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Research Article

Blood Flow Capacity Assessment of End-to-Side Arterial Anastomosis In Vivo in Rats

Maxim Sergeevich Staroverov[1,2] Vladimir Victorovich Krylov[1,2,3] Victor Alexandrovich Lukyanchikov[1,3,4] Egor Andreevich Orlov[5] Alexey Alexandrovich Veselkov[6] Sergey Segreevich Dydykin[7] Vladislav Dmitrievich Shatdler[1]

[1] Department of Fundamental Neurosurgery, Pirogov Russian National Research Medical University, Moscow, Russia
[2] Federal Center of Brain Research and Neurotechnologies of the FMBA, Department of Neurosurgery, Moscow, Russia
[3] Department of Neurosurgery, Sklifosovsky Scientific Research Institute of Emergency Care, Moscow, Russia
[4] Department of Neurosurgery, Research Center of Neurology, Moscow, Russia
[5] Dietrich-Bonhoeffer-Klinikum, Klinik Neurochirurgie, Neubrandenburg, Germany
[6] Department of Neurosurgery, Burdenko Neurosurgery Institute, Moscow, Russia
[7] Department of Topographic Anatomy and Operative Surgery, Sechenov University, Moscow, Russia

Address for correspondence Maxim Sergeevich Staroverov, MD, Department of Fundamental Neurosurgery, Pirogov Russian National Research Medical University, Ostrovityanova Street No. 1, B6, Moscow, Russia 117997 (e-mail: staroverov1995@gmail.com).

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Abstract

Introduction The aim of this article was to assess the flow capacity of end-to-side arterial anastomosis depending on the method of its implementation.

Materials and Methods The study was conducted on 30 live Wistar rats in vivo, which were randomly divided into three groups. In each group of animals, an end-to-side microanastamosis was performed using three methods of donor artery preparation: 45 degrees (group A), 90 degrees (group B), and arteriotomy according to the "fish mouth" type (group C). The determination of flow capacity of anastomosis by measuring the blood volume flow with transonic flowmeter was performed.

Results The obtained average values after the anastomosis were, respectively, 7.335 mL/s (standard deviation [SD]: 2.0771; min: 4.05; max: 10.85), 7.36 mL/s (SD: 0.836 mi: 6.15; max: 8.75), and 6.37 mL/s (SD: 1.247; min: 5.05; max: 9.05). No statistically significant difference in the blood volume flow velocity between all types of anastomoses was obtained ($p = 0.251$).

Conclusion The flow capacity of end-to-side arterial anastomosis does not depend on the chosen method of anastomosis.

Keywords
- flow capacity of end-to-side arterial anastomosis
- blood volume flow velocity
- cerebral revascularization
- arteriotomy in end-to-side anastomosis
- end-to-side anastomosis

Introduction

The use of microarterial anastomoses in neurosurgery has been known since 1967, when Yaşargil first used microanastomosis for the purpose of revascularization of the brain. Yaşargil was the first to introduce several methods, the choice of which was due to the difference in the calibers of the anastomosed vessels. With an approximate equality of diameters, it was necessary to perform an anastomosis at an angle of 90 degrees; if the diameters did not match, the anastomosis was made at an angle

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of 45 to 90 degrees, while the more the diameters did not match, the longer the arteriotomy should be performed.[1] Over time and the development of microsurgery and microneurosurgery, the methods of performing microvascular anastomoses have been improved and modified.[2–7] At present time, leading neurosurgeons specializing in brain revascularization use arteriotomy to perform end-to-side microarterial anastomosis of donor artery of fish-mouth type.[5,8–11]

The mathematical confirmation of the high efficiency of fish-mouth method was presented by Abdulrauf, who studied the geometric dependence of the anastomosis surface area on the method of arteriotomy on a donor artery.[8] When a donor is incised using a fish-mouth method, the anastomosis cross-sectional area increases four times as compared with that when a donor is incised at a 90-degree angle, while the length of the arteriotomy on the recipient doubles. However, clinical and instrumental confirmation of this mathematical hypothesis has not been described in the available world literature.

Study Objective

The aim of this article was to assess the flow capacity of end-to-side arterial anastomosis depending on the method of donor preparation.

Materials and Methods

All experiments were performed according to the main ethical principles of biomedical experiments on animals, as well as the rules of the Sechenov First Moscow State Medical University Ethics Committee.

