International Heart and Vascular Disease Journal. Volume 14, № 51, September 2026
ISSN: 2311-1623 (Print)
ISSN: 2311-1631 (Online)
http://www.heart-vdj.com

Morphological substrate of atrial fibrillation onset in euthyroid sick syndrome: an experimental study

Rakhmatullov R.F.[1], Kondratyeva K.P.[1], Sheina A.E.[1], Dementyeva R.E.[1], Rakhmatullov F.K.[1], Melnikova L.V.[2]

[1] Penza State University, Penza, Russia.
[2] Russian Medical Academy of Continuing Professional Education of the Ministry of Health of the Russian Federation, Moscow, Russia.

AUTHORS

Ruslan F. Rakhmatullov, PHD, MD, associate professor, Department of Internal Medicine, Penza State University, Penza, Russia. ORCID: 0000-0002-2157-544X

Kristina P. Kondratyeva, senior lecturer, Department of Internal Medicine, Penza State University, Penza, Russia. ORCID: 0000-0002-8540-2054

Alina E. Sheina, senior lecturer, Department of Internal Medicine, Penza State University, Penza, Russia. ORCID: 0000-0002-9373-7268

Renata E. Dementyeva*, PHD, MD, associate professor, Department of Internal Medicine, Penza State University, Penza, Russia. ORCID: 0000-0003-1497-5338

Fagim K. Rakhmatullov, PHD, MD, professor, Head of the Department of Internal Medicine, Penza State University, Penza, Russia. ORCID: 0000-0002-2587-8325

Lyudmila V. Melnikova, PHD, MD, professor, Department of General Medical Practice and Polyclinic Therapy, Russian Medical Academy of Continuing Professional Education" of the Ministry of Health of the Russian Federation, Moscow, Russia. ORCID: 0000-0003-4688-1272

Atrial fibrillation (AF) is one of the most common tachyarrhythmias encountered in clinical practice. AF is associated with structural and electrical remodeling of the atrial myocardium. Atrial fibrosis, indicative of structural remodeling, is a complex multifactorial process that contributes to the initiation and maintenance of AF. Although the relationship between AF and fibrosis has been actively investigated for a considerable time in clinical and pathomorphological studies, this process is extremely complex and involves intricate neurohumoral, cellular, and molecular interactions that are not limited to the atria. The mechanisms by which atrial fibrosis contributes to

* Corresponding author. Tel./Тел. +7 963-109-57-82. E-mail: Rdementyeva@gmail.com

DOI: 10.24412/2311-1623-2026-50-57-65

the development of AF in euthyroid sick syndrome (ESS) remain insufficiently studied, which prompted this investigation.

Objective—to study the morphological substrate of AF onset in ESS in an experimental setting using outbred rats.

Materials and methods. The experiment was performed on 146 outbred rats. Hypothyroidism was induced by thiamazole administration, followed by restoration of euthyroidism with levothyroxine sodium under thyroid hormone level monitoring. Based on the assessment of thyroid hormone levels, the following types of hypothalamic-pituitary-thyroid axis response were identified: euthyroidism, thyrotoxicosis, and ESS. In animals with euthyroidism and ESS, the percentage of atrial fibrosis was evaluated.

Results. Regression analysis revealed that in euthyroid animals, the percentage of fibrosis in the zone of the inferior pulmonary veins had the greatest influence on the frequency of AF paroxysms ($\beta = 0.734$, $p = 0.015$). Multiple regression results demonstrated that the maximum influence on AF paroxysm frequency in ESS-1 was exerted by the percentage of fibrosis in the inferior and superior pulmonary veins (ISPV) ($p = 0.002$); in ESS-2, by fibrosis in the ISPV ($p = 0.021$) and the right atrium ($p = 0.015$); and in ESS-3, by fibrosis in the ISPV ($p = 0.023$) and the right atrium ($p = 0.040$). Fibrosis was established as a morphological substrate of AF in ESS. It was found that the atrial fibrosis area in euthyroidism was 18.8%, whereas in ESS types 1, 2, and 3 it was 36.6%, 35.8%, and 38.3%, respectively.

Conclusion. This study demonstrated that varying atrial fibrosis areas in the setting of ESS constitute a morphological substrate for the formation of re-entrant excitation circuits in AF. The fibrosis area in ESS was found to be twice as large as that in euthyroidism.

Keywords: euthyroid sick syndrome, fibrosis, atrial fibrillation, atrial remodeling, thyroid gland, thyroid hormones.

Conflict of interest: none declared.

Received: 26.05.2026
Accepted: 06.08.2026

For citation: Rakhmatullov RF, Kondratyeva KP, Sheina AE et al. Morphological substrate of atrial fibrillation onset in euthyroid sick syndrome: An experimental study. International Heart and Vascular Disease Journal. 2026; 14(51): 41-49. DOI: DOI: 10.24412/2311-1623-2026-51-54-64

Introduction

Atrial fibrillation (AF) is one of the most persistent and widespread cardiac rhythm disorders. Its onset and subsequent maintenance are pathogenetically linked to structural atrial remodeling. Longterm followup of patients with AF helps to determine to what extent changes in their clinical status correspond to the degree of progression of structural changes in the atria [1, 2]. However, a close relationship between characteristic atrial alterations and the clinical picture of the disease is not observed in every case. The results of numerous clinical and experimental studies, based on repeated atrial myocardial biopsies, indicate that an increase in the percentage of fibrosis is accompanied by a higher frequency and longer duration of arrhythmia [3, 4]. At the same time, the specific mechanisms by which atrial fibrosis contributes to the development of AF in euthyroid sick syndrome (ESS) remain insufficiently investigated [5]. Given the limited number of studies on the pathogenesis of AF in ESS, we considered it appropriate to explore this issue in an experimental setting using outbred rats [6, 7].

