Neurosurgery

Characteristics of unruptured compared to ruptured intracranial aneurysms: a multicentre case-control study

--Manuscript Draft--

Manuscript Number:	NEU-D-16-01275R2
Article Type:	Research-Human-Clinical Studies
Section/Category:	Cerebrovascular
Corresponding Author:	Isabel Charlotte Hostettler, M.D. University College London Institute of Neurology London, London UNITED KINGDOM
Order of Authors:	Isabel Charlotte Hostettler, M.D.
	Varinder S Alg, MBBS
	Nichole Shahi, MD
	Fatima Jichi, PhD
	Stephen Bonner, PhD
	Daniel Walsh, PhD
	Diederik Bulters, FRCS
	Neil Kitchen, PhD
	Martin M Brown, FRCP, Professor
	Henry Houlden, PhD, Professor
	Joan Grieve, MD
	David J Werring, PhD, FRCP, Professor
Manuscript Region of Origin:	UNITED KINGDOM
Abstract:	ABSTRACT Background Only a minority of intracranial aneurysms rupture to cause subarachnoid haemorrhage. Objective We tested the hypothesis that unruptured aneurysms have different characteristics and risk factor profiles compared to ruptured aneurysms. Methods We recruited patients with unruptured aneurysms or aneurysmal subarachnoid haemorrhage at 22 UK hospitals between 2011-2014. Demographic, clinical, and imaging data were collected using standardized case report forms. We compared risk factors using multivariable logistic regression. Results 2334 patients (1729 with aneurysmal subarachnoid haemorrhage, 605 with unruptured aneurysms) were included (mean age 54.22 years). In multivariable analyses, the following variables were independently associated with rupture status: black ethnicity (OR 2.42; 95% CI 1.29-4.56; compared to white); aneurysm location (anterior cerebral artery/anterior communicating artery [OR 3.21; 95% CI 2.34-4.40], posterior communicating artery [OR 3.92; 95% CI 2.67-5.74], or posterior circulation [OR 3.12; 95% CI 2.08-4.70], compared to middle cerebral artery). The following variables were inversely associated with rupture status: antihypertensive medication (OR 0.65; 95% CI 0.49-0.84), hypercholesterolemia (0.64 OR; 95% CI 0.48-0.85), aspirin use (OR 0.28; 95% CI 0.20-0.40), internal carotid artery location (OR 0.53; 95% CI 0.38-0.75), and aneurysm size (per mm increase) (OR 0.76; 95% CI 0.69-0.84). Conclusion We show substantial differences in patient and aneurysm characteristics between ruptured and unruptured aneurysms. These findings support the hypothesis that different pathological mechanisms are involved in the formation of ruptured aneurysms and incidentally detected unruptured aneurysms. The potential protective effect of aspirin in the two cohorts might justify randomized prevention trials in patients with unruptured aneurysms.

Additional Information:	
Question	**Response**
Significance of the Work: Please include a brief statement summarizing the significance of the work and in particular how it differs from and advances existing literature.	Aneurysmal subarachnoid haemorrhage (SAH) is a devastating subset of stroke, occurring in relatively young people with a high mortality rate. Understanding risk factors may be helpful in identifying patients at high risk for aneurysmal SAH and consequently prevention. In our large multicentre case-control study, we have identified differing potential risk factors when comparing unruptured intracranial aneurysms with aneurysms causing SAH; in particular, aspirin use was found to be protective, independent of confounding by indication, and might merit further investigation. Our findings are relevant for neurologists and neurosurgeons when making clinical decisions in patients with an unruptured intracranial aneurysm.
Compliance with Research Reporting Guidelines: *Neurosurgery* endorses several reporting guidelines and requires authors to submit their research articles in accordance with the appropriate guideline statement(s) and checklist(s). Completed applicable checklists and flow diagrams must be included with submissions. Research articles that must be submitted according to the appropriate reporting guideline(s) include, but are not limited to: randomized trials, systematic reviews, meta-analyses of interventions, meta-analyses of observational studies, diagnostic accuracy studies, and observational epidemiological studies (eg, case series, cohort, case-control, and cross-sectional studies). Consult the EQUATOR Network, which maintains a useful, up-to-date list of guidelines as they are published, with links to articles and checklists: http://www.equator-network.org. Please confirm below that information is reported according to the relevant reporting guideline(s) and any required materials are included with the submission:	Yes - Submission Adheres to Appropriate Reporting Guideline(s) and Applicable Checklists/Materials Are Included
Please indicate which reporting guideline(s) the study adheres to (eg, STROBE, PRISMA, CONSORT). as follow-up to "**Compliance with Research Reporting Guidelines**: *Neurosurgery* endorses several reporting guidelines and requires authors to submit their research articles in accordance with the appropriate guideline statement(s) and checklist(s). Completed applicable checklists and flow diagrams must be included with submissions. Research articles that must be submitted according to the appropriate reporting guideline(s) include, but are not limited to: randomized trials, systematic reviews, meta-analyses of interventions, meta-analyses of observational studies, diagnostic accuracy studies, and	STROBE

observational epidemiological studies (eg, case series, cohort, case-control, and cross-sectional studies). Consult the EQUATOR Network, which maintains a useful, up-to-date list of guidelines as they are published, with links to articles and checklists: http://www.equator-network.org. Please confirm below that information is reported according to the relevant reporting guideline(s) and any required materials are included with the submission:"	
Statistical Analysis: For manuscripts that report statistics, the Editor requires that the authors provide evidence of statistical consultation or expertise. If your article includes statistics, has the information reported been evaluated by an expert?	Yes
IRB/Ethics Approval: Please indicate if your study has received institutional review board/ethics approval. If yes, these materials are readily available should the Editor request them.	Yes

STROKE RESEARCH CENTRE
UCL INSTITUTE OF NEUROLOGY

RUSSELL SQUARE HOUSE
10-12 RUSSELL SQUARE
LONDON
WC1B 5EH

STROKE RESEARCH CENTRE

UCL

Direct Line: 020 3448 4753
email: d.werring@ucl.ac.uk

Dr David Werring PhD FRCP
Professor of Clinical Neurology

20th September 2016

Dr Nelson M. Oyesiku, MD PhD, FACS, Editor-in-Chief
Editor, *Neurosurgery*

Dear Dr Oyesiku,

Re: Characteristics of unruptured compared to ruptured intracranial aneurysms: a multicentre case-control study

We would be grateful if the above paper could be considered for publication in Neurosurgery as an article. Aneurysmal subarachnoid haemorrhage (SAH) is a devastating subset of stroke, occurring in relatively young people with a high mortality rate. Understanding risk factors may be helpful in identifying patients at high risk for aneurysmal SAH and consequently prevention. In our large multicentre case-control study, we have identified differing potential risk factors when comparing unruptured intracranial aneurysms with aneurysms causing SAH; in particular, aspirin use was found to be protective, independent of confounding by indication, and might merit further investigation. Our findings are relevant for neurologists and neurosurgeons when making clinical decisions in patients with an unruptured intracranial aneurysm.

I take full responsibility for the data, the analyses and interpretation, and the conduct of the research; I have full access to all of the data; the author has the right to publish any and all data separate and apart from any sponsor. A regional review board has approved the use of human subjects for this study.

All authors have read and approved the submitted manuscript; the manuscript has not been submitted elsewhere nor published elsewhere in whole or in part. I sign for and accept responsibility for this material on behalf of all co-authors. None of the authors have any conflict of interest to report.

This study was conducted in compliance with the current version of the Declaration of Helsinki, and GCP as well as all national legal and regulatory requirements.

The GOSH study is funded by the Stroke Association. This work was partly undertaken at UCLH/UCL who received a proportion of funding from the Department of Health's NIHR Biomedical Research Centres funding scheme.

Yours sincerely,

David Werring PhD FRCP FESO
Professor of Clinical Neurology
Consultant Neurologist

UCL Institute of Neurology • National Hospital for Neurology & Neurosurgery • Queen Square • London WC1N 3BG
☎ +44 (0)20 7692 2362 • Fax: +44 (0)20 7278 5069 • http://www.ucl.ac.uk/ion/
Director: Professor Michael G Hanna BSc(Hons) MBChB(Hons) MD FRCP(UK)
Institute Secretary: R P Walker BSc (Econ)

RESPONSE TO REVIEWERS

Dear reviewers

RE: Characteristics of unruptured compared to ruptured intracranial aneurysms: a multicentre case-control study

We are grateful for the opportunity to resubmit a secondary revised version. Our itemized point-by-point responses are given below, and are underlined in our revised manuscript.