The study was conducted in vivo on 30 Wistar rats with an average weight of 309.1 g. (standard deviation [SD]: 17.52; min: 300, max: 355). All of them underwent surgery. Each rat underwent median cervical approach with the mobilization of both common carotid arteries (►**Fig. 1A, B**). After mobilization of the vessels, the values of the blood volume flow velocity in both intact arteries ($V_{f\ int.}$) were registered (►**Fig. 1C**). The determination of flow capacity of anastomosis was performed by measuring the volumetric blood flow velocity with Transonic flowmeter, 1 and 1.5 mm probes.

After mobilization, the recipient artery (one of the carotid arteries) was clipped from the proximal and distal ends. The donor artery (another carotid artery) was clipped from the proximal end; its distal end was ligated directly proximal to the carotid bifurcation. Then a distal transection of the donor artery was made. The preparation of the anastomosing end was performed depending on the chosen method. Three methods for preparing a donor artery (respectively, the laboratory animals were divided into three groups, each included 10 rats) were used: the first group underwent the arteriotomy at the angle of 90 degrees (group A), the second group underwent the fish-mouth arteriotomy (group B), and the third group underwent the arteriotomy at the angle of 45 to 60 degrees (group C). After the arteriotomy on the recipient, the vascular end-to-side anastomosis was performed according to the diameter of the prepared end of the donor (►**Fig. 2**).

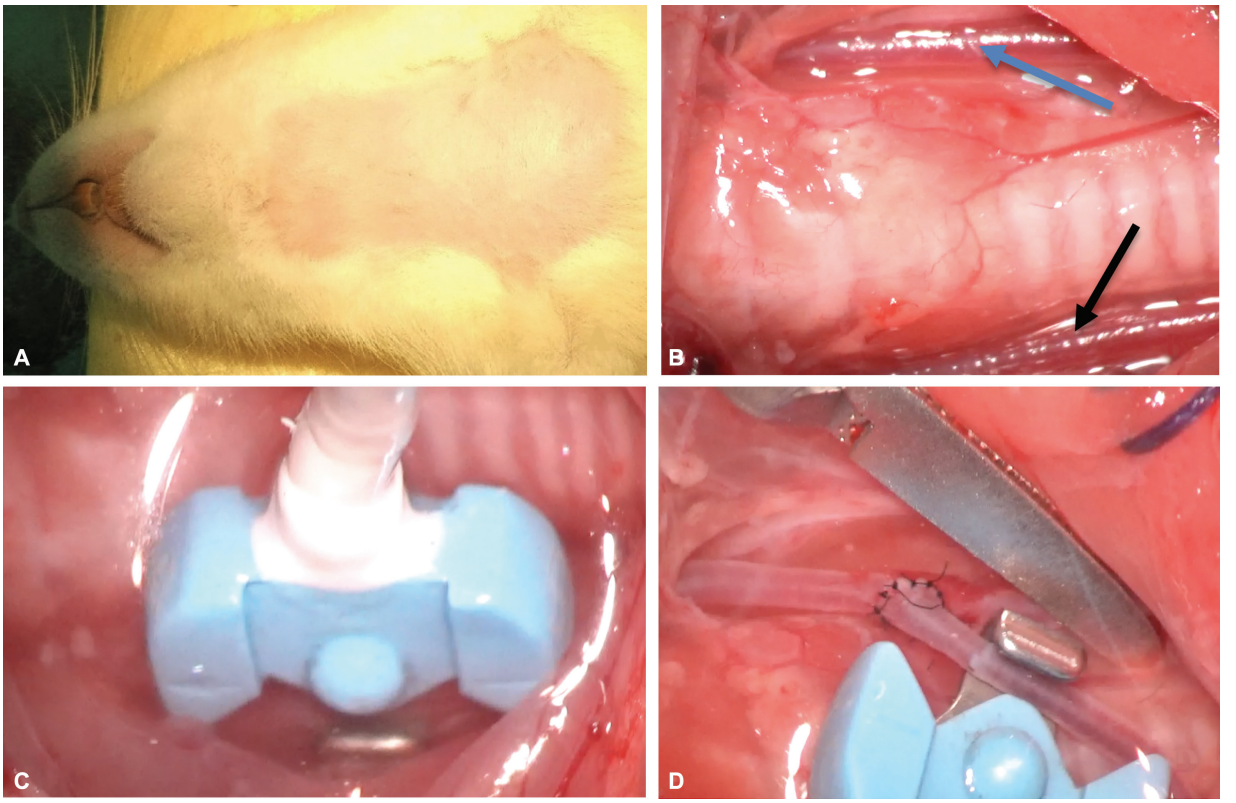

Fig. 1 (**A**) Operational field prepared for work, Wistar rat; (**B**) mobilized carotid arteries: right (blue arrow) and left (black arrow); (**C**) measurement of blood volume flow velocity in the intact an artery; (**D**) measurement of blood volume flow velocity in the donor artery after anastomosis.