Aim—to study the morphological substrate of AF onset in ESS in an experimental study on outbred rats.

Materials and methods

The study was performed on 146 outbred rats (50 males and 96 females) weighing 180–200 g, kept on a standard diet. The experiment consisted of four stages.

Stage 1—an electrocardiogram (ECG) was recorded in standard lead II using a PolySpectrum device for 10 minutes. The duration of ECG intervals and complexes was then assessed. Cardiac rhythm disturbances were evaluated by the average number of extrasystoles and AF paroxysms on the ECG.

Blood samples were collected from the saphenous vein into tubes containing heparin (25 IU/mL) or 3.8% sodium citrate solution. Subsequently, the levels of thyroidstimulating hormone (TSH), free triiodothyronine (fT3), free thyroxine (fT4), and reverse triiodothyronine (rT3) were measured by enzymelinked immunosorbent assay. The fT3/rT3 ratio was calculated. Normally, the optimal fT3/rT3 ratio ranges from 10 to

15, and a decrease below 10 indicates the presence of ESS. Based on thyroid hormone levels, three types of ESS were distinguished: low fT3—ESS type 1; low fT3 and low fT4—ESS type 2; high fT4—ESS type 3.

Stage 2—to induce hypothyroidism, thiamazole was administered at a dose of 2.5 mg/100 g body weight for 3 weeks. Thyroid hormone levels were then reassessed. Baseline values were: TSH—1.8±0.08 mIU/L, fT3—4.5±0.09 pmol/L, fT4—17.7±0.36 pmol/L; after achieving hypothyroidism, the corresponding values were 9.4±0.2 ($p<0.001$), 0.05±0.001 ($p<0.001$), and 0.0271±0.0006 ($p<0.001$). The baseline number of AF paroxysms per 10 minutes was 3.5±0.285 episodes, with arrhythmia duration of 13.3±0.391 seconds; in ESS1—5.6±0.320 episodes and 17.2±0.592 seconds; in ESS2—6.7±0.252 episodes and 19.2±0.691 seconds; in ESS3—7.8±0.257 episodes and 26.3±0.230 seconds.

Stage 3—to restore euthyroidism, levothyroxine sodium (Euthyrox) was given at a dose of 1.5 μg/kg for 3 weeks, followed by reassessment of thyroid hormone levels. Based on the hormone levels measured, six types of hypothalamicpituitarythyroid axis response to Euthyrox at 1.5 μg/kg were identified (Table 1).

Table 1. **Types of hypothalamicpituitarythyroid axis response to Euthyrox**

Response type	n, (%)	TSH (mIU/L)	fT4 (pmol/L)	fT3 (pmol/L)	fT3/rT3
1st—ET	42 (28.8%)	1.88 [0.24; 3.39]	17.3 [10.9: 25.7]	4.47 [2.64; 6.36]	> 10
2nd—TT	15 (10.3%)	0.023 [0.003; 0.035]	56.7 [32.9; 75.8]	12.4 [7.38; 18.42]	> 10
3rd – ST	22 (15.1%)	0.022 [0.0024; 0.0416]	18.9 [10.7; 25.7]	4.4 [2.48; 6.24]	> 10
4th—ESS1	20 (13.7%)	2.16 [0.31; 4.1]	18.8 [10.82; 25.33]	2.3 [1.81; 2.88]	< 10
5th—ESS2	24 (16.4%)	1.76 [0.43; 3.2]	9.1 [8.5; 9.79]	2.44 [2.03; 2.87]	< 10
6th—ESS3	23 (15.8%)	1.78 [0.42; 3.45]	23.62 [22.91; 24.47]	4.7 [3.11; 6.56]	< 10

Note. n (%)—number and percentage of animals studied; 1st type (ET)—first euthyroid type of hypothalamicpituitarythyroid axis response to Euthyrox; 2nd type (TT)—second (thyrotoxic) type of response; 3rd type (ST)—third (subclinical thyrotoxic) type of response; 4th, 5th, and 6th types (ESS1, ESS2, ESS3)—euthyroid sick syndrome types 1, 2, and 3, respectively. Values are presented as median [interquartile range].

Stage 4 of the experimental study included rats with euthyroidism and ESS types 1, 2, and 3. The animals were anesthetized with a mixture of clisazine, zolazepam, and tiletamine, and then decapitated. The heart was excised, washed free of blood, and freed from connective tissue. The objects of study were thin myocardial strips excised from the orifices of the superior pulmonary veins (SPV), from the area between the orifices of the inferior and superior pulmonary veins (ISPV), from the area between the orifices of the inferior pulmonary veins (IPV), from the wall of the right atrium (RA), and from the Bachmann›s bundle (BB). The paraffin sections obtained were stained with hematoxylin and by Van Gieson›s method with counterstaining of nuclei with hematoxylin. Under a microscope, in 10 fields of view at ×200 magnification, the type and area of fibrosis were assessed as a percentage.

Table 2 shows the number and percentage of specimens examined in euthyroidism, ESS1, ESS2, and ESS3.

