Supplemental Material

Exploring the relationship between embolic acute stroke distribution and supra-aortic vessel patency: Key findings from an in vitro model study

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Supplemental Material

Materials and Methods

Clot production

Fresh venous blood was collected from sheep for thrombus analog preparation (Ash Stream Ltd, Hollymount, Ireland). The blood was mixed with ACPD (adenine citrate phosphate dextrose) anticoagulant solution on collection. All thrombus analogs were prepared within 5 hours of blood collection (Cerenovus, Galway, Ireland). The blood was centrifuged at 320 g for 10 min to form a top layer of platelet-rich plasma (PRP). After isolation of the PRP, the remaining blood mixture was centrifuged again at 1300 g to separate the remaining plasma, buffy coat, and RBC. The buffy layer and plasma were discarded to leave the RBC beneath. Platelet-contracted thrombus analogs were formed by mixing the PRP fraction and the RBC in controlled ratios and then mixing in 2.06% calcium chloride solution at a ratio of calcium chloride to plasma of 1:9. The calcium chloride reversed the anticoagulant and began the clotting process. Four blood mixtures with hematocrits (HCT) of 70, 75, 80, and 84% were created in this way and transformed into four different thrombus analogs in cylindrical molds. Molds with a diameter of 5 and 6 mm were used for each type of thrombus analogue. The blood mixtures were incubated at 37 °C for 1 hour, during which time the blood coagulated and started to contract. The analogs were further matured overnight at ambient temperature to allow maximum contraction and let the serum separate out. After the contraction, the diameter of the clots was about 3 - 4 mm. Contraction was checked and confirmed gravimetrically by weighing the solid and liquid phases in the clot molds. Clot analogs were preserved in their own serum at 3°C until use. The clots were 10 to 15 cm long when unmolded and cut into a length of 1 cm before each injection. Experiments were carried out within 24 hours of clot production.

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Table S1 Fragmentation of red blood cell (RBC)-rich clots by batch.

	Batch				
Molds diameter*	84% HCT 6 mm	80% HCT 5 mm	80% HCT 6 mm	75% HCT 5 mm	70% HCT 5 mm
Number of fragments**					
Median	5	4	5	4	4
IQR	(4 - 7)	(3 - 5)	(4 - 7)	(3 - 5)	(3 - 5)
Min – max	2 - 7	1 - 8	2 - 10	2 - 6	2 - 6

*Molds of two different diameters were used (See Material and Methods). **Macroscopic emboli that anchored in an intracranial vessel. HCT: Hematocrit; IQR: Interquartile range. Bonferroni corrected p-values from pairwise comparison of the distribution of the number of fragments by batch were not indicative of any systematic differences.

Table S2 Sequence of embolus lodges for 406 total emboli in the four anatomical conditions of the supra-aortic vessels tested

	Lodge number								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	Total
Total fragments	104	103	90	61	28	14	5	1	406
	Emboli lodged ipsilateral to clot injection								
MCA: M1 segment	71 (68.3)	18 (17.5)	12 (13.3)	-	-	-	-	1 (100)	102
MCA: M2 segment	26 (25)	15 (14.6)	5 (5.6)	-	-	-	-	-	46
MCA: M3 segment	3 (2.9)	5 (4.9)	2 (2.2)	1 (1.6)	-	-	-	-	11
Terminal ICA	3 (2.9)	5 (4.9)	3 (3.3)	-	-	-	-	-	11
Floating ICA thrombus	-	3 (2.9)	5 (5.6)	5 (8.2)	1 (3.6)	1 (7.1)	1 (20)	-	16
ACA: A1 segment	-	16 (15.5)	21 (23.3)	13 (21.3)	1 (3.6)	1 (7.1)	-	-	52
ACA: A2 segment	-	21 (20.4)	9 (10)	10 (16.4)	4 (14.3)	2 (14.3)	1 (20)	-	47
PCA	1 (1)	11 (10.7)	20 (22.2)	12 (19.7)	9 (32.1)	3 (21.4)	-	-	56
	Emboli lodged contralateral to clot injection								
MCA: M1 segment	-	-	-	1 (1.6)	-	-	-	-	1
MCA: M2 segment	-	2 (1.9)	6 (6.7)	5 (8.2)	1 (3.6)	5 (35.7)	-	-	19
MCA: M3 segment	-	-	1 (1.1)	4 (6.6)	5 (18)	1 (7.1)	2 (40)	-	13
ACA: A1 segment	-	1 (0.9)	-	-	-	-	1 (20)	-	2
ACA: A2 segment	-	4 (3.9)	4 (4.4)	7 (11.5)	6 (21.4)	1 (7.1)	-	-	22
ACoA	-	2 (1.9)	1 (1.1)	3 (5)	-	-	-	-	6
PCA	-	-	1 (1.1)	-	1 (3.6)	-	-	-	2