Reviewer 1:

This revised manuscript, which describes a case control study identifying differences in ruptured and unruptured aneurysms, has been revised to adequately address our comments. This new version better defines the limitations of their study, especially regarding potential biases in the study groups. Even with its flaws, this study contributes to our current body of data and will further discussion on differences between ruptured and unruptured aneurysms.

We thank the reviewer for the useful comments and improvement on our manuscript.

Reviewer 2:

The authors did a great job addressing the reviewer's concerns.

We thank the reviewer for the useful comments and improvement on our manuscript.

Reviewer 3:

1. The manuscript has now been improved by deleting obvious concerns and faults. However, the study design and patient selection predisposes yet for misconceptions which are not necessarily true but preferably association between different confounding factors which are difficult to be controlled for. Further analyses are needed.

We thank the reviewer for the useful comments and improvement on our manuscript. We have checked that we have undertaken all of the analyses suggested (see our response to point 4, below).

2. The study consists of 2334 patients (1729 with aneurysmal subarachnoid haemorrhage, 605 with unruptured aneurysms (UIAs)) were included (mean age 54.22 years). Patients with UIAs had clearly larger aneurysm than has previously been published (see ref 1. Vlak et al.) meaning that this population with UIAs is a more selected one than in previous studies.

We agree with this point. Indeed, we had a larger mean UIA size compared to Vlak et al. which could indicate selection bias, a limitation of any hospital-based study. However, an important

strength of our study compared to the Vlak et al study is that we had less missing data: in our study, 605 UIA cohort, aneurysm size was available in 544 patients (90%); in comparison, the meta-analysis of Vlak et al., of 1450 patients included in the meta-analysis UIA aneurysm size was only available in 368 (25%). We have added a statement in the discussion (Page 6, lines 178-182).

3. Patients with high BP values, long-term hypertension and large aneurysms die more likely than survive after SAH and do not reach treatment in secondary and tertiary clinics. For this issue there is only one study (Juvela S. Prehemorrhage risk factors for fatal intracranial aneurysm rupture. Stroke 2003;34:1852-1857). Some autopsy studies also suggest that patients with ruptured MCA aneurysms with space occupying are more common autopsy studies than in clinical studies. So patients with hypertension, large aneurysms and MCA aneurysms may be underestimated in ruptured aneurysm group.

We agree that our study cannot include patients with severe SAH who die before reaching hospital, and that these patients might have different characteristics to those included (e.g larger aneurysms or co-morbidities), potentially introducing selection bias. This is a limitation of any study on ruptured aneurysms, which we acknowledge and discuss (Page 6, lines 200-202).

4. Patients with UIAs were older, had more diseases and medications (hypertension and antihypertensive medication, diabetes, hypercholesterolemia and statin use, previous stroke, coronary artery disease, cardiac disease, and because of several vascular diseases and risk factors also aspirin use. All these factors were more common in patients with UIAs than in those with SAH. Authors have picked aspirin as a "protecting factor" for rupture. Only in Table 3 were odds ratios adjusted for one of these confounders, previous stroke, which had also a lower association with SAH! By which mechanism? The reason seems that in cross-sectional retrospective study factors may associate without a causal relationship. Very sick patients likely die of other diseases than of aneurysm rupture. Because authors have interviewed and collected data from patients authors should present also indications of aspirin treatment. Aspirin was used likely for vascular diseases which shorten life-expectancy.

We thank the reviewer for addressing this important point. We can clarify that aspirin was shown to be "protective" in a pre-planned and systematic analysis, described in our methods, and was not specifically "picked". We adjusted for all of the above-mentioned factors in a multivariable backward stepwise logistic regression analysis: diabetes, statin use, cardio-vascular disease such as myocardial infarction, angina, and PVD. On page 7, lines 227-229 we mention this: "even after fully adjusting for potential confounders (ischemic heart disease, peripheral vascular disease, and diabetes)…". We agree that a cross-sectional study cannot prove causation, or mechanisms of association. We agree that association between previous stroke and unruptured aneurysms is unlikely to be causal; for example, one possibility is that patients with a stroke have more careful management of their vascular risk factors, which might reduce future rupture risk.

Unfortunately, we cannot provide the detailed information about aspirin indication as requested by the reviewer. Nevertheless, the assumption can be made that patients either received aspirin due to an underlying cardiovascular condition (MI [35 on aspirin], angina [29 on aspirin], PVD [12 on aspirin], previous ischemic stroke [51 on aspirin], DM [34 on aspirin]) or as primary prevention (it has become a widespread practice of GP's to start

patients above a certain age on aspirin). One hypothesis why patients with UIA use aspirin more frequently is that there are actively investigated for other potential risk factors, i.e. cardiovascular risk factors. Therefore, these patients could potentially have higher levels of aspirin use.

5. I am a little bit surprised that authors have not noted possibility of Berkson's bias in their study. A classic example is association of lung cancer both with smoking and yellow fingers. Yellow fingers may associate with lung cancer more significantly than smoking yes/no. I think that nobody can insist yellow fingers to cause lung cancer because it is not biologically plausible. This same seems to be here when authors assume that aspirin may prevent aneurysm bleeding. The only real treatment method is occlusion of aneurysm.

We agree that Berkson's bias might affect our results, as in any study where cases and controls are recruited from a hospital (so that controls may not reflect the full population outside hospital). It is also possible that some of our associations are not causal, as in the example given by the reviewer. However, we have performed careful and systematic adjustment for all potential confounding factors to minimise the effect of potential bias and confounding. Our discussion clearly acknowledges the potential selection bias in our hospital-based study.

We also agree with the reviewers that the only definitive treatment of an UIA is its occlusion. However, our data and several other studies report an interesting potential protective effect of aspirin on aneurysm rupture[1-3]. Aneurysm wall inflammation might be reduced by the anti-inflammatory properties of aspirin, which might attenuate growth and reduce rupture risk[2]. To prove or disprove this hypothesis, a randomized trial would be needed. Indeed, we note that such trials are planned (e.g. clinicaltrials.gov NCT03063541). Although we agree that we cannot conclude that aspirin can prevent UIA rupture, we think our data are nevertheless of interest in supporting further investigation of this idea.

Therefore, it is unlikely that Berkson's bias accounts for the possible effect of aspirin. The only way to definitively conclude whether aspirin does or does not have a protective effect is through a randomized controlled trial, which is beyond the scope of our study.

6. Authors should reanalyze results of Table 3 adjusting all above mentioned cardiovascular diseases and risk factors for association between aneurysm groups to see whether aspirin use and hypercholesterolemia are true independent factors. It seems that aspirin use is a proxy of several other factors since, e.g. patients with diabetes commonly use aspirin to prevent ischemic diseases. If some diseases/factors are too uncommon to be tested factors these can be combined as factors such as cardiovascular diseases and cardiovascular risk factors.

We agree that it is important to adjust for all of these factors (confounding by indication). We have therefore included all of them in our analyses (see page 7, lines 227-229 where we mention, "even after fully adjusting for potential confounders (ischaemic heart disease, peripheral vascular disease, and diabetes)". For the purposes of clarity, and in line with standard statistical practice, some of these factors which were not statistically significant do not appear in the final models in Table 2 and Table 3.

7. Authors state in Discussion: "By contrast, the majority of stable unruptured aneurysms would be expected to have low rupture rates, even if this increases with increasing aneurysm size, consistent with observational data reporting that only a very

small minority of incidentally detected unruptured aneurysms will rupture to cause aneurysmal SAH (incidence 9 in 206 100,000)." This statement is not true and most UIAs are smaller than those in this study. Incidence rate is right but UIAs grow with a rate of 3-4% per year in a follow-up of even low-risk UIAs. These do not likely rupture because patients are dying of unrelated causes. However, if UIA is growing the rupture risk is clearly higher and more than 50% of growing UIAs are rupturing during follow-up (Juvela S, Poussa K, Porras M. Factors affecting formation and growth of intracranial aneurysms: a long-term follow-up study. Stroke 2001;32:485-491). This should be noted and cited in discussion since risky UIAs were not selected by aneurysm occlusion at baseline of follow-up n this study. This study also had highest study quality and lowest study bias according to recent meta-analyses of UIA growth (Brinjikji W, Zhu YQ, Lanzino G, Cloft HJ, Murad MH, Wang Z, Kallmes DF. Risk factors for growth of intracranial aneurysms: a systematic review and meta-analysis. Am J Neuroradiol 2016;37:615-620; and Backes D, Rinkel GJ, Laban KG, Algra A, Vergouwen MD. Patient- and aneurysm-specific risk factors for intracranial aneurysm growth: systematic review and meta-analysis. Stroke 2016;47:951-957).

