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ORIGINAL RESEARCH

Secondary Ischemia Assessment in Murine and Rat Preclinical Subarachnoid Hemorrhage Models: A Systematic Review

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BACKGROUND: Delayed cerebral ischemia represents a significant contributor to death and disability following aneurysmal subarachnoid hemorrhage. Although preclinical models have shown promising results, clinical trials have consistently failed to replicate the success of therapeutic strategies. The lack of standardized experimental setups and outcome assessments, particularly regarding secondary vasospastic/ischemic events, may be partly responsible for the translational failure. The study aims to delineate the procedural characteristics and assessment modalities of secondary vasospastic and ischemic events, serving as surrogates for clinically relevant delayed cerebral ischemia, in recent rat and murine subarachnoid hemorrhage models.

METHODS AND RESULTS: We conducted a systematic review of rat and murine in vivo subarachnoid hemorrhage studies (published: 2016–2020) using delayed cerebral ischemia/vasospasm as outcome parameters. Our analysis included 102 eligible studies. In murine studies (n=30), the endovascular perforation model was predominantly used, while rat studies primarily employed intracisternal blood injection to mimic subarachnoid hemorrhage. Particularly, the injection models exhibited considerable variation in injection volume, rate, and cerebrospinal fluid withdrawal. Peri-interventional monitoring was generally inadequately reported across all models, with body temperature and blood pressure being the most frequently documented parameters (62% and 34%, respectively). Vasospastic events were mainly assessed through microscopy of large cerebral arteries. In 90% of the rat and 86% of the murine studies, only male animals were used.

CONCLUSIONS: Our study underscores the substantial heterogeneity in procedural characteristics and outcome assessments of experimental subarachnoid hemorrhage research. To address these challenges, drafting guidelines for standardization and ensuring rigorous control of methodological and experimental quality by funders and journals are essential.

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Aneurysmal subarachnoid hemorrhage (aSAH) remains a stroke subtype with particularly poor outcomes. More than 10% of patients die before reaching the hospital, and, despite advances in treatment regimens, the case-fatality rate remains strikingly high, at ≈35%.[1,2] More than one third of survivors report poor quality of life due to permanent physical or mental disabilities.[3] Although aSAH makes up only about 5% of total strokes, considering the bad outcome rates and the relatively young age of those affected, aSAH contributes to a large number of stroke-related deaths and disability-adjusted life-years on the global scale.[4,5]

Apart from the acute bleeding event, secondary ischemic complications have an extraordinary

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RESEARCH PERSPECTIVE

What Is New?

- We provide data on the substantial heterogeneity in experimental subarachnoid hemorrhage research (including procedural characteristics, but also outcome assessment).
- Standardized definition of outcome parameters as well as certain procedural aspects may improve scientific quality.

What Question Should Be Addressed Next?

- Particularly with respect to detection of secondary ischemia, reliable models for preclinical research are urgently needed.

Nonstandard Abbreviations and Acronyms

aSAH	aneurysmal subarachnoid hemorrhage
CBF	cerebral blood flow
DCI	delayed cerebral ischemia

influence on clinical outcome: Delayed cerebral ischemia (DCI) and vasospasm are the main determinants of death and morbidity within the subacute phase after aSAH.[6] Despite extensive preclinical research, attempts to reproduce an improvement in outcomes have failed in clinical trials. In this regard, clazosentan is one of the most famous therapeutics with promising preclinical results, which finally failed to prove clinical consistency.[7] However, there are recent data that the lack of therapeutic success may have resulted from underdosing the medication.[8]

Lack of clear definitions for DCI and vasospasm may be partly responsible for creating challenges in clinical translation. While DCI represents an umbrella term for secondary ischemic complications following subarachnoid hemorrhage (SAH), vasospasm is commonly defined as the pathological constriction of cerebral arteries, which then leads to ischemia. As terms are often used synonymously and vasospasm itself is referred to as a main cause of DCI, terminology appears to be indistinct.[9] To overcome previous impreciseness, a multidisciplinary research team proposed a clinical definition for DCI in 2010 with cerebral infarction in computed tomography, magnetic resonance imaging, or autopsy and neurological impairment being the 2 obligatory outcome measures.[10] Vasospasm could also be an outcome parameter if "interpreted in conjunction with DCI or functional outcome." The "old" hypothesis of DCI because of vasospasm has also been challenged by various studies showing failure in DCI prevention despite successful vasospasm treatment.[7,11] Currently, a multifactorial cause is commonly proposed: DCI is thought to be the result of a multitude of pathophysiological events such as macro- and microscopic vasospasm, neuroinflammation, and microthrombosis.[6,12]

With regard to the experimental setting, there are even more issues. The clinically predominant term of DCI is defined by a neurological deterioration, which sometimes is hardly detectable in animals. Therefore, we adhere to the suggestions of van Lieshout et al and define the pathology to be detected as "secondary ischemia."[13] However, in addition to terminological inaccuracy, heterogeneous examination of secondary ischemia may lead to failure in clinical translation. Although there has been a significant standardization in some procedural aspects, with the vast majority of later research using mice or rats for trials, experimental setup still may diverge drastically.[14,15] Over the past decades, experimental SAH has mainly been induced by endovascular perforation of large intracerebral arteries or by intracisternal blood injection.[16,17] However, the models used differ fundamentally between each other, and even within the same method, there are many variables that may influence outcome parameters. Differences in procedural characteristics, such as filament caliber (perforation models) or injection locus, volume and frequency (injection models) contribute to challenges in comparing results. Other variables that affect outcomes include animal characteristics, configuration of experimental groups, anesthesia, perinterventional monitoring, confirmation of SAH, sham procedure, and outcome assessment.

