessment of the psychoemotional state of patients who did and did not underestimate their satisfaction with the treatment result (M±SD).

Indicators of psychoemotional state	Patients who did not underestimate their satisfaction	Patients who underestimated their satisfaction	p (Mann-Whitney test)
Anxiety (HADS)	5.87±3.50	7.78±2.59	p=0.02
Depression (HADS)	3.74±2.84	5.22±3.32	p=0.04

Statistical significance of differences between independent quantitative indicator samples was assessed using the Mann-Whitney test; HADS, Hospital Anxiety and Depression Scale.
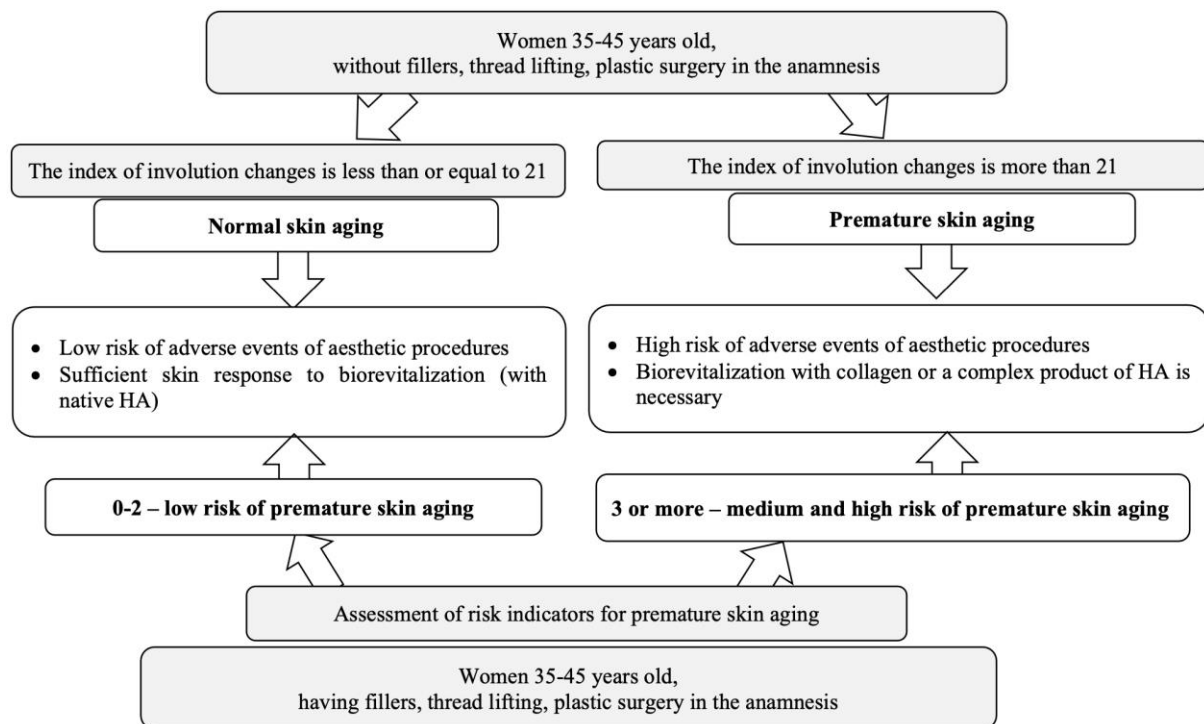
Figure 6. The algorithm of managing patients with involutional changes in the skin.

The results of the histological examination were comparable with the data of the clinical assessment and the data on the change in skin thickness. Pronounced morphological changes were noted in patients with normal skin aging after the use of native HA and in patients with premature skin aging after the use of a collagen preparation and a complex preparation based on HA. Morphological changes of the skin in patients with premature aging after the use of native HA were not substantial. After using placebo, no morphological changes in the skin were observed.

When assessing the psychoemotional state of patients based on the HADS, no statistically significant changes were noted before and after injection procedure. However, an increase in the levels of anxiety and depression was noted in patients with premature skin aging and in patients with CTD (*Tables* 5 and 6).

We also discovered that patients with increased level of anxiety and depression were more likely to underestimate the treatment outcome (which was established by the difference between the GAIS assessment by the patient and the GAIS assessment by the doctor) (*Table* 7).

Discussion

Numerous studies demonstrated contributions of various internal and external factors to the development of aging. Among external factors, the role of UV radiation, air pollution, smoking, and dietary patterns are discussed most often. Internal factors include gender, ethnicity, nutrient deficiency, overweight, increased oxidative stress, neuroendocrine disorders, chronic inflammation, telomere shortening, and genetic factors [19-28]. The role of CTD was shown in the development of signs of premature skin aging as well [2].