We thank the reviewer for this comment and agree that the wording of our sentence was not fully clear ("By contrast, most stable unruptured aneurysms would be expected to have low rupture rates, even if this increases with increasing aneurysm size"). We have changed the wording and included a new sentence focused on the increase in size of a specific unruptured aneurysm. The reference has been added. Please see:

- Page 6, lines 197-200.

8. Comments on evidence of aspirin for treatment of UIAs are not based on unbiased high grade studies. Author has cited one post hoc study of ISUIA. One must remember that ISUIA was a highly selected patient population study with a low rupture risk UIAs. At baseline already 58% were excluded from follow-up because of an aneurysm occlusion and additional 32% were treated during follow-up before a possible rupture (see refs 22 and 23). If 71% of patients with UIAs are excluded totally or partially from a short-term follow-up, the remaining patients are likely similar to those patients with UIAs in the present study, i.e. patients with several cardiovascular diseases or risk factors using aspirin and statins and thought not to be benefit from aneurysm treatment. Patient with risk factors for cardiovascular diseases and aspirin use likely die untimely and likely not from aneurysm rupture because of a shorter life-expectancy. Post hoc study used logistic regression which cannot also discriminate timing of deaths. These results cannot be generalized to all patients with UIAs.

We agree with the reviewer that the level of evidence for the effectiveness of aspirin on prevention of aneurysm rupture is low. However, evidence exists that aspirin harbors a preventive effect (see our comment to point 5, above). The only way to definitely approach this is to conduct a randomized trial on patients with UIA and aspirin intake. We agree that the ISUIA data cannot be generalized to all populations of patients with UIAs.

9. Authors underestimate risks of aspirin stating that it is safe. In a primary prevention study aspirin use decreased risk of myocardial infarction but increased 2-fold risk of hemorrhagic stroke, most likely primary intracerebral hemorrhage (ICH)(Steering Committee of the Physicians' Health Study Research Group. Final report on the aspirin component of the ongoing Physicians' Health Study. N Engl J Med. 1989;321:129-135). In addition, pre-ictal use of aspirin increases case fatality after ICH (Thompson BB, Béjot

Y, Caso V, Castillo J, Christensen H, Flaherty ML, et al. Neurology 2010; 75: 1333-1342).

Patients with SAH have also used more aspirin before rupture than patients with other acute diseases (Juvela S, Hillbom M, Numminen H, Koskinen P. Cigarette smoking and alcohol consumption as risk factors for aneurysmal subarachnoid hemorrhage. Stroke 1993; 24: 639-646). Pre-SAH use of aspirin increases risk for early rebleeding (Juvela S. Aspirin and delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage. J Neurosurg 1995; 82: 945-952; and Toussaint L, G III, Friedman JA, Wijdicks EFM, Piepgras DG, Pichelmann MA, McIvere JI, et al. Influence of aspirin on outcome following aneurysmal subarachnoid hemorrhage. J Neurosurg 2004;101:921-925).

We thank the reviewer for his useful comments regarding the safety of aspirin; we have tried to implement them into our discussion. We have added a statement regarding the potential adverse effects of aspirin on intracerebral haemorrhage and SAH outcome (Page 8, 238-239). We agree that no strong evidence exists on the net clinical benefit of aspirin in preventing aneurysm rupture, potential increase in hemorrhage severity or increase in frequency of hemorrhagic stroke. Only a randomized controlled trial would give us a definitive answer to this question.

- *Page 7/8, lines 236-239*
- *Page 8, lines 245-246*
- *Page 10, lines 319-320*

10. It is yet highly doubtful whether aspirin really prevents SAH. These aspects make it difficult to estimate whether (low risk) UIAs should be studied in a placebo-controlled randomized trial to see prevention of bleeding. Study may need to continue decades to see effect and compliance of aspirin/placebo intake for a long-term time may be modest when risks of gastrointestinal and intracranial bleedings are noted by patients. It is well-known that appropriate completion of a randomized trial of any treatment is a time consuming and includes several difficulties. Recommendation of such trial without careful evaluation is not reasonable.

We agree with the reviewer that randomized treatment trials are time consuming, cost-consuming and might be very challenging due to the length of follow up. We note that a small proof-of-concept randomized trial has already been conducted[2] and that others are planned (clinicaltrials.gov NCT03063541).

Once again, we are most grateful for the carefully considered, thorough and constructive comments from the reviewer. We hope that the paper will be considered of interest to the readers of Neurosurgery and suitable for publication.

Please contact us if further information is required.

Yours sincerely,

The author and co-authors

1. Chalouhi N, Starke RM, Correa T, Jabbour PM, Zanaty M, Brown RD, Jr., et al. Differential sex response to aspirin in decreasing aneurysm rupture in humans and mice. *Hypertension*. 2016;68:411-417
2. Hasan DM, Chalouhi N, Jabbour P, Dumont AS, Kung DK, Magnotta VA, et al. Evidence that acetylsalicylic acid attenuates inflammation in the walls of human cerebral aneurysms: Preliminary results. *Journal of the American Heart Association*. 2013;2:e000019
3. Hasan DM, Mahaney KB, Brown RD, Jr., Meissner I, Piepgras DG, Huston J, et al. Aspirin as a promising agent for decreasing incidence of cerebral aneurysm rupture. *Stroke; a journal of cerebral circulation*. 2011;42:3156-3162

Characteristics of unruptured compared to ruptured intracranial aneurysms: a multicentre case-control study

Isabel C Hostettler MD[1], Varinder S Alg MBBS[1], Nichole Shahi MD[1], Fatima Jichi PhD[2], Stephen Bonner PhD[3], Daniel Walsh PhD[4], Diederik Bulters FRCS[5], Neil Kitchen PhD[6], Martin M Brown FRCP[1], Henry Houlden PhD[7], Joan Grieve MD[6], David J Werring PhD FRCP[1,#] on behalf of the Genetics and Observational Subarachnoid Haemorrhage (GOSH) Study investigators

[1] *Stroke Research Centre, University College London, Institute of Neurology, London, UK*

[2] *Biostatistics Group, University College London Research Support Centre, University College London, UK*

[3] *Department of Anaesthesia, The James Cook University Hospital, Middlesbrough, UK*

[4] *Department of Neurosurgery, King's College Hospital NHS Foundation Trust, London, UK*

[5] *Department of Neurosurgery, University Hospital Southampton NHS Foundation Trust, Southampton UK*

[6] *Department of Neurosurgery, The National Hospital of Neurology and Neurosurgery, London, UK*

[7] *Neurogenetics Laboratory, The National Hospital of Neurology and Neurosurgery, London, UK*

Corresponding author: Professor David Werring, FRCP, PhD, National Hospital of Neurology and Neurosurgery, Institute of Neurology, University College London, Queen Square, WC1N London, United Kingdom, Phone: +44 20 3447 5994, Fax: +44 20 7833 8613, Email: d.werring@ucl.ac.uk

Previous presentations

This study has been presented as a poster at the European Stroke Organisation Conference (ESOC), in Barcelona, Spain 05/2016.

Financial Disclosure

The GOSH study is funded by the Stroke Association. This work was partly undertaken at UCLH/UCL who received a proportion of funding from the Department of Health's NIHR Biomedical Research Centres funding scheme.

Acknowledgments

We thank the many NIHR Clinical Research Network Practitioners and the investigators at participating centres for their assistance with this study, and the patients for taking part.