Only some studies have examined characteristics in experimental SAH research. Although findings from these studies suggest a trend toward rat or mice and an endovascular or injection model, yet there are few studies, which exclusively assess current literature with particular regard to secondary ischemia examination. Of note, most of the studies are >10 years old.[14,18,19] All of them, to a certain extent, addressed the lack of reliability with respect to the reproduction of DCI. Recently, Oka et al analyzed preclinical SAH studies with regard to the models´ propensity to induce DCI, with sobering results.[20] Despite the critical data, historical models continue to be used to examine correlates of DCI without questioning the adequateness of the specific model. Both scientific and ethical concerns (concerning the justification of animal experiments) support this fact.

Here, we analyze the recent literature on experimental rat and murine SAH models examining events

of secondary ischemia and focus on the heterogeneity in modeling but also in defining the pathology and the related technical details, such as the specific point in time of the assessment. The purpose of this study was to display procedural characteristics of rat and murine in vivo SAH models for secondary ischemia assessment in the recent literature.

METHODS

Strengthening the Reporting of Observational Studies in Epidemiology (https://www.strobe-statement.org/) were implemented creating this article.[21] Before data analysis, our study was registered at the International Prospective Register of Systematic Reviews (https://www.crd.york.ac.uk/prospero/; CRD42022337279). No ethics approval was required, as only data from animal studies were secondarily analyzed. Data are available from the corresponding author upon request.

Search Strategy

We searched PubMed and Scopus using the following search terms: ("subarachnoid hemorrhage" OR SAH) AND (rat OR mouse OR mice OR murine) AND (vasospasm OR DCI OR "delayed ischemic deficit" OR DIND OR "delayed ischemic neurologic deficit"). The search terms were defined by a prior screening of the data banks. Further, advanced search strategies broadened the search terms, allowing a comprehensive analysis. A time filter was set for January 2016 to December 2020.

Eligibility Criteria

Only articles that met the following predefined inclusion criteria were considered for further analysis:

- In vivo induction of SAH
- Rat or mouse
- Secondary ischemia/vasospasm/ DCI as an outcome parameter
- Original research article
- Published 2016–2020
- Written in English

Literature Selection

Initially, duplicates were identified. Publications were screened by title and abstract. The first author (E.F.) and last author (A.H.) screened articles independently and subsequently compared results. Full texts of matching articles underwent further analyses. Any discrepancies in literature selection were discussed, and the authors mutually decided on inclusion or exclusion of studies.

Data Extraction

E.F. extracted the data, which were thereafter validated by A.H. Parameters would be considered not reported if it was not clearly stated in the article or not conducted if only a reference without further context was provided. For each study, baseline data such as SAH induction model, strain, group size, sex, weight, and age of animals were collected. Additionally, impact factors of journals were determined using *Journal Citation Reports* (Clarivate, 2022). The country of publication was defined by the last author's country.

The most frequent models (n>5) were selected for more detailed analyses. For the perforation models, we documented type and caliber of perforation device. Injection models were divided into single and double injection and data concerning injection locus, volume, and type of blood were extracted. Speed of injection (μl/min), relative injection volume (μL/g body weight), and ratio of cerebrospinal fluid extracted to blood volume injected were calculated using numbers given in the articles. Specifics on anesthesia, peri-interventional monitoring, methods, and time point for SAH-confirmation were gathered. For outcome assessment, we documented details on method, parameter, locus, and time point of examination as well as evaluation of neurological status. Neurological tests were assigned into 3 categories: sensorimotor, memory and orientation, or general condition.

We defined *secondary ischemia* as the umbrella term for many vasospastic and ischemic events assessed in the studies analyzed. Although *secondary ischemia* does not represent an established generic term, we use the term to consolidate the various differently named outcome parameters.

Statistical Analysis

Categorial variables are represented as total number of reports and corresponding percentages. For continuous variables, means with standard deviation were calculated. All data analyses and graphs were created using GraphPad Prism version 9.4.1 for Windows (GraphPad Software, San Diego, CA; www.graphpad.com).

RESULTS

Search Results

Initially, 345 articles were found in PubMed and Scopus. After identification of duplicates, we screened 195 abstracts. Afterwards, 127 publications were screened in full text, of which 102 proved eligible. The literature selection process and reasons for exclusion of studies are depicted in Figure 1.

