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Research Article

Optimized Therapy for Sarcopenia in Older Patients with Knee Osteoarthritis Using Parenteral Chondroitin Sulphate Combination a Prospective Cohort Observational Study

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Abstract

Purpose: This study aimed to examine the short-term efficacy and safety of parenteral chondroitin sulphate as an optimised therapy for sarcopenia in older patients with knee osteoarthritis. **Methods:** A prospective cohort observational trial was conducted in the Russian Federation on 260 patients with symptomatic knee osteoarthritis and sarcopenia who were pretreated with sarcopenia and pain therapies. Patients were randomly allocated to receive optimised (sarcopenia therapy + pain therapy + chondroitin sulphate) or standard treatment (sarcopenia therapy + pain therapy) for 60 d and then followed up for 30 d. The primary outcome was the visual analogue scale pain intensity score and secondary outcomes included a simple questionnaire to rapidly diagnose sarcopenia, results of muscle strength tests (dynamometry, '5x sit-to-stand test') and quantitative indicators of muscle mass (dual-energy X-ray absorptiometry). **Results:** Optimised treatment group (n=170) had significantly better outcomes, which were maintained during the 30-d follow-up period. Differences in visual analogue scale pain intensity scores were detected at 90 d ($p<0,001$). **Conclusions:** These results suggest that parenteral chondroitin sulphate (Chondroguard®) is a promising strategy to complete sarcopenia treatment in patients with knee osteoarthritis through an increased antioxidant and anti-inflammatory activity, reduced proinflammatory activity, and preservation of mitochondrial function of skeletal muscle myocytes.

Keywords: Sarcopenia; Older patients; Osteoarthritis; Efficacy; Safety; Chondroitin sulphate

Introduction

A gradual decrease in muscle fibre size and number, resulting in a decrease in muscle mass by approximately 40% from 20 to 80 years of age, has been noted in various studies as a major clinical problem associated with aging [1,2]. Sarcopenia is characterised by decreased muscle mass, strength, and physical performance with age and has become an important public health problem worldwide. Recent studies have shown that sarcopenia is associated with diseases such as cancer, heart failure, chronic obstructive pulmonary disease, rheumatoid arthritis, and neurodegenerative disorders [3]. In addition, sarcopenia is associated with health deterioration, including increased mortality, extended admission, and a greater need for rehabilitative care after patients are discharged from hospital [4]. The prevalence rate of sarcopenia in older patients with hip fractures varies from 21 to 74% in men and 12 to 68% in women [5-9].

Manifestation of sarcopenia in the older patients results from a combination of internal factors, including changes in energy supply at the molecular and cellular levels, as well as external and environmental factors, such as diet and physical exercise [10]. Aging-related anorexia, chronic malnutrition, and decreased physical activity may contribute to the development of sarcopenia and accelerate the development of the 'frailty' syndrome in the older patients [11]. An important characteristic of sarcopenia is its first and foremost effect on type II muscle fibres (reduction in muscle fibre size) and anaerobic (white) fibres, which are responsible for actions requiring strength (e.g., when performing the chair rise test). Sarcopenia in older people is associated with a fast-to-slow fibre type shift, mostly affecting the IIx fibres. In contrast, type I or oxidative (red) fibres retained their function during normal aging. Type I muscle fibre size was largely unaffected, which can be explained by the age-related remodelling of motor units that results mostly in the denervation of type II muscle fibres with collateral re-innervation of type I muscle fibres. In sarcopenia, the loss of strength is primarily due to an age-related reduction in muscle mass. The peroxisome proliferator-activated receptor gamma coactivator 1-alpha protects type I fibres from atrophy while the transforming growth factor beta (TGFβ) family and the nuclear factor kappaB (NF-κB) predominantly affect type IIx fibres [12].Sarcopenia has a complex multifactorial pathogenesis involving age-related changes in neuromuscular function, skeletal muscle protein metabolism, hormone levels, tissue sensitivity, a chronic proinflammatory state, and oxidative stress.

Various studies suggest that oxidative stress contributes to sarcopenia development in the older patients, because skeletal muscle and nerve tissue do not have a high capacity for regeneration in contrast to tissues with high mitotic activity. Active oxygen species (AOS), such as superoxide oxygen radicals, hydrogen peroxide, and hydroxyl radicals, are produced due to inflammation or infection and can cause nucleic acid oxidation, chromosome breakdown, cell membrane lipid peroxidation, and damage to collagen, proteins, and enzymes. An increase in AOS is indicative of breakdown in the mitochondrial respiratory chain and/or inadequacy of antioxidant cellular defence mechanisms. Age-related changes in the skeletal muscles are due to the accumulation of lipofuscin, which may be formed as a result of oxidative lipid polymerisation, increased lipid peroxidation, and increased mitochondrial deoxyribonucleic acid (DNA) deletions [13,14]. Proinflammatory cytokines may be involved in the pathogenesis of sarcopenia [15]. Interleukin (IL)-6 is involved in the induction of acute-phase inflammatory proteins, and is associated with increased inflammation and protein catabolism in sarcopenia [15]. Current studies also indicate a significant role for other circulating factors, such as weak apoptosis inducer (TWEAK), tumour necrosis factor (TNF), IL-18, leptin, insulin-like growth factor 1, insulin, and adiponectin, in the development of sarcopenia. Several studies report that antioxidant micronutrients potentially play a role in the pathogenesis of oxidative stress and sarcopenia [15]. Carotenoids and vitamin E are strong antioxidants that play important roles in aging process [16].

Vitamin D deficiency contributes to sarcopenia development and its administration improves the quality of skeletal muscle functions (e.g., as gait speed and hand clenching strength) [17]. The negative impact of vitamin D deficiency in the older patients is associated with an increased risk of falls and fractures and mortality rate [18].

Several studies have demonstrated a correlation between obesity and sarcopenia, the so-called 'sarcopenic obesity', due to the increased inflammatory burden of obesity [19,20]. In obesity, adipose tissue is characterised by a chronic inflammatory state due to the release of multiple proinflammatory cytokines, including TNFα, IL-6, and IL-1β, factors responsible for insulin resistance in adipose tissue along with skeletal muscle loss [21].Studies on potential peripheral blood biomarkers for sarcopenia are still scarce [22]. Studies have proposed a combination of complementary biomarkers (imaging and serum) and functional tests that together constitute an ideal panel of markers for diagnosing sarcopenia [23,24] and may be used in clinical trials to assess the effectiveness and safety of new medications. According to clinical recommendations ('Aging Asthenia', Ministry of Health of the Russian Federation, 2020), when deciding on drug therapy in older and senile patients, considering the presence of chronic and/or acute diseases, geriatric syndromes (especially aging asthenia), results of functional status assessment, occurrence of cognitive and emotional disorders, and social problems of aging asthenia syndrome, and sarcopenia is necessary.Osteoarthritis (OA) and chronic pain syndrome in older

patients accelerates the progression of sarcopenia and requires optimisation of pharmacotherapeutic approaches. Inhibition of aging skeletal muscle and satellite cells, chronic elevation of the senescence-associated secretory phenotype (SASP), and inflammatory processes associated with impaired homeostasis in skeletal muscles, may be considered as potential preventive drug targets y for sarcopenia treatment. The Active Pharmaceutical Ingredient (API) of chondroitin sulphate (CS, Chondroguard®, CS-BIOACTIVE© 'Bioiberica S.A.U.', Spain) has anti-inflammatory and myoprotective effects at the level of skeletal muscle myocytes and satellite cells in sarcopenia treatment [25]. In the Russian Federation (RF), CS is recommended for patients aged > 60 years with joint pain but have contraindications for nonsteroidal anti-inflammatory drugs (NSAIDs). Managing the progression of geriatric syndrome in older patients with OA and sarcopenia needs the development of optimal drug therapies.This study aimed to assess the short-term efficacy and safety of optimised sarcopenia therapy in older patients with knee OA (KOA) using parenteral form of API of CS (as part of Chondroguard® medicinal product) (CS-BIOACTIVE© 'Bioiberica S.A.U.', Spain; ZAO «PharmFirma «Sotex», Russia).

Results

Patient characteristics

After assessing eligibility, 293 patients were recruited, and 33 patients were excluded (Figure 1). The study included 260 patients (59 men; 201 women; average age: 77.2±4.8 years) with radiologically confirmed KOA and exacerbation of pain syndrome, divided into two groups: Group 1 (n=170); Group 2 (n=90) according to the clinical and anamnestic characteristics and meeting the I/E criteria of the study. Patients were randomly allocated into two groups with no statistically significant differences in age, sex, BMI, duration of postmenopause in the women, mean number of comorbid diseases, Charlson comorbidity index, frequency of arterial hypertension and diabetes mellitus, OA duration, VAS pain intensity score (at least 60 mm), duration of knee pain exacerbation, and frequency of radiological OA distribution in grades (Kellgren–Lawrence grades 2 and 3).