 PLEASE SAVE THIS TO YOUR HARD DRIVE UNDER A DIFFERENT FILE NAME AFTER YOU FILL IT OUT

STROBE Statement—checklist of items that should be included in reports of observational studies

Please fill out the page numbers on this form and upload the file as a supplemental file when you submit your revision

Manuscript Number_____ ID: NEUROSURGERY

	Item No	Recommendation	Indicate page number ↓ (Or n/a if not applicable)
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	2
Objectives	3	State specific objectives, including any prespecified hypotheses	2
Methods			
Study design	4	Present key elements of study design early in the paper	2
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	2, 3
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	2, 3
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	n/a
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	3
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	3
Bias	9	Describe any efforts to address potential sources of bias	3
Study size	10	Explain how the study size was arrived at	2-4
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	4, 5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	3
		(*b*) Describe any methods used to examine subgroups and interactions	3
		(*c*) Explain how missing data were addressed	3
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	n/a
		(*e*) Describe any sensitivity analyses	3
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	2, 3
		(b) Give reasons for non-participation at each stage	2, 3
		(c) Consider use of a flow diagram	n/a
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	3, 4, Tbl 1
		(b) Indicate number of participants with missing data for each variable of interest	5
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	n/a
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	n/a
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	3,4
		Cross-sectional study—Report numbers of outcome events or summary measures	n/a
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95%	

		confidence interval). Make clear which confounders were adjusted for and why they were included	4, 5
		(*b*) Report category boundaries when continuous variables were categorized	4
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	5
Discussion			
Key results	18	Summarise key results with reference to study objectives	5, 6
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	8, 9
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	6-8
Generalisability	21	Discuss the generalisability (external validity) of the study results	9
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Cover lette

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

ABSTRACT

Background Only a minority of intracranial aneurysms rupture to cause subarachnoid haemorrhage.

Objective We tested the hypothesis that unruptured aneurysms have different characteristics and risk factor profiles compared to ruptured aneurysms.

Methods We recruited patients with unruptured aneurysms or aneurysmal subarachnoid haemorrhage at 22 UK hospitals between 2011-2014. Demographic, clinical, and imaging data were collected using standardized case report forms. We compared risk factors using multivariable logistic regression.

Results 2334 patients (1729 with aneurysmal subarachnoid haemorrhage, 605 with unruptured aneurysms) were included (mean age 54.22 years). In multivariable analyses, the following variables were independently associated with rupture status: black ethnicity (OR 2.42; 95% CI 1.29-4.56; compared to white); aneurysm location (anterior cerebral artery/anterior communicating artery [OR 3.21; 95% CI 2.34-4.40], posterior communicating artery [OR 3.92; 95% CI 2.67-5.74], or posterior circulation [OR 3.12; 95% CI 2.08-4.70], compared to middle cerebral artery). The following variables were inversely associated with rupture status: antihypertensive medication (OR 0.65; 95% CI 0.49-0.84), hypercholesterolemia (0.64 OR; 95% CI 0.48-0.85), aspirin use (OR 0.28; 95% CI 0.20-0.40), internal carotid artery location (OR 0.53; 95% CI 0.38-0.75), and aneurysm size (per mm increase) (OR 0.76; 95% CI 0.69-0.84).

Conclusion We show substantial differences in patient and aneurysm characteristics between ruptured and unruptured aneurysms. These findings support the hypothesis that different pathological mechanisms are involved in the formation of ruptured aneurysms and incidentally detected unruptured aneurysms. The potential protective effect of aspirin in the two cohorts might justify randomized prevention trials in patients with unruptured aneurysms.

Running Title

Characteristics of aneurysm types

Key words

Characteristics, predisposition to rupture, risk factors, subarachnoid haemorrhage, unruptured intracranial aneurysms.

INTRODUCTION

About 3 % of the population have an unruptured intracranial aneurysm[1] but only a minority rupture to cause a subarachnoid haemorrhage (SAH); the overall risk of subarachnoid haemorrhage for unruptured intracranial aneurysms is about 1% per year[2]. SAH causes 5% of all strokes[3], yet, with young age of onset and approximately 50% mortality, it substantially reduces productive life-years[4-6] despite improvements in risk stratification, imaging, surgical and intensive care treatment. Up to 76% of patients who survive have permanent cognitive deficits, and only 6-17% of survivors return to employment[7]. Hence, predicting (and reducing) the risk of rupture of an intracranial aneurysm is an important clinical and socio-economic need[8].

Previous studies assumed common causal mechanisms for ruptured and unuruptured aneurysms, but it is also possible that their underlying risk factors and pathophysiological mechanisms are different. For example, several studies report that ruptured aneurysms causing SAH are smaller than unruptured aneurysms, suggesting that they might form rapidly and quickly rupture, potentially due to different underlying mechanisms[9-14].

Although many potential risk factors are associated with intracranial aneurysms (e.g. aneurysm location, aneurysm size, smoking, number of aneurysms, age, female sex, hypertension, hypercholesterolemia, previous history of SAH, heart disease, and aspirin use[2, 8, 15-28]), most show inconsistent results,[29, 30] which might in part relate to rupture status. We therefore tested the hypothesis that incidentally detected unruptured aneurysms have different characteristics and risk factor profiles compared to aneurysms that cause SAH.

METHODS

Patients

We collected data from patients with aneurysmal SAH or unruptured aneurysm without previous SAH enrolled in the Genetic and Observational Subarachnoid Haemorrhage (GOSH) study (designed to examine the genetic and clinical characteristics of patients with ruptured and unruptured aneurysms), which recruited at 22 neurosurgical centres in the UK between 2011-2014. Written informed consent was obtained from participants, or from a representative in case of lack of capacity. We excluded patients with perimesencephalic SAH (63 patients) (defined by the characteristic distribution of blood (i.e. mainly or only in the cisterns around the midbrain) and absence of an identified intracranial aneurysm by the local

principle investigator)[31-33] and SAH due to trauma or mycotic aneurysms. Recruitment was as inpatient or from outpatient neurovascular clinics following either new diagnosis or previous diagnosis as an inpatient. Standardised case report forms were completed by trained stroke research practitioners. Medical history was obtained from patient self-reporting and/or available medical records. Hypertension, hypercholesterolemia and diabetes mellitus were defined as present if the patient or medical records indicated hypertension, hypercholesterolemia or hyperglycaemia for which either drug treatment, lifestyle or other advice had been provided. We defined antihypertensive drug use, statin use, and aspirin use by patient self-reporting or available documentation on regular intake at the time of either admission with aneurysmal SAH or of being diagnosed with an unruptured aneurysm. Recreational drug use was assessed for cocaine, cannabis, amphetamine and opiates by patient self-reporting or from relatives. Smoking and alcohol use were defined as on-going use. Family history of aneurysmal SAH and intracranial aneurysm was defined as presence of at least one relative with the relevant disorder. Internal carotid artery location was considered as an aneurysm in the cavernous segment of the internal carotid artery and onwards. The study was approved by a Research Ethics committee.

Statistical analysis

Demographic, clinical and radiological data were first assessed in univariable analysis comparing patients with aneurysmal SAH to those with unruptured aneurysm [Table 1]. Variables for multivariable analysis were chosen based on univariable analysis, as well as previous studies and biological plausibility. We conducted a multivariable backward stepwise logistic regression analysis to identify factors independently associated with aneurysmal SAH. We excluded family history of SAH or unruptured aneurysm and previous history of stroke from the multivariable analysis, due to potential selection bias in the unruptured aneurysm group[16]. A sensitivity analysis was conducted including these variables. As a further sensitivity analysis, we used multiple imputation for missing data values. The level of statistical significance was set at 5% (p-value=0.05). Statistical analysis was performed using STATA 13 (StataCorp. 2011. *Stata Statistical Software: Release 13*. College Station, TX: StataCorp LP).

RESULTS

Baseline characteristics are summarized in Table 1. We included 2334 patients: 1729 with ruptured intracranial aneurysms (1215 females [70.3%], 514 males [29.7%], mean age 53.24,

12.70 SD) and 605 patients with unruptured aneurysms (425 females [70.25%], 180 males [29.75%] mean age 57.03, 12.17 SD).