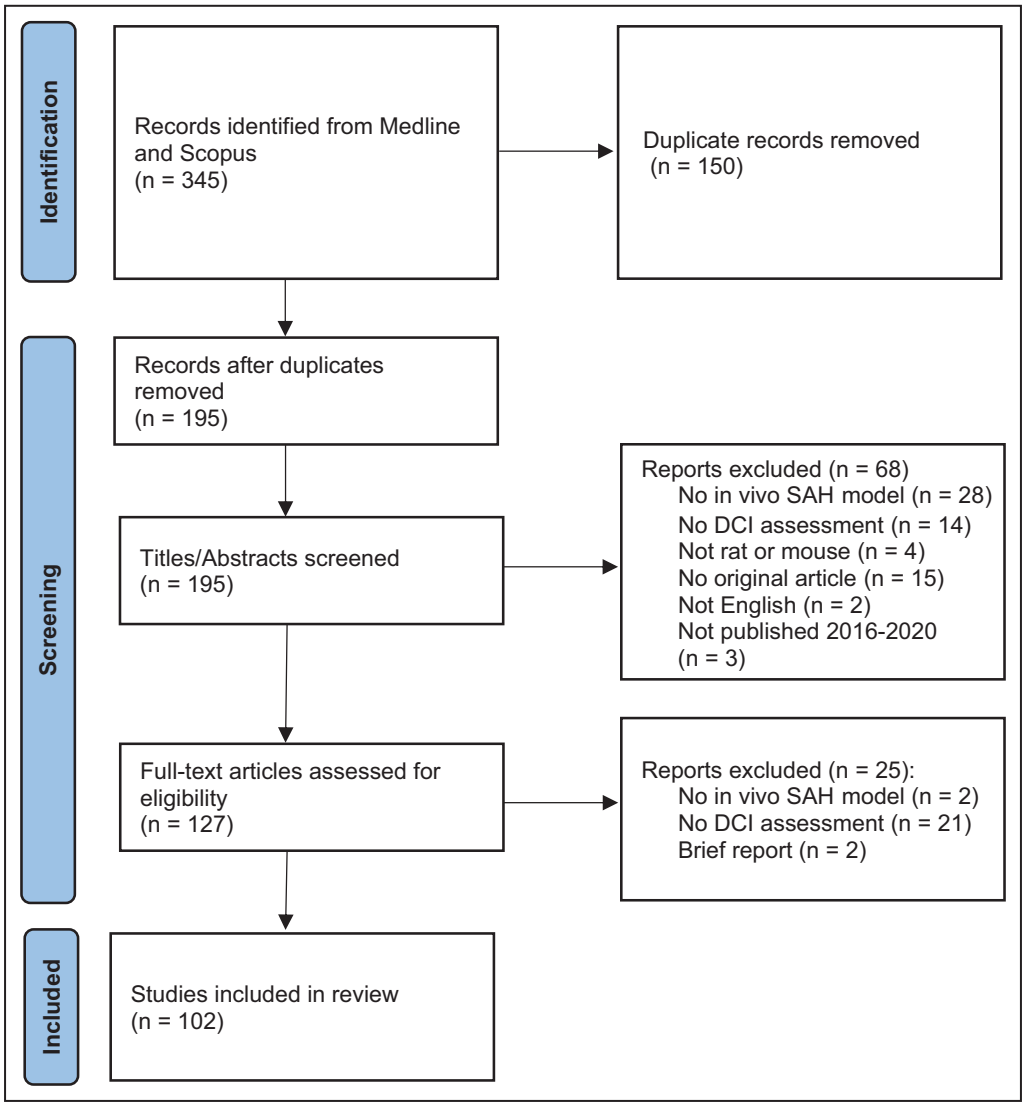

Figure 1. Systematic review flow diagram.
DCI indicates delayed cerebral ischemia; and SAH, subarachnoid hemorrhage.

Publication Data

Most studies included in our review were published by Asian research teams (66.7%), with China providing almost 40% of all publications. The mean impact factor was 3.5 (±1.9).

Basic Experimental Characteristics

Of 102 trials, 71% (n=72) used rat and 29% (n=30) murine models. Most popular strains were Sprague–Dawley rats (86% of the rat models) and C57BL mice (97% of the murine models). The vast majority of research groups used male animals for experiments. Only 10% of rat and 14% of mouse studies used female animals or mixed groups. Most rat studies used animals ranging from 200 to 350 g, and the majority of mice weighed 20 to 30 g. The mean weight range was 62.6 (±41.5 g) in rats and 4.4 (±1.5 g) in mice. For exact numbers and percentages, see Table 1.

We identified 4 categories of SAH induction models: endovascular perforation of intracerebral arteries, single or double intracisternal injection, and other methods. In rat studies, SAH was most commonly mimicked by intracisternal blood injection: 38% (n=27) of studies performed double injection, 35% (n=25) single injection, and 24% (n=17) endovascular perforation. On the contrary, in 63% (n=19) of murine experiments, SAH was induced via endovascular perforation and only 23% (n=7) by single injection. Less common methods were combinations of unilateral common carotid artery occlusion with either endovascular perforation (n=2) or double injection (n=1) in rats and laser puncture of pial arterioles (n=1), puncture of cisterna magna veins (n=1), and the double injection model (n=1) in mouse studies.