Table 2. **Number and percentage of specimens examined in euthyroidism and ESS**

Parameter	ET, n (%)	ESS1, n (%)	ESS2, n (%)	ESS3, n (%)	Total, n (%)
SPV, n (%)	15 (35.7)	9 (45.0)	12 (50.0)	12 (52.2)	48 (45.7)
ISPV, n (%)	12 (28.6)	11 (55.0)	11 (45.8)	11 (47.8)	45 (44.3)
IPV, n (%)	13 (31.0)	10 (50.0)	9 (37.5)	9 (39.1)	41 (39.4)
RA, n (%)	11 (26.2)	9 (45.0)	13 (54.2)	8 (34.8)	41 (40.0)
BB, n (%)	12(28.6)	10 (50.0)	8 (33.3)	12 (52.2)	42 (41.0)
Total, n (%)	105 (30.0)	69 (49.0)	77 (44.2)	75 (45.2)	326 (42.1)

Note. n (%) ET—number and percentage of specimens examined in euthyroidism (ET); n (%) ESS1,2,3—number and percentage of specimens examined in euthyroid sick syndrome types 1, 2, and 3; n (%)—number and percentage of specimens examined from the orifices of the SPV, from the ISPV area, from the IPV area, from the RA, and from the BB.

Microscopy analysis revealed that in outbred rats, the atria exhibited perivascular fibrosis, perimuscular fibrosis, smallfocal fibrosis, and meshlike (encircling) fibrosis.

Statistical analysis

Statistical processing of the obtained results was performed using Statistica 13.3 software. For each sample, the arithmetic mean (M) and standard deviation (m) were calculated. The distribution type was assessed using the Kolmogorov–Smirnov test. To calculate statistical significance in two related groups, Student's ttest was used, while in two unrelated groups, the Mann–Whitney U test was applied. Multivariate analysis was used to identify independent predictors of atrial fibrillation recurrence. To evaluate the influence of parameters, ROC analysis with the area under the curve was employed. Threshold values for quantitative predictors were established

DOI: 10.24412/2311-1623-2026-50-57-65

based on ROC analysis at the optimal sensitivity/specificity ratio. Differences were considered significant at $p < 0.05$.

Ethical review

The experimental study was conducted in accordance with the fundamental rules set forth in the basic documents governing experiments on laboratory animals and their housing conditions (Helsinki Declaration, 2000; GOST 330442014; Rules of Good Laboratory Practice of the Eurasian Economic Union in the Field of Drug Circulation). The experimental study was approved by the Local Ethics Committee at LLC CDKI (minutes of meeting No. 823 dated 11 September 2023).

Results

Depending on the type of ESS, the results of the assessment of the percentage of atrial fibrosis are presented in three parts.

1. Results of atrial fibrosis assessment in ESS type 1.

Table 3 presents a comparative evaluation of the percentage of fibrosis in the studied atrial zones in euthyroid (ET) and ESS1 rats.

Table 3. **Percentage of atrial fibrosis in euthyroidism (ET) and ESS1 (M±m)**

Parameter	ET, (n=42)	ESS1, (n=20)	p
Fibrosis in LA between superior PVs, %	4.4±0.147	5.1±0.252	=0.006
Fibrosis in LA between superior and inferior PVs, %	3.8±0.156	12.4±0.692	<0.001
Fibrosis in LA between inferior PVs, %	3.4±0.161	4.0±0.232	=0.193
Fibrosis in RA, %	3.9±0.159	11.4±0.622	<0.001
Fibrosis in BB, %	3.8±0.112	4.8±0.188	<0.001
Total atrial fibrosis, %	18.8±0.503	36.6±1.429	<0.001
Number of extrasystoles per 10 min	10.5±0.489	15.7±1.281	<0.001
Number of AF paroxysms per 10 min	3.2±0.212	5.8±0.416	<0.001
AF duration, s	5.6±0.463	7.7±0.771	=0.016

Note. Data are presented as arithmetic mean ± standard error of the mean (M±m). ESS1—euthyroid sick syndrome with low fT3 levels.

Analysis revealed that in ET animals, the percentage of fibrosis in the ISPV zone was 14.6% lower than in the SPV zone (3.8±0.156 and 4.4±0.147, respectively, p=0.004). In the IPV zone, it was 18.2% lower than in the ISPV zone (3.4±0.161 and 3.8±0.156, respectively, p<0.001). In the RA, it was 10.7% higher than in the IPV zone (3.9±0.159 and 3.4±0.161, respectively, p=0.033). No significant differences were found between the RA and BB (p>0.05).

Analysis of the percentage of atrial fibrosis in ESS1 rats showed a significant influence of the thyroid component. It was found that fibrosis in the ISPV zone was 58.7% greater than in the SPV zone (5.1±0.252 and 12.4±0.692, respectively, p<0.001). In the IPV area, it was 67.5% lower than in the ISPV zone (12.4±0.692 and 4.0±0.232, respectively, p<0.001). In the RA, it exceeded that in the IPV zone by 64.7% (4.0±0.232 and 11.4±0.622, respectively, p<0.001). In the BB, it was 58.2% less than in the RA (11.4±0.622 and 4.8±0.188, respectively, p<0.001).

When comparing the percentage of fibrosis in the studied zones between ET and ESS1 rats, significant differences were also found. It was revealed that in ESS1, the percentage of fibrosis in the SPV zone was greater by 14.3% (p=0.006), in the ISPV zone by 69.7% (p<0.001), in the IPV zone by 10.9% (p=0.193), in the RA by 65.6% (p<0.001), and in the BB by 17.1% (p<0.001). As can be seen from the data obtained, the most diagnostically significant finding was an almost threefold increase in the percentage of fibrosis in the ISPV zone and the RA.

To evaluate the multiple regression of the number of AF paroxysms with the percentage of fibrosis in the studied atrial zones in ET rats, an analysis was performed, as presented in Table 4.