Associations of potential risk factors with incidental intracranial aneurysms and aneurysmal SAH

In univariable analysis factors associated with aneurysmal SAH as opposed to incidentally-detected unruptured aneurysms were: hypertension not treated (OR 2.54; 1.65-3.93 95% CI; p <0.001), ethnicity (black ethnicity (compared to the baseline group [white ethnicity]), OR 2.31; 1.37-3.90 95% CI; p=0.003); smoking (OR 1.31; 1.09-1.59 95% CI; p=0.005); recreational drug use (OR 1.73; 1.09-2.90 95% CI; p=0.02); aneurysm location (anterior cerebral artery and anterior communicating artery, posterior communicating artery, and posterior circulation (compared to aneurysms of the middle cerebral artery) with location in the anterior cerebral artery or anterior communicating artery having the largest impact [OR 3.50; 2.64-4.64 95% CI]) [Table 1]. Factors that were inversely associated with aneurysmal SAH (i.e. which were apparently protective) were: older age at diagnosis (as a continuous variable OR 0.98; 0.97-0.98 95% CI; p <0.001; as a categorical variable by every 10 year increase of age the odds ratio of aneurysm rupture status was 0.21; 0.14-0.26 95% CI); hypertension (OR 0.53; 0.44-0.64 95% CI; p <0.001); anti-hypertensive medication use (OR 0.45; 0.37-0.54 95% CI; p <0.001); hypercholesterolemia (OR 0.45; 0.37-0.55 95% CI; p <0.001); statin medication use (OR 0.40; 0.33-0.50 95% CI; p <0.001); diabetes mellitus (OR 0.60; 0.40-0.90 95% CI; p=0.013); previous stroke (OR 0.18; 0.12-0.26 95% CI; p <0.001); angina (OR 0.45; 0.27-0.77 95% CI; p=0.002); heart disease (OR 0.53; 0.35-0.81 95% CI; p=0.003); use of aspirin (OR 0.22; 0.17-0.28 95% CI; p <0.001), positive family history of intracranial aneurysm or SAH (OR 0.63; 0.49-0.80 95% CI; p <0.001; [positive family history of aneurysmal SAH separately (OR 0.79; 0.69-0.90 05% CI; p=0.001), positive family history of unruptured intracranial aneurysm separately (OR 0.66; 0.54-0.81 95% CI; p <0.001]), location of aneurysm in the internal carotid artery (OR 0.47; 95% CI 0.35-0.62); and aneurysm size (OR 0.91; 0.90-0.93 95% CI; p <0.001; 406 missing values). For the univariable analysis we also analysed aneurysm size as a categorical variable dividing it into 5mm step categories, which also showed that larger aneurysms are independently associated with a decreased risk of aneurysmal SAH (coefficient -0.089; -0.11-(-0.07) 95% CI; p <0.001.

Most aneurysms were smaller than 7.0 mm. Unruptured aneurysms tended to be larger (mean aneurysm size 9.0mm, SD 6.3mm; median 7.0mm) compared to ruptured aneurysms (mean

aneurysm size 6.6mm, 4.2mm SD; median 5.7mm). The mean duration of antihypertensive treatment was 8.12 years (7.81 SD; median 5); however, this was not associated with aneurysmal SAH in the univariable analysis (OR 1.00; 0.98-1.02 95% CI; p=0.977).

Of 119 patients with recreational drug use, 93 reported consumption of cannabis, 30 cocaine, 17 ecstasy, and 5 opiates. Forty-one patients had consumed multiple drugs.

In the multivariable regression analysis, following factors were independently associated with aneurysmal SAH in the final adjusted model [Table 2]: black ethnicity compared to white ethnicity (OR 2.42; 1.29-4.56 95 % CI; p=0.013 overall categorical variable); and aneurysm location (location in the posterior communicating artery had the largest influence [OR 3.92; 2.67-5.74 95% CI]; p <0.001 overall categorical variable; internal carotid artery location was inversely associated with aneurysmal SAH status). Independent factors inversely associated with aneurysmal SAH status were: treatment with antihypertensive medication (OR: 0.65; 0.49-0.84 95% CI; p=0.001); hypercholesterolemia (OR 0.64; 0.48-0.85 95% CI; p=0.002); aspirin use (OR 0.28; 0.20-0.40 95% CI; p <0.001); increasing aneurysm size (OR 0.76, 0.69-0.84 95% CI; p <0.001), and internal carotid artery location (OR 0.53; 0.38-0.75 95% CI).

In a sensitivity analysis including family history of aneurysm and previous history of stroke in the final model, the associations with aneurysmal SAH did not significantly change; Asian ethnicity became a significant predictor for aneurysmal SAH ([OR 2.13; 1.02-4.46 95% CI], p=0.012 overall categorical variable), and the model's prediction increased (likelihood ratio test p <0.001, Pseudo R2 increasing from 0.18 to 0.20) [Table 3]. A further sensitivity analysis using multiple imputation for missing data in the variables aneurysm size (406 missing values) and aneurysm location (148 missing values) showed no change in OR (data not shown).

DISCUSSION

In this large observational study, we show patients with incidentally detected unruptured intracranial aneurysms have different patient and aneurysm characteristics and potential risk factors compared to patients with ruptured aneurysms. Certain aneurysm locations (anterior cerebral artery and anterior communication artery, posterior communicating artery, and posterior circulation) and black ethnicity were independently associated with aneurysmal

SAH, compared to patients with an unruptured aneurysm. In addition, we found that location in the internal carotid artery is inversely associated with aneurysmal SAH. We also demonstrated antihypertensive medication use, aspirin use, hypercholesterolemia, and larger aneurysm size are independently associated with a decreased risk of aneurysmal SAH compared to the population of incidental intracranial aneurysms.

In our cohort, incidentally detected unruptured aneurysms were larger compared to those with aneurysmal SAH. Our findings are consistent with other studies that show the majority of ruptured aneurysms are less than 10 mm[12, 14, 34, 35]. Indeed, in our study the size of unruptured aneurysms was larger than that reported in a previous meta-analysis[1]. This could indicate selection bias, a limitation of any hospital-based study. However, an important strength is that we have data on unruptured aneurysm size in 544 patients (90%); in the meta-analysis this was only available in 368 patients (25%)[1].

Also in line with our data, is the International Study of Unruptured Intracranial Aneurysms (ISUIA), a lower risk of rupture was reported in intracranial aneurysms with a diameter of less than 10 mm; this observation that small unruptured aneurysms are at lower risk of future rupture than large unruptured aneurysms has been convincingly shown in other large prospective studies[20, 36, 37]. One explanation for this apparent inconsistency is that aneurysms that rupture to cause SAH and incidentally detected unruptured aneurysms have different pathological mechanisms making them behave differently[38]. Most intracranial aneurysms are considered to form over days to weeks, at which point they either rupture or stabilise due to remodelling of the arterial wall[10, 11]. Whether early rupture or stabilisation occurs might be because of different underlying pathological haemodynamic and inflammatory mechanisms[39]. Indeed, differences have been described in the histology of ruptured compared to unruptured aneurysms[9, 10]. Our data are consistent with this hypothesis, in which rapidly developing aneurysms that rupture are expected to be small. By contrast, the majority of stable unruptured aneurysms would be expected to have low rupture rates, even if rupture rate is higher in larger aneurysm compared to smaller aneurysms[1, 3, 39, 40]. Nevertheless, patients whose unruptured aneurysm increase in size during follow-up are at high risk of rupture and warrant further treatment[41, 42]. An alternate explanation is that our results are due to selection bias. One could hypothesise that larger ruptured aneurysms were excluded due to prehospital death if they suffered more severe haemorrhages, while smaller unruptured aneurysms were excluded as they may not have been referred to tertiary care. Therefore, only tentative

conclusions can be drawn from this result and its meaning must be considered carefully. This could only be resolved in a longitudinal prospective large trial. However, a population of patients with unruptured aneurysms as in ISUIA and other studies would not suffice and it would require a healthy population with no prior diagnosis of an aneurysm. Due to the relatively low incidence of aneurysms and particularly SAH these would be logistically challenging.

The large sample size allowed us to investigate not only risk factors but also their treatments. A previous study has found a linear relationship between increase in systemic arterial pressure and pressure in the aneurysm sac[43]. This finding supports the hypothesis that a rapid increase in blood pressure rather than a chronic increased blood pressure lead to aneurysm rupture. Patients with untreated hypertension had a higher OR for rupture. Furthermore, treatment rather than duration of treatment, appears to be associated with a decrease in aneurysmal SAH. These findings are most consistent with a rapid benefit from antihypertensive treatment, rather than a long-term remodelling of the aneurysm wall. The potentially protective effect of treatment is consistent with the hypothesis that a sudden rise in blood pressure might trigger the rapid formation of aneurysms that are prone to rupture early. Different antihypertensive drugs with different mechanisms of action might have differing influences on aneurysm formation and rupture other than purely through blood pressure control[44]. However, we did not have data on specific antihypertensive agents, which should ideally be addressed in further prospective studies.