Table 1. Basic Experimental Characteristics of Included Studies

Parameter	Category	Species	
		Rat	Mouse
		n (%)*	
Number of studies		72 (70.6)	30 (29.4)
Strain	Reported	72 (100)	30 (100)
	Sprague–Dawley	62 (86.1)	…
	Wistar	10 (13.9)	…
	C57BL/6	…	29 (96.7)
	FVB	…	1 (3.3)
Weight	Reported	70 (97.2)	15 (50)
	Most frequent weight range		
	200–350 g	50 (71.4)	…
	20–30 g	…	13 (86.7)
	Mean range±SD†	62.59±41.5	4.4±1.45
Number of animals per study	Reported	62 (86.1)	18 (60)
	Mean±SD	68.6±49	57.5±38.6
Sex	Reported	69 (95.8)	28 (93.3)
	Male	62 (89.9)	24 (85.7)
	Female	4 (5.8)	2 (7.1)
	Mixed groups	3 (4.4)	2 (7.1)
Model	Endovascular	17 (23.6)	19 (63.3)
	Single injection	25 (34.7)	7 (23.3)
	Double injection	27 (37.5)	1 (3.3)
	Other	3 (4.2)	3 (10)

C57BL/6=C57 black 6. FVB indicates Friend virus B.

*Percentages for reporting numbers are represented as proportion of total number of studies included for each species. Percentages for specific characteristics are represented as proportion of reporting numbers.

†Difference between heaviest and lightest animal per study. Weight range was considered 0 if the mean weight of animals was documented.

There were only male animals in 90% of the rat studies and 86% of the murine models. None of the studies that included female animals provided information on the hormonal status, previous ovariectomy, or peri-interventional estrous cycle.

Anesthesia and Peri-Interventional Monitoring

In endovascular perforation models, about half of reported anesthesia was performed using anesthetic gas. Combinations of dissociative anesthetics with an α_2-adrenergic agonist, opioid, or benzodiazepine were most commonly administered to animals undergoing intracisternal injection. Other frequent substances used in injection models included anesthetic gas, chloral hydrate, and barbiturates (for details, please see Table S1).

Reporting of peri-interventional monitoring was poor throughout all species and models. In murine endovascular studies, at least 1 monitoring parameter was reported in 69% of studies. Other models had reporting rates <50%. Of all injection and endovascular models reporting peri-interventional monitoring, body temperature (62.2%) and blood pressure (34%) were the most frequently documented parameters. For details, please see Table 2.

Procedural Characteristics of Endovascular Perforation Models

Seventy-one percent of the rat studies reported the filament type used. Of those, 75% employed nylon or prolene monofilaments. In rat experiments, the filament caliber was 4–0 in 90% of the procedures. In all of the mouse studies reporting filament material, nylon or prolene monofilaments were used for perforation. The filament caliber was 5–0 in 87% of reported cases.

Almost 90% of rat and murine studies reported some sort of sham procedure. Except for 2 mouse experiments, all sham procedures were done by endovascular intervention without perforation of intracerebral arteries.

Verification of SAH following the endovascular procedure was documented in 82% of the rat and 84% of the mouse studies. Mostly, SAH was confirmed by postmortem identification of abnormal blood volume and blood clots in the basal cisterns. Other methods for SAH verification included decrease in cerebral blood flow (CBF) and increase in intracerebral pressure immediately after endovascular perforation (see Table 2). In only 5 of 19 studies using the endovascular murine model and 4 of 17 studies applying the rat endovascular model were peri-interventional measures to assure the severity of SAH (in terms of intracerebral pressure rise and CBF decrease) taken.

Procedural Characteristics of Injection Models

In rat models, the injection locus was predominantly the cisterna magna (89% in the double-injection model, 64% in the single-injection model). The remaining injections were carried out in the chiasmatic cisterns. In rat experiments, all injections were performed using autologous blood. In murine models, both the cisterna magna and chiasmatic cisterns were equally popular injection sites. However, unlike in rat studies, 71% of mice that received a single injection were given donor blood.

The ratio of injected blood volume relative to body weight (μL/g) was either reported or could be calculated in 92% of single-injection rat models and 89% of double-injection rat models. The most common ratio administered intracisternally ranged from ≈0.5 to 1.0 μL blood/g body weight, accounting for 61% of single

Table 2. Procedural Characteristics of Rat and Murine Endovascular Perforations Models of SAH

		Model	
		Rat endovascular (n=17)	Mouse endovascular (n=19)
Parameter	Category	n (%)*	
Filament type	Reported	12 (70.6)	15 (79)
	Nylon or prolene monofilament	9 (75)	15 (100)
	Wire	3 (25)	
Caliber of nylon and prolene monofilaments	Reported	9 (100)	15 (100)
	3–0	1 (11.1)	
	4–0	8 (88.9)	1 (6.7)
	5–0		13 (86.7)
	6–0		1 (6.7)
Sham procedure	Reported	15 (88.2)	17 (89.5)
	Endovascular intervention without perforation of intracerebral arteries	15 (100)	15 (88.2)
	Without further description		2 (11.8)
Verification of SAH†	Reported	14 (82.4)	16 (84.2)
	Postmortem inspection‡	12 (85.7)	9 (56.3)
	Intraoperative CBF-decrease	4 (28.6)	3 (18.8)
	Intraoperative ICP-increase	1 (7.1)	4 (25)
	Postinterventional MRI		3 (18.8)
	Without further description		1 (6.3)

CBF indicates cerebral blood flow; ICP, intracranial pressure; MRI, magnetic resonance imaging; and SAH, subarachnoid hemorrhage.

