Systematic review and meta-analysis of intracranial in-vivo animal studies of early brain injury (EBI) and delayed cerebral vasospasm (DCVS) after subarachnoid haemorrhage (SAH).

Aneurysmal subarachnoid haemorrhage (SAH) is a life threatening condition leading to stroke, permanent neurological damage, and death. Every second patient suffers permanent disability, and estimated lifetime costs are more than double those of an ischemic stroke [1]. For many decades, poor outcome was attributed to delayed cerebral vasospasm (DCVS). In accordance with the paradigm that SAH gives rise to DCVS, which in turn provokes cerebral ischemia, research efforts have focused on attenuation of DCVS.

Recent clinical trials demonstrated successful reduction of DCVS without corresponding improvements in patient outcome [2, 3]. These findings, and a growing body of clinical and experimental evidence, have demonstrated that DCVS is not solely responsible for brain injury following SAH [4-7] and led to increased interest in the phenomenon of early brain injury (EBI) after SAH. The change of focus was interpreted as a paradigm shift from DCVS to EBI [8]. Animal models reflecting the early events after SAH will be of utmost importance in elucidating the complex pathophysiological concepts that are pooled in the umbrella term EBI [9].

The primary aim of this project is to identify and analyse most consistent and reliable acute SAH models, associated parameters and endpoints for the study of EBI after SAH. In addition we aim to update, analyse and complete our existing data collection to generate empirical evidence to define standard experimental parameters for SAH models of DCVS [10]. Implementation of more standardized experimental techniques and associated parameters could increase the comparability among laboratories, facilitate interpretation of results, and increase the relevance of future studies in this field of research. We use a systematic approach to establish a large dataset of experimental studies using models of subarachnoid haemorrhage for both the study of EBI and DCVS.

Search strategy. The literature is searched to identify basic animal models of experimental SAH, their refinements/technical modifications, and studies conducted applying these models. We search EMBASE and PUBMED (from 1969) up to date using the key words “murine”, “rat”, “rabbit”, “canine”, “primate”, “cat”, “pig”, and “goat” in combination with “subarachnoid hemorrhage” using the Boolean operator [AND]. The search will be restricted to “animals”. Two investigators will screen titles and abstracts and provide full text of selected studies. Discrepancies in study selection of these two authors will be discussed with a third author. For identification of various SAH techniques and endpoints used with the animal models, citations from the reference lists are checked for references to additional models.

Eligibility criteria. We consider in vivo experimental mouse, rat, rabbit, cat, dog, pig, goat, and nonhuman primate SAH studies and models investigating EBI injury and DCVS. Studies published in languages other than English, in vitro experiments, studies on extracranial vessels, as well as studies with agents causing brain injury or vasoconstriction other than whole blood are not evaluated.

Analyzed features. From each eligible study we record authors, journal name, publication year, affiliation details (nationality and institute) total animal sample size, experimental purpose (study of pathophysiology or treatment approach), SAH technique and EBI and DCVS monitoring approach, and time course and peak onset of EBI and DCVS and study quality. Many of the
analyzed parameters regarding experimental studies for DCVS after SAH are already recorded for the time period from 1969 – 2007 [10]. Details of analyzed features are outlined as follows:

**Details of animals used:** Age, weight, strain, and sex.

**Details of anesthesia, analgesia, monitoring, postoperative care, and sacrifice:** Anesthesia (injectable (IM, IP, SC, IV single dose, continuous), inhalant, ventilated, spontaneous breathing) use of perioperative analgesia, morbidity and mortality, monitoring depth of anesthesia, oxygen supply y/n, arterial blood gas status (pH, pCO2, pO2, SO2), hematological parameters (Hematocrit, Haemoglobin, Glucose, Na+, K+ ), postoperative pain control, control of body temperature, body weight, heart rate (ECG?), respiratory rate, intracranial pressure (location supra- infratentorial, intraparenchymal, or intraventricular), cerebral blood flow (method, intraparenchymal, surface probe, arterial blood pressure (mean, diastolic systolic), information regarding housing and husbandry (food, light/dark cycle, temperature, food and water ad libitum, specific pathogen free (SPF), breeder mentioned, number of animals/cage). Method of killing (KCl, pentobarbital, others) and perfusion-fixation (perfusion pressure, solution (saline, heparin y/n, followed by formal/paraformalin (percentage), volume).

**Details of SAH induction:** General technique (T1: Intracisternal blood injection, T2: Craniotomy and clot placement, T3: Endovascular vessel puncture, T4: Craniotomy and extravascular vessel puncture, T5: Closed cranium vessel puncture, T6: Closed cranium vessel rupture, T7: Blood shunt), Injection site (supratentorial, infratentorial, transclival, transorbital, angle, depth, stereotaxic coordinates, anatomical landmarks), injection device (tube, type of needle, size), open/transcutaneous blood injection, injection time, manual/pump injection, injection pressure, injection volume (weight adapted, fixed volume), time frame between multiple injections, heparinized/non-heparinized or arterial/venous blood, head tilting position after blood injection (angle, duration), filament size.

**Details of endpoints:** General method (M1: Histology and cast method, M2: Angiography, M3: Cerebral blood flow measurement, M4: Direct observation, M5: Cell death and Degeneration, M6: Cortical spreading depolarization, M7: SAH grading, M8: Neurological impairment). Digital subtraction angiography, magnetic resonance imaging, computed tomography, histological analysis: cross section diameter, thickness of the wall, and cross section area of BA, MCA, ACA, corrugation coefficient of internal elastic lamina, endothelial detachment. DCVS grading (time-point), grading no/mild, moderate, and severe vasospasm (percentage of baseline), transcranial Doppler, Clinical outcome evaluation incl. time point of assessment (early, late, neurobehavioral testing, animal specific, neurological deficits grading scale), SAH bleeding/grading scale (time point), apoptotic index neuronal/endothelial cells (time point after SAH, percentage, quantitative or semi-quantitative, grading, regions hippocampus (CA1-CA3), cortex, cerebellum, cerebral arteries, micro-thromboembolism (quantitative or semi-quantitative, regions hippocampus (CA1-CA3, dentate gyrus, other regions?), cortex, cerebellum), BBB (Evans Blue, semi-quantitative), and brain water content (percentage), mortality and morbidity.

**Details of study quality:** Publication in peer-reviewed journal, random allocation to experimental groups, monitoring of physiologic parameters, blinded outcome assessment, group size based on a-priori power calculation, report of eligibility criteria and drop-outs, compliance with animal welfare regulations, statement of potential conflict of interests.
References