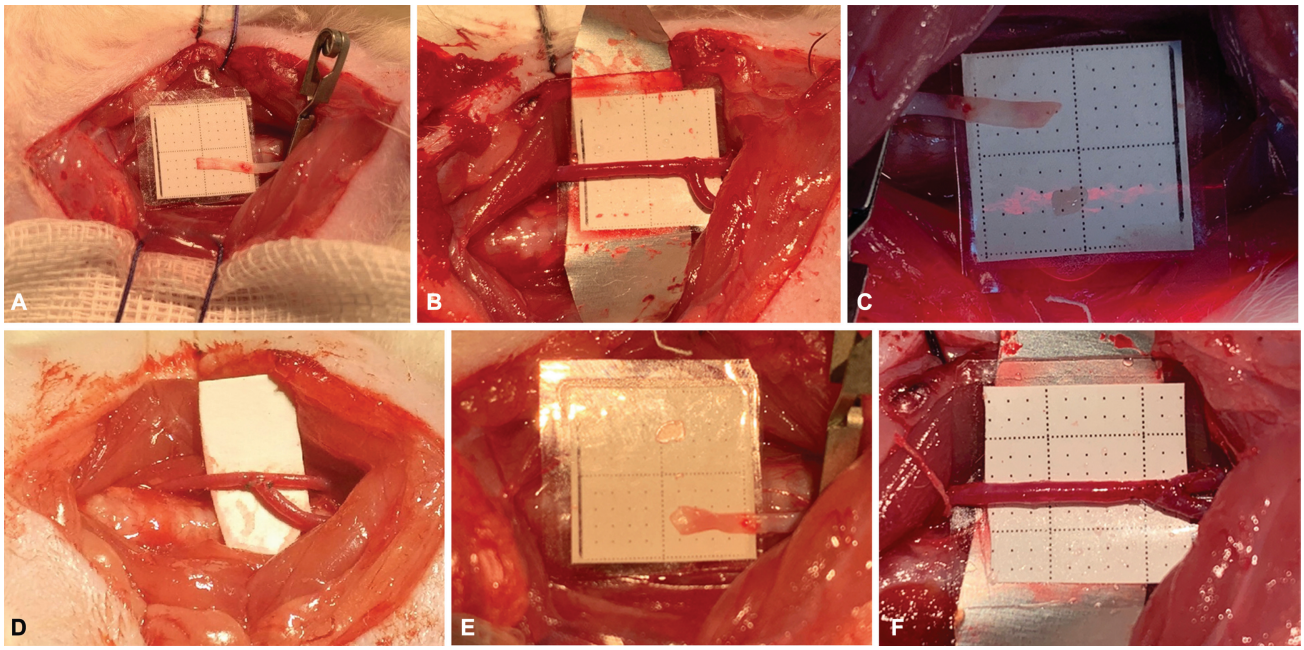

Fig. 2 (**A**) The donor artery prepared for the anastomosis at the angle of 90 degrees; (**B**) the anastomosis performed at the angle of 90 degrees; (**C**) the donor artery prepared for the anastomosis at the angle of 45 to 60 degrees; (**D**) the anastomosis performed at the angle of 45 to 60 degrees; (**E**) the donor artery prepared using the fish mouth method; (**F**) the anastomosis performed according to the method of fish mouth.

Table 1 Brown–Forsythe test to determine the equality of variances

Variable	Brown–Forsythe test of homogeneous variances							
	SS effect	df effect	MS effect	SS error	df error	MS error	F	*p*-Value
Data	4.446167	2	2.223083	23.3610	27	0.865222	2.569378	0.095174

Abbreviations: MS, mean square; SS, sum of squares.

