SUPPLEMENTAL MATERIAL

Supplemental Methods

Inclusion/Exclusion Criteria

Inclusion criteria were diagnosis of a ruptured or unruptured brain aneurysm for which microsurgical clipping was performed in consenting patients between 18 and 79 years of age. Exclusion criteria included traumatic or mycotic aneurysms; aneurysms in patients with adult polycystic kidney disease; patients with concurrent degenerative connective tissue disorders; aneurysms associated with arteriovenous malformations; and patients with aneurysms that were not treated with surgical clipping as part of their standard of care during the hospitalization.

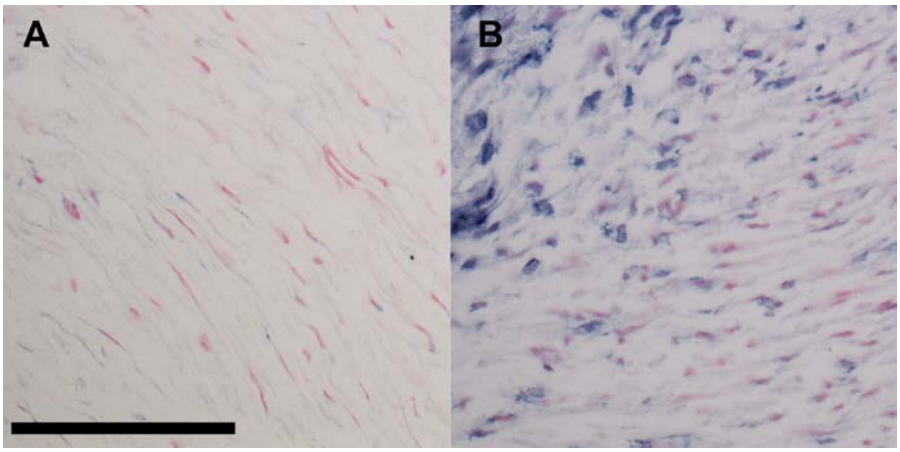
Histology Protocol

Tissue specimens were sectioned 8 µm thick, fixed with acetone, and blocked in 5% bovine serum, 10 mg/ml BSA in 50 mM Tris, 100 mM NaCl (TBS), pH 7.5 for 2 h. The staining of human MPO and macrophage (anti-calprotectin) anitgens was performed using the corresponding mouse anti-human monoclonal antibodies (2C7 and MAC387, dilution: 1:100, AbCam, Cambridge MA). Mouse monoclonal antibody binding was detected using anti-mouse monoclonal alkaline phosphatase-conjugated secondary antibody staining with subsequent NBT/BCIP colorimetric detection (Roche Applied Science, Indianapolis IN). Sections were counter-stained with nuclear fast red. Three non-consecutive sections were examined using light microscopy. The collected RGB JPEG images (magnification: 30x) were color split and the corresponding blue channel images were segmented using IP Lab Spectrum software (BD Biosciences Bioimaging, Rockville MD). The MPO staining was considered positive if > 5 alkaline-phosphatase positive cells were present in at least one field with an area of 400 μm^2. Representative images are shown in Supplemental Figure I.

When the aneurysm specimens were sufficiently large, they were carefully cut in half along the long axis of the dome for both histology and MPO activity analysis. The analysis of MPO activity

was performed by using a commercially available MPO kit (Fluoro MPO, Cell Technology Inc., Mountain View CA). A typical assay included homogenization of 1-10 mg of saline-rinsed, rapidly defrosted tissue in a vial containing a slurry of 300 mg sterile glass beads (1mm diameter) suspended in 0.5 ml of 0.5% solution of hexadecyltrimethylammonium bromide, 10mM N-ethylmaleimide in 0.1 M potassium phosphate, pH 6.5. The homogenization was performed using a mini-BeadBeater (BioSpec Products, Inc. Bartlesville OK) 12 cycles (30 s each) with 1 min cooling on ice in between the cycles. The final disruption was performed by using three freeze-thaw cycles. The samples were cleared by centrifugation (8000xg, 5min) and the activity of MPO was determined in the supernatant using a fluorescent analog of MPO substrate in the presence of hydrogen peroxide. The rates of fluorescence increase was determined using a kinetic assay (λ_{ex}= 550 nm, λ_{em}= 600nm) of an MPO standard solution (Cell Technology Inc.) to generate calibration curves. The protein content in the homogenized samples was determined by using BCA kit (Bio-Rad Inc, Hercules CA).

Supplemental Figure



Supplemental Figure I. A- a representative MPO- negative field, B- an MPO-positive field. Bar=100 µm

Supplemental Results

Supplemental Table I – Results of human aneurysm tissue histology

An No	Pt No	Age (years)	Sex	Rup-tured	Location	D (mm)	Familial SAH	HTN	Aneurysm Morphology	MPO	ARR (%)
1	1	56	M	N	ICA Terminus	7	N	Y	Berry	N	0.76
2	2	50	F	N	MCA	5	N	Y	Berry	N	0.62
3	3	54	F	N	MCA	11	Y	N	Irregular*	Y	2.49
4	4	71	F	N	ACA	9	Y	Y	Berry*	Y	3.38
5	5	44	F	N	MCA	4	N	N	Berry	N	0.46
6	6	57	F	N	AComm	4	N	Y	Berry	N	1.05
7	7	54	F	N	ICA Terminus	1	N	Y	Berry	N	0.33
8	7	54	F	N	MCA	5	N	Y	Berry	N	0.62
9	8	73	F	N	MCA	20	N	Y	Berry	Y	17.07
10	9	67	M	Y	MCA	20	Unk	Y	Berry	Y	-
11	10	54	F	N	MCA	7	Y	Y	Irregular	Y	1.40
12	11	68	M	N	MCA	9	N	Y	Berry**	N	2.02
13	12	75	F	Y	MCA	7	Y	Y	Berry	Y	-
14	13	52	M	N	PComm	8	Unk	N	Berry	Y	2.07
15	14	63	F	N	MCA	8	Unk	Y	Irregular	Y	1.40
16	15	50	F	N	MCA	14	N	N	Berry	Y	2.49
17	15	50	F	N	PComm	3	N	N	Berry	N	0.92
18	16	29	F	N	MCA	14	N	N	Berry	N	2.49
19	16	29	F	Y	PComm	5	N	N	Berry	Y	-
20	17	46	F	N	MCA	10	N	Y	Irregular	Y	3.35
21	18	44	F	N	MCA	6	N	N	Berry	N	0.46
22	19	40	M	N	MCA	9	N	Y	Irregular	Y	1.40
23	19	40	M	N	ATA	3	N	Y	Berry	Y	0.62

Abbreviations: D = diameter, SAH = subarachnoid hemorrhage, HTN = hypertension, MPO= myeloperoxidase, ARR=5-year aneurysm rupture risk (for unruptured aneurysms) estimated using PHASES model, ICA=internal carotid artery, ACA=anterior cerebral artery, AComm=anterior communicating artery, MCA=middle cerebral artery, PComm=posterior communicating artery, Unk=unknown, ATA = anterior temporal artery; Unk=Unkown; * -

indicates documented aneurysm growth during observation; ** indicates patient had prior history of SAH.