Table 4. **Results of multiple regression of the number of AF paroxysms with the percentage of fibrosis in the studied atrial zones in ET rats**

Parameter	Number of AF paroxysms		
Multiple correlation coefficient (R)	0.910		
Coefficient of determination (R^2)	0.804		
Fisher's Ftest value	32.9		p<0.001
Fibrosis localization / Statistical indices	β	t	p
SPV, %	-0.124	-0.502	0.619
ISPV, %	-0.128	-0.356	0.724
IPV, %	0.734	2.6	0.015
RA, %	0.659	1.9	0.066
BB, %	-0.231	-0.659	0.514

Note. β—regression coefficient estimate for the variable; t—Student's ttest.

The multiple correlation coefficient (R) of 0.910 indicates a strong relationship between the num-

ber of AF paroxysms and the percentage of fibrosis in the studied atrial zones in ET rats. The leading statistical indicator is the coefficient of determination (R^2), equal to 0.804 ≈ 80.4%, which shows the proportion of variation in AF paroxysm frequency caused by the independent predictors—the atrial fibrosis zones. Fisher's Ftest (F) and the significance level are used to evaluate the regression. An Fvalue of 32.9 and a significance level (p) of less than 0.001 indicate the adequacy of the regression model.

For comparative assessment of the impact of each component of the fibrosis percentage in the studied atrial zones, standardized β coefficients (regression coefficients) were used, by the magnitude of which the influence of each fibrosis zone on the increase in AF paroxysm frequency was evaluated. According to the multiple regression results, the greatest influence on the frequency of AF paroxysms in euthyroidism is exerted by the percentage of fibrosis in the IPV zone (β = 0.734, p = 0.015).

As noted above, in ET rats, the fibrosis zone in the ISPV accounts for 3.8%, in the IPV—3.4%, and in the RA and BB—3.9% and 3.8%, respectively. These data suggest that the reduction in the percentage of fibrosis in the IPV zone compared with the ISPV, RA, and BB creates an anatomical substrate for dispersion of refractory periods and reentrant excitation. The regression coefficient assessment showed that the percentage of fibrosis in the IPV zone has the greatest influence on AF paroxysm frequency (β = 0.734, p = 0.015). Similar results have been obtained in electrophysiological studies [8]. It was noted that the effective refractory period of the atria in the IPV zone is significantly shorter (p<0.05) than in the ISPV zone, RA, and BB.

To determine the threshold values of atrial fibrosis in the SPV, ISPV, IPV, RA, and BB, ROC analysis was performed. Figure 1 presents the results of ROC analysis of the number of AF paroxysms with the percentage of fibrosis in the studied atrial zones in ET rats.

In euthyroidism, the threshold percentage of fibrosis area in the studied atrial zones can predict the onset or increased frequency of AF paroxysms. The studies established that the cutoff value of atrial fibrosis in the SPV zone was 4.42%, with a sensitivity of 87.5% and specificity of 75.0% (AUC = 0.935, 95% confidence interval (CI) 0.865–1.0, p < 0.05). The threshold value of atrial fibrosis in the ISPV zone was >4.07% with a sensitivity of 75.0% and specificity of 91.7% (AUC = 0.940, 95% CI 0.874–1.0, p < 0.05). The cutoff value of atrial fibrosis in the IPV zone was > 4.05% with a sensitivity of 62.5% and specificity of 95.7% (AUC = 0.938, 95% CI 0.868–1.0, p < 0.05). The threshold value of atrial fibrosis in the RA was >4.08% with a sensitivity of 81.3% and specificity of 87.5% (AUC = 0.945, 95% CI 0.882–1.0, p < 0.05). The cutoff value of fibrosis in the BB was >3.6% with a sensitivity of 93.8% and specificity of 75.0% (AUC = 0.940, 95% CI 0.874–1.0, p < 0.05).

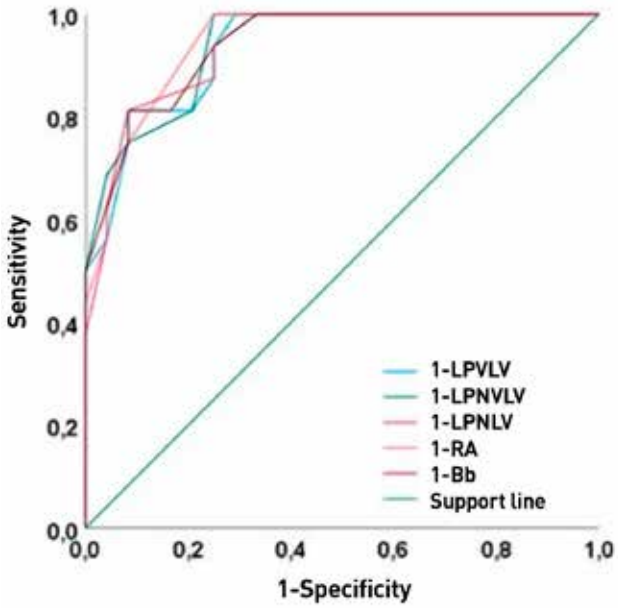

Fig. 1. ROC curves reflecting the threshold nature of the fibrosis percentage in the studied atrial zones in ET rats

Table 5. **Results of multiple regression of the number of AF paroxysms with the percentage of fibrosis in the studied atrial zones in ESS1 rats**

Parameter	Number of AF paroxysms		
Multiple correlation coefficient (R)	0,989		
Coefficient of determination (R2)	0,971		
Fisher's Ftest value (F)	115,4		p <0,001
Fibrosis localization / Statistical indices	β	t	p
SPV, %	0,170	0,917	0,377
ISPV, %	1,205	4,1	0,002
IPV, %	-0,181	-1,480	0,165
RA, %	-0,435	-1,483	0,164
BB, %	0,212	1,175	0,263

Note. β—regression coefficient estimate for the variable; t—Student›s ttest.