After performing the anastomosis, V_f was measured in the donor artery with the occluded and open proximal end of the recipient artery (►**Fig. 1D**). The V_f in the donor artery with the occluded proximal end of the recipient represents the blood flow coming through the anastomosis exclusively from the donor artery. After completing the anastomosis, due to the retrograde blood flow entering the proximal end of the recipient from the donor artery, which could affect the final volume of blood flow, the proximal end of the recipient artery was occluded before measuring the volume flow, and then the blood volume flow was measured in the donor artery (►**Fig. 1D**).

The anastomosis patency was controlled by a visual inspection, Acland's test, ultrasound Doppler flowmetry. In case of insufficiency or suspected insufficiency of the anastomosis, the animal was withdrawn from the study. There were two failed anastomoses in the study (1 in group B, 1 in group C) due to the arterial thrombosis. The absence of changes in hemodynamic parameters, such as arterial spasm, changes in blood pressure, which could affect the reliability of the results, was checked by measuring V_f in the recipient artery, while the donor artery was turned off from the anastomosis. The data obtained were compared with V_f in the recipient before the anastomosis.

During the obtained data analysis, general scientific methods of generalization, statistical analysis (using certified statistical processing programs), and tabular and graphical methods of data presentation were used. To determine the difference between the volumetric blood flow velocities in the three groups after the anastomosis, one-way analysis of variance was used. A normal distribution of variances was observed in the group (►**Table 1**).

Results

V_f in the donor artery with the active proximal end of the recipient in 100% of cases in all three groups was 0.2 to 0.4 mL/s. This fact indicates that the donor artery does not function with active magistral blood flow through the recipient artery, which was also confirmed by the presence of complete thrombosis of the donor artery during revision of the anastomosis 1 week after the intervention.

All of the study groups had a normal distribution according to the characteristics of number of sutures and clamp time. The mean number of sutures was (N): 7,7 in group A (SD: 0.67; min: 6; max: 9); 12.9 in group B (SD: 1.1; min: 8; max: 9), 10,0 in group C (SD: 1.15; min: 8; max: 12).

The mean clamp time (minutes) was 17.2 in group A (SD: 2.35; min: 15; max: 22); 23.2 in group B (SD: 2.09; min: 20; max: 26); 20.7 in group B (SD: 1.56; min: 19; max: 24).

Average $V_{f\ int.}$ for group A (the anastomosis at the angle of 90 degrees) was 7.725 mL/min (SD: 1.879; min: 4.25; max: 10.9) for a donor artery and 8.525 mL/min (SD: 2.2709; min: 5.35; max: 12.3) in the recipient artery. The arteriotomy on the recipient artery was equal to one and a half diameters of the donor artery. Average V_f for the donor artery after the anastomosis was 7.335 mL/s (SD: 2.0771; min: 4.05; max: 10.85).

Average $V_{f\ int.}$ for group B (the anastomosis was performed according to the "fish-mouth" method) was 7.945 mL/s (SD: 1; min: 6.35; max: 10) for the donor artery and 8.14 mL/s (SD: 1.0476; min: 6.7; max: 9.85) in the recipient artery. The arteriotomy on the recipient artery was equal to the three diameters of the donor artery. Average V_f for the donor artery after the anastomosis was 7.36 mL/s (SD: 0.836 min: 6.15; max: 8.75).

Average $V_{f\ int.}$ for group C (the anastomosis at an angle of 45–60 degrees) was 6.92 (SD: 1.645 min: 5.1; max: 10) mL/s for the donor artery and 8.065 mL/s (SD: 1.292; min: 6.35; max: 10.9) in the recipient artery. The arteriotomy on the recipient artery was equal to one and a half diameters of the donor artery. Average V_f for the donor artery after the anastomosis was 6.37 mL/s (SD: 1.247; min: 5.05; max: 9.05).

Thus, in all of study groups, a normal distribution was observed according to the characteristics of intact blood volume flow (►**Fig. 3**).

During the analysis, it was assumed as the null hypothesis that the type of anastomosis does not affect the blood volume flow. The significance level in the analysis was 0.25114 ($p = 0.25114$); therefore, no statistically significant differences between the methods were detected (►**Fig. 4**).