*Percentages for reporting numbers are represented as proportion of total number of studies included for each model. Percentages for specific characteristics are represented as proportion of reporting numbers.

†Parameter includes multiple mentions.

‡Macro- or microscopic assessment of abnormal blood and clot formation in subarachnoid spaces.

injections and 73% of double injections. However, a significant proportion of studies used a higher blood volume per body weight: 26% of single injection and 18% of double injection rat models had a blood-to-body weight ratio of 1 to 1.5.

Cerebrospinal fluid was withdrawn before blood injection in 44% of rat single injections, 56% of rat double injections, and in none of the mouse injection models. In most cases, less cerebrospinal fluid was withdrawn than blood injected into the cisterns. Nevertheless, 50% of rat single-injection and 27% of rat double-injection studies reporting cerebrospinal fluid withdrawal took equal or larger amounts of cerebrospinal fluid than injected blood volume.

The second injections in the rat double hemorrhage models were carried out 1 (31%) or 2 days (69%) after the initial surgery.

Sham surgeries were reported in around two thirds of rat injection models. Close to 90% of murine injection models documented sham procedures. Intracisternal injection of saline in equal amounts of blood volume in the SAH groups was the most common sham method throughout all species. Of those studies reporting sham procedures, surgical opening of the cistern without injection was performed in almost 40% of rat single injections, 25% of rat double injections, and 50% of mouse single injections. For details, please see Table S2. Information on postoperative analgesia was provided in only 10 studies (for details, see Table S3).

Assessment of Secondary Ischemia Following Experimental SAH

In most studies, the representative surrogate parameters determined were cerebral artery diameter and wall thickness. Most commonly, the basilar artery was used for measurements of the vessel parameters. The prevailing method throughout all models was microscopy, which was carried out post mortem in most studies. Only in rat injection models, contractility challenges and verification of ischemia through CBF-measurements or imaging studies were other frequently reported methods. Additionally, some studies reported rare methods for ischemia verification, such as tissue oxygen pressure measurement, single photon emission computed tomography, and digital subtraction angiography (each mentioned once).

The point in time of outcome assessment was quite heterogeneous. In endovascular models and rat injection studies, most measurements took place between

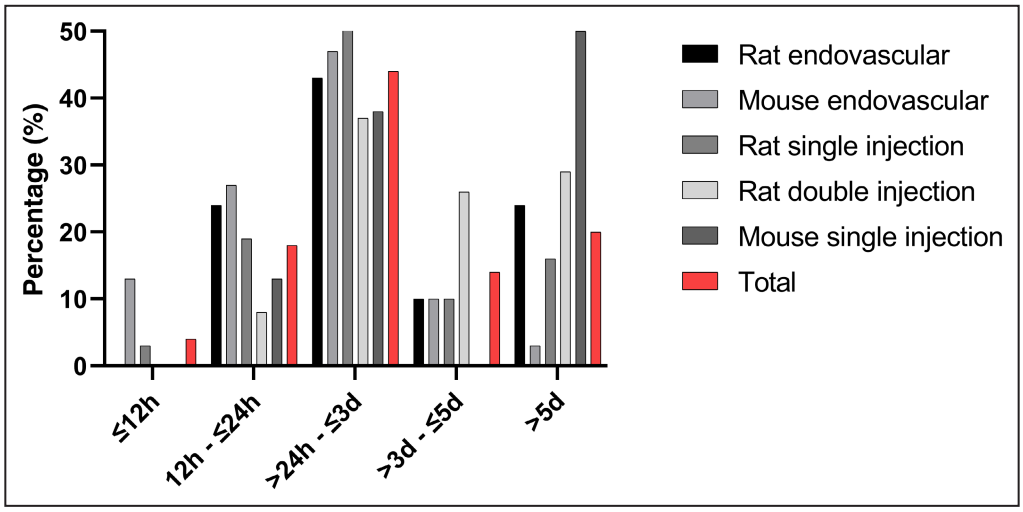

Figure 2. Point in time of assessment of ischemic complications following SAH.
SAH indicates subarachnoid hemorrhage.

days 1 and 3 after SAH. In contrast, in 25% of the endovascular models, measures with respect to secondary ischemia were carried out 12 to 24 hours after SAH. However, the time of outcome assessment varied widely with far later time points: 50% of measurements in the murine single-injection, 29% in the rat double-injection, 24% in the rat endovascular, and 16% in the rat single-injection model were carried out after >5 days after SAH (for details, please see Figure 2 and Table 3).

Neurological examination of animals was reported in 76.5% of endovascular rat and 84.2% of endovascular mouse studies. Injection models had poorer reporting rates with 56% of rat single-injection, 44.4% of rat double-injection, and 57.1% of mouse single-injection studies documenting neurological assessment. Among all models, most popular tests were the ones assessing sensorimotor function. Only few studies carried out tests for memory and orientation.