Our study confirmed the role of CTD as a possible indicator of premature skin aging. In addition, we revealed other predictors that could be used to assess the risk of premature skin aging. Such predictors include: the presence autonomic vascular dystonia, varicose veins, spinal hernia and/or visceroptosis, joint hypermobility syndrome, and arachnodactyly. Also, the risk of premature skin aging was noted in patients with a tired morphotype of facial aging, skin hyperelasticity, and thin easily wounded skin. At the same time, we detected no significant difference in the presence of other described risk factors for skin aging in patients with normal and premature skin aging patterns.

Depending on the prevailing factor affecting the aging process, different authors suggested various methods of correcting age-related changes. These include the appointment of antioxidants, stem cell therapy, appointment of retinoids, hormone replacement therapy, telomere modification, dietary restrictions, and anti-inflammatory therapy [29].

Among the methods used to correct age-related skin changes, the most studied are mesotherapy and biorevitalization methods. These methods involve intradermal injections preparations generating a rejuvenating effect. Pathogenetically, injected preparations can act in different ways. For instance, HA creates conditions for the vital activity of fibroblasts; an addition of nutrients to the preparation increases its bioavailability in the skin; collagen is capable of changing the rigidity of fibroblasts converting them to a younger and synthetically active phenotype [30].

Our study demonstrated the need to choose a preparation for the correction of involutional changes in the skin (biorevitalization) depending on the rate of aging. E.g., we confirmed high clinical efficacy of the preparation based on native HA in patients with normal skin aging. Therefore, it is sufficient for patients with normal aging to use preparation based on native HA to achieve

pronounced clinical efficacy according to objective and subjective research methods.

Patients with premature skin aging demonstrated low clinical efficacy of the preparation with native HA. Patients with premature skin aging needed a complex preparation based on HA (in our case, Teosyal Redensity 1) or collagen (in our case, 7% Collost® gel). However, when assessing the clinical outcome, it is necessary to keep in mind that the psychoemotional state of the patient may influence its subjective assessment, which was also shown in our study.

In general, it is possible to present the management of a patient with age-related skin changes in the form of an algorithm (*Figure* 6).

Conclusion

The presented study shows the role of CTD in assessing the risk of premature skin aging. Predictors were identified (somatic pathology, type of facial aging, physical examination data of the patient, etc.) that could be used to assess the risk of premature skin aging.

In patients with premature aging, preparation with native HA (as a biorevitalizant) had a low clinical efficacy vs. placebo. A collagen-based preparation and a complex HA-based preparation exhibited high clinical efficacy.

Also, patients with premature skin aging had a higher score of anxiety and depression, while patients with a high level of anxiety and depression were more likely to underestimate their satisfaction with the results of the aesthetic treatment.

Conflict of interest
Authors declare no conflicts of interest.

Ethical approval

All prospective subjects gave written informed consent to participate in the study. The study was conducted in compliance with the Declaration of Helsinki of the World Medical Association and approved by the Ethics Committee at Pirogov Russian National Research Medical University.

References

1. Demidov RO, Lapshina SA, Yakupova SP, Mukhina RG. Connective tissue dysplasia: Current approaches to the clinic, diagnosis and treatment. *Practical medicine* 2015; (4-2(89)): 37-40. Russian. https://www.elibrary.ru/item.asp?id=23765084.

2. Kononova NYu, Chernysheva TE, Styazhkina SN. Is connective tissue dysplasia a predictor of premature aging? (The results of the five-year monitoring). *Medical news of the north caucasus* 2016; 11(2-2): 326-330. Russian. https://doi.org/10.14300/mnnc.2016.11068.

3. Bielach-Bazyluk A, Zbroch E, Mysliwiec H, Rydzewska-Rosolowska A, Kakareko K, Flisiak I, et al. Sirtuin 1 and skin: Implications in intrinsic and extrinsic aging – A systematic review. *Cells* 2021; 10(4): 813. https://doi.org/10.3390/cells10040813.

4. Kim HS, Park SY, Moon SH, Lee JD, Kim S. Autophagy in human skin fibroblasts: Impact of age. *Int J Mol Sci* 2018; 19(8): 2254. https://doi.org/10.3390/ijms19082254.

5. Coppedè F. The epidemiology of premature aging and associated comorbidities. *Clin Interv Aging* 2013; 8: 1023-1032. https://doi.org/10.2147/CIA.S37213.