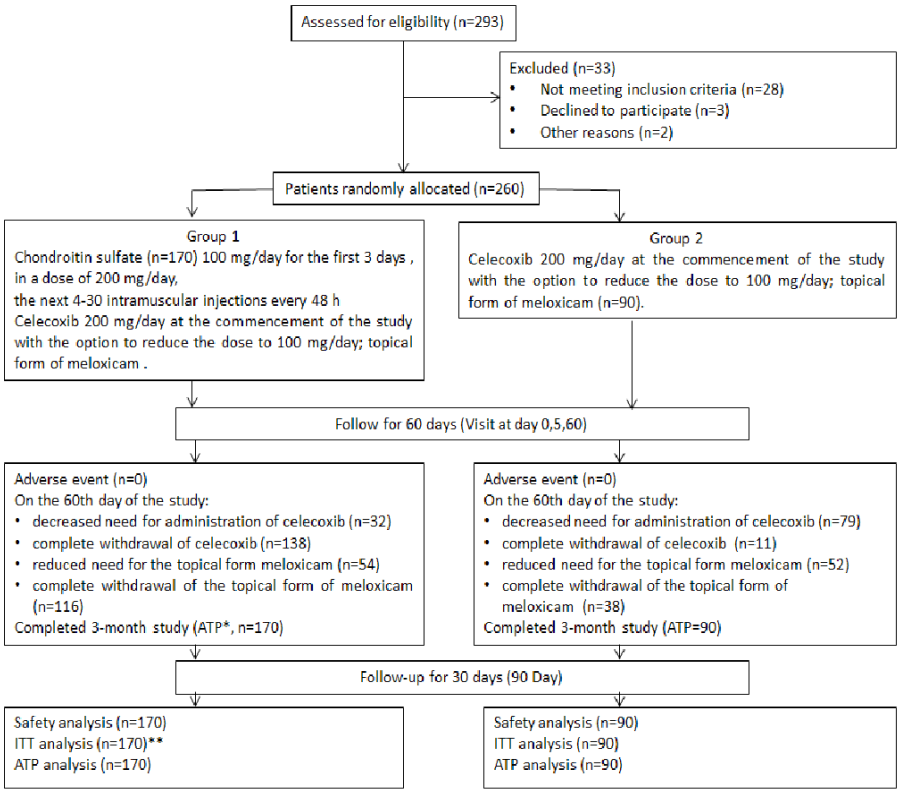


Figure 1: Recruitment Flowchart. *PP – Per Protocol population; **ITT - Intention-to-treat population.

At baseline, all patients (n=260) were verified to have moderate pain (VAS pain intensity score in the range of 63 to 74, Group 1; in the range of 65 to 72, Group 2); sarcopenia, according to mean SARC-F questionnaire score (≥4 points), muscle strength test results (best dynamometer score less than 16 kg in women and 26 kg in men; '5x sit-to-stand test' >12 s) and quantitative muscle mass scores (DXA less than 5.5 kg/m² for women and less than 7.0 kg/m² for men). On the day 0, no intergroup or intragroup statistically significant differences in mean VAS scores were found between men and women. On day 0, both groups showed statistically significant differences in the average SARC-F scores between men and women; women had higher SARC-F scores than men (p<0,001). As per inclusion in the study, significantly higher mean DXA appendicular muscle index values were found in men compared to women in both groups (p=0.0001); although, the mean values for the '5x sit-to-stand test' in men compared to women in both groups were not significantly different. As of inclusion in the study, no significant differences between the mean values of the SARC-F questionnaire score, the muscle strength, and muscle mass tests were recorded when men and women in both groups were compared separately (Table 1).

Characteristics	1 group (n[1]=170)		U-test/p[2], φemp/p[3]	2 group (n=90)		U-test/p, φemp/p[3]		
	Men (n=38)	Women (n=132)	Group 1 women/men	Men (n=21)	Women (n=69)	Group 2 women/men	Group 1/ Group 2/ men	Group 1/ Group 2/ women
Average age, years (Me, Min-Max)	77,5(68-87)	77(68-87)	0,178/0,859	77(68-87)	77(68-87)	0,453/0,650	0,372/0,709	0,142/0,887
Women, %	22,4	77,6	0,553/p>0,05	23,3	76,7	0,553/p>0,05	0,011/p>0,05	0,011/p>0,05
Body mass index, kg/m (Me, Min-Max)	28,5(24,2-31,2)	26,9(24-31,2)	1,414/0,157	28,2(26,6-29,6)	28,5(24,2-29,8)	0,539/0,589	0,712/0,476	2,198/0,08
Duration of postmenopause, years (Me, Min-Max)	-	11,5 (8-16)	-	-	12,5 (7-17)	-	-	0,94/0,345
Arterial hypertension, n/%	24 (16,4)	122 (83,6)	0,00003/ p<0,05	13 (14,4)	68 (75,5)	0,00002/ p<0,05	1,0000/p>0,05	0,128/p>0,05
Diabetes mellitus, n/%	9 (23,7)	29 (76,3)	0,8272/ p>0,05	4 (21)	15 (79)	1,0000/p>0,05	0,7541/p>0,05	0,0000/ p<0,05
Average number of comorbid conditions (Me, Min-Max)	5 (3-6)	5 (3-6)	0,151/0,880	5 (3-6)	5 (3-6)	0,199/0,842	0,309/0,757	0,012/0,990
Charlson Comorbidity Index (Me, Min-Max)	5 (4-7)	5 (4-7)	1,087/0,277	5 (4-7)	5 (4-7)	0,083/0,934	0,083/0,934	0,873/0,383
Duration of osteoarthritis, years (Me, Min-Max)	10,5 (6-14)	12 (9-15)	1,457/0,145	10 (7-14)	10 (7-13)	1,913/ 0,056	1,456/ 0,145	1,089/0,276
Duration of knee joint pain exacerbation, days (Me, Min-Max)	14 (11-17)	14 (11-17)	0,193/ 0,847	14 (12-19)	14 (12-19)	0,057/0,954	1,749/0,080	1,137/0,171
Kellgren-Lawrence radiological grade of osteoarthritis, n/% 2	32 (34)	62 (66)	0,00004/ p<0,05	15 (29,4)	36 (70,6)	0,1384/p>0,05	0,3155/p>0,05	0,4911/p>0,05

3	6 (7,9)	70 (92,1)	0,00004/ p<0,05	6 (15,4)	33 (84,6)	0,1384/p>0,05	0,3155/p>0,05	0,4911/p>0,05
Pain intensity according to VAS score, mm (Me, Min-Max)	68,5 (63-74)	69 (65-72)	0,48/ 0,631	68,5 (62-74)	69 (63-74)	0,59/ 0,557	0,16/ 0,874	0,38/ 0,699
Assessment SARC-F[5], scores (Me, Min-Max)	5 (4-6)	5,5 (4-7)	3,62/ 0,0003	5 (4-6)	6 (4-7)	2,90/ 0,004	0,07/ 0,943	0,14/ 0,889
Dynamometry, kg (Me, Min-Max)	21,5 (18-25)	12,5 (10-15)	*	20 (16-25)	12 (9-15)	*	1,44/0,149	0,30/0,762
"5x Sit-To-Stand Test", s.(Me, Min-Max)	17 (15-20)	18 (14-22)	1,69/0,089	18 (14-20)	19 (14-22)	1,59/0,111	0,29/0,769	0,14/0,886
DXA appendicular muscle index[6], kg/ m^2(Me, Min-Max)	6 (4-7)	4 (3-5)	6,65/<0,001	5 (4-7)	4 (3-5)	4,44/0,0001	1,65/0,099	1,68/0,093
Serum albumin, g/l (Me, Min-Max)	39,5(34-44)	35(28-42)	5,1/<0,001	40(34-47)	35(30-40)	2,95/0,0003	1,02/0,307	0,14/0,886
CRP[7], mg/L (Me, Min-Max)	2,25(1,36-2,76)	2,64 (1,04-3,04)	3,09/0,002	2,12(1,32-2,72)	2,34(1,8-2,7)	1,95/0,05	0,13/0,893	0,90/0,368
IL–6[8], pg/mL (Me, Min-Max)	1,78(0,92-2,58)	2,18 (1,14-2,45)	2,25/0,024	1,97(1,1-2,44)	2,36(1,22-2,88)	2,57/0,01	0,32/0,752	0,54/0,586
IL-1β[9], pg/mL (Me, Min-Max)	0,5(0,1-0,8)	1,2 (0,3-1,7)	6,74/<0,001	0,45(0,1-0,8)	1,1(0,4-1,5)	5,69/<0,001	0,35/0,728	0,87/0,383
TNFα[10], pg/mL (Me, Min-Max)	8,3 (2,3-14,1)	7,9 (1,7-14,1)	0,58/0,564	8,1(2,1-13,8)	8,1(1,6-13,8)	0,37/0,714	0,33/0,739	0,82/0,414
Leptin, ng/mL (Me, Min-Max)	9,4(4,1-16,4)	18,2(6,2-25,5)	4,85/<0,001	9,1(3,8-15,6)	17,8(6,8-24,8)	3,68/0,0002	0,69/0,486	0,91/0,361
25(OH)D3[11], ng/mL (Me, Min-Max)	15,8(10,5-18,1)	10,5 (8,8-15,7)	5,1/<0,001	14,2(10,9-17,8)	11,7(8,4-15,2)	4,24/0,00001	0,14/0,887	1,26/0,207
Total carotenoids, mcM/L (Me, Min-Max)	1,6 (1,4-1,7)	1,9 (1,2-2,3)	4,29/0,00001	1,5 (1,4-1,7)	2(1,3-2,2)	3,59/0,0003	0,09/0,924	0,95/0,339
Vitamin C, mcM/L (Me, Min-Max)	57,7(36,6-78,4)	51,7 (38,9-83,9)	0,94/0,349	52,8(39,4-82,1)	51,8(37,8-72,6)	0,07/0,943	0,49/0,624	0,92/0,357
α- tocopherol, mcM/L (Me, Min-Max)	13,6(9,6-20,2)	22,8 (16,7-27,5)	8,1/<0,001	14,15(10,2-19,5)	20,9(17,2-27,3)	5,92/<0,001	0,32/0,751	1,13/0,257

Notes: 1n – number of patients in the group; 2U-test/ p – value of Mann-Whitney U-test/probability of intergroup or within-group differences in mean Mann-Whitney U-test scores between Groups 1/2 patients (men/women), in patients within Group 1 or Group 2; 3φemp/p – Fisher's exact test value /probability of differences in characteristic values of frequency of occurrence of a symptom according to Fisher's exact test in patients of Groups 1/2; 4VAS pain intensity score – visual analogue scale; 5SARC-F – a simple questionnaire to rapidly diagnose sarcopenia; 6DXA – dual X-ray absorptiometry; 7CRP – C-reactive protein; 8IL-6 – interleukin 6; 9IL-1β – interleukin 1beta; 10TNFα – tumour necrosis factor α; 1125(OH)D – 25-Hydroxyvitamin D; the data are presented as Me (Min-Max) – median (Minimum-Maximum); * no within-group comparison is made for dynamometry between men and women because of significant baseline differences in individual values.