We found that regular aspirin use was significantly less common in patients with SAH than in patients with unruptured aneurysms even after fully adjusting for potential confounders (ischaemic heart disease, peripheral vascular disease, and diabetes; OR 0.28; 95% CI 0.20-0.40; $p<0.001$). This could mean that aspirin use protects against SAH in patients with unruptured aneurysms. Although it is possible that the use of aspirin results in more severe SAH, which might be excluded from our study (e.g. if patients die before reaching hospital), we did not find any association between aspirin use and severity of SAH (data not shown). Thus, this seems an unlikely explanation for the higher proportion of patients taking aspirin in the unruptured aneurysm group (25.45% versus 6.94% in the aneurysmal SAH group). A recent study also showed no association of aspirin or anticoagulation with mortality or complications; on the contrary, aspirin was associated with a shorter hospital stay[45]; other studies showed a potential decrease in ischaemic events after SAH and increase in

asymptomatic survival[46, 47]. However, further studies suggest aspirin given preventively might increase the risk of intracerebral haemorrhage and rebleeding after SAH[46, 48, 49]. Furthermore, our findings are in keeping with prospective data from the ISUIA and other studies, which showed a protective effect for regular aspirin use on intracranial aneurysm rupture[25, 47]. Aspirin could potentially reduce the risk of aneurysm rupture by inhibiting inflammatory mediators (e.g. matrix metalloproteinases and tumor necrosis factor-alpha)[50]. Our findings suggest that unruptured aneurysms are not a contraindication to antiplatelet therapy for patients with a clear indication. Due to lack of definitive strong evidence, neither for a protective effect nor harmful effect, this requires further research. Indeed, our data strengthen the case for randomised trials testing the benefit of regular aspirin use on aneurysm rupture. We cannot fully explain the reason for aspirin use being higher in the unruptured aneurysm group; apart from a true biological effect, another possibility is that patients found to have an unruptured aneurysm are investigated for additional diseases that lead to aspirin prescription (e.g. hypertension, hypercholesterolaemia or previous stroke).

Our study also suggests that hypercholesterolaemia could have a protective effect on aneurysm rupture (OR 0.64; 95% CI 0.48-0.85; p=0.002). Previous smaller studies found a similar relationship, but were unable to determine whether this finding might be due to statin treatment rather than the underlying hypercholesterolaemia[16, 51]. We were able to adjust for use of statins, and showed that hypercholesterolaemia was a protective factor for aneurysmal SAH, independent of the use of statin medications. The mechanism remains unclear. We suggest that part of the effect could emerge through stabilisation of the aneurysm wall and so preventing newly formed aneurysm from early rupture.

We found black ethnicity to be independently associated with aneurysmal SAH, but whether this is due to genetic or environmental factors remains uncertain. Greater risk of aneurysmal SAH in black patients compared to white patients has been described before[52]. Another study found that patients who underwent treatment for unruptured aneurysms generally had higher socioeconomic status and were more likely to be white, female, or insured, suggesting the findings to be due to social implemented reasons rather than based on genetic differences[53].

Our study has important strengths. We included a large sample from multiple neurosurgical centres throughout the UK. The participants with aneurysmal SAH and unruptured aneurysms were recruited from the same hospitals over the same time-period using standardized

inclusion criteria and report questionnaire with standardized definitions of all risk factors. Moreover, the unruptured aneurysm group is free of the disease (aneurysmal SAH). Our large sample size allowed us to undertake a multivariable analysis including many clinical and anatomical factors in our model.

Our study also has limitations. Selection bias towards patients with unruptured intracranial aneurysms with a family history of intracranial aneurysm or aneurysmal SAH, or with a previous stroke has been suggested (as these patients will be more likely to undergo brain imaging)[16]. Indeed, common reasons for intracranial aneurysm screening were positive family history for aneurysmal SAH or unruptured aneurysm, or a previous history of stroke; 18.35% of the unruptured aneurysm patients had a positive family history of SAH or intracranial aneurysms compared to 12.32% with aneurysmal SAH ($p < 0.001$). 11.9% of the patients with unruptured aneurysms had a previous history of stroke compared to 2.3% of those with aneurysmal SAH ($p < 0.001$). Nevertheless, a sensitivity analysis controlling for these variables did not affect our findings [Table 3]. As the aim of our study was not to compare "risk factors" between two groups with the same underlying disease but to evaluate for differing characteristics and potential risk factors in two different diseases, selection bias should be negligible. The study was designed to examine the different genetic and clinical characteristics of patients with ruptured and unruptured aneurysms; we are here presenting the large amount of clinical data collected to explore clinical differences between these cohorts. Another limitation arises from the fact that patients who are diagnosed with an unruptured intracranial aneurysm might not be seen by a neurologist or neurosurgeon due to their advanced age or significant comorbidities. These patients could not be included in our study.
We did not explicitly collect the information about why unruptured intracranial aneurysms were diagnosed, apart from the small symptomatic number (9 patients). The study also has potential for bias towards aneurysmal SAH survivors; patients who died before they could be recruited, or in whom no informant could provide consent, were excluded from our study. Another potential limitation of our study is the case-control design preventing any direct inferences on causality. However, an observational natural history study in a similar population of untreated intracranial aneurysms is unlikely to be either feasible or ethically acceptable; not all diagnosed unruptured aneurysms can be left untreated and be followed-up, which also introduces selection bias. Although it would be highly desirable that diagnosed unruptured aneurysms result from random screening of the population this is unlikely to

happen without large scale intracranial imaging studies, which are likely to be logistically, financially and ethically challenging.

CONCLUSION

We found that patients with aneurysmal SAH most likely have a different risk factor profile in comparison to patients with incidentally detected unruptured aneurysms. Aneurysm location and black ethnicity are associated with aneurysmal SAH in comparison to incidentally detected unruptured aneurysms. Antihypertensive medication, aspirin use, hypercholesterolemia, aneurysm location on the internal carotid artery, and aneurysm size are associated with unruptured intracranial aneurysms compared to aneurysmal SAH. These findings support the hypothesis that risk factors for the type of aneurysm that ruptures early are different to unruptured aneurysms or to those that rupture later. The large potentially protective effect of aspirin use justifies randomized clinical trials to prevent SAH in patients with unruptured aneurysms offering an alternative treatment option in small aneurysms, where the decision for invasive treatment is difficult. This would also hopefully resolve the controversial evidence on protective or harmful effects of aspirin.

REFERENCES

1. Vlak MH, Algra A, Brandenburg R, Rinkel GJ. Prevalence of unruptured intracranial aneurysms, with emphasis on sex, age, comorbidity, country, and time period: a systematic review and meta-analysis. *The Lancet. Neurology.* Jul 2011;10(7):626-636.

2. Wermer MJ, van der Schaaf IC, Algra A, Rinkel GJ. Risk of rupture of unruptured intracranial aneurysms in relation to patient and aneurysm characteristics: an updated meta-analysis. *Stroke; a journal of cerebral circulation.* Apr 2007;38(4):1404-1410.

3. Steiner T, Juvela S, Unterberg A, et al. European Stroke Organization guidelines for the management of intracranial aneurysms and subarachnoid haemorrhage. *Cerebrovascular diseases.* 2013;35(2):93-112.

4. Suarez JI, Tarr RW, Selman WR. Aneurysmal subarachnoid hemorrhage. *N Engl J Med.* Jan 26 2006;354(4):387-396.

5. Johnston SC, Selvin S, Gress DR. The burden, trends, and demographics of mortality from subarachnoid hemorrhage. *Neurology.* May 1998;50(5):1413-1418.

6. Hackett ML, Anderson CS. Health outcomes 1 year after subarachnoid hemorrhage: An international population-based study. The Australian Cooperative Research on Subarachnoid Hemorrhage Study Group. *Neurology.* Sep 12 2000;55(5):658-662.

7. Al-Khindi T, Macdonald RL, Schweizer TA. Cognitive and functional outcome after aneurysmal subarachnoid hemorrhage. *Stroke; a journal of cerebral circulation.* Aug 2010;41(8):e519-536.

8. de Rooij NK, Linn FH, van der Plas JA, Algra A, Rinkel GJ. Incidence of subarachnoid haemorrhage: a systematic review with emphasis on region, age, gender and time trends. *Journal of neurology, neurosurgery, and psychiatry.* Dec 2007;78(12):1365-1372.

9. Costalat V, Sanchez M, Ambard D, et al. Biomechanical wall properties of human intracranial aneurysms resected following surgical clipping (IRRAs Project). *Journal of biomechanics.* Oct 13 2011;44(15):2685-2691.