Analysis of the obtained data showed that the correlation coefficient R = 0.989 indicates a relationship

DOI: 10.24412/2311-1623-2026-50-57-65

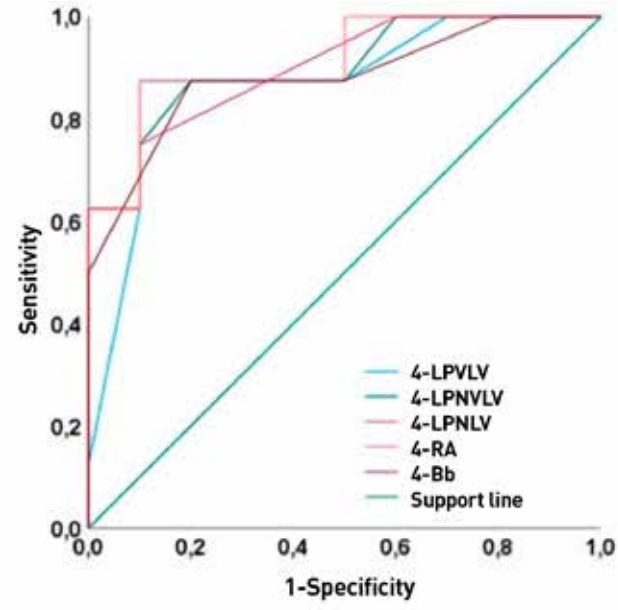

Fig. 2. ROC curves reflecting the threshold nature of the fibrosis percentage in the studied atrial zones in ESS1 rats

between AF frequency and the percentage of fibrosis in the assessed atrial regions. The coefficient of determination (R^2) = 0.971 ≈ 97.1% determines the proportion of variation in AF frequency caused by atrial fibrosis zones. The F test value of 115.4 and a significance level of $p < 0.001$ demonstrate the adequacy of the regression model. The multiple regression results revealed that the maximum influence on AF paroxysm frequency in ESS1 is exerted by the percentage of fibrosis in the ISPV ($\beta = 1.205$, $p = 0.002$).

Figure 2 presents the results of ROC analysis of the number of AF paroxysms with the percentage of fibrosis in the studied atrial zones in ESS1 rats.

The results of the conducted studies suggest that in ESS1, the threshold value of atrial fibrosis can predict AF paroxysms. It was found that the cutoff value of atrial fibrosis in the SPV zone was 5.1%, with a sensitivity of 87.5% and specificity of 90.0% (AUC = 0.875, 95% CI 0.699–1.0, $p < 0.05$). In the ISPV zone, it was 12.5%, with a sensitivity of 75.0% and specificity of 90.0% (AUC = 0.900, 95% CI 0.750–1.0, $p < 0.05$). In the IPV area, it was 4.2%, with a sensitivity of 77.0% and specificity of 90.5% (AUC = 0.902, 95% CI 0.755–1.0, $p < 0.05$). In the RA, it was 11.5%, with a sensitivity of 87.5% and specificity of 90.2% (AUC = 0.913, 95% CI 0.773–1.0, $p < 0.05$). In the BB, it was 4.75%, with a sensitivity of 87.2% and specificity of 80.0% (AUC = 0.881, 95% CI 0.711–1.0, $p < 0.05$).

2. Results of atrial fibrosis assessment in ESS type 2. A comparative evaluation of the percentage of fibrosis in the studied atrial zones in ET and ESS2 rats is presented in Table 6.

Table 6. **Percentage of atrial fibrosis in euthyroidism (ET) and ESS2 (M±m)**

Parameter	ET, (n=42)	ESS2 (n=24)	p
Fibrosis in LA between superior PVs, %	4.4±0.147	3.6±0.335	=0.179
Fibrosis in LA between superior and inferior PVs, %	3.8±0.156	11.7±0.649	<0.001
Fibrosis in LA between inferior PVs, %	3.4±0.161	3.5±0.152	0.506
Fibrosis in RA, %	3.9±0.159	12.4±0.599	<0.001
Fibrosis in BB, %	3.8±0.112	4.5±0.302	0.0001
Total atrial fibrosis, %	18.8±0.503	35.8±1.526	<0.001
Number of extrasystoles per 10 min	10.5±0.489	21.3±1.170	<0.001
Number of AF paroxysms per 10 min	3.2±0.212	7.6±0.520	<0.001
AF duration, s	5.6±0.463	10.6±0.477	<0.001

Note. Data are presented as arithmetic mean ± standard error of the mean (M±m). ESS2—euthyroid sick syndrome with low fT3 and low fT4 levels.

Similar data were obtained when analyzing the studied atrial zones in ESS2 rats. In the ISPV zone, the percentage of fibrosis was 68.8% higher than in the SPV zone (11.7±0.649 and 3.6±0.335, respectively, $p<0.001$). In the IPV zone, it was 69.9% lower than in the ISPV zone (3.5±0.152 and 11.7±0.649, respectively, $p<0.001$). In the RA, it exceeded that in the IPV zone by 71.7% (3.5±0.152 and 12.4±0.599, respectively, $p<0.001$). In the BB, it was 64.2% less than in the RA (12.4±0.599 and 4.5±0.302, respectively, $p<0.001$).