Table 1: Baseline characteristics of all patients.

Main findings of the Study

Additional administration of parenteral CS for pain and sarcopenia therapy, in older patients of both sexes, resulted in the following changes in the pain dynamics and sarcopenia screening scores: statistically significant decrease in mean values of VAS pain intensity score (decrease of pain intensity to mild pain level according to VAS pain intensity score within the range of 5 to 44 mm), SARC-F questionnaire, and '5x sit-to-stand test' score. There was significant increase in the mean values of muscle strength tests (dynamometry, DXA appendicular muscle index) in men and women in Group 1 compared to the same values in this group at day 0. Also, the administration of additional CS therapy regimen to older patients with OA and sarcopenia along with the pain syndrome, revealed statistically significant within-group differences in the mean values of sarcopenia screening scores between men and women: higher mean values of the SARC-F questionnaire score and the '5x sit-to-stand test' score, along with lower mean values of the muscle strength tests (dynamometry, DXA appendicular muscle index) in women compared to that in men in Group 1. Administration of an additional CS therapy regimen to older patients with OA and sarcopenia along with pain revealed no within-group statistically significant differences in the mean VAS pain intensity scores between men and women (Table 2).

The standard therapy regimen for sarcopenia in patients with OA and pain syndrome (inter alia, peroral celecoxib NSAID) in Group 2, demonstrated the following dynamics of pain intensity and sarcopenia screening scores in older patients: statistically significant decrease in mean values of VAS pain intensity score (moderate decrease in pain severity in the range 45 to 74 mm) and SARC-F questionnaire and '5x sit-to-stand test' score in patients of both sexes with a significant increase in mean values of muscle strength and weight tests (dynamometry, DXA appendicular muscle index) in women in Group 2, but no significant difference in muscle strength and mass scores in men compared to those in Group 2 on day 0. In Group 2, older patients with OA and sarcopenia who received standard therapy regimen showed a less pronounced decrease in the mean VAS pain intensity score, SARC-F score, and '5x sit-to-stand test'. A less pronounced increase in the mean values of the muscle strength tests and mass index (dynamometry, DXA appendicular muscle index) in Group 2 men was observed compared to that in men in Group 1. In Group 2 women, similar comparative dynamics of pain intensity scores and sarcopenia screening were registered, with no significant differences when compared to those of women in Group 1. Also, administration of the standard therapy regimen for sarcopenia in older patients with OA and pain syndrome revealed statistically significant within-group differences in the mean sarcopenia screening value scores in men and women: higher mean scores in the SARC-F questionnaire and lower mean values for the muscle strength tests (DXA appendicular muscle index) in women compared to men in Group 2, with no statistically significant differences in VAS pain intensity score and '5x sit-to-stand test' and pain intensity. Statistically significant differences of VAS pain intensity score and '5x sit-to-stand test' were observed in men and women in Groups 1 and 2 by day 5 and persisted until day 60 (Table 2,3).

Characteristic values	Group 1 (n1=170)						U-test/p[2]									U-test/p					
	Visit «5» day		Visit «60» day		Visit «90» day		Visit «5» day	Visit «0» day/Visit «5» day	Visit «0» day/Visit «5» day	Visit «60» day	Visit «0» day/ Visit «60» day	Visit «0» day/Visit «60» day	Visit «90» day	Visit «0» day/Visit «90» day	Visit «0» day/Visit «90» day	Group 1/ group 2 Visit «5» day	Group 1/ group 2 Visit «5» day	Group 1/ group 2 Visit «60» day	Group 1/ group 2 Visit «60» day	Group 1/ group 2 Visit «90» day	Group 1/ group 2 Visit «90» day
	Men (n=38) Me(Min-Max)	Women (n=132) Me(Min-Max)	Men(n=38) Me(Min-max)	Women (n=132) Me(Min-Max)	Men (n=38) Me(Min-Max)	Women (n=132) Me(Min-Max)	women/ men	women	men	women/ men	women	men	women/ men	women	men	women	men	women	men	Women	men
Pain intensity according to VAS score[3], mm	60,5(58-63)	59(57-62)	41(37-44)	42 (35-49)	42(40-43)	42(40-43)	2,92/0,003	14,04/< 0,001	7,50/< 0,001	0,66/ 0,511	14,04/< 0,001	7,5/< 0,001	0,36/ 0,715	14,04/< 0,001	7,50/< 0,001	11,63/< 0,001	6,21/< 0,001	11,63/< 0,001	6,32/< 0,001	11,63/< 0,001	6,32/< 0,001
Group 2 (n=90)							U-test/p														
	Men (n=21) Me(Min-Max)	Women (n=69) Me(Min-Max)	Men (n=21) Me(Min-Max)	Women (n=69) Me(Min-Max)	Men (n=21) Me(Min-Max)	Women (n=69) Me(Min-Max)	women/ men	women	men	women/ men	women	men	women/ men	women	men	-	-	-	-	-	-
Pain intensity according to VAS score, mm	68(64-72)	67(64-70)	47 (44-51)	47 (45-50)	52(51-54)	54(53-55)	1,67/0,09	6,46/< 0,001	1,87/0,06	1,13/0,258	10,14/<0,001	5,55/< 0,001	0,95/0,340	10,14/< 0,001	5,55/< 0,001	-	-	-	-	-	-

Notes:1n – number of patients in the group; 2U-test/ p – value of Mann–Whitney U-test/probability of differences in the mean characteristic values according to Mann–Whitney U-test in patients of Group 1 (men/women), prior to administration/ after administration of chondroitin sulphate (Chondroguard®), in patients of Group 2 (men/women), prior to administration/ after administration of Celecoxib, probability of intergroup differences in mean Mann-Whitney U-test scores between patients of Groups 1/2 (men, women); 3VAS pain intensity score – visual analogue scale; the data are presented as Me (Min-Max) – median (Minimum-Maximum).

Table 2: Changes in the VAS pain intensity score (main outcome).

Characteristic values	Group 1 (n[1]=170)				U-test/p[2]						U-test/p			
	Visit «5» day		Visit «60» day		Visit «5» day	Visit «0» day/Visit «5» day	Visit «0» day/Visit «5» day	Visit «60» day	Visit «0» day/Visit «60» day	Visit «0» day/Visit «60» day	Group 1/group 2 Visit «5» day	Group 1/group 2 Visit «5» day	Group 1/group 2 Visit «60» day	Group 1/group 2 Visit «60» day
	Men (n=38) Me(Min-Max)	Women (n=132) Me(Min-Max)	Men (n=38) Me(Min-Max)	Women (n=132) Me(Min-Max)	women/men	women	men	women/men	women	men	Women	men	women	men
Assessment SARC-F[3], scores	4(3-5)	5(3-6)	2 (1-3)	3 (2-5)	1,94/0,05	10,67/<0,001	3,11/0,002	6,35/<0,001	12,26/<0,001	7,5/<0,001	1,50/0,13	0,33/0,74	7,86/<0,001	5,48/<0,001
Dynamometry, kg	22(18-25)	12(10-15)	23 (20-26)	13,5 (11-16)	*	1,39/0,16	1,44/0,15	*	5,87/<0,001	3,06/0,002	2,72/0,01	4,52/<0,001	0,09/0,924	3,18/0,002
"5x Sit-To-Stand Test", s.	15(14-16)	14(13-15)	14 (12-16)	16 (13-20)	2,01/0,04	14,04/<0,001	7,50/<0,001	4,33/0,0001	6,99/<0,001	6,73/<0,001	10,68/<0,001	6,32/<0,001	3,76/0,0002	4,24/0,0001
DXA appendicular muscle index[4], kg/m²	6(4-7)	5(4-6)	7 (6-8)	7 (6-7)	7,10/<0,001	10,67/<0,001	4,75/<0,001	2,67/ 0,007	7,39/<0,001	7,04/<0,001	7,49/<0,001	4,68/<0,001	10,68/<0,001	4,21/0,0001
Group 2 (n=90)					U-test/p									
	Men (n=21) Me(Min-Max)	Women (n=69) Me(Min-Max)	Men (n=21) Me(Min-Max)	Women (n=69) Me(Min-Max)	women/men	women	men	women/men	women	men	-	-	-	-
Assessment SARC-F, scores	4(3-5)	4(3-6)	4 (3-5)	5 (3-5)	0,14/0,89	6,68/<0,001	2,91/0,004	4,12/0,0001	5,67/<0,001	4,11/0,0001	-	-	-	-
Dynamometry, kg	20(16-25)	13(9-15)	21 (18-25)	13 (10-15)	*	0,66/0,51	1,59/0,11	*	3,96/0,0001	0,62/0,538	-	-	-	-
"5x Sit-To-Stand Test", s.	17 (15-20)	17(15-19)	16 (14-17)	17 (14-20)	0,52/0,61	3,07/0,002	2,63/0,009	1,86/0,063	3,16/0,002	2,60/0,009	-	-	-	-
DXA appendicular muscle index, kg/m²	5(3-7)	5(4-6)	6 (4-7)	5 (4-6)	4,56/<0,001	0,09/0,93	1,57/0,12	3,29/0,001	2,876/0,004	1,81/0,070	-	-	-	-

Notes:[1]n – number of patients in the group; 2U-test/ p – value of Mann–Whitney U-test/probability of differences in the mean characteristic values according to Mann—Whitney U-test in patients of Group 1 (men/women), prior to administration/ after administration of chondroitin sulphate(Chondroguard®), in patients of Group 2 (men/women), prior to administration/ after administration of Celecoxib, probability of intergroup differences in mean Mann–Whitney U-test scores between patients of Groups 1/2 (men, women); 3SARC-F – a simple questionnaire to rapidly diagnose sarcopenia; 4DXA - dual X-ray absorptiometry; the data are presented as Me (Min-Max) – median (Minimum-Maximum); * - no within-group comparison is made for dynamometry between men and women because of significant baseline differences in individual values

Table 3: Changes in the secondary outcomes: SARC-F questionnaire score, results of muscle strength tests (dynamometry, '5x sit-to-stand test') and quantitative indicators of muscle mass (DXA, dual-energy X-ray absorptiometry).