10. Kataoka K, Taneda M, Asai T, Kinoshita A, Ito M, Kuroda R. Structural fragility and inflammatory response of ruptured cerebral aneurysms. A comparative study between ruptured and unruptured cerebral aneurysms. *Stroke; a journal of cerebral circulation.* Jul 1999;30(7):1396-1401.

11. Sato K, Yoshimoto Y. Risk profile of intracranial aneurysms: rupture rate is not constant after formation. *Stroke; a journal of cerebral circulation.* Dec 2011;42(12):3376-3381.

12. Joo SW, Lee SI, Noh SJ, Jeong YG, Kim MS, Jeong YT. What Is the Significance of a Large Number of Ruptured Aneurysms Smaller than 7 mm in Diameter? *Journal of Korean Neurosurgical Society.* Feb 2009;45(2):85-89.

13. Park DH, Kang SH, Lee JB, et al. Angiographic features, surgical management and outcomes of proximal middle cerebral artery aneurysms. *Clinical neurology and neurosurgery.* Jun 2008;110(6):544-551.

14. Rinkel GJ, Djibuti M, Algra A, van Gijn J. Prevalence and risk of rupture of intracranial aneurysms: a systematic review. *Stroke; a journal of cerebral circulation.* Jan 1998;29(1):251-256.

15. Korja M, Silventoinen K, Laatikainen T, et al. Risk factors and their combined effects on the incidence rate of subarachnoid hemorrhage--a population-based cohort study. *PloS one.* 2013;8(9):e73760.

16. Vlak MH, Rinkel GJ, Greebe P, Algra A. Risk of rupture of an intracranial aneurysm based on patient characteristics: a case-control study. *Stroke; a journal of cerebral circulation.* May 2013;44(5):1256-1259.

17. Sonobe M, Yamazaki T, Yonekura M, Kikuchi H. Small unruptured intracranial aneurysm verification study: SUAVe study, Japan. *Stroke; a journal of cerebral circulation.* Sep 2010;41(9):1969-1977.

18. Ishibashi T, Murayama Y, Urashima M, et al. Unruptured intracranial aneurysms: incidence of rupture and risk factors. *Stroke; a journal of cerebral circulation.* Jan 2009;40(1):313-316.

19. Juvela S, Poussa K, Lehto H, Porras M. Natural history of unruptured intracranial aneurysms: a long-term follow-up study. *Stroke; a journal of cerebral circulation.* Sep 2013;44(9):2414-2421.

20. Wiebers DO, Whisnant JP, Huston J, 3rd, et al. Unruptured intracranial aneurysms: natural history, clinical outcome, and risks of surgical and endovascular treatment. *Lancet.* Jul 12 2003;362(9378):103-110.

21. Greving JP, Wermer MJ, Brown RD, Jr., et al. Development of the PHASES score for prediction of risk of rupture of intracranial aneurysms: a pooled analysis of six prospective cohort studies. *The Lancet. Neurology.* Jan 2014;13(1):59-66.

22. Woo D, Khoury J, Haverbusch MM, et al. Smoking and family history and risk of aneurysmal subarachnoid hemorrhage. *Neurology.* Jan 6 2009;72(1):69-72.

23. Investigators UJ, Morita A, Kirino T, et al. The natural course of unruptured cerebral aneurysms in a Japanese cohort. *N Engl J Med.* Jun 28 2012;366(26):2474-2482.

24. Vlak MH, Rinkel GJ, Greebe P, Algra A. Independent risk factors for intracranial aneurysms and their joint effect: a case-control study. *Stroke; a journal of cerebral circulation.* Apr 2013;44(4):984-987.

25. Hasan DM, Mahaney KB, Brown RD, Jr., et al. Aspirin as a promising agent for decreasing incidence of cerebral aneurysm rupture. *Stroke; a journal of cerebral circulation.* Nov 2011;42(11):3156-3162.

26. Etminan N, Beseoglu K, Steiger HJ, Hanggi D. The impact of hypertension and nicotine on the size of ruptured intracranial aneurysms. *Journal of neurology, neurosurgery, and psychiatry.* Jan 2011;82(1):4-7.

27. Tada Y, Wada K, Shimada K, et al. Roles of hypertension in the rupture of intracranial aneurysms. *Stroke; a journal of cerebral circulation.* Feb 2014;45(2):579-586.

28. Gross BA, Rosalind Lai PM, Frerichs KU, Du R. Aspirin and aneurysmal subarachnoid hemorrhage. *World neurosurgery.* Dec 2014;82(6):1127-1130.

29. Schievink WI. Intracranial aneurysms. *N Engl J Med.* Jan 2 1997;336(1):28-40.

30. Wermer MJ, Greebe P, Algra A, Rinkel GJ. Incidence of recurrent subarachnoid hemorrhage after clipping for ruptured intracranial aneurysms. *Stroke; a journal of cerebral circulation.* Nov 2005;36(11):2394-2399.

31. Kapadia A, Schweizer TA, Spears J, Cusimano M, Macdonald RL. Nonaneurysmal Perimesencephalic Subarachnoid Hemorrhage: Diagnosis, Pathophysiology, Clinical Characteristics, and Long-Term Outcome. *World neurosurgery.* Dec 2014;82(6):1131-1143.

32. Kong Y, Zhang JH, Qin X. Perimesencephalic subarachnoid hemorrhage: risk factors, clinical presentations, and outcome. *Acta neurochirurgica. Supplement.* 2011;110(Pt 1):197-201.

33. Sarabia R, Lagares A, Fernandez-Alen JA, et al. Idiopathic subarachnoid hemorrhage: a multicentre series of 220 patients. *Neurocirugia.* Dec 2010;21(6):441-451.

34. Connolly ES, Jr., Mohr JP, Solomon RA. Unruptured intracranial aneurysms. *N Engl J Med.* May 6 1999;340(18):1440-1441; author reply 1441-1442.

35. King JT, Jr., Berlin JA, Flamm ES. Morbidity and mortality from elective surgery for asymptomatic, unruptured, intracranial aneurysms: a meta-analysis. *Journal of neurosurgery.* Dec 1994;81(6):837-842.

36. Forget TR, Jr., Benitez R, Veznedaroglu E, et al. A review of size and location of ruptured intracranial aneurysms. *Neurosurgery.* Dec 2001;49(6):1322-1325; discussion 1325-1326.

37. Unruptured intracranial aneurysms--risk of rupture and risks of surgical intervention. International Study of Unruptured Intracranial Aneurysms Investigators. *N Engl J Med.* Dec 10 1998;339(24):1725-1733.

38. Kataoka K, Taneda M, Asai T, Yamada Y. Difference in nature of ruptured and unruptured cerebral aneurysms. *Lancet.* Jan 15 2000;355(9199):203.

39. Zanaty M, Daou B, Chalouhi N, Starke RM, Jabbour P, Hasan D. Evidence That a Subset of Aneurysms Less Than 7 mm Warrant Treatment. *Journal of the American Heart Association.* Aug 10 2016;5(8).

40. Thompson BG, Brown RD, Jr., Amin-Hanjani S, et al. Guidelines for the Management of Patients With Unruptured Intracranial Aneurysms: A Guideline for Healthcare Professionals From the American Heart Association/American Stroke Association. *Stroke; a journal of cerebral circulation.* Aug 2015;46(8):2368-2400.

41. Juvela S, Poussa K, Porras M. Factors affecting formation and growth of intracranial aneurysms: a long-term follow-up study. *Stroke; a journal of cerebral circulation.* Feb 2001;32(2):485-491.

42. Brinjikji W, Zhu YQ, Lanzino G, et al. Risk Factors for Growth of Intracranial Aneurysms: A Systematic Review and Meta-Analysis. *AJNR. American journal of neuroradiology.* Apr 2016;37(4):615-620.

43. Hasan DM, Hindman BJ, Todd MM. Pressure changes within the sac of human cerebral aneurysms in response to artificially induced transient increases in systemic blood pressure. *Hypertension.* Aug 2015;66(2):324-331.

44. Vergouwen MD, Vermeulen M, de Haan RJ, Levi M, Roos YB. Dihydropyridine calcium antagonists increase fibrinolytic activity: a systematic review. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism.* Jul 2007;27(7):1293-1308.

45. Dasenbrock HH, Yan SC, Gross BA, et al. The impact of aspirin and anticoagulant usage on outcomes after aneurysmal subarachnoid hemorrhage: a Nationwide Inpatient Sample analysis. *Journal of neurosurgery.* Apr 8 2016:1-11.