The results of multiple regression of AF paroxysms with the percentage of fibrosis in the studied atrial zones in ESS2 rats are presented in table 7.

Table 7. **Results of multiple regression of the number of AF paroxysms with the percentage of fibrosis in the studied atrial zones in ESS2 rats**

Parameter	Number of AF paroxysms		
Multiple correlation coefficient (R)	0,982		
Coefficient of determination (R2)	0,963		
Fisher's Ftest value	82,9		p<0,001
Fibrosis localization / Statistical indices	ß	t	p
SPV, %	0,329	1,786	0,093
ISPV, %	0,451	2,6	0,021
IPV, %	-0,041	-0,353	0,729
RA, %	0,532	2,7	0,015
BB, %	-0,278	-1,440	0,169

Note. β—regression coefficient estimate for the variable; t—Student›s ttest.

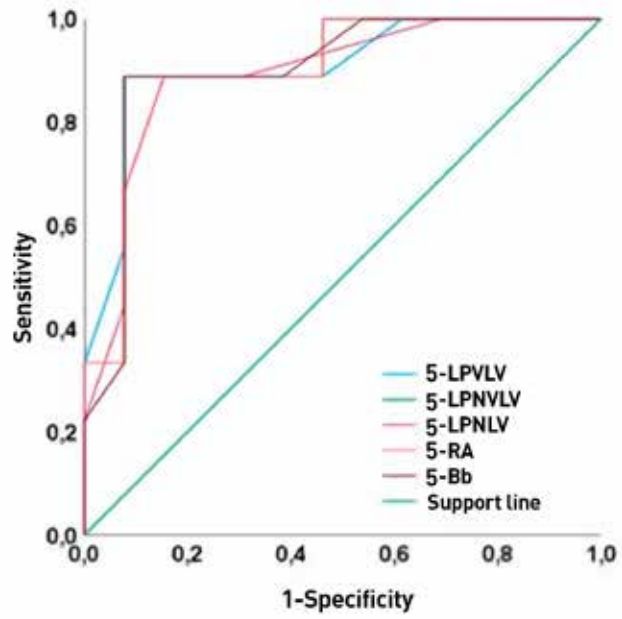

Fig. 3. ROC curves reflecting the threshold nature of the fibrosis percentage in the studied atrial zones in ESS2 rats

A correlation coefficient R of 0.982 indicates a strong relationship between AF paroxysms and the percentage of atrial fibrosis. The coefficient of determination R^2 = 0.963 ≈ 96.3% determines the proportion of variation in AF paroxysms caused by atrial fibrosis zones. Fisher's F test value of 82.9 and a significance level of p < 0.001 demonstrate the adequacy of the regression model. The multiple regression data established that the predominant influence on AF frequency in ESS2 is exerted by the percentage of fibrosis in the ISPV (ß = 0.451, p = 0.021) and in the RA (β = 0.532, p = 0.015).

The results of ROC analysis of the number of AF paroxysms with the percentage of fibrosis in the studied atrial zones in ESS2 rats are presented in Figure. 3.
As can be seen from the data presented, in ESS2, the threshold value of atrial fibrosis in the SPV, ISPV, IPV, RA, and BB zones allows predicting the frequency of AF. In the SPV zone, it is >3.62%, with a sensitivity of 88.9% and specificity of 92.3% (AUC = 0.906, 95% CI 0.770–1.0, p < 0.05). In the ISPV zone, it is >12.1%, with a sensitivity of 88.6% and specificity of 92.0% (AUC = 0.904, 95% CI 0.772–1.0, p < 0.05). In the ISPV zone, it is >12.1%, with a sensitivity of 88.6% and specificity of 92.0% (AUC = 0.904, 95% CI 0.772–1.0, p < 0.05). In the IPV zone, it is >3.8%, with a sensitivity of 66.7% and specificity of 90.4% (AUC = 0.893, 95% CI 0.751–1.0, p < 0.05). In the RA, it is >12.8%, with a sensitivity of 69.5% and specificity of 91.7% (AUC = 0.897, 95% CI 0.761–1.0, p < 0.05). In the BB, it is >4.6%, with a sensitivity of 77.8% and specificity of 93.2% (AUC = 0.897, 95% CI 0.764–1.0, p < 0.05).

3. Results of atrial fibrosis assessment in ESS type 3. A comparative evaluation of the percentage of fibrosis in the studied atrial zones in ET and ESS3 rats is presented in table 8.

Table 8. **Percentage of atrial fibrosis in euthyroidism (ET) and ESS3, (M±m)**

Parameter	ET. (n=42)	ESS3. (n=23)	p
Fibrosis in LA between superior PVs. %	4.4±0.147	4.2±0.216	=0.410
Fibrosis in LA between superior and inferior PVs. %	3.8±0.156	5.6±0.300	<0.001
Fibrosis in LA between inferior PVs. %	3.4±0.161	11.6±0.642	<0.001
Fibrosis in RA. %	3.9±0.159	5.9±0.425	<0.001
Fibrosis in BB. %	3.8±0.112	10.6±0.726	<0.001
Total atrial fibrosis. %	18.8±0.503	38.3±1.59	<0.001
Number of extrasystoles per 10 min	10.5±0.489	17.0±1.114	<0.001
Number of AF paroxysms per 10 min	3.2±0.212	4.2±0.286	=0.006
AF duration. s	5.6±0.463	5.7±0.377	=0.896

Note. Data are presented as arithmetic mean ± standard error of the mean (M±m). ESS3—euthyroid sick syndrome with high fT4 levels.