Optimised treatment group (n=170) obtained significantly better outcomes that were maintained during the 30-d follow-up: differences in mean of VAS pain intensity score (main outcome) on day 90 (p<0,001) were identified for Group 1/2 (men, women), see Figure 2.

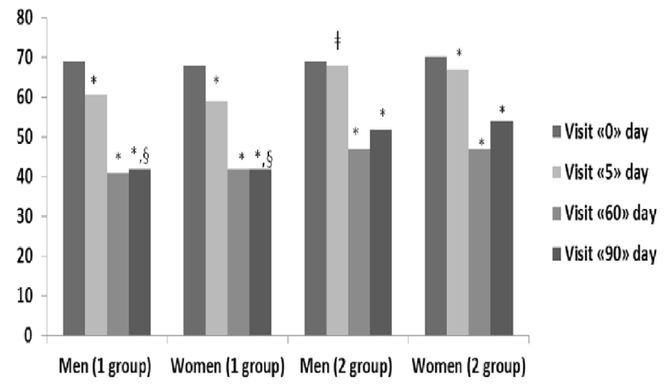


Figure 2: Changes in the VAS pain intensity score (main outcome). * - p<0,001 – probability of differences in the mean characteristic values according to Mann–Whitney U-test in patients of Group 1 (men, women), prior to administration/ after administration of chondroitin sulphate (Chondroguard®), in patients of Group 2 (men/women), prior to administration/ after administration of Celecoxib on visit days 0, 5, 60, (treatment) and 90 (the 30-d follow-up); ǂ - p=0,06, probability of differences in the mean characteristic values according to Mann–Whitney U-test in patients of Group 2 (men), prior to administration/ after administration of Celecoxib on visit days 0/ 5; § - p<0,001 – probability of intergroup differences in mean Mann–Whitney U-test scores between patients of Groups 1/2 (men, women) on day 90 (the 30-d follow-up).

Statistically significant differences in SARC-F questionnaire were not observed in men and women in Groups 1 and 2 on day 5, whereas they were detected on day 60. Significant dynamometry mean values were found in men and women in Group 1 on day 5 while taking CS compared to those in Group 2, but on day 60, statistically significant differences in these parameters were found only in men in Groups 1 and 2 (Table 3).

Additional Findings Of The Study

On day 0 in all patients (n=260), the following laboratory blood parameters changes associated with the development of sarcopenia in older patients with KOA were observed: statistically significant decrease in blood serum albumin concentration, 25(OH)D3, carotenoid levels, plasma α-tocopherol; statistically significant increase in vitamin C concentration and systemic inflammatory blood indicators – CRP concentration, IL-6, IL-1β, TNFα, and leptin compared to the average values of similar indices in older persons of both sexes without sarcopenia (normal ranges of laboratory test values are presented in subsection 'Methods for outcome recording'). A more significant decrease in blood serum albumin and 25(OH)D3 was also observed in older women with sarcopenia compared to those in older men with sarcopenia and OA. within addition, there was significant decrease in blood plasma total carotenoids and α-tocopherol levels in older men with sarcopenia compared to those of elderly women with sarcopenia and OA. Furthermore, statistically significant differences in the blood CRP, IL-6, IL-1β, and leptin concentrations of older men and women with sarcopenia were found. No statistically significant differences in TNFα and vitamin C levels were observed in men and women of both groups (Table 1).

Administration of CS in addition to the standard sarcopenia therapy regimen revealed the following changes in the blood values associated with the development of OA in older patients of both sexes: statistically significant increase in blood serum albumin, 25(OH) D3, and total plasma carotenoids concentrations; statistically significant decrease in CRP, IL-6, IL-1β, TNFα, and leptin blood levels on days 5 and 60 as compared to similar parameters in patients in Group 1, except for blood TNFα level of women on day 60; also, a significant decrease in vitamin C blood levels in older women with sarcopenia was observed on day 60 upon administration of CS in addition to the standard regimen as compared to vitamin C blood levels in patients on day 0 in Group 1, but no similar effect was

observed in older men with sarcopenia; a statistically significant increase in blood α-tocopherol concentrations in older men were observed on days 5 and 60 upon administration of the standard therapy regimen and CS but there was no significant difference in older women in Group 1 after the treatment. The study revealed the absence of significant differences in the TNFα blood levels between men and women upon administration of the standard therapy regimen and CS on day 5 but significant differences on day 60 (Table 4).

Characteristic values	Group 1 (n[1]=170)				U-test/p[2]						U-test/p			
	Visit «5» day		Visit «60» day		Visit «5» day	Visit «0» day/ Visit «5» day	Visit «0» day/ Visit «5» day	Visit «60» day	Visit «0» day/ Visit «60» day	Visit «0» day/ Visit «60» day	Group 1/group 2	Group 1/group 2	Group 1/group 2	Group 1/group 2
											Visit «5» day	Visit «5» day	Visit «60» day	Visit «60» day
	Men (n=38) Me(Min-Max)	Women (n=132) Me(Min-Max)	Men (n=38) Me(Min-Max)	Women (n=132) Me(Min-Max)	women/men	women	men	women/men	women	men	Women	men	women	men
Serum albumin, g/L	45(40-48)	41(37-45)	46(44-48)	43,5(41-45)	6,12/<0,001	10,7/<0,001	6,02/<0,001	8,28/<0,001	14,04/<0,001	7,50/<0,001	1,08/0,279	3,05/0,002	8,59/<0,001	6,32/<0,001
CRP[3], mg/L	1,86 (1,25-2,02)	1,81 (1,07-2,25)	1,62(1,3-1,9)	1,64(1,4-1,8)	1,22/0,221	7,54/<0,001	3,51/0,0004	0,59/0,56	8,11/<0,001	5,51/<0,001	5,13/<0,001	2,01/0,044	5,15/<0,001	3,23/0,0012
IL–6[4], pg/mL	1,43 (0,95-1,55)	1,58 (1,42-1,68)	1,32(1,2-1,5)	1,48(1,3-1,6)	5,21/<0,001	11,28/<0,001	3,39/0,0007	7,51/<0,001	14,04/<0,001	7,50/<0,001	6,58/<0,001	2,38/ 0,017	11,15/<0,001	3,66/0,0003
IL-1β[5], pg/mL	0,25 (0,2-0,5)	0,8 (0,2-1,2)	0,2(0,2-0,4)	0,4(0,3;0,6)	6,94/<0,001	6,21/<0,001	3,78/0,0002	7,79/<0,001	14,04/<0,001	6,83/<0,001	3,04/0,002	2,01/0,044	9,72/<0,001	2,26/0,02
TNFα[6], pg/ mL	6,4 (2,4-8,1)	7,9(1,7-14,1)	5,5(4,0-7,2)	7,25(5,4-8,0)	1,10/0,269	4,11/0,00001	2,77/0,005	5,13/<0,001	1,72/0,09	3,01/0,003	1,45/0,148	0,87/0,385	1,75/0,079	1,28/0,199
Leptin, ng/ mL	5,8 (3,8-13,4)	10,4(4,6-16,8)	6,75(5,3-8,2)	8,4(7,2-11,2)	4,64/<0,001	6,80/<0,001	2,76/0,006	5,85/<0,001	12,28/<0,001	5,13/<0,001	4,48/<0,001	1,47/0,141	6,09/<0,001	3,09/0,002
25(OH)D3[7], ng/ml	15,8(10,5-18,1)	12,65(8,8-15,7)	18,2 (14,7-21,2)	15,2 (11,3-18,6)	7,67/<0,001	5,17/<0,001	4,03/0,0001	6,61/<0,001	10,5/<0,001	5,26/<0,001	5,64/<0,001	2,55/0,011	8,59/<0,001	4,10/<0,001
Total carotenoids, mcM/L	1,7(1,6-1,9)	1,8(1,6-2,0)	1,85 (1,6-2,0)	2,15 (1,7-2,4)	2,24/ 0,025	0,97/0,851	5,71/<0,001	6,24/<0,001	6,0/<0,001	4,93/<0,001	6,32/<0,001	4,10/0,00001	4,15/0,00001	3,66/0,0003
Vitamin C, mcM/L	51,3(39,8-64,6)	50,9(42-61,6)	47,6 (35,6-72,9)	49,7 (32,4-68,3)	0,92/0,87	0,82/0,94	0,93/0,88	0,01/0,988	5,23/<0,001	1,59/0,109	0,92/0,84	0,86/0,92	3,59/0,0003	1,09/0,275
α-tocopherol, mcM/L	16,7(14-18,6)	21,3(19,8-23)	16,8 (13,4-20,5)	22,2 (18,4- 25,4)	9,38/<0,001	0,54/0,58	2,35/0,02	8,07/<0,001	0,05/0,961	2,79/0,005	0,81/0,42	2,89/0,004	0,84/0,399	2,83/0,005
Group 2 (n=90)					**U-test/p**									
	Men (n=21) Me(Min-Max)	**Women (n=69) Me(Min-Max)**	**Men (n=21) Me(Min-Max)**	**Women (n=69) Me(Min-Max)**	**women/men**	**women**	**men**	**women/men**	**women**	**men**	-	-	-	-
Serum albumin, g/L	41,5 (35-48)	42 (35-45)	40(38-41)	41(39-43)	1,05/0,292	2,37/0,018	1,04/0,296	2,07/0,04	10,14/<0,001	0,62/0,54	-	-	-	-
CRP, mg/L	1,88 (1,28-2,54)	2,32(1,0-2,52)	1,78(1,68-2,12)	1,72(1,58-2)	1,46/0,143	4,49/<0,001	1,41/0,159	0,19/0,85	6,79/<0,001	4,34/0,00001	-	-	-	-
IL–6, pg/mL	1,78(1,0-2,2)	1,98 (1,15-2,52)	1,64(1,32-2)	1,96(1,8-2,2)	2,44/0,015	2,77/ 0,005	1,42/0,155	4,14/0,00001	0,36/0,71	1,67/0,09	-	-	-	-
IL-1β, pg/mL	0,4 (0,1-0,6)	0,9 (0,3-1,3)	0,3(0,1-0,6)	0,8(0,5-1,2)	5,79/<0,001	2,55/0,012	1,19/0,232	6,91/<0,001	6,79/<0,001	2,11/0,03	-	-	-	-
TNFα, pg/mL	7,8 (2,0-12,9)	7,9 (1,5-12,6)	5,7 (3,4-8,8)	6,7(4,8-9,8)	0,59/0,557	1,45/0,148	2,03/0,04	1,84/0,064	0,97/0,332	0,82/0,413	-	-	-	-
Leptin, ng/mL	8,2 (3,8-14,5)	16,4 (5,4-20,8)	8,4(6,2-11,0)	13,6(6,2-16,0)	3,45/0,0005	2,07/0,038	1,04/0,296	3,46/0,0005	5,52/<0,001	1,65/0,099	-	-	-	-
25(OH)D3, ng/ mL	14,8 (10,9-17,8)	11,4(10,6-13)	15,6(13,0-18,0)	11,5(8,6-16,2)	6,91/<0,001	1,16/0,246	0,94/0,345	5,11/<0,001	1,34/0,179	0,89/0,372	-	-	-	-
Total carotenoids, mcM/L	1,5(1,4-1,7)	1,9(1,4-2,2)	1,6 (1,4-1,8)	2 (1,5-2,4)	6,91/<0,001	1,99/0,045	1,16/0,242	3,77/0,0002	1,69/0,089	2,16/0,031	-	-	-	-
Vitamin C, mcM/L	52,4 (45,8-57,5)	49,8(47,6-58,4)	50,8(38,2-78,8)	52,8 (39,4-82,1)	3,16/0,001	4,69/<0,001	2,78/0,005	0,21/0,83	1,26/0,207	0,76/0,443	-	-	-	-
α-tocopherol, mcM/L	15,4(12,4-17,2)	21,8(19,2-33,4)	13,8 (11,4-19,8)	21,4 (17,8-26,4)	6,91/<0,001	0,78/0,437	0,26/0,79	6,22/<0,001	0,27/0,79	0,47/0,635	-	-	-	-