6. Aliper AM, Csoka AB, Buzdin A, Jetka T, Roumiantsev S, Moskalev A, et al. Signaling pathway activation drift during aging: Hutchinson-Gilford Progeria Syndrome fibroblasts are comparable to normal middle-age

7. and old-age cells. *Aging (Albany NY)* 2015; 7(1): 26-37. https://doi.org/10.18632/aging.100717.

7. Voytenko VP, Polyukhov AM, Barbaruk LG. Biological age as a key problem of gerontology. In: Gerontology and geriatrics: Biological age, heredity and aging. Kyiv: Institute of Gerontology. 1984: 5-15. Russian.

8. Proshhaev KI, Zhernakova NI, Kapustin RF, Pozdnjakova NM, Martines Garses HK, Duke Kalderon YuF. Human biological age and premature aging in an anthropological context. *The Scientific Notes of the Pavlov University* 2011; 18(2): 14-16. Russian. https://www.elibrary.ru/item.asp?id=22002856.

9. Clatici VG, Racoceanu D, Dalle C, Voicu C, Tomas-Aragones L, Marron SE et al. Perceived age and life style. The specific contributions of seven factors involved in health and beauty. *Maedica (Bucur)* 2017; 12(3): 191-201. https://pubmed.ncbi.nlm.nih.gov/29218067.

10. Ansary TM, Hossain MR, Kamiya K, Komine M. Ohtsuki M. Inflammatory molecules associated with ultraviolet radiation-mediated skin aging. *Int J Mol Sci* 2021; 22(8): 3974. https://doi.org/10.3390/ijms22083974.

11. Orioli D, Dellambra E. Epigenetic regulation of skin cells in natural aging and premature aging diseases. *Cells* 2018; 7(12): 268. https://doi.org/10.3390/cells7120268.

12. Lemperle G, Holmes RE, Cohen SR, Lemperle SM. A classification of facial wrinkles. *Plast Reconstr Surg* 2001; 108(6): 1735-1750. https://doi.org/10.1097/00006534-200111000-00048.

13. MERZ-AESTHETICS: website. https://merz-aesthetics.ru.

14. Lyakhovetsky BI, Glazkova LK, Peretolchina TF, Bochkaryov YuM. Connective tissue dysplasia in the practice of a dermatologist and a cosmetologist: Education and methodology guidelines for the graduate professional education of physicians. Yekaterinburg: UGMA, 2012; 48 p. Russian.

15. Martynov AI, Nechaeva GI. Guidelines of the Russian scientific medical society of internal medicine on the diagnosis, treatment and rehabilitation of patients with the connective tissue dysplasia (first edition). *Medical News of the North Caucasus* 2018; 13(1): 137-209. Russian. https://doi.org/10.14300/mnnc.2018.13037.

16. Snijkers JTW, van den Oever W, Weerts ZZRM, Vork L, Mujagic Z, Leue C, et al. Examining the optimal cutoff values of HADS, PHQ-9 and GAD-7 as screening instruments for depression and anxiety in irritable bowel syndrome. *Neurogastroenterol Motil* 2021; 33(12): e14161. https://doi.org/10.1111/nmo.14161.

17. Werschler WP, Werschler PS. Long-term efficacy of micro-focused ultrasound with visualization for lifting and tightening lax facial and neck skin using a customized vectoring treatment method. *J Clin Aesthet Dermatol* 2016; 9(2): 27-33. https://pubmed.ncbi.nlm.nih.gov/27047630.

18. Potekaev NN, Borzykh OB, Karpova EI, Petrova MM, Shnayder NA, Zatolokina MA, et al. A New approach toward the management of patients with premature skin aging using the predictor effect. *Cosmetics* 2023; 10(2): 49. Russian. https://doi.org/10.3390/cosmetics10020049.

19. Wong QYA, Chew FT. Defining skin aging and its risk factors: A systematic review and meta-analysis. *Sci Rep* 2021; 11(1): 22075. https://doi.org/10.1038/s41598-021-01573-z.

20. Martic I, Jansen-Dürr P, Cavinato M. Effects of air pollution on cellular senescence and skin aging. *Cells* 2022; 11(14): 2220. https://doi.org/10.3390/cells11142220.

21. Nanzadsuren T, Myatav T, Dorjkhuu A, Ganbat M, Batbold C, Batsuuri B, et al. Skin aging risk factors: A nationwide population study in Mongolia risk factors of skin aging. *PLoS One* 2022; 17(1): e0249506. https://doi.org/10.1371/journal.pone.0249506.