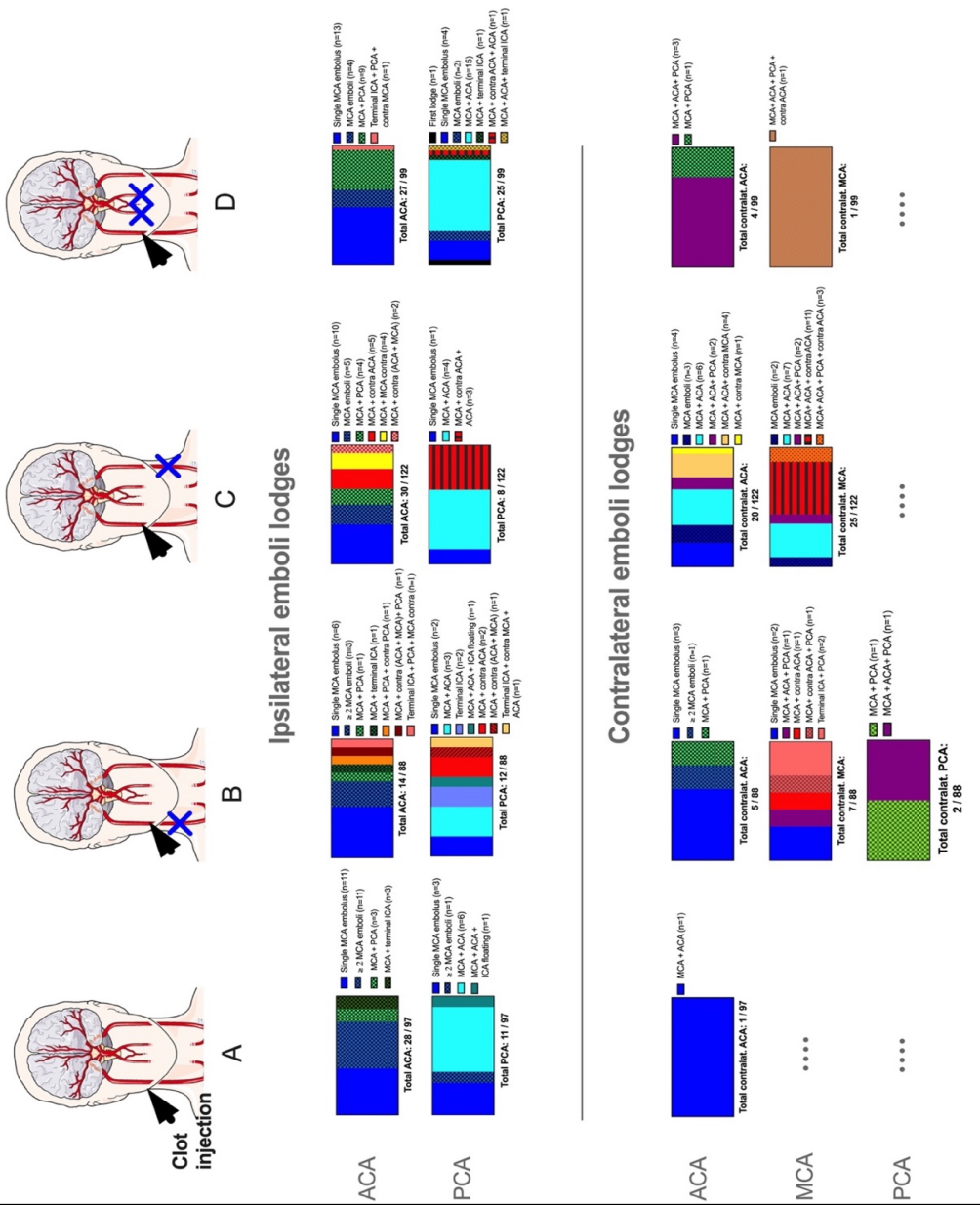
Data in parentheses are percentages. MCA: middle cerebral artery; M1, M2, and M3 segments of the MCA; ICA: internal carotid artery; ACA: anterior cerebral artery; A1 and A2 segments of the ACA; ACoA: Anterior communicating artery; PCA: posterior cerebral artery.