Notes:1n – number of patients in the group; 2U-test/ p – value of Mann–Whitney U-test/probability of differences in the mean characteristic values according to Mann–Whitney U-test in patients of Group 1 (men/women), prior to administration/ after administration of chondroitin sulphate(Chondroguard®), in patients of Group 2 (men/women), prior to administration/ after administration of Celecoxib, probability of intergroup differences in mean Mann–Whitney U-test scores between patients of Groups 1/2 (men, women); 3CRP - C-reactive protein; 4IL-6 – interleukin 6; 5IL-1β – interleukin 1beta; 6TNFα – tumour necrosis factor α; 725(OH)D – 25-Hydroxyvitamin D; the data are presented as Me (Min-Max) – median (Minimum-Maximum).

Table 4: Changes in blood parameters (albumin, CRP, TNFα, IL-1β, IL-6, leptin, 25(OH)D3, vitamin C, total carotenoids, and α-tocopherol).

Standard therapy regimen for sarcopenia (inter alia, NSAID peroral celecoxib) in older patients of both sex in Group 2 demonstrated neither statistically significant increase in blood plasma 25(OH)D3, and α-tocopherol on days 5 and 60, nor significant decrease in blood vitamin C concentrations on day 60 compared to those in Group 2. Meanwhile, the following laboratory blood parameters changes associated with the development of sarcopenia in older women with OA in Group 2 were observed: statistically significant increase in blood serum albumin level; a significant decrease in blood CRP, IL-6, IL-1β, and leptin concentrations; statistically significant decrease in blood vitamin C concentrations on day 5 but no statistically significant changes in TNFα level and total carotenoids in blood compared to the same parameters on day 0. In the context of the standard therapy regimen in older men in Group 2 with sarcopenia and OA, a statistically significant decrease in blood TNFα level on day 5; a significant increase in the total carotenoids concentration in the blood on day 60, no significant differences in blood serum albumin, CRP, IL-6, IL-1β, and leptin on day 5; and no significant differences in blood serum albumin, IL-6, and leptin on day 60 compared to the same parameters on day 0 (Table 4).

Administration of standard sarcopenia therapy regimen to older patients with OA and pain syndrome revealed statistically significant within-group differences in mean values of sarcopenia laboratory test parameters in men and women: higher mean 25(OH)D blood levels and lower mean plasma levels of IL-6, IL-1β, leptin, total carotenoids, and α-tocopherol in men compared to women in Group 2 on days 5 and 60; whereas, there were no within-group differences in mean values of CRP and TNFα on days 5 and 60, and serum albumin on day 5; but there were statistically significant differences on day 60; statistically significant differences in blood vitamin C levels on day 5 with no within-group differences in mean values on day 60 in men and women of Group 2 (Table 4).In comparison to the administration of standard therapy regimen in older women with sarcopenia and OA, those administered standard therapy regimen and CS therapy (Chondroguard®) had statistically significant differences in the following laboratory blood parameters: significantly lower concentrations of 25(OH)D and total blood carotenoids; higher CRP, IL-6, IL-1β, and leptin levels on days 5 and 60; higher blood vitamin C levels in Group 2 females on day 60; but no differences in mean values of vitamin C on day 5 compared to those in Group 1 females; no significant differences in blood plasma TNFα and α-tocopherol levels in older women in Groups 1 and 2 on days 5 and 60; no significant differences in blood serum albumin concentration on day 5 but significant differences in blood serum albumin concentration in older women in Groups 1 and 2 on day 60 (Table 4). Compared to older men with sarcopenia and OA on standard therapy regimen, those on standard therapy regimen and CS therapy (Chondroguard®) had statistically significant differences in the following laboratory parameters: significantly lower blood serum albumin concentration, 25(OH)D levels, total carotenoids, and α-tocopherol levels; higher blood CRP, IL-6, and IL-1β in Group 2 men compared to Group 1 men; no significant differences in blood TNFα and vitamin C levels in older men upon administration of the corresponding therapy in Group 1 and Group 2 on days 5 and 60; no significant differences in leptin concentrations on day 5 but significant differences in leptin concentrations in older men in Groups 1 and 2 on day 60 (Table 4). There was a decreased

need for administration or complete withdrawal of celecoxib on day 60 in 32 (18.8%) and 138 (81.2%) patients, respectively in Group 1; compared to 79 [87.8%] and 11 [12.2%] on day 60 in Group 2 patients, respectively, φemp: 11.74 /<0.01. Similarly, there was reduced need for or complete withdrawal of the topical form of meloxicam on day 60 in 54 (31.8%) and 116 (68.2%) patients in Group 1, respectively; compared to 52 [57.8%] and 38 [42.2%] on day 60 in in Group 2 patients, respectively, φemp:4.06/<0.01.

Adverse Events

No adverse events were reported upon the administration of parenteral CS in a prospective cohort observational study.

Discussion

The differences detected in the dynamics of clinical scores and laboratory parameters in men and women in the context of different therapy regimens may be explained by the pharmacological effects of CS and the molecular mechanisms at the skeletal muscle and nerve fibre level in older patients with sarcopenia. Improvement of VAS pain intensity score, SARC-F questionnaire, '5x sit-to-stand test' scores, muscle strength, and muscle mass indices upon administration of CS; and the standard sarcopenia therapy regimen in older patients of both sexes with KOA may be due to the myoprotective pharmacological effect of CS at the molecular level. This involves the regulation of transcriptomic, metabolomic, and proteomic events of cellular aging, inter alia, adipose tissue accumulation, insulin resistance and low-level inflammation, protein metabolism imbalance, oxidative stress, mitochondrial dysfunction, and regenerative capacity of surrounding cells. Administration of the parenteral form of CS contributes to the restoration of the degree of sulfation and binding of CS molecules to proteins expressed in the extra- and pericellular spaces of skeletal muscle myocytes, which are subject to changes with aging and in the presence of OA, and determines the differentiation of myoblasts at the stage of extensive formation of myotube syncytium [26]. During the aging process, various stressors activate or inhibit numerous cellular signalling cascades, ultimately contributing to the progression of sarcopenia. The development of sarcopenia is associated with various molecules involved in the processes affecting fibre size, mitochondrial homeostasis, and apoptosis. The loss of myocytes is associated with several proteolytic systems, including calpain, ubiquitin-proteasome, and lysosome-autophagy. Researchers have previously identified defects in signalling via the molecular pathways of Akt-mTOR (a molecular target of rapamycin action) and RhoA-SRF (blood serum response factor) in sarcopenic muscles.