In the ISPV zone, the percentage of fibrosis was 23.6% higher than in the SPV zone (5.6±0.300 and 4.2±0.216, respectively, p<0.001). In the IPV zone, it was 52.2% higher than in the ISPV zone (11.6±0.642 and 5.6±0.300, respectively, p<0.001). In the RA, it was 7.7% lower than in the IPV zone (5.9±0.425 and 11.6±0.642, respectively, p<0.001). In the BB, it was 59.9% higher than in the RA (10.6±0.726 and 5.9±0.425, respectively, p<0.001).

Differences between the groups of rats with ET and ESS3 were statistically significant (p<0.05). In the ISPV zone, the percentage of fibrosis was 32.4% higher (p<0.001), in the IPV zone—69.1% higher (p<0.001), in the RA—33.9% higher (p<0.001), and in the BB—62.8% higher (p<0.001).

The results of multiple regression of AF paroxysms with the percentage of fibrosis in the studied atrial zones in ESS3 rats are presented in table 9.

The results of regression analysis indicate that the correlation coefficient R = 0.982 points to a relationship between AF frequency and the zones and percentage of atrial fibrosis. The coefficient of determination R^2 = 0.976 ≈ 97.6% determines

DOI: 10.24412/2311-1623-2026-50-57-65

Table 9. **Results of multiple regression of the number of AF paroxysms with the percentage of fibrosis in the studied atrial zones in ESS3 rats**

Parameter	Number of AF paroxysms		
Multiple correlation coefficient (R)	0.988		
Coefficient of determination (R2)	0.976		
Fisher's F test value (F)	126.3		p<0.001
Fibrosis localization / Statistical indices	β	t	p
SPV. %	-0.350	-1.262	0.226
ISPV. %	0.607	2.5	0.023
IPV. %	0.155	0.481	0.637
RA. %	0.670	2.2	0.040
BB. %	-0.092	-0.326	0.749

Note. β—regression coefficient estimate for the variable; t—Student›s ttest.

the proportion of variation in AF frequency attributable to the percentage of atrial fibrosis. The adequacy of the regression model is indicated by Fisher›s Ftest value of 126.3 and a significance level of $p < 0.001$. According to the multiple regression data, it was revealed that the predominant influence on AF frequency in ESS3 is exerted by the percentage of fibrosis in the ISPV ($\beta = 2.5$, $p = 0.023$) and in the RA ($\beta = 2.2$, $p = 0.040$).

The results of ROC analysis of the number of AF paroxysms with the percentage of fibrosis in the studied atrial zones in ESS3 rats are shown in Figure 4.

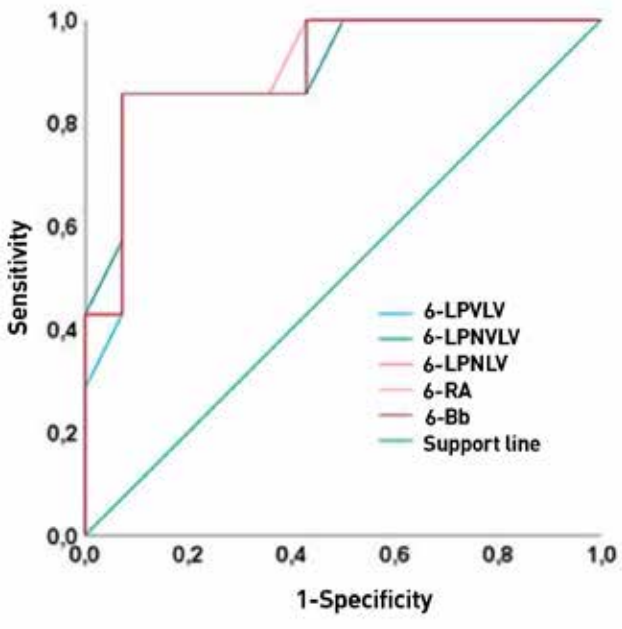

Fig. 4. ROC curves reflecting the threshold nature of the fibrosis percentage in the studied atrial zones in ESS3 rats

In the SPV zone, the threshold level of fibrosis is >4.2%, with a sensitivity of 85.7% and specificity of 78.6% (AUC = 0.903, 95% CI 0.763–1.0, $p < 0.05$). In the ISPV zone, it is >6.1%, with a sensitivity of 71.4% and specificity of 92.9% (AUC = 0.908, 95% CI 0.768–1.0, $p < 0.05$). In the IPV zone, it is >11.9%, with a sensitivity of 85.7% and specificity of 92.6% (AUC = 0.908, 95% CI 0.772–1.0, $p < 0.05$). In the RA, it is >6.4%, with a sensitivity of 85.7% and specificity of 92.1% (AUC = 0.913, 95% CI 0.783–1.0, $p < 0.05$). In the BB, it is >11.1%, with a sensitivity of 85.7% and specificity of 86.0% (AUC = 0.908, 95% CI 0.770–1.0, $p < 0.05$).

Discussion

AF is the most common cardiac arrhythmia, associated with increased cardiovascular morbidity and mortality. Its pathophysiology is based on electrical and structural remodeling occurring in the atrial myocardium [9, 10].

The thyroid gland and the heart share a common embryonic origin, with the heart being an important target organ for thyroid hormones. Various animal models and cellular studies have shown that thyroid hormones are involved in the formation of fibrosis in AF. In this article, we focused on the results of an experimental study in rats linking atrial fibrosis to AF in ESS [11].