Table S3 Sequence of anchoring of the 406 emboli by the anatomical supra-aortic vessel conditions tested.

Sequential embolus locations	Total (n = 406)	**Patent SAV (n = 97)**	**Contralateral CA occluded (n = 122)**	**Ipsilateral CA occluded (n = 88)**	**Vertebral arteries occluded (n = 99)**
First lodge	104	29	24	25	26
MCA	100	28	24	23	25
Terminal ICA	3	1	-	2	-
Ipsilat. PCA	1	-	-	-	1
Second lodge	103	29	24	24	26
MCA	38	14	9	6	9
ACA	37	10	10	5	12
PCA	11	2	1	4	4
Contralat. ACA	7	-	4	3	-
Terminal ICA	5	3	-	1	1
Floating ICA thrombus	3	-	-	3	-
Contralat. MCA	2	-	-	2	-
Third lodge	90	23	24	18	25
ACA	30	11	9	4	6
PCA	20	5	2	2	11
MCA	19	5	4	4	6
Contralat. MCA	7	-	5	2	-
Contralat. ACA	5	-	4	-	1
Floating ICA thrombus	5	2	-	3	-
Terminal ICA	3	-	-	2	1
Contralat. PCA	1	-	-	1	-
Fourth lodge	61	15	23	11	12
ACA	23	7	6	3	7
PCA	12	4	2	2	4
Contralat. MCA	10	0	7	3	0
Contralat. **ACA**	10	1	7	1	1
Floating ICA thrombus	5	3	0	2	0
MCA	1	-	1	-	-
Fifth lodge	28	1	14	6	7
PCA	9	-	2	3	4
Contralat. MCA	6	-	6	-	-
Contralat. ACA	6	-	4	-	2
ACA	5	-	2	2	1
Floating ICA thrombus	1	1	-	-	-
Contralat. PCA	1	-	-	1	-
Sixth lodge	14	-	8	3	3
Contralat. MCA	6	-	5	-	1
ACA	3	-	2	-	1
PCA	3	-	1	1	1
Floating ICA thrombus	1	-	-	1	-
Contralat. ACA	1	-	-	1	-
Seventh lodge	5	-	4	1	-
Contralat. MCA	2	-	2	-	-
ACA	1	-	1	-	-
Floating ICA thrombus	1	-	-	1	-
Contralat. ACA	1	-	1	-	-
Eighth lodge	1	-	1	-	-
MCA	1	-	1	-	-

Unless otherwise indicated, embolus location refers to the side ipsilateral to clot injection (right carotid artery). The contralateral anterior cerebral artery (ACA) included emboli entrapped in the anterior communicating artery (ACoA). Contralat: contralateral; Ipsilat: ipsilateral; MCA: middle cerebral artery; CA: carotid artery; PCA: posterior cerebral artery.

Figure S1. *Embolus lodges prior to an ipsilateral ACA or PCA embolism for each SAV condition.*



At top, the four supra-aortic vessel conditions tested **A**: baseline, all SAV patent during embolism; **B:** embolic shower from a carotid occlusion; **C:** embolic shower contralateral to a carotid artery occlusion; and **D:** embolic shower during occlusion of both vertebral arteries. Below, the corresponding embolus lodge history for each SAV condition. These histories show the combination of embolus locations that preceded a specific ipsilateral or contralateral event. In parentheses, the number of emboli observed for each combination. MCA: middle cerebral artery; ACA: anterior cerebral artery; PCA: posterior cerebral artery; contralat: contralateral.