Some aging stimuli activate the aging process of satellite cells, leading to the loss of muscle repair ability when damaged. Many such stimuli trigger activation of the p53-p21Cip1 and p16Ink4a pathways, which contribute to temporary cell cycle arrest by inhibiting the cyclin-dependent kinases CDK2 and CDK4/6, respectively. Inflammation and SASP formation in aging myocytes converge at the level of activation of transmission of signals of nuclear factor kappa-light-chain-enhancer of activated B cells, and NF-κB. Therefore, this stimulates increased expression of muscle ubiquitin protein ligase (muscle RING-finger protein-1, MuRF1). Triggered aging process would cause mitochondrial dysfunction through deactivation of the peroxisome proliferator-activated receptor-gamma coactivator 1-α (PGC-1α) axis, which is activated by proliferator of sirtuin 1 and peroxisome, which is responsible for maintaining mitochondrial quality control. The accumulation of damaged mitochondrial DNA due to imbalanced mitochondrial dynamics is eliminated by mitophagy, which becomes ineffective under aging conditions and causes apoptosis. The inhibition of satellite cell aging as a preventive strategy can be considered an alternative therapy regimen. The blockade of satellite cell aging, increase in antioxidant activity, enhancement of anti-inflammatory action, decrease in proinflammatory activity, and preservation of mitochondrial function in myocytes may be considered promising strategies for sarcopenia therapy regimens in older patients [27].

Therefore, the goal of sarcopenia therapy regimen optimisation in older patients with OA and pain is the administration of CS, whose molecular mechanism of action is related to activation of CD44 receptor on the surface of cells (chondrocytes, leukocytes, presumably myocytes), which leads to inhibition of IKKβ signal protein kinase within the cell. Inhibition of IKKβ in the cell cytosol is accompanied by a lack of phosphorylation of the IkBα protein, which would not degrade in the proteasome, nor allow the active form of NF-kB, a key intracellular regulator of inflammation. Administration of CS prevents both the formation of the active form of NF-kB, the entry into the cell nucleus, and the activation of proinflammatory response genes, inter alia, genes of chemokines and cytokines (IL1β, IL-6, TNFα). The current study demonstrated that the blockade of the NF-kB cascade in cells under the influence of CS would significantly reduce the release of proinflammatory cytokines into the blood. A decrease in proinflammatory cytokines contributes to the inhibition of myocyte and satellite cell degradation. Simultaneously, NF-kB blockade reduces the expression of TLR2 and TLR4 toll-like receptors in satellite cells and leukocytes. The inhibition of the CD97 receptor contributes to the inhibition of leukocyte activation during inflammation in sarcopenia in older patients with OA.Protein dyscrasia is considered the front-end process that mediates age-related loss of muscle mass. Muscle atrophy occurs when the muscle is unused, immobilized, and/or denervated, as well as a result of starvation, which decreases muscle protein synthesis [28,29].

In this study, all patients with OA and sarcopenia had decreased blood levels of serum albumin (synthesised by the liver and

associated with skeletal muscle function); this is consistent with the results of a study on its concentrations at different age [30]. Blood serum albumin is stored extracellularly in skeletal muscles and immediately penetrates myocytes after physical exercise [31]. Aging disturbs the coordinated balance between protein synthesis and degradation by activating multiple signalling pathways: muscle atrophy F-box protein (MAFbx, muscle atrophy F-box) and MuRF1. The function of muscle-specific ubiquitin E3 ligases is enhanced by activation of the FoxO (forkhead box protein O1) transcription factor. Signalling of the insulin-like growth factor receptor 1 and IGF-1/PI3K-Akt systems is responsible for muscle protein synthesis [32]. Skeletal muscle hypertrophy is promoted by mTOR and glycogen synthase kinase 3β activation, while Akt inhibits protein degradation through FoxO-mediated proteasomal activity [33]. MAFbx and MuRF1 activities are suppressed by the FoxO3A transcription factor [34,35]. An increase in skeletal muscle mass through IGF-1/Akt/mTOR signalling and suppression of protein degradation by inhibition of atrogens may become key therapeutic intervention strategies for sarcopenia treatment in older patients [29]. Recent studies have shown that the partial inhibition of mTORC1 helps slow sarcopenia progression [36]. In contrast to Akt/mTOR signalling, AMPK regulates muscle fibre protein levels by inhibiting mTORC1. AMPK directly activates FoxO-dependent protein degradation [37].

The loss of muscle mass may be associated with increased levels of myostatin, a myokine involved in the reverse regulation of myogenesis. Myostatin is a member of the transforming growth factor beta (TGF-β) superfamily and binds to activin type II receptors on muscle fibres, resulting in the activation of Smad signalling molecules, which can inactivate Akt signalling and its downstream effectors, such as mTOR and FoxO [35,38]. Pharmacological regulation of the PKC/PI3K/Akt pathobiochemical pathway by enhancing Akt phosphorylation with the use of CS (Chondroguard® medicinal product) is a promising myoprotection strategy, as it can help reduce myostatin levels in the skeletal muscles of older patients with sarcopenia [39]. The increase in serum albumin levels, most pronounced after the administration of standard treatment regimen in combination with CS, is indicative of a decrease in catabolism and an increase in antioxidant capacity in skeletal muscles, which is beneficial for muscle mass in older patients with sarcopenia [40]. When CS is added to the sarcopenia therapy regimen, a consequence of the increase in serum albumin concentration is an increase in the bound fraction of hormones, including androgens, affecting not only muscle mass and skeletal muscle function but also leading to increase in satellite cells and nuclei in myocytes [41].Another mechanism mediated by CS may be the activation of the phosphatidylinositol-3-kinase pathway by blood serum albumin when its levels increase, leading to muscle hypertrophy and increased muscle mass [42].Activation of PGC-1α/sirtuin 1 (Sirt1) signalling pathway can prevent muscle atrophy by inhibiting FoxO3 and NF-κB activity [43]. In addition to regulating protein degradation, the PCG-1 family regulates mitochondrial biogenesis and function [44]. Physical exercise, included in the standard management regimen for patients with KOA and sarcopenia in Groups 1 and 2, can increase PCG-1 levels in skeletal muscles and prevent atrophy [45]. Activation of PCG-1α and its coactivators not only suppresses the activity of atrogen proteins, but also compensates for mitochondrial dysfunction in sarcopenia. CS as part of an optimised sarcopenia therapy reduces the activity of NF-κB and activates the PGC-1α/Sirt1 signalling pathway through a feedback mechanism.

Oxidative stress activation and chronic mild inflammation accompany sarcopenia and alter mitochondrial function via several mechanisms [46]. Evidence of an association between sarcopenia and age-specific inflammation has been reported. Several studies have reported elevated levels of circulating proinflammatory markers, such as IL-6 and TNFα, in older patients with sarcopenia and evidence of decreased muscle mass and strength [47,48]. In this study, elevated plasma levels of IL-6 and CRP in patients with sarcopenia were accompanied by decreased physical performance in older patients, which is consistent with findings of others [49]. Increased muscle catabolism caused by higher levels of inflammatory markers in patients with OA and sarcopenia may be a possible driver of this relationship [50]. Some experimental studies have shown that administration of TNFα and IL-6 to rats leads to proteolysis and skeletal muscle atrophy. In addition, a negative correlation was reported between the rate of protein synthesis in skeletal muscles and levels of CRP, IL-6, and type 2 receptor for TNFα, which also coincides with the dynamics of albumin and proinflammatory cytokines, indicated by CRP in patients in both groups in our study prior to the administered therapy [51]. Blockade of NFκB-mediated IL-6 secretion is one of possible mechanisms of CS action in reducing the intensity of main clinical manifestations of sarcopenia. This effect results in an increase in the synthesis and decrease in the decay of muscle proteins, in the reduced expression of myostatin, a muscle growth and muscle atrophy protein (F-box-1, atrogin-1) suppressing factor, thus reducing protein catabolism and increasing anabolism in skeletal muscles. The effect of decreased IL-6 activity is consistent with the results of previous studies [47-54]. The pronounced effect of the standard treatment regimen in combination with reduced TNFα activity in the blood was accompanied by an increase in muscle strength found in our study. Based on the results of assessment thereof, as confirmed by the findings in other studies in which blood TNFα level can predict the reduction in the muscle strength [55]. Significant reduction in blood IL-6 and TNFα levels upon adding CS to the standard treatment regimen also resulted in the elimination of insulin resistance by increasing calcium

absorption, accelerating protein synthesis in muscle fibres, and limiting the sarcopenia progression. Recent studies have shown that inflammatory responses can lead to sarcopenia by triggering mitochondrial abnormalities that affect the mitochondrial number or synthesis of mitochondria [56]. Therefore, a significant decrease in the activity of inflammatory reactions by reducing the level of proinflammatory cytokines, along with the administration of standard treatment regimen and CS, may have a chondroprotective effect in terms of the elimination of mitochondrial dysfunction.

The regulation of the activity of proinflammatory cytokines (demonstrated by statistically significant reduction of IL-1β, IL-6 levels) of CS by influencing the expression density of toll-like receptor 4 (TLR4), a change of which may increase the expression of microRNA-146a that aberrantly binds NF-kB [46].Administration of CS and standard sarcopenia therapy regimen in older patients revealed higher average scores on the SARC-F questionnaire and '5x sit-to-stand test' with lower average values of muscle strength tests (dynamometry, appendicular muscle index using DXA) in women compared to men in Group 1, which may be related to the different initial expression of key molecular drivers of sarcopenia in elder men and women.Numerous studies have shown that leptin is involved in glucose metabolism through its effects on pancreatic beta cells, the immune system, and skeletal muscle myocytes, and regulates inflammatory processes and insulin resistance [57,58]. The decrease in blood leptin levels found in this study, most pronounced upon administration of standard treatment regimen and CS in women with OA and sarcopenia, may be associated with a simultaneous significant decrease in proinflammatory cytokine release (IL-6, TNFα), which would significantly reduce the intensity of insulin resistance and prevent loss of muscle mass and strength [59,60]. The mechanism of action of CS suggests the elimination of myocellular lipid accumulation and lipotoxicity in skeletal muscles owing to the effective reduction in blood leptin levels and, consequently, the limitation of sarcopenic obesity [61-64].