22. Cao C, Xiao Z, Wu Y, Ge C. Diet and skin aging-from the perspective of food nutrition. *Nutrients* 2020; 12(3): 870. https://doi.org/10.3390/nu12030870.

23. Umbayev B, Askarova S, Almabayeva A, Saliev T, Masoud AR, Bulanin D. Galactose-induced skin aging: The role of oxidative stress. *Oxid Med*

Cell Longev 2020; 2020: 7145656. https://doi.org/10.1155/2020/7145656.

24. Bocheva G, Slominski RM, Slominski AT. The Impact of vitamin D on skin aging. *Int J Mol Sci* 2021; 22(16): 9097. https://doi.org/10.3390/ijms22169097.

25. Bocheva G, Slominski RM, Slominski AT. Neuroendocrine aspects of skin aging. *Int J Mol Sci* 2019; 20(11): 2798. https://doi.org/10.3390/ijms20112798.

26. Lee H, Hong Y, Kim M. Structural and functional changes and possible molecular mechanisms in aged skin. *Int J Mol Sci* 2021; 22(22): 12489. https://doi.org/10.3390/ijms222212489.

27. Fatima I, Chen G, Botchkareva NV, Sharov AA, Thornton D, Wilkinson HN, et al. Skin aging in long-lived naked mole-rats is accompanied by increased expression of longevity-associated and tumor suppressor genes. *J Invest Dermatol* 2022; 142(11): 2853-2863.e4. https://doi.org/10.1016/j.jid.2022.04.028.

28. Alameda JP, Ramírez Á, García-Fernández RA, Navarro M, Page A, Segovia JC, et al. Premature aging and cancer development in transgenic mice lacking functional CYLD. *Aging (Albany NY)* 2019; 11(1): 127-159. https://doi.org/10.18632/aging.

29. Zhang S, Duan E. Fighting against skin aging: The way from bench to bedside. *Cell Transplant* 2018; 27(5): 729-738. https://doi.org/10.1177/0963689717725755.

30. Kanta J. Collagen matrix as a tool in studying fibroblastic cell behavior. *Cell Adh Migr* 2015; 9(4): 308-316. https://doi.org/10.1080/19336918.2015.1005469.

Authors:

Nikolai N. Potekaev – MD, DSc, Professor, Head of the Department of Skin Diseases and Cosmetology, Pirogov Russian National Research Medical University; Director of Moscow Research and Practical Center for Dermatology and Cosmetology, Moscow, Russia. http://orcid.org/0000-0002-9578-5490.

Olga B. Borzykh – MD, PhD, Researcher, Molecular and Cellular Technology Center for Collective Use, Voino-Yasenetsky Krasnoyarsk State Medical University, Krasnoyarsk, Russia. http://orcid.org/0000-0002-3651-4703.

Elena I. Karpova – MD, DSc, Department of Skin Diseases and Cosmetology, Pirogov Russian National Research Medical University, Moscow, Russia. https://orcid.org/0000-0003-0510-1022.

Marina M. Petrova – MD, DSc, Professor, Head of the Department of Outpatient Treatment and General Practice with a graduate study course, Voino-Yasenetsky Krasnoyarsk State Medical University, Krasnoyarsk, Russia. http://orcid.org/0000-0002-8493-0058.

Natalia A. Shnayder – MD, DSc, Professor, Lead Researcher, Department of Personalized Psychiatry and Neurology, Bekhterev National Medical Research Center for Psychiatry and Neurology, St. Petersburg; Molecular and Cellular Technology Center for Collective Use, Voino-Yasenetsky Krasnoyarsk State Medical University, Krasnoyarsk, Russia. http://orcid.org/0000-0002-2840-837X.

Diana V. Dmitrenko – MD, DSc, Professor, Head of the Department of Medical Genetics and Clinical Neurophysiology, Graduate School, Voino-Yasenetsky Krasnoyarsk State Medical University, Krasnoyarsk, Russia. http://orcid.org/0000-0003-4639-6365.

Maria A. Zatolokina – MD, DSc, Professor, Department of Histology, Cytology and Embryology, School of Medicine, Kursk State Medical University, Kursk, Russia. http://orcid.org/0000-0002-9553-1597.

Olga M. Demina – MD, PhD, Associate Professor, Department of Skin Diseases and Cosmetology, Pirogov Russian National Research Medical University, Moscow, Russia. https://orcid.org/0000-0001-9406-2787.