46. Juvela S. Aspirin and delayed cerebral ischemia after aneurysmal subarachnoid hemorrhage. *Journal of neurosurgery.* Jun 1995;82(6):945-952.

47. Chalouhi N, Starke RM, Correa T, et al. Differential Sex Response to Aspirin in Decreasing Aneurysm Rupture in Humans and Mice. *Hypertension.* Aug 2016;68(2):411-417.

48. Final report on the aspirin component of the ongoing Physicians' Health Study. Steering Committee of the Physicians' Health Study Research Group. *N Engl J Med.* Jul 20 1989;321(3):129-135.

49. Toussaint LG, 3rd, Friedman JA, Wijdicks EF, et al. Influence of aspirin on outcome following aneurysmal subarachnoid hemorrhage. *Journal of neurosurgery.* Dec 2004;101(6):921-925.

50. Hasan DM, Chalouhi N, Jabbour P, et al. Evidence that acetylsalicylic acid attenuates inflammation in the walls of human cerebral aneurysms: preliminary results. *Journal of the American Heart Association.* Feb 22 2013;2(1):e000019.

51. Inagawa T. Risk factors for the formation and rupture of intracranial saccular aneurysms in Shimane, Japan. *World neurosurgery.* Mar 2010;73(3):155-164; discussion e123.

52. Broderick JP, Brott T, Tomsick T, Huster G, Miller R. The risk of subarachnoid and intracerebral hemorrhages in blacks as compared with whites. *N Engl J Med.* Mar 12 1992;326(11):733-736.

53. Brinjikji W, Rabinstein AA, Lanzino G, Cloft HJ. Racial and ethnic disparities in the treatment of unruptured intracranial aneurysms: a study of the Nationwide Inpatient Sample 2001-2009. *Stroke; a journal of cerebral circulation.* Dec 2012;43(12):3200-3206.

Table 1: Characteristics of the cohort and results of univariable analysis

Variable	Ruptured aneurysm	Unruptured aneurysm	OR; 95% CI	p-value
Age, (mean) y	53.2 (12.7 SD)	57.0 (12.2 SD)	0.98; 0.97-0.98	<0.001
Gender			1.0; 0.82-1.22	0.99
Male	514 (29.7%)	180 (29.8%)		
Female	1215 (70.3%)	425 (70.3%)		
Ethnicity				0.002
-White	1484 (87.91)	551 (93.39%)		
- Mixed	26 (1.54%)	5 (0.85%)	1.93; 0.74-5.05	
- Asian	72 (4.27%)	17 (2.88%)	1.57; 0.92-2.69	
- Black	106 (6.28%)	17 (2.88%)	2.32; 1.37-3.90	
Family history				
Positive Family History SAH/UIA	213 (12.3%)	111 (18.4%)	0.63; 0.49-0.80	<0.001
Positive Family History of SAH	191 (11.1%)	98 (16.2%)	0.79; 0.69-0.90	0.001
Positive Family History of UIA	25 (1.5%)	19 (3.1%)	0.66; 0.54-0.81	<0.001
Smoker	765 (44.3%)	228 (37.7%)	1.31; 1.09-1.59	0.005
Drinker	1168 (67.6%)	392 (64.8%)	1.13; 0.93-1.37	0.215
Recreational drug use	99 (5.7%)	20 (3.3%)	1.78; 1.09-2.90	0.02
Medical History				

Hypertension	542 (31.4%)	281 (46.5%)	0.53; 0.44-0.64	<0.001
Hypertension not treated	123 (80.9%)	29 (19.1%)	2.54; 1.65-3.93	<0.001
Antihypertensive Medication	425 (24.6%)	255 (42.2%)	0.45; 0.37-0.54	<0.001
Hypercholesterolemia	350 (20.2%)	218 (36.0%)	0.45; 0.37-0.55	<0.001
Statin medication	273 (15.8%)	192 (31.7%)	0.40; 0.33-0.50	<0.001
Diabetes Mellitus	69 (4.0%)	39 (6.5%)	0.60; 0.40-0.90	0.013
Previous Stroke	40 (2.3%)	72 (11.9%)	0.18; 0.12-0.26	<0.001
Previous ICH	9 (0.5%)	4 (0.7%)	0.79; 0.24-2.56	0.75
History of Myocardial infarction	41 (2.4%)	19 (3.1%)	0.75; 0.43-1.30	0.304
Coronary artery disease (Angina)	33 (1.9%)	25 (4.13%)	0.45; 0.27-0.77	0.002
Cardiac disease (myocardial infarction and angina)	58 (3.4%)	37 (6.1%)	0.53; 0.35-0.81	0.003
Peripheral vascular disease	25 (1.5%)	15 (2.5%)	0.58; 0.30-1.10	0.09
Aspirin	120 (6.94%)	154 (25.45%)	0.22; 0.17-0.28	<0.001
Aneurysm location				<0.001
- MCA	341 (21.04%)	194 (34.34%)		
- ICA	135 (8.33%)	164 (29.03%)	0.47; 0.35-0.62	
- ACA/Acom	572 (35.29%)	93 (16.46%)	3.50; 2.64-4.64	
- Pcom	359 (22.15%)	63 (11.15%)	3.24; 2.35-	

			4.47	
- Posterior circulation	214 (13.20%)	51 (9.03%)	2.39; 1.68-3.40	
Aneurysm size (mean) mm	6.6 (4.16 SD)	9.0 (6.3 SD)	0.91; 0.90-0.93	<0.001
Multiple aneurysms	447 (25.9%)	161 (26.6%)	0.96; 0.78-1.19	0.714

SD = standard deviation; ICH = intracerebral haemorrhage; MCA = middle cerebral artery; ICA = internal carotid artery; ACA = anterior cerebral artery; Acom = anterior communicating artery; Pcom = posterior communicating artery.

For aneurysm location there were 40 missing values in the ruptured and 108 in the unruptured group

For aneurysm size there were 345 missing values in the ruptured and 61 in the unruptured group

Table 2: Multivariable analysis model evaluating differences between unruptured and ruptured intracranial aneurysms

	Odds Ratio	95% CI	*P* value
Age	1.0	0.98-1.01	0.365
Sex (male vs female)	0.86	0.66-1.11	0.242
Ethnicity			0.013
- white (reference)			
- black	2.42	1.29-4.56	
- asian	2.00	0.98-4.08	
- mixed	1.26	0.45-3.53	
Smoker	1.22	0.96-1.56	0.104
Use of antihypertensive medication	0.65	0.49-0.84	0.001
Hypercholesterolemia	0.64	0.48-0.85	0.002
Aspirin use	0.28	0.20-0.40	<0.001
Aneurysm location			
- MCA (reference)			
- ICA	0.53	0.38-0.75	
- ACA/Acom	3.21	2.34-4.40	
- Pcom	3.92	2.67-5.74	
- Posterior circulation	3.12	2.08-4.70	
Aneurysm size	0.76	0.69-0.84	<0.001

MCA = middle cerebral artery; ICA = internal carotid artery; ACA = anterior cerebral artery; Acom = anterior communicating artery; Pcom = posterior communicating artery.

Table 3: Multivariable analysis model, sensitivity analysis including positive family history of SAH or unruptured intracranial aneurysm and previous history of stroke

	Odds Ratio	95% CI	*P* value
Age	1.0	0.98-1.01	0.392
Sex	0.83	0.64-1.08	0.171
Ethnicity			0.012
- white (reference)			
- black	2.41	1.27-4.56	
- asian	2.13	1.02-4.46	
- mixed	1.20	0.43-3.38	
Antihypertensive medication	0.69	0.52-0.90	0.007
Hypercholesterolemia	0.69	0.52-0.92	0.011
Aspirin use	0.32	0.22-0.45	<0.001
Aneurysm location			<0.001
- MCA (reference)			
- ICA	0.533	0.38-0.75	
- ACA/Acom	3.23	2.34-4.44	
- Pcom	4.07	2.75-6.01	
- Posterior circulation	3.12	2.06-4.72	
Aneurysm size	0.76	0.69-0.84	<0.001
Positive Family History	0.61	0.44-0.85	<0.001
Previous Stroke	0.24	0.14-0.41	<0.001

MCA = middle cerebral artery; ICA = internal carotid artery; ACA = anterior cerebral artery; Acom = anterior communicating artery; Pcom = posterior communicating artery.