Population studies have shown that low blood levels of carotenoids and α-tocopherol in older individuals are associated with decreased thigh and lower leg muscle strength. A diet rich in carotenoids reduces the severity of skeletal muscle atrophy [65]. Carotenoids and α-tocopherol are strong tissue antioxidants [66]. This study adds sarcopenia to other conditions that are associated with low blood levels of carotenoids and α-tocopherol. Low carotenoid levels are thought to lead to loss of muscle mass and subsequent reduction in muscle strength [67]. Our study showed that the skeletal muscle strength of men and women with OA, sarcopenia, and low total blood plasma carotenoid levels are at a higher risk. Administration of combined CS and standard treatment regimen for sarcopenia in older patients of both sexes, resulted in statistically significant increase in total blood plasma carotenoid concentrations in patients of both sexes, as well as in a significant increase in α-tocopherol concentration in men compared to those of male patients in the standard treatment group for sarcopenia. Statistically significant dynamics of α-tocopherol levels in older men, who took CS in addition to administration of standard treatment for sarcopenia, was associated with lower baseline levels of this antioxidant in the blood of older men. In vitro studies have shown synergistic antioxidant and anti-inflammatory effects of CS and α-tocopherol in sarcopenia treatment [68]. The increase in α-tocopherol concentration in blood upon adding CS in sarcopenia therapy and the improvement of muscle strength and mass may be connected with the mechanism of elimination of high mitochondrial membrane fluidity and increase in cytochrome oxidase and NADH-coenzyme Q1 reductase synthesis, and to increase reduced and oxidised glutathione ratio in skeletal muscle cells [69].

A recent study showed that higher dietary intake of antioxidants, including vitamin C, is inversely correlated with sarcopenia in women [70]. Water-soluble vitamin C (ascorbic acid) performs several mechanistic functions related to skeletal muscle metabolism and physiology and may prevent age-related skeletal muscle loss. The mechanisms of vitamin C action in skeletal muscle physiology include carnitine and collagen synthesis, and recent animal studies have further clarified the role of vitamin C deficiency [71-73]. Since vitamin C is an electron donor, it can reduce oxidative damage to muscles and the concentration of proinflammatory cytokines in the bloodstream [74]. Despite the knowledge of the mechanisms by which vitamin C can affect skeletal muscle physiology in the course of aging, the mechanism of action of vitamin C in relation to skeletal muscle mass is poorly known [49-78]. In this study of older women with sarcopenia after standard treatment regimen and CS therapy, lower vitamin C blood levels were noted than in patients subjected to standard treatment regimen alone, whereas there were no significant differences in vitamin C levels in the blood of older men after administration of standard treatment regimen alone or in combination with CS. The reason for the different dynamics of blood plasma vitamin C concentrations in older women and men is probably the difference in carnitine metabolism levels, which are necessary for the metabolism of long-chain fatty acids during physical activity, and collagen in skeletal muscles, which is a key structural component of skeletal muscle and tendon cells. The key molecular drivers of the reduction in sarcopenia severity upon adding CS and restoration of blood plasma vitamin C levels may lead to decreased activity of the ubiquitin ligase atrogin-1/MAFbx and MuRF1F protein in skeletal muscles with a reduction in AOS formation [79].

Administration of combined CS and standard treatment regimen for sarcopenia in older patients of both sexes produced a statistically significant increase in 25(OH)D concentration in blood plasma

compared to similar values in patients offered standard treatment. A decrease in muscle mass can lead to a decrease in the blood 25(OH)D concentration, which contributes to a decrease in muscle strength that occurs much faster than the accompanying loss in muscle mass [80]. This provides evidence of a relationship between the development of sarcopenia and vitamin D levels in the blood of older patients. Histological sections of 25(OH) D-deficient individuals show atrophy of muscle fibres, enlarged interfibrillar spaces, fatty infiltration, fibrosis, and deposition of glycogen granules, which together represent the metabolic and morphological basis of reduced muscle function [81]. Individuals with low blood vitamin D levels showed preferential atrophy of type 2 muscle fibres along with decreased muscle strength and proximal muscle weakness, which is consistent with findings in our study related to the assessment of sarcopenia screening in men and women for inclusion in our study. These changes suggest denervation of the motor neuromuscular units and conversion of fast type 2 muscle fibres to slow type 1 fibres, resulting in the loss of the muscle quality necessary for daily activities [82]. Decreased blood levels of 25(OH)D in older patients and loss of muscle mass are associated with age-related changes [83,84]. Aging is accompanied by a decrease in vitamin D receptor (VDR) expression and the biologically active form of the vitamin [85]. Reduced blood levels of vitamin D in the older persons can lead to decreased VDR expression due to reduced stimulation, thus suppressing receptor expression [86-88]. Over time, these processes would interfere with protein synthesis in myocytes, causing the depletion of type 2 fibres, muscle strength reduction, and eventually sarcopenia development [89]. The presence of the VDR in skeletal muscle myocytes indicates a relationship between blood 25(OH)D concentration, protein synthesis in skeletal muscles, and/or changes in contractility. Chronic inflammation is another key factor in the development of sarcopenia, and vitamin D plays an active role in reducing inflammation [3].

Consequently, restoring adequate blood levels of 25(OH)D while increasing serum albumin levels in older men and women with OA and sarcopenia upon adding CS therapy promotes increased VDR density; thus, implementing the 25(OH)D effect in terms of increased protein synthesis in skeletal muscles with an increase in muscle strength and contractility, and a decrease in skeletal muscle inflammation in older patients with OA.

In addition, CS has anti-inflammatory and anabolic effects on OA and sarcopenia at the subchondral bone level by increasing the expression level of osteoprotegerin and decreasing the expression level of the receptor activator of the nuclear factor-kappa B ligand (RANKL) gene, thereby increasing the RANKL mRNA ratio. The effect of CS on osteoprotegerin mRNA is related to the fact that osteoprotegerin contains a heparin-binding domain that binds to some glycosaminoglycans [90]. Therefore, CS can bind to the heparin domain of osteoprotegerin, thereby increasing its bioavailability and preventing its breakdown. Recent studies have shown that osteoprotegerin modulates the half-life of membrane RANKL, increasing its breakdown via internalisation [91]. RANKL-independent mechanisms regulate CS metabolism in the subchondral bone. Small proteoglycans, such as decorin and biglycan, are composed of CS chains [92] and they isolate TGFβ released by osteoblasts in OA [93], thereby suppressing the direct stimulatory effect of TGFβ on osteoclast formation [94,95].

Limitations. This study has several limitations. This was a prospective cohort observational study with small sample size, which raises uncertainty about its generalizability to all populations. Therefore, large-scale, multicentre investigations are necessary to corroborate the findings. One limitation of observational studies is the random assignment of participants using a random number table, which can cause systematic errors. A multicentre double-blind randomisation protocol may provide a more scientific assessment of the efficacy and safety of an optimised therapy for sarcopenia in older patients with KOA, in addition to the use of parenteral CS.

Materials and Methods

Study Design

This prospective cohort observational study investigated the short-term efficacy and safety of optimised sarcopenia therapy using the parenteral form of API for CS in older patients with KOA.

Inclusion/Exclusion Criteria

Inclusion Criteria: patients of either sex over 65 years of age with radiologically confirmed KOA and exacerbation of pain syndrome (at least 60 mm on the visual analogue scale [VAS]) who had received sarcopenia therapy + pain therapy (various NSAIDs: per os + a topical form) for at least 10 d before inclusion into the study and with confirmed sarcopenia (4 or more points on the simple questionnaire to rapidly diagnose sarcopenia [SARC-F]; best dynamometry score less than 16 kg in women and 26 kg in men; '5x sit-to-stand test' over 12 s; dual-energy X-ray absorptiometry (DXA) skeletal muscle index below 5.5 kg/m² in women and less than 7.0 kg/m2 in men); informed consent to participate in study; signed 'Awareness Consent'.

Exclusion Criteria: patients with autoimmune, oncological, or blood diseases, psychiatric disorders, moderate to severe cognitive impairment, Parkinson's disease, exacerbations of cardiovascular diseases, high cardiovascular risk, chronic kidney disease with glomerular filtration rate less than 50 mL/min, decompensated stage of diabetes and thyroid diseases, liver failure, senile asthenia, hyperhydration, dyspepsia complaints, exacerbation of gastric or 12-gastric ulcer disease within the past

year, chronic erosive gastritis, patients who have had COVID-19 within the past 6 months, patients receiving oral forms of CS and/or glucosamine, diacerein, unsaponifiable avocado and soy compounds, patients with individual intolerance to CS, NSAIDs, diacerein, unsaponifiable avocado and soy compounds or have participated in another clinical study on Symptomatic Slow Acting Drugs for Osteoarthritis (SYSADOA) within 60 d prior to the commencement of the study.

Study Conditions

This study was performed at the clinic of the Russian Gerontological Scientific Clinical Centre attached to the N.I. Pirogov Russian National Research Medical University (Moscow, Russia), Federal State Budgetary Scientific Institution 'Scientific Research Institute of Rheumatology named after V.A. Nasonova' (Moscow, Russia), LLC Medical Centre 'Novomedicina' (Rostov-on-Don). The study was conducted between January 2021 and March 2022.