DISCUSSION

Our data demonstrate that techniques for induction of experimental SAH vary largely. Further, the experimental end points (particularly with respect to assessment of secondary ischemia) are heterogeneous. Even the type of surrogate parameter under consideration differs substantially; morphological criteria such as vessel diameter or vessel wall thickness but also imaging measures or neuromonitoring serve as substitutes for secondary ischemia. Time of end point assessment for secondary ischemia oscillated widely even within the same species/method of induction.

Further, basic procedural characteristics of SAH induction differed significantly particularly in rat and murine injection models. Throughout all models, reporting of peri-interventional monitoring was poor, and anesthesia was conducted with a multitude of different substances.

There are only few current systematic reviews examining experimental characteristics in preclinical SAH research. In a 2018 study, Marbacher et al analyzed in vivo models of SAH, which assessed early brain injury or delayed cerebral vasospasm published 2000 to 2014.[15] Goursaud et al published a systematic review of rat and murine in vivo SAH models assessing vasospasm, cerebral ischemia, or neurological impairment in 2021.[16] However, the number of articles analyzed is lower compared with our sample (only 1 databank search, exclusion of studies with therapeutic agents) and fewer procedural details have been assessed. In accordance with the results of both reviews but also in line with previous data,[14–16,18,19] we documented a wide heterogeneity of models and specific techniques and methods (and point in time) of outcome assessment. Furthermore, the definitions of primary end points related to secondary ischemia exhibit substantial differences. The most common surrogate parameter for vasospastic events/secondary ischemia were morphological criteria such as vessel wall diameter or thickness. In contrast, the clinical definition for DCI constantly shifts toward a functionally dominated term.

Female or mixed-sex groups were severely underrepresented in both our and Goursaud's review (11% and 0%). Those findings indicate a high risk of sex bias in preclinical SAH research. Given the fact that aSAH is more common in women,[22] it is astonishing that the overwhelming majority of animals used are male. The phenomenon of male experimental groups is common in neuroscience, resulting in substantial experimental bias.[23,24] Furthermore, SAH has a sex-dependent aspect, as demonstrated in both clinical and experimental data.[25,26] Certainly, the use of

Table 3. Assessment of Secondary Ischemic Events in Rat and Murine Models of SAH

		Model				
		Rat endovascular	Mouse endovascular	Rat single injection	Rat double injection	Mouse single injection
		(n=17)	(n=19)	(n=25)	(n=27)	(n=7)
Parameter	Category	n (%)*				
Outcome parameter†	Reported	17 (100)	19 (100)	25 (100)	27 (100)	7 (100)
	Diameter, cross-sectional area, circumference	12 (70)	11 (57.9)	15 (60)	22 (81.5)	3 (42.9)
	Wall thickness	6 (35.3)	1 (5.3)	9 (36)	9 (33.3)	1 (14.3)
	Wall thickness/diameter ratio and other complex calculations	1 (5.9)	3 (15.8)			4 (57.1)
	Microthrombi	3 (17.6)	2 (10.5)	1 (4)		
	Cerebral infarction/ hypoperfusion/hypoxia‡	1 (5.9)	6 (31.6)	3 (12)	3 (11.1)	1 (14.3)
	Vessel contractility	2 (11.8)		5 (20)	7 (25.9)	
Region of interest†	Reported	17 (100)	19 (100)	25 (100)	27 (100)	7 (100)
	Basilar artery	13 (76.5)	4 (21.1)	17 (68)	26 (96.3)	1 (14.3)
	Medial cerebral artery	2 (11.8)	6 (66.7)	2 (8)		4 (57.1)
	Anterior cerebral artery			3 (12)		
	Posterior cerebral artery			1 (4)		
	Multiple locations of anterior circulation	3 (17.6)	5 (26.3)	4 (16)	1 (3.7)	1 (14.3)
	Multiple locations of posterior circulation	1 (5.9)				
	Arterioles	1 (5.9)	1 (5.3)	2 (8)	4 (14.8)	1 (14.3)
	Capillaries		1 (5.3)			1 (14.3)
	Whole brain scan, brain slices, parenchyma surface	3 (17.6)	7 (36.8)	3 (12)	3 (11.1)	
Assessment method†	Reported	17 (100)	19 (100)	25 (100)	27 (100)	7 (100)
	In vivo	1 (5.9)	6 (31.6)	2 (8)	2 (7.4)	1 (14.3)
	Post mortem	13 (76.5)	11 (57.9)	20 (80)	22 (81.5)	6 (85.7)
	In vivo and post mortem	3 (17.6)	2 (10.5)	3 (12)	3 (11.1)	
	Microscopy	16 (94.1)	13 (68.4)	18 (72)	21 (77.8)	7 (100)
	MRI	1 (5.9)	4 (21.1)	1 (4)	3 (11.1)	
	CT		2 (10.5)	1 (4)		
	SPECT				1 (3.7)	
	Ultrasound			1 (4)		
	Contractility challenge	3 (17.6)	1 (5.3)	5 (20)	8 (29.6)	
	CBF	2 (11.8)	1 (5.3)	1 (4)		
	DSA		1 (5.3)			
	ptO_2			1 (4)		
Neuroscore†	Reported	13 (76.5)	16 (84.2)	14 (56)	12 (44.4)	4 (57.1)
	Sensorimotor	13 (100)	16 (100)	11 (78.6)	9 (75)	1 (25)
	Memory and orientation	2 (15.4)			1 (8.3)	2 (50)
	General condition	1 (7.7)	5 (31.3)	5 (35.7)	3 (25)	1 (25)

CBF indicates cerebral blood flow; CT, computed tomography; DSA, digital subtraction angiography; MRI, magnetic resonance imaging; ptO_2, tissue partial oxygen pressure; SAH, subarachnoid hemorrhage; and SPECT, single photon emission computed tomography.