It should be noted that the blood flow in the recipient after anastomosis did not increase above the initial one, regardless of the abilities of the donor and did not exceed the initial speed parameters for the intact recipient. To assess the efficiency and long-term performance of the anastomosis, we proposed the coefficient "*effective potential of anastomosis*" (EPA), which is calculated according to the formula:

$$EPA = \frac{\text{Vf donor after anastomosis}}{\text{Vf intact donor}} \times 100\%$$

The anastomosis potential was calculated as the ratio of the blood volume flow in the donor artery after the anastomosis to $V_{f\ int.\ donor}$. EPA reflects the maximum volumetric blood flow that can be provided by this type of anastomosis in points from 0 to 1 (0% to 100%), where the highest score is taken as one.

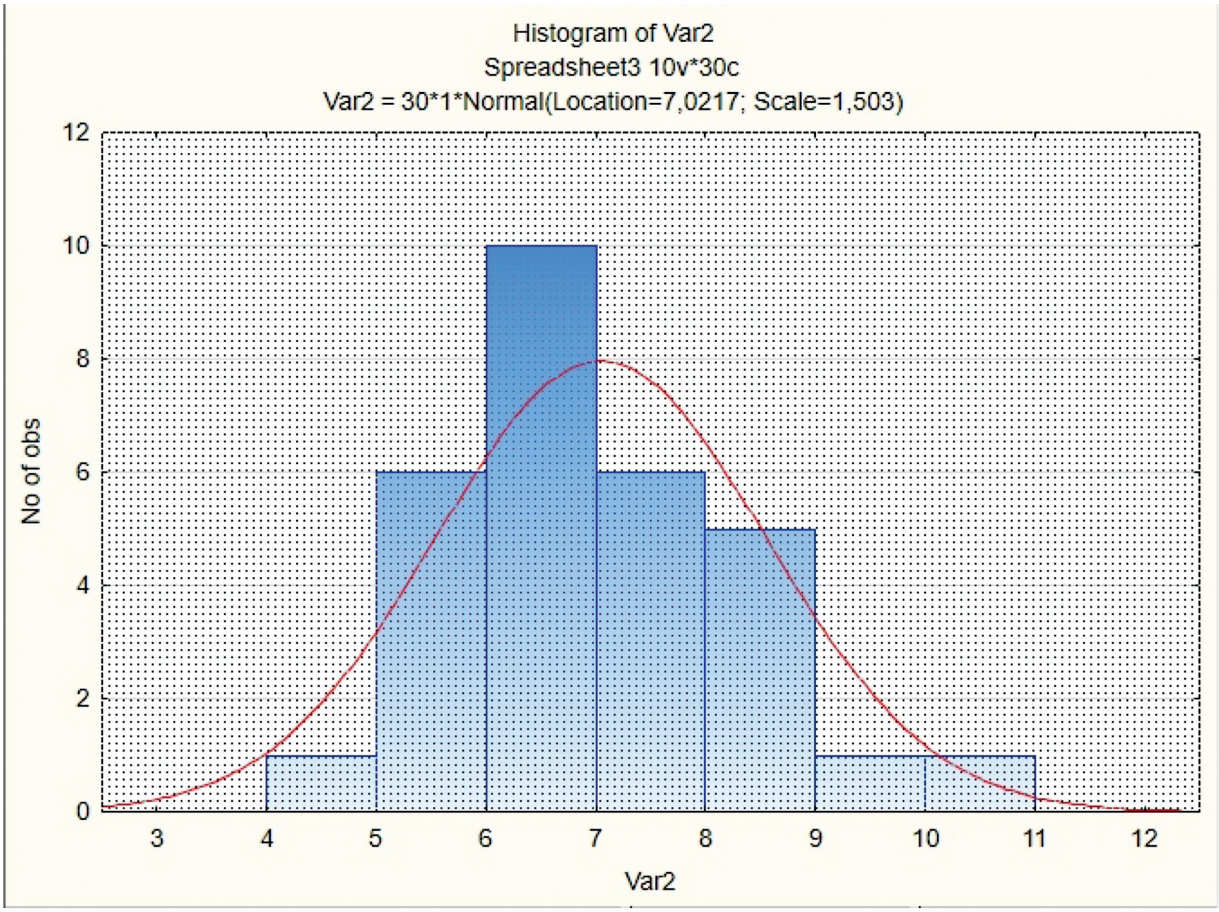

Fig. 3 Distribution according to characteristics of intact blood flow velocity.