Duration of the Study

Patients were treated for 60 d and followed-up over 30 d after completion of the treatment. Upon introduction of the CS (Chondroguard®) treatment regimen, the comparative assessment of therapy efficacy and safety in patients with KOA and sarcopenia was carried out during four patient visits with completion of 'patient clinical report form' with the main sections of the Study Protocol: 'day 0 visit' ('0' day of the study) – study commencement, 'day 5 visit', 'day 60 visit', 'day 90 visit'. At each visit, pain intensity according to VAS score, severity of sarcopenia according to SARC-F scale, results of dynamometry, and '5x sit-to-stand test' were assessed. Patients filled out their daily self-monitoring diaries, which included information on pain intensity according to the VAS score, sarcopenia severity according to the SARC-F scale, noted the use of celecoxib with the indication of the dose, and the need for the use of topical NSAID. At visits for 'day 0', 'day 5', and 'day 60' the assessed parameters were skeletal muscle index in patients measured by DXA, blood serum total albumin, C-reactive protein (CRP), TNFα, IL-1β, IL-6, leptin levels, blood levels of 25(OH)D3, vitamin C, total carotenoids, and α-tocopherol.

Description of the Medical Intervention

The study included 260 patients with radiologically confirmed KOA, exacerbation of pain syndrome (at least 60 mm on the VAS), and sarcopenia who were randomly allocated (random number table) into two groups: Group 1 (n=170) and Group 2 (n=90). Patients in both groups received standard sarcopenia therapy regimen, including administration of vitamin D 2,000 IU/day, an individual diet containing 1.5 g of protein per kg human body mass, at least five servings of fruits and vegetables daily, 3 g of leucine, a complex of physical exercises (balance and equilibration training and isometric exercises to increase muscle endurance) at the rate of 175 min a week in accordance with exercise programs for patients with sarcopenia in Methodological Recommendations 'General principles of pharmacotherapy in older and senile patients' (2019). For pain therapy, patients received celecoxib at a dose of 200 mg/day at the commencement of the study, with the option of reducing the dose to 100 mg/day or refusing the administration for pain relief, or a topical form of meloxicam with the option to refuse the administration at the patient's discretion if not needed. Group 1 patients received parenteral CS (Chondroguard®) subject to the following regimen: for the first 3 d the preparation was administered as intramuscular injections in a dose of 100 mg/day; and the next 4–30 injections in a dose of 200 mg/day. Intramuscular injections were administered once every 48 h. The doses and regimens of all drugs in the study corresponded to the official medical instructions for the use of drugs in patients with osteoarthritis in the Russian Federation. Analysis of efficacy and safety were carried out during visits for 'day 0', 'day 5', 'day 60', and 'day 90' (main outcome, VAS pain intensity) of the study on the basis of 'patient clinical report form' with main sections of the study protocol. The 30-d follow-up period in Groups 1 and 2 was used to evaluate the aftereffects of parenteral CS on sarcopenia and osteoarthritis.

Main Outcome of the Study

The primary outcome was the VAS pain intensity score and secondary outcomes included SARC-F questionnaire score, results of muscle strength tests (dynamometry, '5x sit-to-stand test') and quantitative indicators of muscle mass (DXA, dual-energy X-ray absorptiometry).

Additional Study Outcomes

Additional expected outcomes of the study are characteristic values of blood serum albumin, CRP, TNFα, IL-1β, IL-6, and leptin blood concentrations, 25(OH)D3 levels, vitamin C, total carotenoids, and α-tocopherol blood levels. These characteristic values make it possible to assess anti-inflammatory, immunomodulatory, antioxidant, antiresorptive effects and detail the mechanism of action of parenteral CS in the treatment of sarcopenia in older patients with KOA. Moreover, the results will help in the assessment of the patients' need to take celecoxib and the topical form of meloxicam, the assessment of safety of sarcopenia therapy regimen with parenteral CS in terms of the frequency, nature, severity, duration of adverse events (AEs) and their correlation with administration of drugs according to the WHO scale and the Naranjo scale.

Analysis in Groups

This study was performed on patients with radiologically confirmed KOA, exacerbation of pain syndrome (at least 60 mm on the VAS), and sarcopenia. The category of patients with KOA and sarcopenia

corresponded to the diagnostic criteria and algorithms of specialised medical care in the Methodological Recommendations 'General principles of pharmacotherapy in older and senile patients' (2019), Clinical Recommendations ('Aging Asthenia', Ministry of Health of the Russian Federation, 2020), 'Chronic pain in older and senile patients' (2020).

Methods for Outcome Recording

The pharmacological effects of parenteral CS were evaluated during visits for 'day 0', 'day 5', and 'day 60' based on the following scores: a) VAS questionnaire score (main outcome, also assessed on 'day 90') and SARC-F scale; b) muscle strength tests (dynamometry, '5x sit-to-stand test'); c) quantitative measures of muscle mass (skeletal muscle index using DEXA STRATOS (DMS, France); d) serum albumin levels (reference values: 35–52 g/L), high-sensitivity CRP (reference values: 0.00–1.00 mg/L), TNFα concentration (reference values: <8.1 pg/mL), IL-1β (reference values: 0–11 pg/mL), IL-6 (reference values: 0–7 pg/mL), leptin (3.7–11.1 ng/mL) in blood on a Cobas 6,000 automatic immunochemistry analyser platform (Roche Diagnostics, Switzerland); e) 25(OH)D3 levels (reference values: 30–100 ng/mL; deficiency levels: 20–30 ng/mL, <20 ng/mL) on a UniCel DxI 800 immunochemiluminescent analyser platform (Beckman Coulter, USA), vitamin C (reference values: >50 μmol/L; deficiency <50 μmol/L), total carotenoids (reference values: 1.4–1.7 μmol/L) and α-tocopherol (reference values: 5.00–18.00 μmol/L) in blood with the use of high-performance liquid chromatography-mass spectrometry (Agilent 6230B TOF LC/MS, USA).

Venous blood samples for patients were collected in the morning, kept at room temperature for 10 min, and then centrifuged at 800 rpm for 10 min at 4°C. The erythrocyte fraction at the bottom of the centrifuge tube was separated from the supernatant, and the first fraction was separated into aliquots and stored at -80°C until analysis. The supernatant corresponding to the plasma fraction was then centrifuged at 3,600 rpm for 20 min at 4°C to obtain platelet-free plasma as supernatant, which was similarly separated into aliquots and stored at -80°C until analysis. Peripheral markers (proinflammatory and oxidative stress parameters) were determined either in the plasma or erythrocyte fraction obtained from the whole blood. To ensure the quality of the cytokine and vitamin determinations, each sample was analysed twice, and the mean value obtained for each sample was used as the final value. Additionally, two quality controls were performed to confirm that the values obtained were within the expected concentration range. The safety of parenteral CS therapy was monitored by assessing the frequency, nature, severity, and duration of adverse events and their correlation with the administration of the medicinal product according to the WHO and Naranjo scales, in accordance with the International Medical Dictionary for Regulatory Activities Terminology (MedDRA).

Ethical Review

The study was conducted in accordance with the Declaration of Helsinki and approved by the Local Ethics Committee of LLC Medical Centre 'Novomedicina' (Russia, Rostov-on-Don) including representatives of Russian Gerontological Scientific Clinical Centre of N.I. Pirogov Russian National Research Medical University (Moscow, Russia) and Federal State Budgetary Scientific Institution 'Scientific Research Institute of Rheumatology named after V.A. Nasonova' (Moscow, Russia) (Ethics approval number: protocol code 13, 2021).

Statistical Analysis

Primary and secondary statistical processing were conducted using STATISTICA 10.0 (StatSoft, Inc., USA, 'XLSTAT' version 2019.3.2 [Addinsoft, USA]). The following stratifying variables (age, weight, height, sex, duration of postmenopause, arterial hypertension, diabetes mellitus, mean number of comorbid conditions, Charlson comorbidity index, duration of OA, VAS pain intensity, duration of knee pain exacerbation, and Kellgren–Lawrence radiological grade of osteoarthritis) were used. The final sample was subjected to consideration for the I/E criteria of the study. The sample size required for 90% statistical power to detect a significant difference in the use of standard and optimized sarcopenia therapy regimens in older patients with KOA was calculated using Statistica 10.0. The normality of the quantitative data distribution was assessed using the Kolmogorov-Smirnov test. Descriptive analysis of the study and assessment of therapeutic efficacy and safety included key statistical indices (median, 25th and 75th percentiles). Categorical variables were analysed using Fisher's exact test, whereas continuous variables were compared using the Mann–Whitney U test. Statistical significance was set at $p<0.05$.

Conclusion

Parenteral CS (Chondroguard®) is a promising strategy to complete the standard sarcopenia therapy regimen in patients with KOA and pain through an increased antioxidant and anti-inflammatory activity, and preservation of mitochondrial function of skeletal muscle myocytes.The development of new molecular strategies and potential therapeutic approaches for the management of sarcopenia is essential to combat this complex geriatric syndrome.

Ethics statement

This study was approved by the Local Ethics Committee of LLC Medical Centre 'Novomedicina' (Rostov-on-Don, Russia), including representatives of Russian Gerontological Scientific Clinical Centre of N.I. Pirogov Russian National Research Medical University (Moscow, Russia) and Federal State Budgetary Scientific

Institution 'Scientific Research Institute of Rheumatology named after V.A. Nasonova' (Moscow, Russia) (Ethics approval number: 2021-13). Patients were consented by an informed consent process that was reviewed by the Local Ethics Committee of LLC Medical Centre 'Novomedicina' (Rostov-on-Don, Russia) and we certify that the study was performed in accordance with the ethical standards as laid down in the Declaration of Helsinki.

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Author contribution statement

Irina V. Sarvilina: conceptualisation, methodology, validation, formal analysis, investigation, resources, and writing the original draft. Olga N. Tkacheva: Conceptualization, methodology, validation, writing – review & editing Alexander M. Lila: Conceptualisation, validation, writing – review & editing, supervision, project administration Olga A. Gromova: Methodolo gy, software, data curation, visualization

Data availability statement

Not applicable.

Declaration of competing interest

The authors declare that they have no competing financial interests or personal relationships that may have influenced the work reported in this study.

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