*Percentages for reporting numbers are represented as proportion of total number of studies included for each model. Percentages for specific characteristics are represented as proportion of reporting numbers.

†Includes multiple mentions.

‡Via cerebral imaging, CBF, or ptO_2.

mixed-sex groups requires thoughtful planning of the experiments considering the final intention of analysis (inclusion of both sexes versus sex-specific analysis with respect to specific outcomes).[27] However, there is sufficient data that outcome variability is not only influenced by sex and may rather be susceptible to other influences.[28,29]

Species-adapted filament caliber for endovascular perforation seems to be largely standardized. The dominating caliber was 4–0 in rat and 5–0 in murine models in both our study and in the review from Marbacher and colleagues. As endovascular perforation of intracerebral arteries is necessarily carried out "blindly," this process strongly depends on the experimenter and potential anatomic variants in animals, leaving filament caliber and material as one of only a few standardizable procedural aspects in endovascular perforation models of SAH. Injection models offer a large number of potentially modifiable procedural aspects. We found relative injection volume to be heterogeneous, which might influence the severity of injury and early and delayed cerebral injury by differences in intracerebral pressure and CBF levels.[30]

In the study of Goursaud et al and in our examination, most frequently documented monitoring parameters were blood pressure and body temperature. Of note, <50% of studies in our review explicitly reported peri-interventional monitoring, although the importance of physiological monitoring is already mentioned in guidelines like Stroke Therapy Academic Industry Roundtable.[31] Basic monitoring provides information about animals' physiological reaction to anesthesia and surgical trauma in the peri-interventional phase, thus giving hints about possible complications deriving directly from the intervention rather than from the induced pathology. In line with experimental ischemic stroke experiments, sophisticated neurovascular monitoring such as CBF and intracerebral pressure measurements plays a crucial role in detecting the severity of the induced pathology while ruling out associated complications (such as ischemia).[32]

Furthermore, we identified 12 different combinations of anesthetic substances, with a slight trend toward inhaled anesthetic agents. Marbacher et al previously highlighted the wide range of anesthetic protocols used in SAH experiments. The choice of anesthetic agents can significantly influence (patho)physiology, with some substances even demonstrating neuroprotective properties following experimental SAH.[33,34] Notably, chloral hydrate was frequently used as the only substance for anesthesia induction in a significant number of studies. However, chloral hydrate does not provide sufficient analgesia in small animals; thus, its use as a sole agent should be omitted.[35–37] To optimize comparability of preclinical results and minimize animal suffering, there is an urgent need for standardization of anesthetic protocols.

Our study reveals that indicators of secondary ischemia are most commonly assessed by measuring the characteristics of large intracerebral vessels (eg, diameter, wall thickness) using postmortem microscopy. This indicates that cerebral vasospasm continues to be extensively investigated in secondary ischemia research. Interestingly, there is a growing focus on other pathologies such as microthrombi or cerebral infarction in preclinical SAH research. The wide range of experimental outcomes concerning secondary ischemia complicates the interpretation of results. Furthermore, most of the assessed parameters do not capture the multifactorial pathogenesis of secondary ischemia, and their applicability to the human setting remains uncertain.

We observed a significant heterogeneity in the timing of outcome assessment related to ischemic or vasospastic events in preclinical SAH models. Even when analyzed separately, no particular point in time emerged as dominant in any specific species or model. It is evident that examining the pathology at different points in time after SAH can significantly affect the results concerning the severity of secondary brain injury and treatment efficacy. It is therefore essential to agree upon a universally applicable definition that represents the outcome parameters for secondary ischemic events after SAH to make studies/results comparable.

The lack of specific neurological assessments could pose ongoing challenges in clinical translation. Our research, along with the studies conducted by Marbacher and Goursaud, highlights the relatively low rates of standardized neurological examinations conducted before and during the subacute phase after SAH.

Numerous preclinical studies offer a valuable opportunity to enhance our understanding of the pathophysiology and treatment approaches for SAH. However, our study reveals significant differences with respect to methodological and procedural characteristics. This lack of standardization poses several challenges:

First (and maybe most importantly), it must be assured that the models used actually do represent the pathology to be examined. In other words, if the clinical phenomenon DCI is the target of treatment, an adequate pathology must be evoked by the model. Oka er al[20] have nicely shown that commonly used animal models (with exception of primates) do not represent the full course and spectrum of complications after SAH, which is seen in clinics. Therefore, the essential conclusion based on these data is that our current modeling is not appropriate at all.

Second, substantial differences in experimental setup and end point assessment among the studies hinders an adequate comparability of preclinical data.