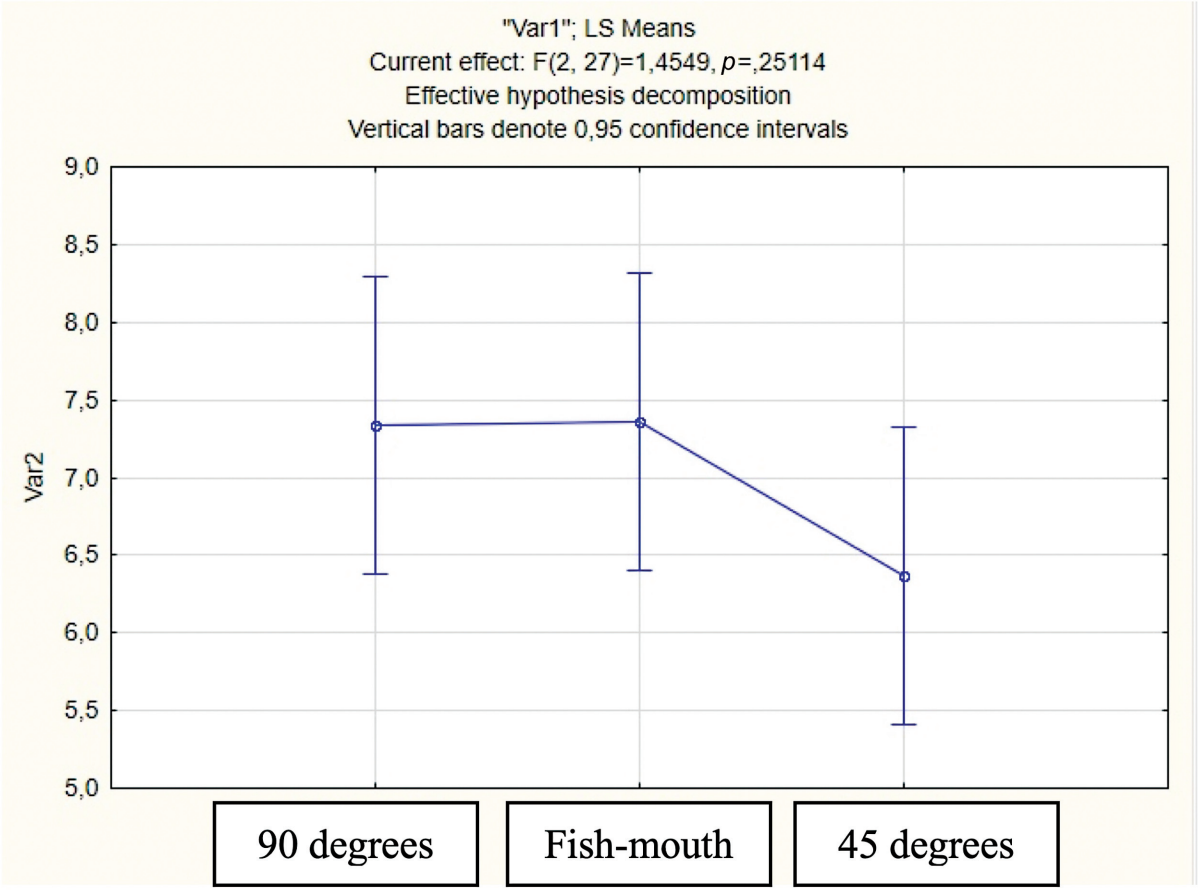

Fig. 4 Testing the hypothesis about the influence of the type of anastomosis on its flow capacity.

In the groups of animals, a normal distribution was observed according to the characteristics: $V_{f\ int.\ recipient}$ and $V_{f\ int.\ donor}$ (►**Fig. 5A** and **B**, respectively).

Thus, in the study group, the normal distribution was observed according to the characteristics of "EPA" (►**Fig. 5B**, ►**Table 2**). $V_{f\ int.\ donor}$ was taken as the potential basis. $EPA_{av.\ for\ 90\ degrees} = 94.298$ (mean square deviation—6.8067, standard error: 2.152; min: 77.70; max: 100). $EPA_{av.\ for\ fish\ mouth} = 94.877$ (mean square deviation—4.824; standard error—1.525; min—83.33; max: 98.10). $EPA_{av.\ for\ 45–60\ degrees} = 93.073$ (mean square deviation—8.05063; standard error —2.545; min—72.50; max: 100).