It was noted that in both ET and ESS animals, the percentage of fibrosis in the ISPV zone was lower than in the SPV zone ($p<0.05$). In the IPV zone, the percentage of fibrosis was lower than in the ISPV zone ($p<0.05$). In the RA, the percentage of fibrosis was higher than in the IPV zone ($p<0.05$). These data suggest that the reduction in the percentage of fibrosis in the IPV zone, compared with the ISPV and RA, creates an anatomical substrate for dispersion of refractory periods and reentrant excitation in AF [12].

Regression analysis revealed that in euthyroid animals, the percentage of fibrosis in the IPV zone had the greatest influence on the frequency of AF paroxysms ($\beta = 0.734$, $p = 0.015$). Similar results have been obtained in electrophysiological studies [13]. It was noted that the effective refractory period of the atria in the IPV zone is significantly shorter ($p<0.05$) than in the ISPV zone and the RA.

Multiple regression results showed that the maximum influence on AF paroxysm frequency in ESS1 was exerted by the percentage of fibrosis in the ISPV

(p = 0.002); in ESS2, by fibrosis in the ISPV (p = 0.021) and the RA (p = 0.015); and in ESS3, by fibrosis in the ISPV (p = 0.023) and the RA (p = 0.040).

Thus, the obtained data indicate that the development of atrial fibrosis is largely responsible for the occurrence of AF in ESS. Experimental studies in rats have shown that different areas of atrial fibrosis constitute the morphological substrate for the formation of reentrant excitation circuits in AF against the background of ESS. The data also showed that the fibrosis area in ESS is twice as large as in euthyroidism.

Conclusion

Based on the experimental study, it has been shown that in ESS, fibrosis is a morphological substrate for the development of AF. The analyses performed revealed a difference in the percentage of atrial fibrosis between euthyroidism and ESS. In euthyroidism, the total percentage of atrial fibrosis was 18.8 %, whereas in ESS type 1 it was 36.6 %, in type 2—35.8 %, and in type 3—38.3 %.

Further development of both experimental models and clinical studies is required to explore the possibilities of minimally invasive diagnosis of atrial myocardial fibrosis for the early detection of the substrate for AF development and reduction of the risk of cardiovascular complications.

Conflict of interest: none declared.

References

1. Raniga D, Goda M, Hattingh L. Left atrial volume index: A predictor of atrial fibrillation recurrence following direct current cardioversion—A systematic review and meta-analysis. Int. J. Cardiol. Heart Vasc. 2024;51:101364. DOI: 10.1016/j.ijcha.2024.101364
2. Gizatulina GP, Martyanova LU, Pavlov AV, et al. Predictors of severe left atrial fibrosis in patients with non-valvular atrial fibrillation. Cardiology. 2020;60(2):47-53. Russian. DOI: 10.18087/cardio.2020.2.n850
3. Moutzouri E, Lyko C, Feller M, et al. Subclinical thyroid function and cardiovascular events in patients with atrial fibrillation. Eur J Endocrinol. 2021;185(3):375-385. DOI: 10.1530/EJE-20-1442
4. Abdu FA, Mohammed AQ, Liu L et al. Low Free Triiodothyronine as a Predictor of Poor Prognosis in Patients With Myocardial Infarction With Non-Obstructive Coronary Arteries. Front Endocrinol (Lausanne). 2021;12:681978. DOI: 10.3389/fendo.2021.681978
5. Ataoğlu HE, Ahbab S, Serez MK, et al. Prognostic significance of high free T4 and low free T3 levels in non-thyroidal illness syndrome. Eur J Intern Med. 2018;57:91-95. DOI: 10.1016/j.ejim.2018.07.018
6. Guo J, Hong Y, Wang Z, et al. Prognostic Value of Thyroid Hormone FT3 in General Patients Admitted to the Intensive Care Unit. Biomed Res Int. 2020;2020:6329548. DOI: 10.1155/2020/6329548
7. Madyanov IV, Kichigin VA. Adaptive thyroid imbalance syndrome. Definition, classification, prevalence in severe somatic diseases. Bulletin of Science and Practice. 2020;6(11):217-225. Russian. DOI: 10.33619/2414-2948/60/26
8. Takawale A, Aguilar M, Bouchrit Y, et al. Mechanisms and Management of Thyroid Disease and Atrial Fibrillation: Impact of Atrial Electrical Remodeling and Cardiac Fibrosis. Cells. 2022;11(24):4047. DOI: 10.3390/cells11244047
9. Giannakoulas G. Clinical implication of thyroid status in patients with atrial fibrillation. Heart Vessels. 2024;39(9):843-844. DOI: 10.1007/s00380-024-02376-8
10. Casis O, Echeazarra L, Sáenz-Díez B, et al. Deciphering the roles of triiodothyronine (T3) and thyroid-stimulating hormone (TSH) on cardiac electrical remodeling in clinical and experimental hypothyroidism. J Physiol Biochem. 2024;80(1):1-9. DOI: 10.1007/s13105-023-01000-z
11. Rakhmatullov RF, Dementeva RE, Sheina AE, et al. Myocardial fibrosis and atrial fibrillation in a biological model for experimental euthyroidism and thyrotoxicosis. Annals of Arrhythmology. 2025;22(2):97-105. Russian. DOI: 10.15275/annaritmol.2025.2.4
12. Gilani N, Wang K, Muncan A, et al. Triiodothyronine maintains cardiac transverse-tubule structure and function. J Mol Cell Cardiol. 2021;160:1-14. DOI: 10.1016/j.yjmcc.2021.06.010
13. Anderson JL, Jacobs V, May HT, et al. Free thyroxine within the normal reference range predicts risk of atrial fibrillation. J Cardiovasc Electrophysiol. 2020;31(1):18–29. DOI: 10.1111/jce.14183