Systematic reviews and meta-analyses heavily rely on scientifically reliable inclusion and exclusion criteria. The enormous methodological heterogeneity limits the interpretation and comparability of experimental results.

Third, in examining the experimental surrogate of DCI, there persists inconsistency in the definition of pathology. The variation of end point definition was widely differing in the type of end point assessed (mechanistic surrogates such as vessel diameter, but also imaging results showing ischemia) and the point in time of assessment.

In summary, the lack of standardization in experimental SAH research poses challenges for comparability, consistency in definition, and ethical considerations in preclinical studies. Translational failure may at least partly result from experimental heterogeneity. Particularly concerning secondary ischemic events after SAH (with the aim to analyze correlates for the clinical term *DCI*), there are substantial doubts if the experimental models available do reflect the further course after SAH in humans at all.[20] Although recent data suggest the occurrence of DCI in the mouse model, this remains a single report with a low case number.[38] It is based, among other things, on broadly technical issues, such as the lack of replication of the clinical phenomenon DCI, as well as the fundamental difficulty of detecting subtle neurological changes in animals, which, as per the clinical definition, is often nearly impossible. Therefore, it is essential to develop alternative models, particularly if the end points of interest do not relate to the acute impairment after SAH.[14,20] Thus, on the one hand, it is a scientific necessity to reconsider the experimental modeling of SAH. On the other hand, it poses an important ethical challenge regarding the fundamental justification of animal experiments and the strain animals undergo during these trials. For instance, considering the high morbidity and death associated with the endovascular perforation model. While the use of organoids in the future may present an alternative strategy, currently, they do not enable a sophisticated analysis of the complex interactions following SAH. Organoids lack crucial features such as cerebral cytoarchitecture and the blood–brain barrier. Consequently, they cannot be considered adequate substitutes for intact, interacting brain tissue.[39,40] However, despite the current absence of a perfect preclinical model to comprehensively analyze SAH (with perhaps the exception of primate models), it is the task of the scientific community to assess the significance of the deployment for various questions, define reasonable end points/readouts, acknowledge weaknesses in the models, and explore new ways to either enhance existing models or develop alternative strategies. Maintaining the status quo, that is, simply continuing with familiar methods, is certainly the least favorable approach from both scientific and ethical perspectives.

Our study has certain limitations. First, we included only research published between 2016 and 2020 in our review. Therefore, our study can only offer an overview of current preclinical SAH research within that specific time frame. The purpose of the temporal limitation was to offer an update on the existing data. Second, only rat and murine studies have been analyzed. Third, the term *secondary ischemia* was used in our study as an umbrella term for many outcome parameters concerning vasospastic and ischemic events.

In conclusion, based on our data but also those of others published more than a decade ago, preclinical murine and rat SAH models lack standardization, and important methodological/procedural aspects (such as peri-interventional monitoring and the inclusion of female animals) are neglected widely. In other words, there is a problem of research rigor. Similar to ischemic stroke research, the adoption of guidelines like the Stroke Therapy Academic Industry Roundtable, Ischaemia Models: Procedural Refinements of In Vivo Experiments, or Animal Research: Reporting of In Vivo Experiments can greatly enhance the quality standards in the preclinical setting.[41–43] A newer initiative addressing both the academic and industrial setting is Enhancing Quality in Preclinical Data (https://quality-preclinical-data.eu/); it provides a broad range of recommendations to improve preclinical research rigor.[44,45] Additionally, specific quality standards for experimental SAH (eg, reporting of severity assessment, application of peri-interventional monitoring) and adequate end points (as well as the exact time of assessment) must be defined in a consensus statement by the community. This must be an effort of the entire community to reflect a consensus rather than individual opinions. Nevertheless, we address some topics for discussion in Data S2. A standardization by no means constitutes a limitation of scientific flexibility; instead, it provides a guideline that is considered the best standard in the community. Deviations from this guideline are naturally possible, but the pressure to provide an adequate explanation for the specific deviation is increased. To express it more positively, a guideline published by the stakeholders in the field may serve as a positive incentive. Finally, it must be stated clearly that most current rodent models do not adequately reflect the clinical phenomenon of DCI. Thus, extrapolations from a preclinical rodent model with respect to DCI must be interpreted critically. We conclude this line of thought with a quote: "Animal research without scientific value is unethical."[46]

CONCLUSIONS

In preclinical models examining secondary ischemia following SAH, there is significant heterogeneity in the

methods of SAH induction and outcome assessment. Additionally, there is inadequate reporting of peri-interventional monitoring and a wide variation in anesthesia protocols. This lack of standardization poses ongoing challenges in the translation of findings to the clinical setting. Therefore, there is a critical need for standardization in preclinical models of SAH research but also the necessity to enhance overall scientific rigor.

ARTICLE INFORMATION

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EF wrote the first draft of the manuscript, analyzed the data, designed the tables, revised the manuscript; UL helped with data interpretation, reviewed the first version of the manuscript critically; HK helped with data interpretation, reviewed the first version of the manuscript critically; AH designed the study, validated the assessed data, checked the analyses, reviewed the first version of the manuscript critically, revised the manuscript.

Supplemental Material

Data S1–S2
Tables S1–S3

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