The proposed EPA was used to determine the relation between the flow capacity of the anastomosis (volumetric blood flow velocity) and the type of anastomosis. The equality of variances was observed in the group (Levene test, ►**Table 2**).

During the analysis, it was accepted for the null hypothesis that the EPA varies depending on the donor preparation method. The significance level during the analysis was $p = 0.877$; therefore, statistically significant differences between the methods could not be found (►**Table 3**, ►**Fig. 6**).

Discussion

The use of microvascular anastomoses is an the most common option of brain revascularization. The indications for revascularization operations of this kind are quite numerous: chronic and acute brain ischemia; safety anastomosis during clipping aneurysms, removal of tumors; interoperative difficulties: thrombosis of large cerebral arteries, etc.[10,12,13] However, the question remains unresolved: which of the methods of vascular anastomosis will ensure the best functioning of the anastomosis and therefore the best neurological outcomes.

In most cases, the surgeon is forced to apply the anastomosis with a difference in the diameter of the vessels. Also, the surgeon is often placed in the condition of time pressure, for example, during the temporary occlusion of the proximal segments of intracranial arteries while making the intra-intracranial bypasses (in situ) or artery reimplantation. In such situations, in our opinion, the application of anastomosis with the fish-mouth donor preparation has several disadvantages. Obviously, the surgeon spends more time preparing the donor artery using this method, which in some situations can be critical. In addition, the excessive angle of the anastomosis casts doubts on the adequate hemodynamics in the area of the anastomosis angle that implies the possibility of inadequate perfusion of distally located tissues, and also increases the possibility of anastomosis dehiscence and the occurrence of hemodynamic aneurysms. It is worth emphasizing that changing the angle to a sharper one was used as a compensatory mechanism when the diameters of the anastomosed vessels did not match to create adequate hemodynamics. In our study,

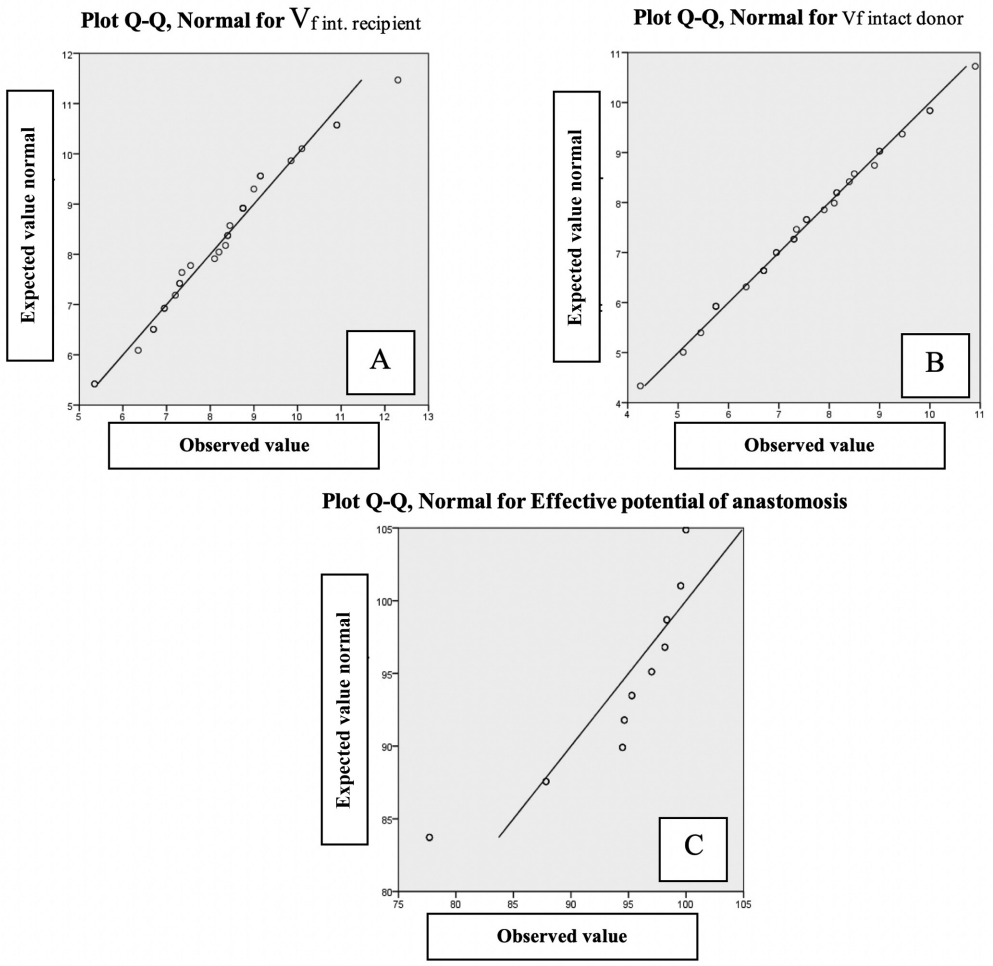

Fig. 5 (A) Normal probability plot for V_f; (B) normal probability plot for $V_{f\ int.\ donor}$; (C) normal probability plot for effective potential of anastomosis.

Table 2 Testing homogeneity of variance for effective potential of anastomosis

Effective potential of anastomosis			
Levene test	df1	df2	Significance (p)
0.202	2	27	0.818

there was no significant difference when applying anastomoses at the angle of 45 to 60 and in the fish-mouth type, relying on this fact, the surgeon has more degrees of freedom for interoperative decisions, taking into account the current potential of the anastomosis.

In addition, at the moment there is no an adequate predictive intraoperation method to assess the quality of the applied anastomosis. The blood flow measurement methods: indocyanine green, contact dopplerography and flowmetry provide only in situ measurement parameters. However, there is a cut-flow index, which, according to the authors' idea, should assess the delayed work of the anastomosis.[14] However, the method itself implies the absence of vascular resistance at one moment of measurement; moreover, the measurement takes place with direct aspiration from the blood vessel, which creates additional force and should increase blood flow. In addition, the measurement is made after the application of the vessel with Papaverine, which initially creates an implausible situation of maximum

Table 3 One-way analysis of variance (ANOVA) for criterion "effective potential of anastomosis"

ANOVA					
	Sum of squares	df	Mean square	F	Significance
Between groups	11.861	2	5.930	0.132	0.877
Within groups	1209.784	27	44.807		
Total	1221.645	29			

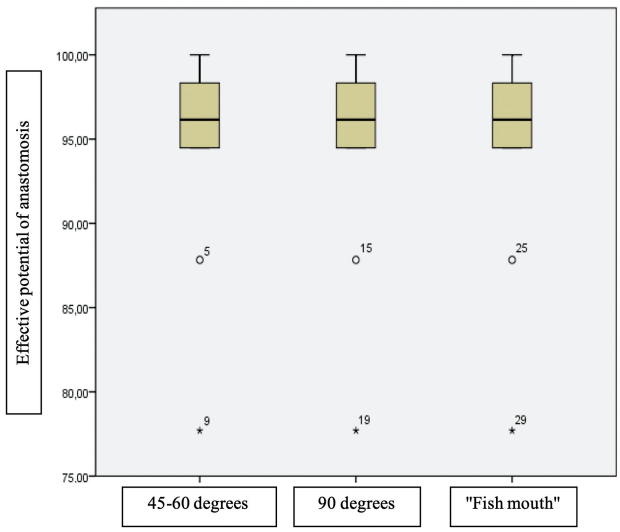

Fig. 6 Box plot for the current potential of the anastomosis depending on the type of donor preparation.

donor performance, despite the fact that the majority of patients in the study were with stenosis (which means that there was a blood flow in the recipient). So, in this index, the following moment is completely omitted: how much blood the recipient can take in after the anastomosis. This fact is critical, because, for example, in our study, in the absence of ligation of the intact proximal end of the recipient, a hemodynamic conflict appeared and the anastomosis was thrombosed in the delayed manner. In our opinion, the EPA could become this indicator, but this idea requires further studies.

Conclusion

There were no statistically significant differences in the volumetric blood flow velocity through the vessels after the micro-anastomoses using three different techniques of preparation of donor artery and recipient arteriotomy was performed.

Note

All experiments were performed according to the main ethical principles of biomedical experiments on animals, as well as the rules of the Sechenov First Moscow State Medical University Ethics Committee.

Authors' Contributions

V.V.K. and S.S.D contributed to the editing of manuscript. V.A.L. designed the study. M.S.S. and E.A.O. conducted the experiment and helped in literature review. A.A.V. and V.D.S. helped in conducting the experiment.

Ethical Approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Funding

None.

Conflict of Interest

None declared.

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